Harvest management affects biomass composition responses of C4 perennial bioenergy grasses in the humid subtropical USA

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Abstract

Elephantgrass (Pennisetum purpureum Schum.) and energycane (Saccharum spp. hybrid) are high-yielding C4 grasses that are attractive biofuel feedstocks in the humid subtropics. Determining appropriate harvest management practices for optimal feedstock chemical composition is an important precursor to their successful use in production systems. In this research, we have investigated the effects of harvest timing and frequency on biomass nutrient, carbohydrate and lignin composition of UF1 and cv. Merkeron elephantgrasses and cv. L 79-1002 energycane. Biomass properties under increased harvest frequency (twice per year) and delayed harvest (once per year after frost) were compared with a control (once per year prior to frost). There were no differences between elephantgrass entries in structural carbohydrates; however, elephantgrasses had greater structural hexose concentration than energycane for single-harvest treatments (avg. 398 vs. 366 mg g$^{-1}$), a trait that is preferred for biofuel production. Delayed harvest of energycane decreased structural hexose compared with the control (374 vs. 357 mg g$^{-1}$) because nonstructural components accumulated in energycane stem as harvest was delayed. Frequent defoliation (2X) increased N, P, and ash concentrations (75% for N and P and 58% for ash) in harvested biomass compared with single-harvest treatments. We conclude that multiple harvests per year increase the harvest period during which feedstock is available for processing, but they do not result in optimal feedstock composition. In contrast, extending the period of feedstock supply by delaying a single harvest to after first freeze did not negatively affect cell wall constituent properties, while it increased length of the harvest period by ~30 days in the southeast USA.

Keywords: biofuel, elephantgrass, energycane, fiber composition, lignin, nitrogen, Pennisetum purpureum, Saccharum spp., structural carbohydrates

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Introduction

Plant structural biomass consists primarily of three major types of polymers: cellulose, hemicellulose, and lignin. These polymers are strongly bonded by noncovalent forces and by covalent cross-linkages (Perez et al., 2002). The cell wall is composed of long crystalline cellulose microfibrils embedded in a matrix of other polysaccharides (Perez et al., 2002). In grasses, the predominant hemicellulosic polysaccharides in cell walls are glucuronorhamnoglucomannan that have a xylose backbone with arabinose and acetyl substitutions and constituents (Vermerris, 2008). Given the abundance of cellulose and hemicellulose in cell walls of perennial grasses, they represent a major source of structural carbohydrates for conversion to bioenergy. Lignin is the third major component of plant structural biomass and limits not only the physical accessibility of cellulose and hemicellulose, but also the activity of cellulolytic enzymes (Jung et al., 2013). This occurs as a result of multiple factors, including shielding cellulose from microbial degradation by providing a surface that cellulolytic enzymes adsorb to irreversibly (Akin, 2007; Vermerris, 2008).

The proportions of cellulose and lignin in biomass affect the yield of biochemical conversion processes. For instance, due to the high lignin concentration in wood, more ethanol can be produced from switchgrass (Panicum virgatum L.) than that from the same weight of wood biomass (McKendry, 2002). Actual concentrations of monomers from cellulose and hemicellulose can be analyzed by procedures established by the National Renewable Energy Laboratory (NREL). These procedures allow for the quantification of sugar monomers from extractives (nonstructural carbohydrates or soluble sugars) and structural carbohydrates, and measurement of acid-soluble and insoluble lignin (Sluiter, 2008a,b).

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The desired composition of biomass for bioenergy is dependent upon the postharvest conversion process used, and in most processes, lower levels of N and ash in the feedstock are preferred (Lewandowski & Heinz, 2003; Waramit et al., 2011). High levels of N and/or ash can reduce thermochemical conversion efficiency and increase wear, while decreasing energy generation when used for co-firing (Shahandeh et al., 2011). Perennial C4 grasses have up to two times greater N-use efficiency than C3 plants (Jakob et al., 2009), and high biomass production with low tissue N concentration makes them important candidate bioenergy grasses.

Harvest management affects not only biomass yield but also its composition (Casler & Boe, 2003; Lewandowski & Heinz, 2003; Adler et al., 2006). Miscanthus (Miscanthus giganteus) and giant reed (Arundo donax L.) showed a gradual decline in stem N concentration with increasing maturity in the United Kingdom (Smith & Slater, 2011). The decline in plant N concentration with increasing maturity has been associated with decreasing leaf proportion and much lower N concentration in stem than in leaves. For instance, average switchgrass leaf N concentration was 13.5 mg g$^{-1}$ compared with 5.7 mg g$^{-1}$ for stem (Shahandeh et al., 2011). Similarly, giant reed and switchgrass leaves contained twofold or greater N when compared with the stem and showed a decline in N and P concentrations from October to December (Kerling et al., 2012).

Elephantgrass (or napiergrass; Pennisetum purpureum Schum.) is indigenous to sub-Saharan Africa in areas of rainfall exceeding 1000 mm. It is in the tribe Paniceae of the Poaceae family (Hanna et al., 2004). Elephantgrass is a robust, creeping rhizomatous plant that perenniates in the tropics and subtropics. Sollenberger et al. (2014) reported that elephantgrass breeding lines developed for biomass averaged 3.77 m in plant height, 271 g in tiller mass, and 7.4 mm in tiller diameter in Florida. Sugarcane (Saccharum spp.) hybrids have a high concentration of cellulose instead of sucrose, and they are a potentially valuable feedstock resource for cellulosic ethanol production. This is why high-cellulose Saccharums are called ‘energycanes (Saccharum spp. hybrid)’ (León et al., 2010). To better understand Saccharum spp. as a biofuel crop, it is useful to note that there are three distinctive types. These include sugarcane (primarily sugar, conventional sugarcane), type I energycane (sugar and fiber), and type II energycane (primarily fiber).

Recent studies on harvest frequency and timing for elephantgrass and energycane grown in Florida, USA, found that two harvests annually negatively affected long-term biomass yield and plant persistence (Na et al., 2015a,b). Harvest management is an important determinant of chemical composition of perennial grasses used for forage (Chaparro & Sollenberger, 1997), and it is reasonable to hypothesize that it affects composition of bioenergy feedstocks as well. Limited data exist describing harvest management effects on chemical composition of perennial grasses used for biofuel feedstocks grown on sandy soils in a subtropical environment. Therefore, the objective of this research was to determine the effect of harvest frequency and timing and grass entry on concentrations of plant structural and nonstructural carbohydrates, lignin, N, P, and ash in harvested biomass.

Materials and methods

Experimental site

The experiment was conducted during 2010 and 2011 at the Plant Science Research and Education Unit (PSREU) at Citra, FL (29.41°N, 82.17°W). The soil was a well-drained Candler sand (hyperthermic, uncoated Lamellic Quartzipsamments). Initial soil characterization (0-20 cm) showed an average soil pH of 7.0, and Mehlich-1 extractable P, K, and Mg of 54, 20, and 123 mg kg$^{-1}$, respectively. These concentrations are considered to be high for P, very low for K, and very high for Mg (Mylavarapu et al., 2009). Monthly average, maximum, and minimum temperatures (Fig. 1) and monthly precipitation (Fig. 2) are shown for the experimental period.

Treatments and experimental design

Treatments included all factorial combinations of three grass entries and three harvest management practices. Each treatment was replicated four times in a split-plot arrangement of a randomized complete block design. Harvest treatment was the main plot and grass entry was the subplot. The three grass entries included two elephantgrasses, cv. Merkeron (Burton, 1989) and a breeding line referred to as UFI1, and cv. L 79-1002 type II energycane (Bischoff et al., 2008). These two species were chosen because earlier work with biomass feedstocks identified them as having the greatest potential in this region (Woodard & Prine, 1993). Merkeron and L 79-1002 were also chosen because they were the cultivars with the largest current presence in the region. Breeding line UFI1 was included because previous research had demonstrated its high yield potential and preferred morphological characteristics (Sollenberger et al., 2014; Na et al., 2015a,b).

Three harvest management treatments were implemented that included different harvest frequency and timing. These were (i) two harvests per year (late July, named 2X-July; November harvest of regrowth after 2X-July harvest, named 2X-Nov), (ii) one harvest per year in fall [at initiation of Merkeron flowering (first entry to flower) and before first freeze; named 1X-Nov], and (iii) one harvest per year in winter (within 1 week following first freeze, with a freeze defined as a temperature of less than 0 °C at 2 m above soil level resulting in

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complete kill of leaves, or at full flowering of Merkeron, whichever happened first; named 1X-Dec). In 2010, harvests occurred on 30 July for 2X-July, 10 November for 1X-Nov and 2X-Nov, and 9 December for 1X-Dec. In 2011, harvest dates were 21 July for 2X-July, 8 November for 1X-Nov and 2X-Nov, and 15 December for 1X-Dec.

Fig. 1 Monthly average and monthly maximum and minimum air temperatures for 2010 and 2011 for the experimental location (available at Florida Automated Weather Network, http://fawn.ifas.ufl.edu), and the 30-year average for Gainesville, Florida (available at Florida Climate Center, http://climatecenter.fsu.edu).

Fig. 2 Monthly rainfall for 2010 and 2011 for the experimental location (available at Florida Automated Weather Network, http://fawn.ifas.ufl.edu), and the 30-year average for Gainesville, Florida (available at Florida Climate Center, http://climatecenter.fsu.edu).
Plot preparation and management

Plots contained six rows, each 6 m long, with 1 m spacing between rows, and were established using aboveground stem pieces planted on December 15, 2009. Thus, the 2010 data are from the establishment year. In both years, N was applied as ammonium sulfate [(NH$_4$)$_2$SO$_4$] at a rate of 150 kg N ha$^{-1}$, and K was applied as muriate of potash (KCl) at a rate of 90 kg K ha$^{-1}$. Nutrients were split applied, with applications of 50 kg N and 45 kg K ha$^{-1}$ in mid-April and 100 kg N and 45 kg K ha$^{-1}$ in mid-May. No P was applied based on soil test results indicating sufficient levels. Limited irrigation was applied to the experiment only at visual signs of significant drought stress, for example, severe leaf rolling. There were five irrigation events in 2010 (total of 60 mm) and three irrigation events in 2011 (total of 50 mm) using a traveling gun irrigation system.

At harvest, four representative tillers (approximately 3 kg fresh weight) were selected from mid-row at least a meter from all borders for determination of plant part proportion, dry matter, and chemical composition of harvested feedstock. Tillers were hand-separated into leaf (blade and sheath) and stem (including inflorescence, if present) components. Leaf : stem ratio data were reported previously (Na et al., 2015b). Samples were dried at 60 °C until constant weight. Stem samples were initially grounded through a hammer mill to reduce particle size. Stem and leaf samples were ground to pass a 1-mm stainless steel screen in a Wiley mill (Model 4 Thomas-Wiley Laboratory Mill, Thomas Scientific, Swedesboro, NJ, USA). Ground samples were transferred to an airtight container immediately.

Biomass fiber analysis

Each component of dried biomass (stem and leaves) was analyzed for chemical composition. A modified NREL procedure was used for compositional analysis (Sluiter, 2008a,b). In the modified NREL procedure, dried biomass was analyzed for nonstructural extractives, structural carbohydrates, and total lignin (Fedenko et al., 2013). For nonstructural extractives, 100 ml of deionized (D.I.) water was added to 1 g of dried sample and autoclaved in sealed pressure tubes (ACE Glass, Inc., Vineland, NJ, USA) at 121 °C for 1 h. Samples were then vacuum-filtered through wet-strengthened 113 filter paper (Whatman, GE healthcare, Little Chalfont, UK). Filtered extractives were collected for nonstructural carbohydrate analysis. The captured structural biomass from extraction was dried at 40 °C and then weighed. A 0.3-g dry subsample was used for two-stage acid hydrolysis; the first stage was a 1-h incubation at 30 °C with concentrated sulfuric acid (72%, Fluka Alaytical; Sigma-Aldrich, St. Louis, MO, USA). In the second stage, sulfuric acid was diluted to 4% by adding D.I. water for 1-h digestion under elevated pressure and temperature (121 °C and 103 kPa). Hydrolyzed samples were vacuum-filtered through a medium-porosity filtering crucible (Coors #60531; CoorsTek, Golden, CO, USA). Two aliquots of filtrate were taken, with one analyzed in duplicate for acid-soluble lignin at a wave-length of 240 nm using a UV–vis spectrophotometer (StellarNet, Inc., Tampa, FL, USA). The second aliquot collected from hydrolyzed samples was neutralized to pH 5.7 using calcium carbonate. Insoluble lignin was determined gravimetrically as total solids remaining in the crucible after vacuum filtration of hydrolyzed biomass. Total lignin was calculated as the sum of acid-soluble and insoluble lignin. Extractives and neutralized hydrolyzed samples were filtered through a 0.22-μm syringe filter and analyzed by HPLC (Perkin-Elmer Flexar system, Waltham, MA, USA) using a refractive index detector and a Bio-Rad Aminex HPX-87H column (300 × 7.8 mm) maintained at 50 °C. Sulfuric acid (HPLC grade, 4 ms) was used as the mobile phase at a flow rate of 0.4 ml min$^{-1}$ with a 10-μl injection and 40-min run time. Perkin-Elmer’s Chromera software was used to quantify peaks (Fedenko, 2011).

Extractives are considered to be the sum of all nonstructural components of plant tissue removed during extraction, of which soluble sugars are a major component. They were determined on a mass balance basis as the difference between initial sample weight and sample weight after hot water extraction (Sluiter, 2008a). Hexoses and pentoses are the sum of six-carbon (6C; glucose and mannose) and five-carbon (5C; xylose and arabinose) structural carbohydrates, respectively. Mannose was never present at minimum quantifiable levels in this study. Total lignin is the sum of acid-soluble and insoluble lignin.

Total nitrogen, phosphorus, and ash

For N and P analyses, a modified Kjeldahl procedure was conducted. Samples were digested using a modification of the aluminum block digestion procedure (Gallaher et al., 1975). Sample weight was 0.25 g, catalyst used was 1.5 g of 9:1 K$_2$SO$_4$: CuSO$_4$, and digestion was conducted for at least 4 h at 375 °C using 6 ml of sulfuric acid and 2 ml hydrogen peroxide. Analysis of digestate was carried out using the Technicon Autoanalyzer and semiautomated colorimetry to determine N and P in the digestate (Hambleton, 1977). Absolute dry matter was determined by oven drying at 105 °C to constant weight and then samples were ashed at 500 °C for 6 h to determine ash concentration.

Statistical analysis

Data were analyzed using mixed-model methods in PROC MIXED (SAS Institute, 2008). In all models, harvest treatment and grass entry were considered fixed effects. Year was considered a repeated measurement (fixed). Block and interactions with block were considered random effects. Because there were two harvests per year in the 2X treatment, samples from both harvests were analyzed separately in the laboratory, and the model effectively contains four levels of the harvest-management treatment, that is, 2X-July, 2X-Nov, 1X-Nov, and 1X-Dec. Leaf, stem, and total aboveground biomass data were analyzed separately for each factor. Total aboveground biomass data were calculated as the weighted average of leaf and stem composition for each factor. Means were compared using the pdiff test of LSMEANS. All means reported in the text are least-square means and were considered different if P ≤ 0.05. Because the effects of greatest interest were harvest-management, entry, and their interaction, and because the harvest-management × entry interaction was significant in most cases, data presented are the means for this interaction. If the
interaction was not significant, only main effect means were compared.

Results

Extractives and total soluble sugars

A harvest management × entry interaction occurred (P = 0.044) for leaf extractives because UF1 elephantgrass showed a greater concentration than other entries in the 1X-Dec harvest, but there were no differences between elephantgrass entries for the other harvest treatments (Table 1). Single-harvest (1X-Nov and 1X-Dec) leaf-extractive concentrations were lower than either 2X-July or 2X-Nov for all grass entries. A harvest management × entry interaction occurred for stem extractives (P < 0.001, Table 1). L 79-1002 stem extractives were greatest in 1X-Dec (338 mg g⁻¹) followed by 1X-Nov (313 mg g⁻¹), while elephantgrass entries had greater stem extractives in 2X-July and 2X-Nov treatments (avg. 275 and 268 mg g⁻¹, respectively) than 1X-

Nov and 1X-Dec (avg. 256 and 252 mg g⁻¹, respectively). In 1X-Dec, L 79-1002 showed the greatest stem extractive concentration followed by UF1 (263 mg g⁻¹) and then Merkeron (241 mg g⁻¹). There also was a harvest management × entry interaction (P < 0.001) for extractive concentration in total aboveground biomass. There were no differences among entries within the 2X-Nov harvest, but L 79-1002 had greater extractive concentration than Merkeron for all other harvest treatments and greater than UF1 in both 1X-Nov and 1X-Dec.

Total soluble sugars in leaf tissue extractives showed a harvest management × entry interaction (P = 0.044) because there were no differences among entries within the 2X-July management, but differences among entries occurred for other harvest managements. One of the elephantgrasses always had the greatest concentration, except for 2X-July, when there were no differences among entries (Table 2). In the stem, a harvest management × entry interaction occurred (P < 0.001) for total soluble sugar concentrations (Table 2). For all three entries, 2X-July total soluble sugar concentration was

Table 1  Effect of grass entry and harvest management interaction on extractives in leaf (P = 0.044), stem (P < 0.001), and total above-ground biomass (P < 0.001). Data are means across four replicates and 2 years (n = 8)

| Harvest management* | 2X-July | 2X-Nov | 1X-Nov | 1X-Dec |
|---------------------|---------|---------|---------|--------|
| Entry               | mg extractives g⁻¹ dry matter |
| **Leaf**            |         |         |         |        |
| L 79-1002           | 199 a¹b¹ | 196 aB | 164 bB | 173 bC |
| Merkeron            | 221 aA  | 226 aA | 194 bA | 187 bB |
| UF1                 | 216 aA  | 225 aA | 197 bA | 201 bA |
| SE                  | 4.0     |         |         |        |
| **Stem**            |         |         |         |        |
| L 79-1002           | 302 bcA | 289 cA | 313 bA | 338 aA |
| Merkeron            | 265 aC  | 262 aC | 250 abC| 241 bC |
| UF1                 | 285 ab  | 274 abB| 262 bB | 263 bB |
| SE                  | 7.9     |         |         |        |
| **Total**           |         |         |         |        |
| L 79-1002           | 267 bA  | 251 cA | 269 bA | 289 aA |
| Merkeron            | 251 ab  | 248 abA| 234 bcB| 229 cC |
| UF1                 | 262 aA  | 256 abA| 244 bB | 254 abB|
| SE                  | 6.0     |         |         |        |

Harvest management treatments were harvested twice per year in July and November (2X-July and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze in December (1X-Dec).

Harvest management means within an entry not followed by the same lower case letter are different (P < 0.05).

Entry means within a harvest management not followed by the same upper case letter are different (P < 0.05).

Table 2  Effect of grass entry and harvest management interaction on total soluble sugars concentration in leaf (P = 0.044), stem (P < 0.001), and total above-ground biomass (P < 0.001). Data are means across four replicates and 2 years (n = 8)

| Harvest management* | 2X-July | 2X-Nov | 1X-Nov | 1X-Dec |
|---------------------|---------|---------|---------|--------|
| Entry               | mg total soluble sugars g⁻¹ dry matter |
| **Leaf**            |         |         |         |        |
| L 79-1002           | 23 b¹A¹ | 32 aB  | 14 cB   | 22 bB  |
| Merkeron            | 26 bA   | 40 aA  | 24 bA   | 22 bB  |
| UF1                 | 26 bA   | 35 aAB | 29 abA  | 33 abA |
| SE                  | 2.9     |         |         |        |
| **Stem**            |         |         |         |        |
| L 79-1002           | 152 cA  | 165 cA | 228 bA  | 280 aA |
| Merkeron            | 97 bB   | 134 aB | 138 aC  | 142 aC |
| UF1                 | 138 bA  | 147 bB | 161 bB  | 189 aB |
| SE                  | 11.2    |         |         |        |
| **Total**           |         |         |         |        |
| L 79-1002           | 109 cA  | 110 cA | 163 bA  | 205 aA |
| Merkeron            | 74 cB   | 96 bB  | 106 abC | 116 aC |
| UF1                 | 102 cA  | 106 cAB| 126 bB  | 167 aB |
| SE                  | 6.9     |         |         |        |

Harvest management treatments were harvested twice per year in July and November (2X-July and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze in December (1X-Dec).

Harvest management means within an entry not followed by the same lower case letter are different (P < 0.05).

Entry means within a harvest management not followed by the same upper case letter are different (P < 0.05).
less than 1X-Dec. The magnitude of this difference varied among entries (84% increase in L 79-1002, 51% in Merkeron, and 36% in UF1, respectively). Across treatments, total soluble sugars in the leaves were several times lower than that in the stem (avg. 27 vs. 164 mg soluble sugars g\(^{-1}\)). In total aboveground biomass, there was a harvest management x entry interaction for soluble sugars (Table 2, \(P < 0.001\)). Because the concentration of leaf soluble sugar was very small and because leaves composed a small percentage of harvested biomass, particularly for 1X-Nov and 1X-Dec, total aboveground biomass soluble sugar concentrations generally followed the same pattern as stem. Concentration of total soluble sugars varied mostly among entries for 1X vs. 2X harvest treatments. Interestingly, among elephantgrass entries, UF1 showed 44% greater soluble sugar concentration in total aboveground biomass compared with Merkeron. Further investigation of UF1 soluble sugar concentration is needed to determine the specific sugars present.

**Structural hexose and pentose**

In the leaves, structural hexose concentration was affected by harvest management and entry (\(P < 0.001\) for both). Entry L 79-1002 showed greater hexose concentration than elephantgrass entries by 7% (Table 3). Structural hexose concentrations were greater in 1X-Nov and 1X-Dec followed by 2X-July and then 2X-Nov. In stem, there was a harvest management x entry interaction (\(P = 0.001\)). Stems of elephantgrass entries had greater structural hexose concentration than those of L 79-1002 for all harvest treatments (Table 3). Structural hexose concentration in L 79-1002 was similar for 2X and 1X-Nov treatments, but it was least for 1X-Dec. Merkeron and UF1 had lowest structural hexose concentration in 2X-Nov, while UF1 had greater hexose concentration in 1X-Nov and then 1X-Dec, but for Merkeron, the 1X-Nov and 1X-Dec treatments were not different.

In total aboveground biomass, harvest management x entry interaction occurred (\(P = 0.001\)). For 2X-Nov, there were no differences among grass entries; however, entry L 79-1002 had lower structural hexose concentration than the elephantgrasses in 2X-July, 1X-Nov, and 1X-Dec (Table 3). Comparing harvest mansagements within an entry, both Merkeron and UF1 had greatest hexose concentration in 1X-Nov and 1X-Dec and least in 2X-Nov, while energycane hexose concentration varied relatively little and was greater in 1X-Nov than in 2X-Nov and 1X-Dec.

Concentration of structural pentose in leaves was affected by harvest management (\(P = 0.001\)). The 2X-July treatment had the lowest pentose concentration compared with other treatments, but there were no differences among the other harvests. There was harvest management x entry interaction for stem pentose concentration (\(P = 0.003\)). The most pronounced difference was that 2X-Nov had the greatest pentose concentration among all entries. For both 2X treatments, L 79-1002 had the greatest pentose concentration compared with the two elephantgrasses (Table 4). There were no differences among entries for the 1X-Nov harvest, while for 1X-Dec, Merkeron had greater pentose concentration than L 79-1002. In total aboveground biomass, structural pentose concentration was affected by harvest management and entry (\(P = 0.001, P < 0.001\), respectively). Entry L 79-1002 had greater pentose concentration than the elephantgrasses; however, the differences among entries were relatively small (4–5 mg of pentose g\(^{-1}\)). The 2X-Nov harvest management had greater pentose concentration than the other defoliation treatments.

**Table 3** Effect of grass entry and harvest management interaction on structural hexose concentration in leaf (\(P = 0.562\)), stem (\(P = 0.001\)), and total above-ground biomass (\(P = 0.001\)). Data are means across four replicates and 2 years (\(n = 8\)).

| Entry   | Harvest management* | 2X-July | 2X-Nov | 1X-Nov | 1X-Dec | Mean |
|---------|----------------------|---------|--------|--------|--------|------|
| Leaf    |                      |         |        |        |        |      |
| L 79-1002 | 371 356 404 395 | 381 A\(^\dagger\) |        |        |        |      |
| Merkeron | 346 334 370 369 | 355 B   |        |        |        |      |
| UF1     | 342 333 369 357 | 350 B   |        |        |        |      |
| Mean    | 353 b\(a\) 341 c 381 a 374 a | 5.2     |        |        |        |      |
| SE      |                      |         |        |        |        |      |
| Stem    |                      |         |        |        |        |      |
| L 79-1002 | 363 a\(B\) 361 aB | 361 aB 361 ab 342 bB |        |        |        |      |
| Merkeron | 400 bA 385 cA 415 aA 406 abA | 7.5     |        |        |        |      |
| UF1     | 397 bA 378 cA 413 aA 394 bA |        |        |        |        |      |
| SE      |                      |         |        |        |        |      |
| Total   |                      |         |        |        |        |      |
| L 79-1002 | 367 ab\(B\) 360 bA | 374 aB 357 bB |        |        |        |      |
| Merkeron | 382 bA 365 cA 403 aA 399 aA |        |        |        |        |      |
| UF1     | 381 bA 362 cA 402 aA 389 bA |        |        |        |        |      |
| SE      |                      |         |        |        |        |      |

\(\dagger\)Entry means across harvest management not followed by the same upper case letter are different (\(P < 0.05\)).

\(\dagger\)Harvest management means across entries not followed by the same lower case letter are different (\(P < 0.05\)).

\(\dagger\)Harvest management means within an entry not followed by the same lower case letter are different (\(P < 0.05\)).

\(\dagger\)Entry means within a harvest management not followed by the same upper case letter are different (\(P < 0.05\)).
between Merkeron and UF1 for 1X-Nov; in contrast, interaction occurred because there were no differences either of the 2X harvest managements (Table 5). The biomass had greater stem lignin concentration than action \( \left(202 \text{ mg lignin g}^{-1}\right) \) compared with 1X-Nov and 1X-Dec. Merkeron generally had greater lignin concentration than energycane.

### Nitrogen, phosphorus, and ash
Nitrogen concentration in the leaves was affected by harvest management and entry \((P < 0.001)\) for both. Energycane leaves had a lower N concentration \((9.9 \text{ mg g}^{-1})\) than elephantgrass entries \((average of 11.5 \text{ mg g}^{-1}, \text{ Table 6})\). Leaf N concentration averaged approximately 37% lower for the single-harvest treat-

### Table 4 Effect of grass entry and harvest management interaction on structural pentose concentration in leaf \((P = 0.977)\), stem \((P = 0.003)\), and total above-ground biomass \((P = 0.139)\). Data are means across four replicates and 2 years \((n = 8)\)

| Harvest management* | 2X-July | 2X-Nov | 1X-Nov | 1X-Dec | Mean |
|---------------------|---------|--------|--------|--------|------|
| **Entry** | mg pentose g\(^{-1}\) dry matter | | | | |
| **Leaf** | | | | | |
| L 79-1002 | 252 | 266 | 273 | 272 | 266A |
| Merkeron | 251 | 266 | 271 | 272 | 265A |
| UF1 | 254 | 273 | 279 | 274 | 270A |
| **Mean** | 252 \(b\) | 268 \(a\) | 274 \(a\) | 273 \(a\) | |
| **SE** | 4.0 | | | | |
| **Stem** | | | | | |
| L 79-1002 | 217 \(b\) \(A\) \(^{\dagger}\) | 244 \(a\) \(A\) | 209 \(b\) \(A\) | 202 \(c\) \(B\) | |
| Merkeron | 206 \(b\B\) | 233 \(a\B\) | 209 \(b\A\) | 213 \(b\A\) | |
| UF1 | 206 \(b\B\) | 230 \(a\B\) | 209 \(b\A\) | 209 \(b\AB\) | |
| **Mean** | 222 \(b\) \(^{\dagger}\) | 248 \(a\) | 227 \(b\) | 222 \(b\) | |
| **SE** | 2.9 | | | | |
| **Total** | | | | | |
| L 79-1002 | 229 | 253 | 228 | 222 | 233 \(A\) \(^{\dagger}\) |
| Merkeron | 221 | 246 | 225 | 225 | 229 \(B\) |
| UF1 | 222 | 246 | 228 | 218 | 228 \(B\) |
| **Mean** | 224 \(b\) \(^{\dagger}\) | 248 \(a\) | 227 \(b\) | 222 \(b\) | |
| **SE** | 2.9 | | | | |

\(^{\dagger}\)Harvest management means across entries not followed by the same lower case letter are different \((P < 0.05)\).

\(^{\dagger}\)Harvest management means within an entry not followed by the same lower case letter are different \((P < 0.05)\).

\(^{\dagger}\)Entry means within a harvest management not followed by the same upper case letter are different \((P < 0.05)\).

\(^{\dagger}\)Entry means across harvest managements not followed by the same upper case letter are different \((P < 0.05)\).

### Lignin
Leaf lignin concentration was affected by harvest management \((P = 0.005)\) and entry \((P < 0.001)\). Entry L 79-1002 leaf lignin concentration was greater than in Merkeron and UF1 by 7–8% \((218, 204, \text{ and } 201 \text{ mg lignin g}^{-1}, \text{ respectively}, \text{ Table 5})\). Single-harvest management \((1X-Nov \text{ and } 1X-Dec; 214 \text{ and } 212 \text{ mg lignin g}^{-1}, \text{ respectively})\) had greater leaf lignin concentration than 2X harvest managements \((2X-July \text{ and } 2X-Nov; 204 \text{ and } 202 \text{ mg lignin g}^{-1}, \text{ respectively})\). For stem lignin concentration, there was a harvest management \(\times\) entry interaction \((P = 0.002)\). For all entries, 1X-Nov and 1X-Dec biomass had greater stem lignin concentration than either of the 2X harvest managements (Table 5). The interaction occurred because there were no differences between Merkeron and UF1 for 1X-Nov; in contrast, within the other harvest management treatments, Merkeron had greater stem lignin concentration than UF1 which had a greater lignin concentration than L 79-1002. In total aboveground biomass, a harvest management \(\times\) entry interaction occurred \((P = 0.002)\). Similar to stem lignin concentration, both the 2X harvest managements generally had lesser lignin concentration compared with 1X-Nov and 1X-Dec. Merkeron generally had greater lignin concentration than energycane.

### Table 5 Effect of grass entry and harvest management interaction on lignin concentration in leaf \((P = 0.069)\), stem \((P = 0.002)\), and total above-ground biomass \((P = 0.002)\). Data are means across four replicates and 2 years \((n = 8)\)

| Harvest management* | 2X-July | 2X-Nov | 1X-Nov | 1X-Dec | Mean |
|---------------------|---------|--------|--------|--------|------|
| **Entry** | mg lignin g\(^{-1}\) dry matter | | | | |
| **Leaf** | | | | | |
| L 79-1002 | 213 | 213 | 226 | 221 | 218 \(A\) \(^{\dagger}\) |
| Merkeron | 199 | 197 | 210 | 211 | 204 \(B\) |
| UF1 | 200 | 197 | 204 | 202 | 201 \(C\) |
| **Mean** | 204 \(b\) \(^{\dagger}\) | 202 \(b\) | 214 \(a\) | 212 \(a\) | |
| **SE** | 2.8 | | | | |
| **Stem** | | | | | |
| L 79-1002 | 167 \(b\) \(^{\dagger}\) \(C\) | 167 \(b\C\) | 182 \(a\) \(B\) | 177 \(a\C\) | |
| Merkeron | 184 \(b\A\) | 182 \(b\A\) | 203 \(a\A\) | 206 \(a\A\) | |
| UF1 | 176 \(b\B\) | 174 \(b\B\) | 199 \(a\A\) | 199 \(a\B\) | |
| **Mean** | 183 \(c\) \(^{\dagger}\) | 186 \(b\AB\) | 195 \(a\B\) | 190 \(ab\C\) | |
| **SE** | 3.5 | | | | |
| **Total** | | | | | |
| L 79-1002 | 183 \(c\) \(^{\dagger}\) | 186 \(b\AB\) | 195 \(a\B\) | 190 \(aba\C\) | |
| Merkeron | 189 \(b\A\) | 188 \(b\A\) | 204 \(a\A\) | 207 \(a\A\) | |
| UF1 | 183 \(b\B\) | 183 \(b\B\) | 201 \(a\A\) | 200 \(a\B\) | |
| **Mean** | 187 \(c\) \(^{\dagger}\) | 190 \(b\AB\) | 197 \(a\B\) | 195 \(aba\C\) | |
| **SE** | 2.9 | | | | |

\(^{\dagger}\)Harvest management means across entries not followed by the same upper case letter are different \((P < 0.05)\).

\(^{\dagger}\)Harvest management means within an entry not followed by the same lower case letter are different \((P < 0.05)\).

\(^{\dagger}\)Entry means within a harvest management not followed by the same upper case letter are different \((P < 0.05)\).

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Table 6  Effect of grass entry and harvest management interaction on nitrogen concentration in leaf ($P = 0.334$), stem ($P = 0.942$), and total above-ground biomass ($P = 0.955$). Data are means across four replicates and 2 years ($n = 8$).

| Harvest management* | Entry | 2X-July | 2X-Nov | 1X-Nov | 1X-Dec | Mean |
|---------------------|-------|---------|--------|--------|--------|------|
| Leaf                | L 79-1002 | 12.5 | 12.9 | 7.4 | 6.6 | 9.9 B† |
|                     | Merkeron | 14.1 | 14.2 | 9.5 | 8.2 | 11.5 A |
|                     | UFI     | 13.0 | 13.7 | 9.5 | 9.5 | 11.4 A |
|                     | Mean    | 13.2 a† | 13.6 a | 8.8 b | 8.1 b | 13.2 |
|                     | SE      | 0.76 | | | | |
| Stem                | L 79-1002 | 5.3 | 4.4 | 3.3 | 3.0 | 4.0 B |
|                     | Merkeron | 7.0 | 5.2 | 4.4 | 3.9 | 5.1 A |
|                     | UFI     | 5.5 | 4.9 | 3.4 | 3.4 | 4.3 B |
|                     | Mean    | 5.9 a | 4.9 b | 3.7 c | 3.5 c | 5.9 |
|                     | SE      | 0.56 | | | | |
| Total               | L 79-1002 | 7.8 | 7.9 | 4.8 | 4.1 | 6.1 B |
|                     | Merkeron | 9.1 | 8.7 | 5.8 | 4.8 | 7.1 A |
|                     | UFI     | 7.6 | 8.0 | 4.6 | 4.2 | 6.1 B |
|                     | Mean    | 8.2 a | 8.2 a | 5.0 b | 4.4 b | 8.2 |
|                     | SE      | 0.58 | | | | |

*Harvest management treatments were harvested twice per year in July and November (2X-July, and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze in December (1X-Dec).

†Entry means across harvest managements not followed by the same upper case letter are different ($P < 0.05$).

‡Harvest management means across entries not followed by the same lower case letter are different ($P < 0.05$).

In stem, N concentration was affected by harvest management ($P = 0.002$) and entry ($P = 0.011$). Merkeron (5.1 mg g⁻¹) N concentration was greater than other entries (4.3 mg g⁻¹ in UFI and 4.0 mg g⁻¹ in L 79-1002, Table 6). The 2X-July treatment, which was harvested about 8 week after the final spring fertilizer application, had greatest N concentration, while 1X-Nov and 1X-Dec harvest management were lowest in N. Similar to stem N, total aboveground biomass N concentration was affected by harvest management ($P < 0.001$) and entry ($P = 0.004$). Merkeron had greatest N concentration. Nitrogen concentration decreased by 39% from 2X-July and 2X-Nov to 1X-Nov and 46% to 1X-Dec.

Phosphorus concentration in leaves, stem, and total aboveground biomass showed similar trends to N concentrations. Phosphorus concentration was affected by harvest management and entry in leaf ($P = 0.002$ and 0.001, respectively), stem ($P < 0.001$ for both), and total aboveground biomass ($P = 0.001$ and <0.001, respectively). In both plant parts and in total biomass, Merkeron had the greatest overall P concentration (Table 7). In leaves, stem, and total plant biomass, P concentration was greater for the 2X treatments compared with 1X-Nov and 1X-Dec. For example, in total aboveground biomass, P concentration in 2X-Nov was approximately twice as great as the single-harvest treatment.

There were harvest management and entry effects on leaf ash concentration ($P = 0.012$ and <0.001, respectively). Entry UFI had the greatest leaf ash concentration followed by Merkeron and then L 79-1002 (Table 8). The 1X-Nov and 1X-Dec harvested biomass had lesser ash concentration (48 and 44 mg g⁻¹, respectively) than 2X-July and 2X-Nov treatments (54 mg g⁻¹ for both). In stem, ash concentration was affected by harvest management ($P < 0.001$). The average of the sin-

Table 7  Effect of grass entry harvest and management interaction on phosphorus concentration in leaf ($P = 0.139$), stem ($P = 0.360$), and total above-ground biomass ($P = 0.348$). Data are means across four replicates and 2 years ($n = 8$).

| Harvest management* | Entry | 2X-July | 2X-Nov | 1X-Nov | 1X-Dec | Mean |
|---------------------|-------|---------|--------|--------|--------|------|
| Leaf                | L 79-1002 | 1.40 | 1.94 | 0.72 | 0.57 | 1.16 B† |
|                     | Merkeron | 1.95 | 2.13 | 1.14 | 1.03 | 1.56 A |
|                     | UFI     | 1.32 | 1.51 | 0.88 | 1.00 | 1.17 B |
|                     | Mean    | 1.56 a† | 1.86 a | 0.91 b | 0.87 b | 1.56 |
|                     | SE      | 0.18 | | | | |
| Stem                | L 79-1002 | 1.30 | 1.44 | 0.70 | 0.63 | 1.02 B |
|                     | Merkeron | 1.64 | 1.99 | 1.21 | 1.09 | 1.48 A |
|                     | UFI     | 1.07 | 1.56 | 0.78 | 0.94 | 1.09 B |
|                     | Mean    | 1.34 b | 1.66 a | 0.89 c | 0.89 c | 1.34 |
|                     | SE      | 0.14 | | | | |
| Total               | L 79-1002 | 1.31 | 1.66 | 0.73 | 0.62 | 1.08 B |
|                     | Merkeron | 1.71 | 2.04 | 1.19 | 1.07 | 1.50 A |
|                     | UFI     | 1.13 | 1.53 | 0.80 | 0.95 | 1.10 B |
|                     | Mean    | 1.38 b | 1.74 a | 0.90 c | 0.88 c | 1.38 |
|                     | SE      | 0.15 | | | | |

*Harvest management treatments were harvested twice per year in July and November (2X-July, and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze in December (1X-Dec).

†Entry means across harvest managements not followed by the same upper case letter are different ($P < 0.05$).

‡Harvest management means across entries not followed by the same lower case letter are different ($P < 0.05$).
Table 8 Effect of grass entry and harvest management interaction on ash concentration in leaf ($P = 0.359$), stem ($P = 0.257$), and total above-ground biomass ($P = 0.853$). Data are means across four replicates and 2 years ($n = 8$)

| Entry          | Harvest management | mg ash g$^{-1}$ dry matter |
|----------------|--------------------|-----------------------------|
|                | 2X-July | 2X-Nov | 1X-Nov | 1X-Dec | Mean |
| Leaf L 79-1002 | 44      | 45     | 33     | 32     | 38   |
| Merkeron      | 59      | 57     | 50     | 43     | 52   |
| UF1           | 60      | 60     | 60     | 56     | 59   |
| Mean          | 54 a‡   | 54 ab  | 48 bc  | 44 c   |      |
| SE            | 3.9     |        |        |        |      |
| Stem L 79-1002| 44      | 43     | 28     | 22     | 34   |
| Merkeron      | 40      | 43     | 26     | 25     | 34   |
| UF1           | 39      | 46     | 25     | 27     | 34   |
| Mean          | 41 a‡   | 44 a   | 26 b   | 24 b   |      |
| SE            | 2.5     |        |        |        |      |
| Total L 79-1002| 44     | 44     | 29     | 24     | 35   |
| Merkeron      | 46      | 48     | 32     | 29     | 39   |
| UF1           | 45      | 51     | 32     | 31     | 40   |
| Mean          | 45 a‡   | 48 a   | 31 b   | 28 b   |      |
| SE            | 2.4     |        |        |        |      |

*Harvest management treatments were harvested twice per year in July and November (2X-July), and 2X-Nov, once per year in November (1X-Nov), and once per year after first freeze in December (1X-Dec).

†Entry means across harvest managements not followed by the same upper case letter are different ($P < 0.05$).

‡Harvest management means across entries not followed by the same lower case letter are different ($P < 0.05$).

Extractives and total soluble sugars

Elephantgrass and energycane extractive concentration responses to harvest management were different. Elephantgrass biomass had greatest extractives concentrations when harvested twice per year, while energycane extractives were greatest when biomass was harvested once per year. This response was due to accumulation of extractives in energycane stems late in the growing season. Because of the preponderance of stem in harvested biomass, especially when harvested once per year, the effects of leaf extractives concentration were small. There are very limited data available for extractives concentration in perennial C4 grasses. An elephantgrass study in Vietnam reported 182 mg g$^{-1}$ of extractives measured using a hot water extraction (Hoa et al., 2008). As observed in the present study, Fedenko et al. (2013) found that total extractives concentration was greater for energycane than elephantgrass when harvested once per year in fall prior to a freeze event (avg. 234 vs. 188 mg g$^{-1}$) due to sugar accumulation in energycane. In Colorado, extractives concentration varied among entries including 164 mg g$^{-1}$ for the C4 perennial switchgrass, 254 mg g$^{-1}$ for the C3 perennial grass tall fescue (Festuca arundinacea L.), and 172 mg g$^{-1}$ for maize stover (Zea may L.; Thammasouk et al., 1997).

Stem was the major contributor of soluble sugars to total aboveground biomass for both grass species in the present study. Although differences existed in soluble sugar concentration in leaves, they contributed marginally to differences on a total harvested biomass basis because leaf proportion was low and leaf abscission occurred late in the growing season (Na et al., 2015b). Single-harvest (1X-Nov and Dec) energycane showed greatest soluble sugar concentration in total harvested biomass because of greater accumulation in the stem, mostly of sucrose. Although energycane stem has a relatively low sugar concentration compared with sugarcane (Saccharum spp. hybrid), it shows measurable levels of soluble sugar accumulation late in the season (Bischoff et al., 2008; Kim & Day, 2011; Tew et al., 2011). Unlike structural carbohydrates, non-cell wall carbohydrates are directly fermentable; however, they are susceptible to microbial degradation during storage (Dien et al., 2006). Moreover, by degradation of free sugars, cellululosic fermentation inhibitors including furfural can be produced (Tran & Chambers, 1986; Zhang et al., 2011). If they cannot be used for conversion to biofuel, they will decrease feedstock quality as they reduce concentration of structural carbohydrates proportionally. In the previous research, L 79-1002 had 101% (first year) and 45% (second year) greater soluble sugar concentration than Merkeron (Fedenko et al., 2013), a similar result to the present study.

Discussion

Extractives and total soluble sugars

Elephantgrass and energycane extractive concentration responses to harvest management were different. Elephantgrass biomass had greatest extractives concentrations when harvested twice per year, while energycane extractives were greatest when biomass was harvested once per year. This response was due to accumulation of extractives in energycane stems late in the growing season. Because of the preponderance of stem in harvested biomass, especially when harvested once per year, the effects of leaf extractives concentration were small. There are very limited data available for extractives concentration in perennial C4 grasses. An elephantgrass study in Vietnam reported 182 mg g$^{-1}$ of extractives measured using a hot water extraction (Hoa et al., 2008). As observed in the present study, Fedenko et al. (2013) found that total extractives concentration was greater for energycane than elephantgrass when harvested once per year in fall prior to a freeze event (avg. 234 vs. 188 mg g$^{-1}$) due to sugar accumulation in energycane. In Colorado, extractives concentration varied among entries including 164 mg g$^{-1}$ for the C4 perennial switchgrass, 254 mg g$^{-1}$ for the C3 perennial grass tall fescue (Festuca arundinacea L.), and 172 mg g$^{-1}$ for maize stover (Zea may L.; Thammasouk et al., 1997).

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Structural hexose and pentose

For lignocellulosic biomass, ethanol fermentation efficiency is determined by the quality of structural carbohydrates (Lu & Mosier, 2008), so it is critical to investigate tissue structural carbohydrates. Energycane

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had greater leaf structural hexose concentration than elephantgrass entries; however, elephantgrass entries showed greater hexose concentration in both stem and total aboveground biomass than energycane. This was due at least in part to an increase in total soluble sugar over time in energycane stem tissue, which resulted in a dilution of the concentration of structural components. A large amount of glucose from cell wall structural components is advantageous for ethanol production because glucose can be converted by most organisms more efficiently to ethanol than most other sugars, especially pentose sugars (Lu & Mosier, 2008). Similar to values observed in the present study, structural hexose concentrations of 347 mg g\(^{-1}\) have been reported for mixtures of C\(_4\) grasses in Minnesota, USA (Gillitzer et al., 2013). Chemical composition of six-carbon structural sugars was 300–337 mg g\(^{-1}\) for switchgrass (Xu et al., 2011). In the prior work at the location of the present study, structural glucose concentration in elephantgrass and energycane was 374 vs. 366 mg g\(^{-1}\) in the first year and 448 vs. 432 mg g\(^{-1}\) in the second year, respectively (Fedenko et al., 2013). Late harvests and increasing maturity have been observed to increase glucose and nonglucose structural sugars in reed canarygrass (Phalaris arundinacea L.) (233–286 mg g\(^{-1}\)) and switchgrass (294–340 mg g\(^{-1}\)) (Dien et al., 2006). This corresponds to what was observed for elephantgrass, but not for energycane, in the present study.

Unlike structural hexose results, energycane showed the greatest pentose concentration in total aboveground biomass. As pentose concentrations increased, structural hexose concentrations decreased in the 2X-Nov harvest for total biomass. As indicated earlier, because of the fermentation inefficiency of pentose by most microorganisms, it is less favorable than hexose (Lu & Mosier, 2008). In switchgrass, the concentration of five-carbon structural sugars ranged from 183 to 196 mg g\(^{-1}\) (Xu et al., 2011). Structural xylose concentration in season-long growth of elephantgrass in Florida was similar to that of energycane (135 and 145 mg g\(^{-1}\), respectively) (Fedenko, 2011). For both reed canarygrass and switchgrass, structural five-carbon sugars increased with increasing maturity (147–191 mg g\(^{-1}\) in reed canarygrass; 210–253 mg g\(^{-1}\) in switchgrass) (Dien et al., 2006). This trend was not observed in the present study. Pentose concentrations of 198 and 187 mg g\(^{-1}\) from structural carbohydrates have been reported for a mixture of C\(_4\) grasses and for a mixture of C\(_3\) grasses, respectively, in Minnesota (Gillitzer et al., 2013). Although there were statistical differences in pentose concentrations in the present study, unlike hexose concentrations, pentose was relatively constant across harvest managements and entries (avg. of 248 mg g\(^{-1}\)) except for 2X-Nov, for which the range was 218–229 mg g\(^{-1}\).

Lignin

Merkeron consistently exhibited the greatest total lignin concentration, and single-harvest per year treatments (1X-Nov and 1X-Dec) showed greater lignin concentration in total aboveground biomass than 2X treatments. Total lignin concentration in the present study is similar to 204–291 mg g\(^{-1}\) reported in a previous study for full-season growth of elephantgrass and energycane (Fedenko et al., 2013). The results of both studies agree that elephantgrass generally has greater lignin concentration than energycane. The reported lignin concentrations are also similar to those for three switchgrasses, which ranged from 214 to 230 mg g\(^{-1}\) for full-season growth (Xu et al., 2011). Prior research with alfalfa (Medicago sativa L.) and pine (Pinus sp.) has shown that greater lignin concentrations decrease pretreatment efficiency relative to lesser concentrations and that even slight changes are sufficient to markedly affect conversion efficiency (Dixon & Chen, 2007; Studer et al., 2011). Therefore, greater lignin concentrations in elephantgrass may negatively affect pretreatment and biofuel production.

Nitrogen, phosphorus, and ash

In general, 2X treatments showed greater N concentration compared with 1X treatments. Merkeron had the greatest N concentration in total aboveground biomass. Similar to responses in the present study, harvest frequency of reed canarygrass in Iowa affected N concentration. When biomass was harvested twice per year (June and fall), N concentration was 13.4 (June) and 8.8 mg g\(^{-1}\) (fall) compared with 8.3 mg g\(^{-1}\) for a single harvest in fall (Tahir et al., 2011). In Japan, elephantgrass that was harvested less frequently had lower N concentrations in both leaves and stem than that harvested more frequently (Hsu et al., 1990). In the same study, the difference between leaf and stem N concentration increased as harvest interval was extended. When plants reached a height of 1 m, N concentrations were 10.8 mg g\(^{-1}\) in leaf vs. 5.0 mg g\(^{-1}\) in stem, and when plants were 2 m tall, N concentrations were 8.6 mg g\(^{-1}\) in leaf vs. 2.6 mg g\(^{-1}\) in stem. Switchgrass biomass N concentration decreased over the season (Madakadze et al., 1999); however, N concentration was relatively constant after September at about 5 mg g\(^{-1}\). Miscanthus N concentration decreased until October and then remained constant for the rest of the season (Heaton et al., 2009). Similarly, delaying harvest from 1X-Nov to 1X-Dec in the present study did not result in a significant decrease in either leaf or stem N, but 1X-Dec N concentration of total aboveground biomass was slightly less than 1X-Nov because of leaf
senescence and the resultant decrease in leaf : stem ratio in 1X-Dec (Na et al., 2015a). When grown with no fertilizer on loamy sands in Georgia, Merkeron elephantgrass had greater overall N concentration than L 79-1002 (3.8 vs. 2.7 mg g\(^{-1}\)) (Knoll et al., 2012), similar to the pattern of response in the present study, but Singh et al. (2015) reported no difference in total above-ground biomass N concentration between L 79-1002 and Merkeron across three sites in Florida.

Similar to N concentration, Merkeron showed the greatest tissue P concentration. The 1X treatments had lesser P concentrations than either of the 2X treatments. Similar results were found for reed canarygrass in Iowa, where P concentration was greater for two harvests per year than for a single harvest (Tahir et al., 2011). Switchgrass P concentration was also affected by harvest management, and decreased slightly from the first to second harvest of a two-harvest per year treatment, and was lowest in the fall harvest of a single-harvest per year treatment (1.3, 1.1, and 0.8 mg g\(^{-1}\), respectively) (Guretzky et al., 2011). Giant reed and switchgrass P concentrations decreased from October to December in Oklahoma (Kering et al., 2012). In switchgrass, leaf N was much greater compared with stem, but leaf and stem P concentrations varied only slightly (Shahandeh et al., 2011).

In the present study, harvest management affected ash concentration. For the 1X treatment, total above-ground elephantgrass biomass ash concentration was lower than that for either harvest of the 2X treatment. The results of the present study are similar to those reported previously for Merkeron and L 79-1002. When 4-year data were averaged, Merkeron had greater ash concentration than L 79-1002 when grown with no fertilizer on loamy sands of the coastal plain of Georgia, USA (45.9 vs. 34.4 mg g\(^{-1}\), respectively) (Knoll et al., 2012). Reed canarygrass responded differently than elephantgrass and energycane, as ash concentration increased slightly over the season (96, 106, and 107 mg g\(^{-1}\) for two harvests per year in June and October, and for one harvest in October, respectively) (Tahir et al., 2011). In switchgrass, ash concentration in Iowa peaked in July (71 mg g\(^{-1}\)) and decreased until fall after which it remained relatively constant between 43 and 45 mg g\(^{-1}\) (Wilson et al., 2013). Limited data for plant part ash concentration indicate that leaf was found to have greater ash than stem (Summers et al., 2001; Bakker & Elbersen, 2005).

In conclusion, harvest frequency (2X vs. single) significantly affects compositional quality of perennial grasses. A single harvest in fall appears to maximize the concentration of cellulose in total biomass, and delaying harvest from 1X-Nov to 1X-Dec had little impact on most response factors. The exception to this was for extractives and soluble sugars in energycane, which increased significantly from 1X-Nov to 1X-Dec harvest. A relatively greater concentration of soluble sugars in energycane reduced the concentrations of the structural components. A major factor affecting concentration differences due to harvest management was differences in leaf:stem ratio because leaves generally had greater N, P, and ash than stem. Later harvests were associated with lesser leaf percentage in total biomass, which caused N, P, and ash to decrease in 1X-Nov and 1X-Dec relative to 2X treatments. Total aboveground biomass N concentration in Merkeron decreased to a greater extent than the other entries because of greater leaf abscission (Na et al., 2015b). The 1X-Nov and 1X-Dec treatments of all entries had lesser concentrations of N and ash, components which can negatively affect some conversion processes, but they had slightly greater lignin concentration than 2X harvests. On the basis of this study results, a single harvest in fall will result in preferred biomass compositional properties, with maximum cellulose concentration of both elephantgrass and energycane. However, unlike energycane, delaying a single harvest of elephantgrass to after a freeze will not compromise cellulose concentration. This study provides needed information on nutrient, carbohydrate, and lignin concentrations and carbohydrate composition in elephantgrass and energycane that will guide decision making regarding biomass harvest frequency and timing for perennial grasses grown for biomass in the southeastern USA.

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