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Yield and Nitrogen Removal of Bioenergy Grasses as Influenced by Nitrogen Rate and Harvest Management in the Coastal Plain Region of North Carolina

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Abstract The agronomic performances of giant miscanthus (*Miscanthus* x *giganteus*) and switchgrass (*Panicum virgatum* L.) grown as bioenergy grasses are still unclear in North Carolina, due to a relatively short period of introduction. The objectives of the study were to compare the biomass yield and annual N removal of perennial bioenergy grasses and the commonly grown coastal bermudagrass [*Cynodon dactylon* (L.) Pers.], and to determine the optimum N rates and harvest practices for switchgrass and miscanthus. A 4-year field trial of the grasses under five annual harvest frequencies (May/Oct, June/Oct, July/Oct, Aug/Oct, and October only) and five annual N rates (0, 67,134, 202, and 268 kg N ha^{-1}) was established at a research farm in Eastern North Carolina in 2011. Across harvest treatments and N rates, greatest biomass was achieved in the second growth year for both miscanthus (19.0 Mg ha^{-1}) and switchgrass (15.9 Mg ha^{-1}). Grasses demonstrated no N response until the second or the third year after crop establishment. Miscanthus reached a yield plateau with a N rate of 134 kg ha^{-1} since achieving plant maturity in 2013, whereas switchgrass demonstrated an increasing fertilizer N response from 134 kg N ha^{-1} in the third growth year (2014) to 268 kg N ha^{-1} in the fourth growth year (2015). The two-cut harvest system is not recommended for bioenergy biomass production in this region because it does not improve biomass yield and increased N removal leads to additional costs.

Keywords Biomass yield · Harvest frequency · Switchgrass · Miscanthus

Introduction

Production of cellulosic biofuels from plant biomass could reduce dependence on fossil fuels. Giant Miscanthus (*Miscanthus* × *giganteus*) and switchgrass (*Panicum virgatum* L.) are emerging as dedicated energy crops due to high biomass yield and low fertilizer requirements [3, 11, 18–20]. Water availability is considered a key factor limiting yield of these grasses [18]. The North Carolina Coastal Plain region lies within a humid, temperate area, which has the potential to produce high biomass yield for bioenergy grasses with proper harvesting and nutrient management. Currently, hay production from coastal bermudagrass [*Cynodon dactylon* (L.) Pers.] is an important part of the local agriculture. Since grasses used for hay and biofuel production share similarities in methods of planting and harvesting, farmers could diversify or alternate their production systems with bioenergy grasses if they bring higher economic returns.

Both miscanthus and switchgrass have high nutrient use efficiencies due to recycling by rhizomes within the plant after senescence [4, 45]. Yield response to N by the two grasses have been well documented and results varied with soil and climate conditions [10, 36, 37, 48]. Cadoux and co-workers [6] reviewed a total of 11 miscanthus studies in Europe that involved N yield response. Five of them had no yield response to N due to high initial soil mineral N content. For example, no N response was found in a 14-year miscanthus yield experiment in England [9]. Schwarz et al. [43] reported a lack of

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response to N by miscanthus over 3 years in Austria. The six studies with N responses in England, Germany, Italy, and Turkey had optimum N rates ranging from 40 to 120 kg ha^{-1} over 3–5 years [1, 5, 10, 12, 29]. In general, the biomass increase from these N inputs was moderate. Nitrogen studies with N^{15} recovered 14, 20, and 26% of the applied N in the aboveground miscanthus biomass during the first three growing seasons in England, which was relatively low compared with other crops [7, 8]. Miguez et al. [34] used statistical models on miscanthus research data from Europe to predict a negligible impact of applied N in the first growing year followed by a relatively small long-term impact in subsequent years.

Miscanthus yield response to N application showed varying results in the USA. Arundale et al. [3] reported the biomass yield of miscanthus after 5 years of establishment increased by 25%, with a linear response up to 202 kg N ha^{-1} year^{-1} across seven locations in Illinois. In a 2-year trial in Oklahoma, no N response was found up to 168 kg N ha^{-1} year^{-1} for miscanthus that was one year old at the start of the experiment [24]. In experiments starting with 1-year-old miscanthus at two North Carolina sites, Haines and co-workers [16] reported no N response up to 135 kg ha^{-1} year^{-1} in 2 of 3 years at one site and in either of 2 years at the second site. Despite multiple miscanthus trials in the Southeastern U.S., there is limited information regarding yield response to N or N use efficiency [13, 26, 37].

Studies on switchgrass N response in the USA have been extensively reported and reviewed. After compiling data from 39 switchgrass field trials, Wullschleger et al. [48] indicated switchgrass responded in general to 100 kg N ha^{-1}. The largest N response of switchgrass was reported in Oklahoma with 448 kg N ha^{-1} year^{-1}over 3 years [47]. Stout and Jung [46] reported N recovery rates of switchgrass ranging from 22 to 57% with 84 kg N ha^{-1} year^{-1} applied in Pennsylvania at four locations over 3 years. In the Northern Appalachian region switchgrass recovered 25–33% of N at applied rates up to180 kg ha^{-1} year^{-1} over 3 years [44]. Liu et al. [30] reported switchgrass recovered on average 31% N from biosolids amendments which contained plant-available N (PAN) rates of 180 kg ha^{-1} on three sites in Virginia. However, some studies reported lower N recovery rates for switchgrass. For example, a multi-site study including South Dakota, New York, Iowa, Oklahoma, and Virginia reported an N recovery rate of switchgrass one year after planting below 10% with up to 112 kg ha^{-1} N year^{-1}applied at all locations over 2 years [36]. Nitrogen response among 19 location and year combinations varied from none to 56 or 112 kg N ha^{-1}year^{-1} and was attributed to variances in soil N content [22].

Timing and frequency of harvests are important factors for bioenergy grass production. Thomason and co-workers [47] reported that harvest frequency played a more important role in switchgrass yield for a 3-year study than applied N rates. Miscanthus and switchgrass are typically harvested in the winter after senescence, allowing aboveground nutrient translocation to rhizomes and reducing nutrient removal [28, 45]. A late harvest, however, might decrease biomass yield due to leaf loss [21, 49]. Some studies have indicated increased biomass yield with two-cut or early harvesting systems [4, 14, 40]. For example, a study in Denmark showed miscanthus harvested in early fall produced approximately 40% more biomass than a late harvest in winter [27]. Switchgrass harvested in Oklahoma by a two-cut system produced 30% more biomass than a one-cut system over 4 years [25]. Although yields might increase, greater removal of nutrients during early or two-cut systems could lead to soil nutrient depletion and require more fertilizer for subsequent growing seasons [15, 25, 30, 35, 40]. Another possible side effect of early harvests is declined biomass yield over time. Perrish [39] reported a 30% miscanthus yield decrease after 3 years of early harvesting in September compared with winter harvesting in Illinois, indicating early harvest might decrease the stand longevity. A study in Italy reported that two-cut systems increased biomass production of switchgrass during the first 2 years but yields declined significantly during the last 2 years of the study due to loss of plant stand [35].

Studies comparing the combined effects of both N fertilization and harvest timing and frequency on miscanthus and switchgrass have not been conducted in the Southeastern U.S. The objectives of the study were to: (i) compare the biomass yield and annual nutrient removal of perennial bioenergy grasses and the commonly grown coastal bermudagrass and (ii) determine the optimum N rates and harvest practices for switchgrass and miscanthus in order to provide guidelines for perennial bioenergy grass productions across the Coastal Plain area of North Carolina and other regions with similar climate and soil properties.

Materials and Methods

Site Description

The experiment was conducted at the NCSU Williamsdale Biofuels Field Laboratory (34°46′ N, 78°6′ W) in Wallace, North Carolina. The site was previously used for fertilized corn production. The soil was a Noboco loamy fine sand (fine-loamy, siliceous, subactive, thermic Oxyaquic Paleudults). On-site weather data during the research period were acquired from the North Carolina State climate office (Raleigh, NC, USA). Throughout the experimental period, average growing season (April to September) temperature was relatively consistent with the 30-year means (Table 1). Annual rainfall for 2012, 2013, and 2015 exceeded the 30-year average, but was 428 mm below the 30-year average in 2014.

Table 1 Monthly precipitation from 2012 to 2015 and 30-year average precipitation and temperature at Wallace, NC

Month	Year				30-year average	
	2012	2013	2014	2015	Precipitation	Temperature
	Precipitation (mm) -					°C
January	78	47	57	84	93	7.4
February	60	112	80	179	86	9.0
March	222	44	111	110	99	12.7
April	45	93	168	201	89	17.1
May	170	70	107	120	100	21.2
June	116	412	126	367	136	25.1
July	283	402	23	68	174	26.8
August	244	148	3	150	176	26.0
September	97	55	30	90	146	23.1
October	55	32	37	192	86	17.5
November	33	78	82	211	78	12.8
December	113	114	86	167	80	8.5

Experimental Design, Sampling, and Analysis

Treatments were arranged in a split-split plot randomized complete block design with three replications. Main plots were three grass species: switchgrass, giant miscanthus, and coastal bermudagrass. Sub plots were five treatments with either single or dual annual harvests (May/Oct, June/Oct, July/Oct, Aug/Oct, and Oct only). Four of the treatments (May/Oct, June/Oct, July/Oct, and Aug/Oct) were harvested twice per year with the first harvest in May, June, July, and Aug, respectively. All of the second harvests for the two-cut treatments and the October-only treatment were cut on the same day, usually after the first killing frost. Each of the harvest treatment sub plots were divided into five sub-sub plots with different annual N rates (0, 67,134, 202, and 268 kg N ha^{-1}) chosen to encompass the entire range of dry matter yield responses for the three grasses. The size of each sub-sub plot was 5 × 5 m. Plugs of miscanthus cv. "Nagara" and switchgrass cv. "Performer" were planted with a transplanter on May 23, 2011. Miscanthus was planted with an in-row spacing of 92 cm and between-row spacing of 100 cm. Switchgrass had equal in-row and between-row spacings of 50 cm. Coastal bermudagrasss cv. "Ranchero Frio" seed was drilled into plots on July 5, 2011 at the rate of 37 kg ha^{-1}. Urea coated with the urease inhibitor NBPT (Agrotain Ultra, Koch Agronomic Services, LLC, Wichita, KS, USA) was broadcast by hand on plots in late March before the shoot emergence each year since 2012. Pre-emergence herbicide S-metolachlor plus atrazine (Bicep II Magnum, Syngenta, Greensboro, NC, USA) and post-emergence herbicide Paraquat (Gramoxone, Syngenta, Greensboro, NC, USA) were used to control weeds wherever necessary each year. A forage plot harvester (Wintersteiger Inc., Salt Lake City, UT, USA) was used to harvest the grasses and record the wet biomass yield. Due to the young plant stand in 2012, miscanthus was not harvested in May or June for the May/Oct or June/Oct harvest treatments. In 2015, the harvest scheme for all the coastal bermudagrass plots was changed to five harvests per year from the same plot (May, June, July, Aug, and Oct) to represent a common harvest practice for bermudagrass hay production in the region.

Plant subsamples from each sub-sub plot were collected at each harvest and dried at 65 °C for three days to determine moisture content and dry biomass yield. Afterward, subsamples were ground to pass a 1-mm sieve (Christy-Turner, Ipswich, Suffolk, UK) small scale hammer mill and analyzed by the NC Department of Agriculture and Consumer Services (NCDA&CS) plant tissue testing laboratory to determine N concentration [31]. In May 2011, before establishment of the research plots, soil samples were collected at each of the three designated blocks to a 1.2 m depth in four segments (0–30, 30–60, 60–90, and 90–120 cm) with a 5-cm diameter hydraulic probe. Three cores were collected from each block and mixed together by depth interval. Soil samples composed of 10 soil cores collected with a 1.7-cm diameter hand probe were taken at the 0–20 cm depth from the zero N sub-sub plots before fertilization in March from 2012 to 2014 and at the end of the study in December 2015.

Soil samples were tested by routine procedures at the North Carolina Department of Agriculture and Consumer Services (NCDA&CS) soil testing laboratory [17]. Nutrient analysis on Mehlich-3 soil extracts were performed using inductively coupled plasma (ICP) [33]; cation exchange capacity (CEC) was determined by summation of basic cations (excluding Na) and buffer acidity [32]; soil pH was determined on a 1:1 soil/water volume ratio [33].

Crop species, harvest treatments, N rates, and years were considered as fixed effects and replications were considered as random effects. Year was treated as a repeated measure. Biomass yield and N removal data were analyzed using the MIXED procedure in SAS version 9.3 [42] to determine main effects and interactions among the fixed factors. The statistical model applied autoregressive structure to account for temporal data collection across years. The SLICE option in the MIXED procedure was used to perform a partitioned analysis for the N rates × crop × year interaction, where year and crop effects were fixed to identify the simple effects of N rates on crop species in certain years. In cases where simple effects of N rate were significant ($P < 0.05$) in a year-crop species combination, biomass yield response to N rates was fit to a linear plateau model using the GLM procedure in SAS as described by Anderson and Nelson [2]. Mean separation of yield and N removal were performed using the DIFF option of the LSMEANS statement in the MIXED procedure at the respective interaction levels. The means were compared by Fisher's protected least significant difference (LSD) at the 5% probability level.

Result and Discussion

Soil Chemical Properties

Soil analysis before crop establishment in 2011 demonstrated most nutrients were concentrated in the 0–30 cm depth, except for soil cations (K, Ca, and Mg) which were more evenly distributed to a depth of 60 cm (Table 2). Throughout the research period, soil P and K levels for samples from the 0–20 cm depth were defined as "high" or "very high" for North Carolina soil tests [17], which means these were not limiting nutrients. The only exception was that soil K concentrations in the switchgrass treatment declined to a "medium" category by the end of 2015. There was no significant decrease in soil P level until the end of the study in 2015, when the P level dropped approximately 50 mg kg^{-1} compared with the values in March 2012.

Biomass Yield

The four-way interaction of year × crop species × harvest treatment × N rate was not significant. Also, none of the two-way or three-way interactions on biomass yield which included both N rate and harvest treatment were significant (Table 3). Therefore, data were pooled over either harvest treatment or N rate.

The impact of the harvest treatments were evidenced by a significant ($P < 0.0001$) year × crop species × harvest treatment interaction (Table 3). Since the year × crop species × N rate interaction was not significant ($P = 0.7728$), the impact of N rate was investigated by the crop-year species combinations with a positive N response identified in Table 4.

Biomass yield differences among crops suggested by the significant crop species × year interaction (Table 3) were evaluated by comparisons between years of species yields averaged across harvest treatments and N rates (Table 5). Miscanthus had the greatest yield among crop species, with values of 19.0 Mg ha^{-1} in 2013, 17.1 Mg ha^{-1} in 2014, and 16.7 Mg ha^{-1} in 2015. Maximum miscanthus yield in 2013 could be an indicator of crop stand maturity that is usually reported at 2 or 3 years after establishment by other studies [10, 19]. The miscanthus yields achieved in this study were higher than or similar to those reported in the Southeastern U.S. [13, 16, 37]. Switchgrass had similar yield to miscanthus in 2012 (13.9 Mg ha^{-1}) and 2013 (15.9 Mg ha^{-1}), but yielded significantly less than miscanthus in 2014 (12.3 Mg ha^{-1}) and 2015 (12.5 Mg ha^{-1}). These combined yields, however, were close to a national average of 14 Mg ha^{-1} reported by Wullschleger et al. [48]. Comparing at the regional scale, the yields were lower than those reported by Palmer et al. (20.1 Mg ha^{-1}) [37] within the same region of this study but higher than the yield of 9.4 Mg ha^{-1} in the Georgia Coastal Plain area [26]. Although not produced for bioenergy, the traditional bermudagrass hay yield was the lowest among grasses in every year, with an average annual yield of 5.4 Mg ha^{-1} (Table 5). A multiple harvest system for bermudagrass adopted in 2015 did not improve yield compared with the one- and two-cut systems used in 2012–2014.

The significant interaction of year × crop species × harvest treatment was evaluated by comparing annual crop species and harvest treatment yields averaged across N rates (Fig. 1). In general, two-cut systems did not produce more biomass yield for all the three grasses than the one-cut treatment throughout the research period. The two-cut system only provided the greatest biomass yield in 2012 for switchgrass and bermudagrass. The two-cut system did not produce significantly greater yield than the one-cut treatment for miscanthus in any year. In 2012, the highest yield of switchgrass was achieved in treatments with dual cuts in July/October and August/October. In 2013, switchgrass under the July/October and August/October harvest treatments still produced the greatest biomass among the two-cut systems but there was no significant difference in yield when compared with the October-only harvest treatment. In 2014 and 2015, the October-only single harvest treatment produced greater switchgrass biomass yield than any of the two-cut treatments. Declines in switchgrass yield over time from the two-cut harvest systems had also been reported by other studies: Thomason et al. [47] and Monti et al. [35] observed switchgrass productivity declined after 2 to 4 years under two- or three-cut harvest management. The yield decline was usually accompanied by a reduction in tiller density, and was attributed to a physiological stress with extra nutrient uptake from the regrowth after the first cut that disrupted the nutrient remobilization to belowground biomass after grass senescence each fall [35, 41]. Furthermore, reduced rainfall in July and August of 2014 and in July 2015 may have limited the production of switchgrass biomass after the first cut (Table 1).

The miscanthus stand was too young to harvest until July 2012 (Fig. 1). From 2013 to 2015, yield of miscanthus under the two-cut harvest systems did not decline relative to the October-only harvest treatment. This indicated miscanthus might be more adapted than switchgrass to the two-cut systems, possibly due to greater nutrient reserves in the belowground biomass or higher nutrient translocation efficiency than in switchgrass. Bermudagrass yields increased under the two-cut treatments, but total annual biomass was less than for miscanthus and switchgrass, especially in 2013 and 2014. When averaged across the 4 years, 88% of the miscanthus and 86% of the switchgrass yields in August/October harvest treatments came from the first harvest. The reduced yields from the

Table 2 Soil chemical properties at the experimental site under coastal bermudagrass (ber), miscanthus (mxg), and switchgrass (sxg)

Year†	Crop	Depth	pH	BS‡	CEC§	Acidity	Mehlich-3-extractable							
							P	K	Ca	Mg	S	Mn	Zn	Cu
		(cm)		(%)	cmol kg^{-1}		mg kg^{-1}							
2011		0–30	5.2	66	2.7	0.9	225	103	245	39	18	7	4	2
		30–60	4.7	61	3.3	1.3	10	109	252	57	89	1	1	0
		60–90	4.5	52	2.7	1.3	4	60	180	46	96	0	0	0
		90–120	4.4	46	2.7	1.5	2	50	163	42	100	0	0	0
2012	ber	0–20	5.8	74	3.9	1.0	293	130	379	81	12	8	6	3
	bxg	0–20	5.8	75	3.9	0.9	339	145	376	79	12	9	6	3
	sxg	0–20	5.7	74	3.5	0.9	328	132	331	72	11	9	6	3
2013	ber	0–20	5.9	76	4.7	1.0	306	146	493	109	18	9	6	6
	mxg	0–20	5.7	72	3.8	1.1	324	92	381	78	16	8	5	6
	sxg	0–20	5.9	77	4.1	1.0	305	117	397	88	24	8	5	6
2014	ber	0–20	6.3	85	4.4	0.7	310	95	515	114	10	9	5	3
	mxg	0–20	6.2	82	4.2	0.8	332	110	464	102	10	9	6	4
	sxg	0–20	6.2	83	4.0	0.7	326	88	443	103	11	8	6	3
2015	ber	0–20	6.0	82	4.7	0.9	275	91	527	119	13	9	5	3
	mxg	0–20	6.0	78	4.3	0.9	271	85	468	96	13	9	5	3
	sxg	0–20	5.9	76	3.9	0.9	262	57	415	90	12	7	4	3

† In 2011, soil samples were taken in May prior to starting the experiment. From 2012 to 2014, soil samples were taken in March before annual fertilization, and in 2015, soil samples were taken in December at the end of the study, from the 0 N experimental sub-sub plots

‡ BS: base saturation = (Ca + Mg + K + Na in cmol kg^{-1})/ CEC × 100%

§ CEC: cation exchange capacity

second harvest in the August/October treatment were associated with a short growing period after the first harvest. From the economic perspective, the efficiency of a second harvest after an initial August harvest might be low because the

Table 3 *F* test and probability values for a mixed model analysis of variance of yield and N removal of as a function of year (Y), crop species (C), harvest treatment (T), and nitrogen rate (N)

Effect	*df*	Biomass yield		N removal	
		F value	*P* value	*F* value	*P* value
C	2	509.50	< .0001	53.31	0.0012
T	4	2.29	0.093	30.1	< .0001
N	4	31.89	< .0001	79.4	< .0001
Y	3	14.23	< .0001	25.72	< .0001
C × T	8	9.27	< .0001	2.39	0.0582
C × N	8	1.62	0.1244	1.44	0.1886
T × N	16	0.61	0.8722	2.45	0.0034
Y × C	5	49.28	< .0001	56.62	< .0001
Y × T	12	4.24	< .0001	14.57	< .0001
Y × N	12	5.29	< .0001	3.76	< .0001
C × T × N	32	0.75	0.8212	0.78	0.7909
Y × C × T	20	2.84	< .0001	11.51	< .0001
Y × C × N	20	0.75	0.7728	1.6	0.049
Y × T × N	48	0.84	0.7626	1.32	0.0817
Y × C × T × N	80	0.54	0.9994	0.87	0.7769

Table 4 *F* test and probability values for simple effects of nitrogen rate (N) on biomass yield when year (Y) and crop species (C) were fixed in the Y × C × N interaction

Crop	Year	*df*	Biomass yield	
			F value	*P* value
Bermudagrass	2012	4	1.75	0.1370
Miscanthus	2012	4	1.06	0.3764
Switchgrass	2012	4	0.59	0.6696
Bermudagrass	2013	4	0.26	0.9004
Miscanthus	2013	4	2.84	0.0237
Switchgrass	2013	4	1.24	0.2936
Bermudagrass	2014	4	1.02	0.3947
Miscanthus	2014	4	10.21	< .0001
Switchgrass	2014	4	4.25	0.0022
Bermudagrass	2015	4	3.80	0.0513
Miscanthus	2015	4	20.84	< .0001
Switchgrass	2015	4	11.93	< .0001

Table 5 Biomass yield averaged across harvest treatments and N rates for each crop species and year. Mean separation of biomass yield was performed within years. Different letters within a given year indicated that mean annual yields were statistically different ($P < 0.05$)

Year	Crop species		
	Miscanthus	Switchgrass	Bermudagrass
		Mg ha^{-1}	
2012	11.8a	13.9a	8.0b
2013	18.9a	15.9a	4.1b
2014	17.0a	12.2b	4.4c
2015	16.7a	12.5b	5.2c

harvesting and bailing comprises a significant part of production cost [35, 38].

Yield Response to Fertilizer N

Yield response curves to applied N rates (Fig. 2) were fit to the year/crop species combinations with significant N effects (Table 4) at the $P = 0.05$ level. Nitrogen applications had significant effects on yield in six of the 12 year-crop species combinations, and most occurred during the later years of the experiment. Consistently, since 2013, miscanthus reached a yield plateau with 134 kg N ha^{-1} (Fig. 2). The slope of the

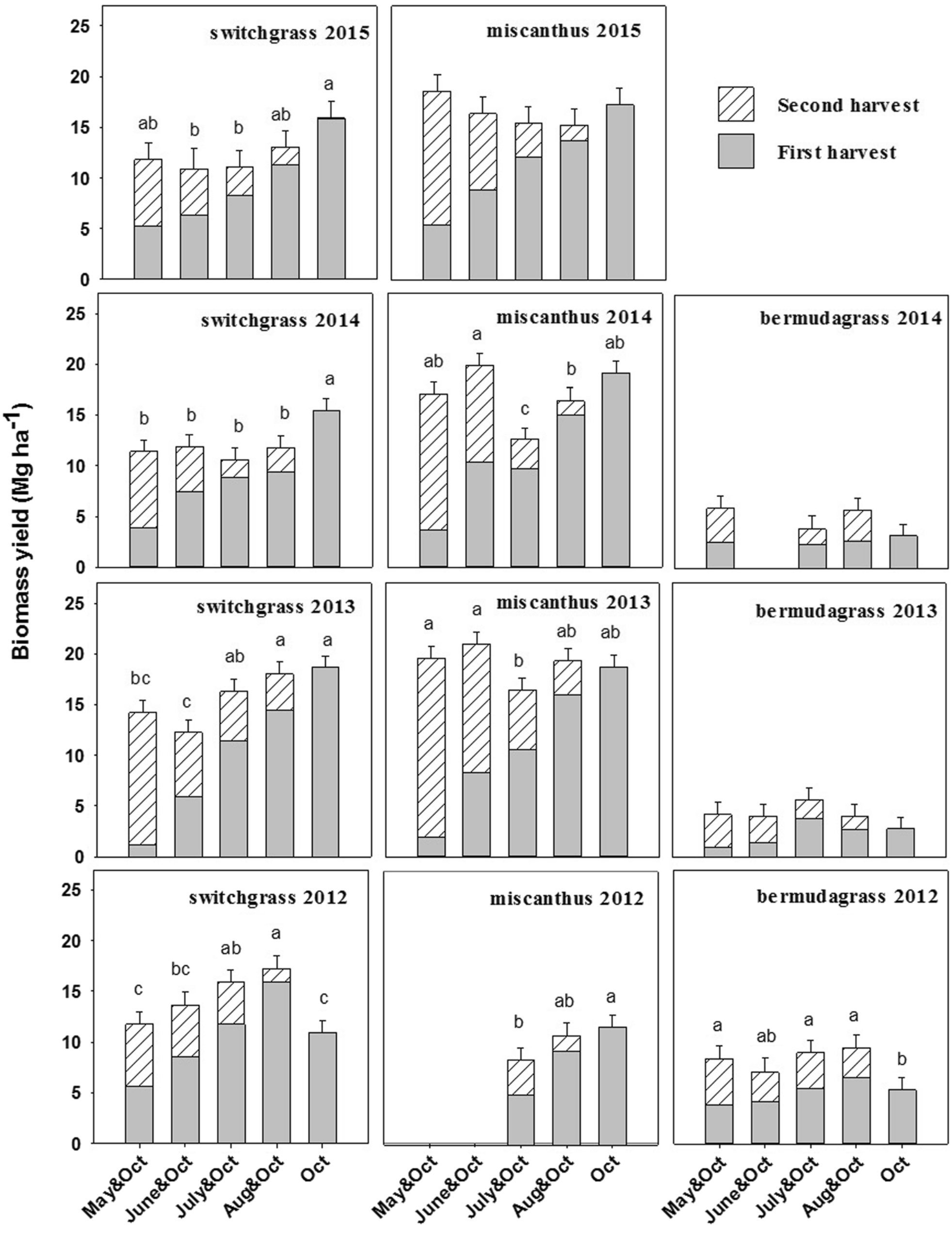

Fig. 1 Biomass yields by year, crop species and harvest treatment averaged across N rates. In 2012, the May/October and June/October treatments for miscanthus were treated as missing values because no harvests were made in either May or June to the young crop stand. In 2014, the June/October harvest treatment was missing for bermudagrass. In 2015, the bermudagrass plots were uniformly harvested for five times so there was no harvest treatment effect. Mean separation of biomass yield was performed within years by crop species. Different letters within a given year and crop species indicate that yields of harvest treatments are statistically different ($P < 0.05$). Error bars are shown as standard errors

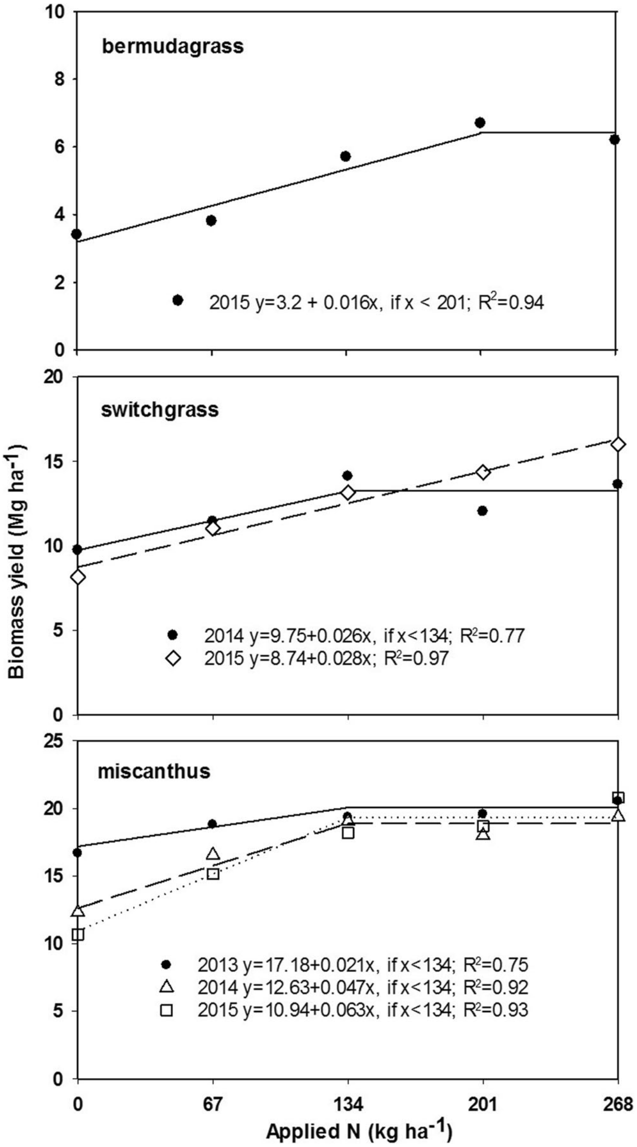

Fig. 2 Linear or linear plateau relationships between biomass yield of grass species and applied N. Only year/crop species combinations with significant responses to N are presented

miscanthus yield response (i.e., biomass produced per unit N fertilizer) increased over time due to a gradual decrease in biomass yield from the unfertilized treatments. Switchgrass did not respond to N applications until 2014. The slopes of switchgrass yield responses were almost parallel in 2014 and 2015. The greater maximum yield in 2015 for switchgrass was obtained with a greater N requirement relative to 2014. The only N response for bermudagrass occurred in 2015 when the harvest scheme was changed to five consecutive cuts per year. All the three grasses showed increasing yield responses to N over time. In contrast to the unfertilized treatment, miscanthus yield increased from 2013 to 2015 by 16, 35, and 41% to a yield plateau of 19.0 Mg ha^{-1}; switchgrass yield increased by 36% to 14.1 Mg ha^{-1} with 134 kg N ha^{-1} in 2014 and by 49% in a linear response up to 268 kg N ha^{-1} in 2015; bermudagrass biomass increased by 49% in 2015 to a yield plateau of 6.7 Mg ha^{-1} when fertilized with 201 kg N ha^{-1}.

The lack of an N response by miscanthus and switchgrass after 1 year of establishment (2012) is in accordance with multiple studies over a wide range of geographical locations [16, 22, 25, 34]. The transition from no response to applied N to increasing dependence on the external N supply coincides with the depletion of native soil N by crop removals over time. This process might be faster in the Southeastern Coastal Plain region where soils have a coarse texture and are low in organic matter content. With relatively large amounts of precipitation in the fall and winter, carry-over effects of applied N are unlikely due to nitrate leaching. A combination of limited

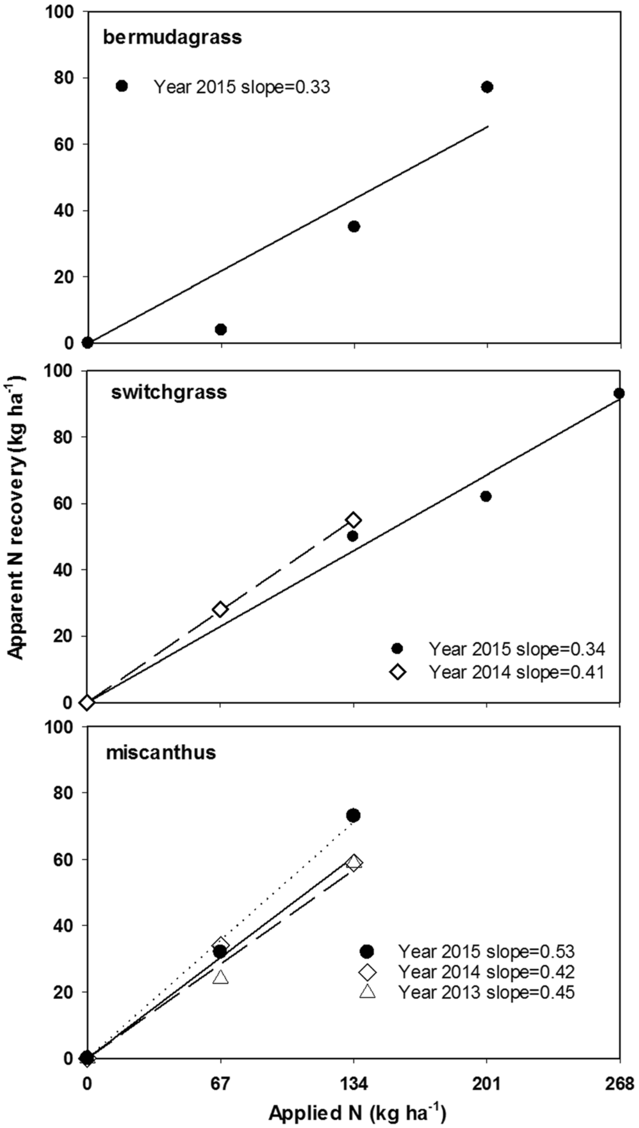

Fig. 3 Apparent N recovery (ANR) (N removal at each N rate – N removal at 0 N rate) of harvested biomass as a function of applied N. The slopes represent the proportions of applied N recovered in aboveground biomass. Fertilizer N treatments beyond the yield plateau with excess N accumulation were excluded

Table 6 Nitrogen removal averaged across harvest treatments by year, crop species and N rate. Mean separation of N removal was performed within years. Upper and lower case letters within a given year are used for row and column comparisons, respectively ($P < 0.05$)

N rate (kg ha^{-1})	Year											
	2012			2013			2014			2015		
	ber[†]	mxg[‡]	sxg[§]	ber	mxg	sxg	ber	mxg	sxg	ber	mxg	sxg
	N removal (kg ha^{-1})											
0	81c	91	108c	40Bb	115Ac	119Ac	22Bb	78Ad	66Ac	50c	76d	69d
67	111ABb	93B	133Ab	48Bab	149Ab	127Ac	35Bb	102Ac	94Ab	54Bc	108Ac	97Acd
134	115Bb	92B	158Aa	56Bab	174Aa	152Aab	44Bab	137Ab	121Aa	85B	149A	119AB
201	140a	109	141ab	63Ca	166Aab	137Bbc	58Ca	143Aab	117Ba	127Ba	161Ab	131ABab
268	154Aa	111B	152Aab	58Bab	188Aa	163Aa	66Ca	162Aa	136Ba	133Ba	207Aa	162Ba

† bermudagrass; ‡ miscanthus; § switchgrass

organic matter and nitrate leaching might lead to an increased response to fertilizer N over time.

Crop N Recovery

Apparent N recovery is defined as the N removal in harvests for a given fertilizer N treatment minus N removal of the treatment without applied N [23]. Apparent N recovery (ANR) of the year-crop species combinations with significant responses to applied N are presented in Fig. 3. The slope of the relation between apparent N recovery and applied N indicates the proportion of fertilizer N removed by the crop and was estimated by linear regression. Miscanthus recovered a higher percentage of applied N (53%) in 2015 than in 2013 (45%) or 2014 (42%), probably due to a shrinking native soil N pool and an expanding root system to intercept more nutrients. Switchgrass recovered similar portions of applied N (41%) to miscanthus in 2014 up to the yield plateau at 134 kg N ha^{-1}. In 2015, however, the percentage of recovered N for switchgrass (34%) was identical to bermudagrass and was considerably lower than miscanthus. The lower percentage of applied N recovery for switchgrass and bermudagrass in 2015 might explain their needs for higher N rates to achieve maximum yields (Fig. 2). The switchgrass N recovery values were notably higher than those reported by Owens et al. [36] at multiple locations across the USA, probably due to differences in harvest managements, climate, and soil types. The combination of the sandy textured soil and humid climate in the Coastal Plain area results in a relatively lower N content in soil than Midwest U.S.

Crop N Removal

Since there were significant interactions for both year × crop species × harvest treatment and year × crop species × N rates interaction (Table 3), crop N removal data were organized by year, crop species and N rates averaged across harvest treatments (Table 6) and by year, crop species and harvest treatment averaged across N rates (Table 7). When analyzed by year × crop species × N rates, the effect of applied N was more pronounced on N removal than on biomass yield (Table 3). This was likely due to plant "luxury N uptake." A pattern of N removal response to N rates can be observed in most year-crop species combinations (Table 6). Although biomass yields for all the three grasses lacked a response to applied N in 2012 (Table 4), there were notable increased N removals up to 201 kg ha^{-1} applied N for bermudagrass and 134 kg N ha^{-1} for switchgrass (Table 6). Lack of increased miscanthus N removal with increasing N rates in 2012 might be due to less developed roots of the young plant stand. In 2013 and 2014, bermudagrass removed significantly less N than miscanthus and switchgrass as a result of substantially lower biomass yields (Fig. 1 and Table 5). With adoption of the five-cut system in 2015, bermudagrass N removal increased 2.7-fold between 0 and 268 kg applied N ha^{-1} (Table 6). The difference in N removal between treatments of 0 and 268 kg N ha^{-1} for miscanthus and switchgrass increased with time from 2013 to 2015 (73, 84, and 131 kg N ha^{-1} for miscanthus and 44, 70, and 91 kg ha^{-1} for switchgrass). Diminishing native soil N supply over time promoted the need for external N supply.

When analyzed by year × crop species × harvest treatments averaged across N rates, at least one of the two-cut treatments removed significantly more N than the Oct only harvest in 9 out 12 year-crop combinations (Table 7). This result was consistent with other studies that included different harvest times or frequencies as a factor [25, 30, 35, 38]. From 2013 to 2015, the two-cut harvest treatments with the most N removal for miscanthus removed 36% (2013), 20% (2014), and 50% (2015) more N than the October-only harvest treatment. The same pattern could also be observed among switchgrass harvest treatments except in 2015 due to a significantly greater yield under the October-only harvest treatment. In general, the greater N removal under two-cut systems was attributed to a greater N concentration in harvested biomass during the summer. Since there was no benefit of increased biomass yield

Table 7 Nitrogen removal averaged across N rate by year, crop species and harvest treatment. Mean separation of N removal was performed within years. Upper and lower case letters within a given year are used for row and column comparisons, respectively ($P < 0.05$)

Harvest treatment	Year										
	2012			2013	2014		2015				
	ber†	mxg‡	sxg§	ber	mxg	sxg	ber	mxg	sxg	mxg	sxg
	N removal (kg ha^{-1})										
May and Oct	224Aa	.	263Aa	58C	171Aab	112Bb	54Ba	134Aa	126Aa	188Aa	132B
June and Oct	107b	.	91 cd	62C	206Aa	149Bab	.	125Aab	100Aab	151a	111
July and Oct	110ABb	98B	158Ab	69B	156Ab	169Aa	57Ba	124Aab	109Aab	158a	126
Aug and Oct	107b	116	117bc	44B	151Ab	143Aab	65Ba	130Aab	116Aa	108b	120
Oct only	55c	89	59d	32B	110Ac	125Ab	26Bb	107Ab	84Ab	93b	100

† bermudagrass; ‡ miscanthus; § switchgrass

when harvested twice per year, the extra N removal under a two-cut systems made it undesirable compared with a single harvest system after senescence; the latter would enable aboveground nutrient translocation to belowground biomass and reduce the N removal at harvest. Since there was a yield decline over time with switchgrass plots under the two-cut systems, a single harvest after senescence would avoid the physiological stress associated with the two-cut harvest systems and improve long-term sustainability.

Conclusions

In the Coastal Plain region of North Carolina, both miscanthus and switchgrass produced significantly greater biomass yields than the conventionally grown coastal bermudagrass during a four-year field trial. The two-cut harvest system, with no advantage in biomass yield and with greater N removal, provided no advantage for bioenergy grass production. There was no response to N fertilizer by miscanthus or switchgrass until the second year after crop establishment. Miscanthus demonstrated a stable yield plateau at N applications of 134 kg ha^{-1} year^{-1} since achieving plant maturity in 2013. A fixed rate of recommended N was not feasible for switchgrass, because N responses increased from 0 to 268 kg ha^{-1} across the 2012–2015 period. An incremental N rate recommendation might be more appropriate for switchgrass production as residual soil N levels decline.

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