Revised: 16 March 2023

RESEARCH ARTICLE

GCB-BIOENERGY BIOPRODUCTS FOR A SUSTAINABLE BIOECONOMY

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Evaluation of eastern gamagrass as dual-purpose complementary bioenergy and forage feedstock to switchgrass

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Funding information National Institute of Food and Agriculture; National Science Foundation

Abstract

Switchgrass (SG) is considered a model bioenergy crop and a warm-season perennial grass (WSPG) that traditionally served as forage feedstock in the United States. To avoid the sole dependence on SG for bioenergy production, evaluation of other crops to diversify the pool of feedstock is needed. We conducted a 3-year field experiment evaluating eastern gamagrass (GG), another WSPG, as complementary feedstock to SG in one- and two-cut systems, with or without intercropping with crimson clover or hairy vetch, and under different nitrogen (N) application rates. Our results showed that GG generally produced lower biomass (by 29.5%), theoretical ethanol potential (TEP, by 2.8%), and theoretical ethanol yield (TEY, by 32.9%) than corresponding SG under the same conditions. However, forage quality measures, namely acid detergent fiber (ADF), crude protein (CP), and elements P, K, Ca, and Mg were significantly higher in GG than those in SG. Nitrogen fertilizer significantly enhanced biomass (by 1.54 Mg ha⁻¹), lignin content (by 2.10 g kg⁻¹), and TEY (787.12Lha⁻¹) in the WSPGs compared to unfertilized treatments. Intercropping with crimson clover or hairy vetch did not significantly increase biomass of the WSPGs, or TEP and TEY in unfertilized plots. This study demonstrated that GG can serve as a complementary crop to SG and could be used as a dual-purpose crop for bioenergy and forage feedstock in farmers' rotations.

K E Y W O R D S

bioenergy, cover crop, eastern gamagrass, forage quality, nitrogen application

1 | INTRODUCTION

The need for renewable sources of energy for the world's growing populations while protecting the environment will remain a major challenge facing humans in the 21st

century (Dincer, 2000; Nazir et al., 2020). Global overdependence on fossil fuels for electricity generation and transportation is the major contributor to global climate change and accompanying environmental degradation (Adebayo & Rjoub, 2022). Bioenergy is appealing as it can

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modulate undesirable impacts of fossil fuel production, as its utilization could slow down projections of greenhouse gas emissions. In addition, the prospects of massive biomass production for bioenergy created expectations of tremendous benefits for the world's agricultural sectors, which could help to diversify and revitalize rural economies (Haberzettl et al., 2021; He et al., 2022; Yacobucci & Schnepf, 2007). However, the early enthusiasm about bioenergy became dampened by the realization of the competition of land for food, feed, and fiber versus bioenergy biomass (He et al., 2022; Lark et al., 2022). Afterall, the feedstocks that were being promoted for biofuels (e.g., grain starch, soybeans, canola, and palm oil) are the same crops cultivated worldwide for food and feed. This dilemma led to the current focus on second-generation biomass feedstock, represented by switchgrass (Panicum virgatum, SG) in the United States and miscanthus in Europe (Heaton et al., 2013; Mehmood et al., 2017; Zegada-Lizarazu et al., 2022).

Switchgrass has been selected by the U.S. Department of Energy (DOE) as the model bioenergy feedstock following exhaustive evaluations of its agronomic traits, including prodigious biomass production (Hui et al., 2018; Keyser et al., 2022; McLaughlin & Kszos, 2005; Mitchell et al., 2012), environmental stress tolerance (Liu et al., 2015), and genomics (Burris et al., 2016; Lovell et al., 2021). As a bioenergy crop, Niu et al. (2015) showed that about 30.1% energy in SG is transformed into biogas and about 57.3% energy is stored in biogas residue, and cumulative gas production of biogas and CH₄ by SG are 268.8 and 135.3 NmL gVS^{-1} . Papa et al. (2015) reported that the total energy produced (as sum of bioethanol plus biomethane) is 8.8 and 10 MJ kg⁻¹ dry matter for switchgrass after mild ionic liquid and pressurized hot water pretreatment, respectively. The total energy potential of SG could be significantly increased with the application of enzymatic hydrolysis (Başar et al., 2020). However, other native warm-season perennial grasses (WSPGs) need to be evaluated to diversify biomass production, consistent with tenets of sustainability.

For example, eastern gamagrass (Tripsacum dactyloides, GG) possesses similar appealing characteristics as SG for bioenergy feedstock use, including high biomass production, environmental protection, and enhancement of soil carbon sequestration (Krizek et al., 2002). GG and other grasses are gradually receiving attention as a potential bioenergy feedstock (Dzantor et al., 2015; He et al., 2022; Li et al., 2018). New studies are needed to understand field performance and biomass yield potential of GG in comparison to SG. One study by Ge et al. (2012) reported that GG produces comparable compositions of cellulose, hemicellulose, and lignin as SG, 10%-17% greater glucose, and 13%-35% more ethanol per gram of biomass than SG. Nevertheless, prospects on the use of GG as bioenergy feedstock remain largely under-explored.

While SG and GG are mostly used as bioenergy crops, they can also be used as forage crops (Keyser et al., 2020; Waramit et al., 2012). The dual use of GG and SG as forage and bioenergy feedstock requires assessment of their quality parameters, including crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF) as well as P, Ca, K, and Mg. CP is a measure of N in forage (Allen et al., 2013). Chemical compositions of biomass feedstock also affect the bioenergy production efficiency and energy generated (Devi et al., 2021). Neutral detergent fiber is a measurement of total cell wall constituents such as hemicellulose, cellulose, lignin, and insoluble ash in a plant while ADF is similar, but does not include hemicellulose (Abaye et al., 2009). Previous studies, mostly focused on SG, found that agricultural practices often have significant impacts on its biomass. Heggenstaller et al. (2009) found that nitrogen (N) application at 140 kg N ha⁻¹ produced higher biomass of SG compared to GG. Waramit et al. (2012) reported that N application increased biomass of SG and GG, but GG reached the maximum yield earlier than SG and other grasses. In a 2-year study, Rushing, Lemus, White, et al. (2019) found consistent biomass yields for GG compared to some other native warm-season grasses. In addition, nutritive values of CP, NDF, and ADF differ between SG and GG, and it is influenced by agricultural practices (Angima et al., 2009; Keyser et al., 2020; Mosali et al., 2013). For example, Jung et al. (1990) reported that CP is increased by 10%-26% with N fertilization for some grasses. Siddineni (2011) found that the content of NDF of GG is significantly lower $(649 \pm 6.8 \text{ kg}^{-1})$ compared to SG $(716 \pm 6.8 \text{ g kg}^{-1})$ indicating a better feed quality of GG. Biomass yield, ADF, and NDF are also found to vary with different harvest treatments (one cut vs. two cuts; McIntosh et al., 2016).

Several other studies compared bioenergy biomass yield in monocultures and in multiple-species polycultures of grasses and legumes. For example, Jungers et al. (2015) reported a 7-year study that compared biomass yield of mono- and polycultures (up to 24 species mix) of bioenergy feedstock. Without N fertilization, monocultures of SG and eight-species mixture of grasses and legumes produced the highest biomass yield. With N fertilization, SG and a four-species mixture of grasses produced the most biomass. Intercropping with legume such as red clover enhances SG biomass and forage quality (Warwick et al., 2016). However, a comprehensive study incorporating cover crop, cut frequency, and other agricultural practices on biomass yield, nutrient value, and forage quality of GG and SG is still lacking.

In this study, we aimed at comparing the field performance, biomass yield, and forage quality of GG and SG to understand best management practices for GG cropping systems and bioenergy and forage feedstock potential. We developed legume intercropping systems for the production of mostly monocultures of SG and GG in one-cut or two-cut system. For 3 years, we studied nine potential agronomic management practices for GG by combining different N application frequencies and with and without legume cover crop (i.e., crimson clover or hairy vetch). The two main objectives were (1) to quantify biomass, feedstock composition, and forage qualities of GG and SG; and (2) to evaluate the effects of N application, cover crop, and cut frequency on biomass yield, feedstock and forage qualities of GG and SG. Our overall goal was to understand whether incorporating GG into farms could diversify growers' portfolios and be used as complementary to SG and its potential dual-purpose benefits for farmers.

2 | MATERIALS AND METHODS

2.1 | Site description

A 3-year (2013–2015) field experiment was conducted at the University of Tennessee Highland Rim Research and Education Center (Latitude 36°28'32.57"N; Longitude 86°49'23.59"W) in Springfield, TN. Soil at this site was Dickson silt loam (a fine-silty, siliceous, semiactive, thermic Glossic Fragiudult with about 8% sand, 75% silt, and 17% sand). Soil organic matter was 2.3%, soil nitrogen content was 0.34%, phosphorus content was 0.87%, potassium content was 4.05%, and pH was 6.4. Prior to these experiments, the site was cultivated with orchardgrass for hay production and it contained patches of johnsongrass. In April 2011, before planting the GG and SG plots, we applied an herbicide burndown application of Roundup® (Gly-4; $2.92 Lha^{-1}$) in the plot area. At different times during the study, we applied more Roundup on GG and Steadfast Q, a mixture of Nicosulfuron (Accent®) and Rimsulfuron (Matrix[®]) mixed with crop oil of $0.01 LL^{-1}$ water, sprayed at the rate of 0.05 L ha⁻¹ in GG plots and at the rate of 0.06 L ha⁻¹ in the SG plots to remove johnsongrass patches (Table 1). The mean annual precipitation at the site is 1284 mm and mean annual temperature is 14.2°C. The field site experienced severe weather with drought and high temperatures during the 2011 season.

2.2 | Experimental design

Although GG and SG are both WSPG, they have different appearances and growth habits. As SG grows in clumps and has flat and narrow leaves, and small and oval-shaped seeds, and GG spends by rhizomes and has larger and GCB-BIOENERGY

TABLE 1 Agricultural practices (treatments) evaluated at the University of Tennessee Highland Rim Research and Education Center.

Treatment ID	Treatment	Description
0	Control	No fertilizer application and no cover crop
0/C	No N application + crimson clover	Crimson clover cover crop seeded at 11.2 kg ha ⁻¹
0/H	No N application + hairy vetch	Hairy vetch cover crop seeded at 22.4 kg ha ⁻¹
Ν	Single N application	84 kg N ha ⁻¹ urea applied at planting
N/C	Single N application + crimson clover	84 kg N ha ⁻¹ urea applied at planting + crimson clover
N/H	Single N application + hairy vetch	84 kg N ha ⁻¹ urea applied at planting + hairy vetch
N/N	Double N application	Urea applied at planting and after the first cut of the two-cut system at 84 kg N ha ⁻¹ each for a total of 168 kg N ha ⁻¹
N/N/C	Double N application + crimson clover	Urea applied at planting and after first cut of the two-cut system at 84 kg N ha^{-1} each for a total of $168 \text{ kg N ha}^{-1} + \text{crimson}$ clover
N/N/H	Double N application + hairy vetch	Urea applied at planting and after first cut of the two-cut system at 84 kg N ha^{-1} each for a total of 168 kg N ha^{-1} + hairy vetch

elongated seeds, different row spacings and seeding rates are required. Seeds of 'Hihglander' variety of GG were purchased from Jimmy May Gamagrass Co., Cave Springs, KY. Cold-stratified seeds were seeded using a corn planter at the rate of 13.4 kg pure live seed (PLS) ha^{-1} at a depth of 3.8 and 77.2 cm row spacing in May, 2011. Seeds of 'Alamo' variety of SG was purchased from Turner Seeds Co., Kansas City, MO. They were seeded with a small seed drill at the rate of 6.72 kg PLS ha⁻¹ on May 19, 2011 at a depth of 0.6 and 19 cm row spacing. On May 10, 2012, the GG plots were reseeded at a rate of 13.4 kg PLS ha⁻¹ to make the plots compatible with current farmer's recommendations and to enhance the GG plots which had an initial low emergence. The experimental data analysis for this study starts in 2013 once the plots were well established.

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Experiments were implemented within $3 \times 6 \text{ m}^2$ plots using a randomized split-plot design with four replications/blocks per treatment. The main treatment factor was grass type (GG or SG) and the second factor for the split plots was cutting frequency (one-cut or two-cut). Nine different agricultural practices/treatments were evaluated including a control (0), cropping with crimson clover (0/C) or hairy vetch (0/H), single N application (N), single N application plus cropping with crimson clover (N/C)or hairy vetch (N/H), double N application (N/N), and double N application plus cropping with crimson clover (N/N/C) or hairy vetch (N/N/H). The detailed description of these nine treatments is provided in Table 1. For each block, we first arranged grass type, then cutting frequency within each grass type. After that, treatments were randomly applied to both GG and SG plots. The total number of plots was 144. Crimson clover and hairy vetch were seeded at 11.2 and 22.4 kg ha⁻¹, respectively, in October of each year. Nitrogen fertilization was surface applied to each plot at the set application rate (Table 1).

2.3 | Biomass harvesting and sample processing

Plots were harvested using a plot forage harvester (Carter Mfg. Co) with flail cutters and a mounted module capable of collecting biomass fresh weights in the field and used to estimate biomass. Harvest was conducted once for the one-cut plots and twice for the twocut plots. The first harvests were conducted on May 31, 2013, May 30, 2014, and May 26, 2015. The harvests at the end of season were conducted on December 3, 2013, October 29, 2014, and November 3, 2015. Subsamples of fresh biomass were dried to constant weight at 70°C using an Oven King industrial capacity dryer (Washington Industrial Corp) to determine dry biomass yield. Portions of dry biomass were sent to the UT Extension Soil Plan and Pest Center for analysis of cellulose and hemicellulose to estimate theoretical ethanol potential (TEP) and theoretical ethanol yield (TEY). The TEP was estimated as follows (Goff et al., 2010):

$$H = [\% Cellulose + (\% Hemicellulose \times 0.07)] \times 172.82$$

(1)

$$P = [\% \text{Hemicellulose} \times 0.93] \times 176.87$$
(2)

$$\text{TEP}(L Mg^{-1}) = [H + P] \times 4.17$$
(3)

where H and P are hexose and pentose carbohydrates, respectively. The TEY (Lha^{-1}) was calculated by multiplying an experimental unit's TEP by its respective biomass yield (Mgha⁻¹) (Goff et al., 2010).

Forage quality variables (acid detergent fiber, ADF; neutral detergent fiber, NDF; acid detergent lignin (ADL), crude protein, CP; lignin; and ash) were determined using near infra-red reflectance spectroscopy (NIR, Model 6500, FOSS North America) (Deaville & Flinn, 2000). Elements of Ca, Mg, P, and K were measured using inductively coupled plasma optical emission spectrometer (ICP-OES, Spectro Arcos FHS16). Relative feed value (RFV) was estimated from NDF and ADF as follows (Holland & Kezar, 1990):

$$RFV = (\% DDM \times \% DMI) / 1.29$$
(4)

where %DDM = $88.9-0.779 \times$ %ADF and %DMI = 120/%NDF.

2.4 | Data analysis

Analysis of variance (split-plot ANOVA) was conducted to test the significant differences between grass types, agricultural practice treatments, years, one-cut versus twocut systems, and their interactions using the generalized linear model procedure (GLM, SAS version 9.3; Hui & Jiang, 1996). Multiple comparisons were conducted using least significant difference (LSD) method when significant effects were detected. Contrasts were also constructed to test if there were significant differences of biomass, and forage quality variables between fertilized versus nonfertilized, single N application versus double N application, no cover crop versus cover crop, and crimson clover versus hairy vetch for biomass yield and forage quality variables.

3 | RESULTS

3.1 | Overall effects of grass type, year, treatment, cutting system, and their interactions on biomass yield, feedstock composition, and forage quality

Aboveground biomass yield, cellulose, hemicellulose, TEP, and TEY showed significant differences between two grass types (Table 2), similar to some previous studies (Backus et al., 2017; Wullschleger et al., 2010). Biomass yield, hemicellulose, lignin content, and TEY significantly varied among the nine agricultural practice treatments. All biomass and composition variables were significantly different among the 3 years, and between the two cutting systems except biomass yield (Table 2). These results are also consistent with other early studies such as Ritchie, et al. (2006) and Maughan (2011). There were significant

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TABLE 2	Significant tests (F values) of the effects of grass type, treatment, year, and their interaction on biomass yield, cellulose,
hemicellulos	e, lignin content, theoretical ethanol potential (TEP), and theoretical ethanol yield (TEY) using analysis of variance (ANOVA).

Source of variation	df	Biomass yield	Cellulose	Hemicellulose	Lignin content	TEP	TEY
Block	3	0.26	0.08	3.64*	0.70	1.51	0.23
Year	2	13.30***	37.70***	75.17***	189.82***	41.60***	17.85***
Grass type	1	148.27***	142.59***	165.87***	3.55	39.02***	160.23***
Treatment	8	8.6***	1.43	3.93***	2.50*	1.23	8.20***
No N versus N	1	45.77***	1.00	0.53	4.32*	0.04	40.98***
Single N versus Double N	1	6.98**	2.78	0.26	0.62	0.18	6.55*
No cover crop versus cover crop	1	0.16	2.91	9.24**	4.33*	0.01	0.11
Clover versus Hairy vetch	1	1.82	0.40	4.95*	6.08*	0.78	1.33
Cut frequency	1	2.22	442.56***	186.81***	327.16***	358.45***	17.63***
Year×Grass type	2	26.97***	7.12***	22.37***	1.45	7.07***	23.43***
Year×Treatment	16	1.46	0.37	0.63	0.67	0.55	1.35
Grass type×Treatment	8	1.11	3.03**	3.23***	2.48*	1.47	1.07

Note: Bold font highlights significant effects: ***p < 0.001, **p < 0.01, and *p < 0.05. Treatment indicates agricultural practice.

interactions between year and grass type on all biomass variables except in lignin content. Grass type and treatment interactively influenced cellulose, hemicellulose, and lignin content. Biomass yield, lignin content, and TEY were significantly increased when N was applied. However, double N application only influenced biomass yield and TEY. Significant differences in the amount of hemicellulose and lignin were found when cover crops were present, and differences vary depending on cover crop species (Table 2).

Forage quality for ADF, NDF, ADL, RFV, CP, ash, P, K, Ca, and Mg contents all varied among the 3 years and the two cutting systems, and ADF, NDF, CP, ash, Ca, and K significantly varied between SG and GG (Table 3). These results were consistent to some previous studies (Backus et al., 2017; Edwards et al., 1999; Keyser et al., 2020). The agricultural practice treatments significantly influenced all variables except NDF, RFV, ash, and K. There were significant interactions between year and grass type for ADF, NDF, ash, Ca, and K, between grass type and treatment for ADF, NDF, ADL, ash, Ca, Mg, and K (Table 3). There were also significant differences in ADL, CP, Mg, and P between no N application and N application, in ADF, CP, and Mg between one and double N application, in ADF, ADL, and P between cover crop and no cover crop, and in ADF, ADL, CP, Mg, and K between crimson clover and hairy vetch. Our results showed that different agricultural practices such as N application and use of cover crop could influence the forage quality of GG and SG, similar to some previous studies (Ge et al., 2012; Habermann et al., 2019; Keyser et al., 2020; Waramit et al., 2012). Nitrogen application not only improved biomass but also TEY, especially double N application.

3.2 | Year of growth impact on biomass yield, feedstock composition, and forage quality

Multiple environment factors and different field management practices result in large variability in the biomass yield of bioenergy crops (Maughan, 2011). In particular, stand age and interannual weather variations have been shown to significantly impact the biomass yield of SG and GG. Likewise, we found significant differences in biomass yield among the 3 years studied as well as in all other variables investigated in this study. Similarly, forage nutritive values also varied among the 3 years. For instance, NDF and Mg increased gradually from 2013 to 2015 while ADL, P, and Ca decreased gradually (Table 5). ADF, RFV, and K did not change in 2013 and 2014, but ADF and RFV were reduced significantly while K was enhanced in 2015. CP was highest in 2013 and then decreased in 2014 and 2015. The changes in nutritive values could be caused by the relatively dry and warm growing season in 2015 (Figure S1) which could influence leaf photosynthesis and decrease forage quality (Habermann et al., 2019).

3.3 Grass type impact on biomass yield, and bioenergy feedstock composition and forage quality

Mean biomass yield of GG (6.23 Mgha⁻¹) was 29.5% lower than SG (8.84 Mgha⁻¹) over the 3 years (Table 4), which was consistent with Backus et al. (2017) observations where SG produced ~7–8 Mgha⁻¹ while GG produced ~4–6 Mgha⁻¹. Switchgrass is considered a cellulosic feedstock and the majority of its dry biomass consist of cellulose, hemicellulose, **TABLE 3** Significant tests (*F* values) of the effects of grass type, treatment, year and their interactions on acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), crude protein (CP), lignin content, ash, Ca, Mg, P, and K contents using analysis of variance (ANOVA).

Source of	46	ADE	NDF	ADI	DEV	CD	Ash	Ca	Ма	D	V
variation	ar	ADF	NDF	ADL	RFV	СР	Ash	Ca	Mg	Р	K
Block	3	0.83	2.6	1.92	1.04	0.30	1.52	0.63	0.42	1.54	0.97
Year	2	21.46***	30.72***	328.84***	5.72*	15.48***	6.49**	76.04***	11.14***	262.71***	42.59***
Grass type	1	94.34***	39.83***	1.56	0.10	197.4 1***	21.20***	175.8***	2.87	3.61	27.97***
Treatment	8	2.94**	1.55	5.11***	0.90	10.52***	1.19	2.46*	3.77***	3.79***	1.40
No N versus N application	1	0.14	0.24	13.47***	0.01	54.27***	2.55	0.26	9.66**	11.69***	0.02
Single N versus Double N application	1	0.59	0.01	5.00*	0.34	8.21**	0.14	0.00	5.49*	0.03	1.65
No cover crop versus Cover crop	1	9.91**	1.23	7.14***	0.15	1.58	0.19	1.93	1.55	1.40	0.04
Clover versus Hairy vetch	1	3.91*	0.94	6.08*	0.18	5.63*	2.52	3.81	5.96*	11.91***	3.97*
Cut frequency	1	1049.7***	1108.1***	186.45***	1493.0***	1050.22***	292.55***	72.81***	110.92***	386.92***	1328.93***
Year × Grass type	2	5.06**	10.78***	0.83	1.32	0.85	4.50*	5.33**	0.84	0.85	26.64***
Year \times Treatment	16	0.38	0.46	0.38	0.37	0.73	0.76	0.87	1.32	1.61	0.87
Grass type × Treatment	8	2.71**	1.97*	4.33***	1.46	1.53	2.16*	3.95***	2.72**	3.00**	2.21*

Note: Bold font highlights significant effects: ***p < 0.001, **p < 0.01, and *p < 0.05. Treatment indicates agricultural practice.

and insoluble structural carbohydrates. While cellulose often makes up 30%-50% of total biomass for SG, GG has higher cellulose content (Ge et al., 2012). Scagline-Mellor et al. (2018) reported that SG had TEY of 450 Lha^{-1} and total ethanol production (TEP) was 3699Lha⁻¹. In this study, we found that GG had higher cellulose but lower hemicellulose, TEP, and TEY, and no difference in lignin content compared to SG (Table 4). The forage nutritive values varied significantly between GG and SG. Compared to SG, GG had higher ADF (by 3.8%), CP (by 29.1%), ash (by 11.3%), Ca (by 32.2%), and K (by 9.4%), and lower NDF (by 2.3%) (Table 5). Forage quality of both SG and GG varies greatly with growth stages as plant nutrients change with plant growth and also among different studies probably due to different agricultural practices such as nitrogen application rates and growing conditions.

3.4 | Nitrogen application impact on biomass yield, feedstock composition, and forage quality

Agricultural practices, including different N application amounts and application times, have been utilized to increase biomass yield and enhance forage quality (Keyser et al., 2020; McLaughlin & Kszos, 2005; Vogel et al., 2002; Warwick et al., 2016). In our study we compared control plots that received no N fertilizer to plots receiving single and double application of urea (Tables 4 and 5). Among all cropping systems, the N/N/C treatment produced the highest biomass yield $(8.68 \text{ Mg ha}^{-1})$, followed by the N/H, N/N/H, N/N, and N treatments which were slightly lower but not significantly different from N/N/C (Table 4). All other treatments produced significantly lower biomass yields. A contrast test indicated that urea, whether single or double application, significantly increased biomass yield by 1.54 Mg ha⁻¹. Double N application treatments further increased biomass yield by 0.69 Mgha⁻¹ compared to single N application treatments. In regard to forage quality, N fertilization increased CP content in GG and SG (Table 5). Nitrogen application increased CP by 1.07% compared to no N application, and double N application increased CP by 0.48% compared to single N application. In this study, there was no effect of N application on ADF, NDF, and RFV while ADL increased in all N applications (Table 5).

3.5 | Cover crop impact on biomass yield, feedstock composition, and forage quality

Incorporating cover crops such as hairy vetch and crimson clover with grasses did not change biomass yield,

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TABLE 4 Multiple comparison of biomass yield, cellulose, hemicellulose, lignin content, theoretical ethanol potential (TEP), and theoretical ethanol yield (TEY) among years, grass types, treatments, cutting systems, and impacts of N applications and incorporating cover crops.

Variable	Biomass Mg ha ⁻¹	Cellulose g kg ⁻¹	Hemicellulose g kg ⁻¹	Lignin g kg ⁻¹	TEP L Mg ⁻¹	TEY L ha ⁻¹
Year						
1 (2013)	7.48b	407.89a	283.62c	45.81a	515.66b	3831.9b
2 (2014)	6.89c	390.74c	294.26b	45.57a	498.72c	3474.4c
3 (2015)	8.24a	399.42b	322.38a	27.60b	523.43a	4317.2a
Grass type						
Switchgrass	8.84a	389.74b	317.26a	40.56	519.68a	4615.9a
Eastern gamagrass	6.23b	408.96a	282.92b	38.77	505.23b	3144.0b
Treatment						
0	6.43b	399.61	302.41abc	35.55d	513.27	3336.5b
0/C	6.74b	398.82	308.12ab	37.38 cd	518.51	3508.5b
0/H	6.36b	403.04	285.61d	41.85ab	506.61	3204.3b
Ν	7.80a	396.11	311.04a	38.21bcd	511.07	3992.4a
N/C	6.74b	401.46	291.83 cd	39.31abcd	507.75	3452.4a
N/H	8.56a	403.69	296.95bcd	42.20a	516.25	4426.0a
N/N	8.19a	396.51	304.00ab	41.06abc	512.53	4223.4a
N/N/C	8.68a	398.80	302.62abc	40.05abc	515.11	4463.9a
N/N/H	8.31a	396.10	298.19bc	41.36ab	511.00	4275.5a
Cut frequency						
One cut	7.69	416.28a	318.31a	48.69a	534.36a	4118.6a
Two cuts	7.37	382.42b	281.86b	30.64b	490.55b	3634.4b
Treatment comparison						
No N versus N	1.54*	-1.71	2.06	2.10*	-0.51	787.12*
Single N versus Double N	0.69*	-3.29	1.66	0.91	1.19	363.45*
No cover crop versus Cover crop	0.09	2.91	-8.60*	2.09*	0.25	40.42
Clover versus Hairy vetch	0.35	1.24	-7.27*	2.89*	-2.51	164.02

Note: 0, 0/C, 0/H, N, N/C, N/H, N/N, N/N/C, and N/N/H indicate control, no N application + crimson clover, no N application + hairy vetch, single N application, single N application + crimson clover, single N application + hairy vetch, double N application, double N application + crimson clover, and double N application + hairy vetch, respectively. Values with different letters indicated significance among years, grass types, treatments, or cutting systems.

TEP, TEY, NDF, RFV, CP, and elemental contents of grasses, but increased lignin content, ADF, and ADL (Table 3). Averaged over the 3 years of the study, incorporating hairy vetch increased SG and GG ADF content by $11.48 \,\mathrm{g \, kg^{-1}}$ over the control treatment. In addition, hairy vetch treatments had the lowest NDF and hemicellulose content, while having the highest cellulose content. Thus, incorporating hairy vetch reduced the digestibility. Furthermore, average CP content with only hairy vetch was 7.54%, which was about the same as the single N application treatments and higher than the control, although crimson clover did not produce the same result. The increased CP with hairy vetch could be caused by increased N availability in the plots due to its N fixation.

3.6 | Cutting frequency impact on biomass yield, feedstock composition, and forage quality

Cutting frequency did not influence total biomass yield but significantly changed nutritive values of these two grasses (Tables 4 and 5). Our results are inconsistent with Rushing, Lemus, White, et al. (2019) which reported higher biomass yield of GG with higher harvest frequency in a 2-year study, meanwhile SG yield was dramatically reduced the second year of the study. We found that the biomass yield in the first harvest of the two-cut system was lower, but the overall total biomass yield was similar between the two cutting systems. It is worth noting that cutting time may have significant impacts on biomass, particularly for GG. For feedstock GCB-BIOENERGY

TABLE 5 Multiple comparison of acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), crude protein (CP), lignin content, ash, Ca, Mg, P, and K contents among years, grass types, treatments, cutting systems, and impacts of N applications and incorporating cover crops. Please see Table 1 for abbreviations of treatments.

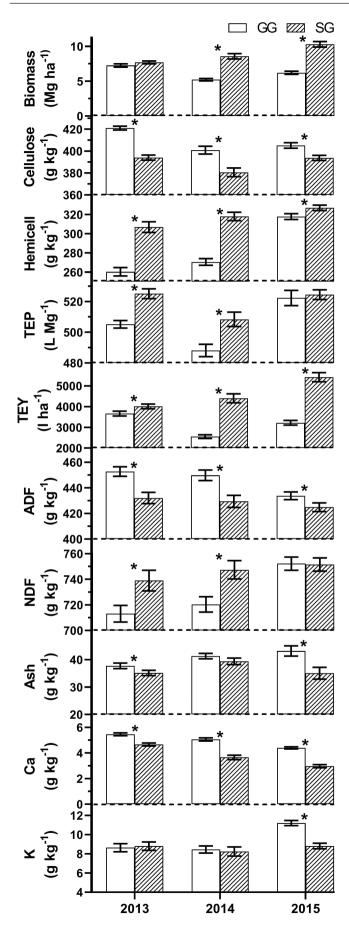
Variable	ADF (gkg ⁻¹)	NDF (gkg ⁻¹)	ADL (g kg ⁻¹)	RFV	CP (%)	Ash (g kg ⁻¹)	Ca (g kg ⁻¹)	Mg (g kg ⁻¹)	P (gkg ⁻¹)	K (g kg ⁻¹)
Year										
1 (2013)	442.44a	726.07c	51.77a	70.47a	8.21a	36.44b	5.06a	2.61c	2.02a	8.72b
2 (2014)	439.63a	733.89b	48.85b	69.91a	7.30b	40.34a	4.36b	2.74b	1.80b	8.35b
3 (2015)	429.39b	751.90a	29.93c	68.96b	7.56b	39.02a	3.68c	2.88a	1.49c	10.01a
Grass type										
Switchgrass	428.84b	745.99a	43.99	69.84	6.72b	36.53b	3.76b	2.70	1.75	8.62b
Eastern gamagrass	445.47a	728.56b	43.04	69.72	8.66a	40.68a	4.97a	2.78	1.79	9.43a
Treatment										
0	432.81 cd	735.22	38.28d	70.35	6.75c	40.94	4.33abc	2.57 cd	1.69d	9.02
0/C	435.73 cd	743.85	41.26 cd	69.08	6.54c	38.56	4.04c	2.51d	1.70 cd	9.14
0/H	444.29a	729.90	45.12ab	69.84	7.61b	39.36	4.65a	2.84ab	1.79ab	8.96
Ν	430.97d	742.87	42.15bc	69.78	7.65b	36.94	4.19c	2.70bcd	1.77abc	9.12
N/C	438.83abc	730.66	43.47abc	70.14	8.08ab	37.39	4.64a	2.76abc	1.79ab	8.93
N/H	443.39ab	740.34	44.77ab	69.06	7.68b	40.57	4.32abc	2.72bc	1.82a	9.37
N/N	436.25bcd	740.25	45.84a	69.51	8.29a	37.10	4.32abc	2.83ab	1.81a	9.00
N/N/C	436.39bcd	739.01	44.52ab	69.68	8.02ab	37.64	4.24bc	2.82ab	1.73bcd	8.41
N/N/H	435.73 cd	733.46	46.23a	70.58	8.56a	38.92	4.60ab	2.93a	1.84a	9.28
Cut frequency										
One cut	464.90a	783.21a	48.67a	62.69a	5.45b	30.89b	3.98b	2.50b	1.59b	6.21b
Two cuts	409.41b	691.36b	38.35b	76.87b	9.92a	46.31a	4.76a	2.99a	1.95a	11.84a
Treatment contrast										
No N versus N application	-0.68	1.44	2.94 ^a	0.04	1.07 ^a	-1.52	0.05	0.15 ^a	0.07 ^a	-0.02
Single N versus Double N application	-1.61	-0.38	2.07 ^a	0.26	0.48 ^a	-0.41	-0.00	0.13 ^a	0.00	-0.24
No cover crop versus Cover crop	5.72 ^a	-3.24	2.14 ^a	-0.15	0.18	0.41	0.13	0.06	0.02	-0.03
Clover versus Hairy vetch	4.15 ^a	-3.27	2.29 ^a	0.19	0.40 ^a	1.75	0.22	0.14 ^a	0.08 ^a	0.38 ^a

Note: 0, 0/C, 0/H, N, N/C, N/H, N/N, N/N/C, and N/N/H indicate control, no N application + crimson clover, no N application + hairy vetch, single N application, single N application + crimson clover, single N application + hairy vetch, double N application, double N application + crimson clover, and double N application + hairy vetch, respectively. Values with different letters indicated significance among years, grass types, treatments, or cutting systems. ^aSignificant effect.

and forage quality, cellulose, hemicellulose, TEP, TEY, lignin, ADF, NDF, and ADL were lower, and CP and P, K, Ca, and Mg were higher in the two-cut system (Table 5). The TEP in the one-cut system was 534.36 LMg⁻¹, 8.9% higher than the two-cut system.

3.7 | Interactive effects of grass type, treatment, year, and cutting system on biomass yield and nutritive values

We found significant interactive effects between year and grass type in biomass yield (Table 2, Figure 1a). There was no significant difference in biomass yield between the two grasses in 2013, but biomass yield of SG was significantly higher than SG in both 2014 and 2015. It indicated that SG can produce more biomass than GG once established. Significant interactive effects were found for cellulose, hemicellulose, TEP, and TEY between year and grass type, and between treatment and grass type (Table 2). Over 3 years, cellulose content of GG was higher than SG, while hemicellulose content of SG was higher than GG in the first 2 years (Figure 1b,c). As a result, TEP of SG was higher in the first 2 years compared to GG. This indicated that the digestibility of SG was higher than GG. Interactive effects between



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FIGURE 1 Changes in biomass yield, cellulose, hemicellulose, theoretical ethanol potential (TEP), theoretical ethanol yield (TEY), acid detergent fiber (ADF), neutral detergent fiber (NDF), ash, Ca, and K contents between eastern gamagrass (GG) and switchgrass (SG) from 2013 to 2015. Error bars indicate standard errors. * indicates significant difference between GG and SG.

year and grass type (GG or SG) were significant for ADF, NDF, ash, Ca, and K (Figure 1f–j). Eastern gamagrass had higher ADF and lower NDF in 2013 and 2014, but similar values in 2015 compared to SG. Differences in ash and K content were small between GG and SG in 2013 and 2014, but larger differences were found in 2015. Our results showed that climate factor such as precipitation might influence the cell wall components of feedstock and element acquisition by grasses.

There was no significant interactive effect between grass type and treatment in biomass yield, but cellulose, hemicellulose, and lignin contents of GG and SG varied among different treatments (Figure 2a-c). The largest differences in cellulose and hemicellulose contents between GG and SG appeared in the treatments without N applications (0, 0/C, and 0/H) (Figure 2a,b). SG had higher lignin content in the 0 treatment than GG (Figure 2c) and SG had the lowest lignin content in the N treatment. Effects of grass type on ADF, NDF, CP, ash, Ca, Mg, P, and K contents varied among different treatments (Figure 2d-k). SG had lower ADF than GG among most of the treatments, but GG had higher CP than SG in most of the treatments, particularly under no N applications. Elements of Ca, Mg, P, and K in GG tended to be higher than in SG. These results indicated that while GG had relative lower biomass compared to SG, it has some better forage quality variables and could be considered as a dual-purpose bioenergy crop.

4 | DISCUSSION

4.1 | Year of growth impact on biomass yield, feedstock composition, and forage quality

We found significant differences in biomass yield and other variables among the 3 years in this study. Variation in biomass yield among years has been reported by many previous studies (e.g., de Koff & Tyler, 2011; Ritchie et al., 2006; Rushing, Lemus, White, et al., 2019). Maughan (2011) found that SG biomass yield averaged $6.6 \pm 3.0 \text{ Mgha}^{-1}$ during the establishment year, increased to $9.1 \pm 5.5 \text{ Mgha}^{-1}$ in the second year, and reached a maximum of $10.9 \pm 5.2 \text{ Mgha}^{-1}$ in the third year, in a meta-analysis including 106 sites from

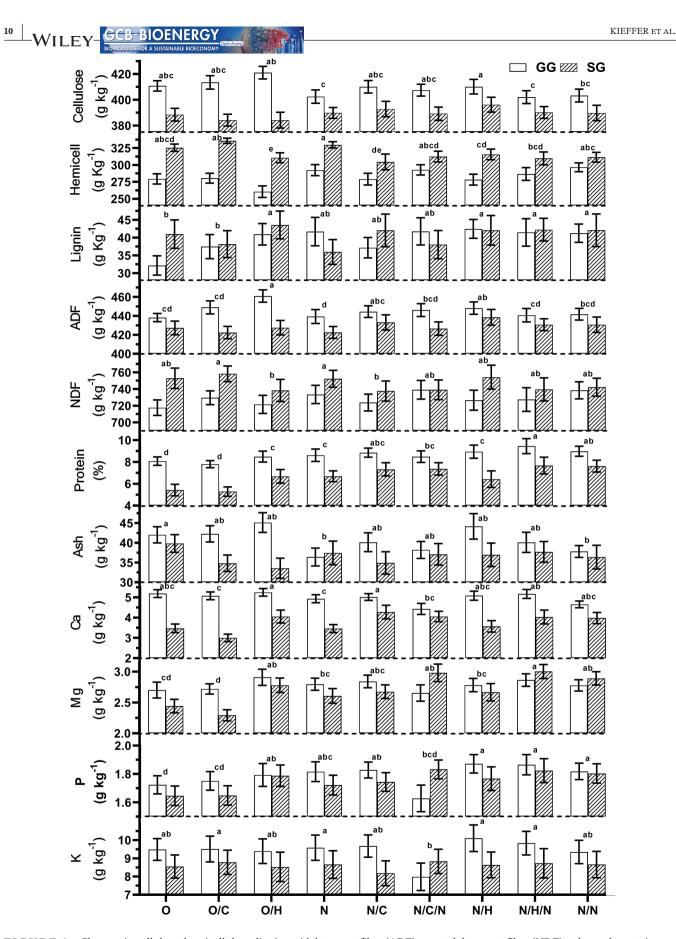


FIGURE 2 Changes in cellulose, hemicellulose, lignin, acid detergent fiber (ADF), neutral detergent fiber (NDF), ash, crude protein, ash, Ca, Mg, P, and K contents among different treatments in eastern gamagrass (GG) and switchgrass (SG). Please see Table 1 for treatment abbreviations. Error bars indicate standard errors. Treatments with the same small letters indicate no significant difference.

45 studies. Alongside stand age, interannual weather variations, particularly early season precipitation greatly affects, and it is a good predictor of, biomass yield for SG crops (Maughan, 2011). The annual biomass yield of GG is also affected by interannual weather variations. For instance, biomass yield ranged from 1.3 to 7.8 Mgha⁻¹ over 9 years (Ritchie et al., 2006). Rushing, Lemus, White, et al. (2019) indicated that year of growth is the main source of variation in dry matter yields in feedstock cropping systems. Interestingly, they also found that GG yields were less affected when compared to other native warm-season grasses. In our study, biomass yield and all feedstock composition variables were affected by year of growth. For example, the highest yield occurred in the third year $(2015, 8.24 \text{ Mg ha}^{-1})$ while the lowest yield occurred in the second year (2014, $6.89 \text{ Mg} \text{ ha}^{-1}$), likely due to less precipitation during the growing season in the second year of the study (Table 4, Figure 1a). Our results concur with Rushing, Lemus, White, et al. (2019) by showing greater SG yield variations compared with GG across the studied years, indicating that yield of GG could be more stable than SG. Irrigation at adequate times may reduce the variation of biomass yield, especially during drought years. This study, together with previous studies, also demonstrated that GG and SG could produce adequate forage quality even under adverse environmental conditions (Burns et al., 1996; Ge et al., 2012).

4.2 | Grass type impact on biomass yield, and bioenergy feedstock composition and forage quality

Our results of lower mean biomass yield of GG than SG were consistent with Backus et al. (2017) who reported that SG produced $\sim 7-8$ Mg ha⁻¹ while GG produced ~4–6 Mg ha⁻¹. Similarly, Temu et al. (2018) reported SG biomass of 7.2 and 5.0 Mg ha⁻¹ for GG. Switchgrass biomass can vary greatly in the literature, for example, ranging from as low as 1 Mg ha^{-1} to as high as 40 Mg ha^{-1} , because of differences in the varieties and ecotypes, agricultural practices, and harvesting stages used across different studies (Wullschleger et al., 2010). In a majority of studies, the biomass yield of SG ranges from 10 to 15 Mg ha^{-1} (Cherney et al., 2017; Scagline-Mellor et al., 2018; Wullschleger et al., 2010), slightly higher than our results. Biomass of GG is mostly found to be lower than SG with a range from 5.4 to 16.4 Mg ha^{-1} (Alderson et al., 2007). Potentially, SG is more sensitive to spring temperatures, and earlier spring growth may increase biomass yield compared to GG. Regarding to feedstock composition, our results of cellulose are comparable, but hemicellulose and lignin contents are higher than those reported by Ge et al. (2012) possibly due to different growing conditions (i.e., dry land

and not irrigated in Arkansas). Forage quality of both SG and GG varies greatly with nitrogen application rates and growing conditions. Switchgrass CP can vary from 3.6% to 22.1% (Angima et al., 2009; Backus et al., 2017; Biermacher et al., 2017; Edwards et al., 1999; Sanderson & Burns, 2010; Waramit et al., 2012). The CP of GG ranges from 4.81 to 14.14 (Angima et al., 2009; Edwards et al., 1999; Keyser et al., 2020). The NDF of GG ranges from 553 to 767 $g kg^{-1}$ (Keyser et al., 2020; Waramit et al., 2012), and of SG from 523 to 770 g kg^{-1} (Backus et al., 2017; Mosali et al., 2013; Sanderson & Burns, 2010; Waramit et al., 2012). ADF ranges from 277.0 to 424.2 gkg^{-1} (Backus et al., 2017; Keyser et al., 2020; Mosali et al., 2013; Sanderson & Burns, 2010). Our results for SG were well within these reported ranges while values for GG were higher for CP and lower for NDF. All these results indicated that even though GG is less productive, it might be easy to ingest and digest and more suitable for use as a dual-purpose crop system where forage feedstock production is preferred.

4.3 | Nitrogen application impact on biomass yield, feedstock composition, and forage quality

This study showed that different N application significantly influence biomass yield. It is known that although SG can tolerate low soil fertility, it responds to N fertilization with significant increases in biomass yield (Berg, 1995; Vogel et al., 2002). In agreement with our results, Angima et al. (2009) found that SG yield increases with N application and reached 8.3 Mgha⁻¹ at 168 kg Nha⁻¹. Several studies have demonstrated that the greatest influence on biomass vield in SG occurs at N fertilization rates between 40 and 70 kg Nha⁻¹ (Holmberg, 2014; McLaughlin & Kszos, 2005; Moyer & Sweeney, 2016; Rushing, Lemus, & Lyles, 2019). In contrast, GG biomass yield continues to increase when the N application rate is increased from $56 \text{ kg} \text{ ha}^{-1}$ to 112 and $168 \text{ kg}\text{ha}^{-1}$ or even up to $336 \text{ kg}\text{ha}^{-1}$ (Brejda et al., 1997; Guretzky et al., 2011; Lemus et al., 2008; Muir et al., 2001; Vogel et al., 2002). Recommended N fertilization rate may vary with location and it can depend on precipitation, cultivar, and harvest management. Overall, SG and GG fertilized with N rates between 56 and 112 kg N ha⁻¹ can produce greater sustainable yields (Lemus et al., 2008).

For forage quality, as demonstrated in this study and several other studies, N fertilization increased CP content in GG and SG (Table 5; Anderson & Akin, 2008; Moyer & Sweeney, 2016; Vogel et al., 2002; Waramit et al., 2012). Brejda et al. (1997) also reported CP concentration increases in response to increasing N application rate. Whereas Keyser et al. (2020) found that N fertilization only has modest influence on pasture quality with CP being slightly higher and NDF slightly lower at 134 versus 67 kg N ha^{-1} . We did not find significances in ADF, NDF, and RFV among N applications (Table 5), but Johnson et al. (2001) indicated a gradual decrease in NDF content as N fertilization increased. Waramit et al. (2012) noted that the NDF concentration in SG and GG was greater in low N application rate treatments (65 kg ha^{-1}) , while higher rates had no consistent effects on NDF concentration. Therefore, the effect of N fertilization on NDF is still not conclusive. But N fertilization could improve forage quality for warm-season grasses through increasing the concentration of CP. Our results indicate that biomass yield and CP could be enhanced when N application rate is applied twice for a total of $168 \text{ kg N} \text{ ha}^{-1}$.

4.4 | Cover crop impact on biomass yield, feedstock composition, and forage quality

Our results of the impacts of cover crop on biomass yield are similar to those of Warwick et al. (2016) which reported intercropping with legumes had little to no effect on yield and forage quality of SG. Nevertheless, a metaanalysis of SG production with and without legumes reported significant higher yields when legumes were present (Wang et al., 2010). For example, some studies have shown as much as a 20% increase in biomass yield with the inclusion of legumes in SG (Ashworth et al., 2012). In our study, the insignificant impacts of cover crops might be due to poor legume establishment in the first year. When compared to grass alone, the incorporation of legumes can increase CP concentration while lowering NDF and ADF (Bonin & Tracy, 2011; Posler et al., 1993). Ashworth et al. (2012) also reported that SG with cover crop treatments was able to produce higher CP plants. Our results and results from these studies indicate that incorporating legumes may have the potential to increase the forage quality of both GG and SG systems while maintaining the biomass yield.

4.5 | Cutting frequency impact on biomass vield, feedstock composition, and forage quality

We found that cutting frequency did not influence biomass yield but had significant impacts on nutritive values. Similar to our results, Moyer and Sweeney (2016) also found that forage quality differed between two-cut and one-cut management systems, and NDF, ADF, and lignin were higher in one-cut system. Our results indicated that forage quality was higher in the two-cut system than in the one-cut system. The quality of SG in the first harvest is

higher because plants contain higher CP and lower NDF and ADF than in the second harvest. This is because biomass quality is often reduced as plants mature (Brooke Stefanik 2018, Thesis). A three-cut system was reported to give 11%-24% higher dry matter yield, higher CP, and lower NDF than the two-cut system (Sanderson, 2008). Although biomass yield was unchanged by the cutting system in our study, two-cut system seems to produce better feedstock and forage quality.

5 CONCLUSIONS

Utilization of bioenergy crops is a necessary endeavor in combating climate change and the over dependence of fossil fuels. We compared the field performance and biomass yield of GG and SG to understand best management practices for GG cropping systems and GG bioenergy potential. We found that: (1) Compared to SG, GG produced 29.5% less biomass yield, had lower TEP, TEY, and NDF, but higher CP, Ca, and K contents and there was no difference in RFV between the two grass types. These results indicate that even though GG is less productive, it might be easy to ingest and digest and more suitable for forage feedstock. (2) Treatments with double N application (N/N), double N application plus hairy vetch (N/N/H), and single N application plus hairy vetch (N/H)mostly produced the highest biomass, ADL, lignin, CP, P, and Ca. Incorporating cover crops plus N application also increased biomass and CP. (3) The cutting systems did not influence biomass yield, but one-cut system produced higher TEP, lignin, ADF, NDF, and ADL and lower CP, P, and K. (4) There was large interannual variability for all variables investigated in this study, indicating that climatic factors such as precipitation played an important role in biomass yield, feedstock, and forage quality. These findings improved our understanding of these two bioenergy and forage crops and provided useful information for farmers to improve the biomass yield and quality.

ACKNOWLEDGMENTS

This research was supported by the USDA projects and the preparation of this manuscript was partially support by the National Science Foundation (NSF) MRI and EiR projects.

CONFLICT OF INTEREST STATEMENT The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data that support the findings of this study can be directly accessed via the figshare online data repository at https://doi.org/10.6084/m9.figshare.22492816.v1.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Kieffer, C., Hui, D., Matamala, R., Li, J., Tyler, D., & Dzantor, E. K. (2023). Evaluation of eastern gamagrass as dualpurpose complementary bioenergy and forage feedstock to switchgrass. *GCB Bioenergy*, *00*, 1–15. https://doi.org/10.1111/gcbb.13054