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Forage Harvest Timing Impact on Biomass Quality from Native Warm-Season Grass Mixtures

David W. McIntosh,* Gary E. Bates, Patrick D. Keyser, Fred L. Allen, Craig A. Harper, John C. Waller, Jessie L. Birckhead, and William M. Backus

ABSTRACT

Biomass production systems using native warm-season grasses can allow for an early-season harvest (for forage) followed by a dormant harvest (for biomass). A study was conducted to investigate the impact of harvest timing and grass species on the chemical composition of harvested forage and biomass. The three-species composite treatments were switchgrass (SG) (*Panicum virgatum* L.); a two-way blend of big bluestem (*Andropogon gerardii* V.) (BB) and indiangrass (*Sorghastrum nutans* L.) (IG); and a three-way mixture of SG, BB, and IG. Harvest treatments were a biomass harvest (BH) in late fall, early-boot (EB) harvest (for forage) followed by BH, or early-seedhead harvest (ESH) (for forage) followed by BH. Forage harvested at EB had greater crude protein and less neutral detergent fiber (NDF) and acid detergent fiber (ADF) compared with ESH ($P = 0.05$). The ADF and NDF content of biomass was greatest for BH and was reduced in biomass regrowth after EB, with the later ESH resulting in the largest decrease in fiber content. Total macronutrient removal of N, P, and K was increased in the dual-use system. Results indicated that it is possible to alter the composition of biomass provided for bioenergy production by taking an early-season forage harvest.

Core Ideas

- Use mixed species native warm-season grasses for forage/biomass production.
- Provide nutritional and quality data on switchgrass and mixed species stands for forage and ethanol production.
- It is possible to alter forage nutritive values/biomass quality with the addition of other grasses with switchgrass.

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The development of renewable bioenergy resources has become increasingly important over the last three decades (Lynd et al., 1991; McLaughlin and Kszos, 2005; Sanderson et al., 1996). Switchgrass has often been a primary species investigated for bioenergy (Lynd et al., 1991; Sanderson et al., 1996), with a single late fall or early winter harvest resulting in the greatest sustainable biomass yield (Parrish and Fike, 2005). There is the potential to remove an early-season forage harvest in a biomass system, which may provide more options to producers from the same crop (Mosali et al., 2013; Sanderson and Adler, 2008). Research has indicated that an early-season forage harvest followed by a fall biomass harvest will result in a reduced biomass yield but will also result in an increase in total yield influenced by the forage harvest timing (McIntosh et al., 2015).

Just as harvest timing influences yield, timing is a major factor influencing forage nutritive value (Ball et al., 2015). In hay production, nutritive value is an important consideration when using native warm-season grasses (NWSGs) in mixture and can be affected by plant maturity (Springer et al., 2001). As harvest is delayed from early to late seedhead production, nutritive value decreases dramatically, making it important to include plant maturity as one of the considerations for hay production instead of yield alone (Waramit et al., 2012). With proper management, switchgrass (SG) in monoculture can produce good nutritive values before maturity causes reduced values, usually after the vegetative stage at late boot when the head is emerging (Mitchell et al., 2001; Richner et al., 2014). Species mixtures that include SG provided good quality forage with increased yields, depending on the management system (Fike et al., 2006; Posler et al., 1993; Sanderson et al., 2006). Suggestions from Guretzky et al. (2011) included using SG in the vegetative stage of growth if used for a dual use, thereby allowing regrowth for biomass if harvested in

D.W. McIntosh, G.E. Bates, and F.L. Allen, Dep. of Plant Sciences, Univ. of Tennessee Institute of Agriculture, 2431 Joe Johnson Drive, 252 Ellington Bldg, Knoxville, TN 37996; P.D. Keyser, C.A. Harper, and J.L. Birckhead, Dep. of Wildlife and Fisheries, Univ. of Tennessee Institute of Agriculture, 2431 Joe Johnson Drive, 274 Ellington Bldg, Knoxville, TN 37996; J.C. Waller and W.M. Backus, Dep. of Animal Science, Univ. of Tennessee Institute of Agriculture, 2506 River Drive, Knoxville, TN 37996. *Corresponding author (dmcintos@utk.edu).

Abbreviations: ADF, acid detergent fiber; BB, big bluestem; BH, biomass harvest; CP, crude protein; DB, digestible biomass; EB, early-boot harvest; ESH, early-seedhead harvest; IG, indiangrass; IVTDMD48h, in vitro true dry matter digestibility at 48 h; NDF, neutral detergent fiber; NIRS, near-infrared spectroscopy; NIRSC, Near-Infrared Spectroscopy Consortium; NWSG, native warm-season grass; SG, switchgrass; TDN, total digestible nutrient.

late fall. Compared with SG, big bluestem (BB) and indiangrass (IG) generally have greater nutrititive value because of their later onset of maturity and leafiness during early summer, which provides more consistent quality forage throughout a majority of the growing season (Ball et al., 2015; Mitchell et al., 2001; Redfearn and Nelson, 2003; Stubbendieck et al., 2002).

The timing of an early-season forage harvest may also affect the chemical composition of the biomass regrowth. For NWSG biomass systems, biofuel yield predictions have usually been based on the cellulosic components where digestibility components of fiber, lignin, and cellulose make up 95% of the information needed (Lorenz et al., 2009). The increased lignin concentrations limit the conversion process by inhibiting sugar and fermentation recovery from biomass (Dien et al., 2006; Sanderson et al., 2006; Vogel and Jung, 2001).

Switchgrass has been a primary species used in biomass research, where under a single fall biomass harvest it is desirable to have high neutral detergent fiber (NDF) and acid detergent fiber (ADF) with less N and ash (Mulkey et al., 2008). More lignin was found in SG biomass harvested in fall after a boot-stage forage harvest compared with a single fall biomass harvest (Richner et al., 2014). Recent work on NWSGs in monoculture and mixtures reported that SG should be included in mixtures because it resulted in greater biomass yield and greater cellulose compared with mixtures with BB and IG (Hong et al., 2013). The addition of BB and IG in the species mixtures produced biomass that contained less lignin than any monoculture (Hong et al., 2013). Vogel et al. (2013) concluded that biomass ideal for biofuel production should have decreased fiber and ash content with greater digestibility to convert the cellulose more efficiently. Digestibility is an important component in the sugar extraction process and correlates with lignin (negatively) and cellulose availability (positively) for fermentation (Chang and Holtzapple, 2000; Lee, 2006).

Typically, NWSGs have been promoted as requiring less inputs when used for biomass production. However, when reviewing recommendations for N fertilization, many differences in results and conclusions surfaced. The only recommendations that were consistent were that fertilization with N is not recommended during the first year due to weed pressure and that an application of N during grass green-up may increase yield and nutrient content (Thomason et al., 2005). In Texas, applications of 168 kg N ha^{-1} made annually during the early growing season were reported to result in the greatest yield with adequate moisture allowing the available nutrients to be used effectively by SG grown for biomass production (Muir et al., 2001).

As NWSG species are combined, additional nutrient replacement may be necessary depending on the harvest management of hay and/or biomass. Fertilizer applications of P and K are based on regular soil testing and applied only when soil test results show low amounts. This can be a problem when replacement fertilizer is not applied if the NWSGs are for hay production because more plant material is removed during harvests. This is particularly relevant with N applications because as forage and biomass removal dramatically increases, removal of soil nutrients also increases (Epstein et al., 1996; Muir et al., 2001; Ocumpaugh et al., 2003). Recently, Seepaul et al. (2014) reported that P and K removal increased with a two-harvest system compared with a single fall biomass harvest. Furthermore, fertilizer recommendations in a dual-use system may need to be increased to produce high-quality forage and biomass in the same system (Brejda, 2000). Other research that focused on forage and biomass systems reported that greater yields remove more nutrients and potentially require more fertilization, but there is limited research on mixed NWSG stands (Guretzky et al., 2011; Propheter and Staggenborg, 2010).

This study was conducted to investigate the impact of forage species and harvest timing on the forage nutritive value and biomass quality of NWSGs. The objectives of this study were to determine (i) the effect of two early-season harvest timings (early-boot [EB] and early-seedhead harvest [ESH]) on forage nutritive values of native grasses in monoculture and mixtures and (ii) the effect of forage harvest timing on biomass quality in a dual-use system. Yield data from this study (forage and biomass) have been previously reported in McIntosh et al. (2015).

MATERIALS AND METHODS

Location

This experiment was conducted from 2010 to 2012 at three locations in Tennessee. The first location was the East Tennessee Research and Education Center in Knoxville (35°54′2″ N, 83°57′36″ W; 274 m elevation) on an Etowah Silt Loam (fine-loamy, siliceous, semiactive, thermic Typic Paleudults) (Soil Survey Staff, 2014). The second location was the Plateau Research and Education Center near Crossville (36°2′38″ N, 85°9′48″ W; 576 m elevation) on a Lily Loam (fine-loamy, siliceous, semiactive, mesic Typic Hapludults) (Soil Survey Staff, 2014). The third location was the Highland Rim Research and Education Center near Springfield (36°28′22″ N, 86°49′7″ W; 201 m elevation) on a Mountview Silt Loam (fine-silty, siliceous, semiactive, thermic Oxyaquic Paleudults) (Soil Survey Staff, 2014).

Establishment

Plots were established in 2008 at Springfield and in 2009 at Knoxville and Crossville. In all three locations, previous field use was pasture and/or hay fields dominated by tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.] with no other management or recent history of research use before this study. In the fall before establishment, 2.24 kg ai ha^{-1} glyphosate [*N*-(phosphonomethyl) glycine] was used to eradicate existing vegetation. A second application of glyphosate at that same rate was made 2 wk before planting. At establishment, BB+IG plots were treated with an application of glyphosate (2.2 kg ai ha^{-1}) and imazapic (0.11 kg ai ha^{-1}) (2-[[(RS)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]]-5-methylnicotinic acid) to provide pre-emergence weed control. The SG plots did not require additional weed control before planting. At establishment, no lime, P, or K fertilizer was required based on soil test results (University of Tennessee Soil, Plant and Pest Center, Nashville, TN). All sites were planted in early May into a conventionally prepared seedbed where ground was tilled and cultipacked before using a no-till plot drill to plant. Plot size at Knoxville was 1.8 by 7.6 m (12.9 m^2) and at Crossville and Springfield was 1.5 by 7.6 m (11.4 m^2).

Treatments

Treatments of NWSG composites were: Treatment 1, 100% SG monoculture; Treatment 2, a two-way blend of 65% BB and 35% IG; and Treatment 3, a three-way mixture of 50% SG, 35% BB, and 15% IG (50:50 ratio of Treatments 1 and 2). Seeds were blended to the appropriate ratios based on mass of pure live seed. Seeding rates were: SG, 6.7 kg ha^{-1}; BB+IG, 5.4 kg BB ha^{-1} and 2.8 kg IG ha^{-1}; and SG+BB+IG, 3.4 kg SG ha^{-1}, 2.7 kg BB ha^{-1}, and 1.4 kg IG ha^{-1} (Bates et al., 2008). The cultivars Alamo SG, Rumsey BB, and OZ-70 IG were used in this study. Alamo is a lowland type SG that has been used in biomass production. Rumsey and OZ-70 are cultivars that were adapted to the southeastern growing conditions and are available through Roundstone Native Seed LLC.

Weed Control

During the study, plots containing SG were mowed twice to reduce weed competition during the establishment year. In the first year after establishment, metsulfuron (14.0 g ai ha^{-1}) (2-[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]-oxomethyl] sulfamoyl]benzoic acid methyl ester) was applied to BB+IG plots for broadleaf weed control. No herbicide treatment was required on the plots containing SG. Once the study was in the second year after establishment, weed control was not necessary.

Fertilization

Plots were fertilized annually with 101 kg N ha^{-1} with urea (46–0-0). The biomass harvest (BH) treatment received one N application at green-up in mid-April, whereas the dual-use treatments received half at green-up and the remaining half after the early-season forage harvest. Lime, P, and K were not required at Knoxville and Crossville. Springfield required a spring application, in Year 2, of 101 kg P ha^{-1} in the form of diammonium phosphate at green-up, and N was adjusted for the N content of the biomass harvest at University of Tennessee Soil, Plant and Pest Center.

Harvest

Harvest treatments were implemented during 2010–2012 and consisted of BH, EB+BH, and ESH+BH. Forage harvest timings were based on the growth stage of SG monoculture. Timing for EB was at stem swell due to the development of the seedhead and flag leaf formation. Typically this occurred from the last week in May to the first week of June, depending on location. At ESH, a seedhead was emerged and fully expanded from the sheath, which corresponded to approximately the last week of June. The average interval between EB and ESH during the course of the study was 27 d. The BH harvest took place after the first killing frost for each location.

Plots were harvested at a 15-cm residual height using a flail-type small-plot harvester (Carter Mfg. Co., Inc.; Swift Machine and Welding Ltd.). A 0.9 by 7.6 m harvest strip was removed from center of the plot area, resulting in a harvested area of 6.9 m^2. Harvested forage was weighed, and a subsample was dried at 60°C in a forced-air oven for 72 h to determine moisture content and, ultimately, yield (Murray and Cowe, 2004). Additional stand density data were collected using a transect method often used in wildlife canopy observations. After reviewing the data, the authors concluded that the visual observations were a reliable substitute for the transect method and therefore did not include these data with the rest of the results. The stands of NWSGs were representative of the monoculture and mixtures at planting. However, we do not have data to represent comparison to the transect data that were not suitable for an agronomic study.

Climatological Data

Rainfall and temperature data were collected by a weather station located at each study site. The 30-yr monthly mean rainfall for each location (ID: USC00404946, East Tennessee Research and Education Unit; ID: USC00402202, Plateau Research and Education Unit; and station ID: USC00408562, Highland Rim Research and Education Unit) indicated that annual totals were greater than or within 15% of the 30-yr mean for the study period (Golden Gate Weather, 2014).

Near Infrared Spectroscopy Analysis

Near-infrared spectroscopy (NIRS) technology (FOSS 5000, FOSS NIRSystems, Inc.) was used to determine forage nutritive values and biomass quality. Equations for the forage nutritive analysis and biomass quality were standardized and checked for accuracy using the grass hay equation developed by the NIRS Forage and Feed Consortium. The NIRS equations provided by the Near-Infrared Spectroscopy Consortium (NIRSC) were expanded with cooperation from this research study to include all the NWSG treatments and harvested material. Original equations for NIRS analysis were developed and compared with the wet chemistry results provided by Dairy One Analytical. These equations were then shared with the NIRSC to expand the grass hay equation, allowing outliers to be included in their database. Samples determined by the NIRSC as necessary additions included the NWSG mixtures for all harvest treatments, and expansion was performed to include biomass material harvested at post-frost senescence. Wet chemistry was performed on the selected samples and spectra recorded into the equation. Win ISI II (Infrasoft International LLC) software was used for NIRS analysis. The Global H statistical test compared the samples against the model and other samples within the database for accurate results, where all forage samples fit the equation with $H < 3.0$ and are reported accordingly (Murray and Cowe, 2004). Although the NIRS analysis is a predictive method, the treatments within this study fit the grass hay equation released by the NIRSC in 2012. This included the biomass material from all NWSG treatments as well as early-season harvests for forage.

Estimated biofuel yield was not reported in this study because mixed NWSG was not part of the current equation provided by the NIRSC to predict ethanol yield and components (Vogel et al., 2011). Current research suggests in vitro true dry matter digestibility at 48 h (IVTDMD48h) could become a leading constituent for estimating biofuel conversion efficiency from switchgrass and possibly other NWSG (Vogel et al., 2011, 2013). However, using data from the quality constituents allowed for a calculated digestible biomass (DB) using yield data in Fig. 2 from McIntosh et al. (2015) multiplied by the IVTDMD48h. Nutrient removal was calculated by converting crude protein (CP) to N by dividing CP by 6.25. The P and K were converted to oxide forms to report appropriate removals. Van Soest et al. (1991) calculations for cellulose (% ADF – % Lignin) and hemicellulose (% NDF – % ADF) were used to determine biomass quality attributes.

Statistical Methods

Dependent variables (CP, ADF, NDF, total digestible nutrients [TDNs], P, K, lignin, cellulose, hemicellulose, digestible biomass, N, P, P_2O_5, K, K_2O, ash, and IVTDMD48h) were analyzed under a randomized complete block design with a factorial arrangement of the three NWSG composites and three harvest

Table 1. Forage nutritive value averaged across three experimental locations (Knoxville, Crossville, and Springfield, TN) and 3 yr (2010–2012).

Harvest treatment†	NWSG treatment‡	Forage nutritive value§ (dry matter basis)					
		CP	ADF	NDF	TDN	P	K
		g kg^{-1}					
EB	SG	106.8b¶	403.1b	684.9b	566.0b	2.7	19.1ab
	BB+IG	114.7a	391.7c	648.2d	578.9a	2.5	19.0b
	SG+BB+IG	106.8b	387.0c	642.8d	584.3a	2.6	19.7a
ESH	SG	86.8c	435.4a	729.8a	529.1c	2.3	16.2d
	BB+IG	93.1c	401.7b	668.3c	567.5b	2.2	18.1c
	SG+BB+IG	88.5cd	409.5b	684.4b	558.5b	2.2	17.9c
LSD		5.1#	9.6	14.2	11.0	ns††	0.7

† EB, early-boot; ESH, early-seedhead.

‡ NWSG, native warm-season grasses. Treatments: BB+IG, two-way blend of big bluestem/indiangrass; SG, switchgrass; SG+BB+IG, three-way mixture of switchgrass/big bluestem/indiangrass.

§ CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; TDN, total digestible nutrient.

¶ Means within a column not sharing a lowercase letter are significantly different for the harvest × NWSG interaction (Fisher's protected LSD, $\alpha = 0.05$).

Crude protein differences reported at the 0.1 probability level ($P = 0.067$).

†† Nonsignificant at the 0.05 probability level.

treatments replicated four times over 3 yr. Data were analyzed using SAS and the MIXED procedure with repeated measures (autoregressive variance structure) over 3 yr (SAS Institute, 2012). Random effects [replication × location (year)] were included in the model, with fixed effects being NWSG and harvest. Based on preliminary analysis, main effect differences in forage and biomass yield for year and location were not significant ($P > 0.05$); therefore, results were pooled over those factors in the subsequent model. These data were separated into forage nutritive values and biomass quality for comparison. Results are presented with the two-way interaction NWSG × harvest. Normality of residuals was assessed by the Shapiro–Wilk test ($W \geq 0.90$). Mean separations were conducted using Fisher's protected LSD with $\alpha = 0.05$.

RESULTS AND DISCUSSION

Forage Nutritive Values

Forage nutritive values were affected by both harvest timing and NWSG species composition. As expected, delaying harvest from EB to ESH caused a reduction in nutritive values. Forage harvested at EB had greater CP (109.4 vs. 89.5 g kg^{-1}; $P < 0.001$) and TDNs (576.4 vs. 552.0 g kg^{-1}; $P < 0.001$) compared with ESH. Crude protein also measured lesser in ADF (393.9 vs. 415.6 g kg^{-1}) and NDF (658.6 vs. 694.2 g kg^{-1}) compared with ESH harvest ($P < 0.001$). This agrees with the paradigm of the decrease in forage nutritive values with advancing plant maturity (Ball et al., 2015).

There were CP differences in the two-way interaction of NWSG × harvest from EB to ESH; however, CP for all NWSG mixtures were greater in the EB harvested forage ($P = 0.067$) (Table 1). The BB+IG and SG+BB+IG were similar in TDN and fiber measurements within forage harvest timing (Table 1). When harvested at EB, BB+IG and the three-way mixture (SG+BB+IG) produced greater TDNs and lesser ADF and NDF than did SG (Table 1). However, CP content was greater for BB+IG at EB (Table 1). When harvested at ESH, CP, ADF, and TDN values were similar for BB+IG and for SG+BB+IG, but NDF were less for BB+IG (Table 1). Switchgrass generally had the least nutritive value, whereas BB+IG was greatest (Table 1).

Forage nutritive values were highly dependent on the plant growth stage at harvest. Forage harvested at EB had greater CP and TDNs compared with ESH for all NWSG (Table 1). Including SG with BB+IG produced forage of similar nutritive value to BB+IG. The addition of SG to BB+IG to produce the three-way mix resulted in improved forage nutritive values over the SG monoculture (Table 1). Regardless of the NWSG chosen to include in a mixture, the greatest forage nutritive values were produced when forage was harvested at EB.

Biomass Quality

Switchgrass harvested after the first frost is currently the standard recommendation for biomass management (Adler et al., 2006). This BH treatment produced the greatest biomass yield of all the harvest treatments in Fig. 2 of McIntosh et al. (2015). Data reported here indicate that biomass from this treatment contained the greatest ADF, NDF, and cellulose and the least N and P (Table 2). Taking an EB harvest decreased the ADF, NDF, and cellulose content of the biomass, with the later harvest timing (ESH) having the larger decrease (Table 2). Forage removal with the EB and ESH resulted in greater N and P in the BH (Table 2).

Biomass from BB+IG and SG+BB+IG harvested only in the fall had lesser ADF and cellulose, as well as greater N and P, compared with SG harvested at BH (Table 2). The NDF level for BB+IG was similar to SG harvested at BH (Table 2). Taking a forage harvest at EB from BB+IG and SG+BB+IG did not affect the fiber content or N, P, or K level in the fall biomass. Where cellulose, lignin, and hemicellulose are the greatest factors influencing the conversion of biomass to liquid fuels changes according to whether the constituents with a harvest treatment could alter the efficiency of conversion techniques (Jacobsen and Wyman, 2000). For the BH, all NWSG treatments (SG, SG+BB+IG, and BB+IG) had ash content between 42.0 and 48.5 g kg^{-1}, with no significant difference due to treatment (Table 2). There were no NWSG × harvest interactions or main effect differences for ash ($P = 0.126$) and lignin ($P = 0.368$) (Table 2). Lignin concentrations were consistent and not different across all NWSG and harvest treatments, ranging from 61.2 to 68.2 g kg^{-1} (Table 2). Hemicellulose concentration was not affected by treatment (NWSG × harvest) (Table 2). When forage harvest was delayed until ESH, however, ADF, NDF, and cellulose decreased (Table 2). Fiber profile similarities between BB+IG and SS+BB+IG were maintained across harvest treatments (Table 2). Biomass cellulose were significantly

Table 2. Biomass quality averaged across three experimental locations (Knoxville, Crossville, and Springfield, TN) and 3 yr (2010–2012).

Harvest treatment†	NWSG treatment‡	Biomass quality§								
		ADF	NDF	N	P	K	Ash	Lignin	Cellulose	Hemicellulose
		g kg^{-1}								
BH	SG	530.0a¶	814.5a	5.6e	0.9f	5.6d	42.0	63.6	466.4a	284.5
	BB+IG	505.0bc	809.7ab	6.8bcd	1.3c	8.8ab	45.6	61.2	443.8b	304.7
	SG+BB+IG	495.9cd	799.1bc	6.5cd	1.3cd	8.4b	47.7	68.2	427.7cd	303.2
EB+BH	SG	503.1bc	788.3c	7.4b	1.1e	6.1d	46.4	67.8	436.2bc	285.2
	BB+IG	507.1b	811.1a	6.3de	1.4bc	8.7ab	43.5	66.9	439.3b	304.0
	SG+BB+IG	490.1de	789.5c	6.6bcd	1.4bc	9.1a	48.5	64.6	425.5cd	299.4
ESH+BH	SG	481.6ef	763.8e	9.1a	1.2d	6.7c	46.8	64.2	417.4de	282.2
	BB+IG	491.4de	788.1cd	7.3bc	1.4ab	9.0ab	48.1	64.5	426.9cd	296.7
	SG+BB+IG	477.5f	777.6d	7.4b	1.5a	9.6a	48.0	65.2	412.3e	300.1
LSD		10.1	10.7	0.1	0.1	0.1	ns#	ns	11.2	ns

† BH, biomass harvest; EB+BH, early-boot plus fall senescence; ESH+BH, early-seedhead plus fall senescence.

‡ NWSG, native warm-season grasses. Treatments: BB+IG, two-way blend of big bluestem/indiangrass; SG, switchgrass; SG+BB+IG, three-way mixture of switchgrass/big bluestem/indiangrass.

§ ADF, acid detergent fiber; NDF, neutral detergent fiber.

¶ Means within a column not sharing a lowercase letter are significantly different for the harvest × NWSG interaction (Fisher's Protected LSD, α = 0.05).

Nonsignificant at the 0.05 probability level.

different between the three NWSG mixtures when harvested at BH, with SG having the greatest level, followed by BB+IG (Table 2). End-of-growing-season cellulose decreased compared with EB when early-season forage growth was removed (Table 2).

Digestible Biomass

Biomass digestibility in vitro was used to estimate the ability of microbes to digest the biomass from the various treatments. There were no differences in biomass IVDMD48h between BH, EB+BH, and ESH+BH for any NWSG treatment (Table 3). Using these data, DB was determined by multiplying biomass yield found in Fig. 2 of McIntosh et al. (2015) by IVTDMD48h (Table 3). The SG monoculture had the greatest DB of 7.4 Mg ha^{-1} at BH, making it a desirable biomass crop (Table 3). Using BB+IG or SG+BB+IG resulted in less DB biomass in the EB+FD and ESH+FD harvest treatments; however, that was not the case for DB at BH, where the three-way mixture was statistically the same as SG (Table 3). This DB relates to plant maturity and time between early-season forage removal and the plants in fall senescence when above-ground material dies.

Total Nutrient Removal

Total macronutrient removal was calculated using previous yield data presented in McIntosh et al. (2015) with the nutrient content data presented in this manuscript. To calculate combined nutrient removal amounts, the CP was converted to N (CP ÷ 6.25) and the P and K to oxide forms of phosphate and potash (Table 1). Then, the respective forage (Table 1) and biomass nutrient contents (Table 2) were multiplied by their respective yield found in Fig. 1 and 2 of McIntosh et al. (2015) to estimate total nutrient removal. These were summed to determine total N, P, and K removal for each treatment (Table 4). The least total nutrient removal for all NWSG harvest treatments occurred at BH (Table 4). When an early-season forage harvest was taken, there was an increase in

Table 3. Digestibility attributes of biomass averaged across three experimental locations (Knoxville, Crossville, and Springfield, TN) and 3 yr (2010–2012).

Harvest treatment†	NWSG treatment‡	Digestibility attributes of biomass		
		Biomass yield	IVTDMD48h§	Digestible biomass¶
		Mg ha^{-1}	g kg^{-1}	Mg ha^{-1}
BH	SG	16.6a#	317.7	5.3a
	BB+IG	7.3de	368.7	2.7cd
	SG+BB+IG	11.7b	432.8	5.1a
EB+BH	SG	11.2bc	351.3	3.9b
	BB+IG	8.3d	381.4	3.2bc
	SG+BB+IG	8.5d	412.5	3.5b
ESH+BH	SG	8.5d	314.9	2.7cd
	BB+IG	5.2e	401.7	2.1d
	SG+BB+IG	8.6cd	410.7	3.5b
LSD		2.7	ns††	1.4

† BH, biomass harvest; EB+BH, early-boot plus fall senescence; ESH+BH, early-seedhead plus fall senescence.

‡ NWSG, native warm-season grasses. Treatments: BB+IG, two-way blend of big bluestem/indiangrass; SG, switchgrass; SG+BB+IG, three-way mixture of switchgrass/big bluestem/indiangrass.

§ In vitro true dry matter digestibility at 48 h.

¶ Digestible biomass calculated by multiplying the percentage of IVTDMD48h by biomass yield of Fig. 2 McIntosh et al. (2015).

Means within a column not sharing a lowercase letter are significantly different for the harvest × NWSG interaction (Fisher's protected LSD, α = 0.05).

†† Nonsignificant at the 0.05 probability level.

Table 4. Nutrient removal for forage, biomass, and combined harvests averaged across three experimental locations (Knoxville, Crossville, and Springfield, TN) and 3 yr (2010–2012).

Harvest treatment†	NWSG treatment‡	Nutrient removal								
		Forage§			Biomass			Total		
		N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O
		kg ha^{-1}								
BH	SG	–	–	–	75.5a¶	34.8a	101.1a	74.6d	34.6c	100.9e
	BB+IG	–	–	–	46.1de	21.1cd	68.1bc	46.2e	21.2d	67.8e
	SG+BB+IG	–	–	–	61.5bc	32.0ab	99.7a	61.9de	31.6cd	99.4e
EB+BH	SG	131.6ab	47.03ab	183.0ab	75.8a	26.2bcd	74.9b	221.2a	80.0a	276.21ab
	BB+IG	86.92c	29.65c	116.0c	42.3de	25.7bcd	69.7b	132.2c	55.2b	185.44d
	SG+BB+IG	113.6bc	38.49bc	156.1bc	52.5cd	26.6abcd	82.6ab	158.6b	63.6b	235.12bc
ESH+BH	SG	147.2a	58.04a	217.3a	74.1ab	25.9bcd	65.6bc	210.4a	78.3a	267.8ab
	BB+IG	109.7bc	39.45bc	165.9bc	35.0e	18.0d	47.6c	139.5bc	56.6b	209.12cd
	SG+BB+IG	143.9a	52.89a	221.0a	52.4cd	27.9abc	80.9ab	203.04a	82.1a	305.3a
LSD		27.5	11.2	44.3	12.0	8.4	20.8	25.8	12.6	41.36

† BH, biomass harvest; EB+BH, early-boot plus fall senescence; ESH+BH, early-seedhead plus fall senescence.

‡ NWSG, native warm-season grasses. Treatments: BB+IG, two-way blend of big bluestem/indiangrass; SG, switchgrass; SG+BB+IG, three-way mixture of switchgrass/big bluestem/indiangrass.

§ Forage yield data used for nutrient removal calculations found in Fig. 1 of McIntosh et al. (2015).

¶ Means within a column not sharing a lowercase letter are significantly different for the harvest × NWSG interaction (Fisher's protected LSD, $\alpha = 0.05$).

N, P, and K removal for all NWSG treatments (Table 4). Total P and K removal was similar between SG and SG+BB+IG, whereas BB+IG removed significantly less N, P, and K, primarily due to lesser yield found in Fig. 1 and 2 of McIntosh et al. (2015). The removal amounts of total P and K for SG were similar to the results reported by de Koff and Abimbola (2015), who found similar yield and concentrations of P and K in SG under similar harvest timings. Additionally, research by Lindsey et al. (2013) in Tennessee determined that, although K decreased for the BH harvest, there were greater concentrations of P and K throughout the growing season (Table 4). As stated in Materials and Methods, soil testing and fertilization occurred on the entire study area, not on an individual plot basis. Therefore, there should be reservations about making major conclusions based on these removal rates.

However, these data indicate that total nutrient removal will be significantly increased if a forage harvest is taken before the biomass harvest. The increased nutrient removal in a dual-use harvest system will increase the importance of routine soil testing for NWSG fields used for both forage and biomass. Taking an early-season forage harvest also significantly increased the total removal of N, P, and K. Data presented here agree with Kimura et al. (2015), which showed that as yield increased with plant maturity the potential for nutrients removed increased over time. Based on removal rates shown in this study, a dual-use system will require significantly greater N, P, and K replacement fertilization than a biomass-alone system. Current recommendations for biomass production in Tennessee are for P and K applications only on soils with low nutrient status with <20 kg P ha^{-1} and <101 kg K ha^{-1} (University of Tennessee Soil, Plant and Pest Center, 2012). An economic analysis of this study can be found in Boyer et al. (2015).

CONCLUSIONS

A dual-use forage and biomass system can be successful, depending on the goals of the producer and on which marketable product is needed. Harvest timing and NWSG in mixture can be used to alter yield and quality characteristics of both the forage and the biomass. Producers will need to consider the economic value of the forage and biomass crop as well as the expenses involved with these harvest scenarios. For alternative energy and fuel production, these data confirm that SG harvested at BH will produce the greatest quality biomass material according to current industry standards. Forage harvest nutritive values of NWSG in mixture can support livestock with adequate nutrition, particularly if the early harvests are made at the EB stage of growth. Delaying harvest until ESH will affect the quality of the forage harvested as well as the yield and chemical characteristics of the biomass. The use of NWSG in mixture can alter quality attributes in both forage and biomass systems and has the potential to become a management practice for manipulating forage and biomass quality.

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REFERENCES

Adler, P.R., M.A. Sanderson, A.A. Boateng, P.J. Weimer, and H.G. Jung. 2006. Biomass yield and biofuel quality of switchgrass harvested in fall or spring. Agron. J. 98:1518–1525. doi:10.2134/agronj2005.0351

Ball, D.M., C.S. Hoveland, and G.D. Lacefield. 2015. Southern forages: Modern concepts for forage crop management. 4th edition, Potash and Phosphate Institute, Norcross, GA.

Bates, G., C. Harper, and F. Allen. 2008. Forage and field crop seeding guide for Tennessee. PB 378. Univ. of Tennessee Ext. Serv., Knoxville.

Boyer, C.N., A.P. Griffith, D.W. McIntosh, G.E. Bates, P.D. Keyser, and B.C. English. 2015. Breakeven price of biomass from switchgrass, big bluestem, and indiangrass in a dual-purpose production system in Tennessee. Biomass Bioenergy 83:284–289. doi:10.1016/j.biombioe.2015.10.006

Brejda, J.J. 2000. Fertilization of native warm-season grasses. In: B.E. Anderson and K.J. Moore, editors, Native warm-season grasses: Research trends and issues. CSSA Spec. Publ. no. 30. CSSA, Madison, WI. p. 177–200.

Chang, V.S., and M.T. Holtzapple. 2000. Fundamental factors affecting biomass enzymatic reactivity. Appl. Biochem. Biotechnol. 84:5–37.

de Koff, J.P., and A. Abimbola. 2015. Changes in nutrient characteristics of switchgrass for bioenergy. Agron. J. 107:2401–2409. doi:10.2134/agronj15.0183

Dien, B.S., H.G. Jung, K.P. Vogel, M.D. Casler, J.F. Lamb, L. Iten, R.B. Mitchell, and G. Sarath. 2006. Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass. Biomass Bioenergy 30:880–891. doi:10.1016/j.biombioe.2006.02.004

Epstein, H.E., W.K. Lauenroth, I.C. Burke, and D.P. Coffin. 1996. Ecological responses of dominant grasses along two climatic gradients in the Great Plains of the United States. Vegetation Sci. 7:777–788. doi:10.2307/3236456

Fike, J.H., D.J. Parrish, D.D. Wolf, J.A. Balasko, J.T. Green, Jr., M. Rasnake, and J.H. Reynolds. 2006. Switchgrass production for the upper southeastern USA: Influence of cultivar and cutting frequency on biomass yields. Biomass Bioenergy 30:207–213. doi:10.1016/j.biombioe.2005.10.008

Golden Gate Weather. 2014. U.S. climate normals. Golden Gate Weather Serv. http://ggweather.com/normals/ (accessed 5 Mar. 2014).

Guretzky, J.A., J.T. Biermacher, B.J. Cook, M.K. Kering, and J. Mosali. 2011. Switchgrass for forage and bioenergy: Harvest and nitrogen rate effects on biomass yields and nutrient composition. Plant Soil 339:69–81. doi:10.1007/s11104-010-0376-4

Hong, C.O., V.N. Owens, D.K. Lee, and A. Boe. 2013. Switchgrass, big bluestem, and indiangrass monocultures and their two-and three-way mixtures for bioenergy in the Northern Great Plains. BioEnergy Res. 6:229–239. doi:10.1007/s12155-012-9252-9

Jacobsen, S.E., and C.E. Wyman. 2000. Cellulose and hemicellulose hydrolysis models for application to current and novel pretreatment processes. Appl. Biochem. Biotechnol. 81:84–86.

Kimura, E., H.P. Collins, and S. Fransen. 2015. Biomass production and nutrient removal by switchgrass under irrigation. Agron. J. 107:204–210. doi:10.2134/agronj14.0259

Lee, R. 2006. Switchgrass as a bioenergy crop. ATTRA–Nat. Sustain. Agric. Info. Serv. www.attra.ncat.org/attra-pub/switchgrass.html (accessed 12 Jan. 2012).

Lindsey, K., A. Johnson, P. Kim, S. Jackson, and N. Labbé. 2013. Monitoring switchgrass composition to optimize harvesting periods for bioenergy and value-added products. Biomass Bioenergy 56:29–37. doi:10.1016/j.biombioe.2013.04.023

Lorenz, A. J., R. P. Anex, A. Isci, J. G. Coors, N. de Leon, and P. J. Weimer. 2009. Forage quality and composition measurements as predictors of ethanol yield from maize (*Zea mays* L.) stover. Biotechnol. Biofuels 2:5. doi:10.1186/1754-6834-2-5

Lynd, L.R., J.H. Cushman, R.J. Nichols, and C.E. Wyman. 1991. Fuel ethanol from cellulosic biomass. Science 251:1318–1323. doi:10.1126/science.251.4999.1318

McIntosh, D.W., G.E. Bates, P. Keyser, F. Allen, C. Harper, J. Waller, M. Backus, and J. Birchhead. 2015. The impact of forage harvest timing on biomass yield from native warm-season grass mixtures. Agron. J. 107:2321–2326. doi:10.2134/agronj15.0251

McLaughlin, S.B., and L.A. Kszos. 2005. Development of switchgrass (*Panicum virgatum*) as a bioenergy feedstock in the United States. Biomass Bioenergy 28:515–535. doi:10.1016/j.biombioe.2004.05.006

Mitchell, R., J. Fritz, K. Moore, L. Moser, K. Vogel, D. Redfearn, and D. Wester. 2001. Predicting forage quality in switchgrass and big bluestem. Agron. J. 93:118–124. doi:10.2134/agronj2001.931118x

Mosali, J., J.T. Biermacher, B. Cook, and J. Blanton. 2013. Bioenergy for cattle and cars: A switchgrass production system that engages cattle producers. Agron. J. 105:960–966. doi:10.2134/agronj2012.0384

Muir, J.P., M.A. Sanderson, W.R. Ocumpaugh, R.M. Jones, and R.L. Reed. 2001. Biomass production of 'Alamo' switchgrass in response to nitrogen, phosphorus, and row spacing. Agron. J. 93:896–901. doi:10.2134/agronj2001.934896x

Mulkey, V.R., V.N. Owens, and D.K. Lee. 2008. Management of warm-season grass mixtures for biomass production in South Dakota USA. Bioresour. Technol. 99:609–617. doi:10.1016/j.biortech.2006.12.035

Murray, I., and I. Cowe. 2004. Sample preparation. In: C.A. Roberts, J.J. Workman, and J.B. Reeves, editors, Near-infrared spectroscopy in agriculture. ASA, CSSA, and SSSA, Madison, WI. p. 75–112.

Ocumpaugh, W., M. Hussey, J. Read, J. Muir, F. Hons, G. Evers, K. Cassida, B. Venuto, J. Grichar, and C. Tischler. 2003. Evaluation of switchgrass cultivars and cultural methods for biomass production in the south central US. Oak Ridge National Laboratory, Oak Ridge, TN.

Parrish, D.J., and J.H. Fike. 2005. The biology and agronomy of switchgrass for biofuels. Crit. Rev. Plant Sci. 24:423–459. doi:10.1080/07352680500316433

Posler, G.L., A.W. Lenssen, and G.L. Fine. 1993. Forage yield, quality, compatibility, and persistence of warm-season grass-legume mixtures. Agron. J. 85:554–560. doi:10.2134/agronj1993.00021962008500030007x

Propheter, J.L., and S. Staggenborg. 2010. Performance of annual and perennial biofuel crops: Nutrient removal during the first two years. Agron. J. 102:798–805. doi:10.2134/agronj2009.0462

Redfearn, D.D., and C.J. Nelson. 2003. Grasses for southern areas. In: R.F. Barnes, C.J. Nelson, M. Collins, and K.J. Moore, editors, Forages: Introduction to grassland agriculture. Wiley-Blackwell, Ames, IA. p. 149–169.

Richner, J.M., R.L. Kallenbach, and C.A. Roberts. 2014. Dual use switchgrass: Managing witchgrass for biomass production and summer forage. Agron. J. 106:1438–1444. doi:10.2134/agronj13.0415

Sanderson, M.A., and P.R. Adler. 2008. Perennial forages as second generation bioenergy crops. Int. J. Mol. Sci. 9(5):768–788. doi:10.3390/ijms9050768

Sanderson, M.A., P.R. Adler, A.A. Boateng, M.D. Casler, and G. Sarath. 2006. Switchgrass as a biofuels feedstock in the USA. Can. J. Plant Sci. 86(Special Issue):1315–1325. doi:10.4141/P06-136

Sanderson, M.A., R.L. Reed, S.B. McLaughlin, S.D. Wullschleger, B.V. Conger, and D.J. Parrish. 1996. Switchgrass as a sustainable bioenergy crop. Bioresour. Technol. 56:83–93. doi:10.1016/0960-8524(95)00176-X

SAS Institute. 2012. SAS system for Windows. Version 9.3. SAS Inst., Cary, NC.

Soil Survey Staff. 2014. Web soil survey. Natl. Soil Surv. Ctr., Lincoln, NE. http://websoilsurvey.sc.egov.usda.gov/app/homepage.htm (accessed 20 Nov. 2014).

Seepaul, R., B. Maccon, K.R. Reddy, and W.B. Evans. 2014. Harvest frequency and nitrogen effects on yield, chemical characteristics, and nutrient removal of switchgrass. Agron. J. 106:1805–1816. doi:10.2134/agronj14.0129

Springer, T.L., G.E. Aiken, and R.W. McNew. 2001. Combining ability of binary mixtures of native, warm-season grasses and legumes. Crop Sci. 41:818–823. doi:10.2135/cropsci2001.413818x

Stubbendieck, J., S. L. Hatch, and C. H. Butterfield. 2002. North American range plants. University of Nebraska Press., Lincoln, NE.

Thomason, W.E., W.R. Raun, G.V. Johnson, C.M. Taliaferro, K.W. Freeman, K.J. Wynn, and R.W. Mullen. 2005. Switchgrass response to harvest frequency and time and rate of applied nitrogen. J. Plant Nutr. 27:1199–1226. doi:10.1081/PLN-120038544

University of Tennessee Plant, Pest and Soil Center. 2012. Soil testing and fertilizer recommendations: Agronomic crops. https://ag.tennessee.edu/spp/Pages/soilfertilizerpubs.aspx (accessed 14 Apr. 2012).

Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and no starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2

Vogel, K.P., R.B. Mitchell, G. Sarath, H.G. Jung, B.S. Dien, and M.D. Casler. 2013. Switchgrass biomass composition altered by six generations of divergent breeding for digestibility. Crop Sci. 53:853–862. doi:10.2135/cropsci2012.09.0542

Vogel, K.P., B.S. Dien, H.G. Jung, M.D. Casler, S.D. Masterson, and R.B. Mitchell. 2011. Quantifying actual and theoretical ethanol yields for switchgrass strains using NIRS analyses. BioEnergy Res. 4:96–110. doi:10.1007/s12155-010-9104-4

Vogel, K.P., and H.G. Jung. 2001. Genetic modification of herbaceous plants for feed and fuel. Crit. Rev. Plant Sci. 20:15–49. doi:10.1080/20013591099173

Waramit, N., K.J. Moore, and S.L. Fales. 2012. Forage quality of native warm-season grasses in response to nitrogen fertilization and harvest date. Anim. Feed Sci. Technol. 174:46–59. doi:10.1016/j.anifeedsci.2012.02.008