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Raymond B. Iglay

Mississippi State University, ray.iglay@msstate.edu

Tara J. Conkling

Mississippi State University

Travis L. DeVault

USDA APHIS Wildlife Services

Jerrold L. Belant

Mississippi State University

James A. Martin

University of Georgia, jmart22@uga.edu

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Forage or Biofuel: Assessing Native Warm-season Grass Production among Seed Mixes and Harvest Frequencies within a Wildlife Conservation Framework

Raymond B. Iglay^{1,*}, Tara J. Conkling^{1,2}, Travis L. DeVault³, Jerrold L. Belant^{1,4}, and James A. Martin⁵

Abstract - Native warm-season grasses (NWSG) are gaining merit as biofuel feedstocks for ethanol production with potential for concomitant production of cattle forage and wildlife habitat provision. However, uncertainty continues regarding optimal production approaches for biofuel yield and forage quality within landscapes of competing wildlife conservation objectives. We used a randomized complete block design of 4 treatments to compare vegetation structure, forage and biomass nutrients, and biomass yield between *Panicum virgatum* (Switchgrass) monocultures and NWSG polycultures harvested once or multiple times near West Point, MS, 2011–2013. Despite taller vegetation and greater biomass in Switchgrass monocultures, NWSG polycultures had greater vegetation structure heterogeneity and plant diversity that could benefit wildlife. However, nutritional content from harvest timings optimal for wildlife conservation (i.e., late dormant season-collected biomass and mid-summer hay samples) demonstrated greater support for biofuel production than quality cattle forage. Future research should consider testing various seed mixes for maximizing biofuel or forage production among multiple site conditions with parallel observations of wildlife use.

Introduction

Recent interests in climate change have fostered development of renewable energy production, including biofuels, as an option to reduce carbon emissions, with the United States setting an estimated production goal of 16 billion gallons per year of cellulosic biofuels by 2022 (Perlack et al. 2011). Primary row and small grain crops used for biofuel production globally include *Zea mays* L. (Corn), *Saccharum officinarum* L. (Sugarcane), and *Triticum aestivum* L. (Wheat). However, these traditional monoculture biofuels may negatively impact carbon sequestration, conservation of biodiversity, and air and water quality. Removal of pre-existing habitat is also detrimental to wildlife populations and diversity (Fargione et al. 2009, Hartman et al. 2011, Knight 2010, Parrish and Fike 2005).

¹Department of Wildlife, Fisheries, and Aquaculture, Mississippi State University, Box 9690, Mississippi State, MS 39762. ²Current address - US Geological Survey, Forest and Rangeland Ecosystem Science Center, Snake River Field Station, Boise, ID 83706. ³United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, 6100 Columbus Avenue, Sandusky, OH 44870. ⁴Current address - College of Environmental Science and Forestry, State University of New York, 1 Forestry Drive, Syracuse, NY 13210. ⁵Warnell School of Forestry and Natural Resources, Savannah River Ecology Lab, The University of Georgia, Warnell 3 Room 320, 180 East Green Street, Athens, GA 30602. *Corresponding author - ray.iglay@msstate.edu.

Row and small grain crops for biofuel production generally occur on arable land currently used for growing food crops (Campbell et al. 2008). Perennial native warm-season grass species including *Panicum virgatum* (Switchgrass), *Andropogon gerardii* (Big Bluestem), and *Sorghastrum nutans* (Indiangrass), however, can be used to generate lignocellulosic biofuels in marginal landscapes (DeVault et al. 2012) that are not necessarily suited for traditional agricultural practices (Gonzalez-Hernandez et al. 2009). Native warm-season grasses (hereafter NWSG) provide wildlife habitat, reduce erosion, and support ecosystem functions such as nutrient cycling (Hong et al. 2013, Samson and Omielan 1992). Unlike traditional agricultural crops, NWSG require minimal supplemental nutrients to establish and manage (Mulkey et al. 2006, Tilman et al. 2006).

Past research demonstrated that NWSG, especially Switchgrass monocultures, can provide high biofuel yields across multiple environments and topographic gradients (David and Ragauskas 2010, Mitchell et al. 2012, Sanderson and Adler 2008, Sanderson et al. 2004). Switchgrass monocultures are the most-frequently studied native warm-season grass species considered for biofuel production (Gonzalez-Hernandez et al. 2009, Vogel et al. 2002, Sarath et al. 2008), but mixed-species plantings of grasses and forbs may offer advantages over monocultures for biofuel production (Gonzalez-Hernandez et al. 2009, Tilman et al. 2006). Mixed species plantings often include perennial plants with diverse adaptations capable of tolerating biotic and abiotic stressors (Gonzalez-Hernandez et al. 2009) and provide biomass yields similar to or greater than Switchgrass monocultures (Adler et al. 2006, 2009; Tilman et al. 2006). Additionally, the viability of monocultures such as Switchgrass for wildlife habitat or other ecosystem services may be limited, especially during breeding season when diverse polycultures may be more useful to nesting birds by providing greater availability of potential nest sites as well as arthropods to feed nestlings (Conkling et al. 2017, Hovick et al. 2014, McCoy and Kurzejeski 2001, Monroe et al. 2016, Sanderson et al. 2004). Therefore, increasing structural heterogeneity within plantings could support diverse grassland bird communities, thereby increasing the appeal of NWSG plantings as alternative land covers for biomass production (Coppedge et al. 2008, Davis and Brittingham 2004, Hovick et al. 2014, Valone and Kelt 1999). However, grassland bird conservation goals could also affect forage and biomass goals by constraining harvest timing (Ball et al. 2007).

Harvesting biofuel crops multiple times per year may generate additional biomass for production, but increased harvest frequency has variable effects on the nutritional quality of the collected forage, thereby affecting its suitability as a biofuel or forage for livestock (Adler et al. 2006, Fike et al. 2006). For example, as cellulose characteristics such as cell-wall constituents including cellulose, hemicellulose, and lignin increase, biofuel production likewise increases but ruminant digestibility decreases (Ball et al. 2001). Alternatively, forage quality can increase with high concentrations of crude protein and digestible dry matter. Considering the differing criteria of biomass for livestock forage and biofuel production, investigation of alternative planting and harvest regimes could inform

landowners of approaches to increase profit on marginal lands to enhance biofuel or forage. Our objectives were to: (1) examine differences in vegetation structure and species composition between monocultures and mixed species plantings and (2) determine the effects of harvest frequency and planting regime on biofuel and forage production. We expected greater quality biomass for biofuel production within Switchgrass monocultures, as biomass production for biofuels may be inversely related to the species diversity of a given plot (Johnson et al. 2010, Tilman et al. 2006). However, we also predicted lower nutritional forage quality including crude protein in Switchgrass monocultures and grasses harvested multiple times in a year compared to NWSG plantings harvested just once per year (Guretzky et al. 2011).

Field-Site Description and Methods

We conducted the study on 16 experimental plots (5.03–8.41 ha) located at B. Bryan Farm near West Point, MS, within the Blackland Prairie region (33°38'53"N, 88°34'43"W). The study site was primarily composed of pastures, row crop agriculture, and grasslands managed for conservation on high alkalinity soils classified as Inceptisols and Vertisols (Barone and Hill 2007). We arranged the study plots in a randomized complete block design with 8 plots planted with a NWSG mixture (Table 1) and 8 plots planted to a Switchgrass monoculture during spring 2010. We conducted no harvests in 2010 or 2011 to allow plants to establish, and did not fertilize the plots throughout the study. Other common species in the seedbank were *Ambrosia artemisiifolia* (Annual Ragweed), *Urochloa platyphylla* (Broadleaf Signalgrass), and *Sesbania* spp. (riverhemp). Plots were harvested once or twice annually as is done for haying and biomass collection, resulting in 4 treatments: single harvest NWSG (“NWSG Single”), multiple harvest NWSG (“NWSG Multiple”), single harvest Switchgrass (“Switchgrass Single”), and multiple harvest Switchgrass (“Switchgrass Multiple”). The first harvest (dormant harvest) occurred

Table 1. Species planted (kg/ha) in at Bryan Farms in Clay County, MS, (2011–2013), for comparing native warm-season grass plots to Switchgrass monoculture plots.

Common name	Species	Planting rate
Big Bluestem	<i>Andropogon gerardii</i> Vitman	2.27
Little Bluestem	<i>Schizachyrium scoparium</i> (Michx.) Nash	4.50
Indiangrass	<i>Sorghastrum nutans</i> (L.) Nash	2.27
Roundhead Lespedeza	<i>Lespedeza capitata</i> Michx.	0.28
Grayhead Coneflower	<i>Ratibida pinnata</i> (Vent.) Barnhart	0.28
Showy Tick Trefoil	<i>Desmodium canadense</i> (L.) DC.	0.28
Tickseed Sunflower ¹	<i>Bidens aristosa</i> (Michx.) Britton	0.28
Illinois Bundleflower	<i>Desmanthus illinoensis</i> (Michx.) MacMill. ex B.L. Rob. & Fernald	0.28
Wild Blue Lupine	<i>Lupinus perennis</i> L.	0.28
Switchgrass – ‘Alamo’ ²	<i>Panicum virgatum</i> L.	10.10

¹Tickseed Sunflower had the greatest establishment of all planted forbs.

²Switchgrass was only planted in Switchgrass monocultures.

in April 2012 before green-up to simulate a winter harvest and was applied to all plots after fields were dry enough to access with equipment. The timing of both harvest treatments helped provide standing cover during grassland bird breeding and overwintering seasons. We were unable to successfully establish Switchgrass in 1 Switchgrass Single and 1 Switchgrass Multiple plot, so we removed these plots from subsequent analyses ($n = 14$ plots for analysis).

We measured maximum height of visual obstruction (VOR) in addition to heights of dominant plant species and species composition data among treatments using Robel-pole and point-intercept methods, respectively (Robel et al. 1970). We used point-intercept methods to characterize plant communities and Robel-pole measurements to index habitat heterogeneity (wildlife conservation benefit) and biomass production (Robel et al. 1970). We employed a geographic information system (i.e., ArcGIS) to overlay 50 m x 50 m grids on each plot, randomly selected 5 grid squares per plot based on preliminary sample-size analysis, and centered a 50-m line transect per square with a random orientation. We recorded VOR in all 4 cardinal directions every 10 m along each transect per month from June 2011 to October 2013 ($n = 5$ subsamples per transect per month; Barone and Hill 2007, Robel et al. 1970). From June–October 2011 and May–October 2012 and 2013, we identified the 3 most common plant species (including standing dead vegetation) and measured heights (cm) at 5-m intervals in addition to bare ground and litter (10 subsamples per transect per month; Caratti 2007). We did not sample species composition from November to April because of winter dormancy and our interest in growing-season biomass production.

Metrics calculated included average height and species frequency of occurrence for species and growth forms (e.g., bare ground and litter [frequency only]), dead grass, forbs, grasses, herbaceous vines, legumes, sedges and rushes, semi-woody vines, woody plants, woody vines] by plot and month for analysis. Frequency of occurrence was also used to calculate species richness and Shannon–Wiener diversity index (H') from a species matrix including all identified species. We used frequency of occurrence by growth form to determine the most common growth forms detected (i.e., growth forms occurring in at least 25% of samples among all transects per plot per month).

We measured biofuel production and cattle forage production among treatments by weighing clipped biomass and hay bales in addition to conducting nutrient analyses. We collected 6 biomass samples from 1-m² plots along each transect, 1 sample per 10 m (i.e., 0 m, 10 m, 20 m, 30 m, 40 m, 50 m) from late March to early April 2014. All vegetation samples were weighed (kg; wet weight) and then frozen until dried at 60 °C in a forced-air oven for 72 hours, then weighed again (dry weight). We used samples from the 10-m and 40-m points on each transect to determine biofuel nutrients and from the 0-m and 50-m points for forage nutrient analysis. Despite similarities in biofuel and forage nutrients, we present separate statistics for each because we submitted separate samples to the nutrient analysis laboratory. Average dry and wet biomass weights (Mg/ha) and average nutrients per transect from biomass samples were calculated. For hay-bale forage nutrient

analysis, we collected sample cores from 5 hay bales per plot in late June 2012 and early July 2013 from June harvests. We weighed (kg) a subsample of hay bales ($n = 1\text{--}16$ bales, mean = 11.6 bales, $\sigma = 4.56$) using a truck scale, correcting for truck and trailer weight, and calculated total biomass weight as the product of the average hay bale weight and total bales per plot (Mg/ha). We only measured hay weights from multiple harvested treatments due to limited availability of a truck scale. All nutrient analyses followed standard protocols and were conducted by the Mississippi State Chemical Laboratory using a 0.5-mm sieve.

We used univariate (GLMM) and multivariate (MGLMM) generalized linear mixed models to investigate vegetation structure and composition directional responses to treatments (Hadfield 2010). We assessed plant height, visual obstruction, growth form, and nutrient responses in a Bayesian model-selection framework, thus avoiding issues associated with multiple hypothesis testing. We used deviance information criterion (DIC) to select the best random structure of our treatment model with random effects (e.g., block, plot, year, month) associated with each observation (*idh* variance structure) or among all observations (Spiegelhalter et al. 2002). We ran 3 fixed-effects models (e.g., treatment by growth form, growth form, and null) with an effective sample size of 5000 from 100,000 iterations after a 50,000-iteration burn-in and thinning interval of 10. We ran each model 3 times to visually assess error (Hadfield 2010) and convergence. We selected the model with the least average DIC value and deemed it to have strong directional response when $\Delta\text{DIC} \geq 4.00$. We also calculated summary statistics of the top model's posterior distribution, including using the time-series standard error because it better represents the standard error of time-series data and an additional statistic representing the proportion of posterior distribution values >0 as a metric of the strength of the directional response when 95% credible intervals overlapped 0. We developed univariate Bayesian models (GLMM) for species richness, Shannon's diversity index, visual obstruction, and nutrient- and biomass-production responses comparing treatment and null models and an added interaction term (treatment \times year) for hay-bale nutrient analysis. For total hay weight per plot, we used paired *t*-tests to compare total weight between years and treatments with block indicating each pair. We further investigated vegetation composition responses to treatments using analysis of similarity (ANOSIM). For ANOSIM, we tested for no difference in species composition among treatments by year using frequency of occurrence data, Bray-Curtis distance measure, and 999 permutations.

Results

We identified 48 plant species (24 forbs, 11 grasses, 4 legumes, 3 herbaceous vines, 3 sedges and rushes, and 1 species each of shrubs, semi-woody vines, and woody vines) among all treatments and years. However, only grasses, forbs, legumes, Switchgrass, bare ground, dead grass, and litter were included in analysis (i.e., growth forms occurring in at least 25% of samples among all transects per plot per month). Indiangrass, Switchgrass, *Schizachyrium scoparium* (Little Bluestem), Broadleaf Signalgrass, and Big Bluestem were the most common species occurring

in at least 20% of transects (Table 2). *Desmanthus illinoensis* (Illinois Bundleflower), Annual Ragweed, *Conyza canadensis* (Horseweed), *Ranunculus fascicularis* (Early Buttercup), *Bidens polylepis* (Tick-seed Sunflower), and *Ipomoea pandurata* (Morning Glory) were common species occurring in 10–19% of transects. When present, Switchgrass and Little Bluestem were the tallest dominant plants on average, followed by Horseweed, Big Bluestem, and Tick-seed Sunflower among all treatments and years (Table 2).

Average height by vegetation growth form differed among treatments, but frequency of occurrence did not (Table 3). Forbs, other grasses, and legumes were taller in NWSG polycultures than either Switchgrass treatment (Table 4). Switchgrass was tallest in Switchgrass Single monocultures. Grasses were also tallest in Switchgrass Single monocultures with similar occurrence among all treatments. Litter and standing dead grass did not demonstrate strong directional responses to treatments (i.e., minimal differences among treatments).

Table 2. The tallest plants on average among Switchgrass monocultures and native warm-season polycultures (NWSG) harvested once (single) or multiple times from 2011 to 2013 and sampled using line transects June to October 2011 and May to October 2012 and 2013 near West Point, MS.

Treatment/species	Common name	Height (cm)		Occurrence
		Mean	SE	
Switchgrass Single				
<i>Panicum virgatum</i>	Switchgrass	113.26	2.90	100%
<i>Urochloa platyphylla</i> (Munro ex C. Wright) R.D. Webster	Broadleaf Signalgrass	8.36	0.95	36%
<i>Ranunculus fascicularis</i> Muhl. ex Bigelow	Early Buttercup	2.11	0.35	16%
<i>Digitaria ciliaris</i> (Retz.) Koeler	Southern Crabgrass	1.02	0.41	4%
<i>Ambrosia artemisiifolia</i> L.	Annual Ragweed	1.00	0.62	2%
Switchgrass Multiple				
<i>Panicum virgatum</i>	Switchgrass	81.96	2.54	96%
<i>Urochloa platyphylla</i>	Broadleaf Signalgrass	12.16	1.05	53%
<i>Digitaria ciliaris</i>	Southern Crabgrass	6.09	1.12	16%
<i>Ranunculus fascicularis</i>	Early Buttercup	3.32	0.43	24%
<i>Schizachyrium scoparium</i>	Little Bluestem	1.57	0.74	2%
NWSG Single				
<i>Sorghastrum nutans</i>	Indiangrass	43.19	2.23	70%
<i>Schizachyrium scoparium</i>	Little Bluestem	40.94	2.15	59%
<i>Andropogon gerardii</i>	Big Bluestem	29.63	2.71	32%
<i>Conyza Canadensis</i> (L.) Cronquist	Horseweed	28.90	2.51	39%
<i>Ambrosia artemisiifolia</i>	Annual Ragweed	20.02	1.88	35%
NWSG Multiple				
<i>Sorghastrum nutans</i>	Indiangrass	47.54	1.99	86%
<i>Schizachyrium scoparium</i>	Little Bluestem	39.02	1.91	67%
<i>Andropogon gerardii</i>	Big Bluestem	37.47	2.68	49%
<i>Bidens polylepis</i>	Tick-seed Sunflower	12.33	1.55	20%
<i>Ambrosia artemisiifolia</i>	Annual Ragweed	11.02	1.41	24%

Table 3. Model results comparing average height and frequency of occurrence of vegetation growth forms in Switchgrass monocultures and native warm-season grasses polycultures harvested once or multiple times near west Point, MS, sampled with point intercepts from June to October 2011 and May to October 2012 and 2013.

Variable	Model	k	DIC	
			Average	Delta
Average height	Growth Form + Treatment:Growth Form	63	52332.30	
	Null	36	52337.14	4.84
	Growth Form	42	52339.21	6.91
Frequency of occurrence	Null	36	34867.96	
	Growth Form	42	34868.07	0.11
	Growth Form + Treatment:Growth Form	63	34868.70	0.74

Table 4. Average height of live and dead growth forms occurring in at least 25% of transects among Switchgrass monocultures and native warm-season grass polycultures (NWSG) harvested once (Single) or more than once (Multiple) in fields near West Point, MS, during June–October 2011 and May–October 2012 and 2013. All responses are in comparison to Switchgrass monocultures harvested multiple times.

Growth form ^A	Treatment	Time-series				95% CI	
		Mean	SE	Mode	Prop > 0	Lower	Upper
Dead Grass	Switchgrass Single	-0.931	0.015	-0.888	0.185	-2.989	1.207
	NWSG Multiple	0.361	0.013	0.206	0.653	-1.536	2.136
	NWSG Single	-1.061	0.014	-1.424	0.133	-3.000	0.783
Forbs	Switchgrass Single	-1.160	0.074	-0.928	0.409	-11.730	8.883
	NWSG Multiple	24.340	0.069	23.510	1.000	14.750	33.800
	NWSG Single	41.350	0.067	40.133	1.000	31.720	50.910
Grass ^B	Switchgrass Single	-9.275	0.067	-10.540	0.021	-18.740	-0.984
	NWSG Multiple	32.480	0.056	31.745	1.000	24.630	41.040
	NWSG Single	34.950	0.060	35.425	1.000	26.500	43.020
Legumes	Switchgrass Single	-0.705	0.077	-2.140	0.452	-11.800	9.826
	NWSG Multiple	20.460	0.070	20.921	1.000	10.560	30.300
	NWSG Single	12.860	0.072	11.388	0.993	2.832	22.570
Switchgrass	Switchgrass Single	31.960	0.038	31.928	1.000	26.870	37.530
	NWSG Multiple	-79.590	0.034	-78.973	0.000	-84.260	-74.940
	NWSG Single	-79.500	0.035	-79.782	0.000	-84.140	-74.660

^AGrowth forms included bare ground and litter (frequency only), dead grass, forbs, grasses, herbaceous vines, legumes, sedges and rushes, semi-woody vines, woody plants, and woody vines.

^BSwitchgrass was not included in the calculation of “Grass” coverage, only other Poaceae including other planted NWSG species were.

Maximum heights of visual obstruction (VOR) differed among treatments ($\Delta\text{DIC} = 4.72$), with tallest VOR in Switchgrass Single plots followed by Switchgrass Multiple, NWSG Single, and NWSG Multiple plots (Table 5). Total wet and dry biomass did not exhibit an apparent treatment effect, but some differentiation was evident with single harvest plots having greater biomass than multiple harvest plots (Tables 6, 7). Total weight of hay harvested for both Multiple treatments increased about 40% from 2012 to 2013 (2.47 Mg/ha in 2012 vs. 3.54 Mg/ha in 2013; $t = 3.03$, $P = 0.023$). Between Multiple treatments across years, Switchgrass monocultures (mean = 4.23 Mg/ha, SE = 0.312) produced over twice the biomass of NWSG plots (mean = 2.08 Mg/ha, SE = 0.154) ($t = -6.06$, $P = 0.002$).

Most forage nutrients in monoculture Switchgrass and NWSG polyculture treatments from biomass samples were similar (Table 6). Dry matter decreased from Switchgrass Single to Switchgrass Multiple to NWSG Single and NWSG Multiple treatments (Table 7). Crude protein was greatest in NWSG Multiple polycultures, least in Switchgrass Single monocultures, and at intermediate levels in Switchgrass Multiple and NWSG Single treatments. Hay bales from Multiple treatment plots had greater fat content in monoculture Switchgrass than NWSG polyculture (Table 8). However, biofuel nutrients did not exhibit any strong directional responses among treatments (Table 7).

Vegetation community species richness ($\Delta\text{DIC} = 167.68$) and Shannon–Wiener diversity ($\Delta\text{DIC} = 206.58$) differed among treatments and were greatest in both harvest types of NWSG polycultures and least in Switchgrass Single monocultures (Table 5). Dissimilarity of vegetation communities among treatments was strong during all 3 years (2011: $R = 0.598$, $P \leq 0.001$; 2012: $R = 0.544$, $P \leq 0.001$; 2013: $R = 0.653$, $P \leq 0.001$).

Table 5. Average height of maximum visual obstruction, species richness and Shannon-Wiener Diversity Index of vegetation communities within Switchgrass monocultures (Switch) and native warm-season grass polycultures (NWSG) in West Point, MS, during June–October 2011 and May–October 2012 and 2013 either harvested once (Single) or Multiple times. All responses are in comparison to Switchgrass monocultures harvested multiple times. CI = confidence interval.

Variable	Treatment	Time-series		Mode	Prop > 0	95% CI	
		Mean	SE			Lower	Upper
Maximum visual	Switchgrass Single	36.84	0.04	36.96	1.00	31.84	42.07
Obstruction height (m)	NWSG Multiple	-19.81	0.03	-19.59	0.00	-24.37	-15.18
	NWSG Single	-7.51	0.03	-8.33	0.00	-12.01	-2.82
Species richness	Switchgrass Single	-0.451	0.003	-0.517	0.020	-0.904	-0.029
	NWSG Multiple	2.140	0.003	2.169	1.000	1.737	2.524
	NWSG Single	2.023	0.003	1.972	1.000	1.615	2.421
Shannon–Wiener diversity index	Switchgrass Single	-0.209	0.001	-0.196	0.001	-0.325	-0.089
	NWSG Multiple	0.616	0.001	0.608	1.000	0.505	0.720
	NWSG Single	0.607	0.001	0.584	1.000	0.498	0.714

Discussion

Planting regimes perpetuated differences in vegetative structure heterogeneity among treatments and had greater influence on plant species richness and diversity than harvest frequencies. Switchgrass monocultures always supported the tallest vegetation, but vertical structure heterogeneity and plant diversity was greater in NWSG polycultures with multiple grass species, forbs, and legumes contributing to overall structure. Biomass production followed VOR trends (Robel et al. 1970), but biomass did not vary significantly among treatments. However, Switchgrass monocultures produced substantially more harvested hay than NWSG polycultures.

Table 6. Model results from multivariate generalized linear mixed models comparing forage and biofuel nutrient analysis results from biomass samples and forage nutrient analysis results from hay core samples in Switchgrass monocultures and native warm-season grass polycultures (NWSG) harvested once or multiple times near West Point, MS. Two biomass samples per nutrient analysis were collected during late March and early April 2014. Hay core samples were sampled in late June 2012 and early July 2013 and only in Switchgrass or NWSG fields harvested multiple times.

Group	Variable	Treatment model DIC	Null model DIC	Δ DIC ¹
Biomass forage nutrients	Dry matter (%)	482.38	498.33	-15.95
	Crude protein (%)	314.75	355.19	-40.44
	Neutral detergent fiber (%)	666.90	666.52	0.38
	Acid detergent fiber (%)	734.62	733.19	1.44
	Crude fiber (%)	742.98	741.50	1.47
	Fat (%)	94.42	93.91	0.51
	Gross energy (MJ)	1531.64	1535.86	-1.22
	Total wet biomass (Mg/ha)	-5.22	-8.64	3.42
	Total dry biomass (Mg/ha)	-1.70	-5.04	3.34
Biomass biofuel nutrients	Dry matter (%)	-5.73	-7.03	1.30
	Moisture (%)	-5.93	-6.36	0.43
	Crude protein (%)	-1.99	-0.99	-1.00
	Starch (ppm)	4.96	4.38	0.58
	Simple sugars (%)	-4.99	-3.25	-1.74
	Water Soluble carbohydrates (%)	-13.47	-9.62	-3.85
	Fructans (%)	-4.13	-1.00	-3.14
	Non-fiber carbohydrates (%)	20.98	18.75	2.23
	Acid-insoluble residue lignin (%)	17.21	16.06	1.15
	Neutral detergent fiber (%)	17.45	16.71	0.73
	Acid detergent fiber (%)	28.68	29.62	-0.94
Hay forage nutrients	Dry matter (%)	98.28	98.46	-0.42
	Ash (%)	281.97	280.83	1.14
	Crude protein (%)	63.73	64.03	-0.30
	Neutral detergent fibers (%)	329.56	327.19	2.37
	Acid detergent fiber (%)	309.24	307.24	1.99
	Crude fiber (%)	276.48	276.72	-0.24
	Fat (%)	-12.78	16.26	-29.11
	Gross Energy (MJ)	851.76	848.54	3.22

¹ Negative Δ DIC indicates better model fit by treatment model than null (Δ DIC = DIC_{Treatment} - DIC_{Null}).

Both Switchgrass monocultures and NWSG polycultures harvested once (dormant season) or multiple (dormant season + growing season; late June/early July) times per year were better suited for biofuel production than cattle forage when managing for grassland birds, according to nutrient analysis. Therefore, the primary advantages of NWSG polycultures over Switchgrass monocultures for biofuel production may be limited to wildlife conservation.

Previous studies have observed plant communities among harvest frequencies in conservation grasslands similar to our NWSG plantings (Jungers et al. 2015, Stahlheber et al. 2016). However, increased cutting frequency can increase light availability, promoting photophilic plant species otherwise deterred by tall vegetation and dense grass canopies (Hautier et al. 2009, Wilson and Tilman 1991). Our study plots were on arable land, previously used for agriculture production.

Table 7. Descriptive statistics of forage and biofuel nutrient analysis results from biomass samples from dormant Switchgrass monocultures and native warm-season polycultures (NWSG) harvested once or multiple times near West Point, MS, collected during late March and early April 2014. Total wet and dry biomass were average weights among samples among plots per treatment.

Material/variable	Switchgrass Multiple Harvest		Switchgrass Single Harvest		NWSG Multiple Harvest		NWSG Single Harvest	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Forage nutrients								
Dry matter (%)	91.33	0.34	91.53	0.29	89.66	0.26	90.38	0.29
Ash (%)	4.68	0.39	3.71	0.35	6.52	0.55	5.14	0.30
Crude protein (%)	2.83	0.17	1.77	0.07	3.29	0.20	2.29	0.10
Neutral detergent fiber (%)	83.81	0.66	89.09	0.44	77.68	0.97	82.06	0.51
Acid detergent fiber (%)	49.79	0.54	55.74	0.41	48.69	1.06	53.76	0.83
Crude fiber (%)	40.88	0.63	46.03	0.49	40.38	1.08	45.06	0.84
Fat (%)	0.25	0.06	0.23	0.07	0.71	0.08	0.45	0.06
Gross energy (MJ)	4111.19	25.70	4123.14	16.37	3925.77	27.38	4009.68	17.14
Total wet biomass (Mg/ha)	4.00	0.65	8.40	3.23	7.07	3.13	7.19	2.88
Total dry biomass (Mg/ha)	2.30	0.30	6.23	2.55	4.55	1.98	4.78	2.10
Biofuel nutrients								
Moisture (%)	7.70	0.20	7.87	0.13	7.40	0.18	7.33	0.25
Dry matter (%)	92.30	0.20	92.13	0.13	92.60	0.18	92.68	0.25
Crude protein (%)	3.17	0.23	2.23	0.38	2.95	0.16	3.13	0.26
Fructose (ppm)	4404.00	129.00	5940.50	825.50	-	-	-	-
Glucose (ppm)	3715.50	77.50	5134.50	1064.50	-	-	-	-
Starch (ppm)	0.60	0.17	1.07	0.43	1.28	0.43	0.85	0.52
Simple sugars (%)	1.40	0.30	1.93	0.38	1.15	0.05	1.48	0.26
Water-soluble carbohydrates (%)	1.73	0.03	1.70	0.06	1.43	0.20	1.08	0.09
Fructans (%)	0.33	0.27	-	-	0.28	0.23	-	-
Non-fiber carbohydrates (%)	4.87	0.61	3.47	0.65	6.25	0.70	4.95	1.56
Acid-insoluble residue lignin (%)	10.79	0.69	11.43	0.85	11.53	1.07	12.32	1.56
Acid detergent fiber (%)	47.37	0.57	51.30	0.46	51.18	1.06	54.78	3.07
Neutral detergent fiber (%)	75.03	0.57	77.23	0.58	74.15	0.58	75.38	1.43

Multiple studies have attributed diverging plant communities in biofuel plant trials to past land use and site conditions (Foster et al. 2003, Grman et al. 2013). However, by conducting our study on homogeneous study plots (i.e., old row crop fields under similar past management and soil conditions), plant community divergence can be attributed more to plant seed mixes and post-planting management (i.e., harvest rate) than to expressed seedbanks despite observing some sub-dominant non-planted species contributing to the study stands.

Predominant seedbank species expressed in plots were more prevalent in Switchgrass monocultures than NWSG polycultures. For example, Broadleaf Signalgrass is a prolific seed producer capable of saturating seedbanks and decreasing the effectiveness of site-preparation techniques. Broadleaf Signalgrass has been a troublesome weed throughout the southeastern United States, even reducing corn productivity over 30% (Alford et al. 2005) and was second in dominance and frequency to Switchgrass in monoculture plots. Multiple-year herbicide applications are often required to adequately prepare sites for NWSG plantings because new plantings are susceptible to weed competition (Temu et al. 2016, Washburn and Barnes 2000). However, a current study demonstrating Switchgrass monocultures as an alternative land cover for airports from Michigan to Mississippi has observed similar issues of seedbanks competing directly with planted Switchgrass even after 2 years of intense site preparation (e.g., 2 applications glyphosate and metsulfuron methyl and 1 application of imazapyr; R.B. Iglay, unpubl. data). Therefore, site-preparation techniques should be explored for marginal land opportunities that can produce grasslands with significant proportions of C_4 grasses for effective ethanol yield (Adler et al. 2009, Stahlheber et al. 2016).

Biomass production in this study was similar to past work with unfertilized fields (Muir et al. 2001, Vogel et al. 2002), but Switchgrass monocultures exceeded

Table 8. Descriptive statistics of forage nutrient analysis results from hay samples in Switchgrass monocultures and native warm-season polycultures (NWSG) planted in spring 2010 and harvested twice in 2012 (dormant harvest in early April and growing season harvest in late June to early July) and in late June to early July 2013 near West Point, MS. Most Switchgrass plants were in the late-boot to early-seedhead stage during harvest.

Variable	2012				2013			
	Switchgrass		NWSG		Switchgrass		NWSG	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Dry matter (%)	96.84	0.08	95.69	0.25	96.41	0.21	95.28	0.18
Ash (%)	6.33	0.38	6.67	0.21	6.33	0.38	7.70	0.33
Crude protein (%)	4.10	0.13	3.64	0.06	3.00	0.08	4.25	0.14
Neutral detergent fibers (%)	74.22	0.37	72.09	0.58	79.28	0.76	69.77	1.30
Acid detergent fiber (%)	39.62	0.46	44.06	0.87	45.44	0.75	43.23	0.69
Crude fiber (%)	34.80	0.25	38.19	0.79	39.01	0.59	35.70	0.47
Fat (%)	1.49	0.04	1.20	0.03	1.00	0.04	0.76	0.10
Gross energy (MJ)	4193.49	23.78	4109.13	17.32	4157.89	41.40	4065.52	30.19

NWSG polycultures in terms of hay production. McIntosh et al. (2015, 2016) investigated similar treatments with fertilized fields and observed greatest biomass yields in fields with Switchgrass when harvested in the fall, but their results also indicated changes in harvest yields between early boot and early seedhead stages. Adler et al. (2009) had observed decreasing biomass yields with increasing species richness but had no comparison to a monoculture, only increasing species richness among Conservation Reserve Program fields. Tilman et al. (2006) compared biofuel production among NWSG polycultures, corn, and soybeans. Switchgrass has taken center stage as an optimal alternative biofuel crop to small grains (Parrish and Fike 2005, Sarath et al. 2008, Vogel et al. 2002) in part due to biomass production but also to the feasibility of biofuel conversion (McLaughlin and Kszos 2005, Mitchell et al. 2008, Parrish and Fike 2005, Sanderson et al. 2006).

Harvest timing could have greater influence on the quality of biomass or forage from NWSG polycultures and Switchgrass monocultures than plant diversity (Ball et al. 2007; McIntosh et al. 2015, 2016). Biomass for biofuel production encourages fall biomass harvests after the first frost to maximize translocation of plant nutrients to roots for storage reserves before harvest (McIntosh et al. 2015, 2016; Muir et al. 2001; Sanderson et al. 1996; Vogel et al. 2002). However, delaying harvest to early spring can allow for additional nutrient loss while maintaining similar caloric content for producing quality biomass feedstock (Johnson and Gresham 2014). Similarly, leached nutrients such as N, K, and S can remain in the system rather than be removed from fall Switchgrass harvests (Gamble et al. 2015, Johnson and Gresham 2014). Concomitantly, delaying harvest to minimize interference with nesting birds would favor biomass production over forage because of increased yield and decreased nutrients (McIntosh et al. 2015, 2016).

Despite having single- and multiple-harvest treatments, we sampled all plots at the same time, at the end of March and early April 2014, for biomass clippings and compared hay harvests from approximately the same midsummer time period between years for multiple-harvest treatments. During summer, many NWSG species experience nutrient lows, especially crude protein. Guretzky et al. (2011) observed poor forage quality for mid-summer Switchgrass harvests in Oklahoma. Crude protein can decrease over 50% in NWSG species such as Little and Big Bluestem from May through late summer and early fall (July–September; Sedivec and Barker 1997). Big Bluestem can maintain 16–18% crude protein through August but drop as low as 6% come September (United States Department of Agriculture Natural Resources Conservation Service 2018).

Late spring harvests of NWSG polycultures and Switchgrass monocultures, between dormant and June harvests of this study, could produce viable biomass feedstocks though the low lignin and acid detergent and neutral detergent fibers make such harvest less than ideal for ruminant intake and digestibility (Ball et al. 2001). Reduced mineral nutrient concentrations benefit ethanol production unless excess lignin inhibits availability of cellulose and hemicellulose during thermochemical conversion (Adler et al. 2006, Chen and Dixon 2007, Sanderson et al. 2007, Sarath et al. 2008). We observed slightly greater (14% increase)

acid-insoluble residue lignin in NWSG polyculture biomass samples compared to Switchgrass monocultures (average values below 12.5%) among treatments. Past studies have observed Switchgrass acid-insoluble lignin levels of 17.8%, with other biofuel crops varying from 6.1% to 29.1% (Mood et al. 2013). However, late spring harvests could be detrimental to nesting birds, thereby decreasing the potential concomitant benefits of biomass production and wildlife conservation (Roth et al. 2005, Perlut et al. 2006).

Greater crude protein in multiple-harvest treatments mimics existing Switchgrass research observing greater nutritional quality of grasses harvested multiple times compared to those harvested just once per year (Guretzky et al. 2011). However, the predominant trend in our forage nutrient analysis was no difference in nutrient load among treatments. Biomass samples among treatments yielded crude protein levels that were lower than poor grass hay requirements for cattle forage, and only average crude protein of NWSG Multiple polycultures met minimum quality standards for silage (Burns et al. 1984, National Research Council 2000). Acid (ADF) and neutral detergent fibers (NDF) were 65–88% greater than primary forage quality standards which recommend below 31% for ADF and below 40% for NDF (Ball et al. 2001, National Research Council 2000). Thus, the only prime forage quality aspect of late dormant season harvested biomass was percent dry matter (National Research Council 2000). Mid-summer hay samples did not provide any additional benefits for cattle forage.

Summer (late June to early July) harvests of unfertilized Switchgrass monocultures and NWSG polycultures were better suited for biofuel production than cattle forage in east-central Mississippi. While Switchgrass monocultures were better suited for maximizing biomass production, NWSG polycultures could provide concomitant benefits of wildlife habitat and biomass for biofuel production, thereby increasing the appeal of converting marginal land for biomass production. Adjusting harvest timing could generate greater nutritional content for cattle (Hedtcke et al. 2014, McIntosh et al. 2016, Sanderson et al. 1999, Trócsányi et al. 2009) while providing additional feedstock supplies for refineries (Anteau et al. 2011, McIntosh et al. 2015, Richard 2010, Sanderson et al. 1999), but more research is needed across ecoregions including an examination of potential detrimental effects on wildlife communities (e.g., harvest practices destroying breeding grassland bird nests; Roth et al. 2005 Perlut et al. 2006). Seed mixes can drive future plant communities if seedbank competition is minimal and could be tailored to meet landowner interests regarding biofuel and forage production.

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