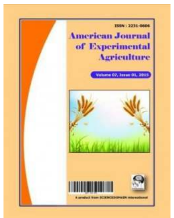

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Growth Response to Nutrient Management during a 3 Year Study of *Miscanthus* x *giganteus*

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Authors' contributions

This work was carried out in collaboration between all authors. Author RR designed the study, wrote the protocol and reviewed the manuscript. Author MM wrote the first draft of the manuscript. Authors RR, MM and AS managed the analyses of the study. Authors RR and MR identified the biomass feedstock species studied in this project. Author MM performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

To accelerate the acclimation of novel crops for bio-energy feedstock supply chain, a pilot study focused on *Miscanthus giganteus* was conducted in the piedmont area of North Carolina to determine region specific productivity. The main aim of the study was to determine the growth response in biomass accumulation during a 3 year establishment period under different nutrient management practices. Growth response was tested through nutrient application at (5) different fertilizer rates: 0 NPK kg ha^{-1} (T_1), 67 NPK kg ha^{-1}, (T_2), 135 NPK kg ha^{-1} (T_3), 202 NPK kg ha^{-1} (T_4), and 269 NPK kg ha^{-1} (T_5). The experimental study took place at NC A&T State University research farm located in Greensboro, NC (Guilford County). "Nutrient Management" *P* (<.0001/<.0001), "Harvest Year" *P* (<.0001/<.0001), and the "Interaction Effect" *P* (0.0002/0.01) were significant factors affecting (*Fresh/Dry*) matter accumulation observed during 3 consecutive years. Fresh matter was shown to dramatically increase in biomass accumulation with fertilizer treatment T_3 during 2013 (17.57±1.74 t ha^{-1}), 2014 (38.51±0.8 t ha^{-1}), and 2015 (45.43±2.91 t ha^{-1})

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harvests as compared to the control treatment T_1. Dry matter followed a similar trend in which yield was shown to significantly increase at treatment application T_3 during 2014 (23.54±0.8 t ha^{-1}), and 2015 (36.15±3.05 t ha^{-1}), as compared to the dry matter yields recovered from treatment application T_1 during 2014 (11.02±1.6 t ha^{-1}), and 2015 (26.76±0.64 t ha^{-1}). Treatment T_3 has produced significantly higher biomass than T_1 & T_2.

Keywords: Biomass; Giant miscanthus; growth response; North Carolina.

ABBREVIATIONS

DOY : *Day of Year*
FY : *Fresh Yield*
DY : *Dry Yield*

HIGHLIGHTS

- *Miscanthus giganteus* is well adapted to the climatic conditions of the piedmont region of N.C.
- Hyper accumulation of above ground biomass can be achieved in a short period under resource constraints.
- There is a positive growth response due to nutrient application at different rates of NPK.

1. INTRODUCTION

Miscanthus is native to tropical and sub-tropical regions of Africa and southern Asia [1]. *Miscanthus* has a unique advantage over traditional C_3 biomass crops (wheat, rice, and potatoes) due to the enhanced capacity of the C_4 photosynthetic pathway which produces large quantities of biomass [2]. Primarily, 3 species are referred to high biomass yielders for *Miscanthus*; *M. giganteus*, *M. sacchariflorus*, and *M. sinensis* [3]. Our research goal was to explore the productivity and resources requirement to produce a viable, non-invasive, high yielding grass species *M. giaganteus*. *M. giaganteus* used in this study is a genetically altered crop which is produced as a sterile hybrid between *M. sinensis* and *M. sacchariflorus* with characterization of being a low input, high biomass yielding, rhizomatous perennial grass, with a growing interest in bio-materials industry.

Miscanthus is an undomesticated crop which under optimized production practices could increase productivity [4]. Potential by-products from pyrolysis of residues could produce bio-liquid, gas, and biochar [5]. In 2007, an estimated 10,000ha of *Miscanthus* biomass was dedicated for heat generation in power stations in the UK [6]. If marketability of *Miscanthus* continues to expand economic competitiveness could equal that of traditional crops cultivated on arable land [7]. By-products from *Miscanthus* biomass could increase its production value in bio-refinery (chemical, manufacturing and power generation) systems. *Miscanthus* has been sold as horse bedding due to high absorbency and bio-degradability characteristics [8]. Another alternative use is as a bio-degradation product in mushroom cultivation [9]. Lastly, by-products recovered through *Miscanthus* operations could help increase dimensional stability to improve the quality of fiberboard [10].

To increase economic viability of *Miscanthus* biomass production, growers focus on nutrient management strategies which increase yields. However, excessive application of fertilizers leads to environmental pollution and reduced profit margins. *Miscanthus* has gained interest over the past few years due to low input requirement, 3 m $year^{-1}$ growth rate, with estimated Drymatter Yield (DY) between 20-25 t ha^{-1} [11]. Average lifespan of well-established *Miscanthus* is estimated between 15–20 years [12]. However, maturity is reached in the third year with DY estimated between 10-30 t ha^{-1}at autumn harvest [13]. Increased biomass accumulation during the first six years of establishment has been noted however, noticeable reductions were observed after seven consecutive production years [6]. DY of over 37 t ha^{-1}during first year establishment have also been reported [14]. Nitrogen loss from fertilizer application has been reported to be comparable to cereal production [15]. Studies conducted have shown that to produce 1.5 kg m^{-2} above ground DY it would require 9.2, 1.3 and 20.4 g m^{-2} of N-P-K [16]. Parallel and, stable growth responses to nutrient application were not found for *M. giganteus* in previously conducted experiments.

A significant increase in biomass production has been noted when N application reached upwards to 110 kg ha^{-1} of N [13]. A (62%) increase in additional biomass accumulation was reported when fertilizer rates increased from 100 to 200 kg N ha^{-1} [14]. However, results have been reported of smaller incremental DY increase of only (0.05%) when N fertilizer application was increased from 0 to 180 kg ha^{-1} of N [17]. DY estimates vary dramatically between consecutive annual harvest's, reports of first year biomass was estimated between (0.7- 2 t ha^{-1}), second year (7.9-15.5 t ha^{-1}) and in the third production year ranged between (17.4-24.5 t ha^{-1}) [18]. Few studies have been conducted on *M. giganteus* in North Carolina. Little available information on the production of *M.* x *giganteus* using a blanket application of N-P-K was found during the literature review.

1.1 Objectives

1.) Assessment of growth response of *M.* x *giganteus* under differential nutrient applications.
2.) Determine biomass productivity response between annual harvests during three consecutive harvest years.

2. METHODOLOGY

2.1 Experimental Site and Trial Establishment

The study was conducted on an Enon Sandy Clay Loam (Taxonomic class: Fine, mixed, active, thermic Ultic Hapludalfs) with (2-4%) slopes and (6-10%) slopes, located in Greensboro, North Carolina (North Carolina Agricultural and Technical State University Research Farm, coordinates: latitude: 36.06733°, longitude: -79.73447, altitude 223 m. The current climate hardiness zone is classified as zone 7_B.

2.2 Site Preparation and Nutrient Management

Miscanthus variety "Freedom" rootstock was obtained from REPREVE Renewables, LCC in Greensboro, USA and was grown using five different fertilizer treatments. The experimental plot layout was a 2x5 factorial design replicated four times. A total of twenty 4 m x 12 m plots were established with 3 m alley. Furrows were opened using a plow and rhizomes were placed 0.60 m apart and covered with soil. Five different fertilizer rates (0 NPK kg ha^{-1} (T_1), 67 NPK kg ha^{-1}, (T_2), 135 NPK kg ha^{-1} (T_3), 202 NPK kg ha^{-1} (T_4), and 269 NPK kg ha^{-1} (T_5)) were Fertilizer was applied in split applications through broadcasting at pre-determined amounts of 17-17-17 N-P-K (N-P2O5-K2O) fertilizer. Pest management was done by applying Biceps in (2013), and a (Fungicide) Diathane was applied in (2014); weeds were manually removed during each growing season to reduce competition pressure. Major phenological developments are listed in (Table 1).

2.3 Biomass Harvesting and Sub-sample Quantification Measurements

Plants were harvested leaving stubs approximately 15 cm from the soil surface. Biomass from 1 M^2 quadrant area was collected from each plot from a randomly chosen location, and the entire sample was immediately weighed on a Mettler Toledo SB16000 scale. To reduce analysis sensitivity to edge effects, samples were not taken adjacent to outside rows or located within close proximity to borders. A subsample (6 stalks) was collected from each quadrant to measure moisture content. Fresh biomass samples were dried at 70℃ to determine the moisture content of each sample, using a Fischer thermotemp oven over a 24 hrs duration. Moisture content was determined and expressed as a percentage of fresh weight [20].

2.4 Statistical Analysis

Growth response was analyzed using PROC MIXED (Mixed Procedure, SAS v9.2, SAS Institute, Cary, NC, U.S.A.), using (4) replications with (5) fertilizer rates "Nutrient Management", and (3) harvest years "Harvest Year" as fixed effects with the corresponding interaction between "Nutrient Management" x "Harvest Years".

3. RESULTS

Miscanthus biomass production was studied for 3 growing seasons from 2013-2015 and the data was tabulated for fresh weight, moisture loss and dry weight. Yield data and statistical analysis are presented in the Table 2.

To determine the optimum dose of fertilizer to aid in efficient nutrient management guidelines for *Miscanthus,* we evaluated a range of fertilizer applications (T_2, T_3, T_4, and T_5) with a control treatment T_1 which received no nutrient application. During the first establishment year we found that pairwise comparisons between treatment applications T_3, T_4, and T_5 produced a significant increase in fresh matter yield compared to the control treatment T_1 (Table 3). In the following growing season (2014) the same trend in growth response was evident, however, during (2015) only treatment comparisons T_3 and T_5 produced significantly greater fresh matter yields compared to the control treatment (T_1) (Table 3). When evaluating yield response between treatment plots and observed fresh matter yields produced we found that pairwise comparisons between treatment applications T_2 and T_3 did not differ significantly during the first production year (2013), however, during (2014), and (2015) treatment T_3 produced significantly higher fresh matter yields as opposed to treatment T_2 (Table 3). When evaluating treatment application T_4 to yields achieved by treatment application T_3 we observed significantly comparable fresh matter yields retrieved during the first and second growing season (2013) (Table 3). However, in latter production years (2015) treatment comparisons T_4 and T_3 were significantly different in fresh matter accumulation in which treatment T_3 produced significantly higher fresh matter yield accumulation (Table 3). Yield response between treatment applications T_4 compared to T_5 produced comparable yields during the first two production years (2013-2014), however, yields were significantly higher for treatment application T_5 during the 2015 growing season (Table 3).

Table 1. Provides a summary of principal cultivation practices and major developmental phases. Day of Year (DOY), and Growing Degree Days (GDD) both production seasons. Asterix indicates non- quantifiable parameter. Growing degree days was calculated as GDD= [(Temp max℃ + Temp min℃)/2]. Thermal base temperature (- 10℃) was determined by a previously determined formula [19]

Event	2013		2014		2015	
	DOY	GDD	DOY	GDD	DOY	GDD
Planting date:	147	*	*	*	*	*
Germination date:	170	303	99	3	86	9
Herbicide application:	163	211	99	3	*	*
Fertilizer application$_1$:	164	227	126	157	118	155
Fertilizer application$_2$:	196	681	198	1,114	146	424
Harvest date:	326	1,835	329	2,185	26	2,318
Tasseling started:	266	1,597	255	2,005	268	2,393
Vegetative state ended:	304	1,835	298	2,206	331	2,452
Predominately brown color:	312	1,846	322	2,204	352	2,468

Table 2. Results of fit statistics and type 3 tests of fixed effects using PROC MIXED for biomass accumulation characteristics of the individual main effects "Nutrient Management" and "Harvest Window" and the interaction effect for *Miscanthus* collected annually for 3 consecutive years. Comparisons for means were pooled across "Nutrient Management" and "Harvest Window"

Type 3 tests of fixed effects		
Effect	**Fresh matter accumulation**	**Dry matter accumulation**
Block	0.40	0.47
Nutrient management	<.0001**	<.0001**
Harvest year	<.0001**	<.0001**
Nutrient management x Harvest year	0.0002*	0.01*
-2 res log likelihood	153.9	141.6
AIC	155.9	143.6
AICC	156.1	143.8
BIC	157.2	144.9

†= indicates a significant deviation between Main Effects "Nutrient Management" and "Harvest Year" at 0.001, and ** indicates highly significant deviation 0.0001 between aforementioned pairwise comparison*

Table 3. Differences of pairwise comparisons using Tukey post hoc analysis for fresh matter accumulation of the interaction effect between "Nutrient Management" and "Harvest Year" for *Miscanthus* collected during the 2013, 2014, and 2015 growing seasons. Individual nutrient management yields obtained at each fertilizer rate during each individual harvest year

Pairwise comparisons	2013 (*p* Value)	2014 (*p* Value)	2015 (*p* Value)
T_1 vs.T_2	0.49	0.002**	0.06
T_1 vs.T_3	0.03*	<.0001**	<.0001**
T_1 vs.T_4	0.04*	<.0001**	0.36
T_1 vs.T_5	0.03*	<.0001**	0.0007**
T_2 vs.T_3	0.14	<.0001**	0.002**
T_2 vs.T_4	0.17	0.0001**	0.32
T_2 vs.T_5	0.14	0.01**	0.06
T_3 vs.T_4	0.91	0.06	0.0002**
T_3 vs.T_5	0.99	0.001**	0.17
T_4 vs.T_5	0.91	0.10	0.007**
Nutrient management	**2013 (t ha^{-1})**	**2014 (t ha^{-1})**	**2015 (t ha^{-1})**
T_1	12.52±1.14	16.24±2.39	33.55±0.74
T_2	13.70±1.19	23.87±2.27	37.91±4.22
T_3	17.57±1.74	38.51±0.80	45.43±2.91
T_4	17.32±2.74	33.98±1.28	36.32±2.63
T_5	16.84±0.03	30.86±2.51	42.29±3.39

†= indicates a significant deviation between Main Effects "Nutrient Management" and "Harvest Year" at 0.001, and ** indicates highly significant deviation 0.0001 between aforementioned pairwise comparison*

Table 4. Differences of Tukey post hoc analysis for dry matter accumulation of the interaction effect between "Nutrient Management" and "Harvest Year" for *Miscanthus* collected during the 2013, 2014, and 2015 growing season. Individual nutrient management yields obtained at each fertilizer rate during each individual harvest year

Pairwise comparisons	2013 (*p* Value)	2014 (*p* Value)	2015 (*p* Value)
T_1 vs.T_2	0.70	0.01*	0.07
T_1 vs.T_3	0.08	<.0001**	<.0001**
T_1 vs.T_4	0.15	<.0001**	0.15
T_1 vs.T_5	0.11	0.001**	0.0004**
T_2 vs.T_3	0.18	0.0002**	0.002*
T_2 vs.T_4	0.29	0.01*	0.71
T_2 vs.T_5	0.22	0.26	0.03*
T_3 vs.T_4	0.77	0.11	0.001**
T_3 vs.T_5	0.92	0.004*	0.30
T_4 vs.T_5	0.84	0.15	0.01
Nutrient management	**2013 (t ha^{-1})**	**2014 (t ha^{-1})**	**2015 (t ha^{-1})**
T_1	9.16±0.80	11.02±1.60	26.76±0.64
T_2	9.57±1.22	15.66±1.31	30.16±3.52
T_3	12.37±0.86	23.54±0.80	36.15±3.05
T_4	11.84±1.64	20.5±1.07	29.95±2.07
T_5	11.71±0.22	18.24±1.51	34.26±2.88

†= indicates a significant deviation between Main Effects "Nutrient Management" and "Harvest Year" at 0.001, and ** indicates highly significant deviation 0.0001 between aforementioned pairwise comparison*

3.1 Dry Matter Accumulation

During the (2013) growing season applying synthetic fertilizer from treatment application (T_2-T_5) did not increase dry matter yield as compared to treatment T_1 (Table 4 above). In the following production year (2014) all plots that received synthetic fertilizer (T_2-T_5) produced significantly greater yield response compared to treatment application T_1. However, during the next growing season (2015) we found that only treatment applications T_5 and T_3 produced a significant increase in dry matter yield (Table 4). When determining differences between increased fertilizer applications between synthetic fertilizer additions we found that treatments T_2 and T_3 produced comparable yields during the first production year (2013). However, during (2014) and (2015) a significant deviation in yield was observed in which T_3 produced higher dry matter yield compared to treatment application T_2 (Table 4). Treatment application T_3 and T_4 produced similar dry matter yields during the first and second production year (2013-2014). However, in (2015) yields retrieved from plots applied with treatment T_3 were significantly greater compared to yields recovered from treatment application T_4 (Table 4). No significant difference was determined between the application of treatments T_4 and T_5 during the first two production years, however, during (2015) T_5 produced a significantly greater yield as opposed to treatment T_4 (Table 4).

4. DISCUSSION

4.1 Comparison with Previous Research

Through the literature the growth response exhibited by *Miscanthus* to nutrient management is often debated. Due to the inconsistency of the reported findings we chose to compare our results with experiments that were conducted in a similar climatic zone. An experiment located in Lexington, KY focused primarily on Nitrogen addition to *Miscanthus* with a harvest window of 185 days growth, however, our experiment during first year growth used a 179 days. Maughn et al. [21] used N application rates 0, 60, and 120 kg $ha^{-1}$for a duration of 185 days produced 15.2,17.9, and 17.6 t ha^{-1} of (DY) for the respective treatments while we found at 179 day duration dry yields of 9.16, 9.57, and 12.37 t ha^{-1} for treatment applications T_1, T_2, and T_3. Researchers from Pulawy, Poland found that during 1st and 2nd year growth at application of 75-50-75 NPK kg ha^{-1} was found to yield 12.05 t $ha^{-1}$and 19.95 t/ha of (DY) when data was pooled between two growing sites [22]. The most relatable treatment to application 75-50-75 would be the 67-67-67 or T_2 treatment applied during our study. We observed first year yields of (9.57 t ha^{-1}) and (15.66 t ha^{-1}) when evaluating biomass produced during first and second establishment years. Researchers in North Carolina conducted a multi-year experiment growing *Miscanthus*, they found a more dramatic increase in yields that received nil-fertilizer application during 1st production year (2.61 t ha^{-1}) and during the 2nd production year (15.78 t ha^{-1}) [23]. Our results found that first and second year yields treated with T_1 were estimated at (9.16 t $ha^{-1}$and 11.02 t ha^{-1}) in the following production year. The same researchers also compared yields during 1st and 2nd production years using fertilizer rate of 135-147-0 N-P-K kg ha^{-1} and the corresponding (DY) was estimated at (3.02 t ha^{-1}) and (15.26 t ha^{-1}) [23]. Our treatment T_3 produced 12.37 t ha^{-1} during the first production year (2013) and 23.54 t ha^{-1} in the following production year (2014), this increase in biomass production could be due to better establishment of rhizomes from 2013 to 2014 and thereby put out more biomass.

5. CONCLUSION

Over the duration of this three year experimental study, we explored growth response due to nutrient management practices involving differential levels of synthetic fertilizer application. We found that dry matter accumulation increased from 2013 to 2014 by (38%), and from 2014 to 2015 by (43%). During 2014, and 2015 we found that treatment application T_3 produced significantly higher dry matter yields compared to the control treatment which increased yields by (53%), and (25%). We also found that dry matter yield increased by (33%) and (16%) during 2014 and 2015 growing seasons when treatment T_3 was applied as opposed to treatment T_2. Depending on the project study outcomes, significant yield for *Miscanthus* can be achieved using 135-135-135 (kg ha^{-1}) of NPK versus no nutrient supplementation. However, application of increased doses of fertilizers did not produce significantly different yield over T_3.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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