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RESEARCH ARTICLE

Harvest and nitrogen effects on bioenergy feedstock quality of grass-legume mixtures on Conservation Reserve Program grasslands

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Abstract

Perennial grass mixtures established on Conservation Reserve Program (CRP) lands can be an important source of feedstock for bioenergy production. This study aimed to evaluate management practices for optimizing the quality of bioenergy feedstock and stand persistence of grass-legume mixtures under diverse environments. A 5-year field study (2008–2012) was conducted to assess the effects of two harvest timings (at anthesis vs after complete senescence) and three nitrogen (N) rates (0, 56, 112 kg N ha^{-1}) on biomass chemical compositions (i.e., cell wall components, ash, volatiles, total carbon, and N contents) and the feedstock energy potential, examined by the theoretical ethanol yield (TEY) and the total TEY (i.e., the product of biomass yield and TEY, L ha^{-1}), of cool-season mixtures in Georgia and Missouri and a warm-season mixture in Kansas. The canonical correlation analysis (CCA) was used to investigate the effect of vegetative species transitions on feedstock quality. Although environmental variations (mainly precipitation) greatly influenced the management effect on chemical compositions, the delayed harvest after senescence generally improved feedstock quality. In particular, the overall cell wall concentrations and TEY of the warm-season mixtures increased by approximately 7%. Additional N supplies improved

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the total TEY of both mixtures by ~1.6–4.2 $L\,ha^{-1}$ per 1.0 $kg\,N\,ha^{-1}$ input but likely lowered the feedstock quality, particularly for the cool-season mixture. The cell wall concentrations of cool-season mixture reduced by approximately 3%–6%. The CCA results indicated that the increased legume compositions (under low N input) likely enhanced lignin but reduced ash concentrations. This field research demonstrated that with proper management, grass-legume mixtures on CRP lands can provide high-quality feedstock for bioenergy productions.

KEYWORDS

bioenergy feedstock quality, canonical correlation analysis, conservation reserve program, cool-season mixtures, harvest management, nitrogen management, warm-season mixtures

1 | INTRODUCTION

Currently, most commercialized biofuels are produced from food-based crops, such as corn and sorghum, mainly in the United States, and sugarcane in Brazil for ethanol production or soybeans for biodiesel production (USDA, 2021). Using these crops as feedstock sources, however, not only competes with food/feed supply (food vs fuel debate) but also increases adverse effects on environmental quality by committing more chemical inputs and field activities. For example, heavy inputs of fertilizers, herbicides, and pesticides, or continuous tillage practices result in increases in the degradation of soil and water qualities (Olsson et al., 2019; Tenenbaum, 2008). Instead, perennial herbaceous crops (e.g., switchgrass) have been considered alternative and sustainable energy sources because they typically require less fertilizer (e.g., N) input and land-disturbing activities that can offer multiple environmental benefits, including the mitigation of soil erosion and greenhouse gas emissions, and increases in soil health, nutrient retention, carbon sequestration, water quality, and biodiversity (Brown & Brown, 2014; Lee et al., 2007; McLaughlin & Walsh, 1998; Monti et al., 2012; Nikièma et al., 2011; Yang et al., 2019). Furthermore, some marginally productive and environmentally sensitive croplands are not suitable for growing annual row crops because of their low economic returns associated with high environmental hazards (e.g., substantial nutrient loss via surface runoff and leaching). These marginal lands are not suggested for continuing food/feed-based commodity crop productions but for other purposes, including perennial bioenergy crop cultivations (Emery et al., 2017; Kim et al., 2018; Milbrandt et al., 2014; Varvel et al., 2008).

About 11% (~86 million ha) of the US mainland is considered marginal (Milbrandt et al., 2014). By 2020, around 9 million ha of the existing marginal land had been enrolled in Conservation Reserve Program (CRP), a land retirement program that was established by the Food Security Act of 1985 to safeguard vulnerable land from further degradation (USDA-FSA, 2020). Under the program, land that was unstainable for intensive management associated with row crops was converted to long-term vegetative cover (e.g., native species). Recently, these CRP lands have been proposed as a potential source of bioenergy feedstock production and could contribute up to 50 million Mg of dry biomass annually (USDOE, 2011). This contribution can help to achieve the goal of increased use of renewable fuels (including cellulosic biofuel, biomass-based diesel, and advanced biofuel) to 36 billion gallons by 2022 to replace petroleum-based transportation fuels, mandated by the US government under the Renewable Fuel Standard program. Furthermore, sustainability and resource use efficiency in CRP land can be improved by planting polyculture (e.g., grass-legume mixtures). Studies showed that polyculture production systems had better yield productivity, resistance in weed invasion, and ecosystem services than monoculture systems (Carlsson et al., 2017; De Deyn et al., 2011; Dhakal & Islam, 2018; Jungers et al., 2015; Nyfeler et al., 2011; Quijas et al., 2010; Sanderson et al., 2012; Suter et al., 2015; Yang et al., 2019). Establishing perennial grass mixtures on CRP lands has shown their potentials for dedicated bioenergy feedstock production (Anderson et al., 2016; Chen et al., 2021; Lee et al., 2018; Mohammed et al., 2014). This production system can also provide long-term opportunities for improving the sustainability of agroecological farming and socio-economic development by offering less effort/cost input and alternative incomes for local farmers and by offsetting the program rental costs (Chen et al., 2021; Zhang et al., 2018).

To ensure a reliable feedstock supply and a sustainable production system, it is critical to optimize nitrogen (N) and harvest management practices for perennial energy crops on CRP lands (Anderson et al., 2016; Guretzky et al., 2011; Hong et al., 2014; Lemus et al., 2008; Mulkey et al., 2006). For grass-legume mixtures, the management optimization is more complicated than for perennial monocultures because each species responded differently to different practices. For example, N applied to grass-legume mixtures may improve the biomass yield of perennial grasses while simultaneously reducing persistence of legumes (Harmoney et al., 2016; Lee et al., 2013; Mallarino & Wedin, 1990). Similarly, harvest management impacts biomass yield, feedstock quality, and the vegetative longevity of the perennial grasses. While anthesis and frequent harvest practices enhance overall biomass yield, the regrowth vigor and feedstock qualities are negatively affected (Anderson et al., 2016; Guretzky et al., 2011; Mohammed et al., 2014; Waramit et al., 2011). Contrastingly, delayed harvest after complete senescence can maximize nutrient translocation to belowground biomass and improve the vegetative persistence and resilience to extreme events, such as drought events (Wayman et al., 2014). Therefore, the optimal management practices must incorporate aspects other than maximizing biomass yield only, especially for bioenergy feedstock production systems on CRP areas. The best management must maintain both vegetative vigor and high feedstock quality for bioenergy productions (Lemus et al., 2008).

A long-term replicated field trial of different perennial grass and legume mixtures in six CRP sites (i.e., Kansas, KS; Oklahoma, OK; North Dakota, ND; Montana, MT; Georgia, GA; Missouri, MO) have been assessed for yield potential and economic feasibility based on different N and harvest management from 2008 to 2013 (Anderson et al., 2016; Lee et al., 2013; Mohammed et al., 2014). The management effect on species compositions was also evaluated for the KS-, MO-, ND-, and MT-CRP sites but not for grass-legume mixtures in GA (Harmoney et al., 2016; Mohammed et al., 2014). These studies concluded that the increased N fertilizer rate can improve biomass yield, mainly by increasing perennial grasses, but actually reducing the legume coverages for all experimental sites. The N-induced yield, however, might not be able to offset the incremental costs of N fertilizers, application, and the total operations. From the industrial standpoint, the feedstock chemical compositions are critical indices to ensure the quantity and quality of the bioenergy products and the conversion efficiency (Brown & Brown, 2014; Jönsson et al., 2013; Li et al., 2016). For instance, glucan, xylan, lignin, and ash contents in biomass are of particular importance in either bio- or thermal-chemical conversion processes; the increased biomass volatiles and biomass carbon concentrations offers important advantages for combustion processes, such as pyrolysis and gasification (Demirbas, 2004; Jönsson et al., 2013; Li et al., 2016). Nevertheless, the effects of the environment and management on biomass compositions of the grass-legume mixtures have not been investigated. Therefore, this study aimed to evaluate (1) the effects of the cultivation environments (GA, MO, KS), species (perennial cool/warm-season grass and legume mixtures), and N (application rates) and harvest management (harvest timing) practices on biomass compositions, especially the critical attributes for bioenergy conversions (i.e., glucan, xylan, lignin, ash, volatiles, overall C and N contents) and (2) the impacts of the vegetative species transition on bioenergy feedstock quality.

2 | MATERIALS AND METHODS

2.1 | Site description

Initially, six locations were identified as potential CRP grassland regions and grass-legume mixtures were established (see Anderson et al., 2016; Lee et al., 2013). Subsequently, three sites with contrasting environmental conditions and species composition were identified for evaluating feedstock quality. These were Oconee County, GA (33.8°N 83.4°W), Boone County, MO (39.0°N 92.2°W), and Ellis County, KS (38.8°N 99.4°W). The predominant species were managed differently among locations. Cool-season grasses comprised of tall fescue [TF, *Schedonorus arundinaceus* (Schreb.) Dumort.], orchardgrass (OR, *Dactylis glomerata* L.), and the lespedeza [LSP, *Kummerowia striata* (Thunb.) Schindl.] legume mixtures were established in GA. Mixtures of tall fescue and the predominant legume of red clover (RC, *Trifolium pratense* L.) were grown in MO. In KS, the warm-season grass-legume mixtures were established comprised of sideoats grama [SO, *Bouteloua curtipendula* (Michx.) Torr.], switchgrass (SW, *Panicum virgatum* L.), little bluestem [LB, *Schizachyrium scoparium* (Michx.) Nash], Indiangrass [IN, *Sorghastrum nutans* (L.) Nash], and yellow sweetclover [YSC, *Melilotus officinalis* (L.) Lam.] (Table 1). Selected environmental conditions at the three sites are shown in Table 1. Weather information, including cumulative precipitation and monthly temperature, from 2008 to 2012 along with 30-year averages (1983–2012) were obtained from the National Oceanic and Atmospheric Administration for Oconee County, GA (Watkinsville 5 SSE station, USW00063850), Boone County, MO (Columbia U of M station, USC00231801), and Ellis County, KS (HAYS 1S station, USC00143527) and shown in Figure 1. Based on the CRP regulations, no fertilization, field management practices, and aboveground biomass harvest were implemented in these research sites prior to the beginning of this study in 2008. In the spring of 2008, the field sites were mowed at a 10-cm height before the first N fertilizer treatment.

TABLE 1 Location, conservation reserve program (CRP) enrollment year, environmental conditions including the 30-year averages of precipitation (Precip.) and temperature (temp.), the selected soil chemical properties and soil classification in top 15 cm of soil, and initial species composition, for each of the four CRP research sites

			30-year average		Soil characteristics and classification								Predominant species	
						SOC	TN	P^a	K^a	Ca^a	Mg^a			
State	Location	CRP Since	Precip. (mm)	Temp. (°C)	pH	g kg^{-1}		mg kg^{-1}				Soil class	Perennial grass	Legume
GA	Oconee County (33.8°N 83.4°W)	1986	1249	15.8	5.5	10.4	0.8	21.1	166.6	614.8	111.6	Kanhapludul	TF, OR	LSP
MO	Boone County (39.0°N 92.2°W)	2004	1056	12.7	5.7	19.0	2.1	53.8	94.9	2126.2	240.6	Epiaqualfs	TF	RC
KS	Ellis County (38.8°N 99.4°W)	1988	588	11.8	7.5	24.3	1.9	11.6	364.4	2973.2	352.5	Argiustolls	SO, SW, LB, IN	YSC

Abbreviations: IN, Indiangrass; LB, little bluestem; LSP, lespedeza; OR, orchardgrass; RC, red clover; SO, sideoats grama; SOC, soil organic carbon (LECO method); SW, switchgrass; TF, tall fescue; TN, total nitrogen (Kjeldahl method); YSC, yellow sweetclover.

[a]Macronutrients determined by the Mehlich-3 method.

2.2 | Experimental design

A full factorial design was used in the experiment, including three N levels (0, 56, 112 kg N ha^{-1}) and two harvest times (at anthesis or after complete senescence), within a randomized complete block with three replicates at each location. For each treatment, the plot size was approximately 0.5-ha. Urea fertilizer (46-0-0) was used as the N-source and broadcasted annually using a farm-scale fertilizer spreader between April and June (see Anderson et al., 2016; Lee et al., 2013). Harvest management was determined by grass species and locations. Entire plots were harvested using a farm-scale harvester at a cutting height of 10- to 15-cm. All harvest events at GA and MO were imposed at anthesis/peak standing crop (PSC) or after senescence/the end of the growing season (EGS). In the GA site, the biomass harvest at PSC was conducted only in the spring (single cut), but the EGS harvesting occurred in both spring and fall (two cuts). Both biomass cuts, spring and fall, were later combined to represent EGS treatment. In MO, the biomass was harvested twice (in the early spring and early fall at anthesis) and combined to represent the PSC treatment. Likewise, the biomass harvested in the late spring and at the end of year was combined to represent the EGS treatment. For the warm-season grass and legume mixtures at KS, the biomass was harvested annually either at PSC or after a killing frost (KF). The harvest timing at PSC for each location was determined based on predominant grasses reaching anthesis. The details of the harvest and fertilizer application dates were shown in Lee et al. (2013) and Anderson et al. (2016). The dry-weight-rank procedure (Gillen & Smith, 1986; Harmoney et al., 2016) was used to evaluate the species compositions of grass-legume mixtures. Estimated compositions were proportionated to the range between 0 and 1.

2.3 | Biomass compositional analysis

The harvested biomass was baled and weighted, and subsamples were collected from bales using an electric core sampler with 5-cm diameter and 50-cm length. Subsamples were dried at 60°C for 48 hours in an air circulated oven for the moisture correction and ground to pass a 2-mm screen in a Wiley mill (Model 4, Thomas Scientific) for the feedstock compositional analysis. Concentrations of glucan, xylan, lignin, and ash in biomass were determined using Fourier transform near-infrared (FT-NIR) spectroscopy coupled with partial least square (PLS) multivariate prediction models developed by the National Renewable Energy Laboratory (NREL). Further details on the laboratory analytical procedures used to measure the chemical composition of the model

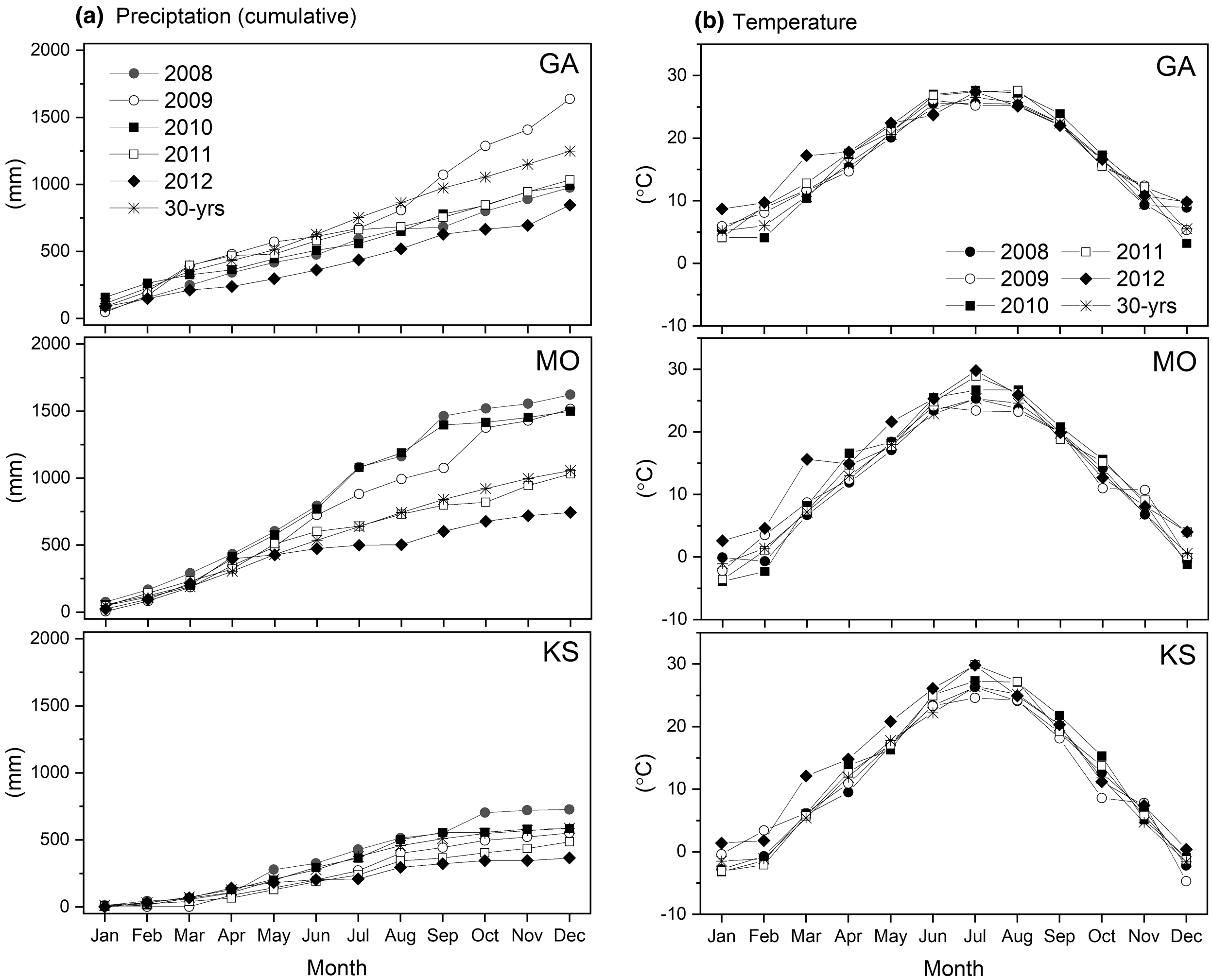

FIGURE 1 Local weather conditions at three experimental sites (GA, MO, and KS) across the 5 years (2008–2012) of study including (a) monthly cumulative precipitation and (b) average monthly temperature and the 30-year monthly average (1983–2012) (data: NOAA).

calibration samples are described in Sluiter et al. (2010). The measured concentrations of glucan and xylan were used to estimate a theoretical ethanol (EtOH) yield (TEY) per dry biomass basis (Liters of EtOH per Mg dry matter, L Mg^{-1}) using equations (Equation 1–3) described in Emerson et al. (2014). The total TEY per hectare of harvested biomass (L ha^{-1}), was estimated by multiplying TEY (L Mg^{-1}) and the harvested biomass yield (Mg ha^{-1}) using Equation 4.

$$\text{C6 EtOH yield}\left(\text{L Mg}^{-1}\right)=\frac{\text{X(g) glucan}}{\text{1(kg) biomass}}\times\frac{1.11\text{(g) }C6}{\text{1(g) glucan}}\times\frac{0.51\text{(g) EtOH}}{1\text{(g) }C6}\times\frac{3.79\text{(L) EtOH}}{2971\text{(g) EtOH}}\times\frac{10^4\text{(kg) biomass}}{\text{1(Mg) biomass}} \tag{1}$$

$$\text{C5 EtOH yield}\left(\text{L Mg}^{-1}\right)=\frac{\text{X(g) xylan}}{\text{1(kg) biomass}}\times\frac{1.1136\text{(g) }C5}{\text{1(g) xylan}}\times\frac{0.51\text{(g) EtOH}}{1\text{(g) }C5}\times\frac{3.79\text{(L) EtOH}}{2971\text{(g) EtOH}}\times\frac{10^4\text{(kg) biomass}}{\text{1(Mg) biomass}} \tag{2}$$

$$\text{TEY}\left(\text{L Mg}^{-1}\right)=\text{C6}+\text{C5 EtOH yield}\left(\text{L Mg}^{-1}\right) \tag{3}$$

$$\text{Total TEY}\left(\text{L ha}^{-1}\right)=\text{TEY}\left(\text{L Mg}^{-1}\right)\times\text{Dry biomass yield}\left(\text{Mg ha}^{-1}\right) \tag{4}$$

FT-IR spectra were also used to predict volatiles, ash, carbon, and nitrogen of samples using PLS 1 models. Model calibration samples comprised of mixed perennial grasses, energy cane, *Miscanthus*, sorghum, and switchgrass were analyzed using a Thermo Anataris II FT-NIR with autosampler attachment (Thermo Scientific) and via proximate and ultimate analyses. Proximate analysis was used to determine the volatiles and additional biomass ash content data using the American Society for Testing and Materials (ASTM) standard D 5142-09 and a LECO Thermogravimetric Analyzer 701 (St. Joseph). Briefly, the dry biomass was placed in a covered crucible to prevent samples from the air during devolatilization. The covered crucible was heated to 950°C for 9 min under UHP nitrogen. The content of volatiles was calculated from the weight loss (ASTM standard E872-82). The biomass ash was measured by heating the dry biomass samples at 750°C under O_2 until a constant weight is reached, and the remaining mass was used to determine the ash content. The ultimate analysis was used for determining biomass C and N contents using the combustion process of dried biomass in a controlled atmosphere according to ASTM D 5373-10, but with a slightly different burn profile as described in the Flour and Plant Tissue Method. During the combustion, biomass-C and -N were converted to CO_2 and NO_x, respectively. The gas products were analyzed for C and N contents using a LECO TruSpec CHN Analyzer.

2.4 | Statistical analysis

Treatment effects on feedstock chemical compositions were analyzed using the three-way, repeated-measures analysis of variance (ANOVA) using the PROC MIXED procedure in SAS (SAS Institute, 2007). The harvest year (5 years), harvest management (two timings), N levels (three rates), and their interactions were considered fixed factors, while the replicates were considered random. The measurement year was considered as the repeated factor, and each plot was used as a subject in the repeated measurement. Each location was analyzed separately because of the diverse species and environmental conditions. The model-predicted residuals were used to assess the normality and homogeneity of residuals to meet the ANOVA assumption using a Shapiro–Wilk test and equal variance test. Proportion values of chemical composition ranging from 0 to 1 were found to have departed from the mean and were subsequently transformed using the arcsin square root transformation (i.e., $\arcsin\sqrt{\text{proportion value}}$). All significant difference were determined at $p \leq 0.05$. Pairwise mean comparisons were made using the Tukey method for p-value adjustment.

In addition, since the transition of vegetations likely influenced feedstock quality, this transition effect on feedstock chemical compositions was investigated using the canonical correlation analysis (CCA). The CCA is a multivariate technique that can simultaneously evaluate the linear interrelationships between two variable sets, namely vegetative species compositions (independent variables/predictors) and feedstock chemical compositions (dependent variables/outcomes). To investigate the simultaneous relationship between several predictors and outcomes, two synthetic variable sets (predictors and outcomes) were created under the CCA process, and the CCA can derive a canonical function by maximizing the correlation between two synthetic variable sets. The PROC CANCORR procedure in SAS was used for the CCA. Two criteria are used to evaluate and establish significance of the CCA-developed canonical functions: 1) the significance of F statistic (p-value <0.001) and (2) that ≥10% of the shared variance in the two variable sets can be explained by the function of interest (Sherry & Henson, 2005). Three indicators have been often used to determine the relative contribution of each original variable to each canonical function, including canonical weights (standardized canonical coefficients), canonical loadings and cross-loadings (structural correlation coefficient, r_s). The canonical weights, however, are subjected to multicollinearity (Liu et al., 2009). In this study, we focused on canonical loadings and cross-loadings as suggested by Kabir et al. (2014) and Liu et al. (2009). The variable was considered to have a significant contribution to the canonical function if its loading was >|0.30| (Kabir et al., 2014).

3 | RESULTS

3.1 | General soil and weather information

Compared with KS-CRP, the soils in GA and MO CRP-sites were more acidic presumably due to lower contents of alkali (K) and earth-alkaline (Ca and Mg) elements (Table 1). Highly weathered soil in GA (Ultisol) also showed lower soil organic carbon and overall fertility compared with the soils in MO (Alfisol) and KS (Mollisol). Monthly cumulative precipitation during the study period (2008–2012) and their 30-year average (1983–2012) for three CPR sites are shown in Figure 1a. The 30-year precipitation averaged 1249-, 1056-, and 588-mm in GA, MO, and KS, respectively. The 5-year average in the study period was generally higher in GA (1097-mm) and MO (1283-mm) than in KS (544-mm). The lower precipitation recorded in 2012 (846-mm in GA; 744-mm in MO; 366-mm in KS) was due to a nationwide drought. Increases in monthly temperature were also observed in the drought year in the three CRP locations (Figure 1b).

3.2 | Overview of species and chemical compositions

The average species and biomass chemical compositions across the 5 years are shown in Table 2. In GA, the 5-year averages of TF, OR, and LSP, a legume species, were 50.8%, 13.4%, and 10.5%, respectively. In MO, 61.5% and

TABLE 2 Descriptive statistics for both vegetative species composition and feedstock chemical compositions of three CRP sites (GA, MO, and KS) in 2008–2012

	GA		MO		KS	
Variables	**Mean (SD)**	**Median (Q1, Q3)**	**Mean (SD)**	**Median (Q1, Q3)**	**Mean (SD)**	**Median (Q1, Q3)**
Species	%					
TF	50.8 (13.1)	51.1 (44.1, 61.4)	61.5 (13.0)	63.0 (53.8, 71.0)	—	—
OR	13.4 (8.9)	14.6 (6.3, 17.9)	—	—	—	—
SO	—	—	—	—	23.9 (13.5)	21.7 (14.1, 36.5)
SW	—	—	—	—	12.3 (8.5)	10.7 (6.4, 16.0)
LB	—	—	—	—	12.9 (8.8)	11.4 (5.3, 20.2)
IN	—	—	—	—	10.7 (8.0)	9.5 (4.0, 15.2)
Legumes[a]	10.5 (8.8)	9.8 (1.8, 18.0)	27.9 (9.0)	28.0 (21.0, 32.5)	14.1 (17.6)	8.1 (0.0, 22.6)
Weed	17.6 (12.8)	15.6 (8.1, 24.3)	7.0 (5.1)	6.0 (3.0, 10.0)	15.9 (20.3)	7.3 (1.8, 20.2)
Compositions	$g\ kg^{-1}$					
Glucan	284.8 (19.0)	285.3 (273.1, 297.8)	285.3 (20.5)	282.7 (270.2, 301.8)	297.6 (23.8)	294.0 (279.0, 313.3)
Xylan	165.6 (13.8)	165.9 (154.3, 174.9)	141.4 (15.1)	143.2 (130.7, 147.9)	203.2 (19.0)	200.0 (187.8, 221.3)
Lignin	153.0 (10.1)	152.9 (145.4, 158.5)	157.4 (14.1)	156.5 (147.0, 168.1)	160.1 (12.2)	159.0 (150.0, 167.3)
Ash-C	76.4 (10.6)	75.7 (68.5, 83.3)	75.5 (10.3)	74.1 (67.5, 83.0)	84.2 (17.0)	83.0 (73.0, 95.5)
Volatile	796.3 (8.4)	795.9 (789.6, 801.2)	780.9 (6.6)	782.2 (775.8, 785.3)	800.2 (11.5)	801.5 (795.0, 807.3)
Ash-P	54.5 (10.2)	55.1 (45.6, 61.8)	67.9 (8.2)	67.0 (62.7, 73.9)	66.1 (17.3)	63.2 (56.6, 70.3)
C	484.6 (6.5)	484.9 (481.1, 488.0)	477.3 (5.2)	477.9 (475.0, 480.7)	472.8 (6.7)	472.5 (469.0, 476.0)
N	14.3 (3.5)	14.3 (11.5, 17.5)	13.1 (2.5)	12.8 (11.3, 14.8)	8.3 (3.0)	9.0 (6.0, 11.0)

Note: Lowercase letters indicate mean separation between locations ($\alpha = 0.05$), organized highest to lowest value for each row.

Abbreviations: Ash-C, the ash based on the chemical compositional analysis; Ash-P, the ash based on the proximate analysis; IN, Indiangrass.; LB, little bluestem; OR, orchardgrass; SO, sideoats grama; SW, switchgrass; TF, tall fescue.

[a]Legumes: lespedeza in GA, red clover in MO, and yellow sweetclover in KS.

27.9% of the canopy were covered by TF and RC, respectively. The warm-season grass and legume mixtures in KS were composed of SO (23.9%), LB (12.9%), SW (12.3%), IN (10.7%), and YSC (14.1%). In GA and MO, the cool-season grass predominant mixtures had lower concentrations of structural components (i.e., glucan, xylan, and lignin), ash, and volatiles than the warm-season grass mixtures in KS ($p < 0.0001$). Conversely, the cool-season grass and legume mixtures had higher concentrations of biomass-C and -N than the warm-season grass predominant field ($p < 0.0001$).

3.3 | Cool-season mixtures

The management effects on vegetative species and chemical composition of the cool-season grass and legume mixtures were evaluated in GA and MO sites. For species compositions in GA, three-way interaction among year, N rate, and harvest timing was only significant for the legume (LSP) content (Table 3); however, no consistent pattern was observed. The two-way interaction between year and harvest timing was significant for both the TF and legume contents. Two harvest regimes did not influence the TF and legume compositions in 2008–2010. In 2011 and 2012, the EGS harvest substantially reduced the TF,

TABLE 3 Analysis of variance (ANOVA) showed the effects of main factors, including year (Y), N rate (N), and harvest timing (HT) and interactions on vegetative species compositions of the cool-season grass and legume mixtures in MO-CRP site with significance level of 0.05

Factors	**TF**	**OR**	**CG**	**Legume**	**Total weed**
Y	****	****	****	****	****
N	**	****	****	****	**
HT	***	*ns*	***	****	ns
Y×N	ns	*	ns	ns	ns
Y×HT	**	ns	ns	**	ns
N×HT	ns	ns	ns	**	ns
Y×N×HT	ns	ns	ns	****	ns

Abbreviations: CG, cool-season grass (sum of TF and OR); Legume, lespedeza predominant; OR, orchardgrass; TF, tall fescue.

Level-1 (*): $0.05 < p < 0.01$; Level-2 (**): $0.01 < p < 0.001$; Level-3 (***): $0.001 < P < 0.0001$; Level-4 (****): $p < 0.0001$; ns: not significant.

conversely increased the legume, compared with the PSC harvest (Table 4). Averages across years and N treatments, EGS harvest reduced the TF and overall cool-season grass contents by approximately 15% and 13%, respectively, but increased legume proportion by 59%. Overall, the proportion of cool-season grass stabilized in the third experimental year (2010) when averaged across treatments; however, the legume proportion substantially declined while weeds increased over the years (Table 4). In contrast, all species were sensitive to different N inputs (Table 3). Increased N rate from 0 to 112 kg-N ha^{-1} increased the overall cool-season grass composition from 54.7% to 70.3% but reduced the legume and weed compositions from 14.9% to 6.7% and from 21.8% to 16.4%, respectively.

Although all analytes related to the chemical composition of feedstock were significantly impacted by the two-way interaction of year and harvest timing in GA (Table 5), only the total TEY showed a consistent pattern between two harvest practices (Table 6). The EGS harvest regime consistently improved the total TEY relative to the PSC harvest in 2008, 2009, and 2012 (Table 6). Averages across years and N rates showed the EGS harvesting not only increased the total TEY by 47% but also improved the overall feedstock quality (i.e., increased TEY and reduced ash concentrations) compared with PSC. Average cell wall components, and TEY tended to be higher in 2008 and 2012 than in other years. For N rate, only the main factor was significant for all quality indicators. Compared with the zero N input, the 112 kg-N ha^{-1} increased the total TEY from 1142.1 to 1365.4 L ha^{-1} (~20% increase) but reduced the overall cell wall compositions (622.3–586.9 g kg^{-1}), volatiles (801.9–792.2 g kg^{-1}), biomass-C (486.1–483.5 g kg^{-1}), and the TEY (339.1–317.3 L Mg^{-1}). Concentrations of both ashes and the biomass-N also increased with increasing N rate (Table 6).

In MO, the responses of the feedstock compositions to three factors and their interactions were similar to the responses in GA. Two-way interaction between year and harvest timing was also significant for all chemical compositions (Table 5), but no consistent trend was shown (Table 7). This interaction effect was likely due to the year variations in compositions. The average across 5 years and three N rates indicated that the EGS harvest regime also substantially improved the total TEY by 47% compared with the PSC harvest. Averages across all treatments showed that higher cell wall concentrations, TEY, and total TEY, corresponding to lower concentrations of both ashes and biomass-N, usually occurred in 2009–2011

TABLE 4 Species composition functional group vegetation of the harvested biomass in the GA CRP land regimes, influenced by year (Y), nitrogen fertilizer rates ($N = 0$, 56, and 112 kg ha^{-1}), harvest timing (HT: PSC vs EGS), and the Y × HT interaction from 2008 to 2012. Lowercase letters indicate mean separation $\alpha = 0.05$ organized highest to lowest value for each column (no mean separations were applied if the variable effect was not significant)

Factor		TF	OR	CG	Legume	Total weed
Y	HT	Composition (%)				
2008	PSC	47.8bcd	4.9	52.7	12.3ab	14.0
	EGS	50.9abcd	0.0	50.9	15.9a	13.3
2009	PSC	65.2a	14.8	80.0	9.5abc	1.3
	EGS	61.4ab	13.2	74.7	6.5bc	2.7
2010	PSC	51.3abc	16.2	67.5	12.5ab	18.8
	EGS	47.8bcd	13.5	61.3	17.1a	20.1
2011	PSC	54.6ab	15.5	70.1	3.0c	24.4
	EGS	35.8d	19.5	55.3	12.7ab	25.9
2012	PSC	55.6ab	16.5	72.1	3.0c	24.9
	EGS	37.7cd	19.7	57.4	12.4ab	30.4
Y mean	2008	49.4b	2.4b	51.8c	14.1a	13.7c
	2009	63.3a	14.0a	77.4a	8.0b	2.0d
	2010	49.6b	14.8a	64.4b	14.8a	19.4bc
	2011	45.2b	17.5a	62.7b	7.8b	25.2ab
	2012	46.6b	18.1a	64.8b	7.7b	27.6a
HT mean	PSC	54.9a	13.6	68.5a	8.1b	16.7
	EGS	46.7b	13.2	59.9b	12.9a	18.5
N mean	0	45.6b	9.1b	54.7b	14.9a	21.8a
	56	52.8a	14.8a	67.7a	9.8b	14.5b
	112	54.1a	16.2a	70.3a	6.7b	16.4b

Abbreviations: CG, cool-season grass (sum of TF and OR); Legume, lespedeza predominant; OR, orchardgrass, TF, tall fescue.

TABLE 5 Analysis of variance (ANOVA) showed the effects of main factors, including year (Y), N rate (N), and harvest timing (HT) and interactions on chemical composition, and proximate and ultimate analytes of cool- and warm-seasons grass and legume mixtures in three CRP sites (GA, MO, and KS) with significance level of 0.05

		Chemical compositions (%)					Proximate and ultimate analysis (%)					
Site	Factors	Glu	Xyl	Lig	Ash-C[a]	Cell wall	Volatile	Ash-P	C	N	TEY	Total TEY
GA	Y	****	****	****	****	****	****	****	****	****	****	****
	N	****	****	**	****	****	****	****	*	****	****	*
	HT	ns	**	ns	**	ns	ns	*	***	*	*	****
	Y×N	ns	ns	**	ns	ns	**	**	***	ns	ns	ns
	Y×HT	****	***	***	****	****	****	****	****	****	****	****
	N×HT	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Y×N×HT	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
MO	Y	****	****	****	****	****	****	****	**	****	****	****
	N	ns	****	***	ns	**	ns	ns	ns	ns	ns	****
	HT	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	****
	Y×N	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Y×HT	****	***	**	****	****	****	**	*	***	****	ns
	N×HT	***	*	ns	*	***	ns	ns	ns	***	***	ns
	Y×N×HT	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
KS	Y	****	****	****	****	****	****	****	****	****	****	****
	N	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	****
	HT	**	****	****	ns	****	*	*	***	****	****	*
	Y×N	ns	****	**	ns	**	ns	ns	****	*	****	**
	Y×HT	****	****	**	*	****	*ns*	*ns*	****	****	****	****
	N×HT	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Y×N×HT	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns

Abbreviations: Ash-C, ash determined by chemical compositional analysis; Ash-P, ash determined by the proximate analysis; C, carbon; Cell wall, the sum of Glu, Xyl, and Lig; Glu, glucan; Lig, lignin; N, nitrogen; TEY, theoretical ethanol yield; Total TEY, TEY times DM yield.; Xyl, xylan.

Level-1 (*): $0.05 < p < 0.01$; Level-2 (**): $0.01 < p < 0.001$; Level-3 (***): $0.001 < p < 0.0001$; Level-4 (****): $p < 0.0001$; ns: not significant.

[a]The MO ash-C data in 2011 did not include for statistical analysis due to substantial number of missing values.

than in 2008 and 2012 (Table 7). Increased N rate (0–112 kg ha^{-1}) also increased the total TEY from 1196.9 to 1670.3 L ha^{-1} (~40% increase) but lowered the feedstock quality by reducing the overall cell wall concentrations from 597.2 to 579.7 g kg^{-1}.

3.4 | Warm-season mixtures

Chemical compositions of the warm-season mixtures in KS were also significantly influenced by the year x harvest timing interaction (Table 5). Although harvest timing impact on feedstock compositions varied from year to year, the biomass harvested after KF generally led to higher cell wall components (i.e., increased xylan and lignin shown in Table 8). Contrastingly, the KF harvest lowered biomass-C and -N concentrations relative to PSC in 2008–2010. Averages across year and N rate showed that the KF harvest increased the overall cell wall concentration and TEY by approximately 7% but reduced the total TEY by 12% compared with PSC (Table 8). On the other hand, the KF regime substantially reduced the concentrations of ash-P and tissue-N by 10% and 40%, respectively. The averaged cell wall components (glucan, lignin), TEY, and total TEY across all treatments showed reduced concentrations in the last two experimental years (2011 and 2012). Both ash-C and ash-P concentrations peaked at 100.3 and 87.4 g kg^{-1}, respectively, in 2012. The total TEY increased over time from 2008 to 2010 but substantially declined in 2011 and 2012. For the N rate, the ANOVA results showed a significant year × N-rate interaction for chemical compositions (Table 5), but no discernible trend (data not shown). The average across years and two harvest regimes showed that only the biomass-N concentrations and total TEY consistently increased with increasing N

TABLE 6 Chemical composition and proximate/ultimate analyses of the harvested biomass in the GA CRP land regimes, influenced by year (Y), nitrogen fertilizer rates ($N = 0$, 56, and 112 kg ha^{-1}), harvest timing (HT: PSC vs EGS), and the Y × HT interaction from 2008 to 2012. Lowercase letters indicate mean separation at $\alpha = 0.05$ organized by highest to lowest value for each column (no mean separations were applied if the variable effect was not significant)

Factor		Chemical compositions (g kg^{-1})					Proximate and ultimate analysis (g kg^{-1})					
Y	HT	Glu	Xyl	Lig	Ash-C	Cell wall	Volatile	Ash-P	C	N	TEY (L mg^{-1})	Total TEY (L ha^{-1})
2008	PSC	298.5b	173.2ab	156.8abc	78.8abc	628.5abc	797.4bc	52.1bcd	482.8b	11.0b	343.6ab	263.6e
	EGS	290.1b	180.4a	161.6a	74.4 cd	632.1ab	796.9bc	49.0 cd	485.5ab	10.7b	342.9abc	719.3d
2009	PSC	286.3b	159.8c	148.8bcd	87.3a	594.8d	790.2 cd	59.0abc	482.9b	16.3a	324.8 cd	893.6 cd
	EGS	285.4b	161.8bc	145.9 cd	84.7ab	593.0d	788.4d	60.4ab	483.1b	15.5a	325.7bcd	1378.5b
2010	PSC	267.0 cd	160.2bc	161.2a	86.2a	588.4d	798.7ab	54.5bcd	489.1a	15.4a	311.2de	1191.5bc
	EGS	283.7b	163.4bc	155.8abc	73.7 cd	602.9 cd	797.5bc	52.7bcd	490.7a	16.0a	325.6bcd	1150.9bcd
2011	PSC	282.6bc	162.4bc	158.9ab	65.6de	603.9bcd	804.8a	46.5d	489.0a	14.9a	324.1d	1496.8b
	EGS	255.2d	156.9c	147.5 cd	75.7bcd	559.6e	799.8ab	53.8bcd	487.8ab	16.9a	300.3e	1321.1bc
2012	PSC	284.1b	157.2c	141.2d	74.2 cd	582.5de	787.5d	67.9a	472.2c	16.1a	321.4d	1194.5bc
	EGS	314.9a	180.4a	152.1abc	63.4e	647.4a	801.9ab	49.2 cd	482.5b	10.2b	360.7a	2849.5a
Y mean	2008	294.3ab	176.8a	159.2a	76.6bc	630.3a	797.2b	50.6b	484.1b	10.9c	343.2a	491.5d
	2009	285.8b	160.8bc	147.3b	86.0a	593.9b	789.3c	59.7a	483.0b	15.9a	325.2b	1136.1c
	2010	275.4c	161.8bc	158.5a	80.0ab	595.7b	798.1ab	53.6ab	489.9a	15.7a	318.4bc	1171.2bc
	2011	268.9c	159.7c	153.2ab	70.7 cd	581.8b	802.3a	50.1b	488.4a	15.9a	312.2c	1408.9bc
	2012	299.5a	168.8ab	146.7b	68.8d	614.9a	794.7b	58.5a	477.3c	13.1b	341.0a	2022.0a
HT mean	PSC	283.7	162.6b	153.4	78.4a	599.6	795.7	56.0a	483.2b	14.8a	325.0b	1008.0b
	EGS	285.9	168.5a	152.6	74.4b	607.0	796.9	53.0b	485.9a	13.8b	331.0a	1483.9a
N mean	0	290.7a	174.7a	156.9a	71.5b	622.3a	801.9a	47.5b	486.1a	12.0c	339.1a	1142.1b
	56	285.9a	164.1b	150.7b	78.1a	600.8b	794.8b	57.4a	484.0ab	14.7b	327.8b	1230.3ab
	112	277.7b	157.9c	151.3b	79.6a	586.9c	792.2b	58.7a	483.5b	16.2a	317.3c	1365.4a

Abbreviations: Ash-C, ash determined by chemical compositional analysis; Ash-P, ash determined by the proximate analysis; C, carbon; Cell wall, the sum of Glu, Xyl, and Lig; Glu, glucan; Lig, lignin; N, nitrogen; TEY, theoretical ethanol yield; Total TEY, TEY times DM yield.; Xyl, xylan.

TABLE 7 Chemical composition and proximate/ultimate analyses of the harvested biomass in the MO CRP land regimes, influenced by year (Y), nitrogen fertilizer rates ($N = 0$, 56, and 112 kg ha^{-1}), harvest timing (HT: PSC vs EGS), and the Y × HT interaction from 2008 to 2012. Lowercase letters indicate mean separation $\alpha = 0.05$ organized highest to lowest value for each column (no mean separations were applied if the variable effect was not significant)

Factor		Chemical compositions (g kg^{-1})					Proximate and ultimate analysis (g kg^{-1})					
Y	HT	Glu	Xyl	Lig	Ash-C	Cell wall	Volatile	Ash-P	C	N	TEY (L mg^{-1})	Total TEY (L ha^{-1})
2008	PSC	283.7 cd	153.8a	149.4b	80.3ab	586.9bcd	776.7bc	68.5abcde	474.0bc	12.7b	318.5ab	1203.9
	EGS	258.5f	130.5bc	145.3b	89.1a	534.2e	773.7c	72.5abc	476.4abc	16.1a	283.1c	1620.6
2009	PSC	278.7de	152.1a	153.2ab	85.3ab	584.0 cd	775.7c	72.0abcd	474.4bc	12.7b	313.6b	1476.9
	EGS	303.6ab	145.5a	168.4a	69.1 cd	617.6a	785.6a	62.8d	482.2a	11.8b	326.8ab	1930.8
2010	PSC	305.4a	139.8ab	160.5ab	63.3d	605.5abc	788.6a	59.7e	478.5abc	10.7b	323.5ab	1385.1
	EGS	310.2a	145.3a	159.9ab	68.7 cd	614.7ab	783.3ab	66.4bcd	477.2abc	11.2b	332.3a	1930.0
2011	PSC	285.8bcde	149.2a	170.8a	N/A	605.0abc	785.2a	63.4 cde	480.3ab	12.0b	315.9ab	1057.0
	EGS	300.8abc	152.4a	161.5ab	N/A	615.1abc	785.9a	63.3cde	479.8ab	12.2b	329.4ab	1964.2
2012	PSC	267.0ef	123.6c	153.8ab	75.2bc	544.2e	775.9c	74.5ab	472.5c	16.0a	283.8c	533.4
	EGS	270.0def	126.4bc	160.7ab	76.5bc	558.0de	778.1bc	76.1a	477.4abc	15.2a	289.4c	886.5
Y mean	2008	271.1c	142.1b	147.3b	84.7a	560.6b	775.2c	70.5ab	475.2b	14.4a	300.8b	1412.3b
	2009	291.2b	148.8ab	160.8a	77.3b	600.8a	780.6b	67.4bc	478.3ab	12.2b	320.2a	1703.9a
	2010	307.9a	142.6ab	160.2a	66.0c	610.1a	785.9a	63.1c	477.8ab	10.9b	327.9a	1657.6ab
	2011	292.7b	150.9a	166.2a	N/A	610.3a	785.5a	63.3c	480.1a	12.1b	322.6a	1510.6ab
	2012	268.1c	125.0c	157.3a	75.8b	551.2b	777.0bc	75.3a	475.0b	15.6a	286.6c	709.9c
HT mean	PSC	283.8	143.7	157.6	76.0	585.2	780.4	67.6	475.9b	12.8	311.1	1131.2b
	EGS	288.6	140.0	159.1	75.9	588.0	781.3	68.2	478.6a	13.3	312.2	1666.4a
N mean	0	283.5	149.7a	164.0a	77.0	597.2a	780.1	67.7	477.4	13.0	315.2	1196.9b
	56	284.7	139.1b	158.7ab	74.2	582.6b	781.7	67.3	478.2	13.1	308.3	1329.3b
	112	290.4	136.8b	152.4b	76.7	579.7b	780.8	68.8	476.2	13.1	311.4	1670.3a

Abbreviations: Ash-C, ash determined by chemical compositional analysis; Ash-P, ash determined by the proximate analysis; C, carbon; Cell wall, the sum of Glu, Xyl, and Lig; Glu, glucan; Lig, lignin; N, nitrogen; TEY, theoretical ethanol yield; Total TEY, TEY times DM yield.; Xyl, xylan.

TABLE 8 Chemical composition and proximate/ultimate analyses of the harvested biomass in KS CRP land regimes, influenced by year (Y), nitrogen fertilizer rates ($N = 0$, 56, and 112 kg ha^{-1}), harvest timing (HT: PSC vs KF), and the Y × HT interaction from 2008 to 2012. Lowercase letters indicate mean separation $\alpha = 0.05$ organized highest to lowest value for each column (no mean separations were applied if the variable effect was not significant)

Factor		Chemical compositions (g kg^{-1})					Proximate and ultimate analysis (g kg^{-1})					
Y	HT	Glu	Xyl	Lig	Ash-C	Cell wall	Volatile	Ash-P	C	N	TEY (L mg^{-1})	Total TEY (L ha^{-1})
2008	PSC	291.1 cd	187.2efg	154.6 cde	87.8abc	632.8c	794.7	69.3	473.1bc	11.0a	348.6c	724.0c
	KF	315.7b	227.6a	173.3a	78.6bc	716.6a	799.3	59.0	465.9d	3.7c	396.1a	370.0d
2009	PSC	293.2c	184.3 fg	146.6e	79.8abc	624.1c	800.0	59.3	473.9bc	9.2a	347.9c	731.6c
	KF	297.0c	214.1b	162.7bc	78.7bc	673.8b	805.4	55.9	469.9 cd	6.5b	372.7b	926.5b
2010	PSC	326.8ab	179.6 g	169.7ab	63.7c	676.1b	808.2	59.0	483.8a	10.2a	368.7b	1143.7a
	KF	336.6a	209.0bc	176.7a	65.0c	722.3a	810.4	52.6	476.5b	4.9bc	397.6a	1133.2a
2011	PSC	272.2e	197.8 cde	146.2e	99.6ab	616.1c	796.9	71.7	466.2d	10.4a	342.7c	230.6de
	KF	281.6 cde	227.3a	160.0 cd	87.2abc	668.9b	806.9	59.1	471.0bcd	6.3b	371.3b	77.2e
2012	PSC	284.1 cde	195.6def	152.2de	85.6abc	631.9c	788.7	89.8	474.2bc	11.0a	349.7c	115.4e
	KF	275.1de	206.5bcd	156.2 cd	103.9a	637.7c	792.1	85.3	472.7bc	9.8a	351.3c	97.6e
Y mean	2008	303.7b	207.7ab	164.1b	83.4bc	674.7b	796.9bc	64.1b	469.6c	7.4b	372.3b	547.0c
	2009	295.4b	199.5c	154.8c	79.6c	648.9c	802.8ab	57.6b	472.1bc	7.9b	360.3c	829.0b
	2010	331.9a	194.6c	173.5a	64.6d	699.2a	809.2a	55.7b	480.2a	7.5b	383.1a	1138.4a
	2011	277.2c	212.8a	153.4c	93.7ab	642.5 cd	801.9ab	65.4b	468.6c	8.3b	357.0c	153.9d
	2012	279.9c	201.3bc	154.4c	100.3a	634.8d	790.3c	87.4a	473.5b	10.4a	350.5c	106.5d
HT Mean	PSC	293.7b	189.1b	154.1b	85.7	636.2b	797.6b	69.7a	474.3a	10.4a	351.5b	589.1a
	KF	301.5a	217.2a	166.0a	82.9	683.9a	802.8a	62.4b	471.3b	6.2b	377.8a	520.9b
N mean	0	298.8	201.8	161.5	84.2	661.4	799.5	65.3	472.4	8.0b	364.5	453.0b
	56	299.9	205.2	158.5	83.8	662.8	800.3	65.2	472.8	8.0ab	367.9	579.3a
	112	294.2	202.5	160.1	84.9	656.0	800.8	67.6	473.2	8.9a	361.7	632.7a

Abbreviations: Ash-C, ash determined by chemical compositional analysis; Ash-P, ash determined by the proximate analysis; C, carbon; Cell wall, the sum of Glu, Xyl, and Lig; Glu, glucan; Lig, lignin; N, nitrogen; TEY, theoretical ethanol yield; Total TEY, TEY times DM yield; Xyl, xylan.

rate. By increasing N rate from 0 to 112 kg N ha^{-1}, the tissue-N concentrations increased from 8.0 to 8.9 g kg^{-1}, and the total TEY improved from 453.0 to 632.7 L ha^{-1} (Table 8).

3.5 | Canonical correlation analysis

The number of CCA functions was based on a set, independent or dependent set, with the least number of variables (i.e., vegetative species composition set in this study). Thus, only 3–6 functions were derived based on the number of species in each location (Table 9). The full model across all canonical functions was significant based on the Wilk's λ criteria of 0.22 (GA), 0.27 (MO), and 0.08 (KS), respectively ($p < 0.0001$ shown in Table 9). The Wilk's λ indicates the variance unexplained by the full model, so the value of 1-λ represents the overall effect size of the model and can be interpreted as r^2 in multiple regressions. For instance, the 1−λ of the model including four CCA functions in GA was 0.78, meaning that the full model can explain about 78% of the variance shared between two variable sets. Likewise, the full CCA models explained 73% and 92% of the variances in MO and KS, respectively. In each model, the R_c^2 showed that first two canonical functions explained substantial variability between predictor and outcome variable sets, and the first function explained 59%, 47% and 78% of the total variability in GA, MO and KS, respectively (Table 10). Thus, we only focused on the first function, as this was deemed adequate for interpreting variability between the two sets of variables. The loadings (r_s) and cross-loadings of the species and chemical composition variable sets are shown in Table 11. In GA, the loadings showed the most important species composition predictors of the chemical compositions was weed (−0.74) followed by TF (0.64), OR (0.60), and legume (−0.40). The cross-loadings also showed the same trend. The energy-rich indicators (i.e., cell wall, volatiles, and biomass-C) were negatively correlated to the biomass-ash and -N in the chemical composition set and the TF and OR compositions in the species composition set. For the MO site, loadings on weed (0.75) was also the most significant predictor of the feedstock compositions/quality, followed by legume (0.49). The contribution of TF, however, was minimal (−0.12). In the chemical composition set, the loadings showed that lignin (0.91) and biomass-C (0.79) were the primarily contributors to the first canonical function. In the KS site, the loadings on the first function indicated that legume (0.93) was the most important variable from the species composition set for predicting feedstock quality. Among all warm-season grasses, however, only SO (−0.54) showed a significant contribution for predicting the chemical composition (and ultimately quality). Three structural cell wall compositions, biomass-ash, and -C had significant loadings (i.e., > |0.30|) among feedstock quality variables.

4 | DISCUSSION

4.1 | Bioenergy feedstock quality

Each conversion technology has different chemical composition requirements to ensure conversion efficiency (Brown & Brown, 2014; Jönsson et al., 2013; Li et al., 2016). For instance, the biochemical conversion technique is commonly used to produce EtOH from the carbohydrate-rich components (i.e., glucan/cellulose and xylan/hemicellulose). For the biochemical process, lignin is considered an undesirable compound along with the ash content because the increased lignin can (1) enhance biomass recalcitrance, (2) inhibit microbial growth by producing toxic compounds (e.g., phenols and aromatics) during the hydrolysis process, and (3) interfere with cellulase enzyme accessibility to the polysaccharides for sugar production, and (4) not be the source for biological transformation (Li et al., 2010, 2016; Palmqvist & Hahn-Hägerdal, 2000a, 2000b; Pu et al., 2013; Studer et al., 2011). The thermochemical process (e.g., combustion, gasification, fast pyrolysis, or hydrothermal liquefaction) can use heat and/or catalysts to covert carbon-rich materials, including lignin, into energy resources (e.g. syngas or hydrocarbon biofuels). Ash components, mainly inorganic compounds, are unfavorable for bio- and thermo-chemical processes because ash can reduce the conversion effectiveness and upgrading performances due to its strong catalytic effect (Bridgwater, 2012; Kenney et al., 2013; Li et al., 2016).

TABLE 9 Wilks' λ test results of canonical correlation analysis between vegetative species and chemical compositions from GA, MO, and KS in 2008–2012

Site	Function no.	Wilks' λ	*F*-statistic	Hypothesis DF	Error DF	*p*-value
GA	4	0.22	5.43	28	286	<0.0001
MO	3	0.27	5.21	21	190	<0.0001
KS	6	0.08	6.08	42	360	<0.0001

Abbreviations: DF, degree of freedom; Function, canonical function.

For the combustion process, high biomass N contents likely increased nitrogen oxide (NO_x) formation, which is considered a deleterious product for the environment (Lewandowski & Kauter, 2003; Prochnow et al., 2009). In this study, the increased concentrations of cell wall components, volatiles, biomass-C, and TEY referred to the improved feedstock quality for energy productions; conversely, the increased biomass-ashes and -N concentrations meant low-quality feedstock.

TABLE 10 Canonical correlation analysis of vegetative species and chemical compositions from GA, MO, and KS in 2008–2012

Site	Function	R_c	R_c^2	F value	DF	p-value
GA	1	0.77	0.59	5.43	28	<0.0001
	2	0.62	0.38	3.27	18	<0.0001
	3	0.34	0.11	1.47	10	0.16
	4	0.23	0.05	1.10	4	0.36
MO	1	0.68	0.47	5.21	21	<0.0001
	2	0.58	0.34	4.47	12	<0.0001
	3	0.48	0.23	4.04	5	0.0029
KS	1	0.88	0.78	6.08	42	<0.0001
	2	0.68	0.46	2.92	30	<0.0001
	3	0.41	0.17	1.53	20	0.07
	4	0.34	0.11	1.25	12	0.25
	5	0.24	0.06	0.86	6	0.53
	6	0.06	0.00	0.13	2	0.87

Abbreviations: DF, degree of freedom; Function, canonical function; R_c, canonical correlation coefficient; R_c^2, squared canonical correlation, meaning that the amount of the variance shared between the variable sets.

4.2 | Environmental effect

The warm-season grasses usually service as a better herbaceous feedstock for bioenergy production than the cool-season grasses by providing higher carbon-rich components and lower ash content (Cherney et al., 1991; Kenney et al., 2013; Hatfield et al., 2009; Sage & Zhu, 2011; van der Weijde et al., 2013; Zhu et al., 2008). This study, however, showed a higher ash content in the warm-season grass predominant biomass (KS) than the cool-season grasses predominant biomass (GA and MO). This opposite trend was attributed to different soil properties and nutrient contents of the CPR cropland. Compared with GA and MO, the KS CRP site has higher alkali and alkaline earth metals (e.g., K, Ca, and Mg) in soil (Table 1). Increased concentrations of alkali/alkaline earth metals in soil likely facilitate the accumulation of metal nutrients in plant tissue, becoming the source of biomass ash (Li et al., 2016). Also, significant year variations in vegetation species and feedstock chemical compositions in each location were due to the changed weather pattern over the years,

TABLE 11 Canonical solution for the first composition scores of the indicators of feedstock quality and vegetative species compositions from GA, MO, and KS in 2008–2012

Variables	GA			MO			KS		
Independent	Coef.	r_s	Cross r_s	Coef.	r_s	Cross r_s	Coef.	r_s	Cross r_s
TF	0.17	0.64	0.49	0.85	−0.12	−0.09	—	—	—
OR	0.55	0.60	0.46	—	—	—	—	—	—
SO	—	—	—	—	—	—	−0.14	−0.54	−0.48
SW	—	—	—	—	—	—	−0.03	0.01	0.01
LB	—	—	—	—	—	—	−0.10	−0.20	−0.17
IN	—	—	—	—	—	—	0.00	−0.15	−0.13
Leg	−0.19	−0.40	−0.30	0.99	0.49	0.34	1.02	0.93	0.82
Weed	−0.66	−0.74	−0.57	0.83	0.75	0.51	0.25	−0.18	−0.16
Dependent									
Glucan	0.86	−0.17	−0.13	0.06	−0.10	−0.07	0.56	0.75	0.66
Xylan	−0.05	−0.63	−0.48	0.16	0.51	0.35	−0.60	−0.52	−0.46
Lignin	−0.14	−0.61	−0.47	0.65	0.91	0.62	0.39	0.58	0.51
Ash-C	0.66	0.64	0.49	−0.02	−0.14	−0.10	−0.01	−0.64	−0.56
Volatile	0.23	−0.73	−0.56	−0.61	0.33	0.22	−0.04	0.29	0.26
C	0.01	−0.38	−0.29	0.68	0.79	0.54	0.07	0.65	0.57
N	1.08	0.73	0.56	0.01	−0.35	−0.24	−0.01	0.01	0.01

Abbreviations: Coef., standardized canonical function coefficient; IN, Indiangrass; LB, little bluestem; OR, orchardgrass; r_s, structure coefficients (loadings), great than |0.30| are underlined; SO, sideoats grama; SW, switchgrass; TF, tall fescue.

especially precipitation (Harmoney et al., 2016; Templeton et al., 2009; Williams et al., 2016). For instance, the nationwide drought event in 2012 led to substantial declines in legume composition in GA shown in this study, and MO and KS reported by Harmoney et al. (2016). Compared with legumes, the perennial grasses were more resistant to water stress by showing a stable biomass yield and coverage proportion. This decline in legume compositions was likely followed by the increase in weed compositions. For feedstock chemical compositions, legumes tended to have higher lignin contents than grasses (Cherney et al., 1988; Jensen et al., 2012). Although legumes only covered 10%–30% of the canopy, the declined legume likely reduced the lignin concentration, especially in GA and KS. Many studies consistently reported that the water-deficit growing condition could reduce structural cell wall compositions by increasing the non-structural carbohydrate in the lignocellulosic feedstock (Hoover et al., 2018). In this study, however, the reduced cell wall composition due to the severe drought was only observed in MO and KS and not GA. Although all three locations were subjected to water stress in 2012, the cumulative precipitation during the growing season (May to Oct) indicated that the MO and KS sites only received 280-mm and 205-mm of rainfall (~50% of the 30-year average), respectively, but the GA site still had 427-mm precipitation (~70% of the 30-year average).

4.3 | Harvest management

For perennial grasses, delaying a harvest until EGS or after KF has been consistently reported to provide multiple benefits, including (1) improvements of stand persistence and regrowth vigor potentials by extending the time for vegetative development and reproductive tiller growth and (2) increases in nutrient use efficiency and feedstock quality by facilitating the nutrient translocation from the aboveground to underground biomass which can be recycled in the following year (Lee et al., 2014; MacAdam & Nelson, 2003; Zumpf et al., 2019). In this study, the delayed harvest also benefited the perennial vigor in MO and KS by improving the overall coverage of perennial grasses except for GA (Table 4; Tables S1 and S2). Since the harvest management in GA consisted of two harvest timing and frequencies (only one harvest at PSC but two harvests at the EGS in the late spring and fall), this confounding management effect on perennial persistence became even more complicated in GA than MO and KS. We hypothesized that this opposite effect was mainly due to the rapid depletion of soil nutrients under the frequent harvest practice. Compared with only one harvest at PSC, the two harvests likely increased biomass nutrient removal from soil and accelerated the nutrient depletion in the soil (Follett & Wilkinson, 1995; Gabrielle et al., 2014; Kering et al., 2013; Minson, 1981; Mullahey et al., 1992; Pedroso et al., 2014). The GA CRP sites already had lower soil fertility than the other locations, so the insufficient nutrient contents can have a severe impact on grass persistence (Table 1). Delayed harvesting has been suggested to improve the feedstock quality through reducing moisture, ash, and N contents in aboveground biomass due to the nutrient translocation to the underground tissues (Mitchell & Schmer, 2012; Ong et al., 2018).

4.4 | Nitrogen management

Our previous study showed that increased N rate typically improved biomass yield for warm- and cool-season grass mixtures (Anderson et al., 2016; Lee et al., 2013; Mohammed et al., 2014). The increased yield likely improved the total TEY, shown in this study. Increased N rate also improved the stand persistence and productivity of the cool-season grasses but consistently declined the legume coverage over the years in our studies (Table 4 and Table S1; Harmoney et al., 2016; Mohammed et al., 2014) and other literature (Mallarino & Wedin, 1990). Compared with the warm-season grasses, the cool-season grasses were highly responsive to the N supply for stand coverage and biomass chemical compositions (Cherney et al., 1991). With more N input, the cool-season grass becomes more competitive than legumes and the annual weed compositions (Table 4 and Table S1; Harmoney et al., 2016). Nitrogen effects on biomass compositions, however, are substantially influenced by environmental variations (weather and soils) and species (Allison et al., 2012; Emery et al., 2020; Heggenstaller et al., 2009; Hong et al., 2014; Waramit et al., 2011). Increased N was consistently reported to increase the overall yield and biomass-N concentrations (Arundale et al., 2015; Gurezky et al., 2011; Ibrahim et al., 2017; Lemus et al., 2008; Mulkey et al., 2008; Murozuka et al., 2014; Vogel et al., 2002). The additional N supply, however, has been reported to have positive (Allison et al., 2012; Arundale et al., 2015; Hong et al., 2014; Lemus et al., 2008; Nazli & Tansi, 2019; Waramit et al., 2011), negative (Hodgson et al., 2010), or no effects (Lee et al., 2007; Ibrahim et al., 2017; Seepaul et al., 2014) on cellulose and lignin concentrations in the monoculture production studies. In the mixture systems, biomass compositions were influenced by the confounding factors of N treatments and species transitions. In GA and MO, increased N input decreased the feedstock quality by increasing the biomass-ash and -N contents and reducing the cell wall compositions. Since legumes have higher lignin than perennial grasses, the reduced lignin can be attributed to the declined legume composition with increasing N input. Many studies reported that the biomass compositions of the warm-season grass seldom responded to N supplies except for the tissue-N concentration (Lee et al., 2007; Ibrahim et al., 2017; Seepaul et al., 2014).The increased tissue-N concentration resulting from more N input was also shown in the KS site of this study and likely has adverse impact on feedstock quality.

4.5 | Species effect on chemical compositions

The CCA was used to study the relationship between species transitions and feedstock quality because this method can avoid the multicollinearity issue for the correlated predictors in regression prediction models, such as multiple linear regression analysis (Kabir et al., 2014; Sherry & Henson, 2005). The CCA analysis can also differentiate the contributions of predictors (species) to the outcomes (compositional analytes). This study showed that feedstock quality was significantly associated with species compositions, and their relationships varied based on the predominant species and the growth environment. For instance, the changed weed compositions substantially influenced the feedstock chemical compositions of cool-season mixtures but negligibly affected the quality of warm-season mixtures. A strong negative association between weed/legume and cool-season grasses demonstrated that their competitive relationship and preferences for management practices. The negative relationship between the cool-season grass and the quality attributes showed that the increased cool-season grass compositions likely reduced the feedstock quality for bioenergy productions, such as declines in cell wall compositions and volatiles. As a dedicated bioenergy crop candidate, the cool-season grass has an attractive yield potential for energy productions (e.g., high total TEY) and early biomass accumulation (i.e., in spring) before the warm-season biomass are ready; however, its nutrient-rich characteristics in plant tissues likely increase biomass-ash and -N contents (Florine et al., 2006; Lee et al., 2013). The KS site also showed plant competitions between the warm-season grasses and legumes. Increased legume compositions likely enhanced lignin (positive relationship) but reduced xylan (negative relationship) contents because legumes usually have higher lignin concentrations but much lower hemicellulose (the predominant compositions of the structural xylan) than grasses (Cherney et al., 1988, 1991; Jarchow et al., 2012; Mohammed et al., 2014). Improved lignin concentration was also associated with increases in glucan (Pearson's correlation coefficient, $r = 0.53$) and declines in ash concentration ($r = -0.81$ for the ash-C data not shown). Although each indicator provided different information regarding quality control, our results showed that the quality attributes of structural xylan and lignin and the overall C content were more sensitive to the transition of species in the grass-legume mixture system.

5 | CONCLUSIONS

Perennial grass-legume mixtures are ideal polyculture production systems for the Conservation Reserve Program (CRP) land, initially designed for soil and water conservation. For grass mixture systems, it could be challenging to optimize the management practices for proving a sustainable feedstock supply and ensuring vegetative longevity. This study showed that different grass mixtures responded to specific practices for feedstock quality. The chemical compositions of cool-season mixtures were highly sensitive to the N supply compared with the warm-season mixtures. Although the increased N input can improve the total theoretical ethanol yield, the additional N input likely reduced the feedstock quality by reducing the concentrations of cell wall components. The warm-season mixtures responded to the harvest timing more than the cool-season mixtures. Delayed harvest after complete senescence consistently improved feedstock quality of the warm-season mixtures by increasing concentrations of glucan, xylan, lignin, and volatiles and reducing the ash and tissue nitrogen contents. The CCA provided a useful tool to identify the effect of vegetative species transitions on feedstock quality. Most of the quality attributes responded to the changes in species compositions (especially legumes), but the biomass glucan concentrations seemed insensitive to this transition in the cool-season mixtures. Perennial grass and legumes/weeds usually showed a competitive relationship, also meaning that these species favored different management practices. The increased legume compositions likely increased the lignin concentrations in biomass. This long-term field research demonstrated that the goal of supplying high-quality feedstock and maintaining stand persistence can be achieved under proper management and a sufficient water supply. In addition, the CCA coupled with the approach of vegetative species identification (e.g., remote-sensing techniques) can be a powerful tool to predict feedstock quality for future studies.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Chemical composition data presented are available in the Bioenergy Feedstock Library (bioenergylibrary.inl.gov).

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