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Title: Effects of Delayed Winter Harvest on Biomass Yield and Quality of Napiergrass and
Energycane

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Abstract

Napiergrass (*Cenchrus purpureus* (Schumach.) Morrone) and energycane (*Saccharum* hyb.) are perennial grasses that are well-suited for biomass production in the southeastern USA. The purpose of this study was to determine the effects of delayed winter harvest on biomass yield and quality of these grasses. The study was conducted on two adjacent sites near Midville, GA. Each site used a split-plot design with four replications, with species as the main plot, and harvest times (December, January, or February) as sub-plots. Dry matter (DM) yields were measured by mechanical harvesting, and a sample of biomass was taken from each harvest for determination of ethanol production by simultaneous saccharification and fermentation (SSF). Biomass moisture, N, P, K, and ash mass fractions were also measured. Energycane DM yields were stable from December (46.8 Mg ha⁻¹) to January (42.9 Mg ha⁻¹), but then declined (36.8 Mg ha⁻¹), while napiergrass yields declined sharply from December (47.0 Mg ha⁻¹) to January (35.0 Mg ha⁻¹). Napiergrass moisture mass fraction was reduced by an average of 18% in February harvests compared to December. Mass fractions of N, K, and ash tended to decrease with later harvesting, but sometimes increased due to changes in biomass composition. Delaying harvest of napiergrass from December to January reduced N removal by an average of 144 kg ha⁻¹, while delaying harvest of energycane to February reduced N removal by an average of 54 kg ha⁻¹. In SSF, later-harvested energycane produced less ethanol per unit of DM while napiergrass was less affected by harvest date.

Keywords: simultaneous saccharification and fermentation; cellulosic ethanol; nitrogen; *Cenchrus purpureus* (Schumach.) Morrone; *Saccharum* hyb.

Abbreviations:

46 DM – dry matter

47 SSF – simultaneous saccharification and fermentation

48

49 **1. Introduction**

50 Napiergrass (*Cenchrus purpureus* (Schumach.) Morrone; formerly *Pennisetum*
51 *purpureum* Schumach.) is a highly productive perennial bunch grass with excellent potential as
52 an energy-producing biomass crop for the southeastern USA [1]. Though it is of tropical origin,
53 several selections have been identified which are able to overwinter successfully in warmer
54 temperate regions where frost kills the above-ground portion of the plant but does not kill the
55 underground rhizomes and roots. The cultivar Merkeron, for example, which was developed at
56 Tifton, GA, has withstood temperatures as low as -18°C [2]. Energycanes are also highly
57 productive bunch grasses with good biomass potential in this region. As hybrids of sugarcane
58 (*Saccharum* spp.) and related species, they were developed specifically for bioenergy purposes.
59 They tend to have greater cold tolerance, a higher proportion of fiber, and a lower proportion of
60 free sugars in the stems than commercial sugarcanes [3]. Both napiergrass and energycane
61 should be suitable for cellulosic ethanol production or for thermal energy applications, but
62 production systems for these crops are still being developed. Relatively little information is
63 available on how different production practices will affect yields and biomass quality of these
64 grasses for specific applications.

65 To maximize yield and stand persistence, Calhoun and Prine [4] suggest that a biomass
66 production system utilizing napiergrass should involve a single harvest at the end of the growing
67 season. A recent study by Na et al. [5] showed that an additional midsummer harvest of
68 energycane or napiergrass is possible, but that the two-harvest system resulted in higher nutrient
69 removal and greater biomass moisture content. The midsummer harvest also caused decreased

stand persistence, with energycane showing greater stand reduction than napiergrass. Thus, for both of these grasses, harvest would most often occur in the late fall or winter after active growth has stopped, or after dormancy has been induced by cold temperatures. Over the winter months the standing biomass can be stored in the field, allowing flexibility in harvest timing and utilization of the biomass. Delaying the harvest may offer additional benefits in biomass quality. Late winter or spring harvest has been shown to greatly reduce biomass moisture mass fraction in several perennial species including reed canarygrass (*Phalaris arundinacea* L.) [6,7], switchgrass (*Panicum virgatum* L.) [8], and *Miscanthus* $\times$ *giganteus* [9] in colder climates. Knoll et al. [10] however reported only a modest reduction in moisture mass fraction of energycane with late winter harvest in Georgia, USA.

Delayed harvest may also reduce the mass fractions of ash and nutrients such as N and K, which improves the quality of the biomass for combustion [11]. For example, Burvall [12] observed that in spring-harvested reed canarygrass, ash mass fraction tended to decrease and ash fusion temperature tended to increase, indicating an improvement in combustion properties. In the fall during senescence, grasses such as switchgrass [13] and *Miscanthus* [14] actively translocate N into the rhizomes. After senescence, various minerals including K are lost from the standing biomass through leaching and leaf drop [6,8,9,14], which also improves the nutrient efficiency of biomass production.

Despite the potential improvements in biomass quality for combustion, one important disadvantage of delayed harvest of perennial grasses is the high potential for loss of total dry matter yield, primarily in the leaf fraction, during the winter. Winter yield reductions have been reported in reed canarygrass [7,15] and *Miscanthus* [14]. Significant losses were also reported in delayed harvests of switchgrass, primarily due to lodging which hindered mechanical harvesting

[8]. However, in that study lodging was primarily due to snowfall over the winter, which is rarely a concern in regions where napiergrass and energycane can be grown. Information is needed for napiergrass and energycane to determine the extent of the tradeoff between improved biomass quality for combustion or fermentation and total dry matter yield losses due to delayed harvest. A recent study by Knoll et al. [10] reported changes in energycane biomass quality with delayed harvest, but yield losses were not quantified due to small plot size. Na et al. [16] recently reported that delayed winter harvest of energycane did not result in significant yield loss, but an average of 30% yield loss was observed for delayed harvest of napiergrass.

The purpose of this study was to determine the effects of delayed winter harvest on the quantity and quality of harvestable biomass of napiergrass and energycane. Cellulosic ethanol production from these grasses harvested at different times was measured directly in the laboratory using a simultaneous saccharification and fermentation (SSF) procedure. The effects of delayed harvest on nutrient removal by these crops were also investigated.

2. Materials and methods

2.1. Experimental design and study site

This study was conducted at the Southeast Georgia Research and Education Center near Midville, GA (32°52'36"N, 82°12'33"W). The soil at this site is a Dothan loamy sand (Fine-loamy, siliceous, thermic Plinthic Kandiudults). On 30 Oct 2008 stem cuttings of napiergrass cultivar Merkeron [2] and energycane L79-1002 [17] were planted horizontally at a depth of 10 cm [18]. The entire field was irrigated during plot establishment, and then the field was divided into two adjacent locations (Site 1 and Site 2). Site 1 was maintained as dryland, while Site 2 received irrigation. Irrigation at Site 2 began in April, around the time the grasses began to show

substantial growth, and ended in October. Irrigation was applied at a rate of approximately 19–25 mm per week unless sufficient rainfall was received. From April through November Site 1 received 594, 420, and 722 mm of rainfall in 2010, 2011, and 2012 respectively. Average rainfall for this period is 737 mm at this location [19]. Adding rainfall and irrigation, Site 2 received a total of 945, 958, and 855 mm rainfall equivalent in the 2010, 2011, and 2012 growing seasons, respectively. Nitrogen fertilizer in the form of granular NH_4NO_3 was surface-applied each year in early spring, around the time the grasses began to show new growth, at a rate of 112 kg ha^{-1} of N. In order to simulate a low-input production system, no P or K was applied during the study period.

Each location was laid out in a split plot design with four replications. Species was the main plot, and harvest time was the subplot. Each main plot consisted of eight rows, 9 m long and 1.8 m apart, with the outermost rows serving as borders. Beginning in December 2010 subplots of two rows each were harvested in December, January, or February (Year 1). This was repeated in the winters of 2011–2012 (Year 2) and 2012–2013 (Year 3). All harvests took place after the first killing freeze. Exact harvest dates are shown in Table 1. The biomass was harvested mechanically with a Champion C1200 tractor-mounted forage harvester (Kemper, Stadthohn, Germany). The fresh yields were recorded using a Cibus TRM tractor-mounted weighing system (Wintersteiger USA, Salt Lake City, UT). A sample (approx. 1 kg) from each subplot was weighed fresh, dried to constant weight in an oven at 60°C , and weighed again to determine dry matter (DM) and moisture mass fractions. The dried samples were then ground in a Wiley mill to pass a 2-mm screen for further analyses.

(Insert Table 1)

2.2. Nutrient analysis of biomass

Mass fractions of nutrients in dried, ground biomass samples were determined at the University of Georgia Agricultural and Environmental Services Laboratories (AESL). Nitrogen mass fraction was determined by dry combustion, and P and K mass fractions by inductively-coupled plasma (ICP) spectrometry. Total nutrient removal was calculated by multiplying nutrient mass fraction by DM yield. Ash mass fraction was determined by weighing a sample of dry biomass, combusting the sample in a Muffle furnace for six hours at 450°C, and then weighing the remaining ash.

2.3. Biomass pretreatment and simultaneous saccharification and fermentation (SSF)

Conversion of biomass to ethanol was conducted by using a benchtop dilute acid pretreatment and SSF procedure as described by Doran-Peterson et al. [20] with minor modifications. Samples of biomass were dried overnight in an oven at 70°C to determine exact moisture content to correct for moisture absorbed during storage (usually around 50 g kg⁻¹). Two grams DM were then placed in a 125-mL Erlenmeyer flask with 10 mL dilute sulfuric acid (17.5 g L⁻¹ H₂SO₄). Based on moisture content of the biomass, enough water was added to make a total volume of 14.7 mL. Each flask was capped with a rubber stopper, which was vented with a hypodermic needle. This was then autoclaved for 1 h at 121°C (liquid cycle). Each pretreatment/SSF was carried out in duplicate. After pretreatment, samples were cooled to room temperature and then the pH was adjusted to 5.0 using approximately 1.7 mL Ca(OH)₂ suspension (100 g L⁻¹) and 0.6 mL 1 mol L⁻¹ citric acid. The pH was checked using pHdriion paper (Micro Essential Laboratory, Inc., Brooklyn, NY), and any further pH adjustments were

made using drops of 5 mol L⁻¹ KOH or HCl. Enzymes were mixed with 10 × YP broth (2 mL flask⁻¹) and then filter-sterilized with a disposable filter system (Corning, Inc., Corning, NY). Each SSF reaction contained 10 filter paper units Celluclast 1.5L cellulase (Novozymes, Franklinton, NC) and 120 cellobiase units Novo 188 cellobiase (Novozymes). Activity of the cellulase was determined using the procedure described by Adney and Baker [21]. Activity of the cellobiase was obtained from the manufacturer. Xylose-fermenting yeast (*Saccharomyces cerevisiae*) strain YRH400 [22] was added to an OD₆₀₀ of 0.5, and a final fermentation volume of 20 mL. The SSF's were placed in an incubator/shaker at 30°C with constant agitation at 150 rpm. Samples (approx. 1 mL) to quantify ethanol were taken every 24 h. Samples were centrifuged to remove insoluble material, and then the supernatant was filtered through a CoStar Spin-X microcentrifuge tube 0.22 µm nylon filter (Corning, Inc.) to remove all remaining yeast cells and to stop fermentation. Samples were then immediately frozen (-20°C) until analysis.

2.4. Quantification of ethanol

Ethanol was quantified by mixing equal volumes of filtered sample with a 20 mL L⁻¹ aqueous solution of isopropanol (internal standard). One to two microliters of this mixture was injected into a GC-17A gas chromatograph (Shimadzu Corp., Kyoto, Japan) fitted with a DB-WAX column (30 m × 0.53 mm ID, 1 µm film thickness; Agilent Technologies, Inc., Santa Clara, CA), with a constant oven temperature of 50°C, injector and detector temperatures of 230°C, and a column flow rate of 2.8 mL min⁻¹ He. The peak area ratio of ethanol:internal standard was used for ethanol quantification based on a standard curve from known concentrations of ethanol. Most SSF reactions reached maximum ethanol concentration by 48 h of fermentation. Some increased slightly at 72 h. The maximum ethanol concentration (g L⁻¹)

from each reaction was used for analysis after converting to g kg⁻¹ (DM basis). These values were multiplied by DM yields to estimate per-hectare ethanol production.

2.5. Data analysis

Data were analyzed in SAS v. 9.2 (SAS Institute, Cary, NC) using the GLIMMIX procedure. Year, location, species, and harvest date were included in the model as fixed effects with all possible interactions. Replication, nested within location, was included as a random effect. Individual plots were identified as subjects in the RANDOM statement to account for repeated measures, and the default covariance structure (VC) was used. To correct for overdispersion, yield, nutrient removal, and NUE data were log-transformed using the DIST=LOGNORMAL option in the MODEL statement. The SLICE and SLICEDIFF options were used to compare treatments within year, species, etc. Significant differences were determined at $\alpha \leq 0.05$, adjusted for multiple comparisons using the TUKEY option. When data were transformed, comparisons were made on the log scale and then the means were back-transformed.

3. Results

3.1. Biomass yield

In Year 1, there was no difference in yields between the two sites, but in subsequent years Site 2 produced more biomass than Site 1. In Year 1, energycane produced an average of 57.3 Mg ha⁻¹ DM in the December harvest, while napiergrass produced 46.0 Mg ha⁻¹ DM in the same harvest. In Year 2, energycane yielded 57.4 Mg ha⁻¹ DM in December at Site 2 and only 26.7 Mg ha⁻¹ at Site 1. Likewise napiergrass also yielded less at Site 1, but the difference between

sites was not as great (54.0 vs. 41.2 Mg ha⁻¹ DM at Sites 2 and 1, respectively). A similar pattern was observed in the Year 3 (Fig. 1). In general, more biomass was recovered in the December harvests than in later harvests, but the two species responded somewhat differently to delayed harvest. Energycane harvests in January were not significantly lower than those in December, but February harvests were lower than in December. In contrast, napiergrass biomass yields tended to decrease sharply between December and January, but then did not decrease further in February (Fig. 1). The average loss of harvestable biomass between December and February was 21.4% for energycane and 25.9% for napiergrass.

(Insert Fig. 1)

3.2. Biomass moisture mass fraction

Overall, location had little to no effect on the moisture mass fraction (w_{H_2O}) in the biomass. However, with the exception of energycane in Year 1, harvest timing generally showed a strong effect. In Year 1, the mean w_{H_2O} of energycane was 633 g kg⁻¹ throughout the harvest season. In Year 2, the w_{H_2O} of energycane biomass did not change between the first and second harvests, but then decreased between the second and third harvests (Fig. 2). In Year 3, the w_{H_2O} of energycane biomass actually increased between December (548 g kg⁻¹) and January (587 g kg⁻¹), and then did not change. Napiergrass showed a steady decline in w_{H_2O} in Year 1, but in Years 2 and 3 the w_{H_2O} of napiergrass increased between the December and January harvests by 35 – 40 g kg⁻¹, and then rapidly declined between the January and February harvests (Fig. 2). Averaged across years, in February harvests, napiergrass biomass contained less moisture than energycane (531 vs. 614 g kg⁻¹, respectively).

(Insert Fig. 2)

3.3. Biomass nutrient and ash mass fractions

Biomass N mass fraction (w_N ; dry basis) varied from year to year, and was sometimes affected by location. In Year 1, location had no effect but in Year 2, w_N was higher at Site 1 for both species. In Year 3, location did not affect napiergrass but energycane again had higher w_N at Site 1. Location did not show a significant interaction effect with harvest time. In the first two years harvest time had no effect on the w_N of energycane biomass, while that of napiergrass decreased from December to January by $2.05 \text{ g kg}^{-1} \text{ DM}$ and $1.68 \text{ g kg}^{-1} \text{ DM}$ in Years 1 and 2, respectively (Fig. 3). In Year 3, w_N of napiergrass showed a steady decrease from December through February ($-1.02 \text{ g kg}^{-1} \text{ DM}$), while the w_N of energycane biomass increased by $2.64 \text{ g kg}^{-1} \text{ DM}$ during the same time (Fig. 3).

In Year 1 napiergrass showed a slight decrease ($-0.203 \text{ g kg}^{-1} \text{ DM}$) in P mass fraction (w_P) from December through February, but in general, w_P was unaffected by harvest time (data not shown). Across all treatments, napiergrass was found to contain slightly greater w_P than energycane (0.975 vs. $0.879 \text{ g kg}^{-1} \text{ DM}$, respectively; data not shown). Likewise, the average K mass fraction (w_K) of napiergrass was greater than that of energycane (1.52 vs. $1.25 \text{ g kg}^{-1} \text{ DM}$, respectively). Energycane w_K was also unaffected by harvest timing in all three years of the test. Napiergrass w_K was only affected in Years 1 and 3. In Year 1 a clear trend of decreasing w_K from December through February was observed. The total decrease was $0.58 \text{ g kg}^{-1} \text{ DM}$. The magnitude of the effect in Year 3 was less, decreasing only $0.23 \text{ g kg}^{-1} \text{ DM}$ from January to February (Fig. 3).

(Insert Fig. 3)

Mass fraction of total ash (w_{ash}) in energycane biomass was not affected by harvest time in Years 1 and 2. In Year 3 there was a slight decrease from December through February from 47.7 to 42.1 g kg⁻¹ DM (Fig. 4). In napiergrass, w_{ash} was affected by harvest time in Years 1 and 3, but different trends were observed. In Year 1, it decreased from 59.4 g kg⁻¹ in December to 44.9 g kg⁻¹ in January. In Year 3, w_{ash} increased from December (46.2 g kg⁻¹) to January (56.0 g kg⁻¹), and then decreased in February (49.2 g kg⁻¹; Fig. 4).

(Insert Fig. 4)

3.4. Biomass nutrient removal

Removal of N in harvested biomass did not differ between locations in Year 1 for either species. In subsequent years, despite the fact that biomass yields were generally lower at Site 1, total N removal was not generally different between locations. The only significant difference was in Year 2, where energycane harvest removed 49.3 kg ha⁻¹ more N at Site 2 than at Site 1 (average of three harvests). Harvest timing had a larger effect on napiergrass than on energycane with respect to N removal. In all three years, N removal by napiergrass dropped sharply between December and January, and then did not change. On average the change was -144.1 kg ha⁻¹ of N (-39.9%). In Years 1 and 2, N removal by energycane decreased between December and February, by an average of -53.7 kg ha⁻¹ of N (-23.9%), but harvest time did not affect N removal by energycane in Year 3. In December, napiergrass harvest removed more N than energycane

(361.3 vs. 220.9 kg ha⁻¹, respectively), but by February harvest napiergrass N removal dropped to 213.1 kg ha⁻¹, not statistically different from that of energycane in December (Table 2).

Total P removal was generally greater at Site 2 than at Site 1, except for napiergrass in Year 1, where P removal at the two locations was similar. Removal of P in harvested biomass generally decreased from December through February for both species, though the harvest effect was not significant for energycane in Year 2. Both grasses removed an average of 43.8 kg ha⁻¹ of P in December harvests, and an average of 31.2 kg ha⁻¹ of P in February.

In Year 2, harvest time did not affect K removal by energycane, but it was greater at Site 2 than at Site 1. In December harvests, napiergrass removed more K than energycane (763.5 vs. 579.6 kg ha⁻¹ of K, respectively). Although K removal of both grasses tended to decrease with harvest date, the effect was stronger for napiergrass. Potassium removal did not tend to differ between species for January and February harvests (Table 2).

(Insert Table 2)

3.5. Ethanol production

In Years 1 and 2, harvest date did not affect the efficiency of SSF to convert napiergrass biomass to ethanol, but in Year 3, delayed harvest reduced the yield of ethanol from 124.8 g kg⁻¹ in December to 107.8 g kg⁻¹ in February (Fig. 5). Conversion of energycane biomass to ethanol was more strongly affected by harvest date than was napiergrass; however, in Year 2 the effect was lower than in Years 1 and 3 (Fig. 5). In Years 1 and 3, December-harvested energycane biomass produced over 100 g kg⁻¹ ethanol, while February-harvested energycane biomass only produced approximately 89.5 g kg⁻¹.

(Insert Fig. 5)

Combining biomass yields with ethanol production in the laboratory, per-hectare ethanol yields for both species were greatest in December and lowest in February. Across years, sites, and species the mean ethanol yields were 6,483; 5,283; and 4,340 L ha⁻¹ for December, January, and February, respectively. Ethanol yield estimates for energycane ranged from 2,655 L ha⁻¹ (Year 2, Site 2, Feb) to 9,223 L ha⁻¹ (Year 1, Site 2, Dec). Napiergrass could produce between 3,826 L ha⁻¹ (Year 1, Site 1, Feb) and 8,066 L ha⁻¹ (Year 3, Site 2, Dec; Fig. 6).

(Insert Fig. 6)

4. Discussion

Loss of harvestable biomass over the winter months showed a slightly different pattern between the two species. Our visual observations suggested that napiergrass drops its leaves soon after senescence, while those of energycane tend to be retained for a longer time. Though amount of dropped leaves was not directly measured, the substantial decrease from December to January in harvested standing biomass of napiergrass supports these visual observations. In a similar study in Florida, Na et al. [16] measured the leaf to stem ratio in delayed harvests of napiergrass and energycane and found that energycane had a greater proportion of leaves than napiergrass in one of two years, but that both species lost leaves as harvest was delayed. Decreases in the quantity of biomass combined with decreases in the fermentability of that biomass suggest that there is no advantage in delaying harvest past December. If there were a

substantial decrease in biomass moisture mass fraction over the winter, then perhaps the decreased yields would be economically acceptable as transportation of wet biomass would be expensive. Deciduous grasses such as switchgrass can dry considerably prior to harvest during the winter season [8], due to natural post-flowering senescence and thinner hollow stems, but energycane and napiergrass are forced into senescence by the onset of freezing temperatures, and they have thick, full stems which prevent substantial drying. Consistent with our results Na et al. [16] reported an increase in DM concentration, or decrease in moisture, of napiergrass biomass in only one out of three winters in their study, and only a slight increase in DM concentration for energycane. Even in February harvests, we observed that over half of the fresh mass of these grasses is water, in agreement with previous observations in a set of nine energycane genotypes [10]. The moisture mass fraction of napiergrass actually increased slightly between December and January in two out of three years, and this was also observed in Year 3 for energycane between January and February. This could be explained by a decrease in the proportion of leaves in the standing biomass, leaving a higher proportion of stalks, which contain more water than leaves, or it could also be due to absorption of water from rain or dew.

In general, delayed harvest caused a decrease in the mass fraction of N, K, and total ash, or had no effect. These results are expected, but occasionally the apparent mass fractions of these components increased. For example, in Year 3, w_{ash} of napiergrass increased from December to January by 9.76 g kg^{-1} . This could possibly be explained by the change in leaf to stem ratios observed during this same time period, which could alter the composition of the biomass. However, leaves typically contain a higher concentration of mineral nutrients than stems in other biomass crops [23]. Further study may be needed to characterize the partitioning of minerals in napiergrass. Likewise, w_{N} of energycane increased between January and

February, also in Year 3, by 2.11 g kg^{-1} . A previous study of energycane [10] showed that a decrease in the free sugar content of the standing biomass caused by fermentation from naturally occurring yeast and bacteria can also cause an apparent increase in the proportion of other components such as N. Although L79-1002 is a high-fiber Type II energycane [24], it still contains a considerable amount of fermentable free sugars [17]. The decrease in fermentability of the energycane biomass with later harvest supports this conclusion.

Major nutrients such as N, P, and K that are removed from the soil by the crop are usually replaced as fertilizer, which adds to the overall cost of production. Delaying harvest may allow some of these nutrients to translocate to the roots or recycle in the leaf litter, possibly benefiting subsequent crops. . On average, delaying energycane harvest from December to February only reduced N removal by about 25 kg ha^{-1} . However, delaying napiergrass harvest to February reduced N removal by approximately 148 kg ha^{-1} , averaged across years and sites. Delaying the harvest of energycane to February reduced removal of P and K by approximately 10 kg ha^{-1} and 147 kg ha^{-1} , respectively, while delaying harvest of napiergrass reduced nutrient removal by 15 kg ha^{-1} for P and 280 kg ha^{-1} for K.

4.1. Conclusions

Napiergrass and energycane are highly productive grasses for bioenergy production in the southeastern USA. Standing biomass of these grasses can be stored in the field into the winter months, but some loss of total yield should be expected with delayed harvest. Only modest reductions in moisture mass fraction were observed with delayed harvest, consistent with a previous study in energycane [10]. Biomass mass fractions of N, K, and ash usually did not change or decreased with later harvest. Occasionally, increases in N, K, or ash mass fractions

were observed likely due to changes in biomass composition from leaf drop or free sugar degradation. Delayed harvest of napiergrass reduced N removal considerably more than for energycane, potentially recycling some of this N for the subsequent crop. Some decreases in the ability of energycane biomass to convert to ethanol by SSF were observed in later harvests, while conversion of napiergrass to ethanol was less affected by harvest date.

Disclaimer

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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Figure Captions:

Fig. 1 – Dry matter (DM) yields of **(a)** energycane and **(b)** napiergrass grown at two adjacent sites near Midville, GA over three years, harvested in December, January, or February. Within sites/years, means with the same letter are not significantly different.

Fig. 2 – Biomass moisture mass fraction (w_{H_2O}) of **(a)** energycane and **(b)** napiergrass harvested near Midville, GA in December, January, or February over three years. Error bars represent one standard error of the mean.

Fig. 3 – Dry biomass mass fractions of **(a,b)** N and **(c,d)** K for **(a,c)** energycane and **(b,d)** napiergrass, harvested in December, January, or February near Midville, GA over three years. Error bars represent one standard error of the mean.

Fig. 4 – Dry biomass ash mass fraction (g kg^{-1} DM) of **(a)** energycane and **(b)** napiergrass harvested in December, January, or February over three years. Error bars represent one standard error of the mean.

Fig. 5 – Ethanol yield (g kg^{-1} DM) from simultaneous saccharification and fermentation (SSF) of **(a)** energycane and **(b)** napiergrass biomass harvested in December, January, or February over three years. Error bars represent one standard error of the mean.

Fig. 6 – Per-hectare ethanol yields of **(a)** energycane and **(b)** napiergrass grown at two adjacent sites near Midville, GA over three years, harvested in December, January, or February. Within sites/years, means with the same letter are not significantly different.

Table 1. Dates of first freeze and biomass harvests at Midville, GA over the three-year study.

	Year 1	Year 2	Year 3
First freeze	Nov. 7, 2010	Nov. 11, 2011	Nov. 25, 2012
Harvest 1	Dec. 8, 2010	Dec. 8, 2011	Dec. 19, 2012
Harvest 2	Jan. 14, 2011	Jan. 12, 2012	Jan. 16, 2013
Harvest 3	Feb. 24, 2011	Feb. 8, 2012	Feb. 20, 2013

Table 2. Annual nutrient removals (N, P, and K), with respect to DM or ethanol production, based on biomass harvest time (December, January, or February) for energycane and napiergrass, averaged across two sites near Midville, GA over three years.

Harvest	Energycane			Napiergrass		
	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
	N removal (kg ha ⁻¹)					
Dec	229.8 a	220.0 a	212.9 a	331.6 a	407.8 a	344.4 a
Jan	200.9 a	198.2 ab	223.1 a	188.6 b	219.8 b	243.1 b
Feb	166.4 b	176.0 b	244.7 a	167.4 b	249.5 b	222.2 b
	P removal (kg ha ⁻¹)					
Dec	46.5 a	36.1 a	38.2 a	45.9 a	49.5 a	46.7 a
Jan	36.5 b	35.3 a	35.6 a	31.7 b	31.2 b	38.0 ab
Feb	31.0 b	32.8 a	26.9 b	26.6 b	37.2 b	32.8 b
	K removal (kg ha ⁻¹)					
Dec	765.9 a	529.0 a	443.8 a	809.0 a	852.0 a	629.5 a
Jan	628.7 b	521.3 a	423.9 a	518.1 b	551.3 b	512.3 b
Feb	504.2 c	471.6 a	322.4 b	389.5 c	647.2 b	415.4 c

Within columns, means with the same letter are not significantly different.

a

b

a **b** **c** **d**

a

b