NUTRIENT CYCLING IN SWITCHGRASS AND SOIL HEALTH AS AFFECTED BY COVER CROPS

By

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Title of Study: NUTRIENT CYCLING IN SWITCHGRASS AND SOIL HEALTH AS AFFECTED BY COVER CROPS

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Abstract: The focus of these studies was nutrient management of specialty crops. Management practices for switchgrass fertilization in production of biofuel feedstock and forage hay are variable depending on climate, harvest timing and cultivar. Switchgrass studies focused on aspects of fertilization, nutrient cycling and harvest timing. Positive trends in yield with increasing rates of nitrogen (N) fertilizer ($P < 0.05$) were observed in some years while no yield differences were found due to P fertilization. Nitrogen use efficiency increased with N rate ($P < 0.05$). Nutrient translocation is a likely contributor to low fertilizer response. Aboveground (AG) biomass (leaves and stems) and belowground (BG) (roots) were analyzed for yield (AG) and nutrient concentrations (AG and BG). Maximum aboveground biomass ranged from 13.7±2.9 to 19.8±1.2 Mg ha^{-1} across years until senescence. Nitrogen concentration of AG decreased as season progressed ($P < 0.0001$). Belowground N increased over time from 3.3 to 13.9 g N kg^{-1} in two of three study years ($P < 0.05$), indicating macronutrients movement from AG to BG. Nutrients stored in roots can be used for regrowth in the following growing season. When assessing biomass quality by harvest timing, tissue-N removal increased from the 0 to the 235 kg N ha^{-1} application rate ($P < 0.05$). Nitrogen removal decreased during subsequent harvests at a fixed N rate ($P \leq 0.0001$). Quality was most impacted by harvesting time. Fibers and most minerals in the biomass increased as accumulated growing degree days (AGDD) increased ($P \leq 0.0001$), but N and total digestible nutrients (TDN) decreased as AGDD increased ($P \leq 0.0001$).

Vegetable growers face challenges of soil quality degradation with conventional tillage. Cover crops add soil organic matter (SOM) to improve soil health parameters and vegetable crop production. Four cover crop treatments were studied, including clean fallow as a control. Gravitational water content, water stable aggregates, and microbial CO_2-C were significantly lower in fallow compared to some cover treatments. Few significant differences in cash crop yields with cover crop combinations were seen in this study. Perhaps treatments had not been in place long enough to significantly improve soil health parameters like SOM.

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CHAPTER I

NITROGEN AND PHOSPHORUS AFFECTING SWITCHGRASS YIELD, NUTRIENT REMOVAL AND NITROGEN USE EFFICIENCY

ABSTRACT: Best management practices for switchgrass fertilization in production of biofuel feedstock and forage hay are variable depending on climate, harvest timing and cultivar. Two studies of nitrogen (N) and phosphorus (P) fertilization were conducted to evaluate the yield response, nutrient removal and nitrogen use efficiency (NUE) in 10+ years old switchgrass stands in Stillwater, OK. Seven rates of N fertilizer were applied ranging from 0 to 235.2 kg N ha^{-1} for 3 consecutive years; and 6 P fertilizer rates ranging from 0 to 84.0 kg P ha^{-1} were tested at a different location of the same field. In the N study, positive linear trends in yield with increasing rates of N fertilizer ($P < 0.05$) were observed in two out of three years. No yield differences were found due to P fertilization. Differences in N, P and potassium (K) removal due to N fertilization were found only in one of the 3 years. Nitrogen concentration in harvested biomass increased as N rates increased. Nitrogen use efficiency increased with N rate ($P < 0.05$), although many NUE values were negative up to a 134.4 kg N ha^{-1} rate. Better understanding of nutrient uptake

and use efficiency, removal with harvests and cycling within the plant could provide more refined fertilization practices in switchgrass production.

INTRODUCTION

Research studies focusing on switchgrass (*Panicum virgatum*) management have been plentiful as the perennial native grass is a candidate for biofuel feedstock. Management practices concerning fertilization, nutrient removal with harvest, harvest timing and nutrient use within the plant have been studied as switchgrass had become an economic and environmental interest in the early 2000s. The majority of fertilization studies for managed switchgrass stands have concentrated on nitrogen (N) since it is the most limiting nutrient for switchgrass.

Nitrogen rate studies have resulted in varying optimal rates for switchgrass depending on the location and cultivar. Recommendations have ranged from 0 in Oklahoma (Thomason et al., 2005) to 168 kg N ha^{-1} in Texas (Muir et al., 2001). Many native grasses have not shown positive yield response to N fertilization (Moore et al., 2000; Thomason et al., 2005; Rushing et al., 2019), and lower rates of applied N have often produced more positive response than higher rates in native grasses (Thomason et al., 2005; Rushing et al., 2019). For switchgrass, this lack of response is due to adaptation in low fertility environments, such as mycorrhizal associations, deep mining of nutrients, and seasonal nutrient translocation (Thomason et al., 2005; Ashworth et al., 2017). Switchgrass' ability to use other sources for N suggests there is low need for fertilization.

Phosphorus (P) is considered the second most-limiting nutrient for switchgrass; response to P fertilization having primarily been shown as luxury consumption of P

(Morris et al., 1982; Moore et al., 2000). Slight positive impacts from P addition alone has been shown in warm-season native grasses (Moore et al., 2000; Kering et al., 2012). Mycorrhizal associations within the root system is cited as the primary reason for the low response to P fertilization, with fungal hyphae enhancing the ability of the plant in uptake of available P (Brejda et al., 1993).

In managed switchgrass production, nutrient removal with harvest is thought to eventually become detrimental to the stand with repeated harvests, even though nutrient removal has been cited as low for switchgrass with harvesting (Kering et al., 2012; Kering et al., 2013). Therefore, fertilization inputs are necessary to replenish nutrients removed with harvests (Rushing et al., 2019). Understanding the type of fertilization practice needed in managed switchgrass with this apparent low fertilization requirement and low nutrient removal with harvest is necessary. Nitrogen use efficiency (NUE) could be a useful parameter to assist in switchgrass production. The difference method, measured as [(yield at N_x – yield at N_0) / fertilizer rate x] where N_x is N fertilizer at a given rate and N_0 is the control plot (Zemenchik and Albrecht, 2002), could be a practical tool to determine NUE. Switchgrass would be expected to have higher NUE as a species using C_4 photosynthesis than C_3 plants (Friesen and Cattani, 2017). The objectives of these studies were (i) to determine the effect of N and P fertilizer rates on biomass yields in single late-season harvest; (ii) to evaluate nutrient removal with harvest; (iii) and to evaluate NUE as affected by N fertilization for switchgrass for forage and biofuel use.

MATERIAL AND METHODS

Experimental design and treatment description

Separate N and P fertilization studies were initiated in 2008, Stillwater, OK (36°08'01.54"N; 97°06'17.16"W for N; 36°08'01.52"; 97°06'10.80"W for P) in a 10+ year old Kanlow switchgrass field, as replicated randomized complete block designs (RCBD) with four replications (n = 4). The soil at the site is a Norge loam (fine silty, mixed, active, and thermic Udic Paleustoll) (Soil Survey Staff, NRCS-USDA). Nitrogen application rates were 0, 33.6, 67.2, 100.8, 134.4, 168.0, 201.6, and 235.2 kg N ha^{-1} and the experiment was repeated in 2009 and 2010. The phosphorus study was conducted in 2008 with rates of 0, 16.8, 33.6, 50.4, 67.2, and 84.0 kg P ha^{-1}. The plot size for both studies was 18.3 m^2 (3.0 m x 6.1 m). A 1.5 m wide separation between plots was used to minimize cross contamination.

Soil sampling and analysis

Soil samples were collected to a depth of 15 cm from each plot prior to fertilization each year (Table 1.1). Samples were oven dried at 65 ^oC for 24 h and ground to pass through a 2-mm sieve. Soil pH was measured with an electrode in a 1:1 soil:water suspension. Plant available N was extracted by 1M KCl and analyzed by a flow injection auto-analyzer (LACHAT QuickChem 8000, Milwaukee, WI) (Kachurina et al., 2000). Plant available P, K, calcium (Ca), and magnesium (Mg) were extracted using a Mehlich-3 solution (Mehlich, 1984). Sulfate-S was extracted by 0.008M calcium phosphate. Micronutrients iron (Fe), zinc (Zn), boron (B), and copper (Cu) were extracted by DPTA-Sorbitol (Hanson et al., 1998) and quantified by an inductively coupled plasma (ICP) spectrometer.

Table 1.1. Average soil pH and plant available nutrients prior to fertilization tested in 2008, 2009, and 2010.

Study	Year	pH	NO_3-N	P	K	SO_4-S	Ca	Mg	Fe	Zn	B	Cu
			……kg ha^{-1}……						…….mg kg^{-1}……			
N	2008	6.3	5.4	16.8	120	8.8	1614	326	51	0.79	0.35	1.4
	Std. dev.†	0.2	0.8	2.9	17	0.9	171	34	9.9	0.2	0.04	0.2
N	2009	6.2	7.1	15.9	115	5.5	1612	324	57	0.77	0.36	1.5
	Std. dev.	0.3	8.6	2.7	20	0.4	172	33	9.6	0.1	0.04	0.2
N	2010	6.4	2.5	16.1	114.6	6.3	1587	315	59	0.75	0.27	1.5
	Std. dev.	0.2	1.0	3.0	13.2	1.8	158	29	12	0.2	0.03	0.2
P	2008	6.2	1.0	14.1	68.3	4.5	989	214	23	0.44	0.18	1.6
	Std. dev.	0.2	0.6	2.8	5.7	0.7	59	16	3.6	0.4	0.01	1.2

†Std. dev. = Standard deviation of the mean (n=4).

Fertilization

In the N study, N was applied as urea or as a combination of urea and Diammonium phosphate (DAP; 18-46-0) to supply P in individual plots when P was deficient based upon soil test. Single applications were used at rates of $\leq$ 67.2 kg N ha^{-1}, and split-applications used at rates $\geq$ 100.8 kg N ha^{-1}. Rates $\leq$ 67.2 kg N ha^{-1} were applied in mid-March (as urea or urea and DAP) and remaining N for $\geq$ 100.8 kg N ha^{-1} rates in early May to reach adequate N. No applications were made before this study, except for stand establishment. For the P study, P as triple superphosphate (TSP; 0-46-0) was broadcast applied in March.

Switchgrass harvest and biomass

A single harvest was performed after senescence in November of each year and analyzed for biomass yield and nutrient composition. Plots were harvested using a flail harvester (Carter Manufacturing Co., Inc., Brookston, IN) and biomass was collected. Yield was measured at harvest with a hanging scale.

Representative subsamples of harvested biomass were dried at 48.9 °C for 24 h, then chopped and ground to pass through a 1-mm sieve. Half a gram of ground plant materials was predigested for 1 h with 10 mL of trace metal grade HNO_3 in a HotBlock™ Environmental Express block digester. Digests were heated to 115 ºC for 2 h and diluted with deionized water to 50 mL (Jones and Case, 1990). Samples were analyzed by an ICP for P and K. Total N was determined with a carbon/nitrogen (C/N) dry combustion analyzer.

Statistical analysis

Regression analyses were performed for average values from replicate data by rate (*x-axis*). Trend analysis was conducted from regression models by level of significance. Lower *P-value* of each model determined the best fit using the PROC REG of SAS ver. 9.4. Equation coefficients of each model were tested for significance as well. Differences in yield and nutrient removal were determined using analysis of variance (ANOVA) and Duncan's Multiple Range Test. Nitrogen use efficiency (NUE) for the N study was calculated for regression analysis. The difference method was used in calculations, as [(yield at N_x – yield at N_0) / fertilizer rate x], where N_x is N fertilizer at a given rate and N_0 is the control plot (Zemenchik and Albrecht, 2002).

RESULTS AND DISCUSSION

Switchgrass yield responses to N and P fertilization

Yields increased as N rate increased in 2008 ($P < 0.01$). On average, yields increased by 15 to 25 kg ha^{-1} (0.015 to 0.025 Mg ha^{-1}) for every 1 kg N ha^{-1} applied (Fig. 1). Standard deviation within replicated plots was 1.2 Mg ha^{-1}. In 2009, no clear yield response to N fertilization was observed due to the high variability among replicated plots. ANOVA showed yields ranging from 8.3 Mg ha^{-1} to 19.5 Mg ha^{-1} with no significant difference, and standard deviation was 4.3 Mg ha^{-1}. In 2010, a significant positive linear trend was observed ($P < 0.0001$) and the biomass yield was increased by 24 to 36 kg ha^{-1} (0.024 to 0.036 Mg ha^{-1}) per kg N ha^{-1} applied (Fig. 1.1). Mean yields ranged from 6.5 to 14.4 Mg ha^{-1}, and the standard deviation was 2.6 Mg ha^{-1}.

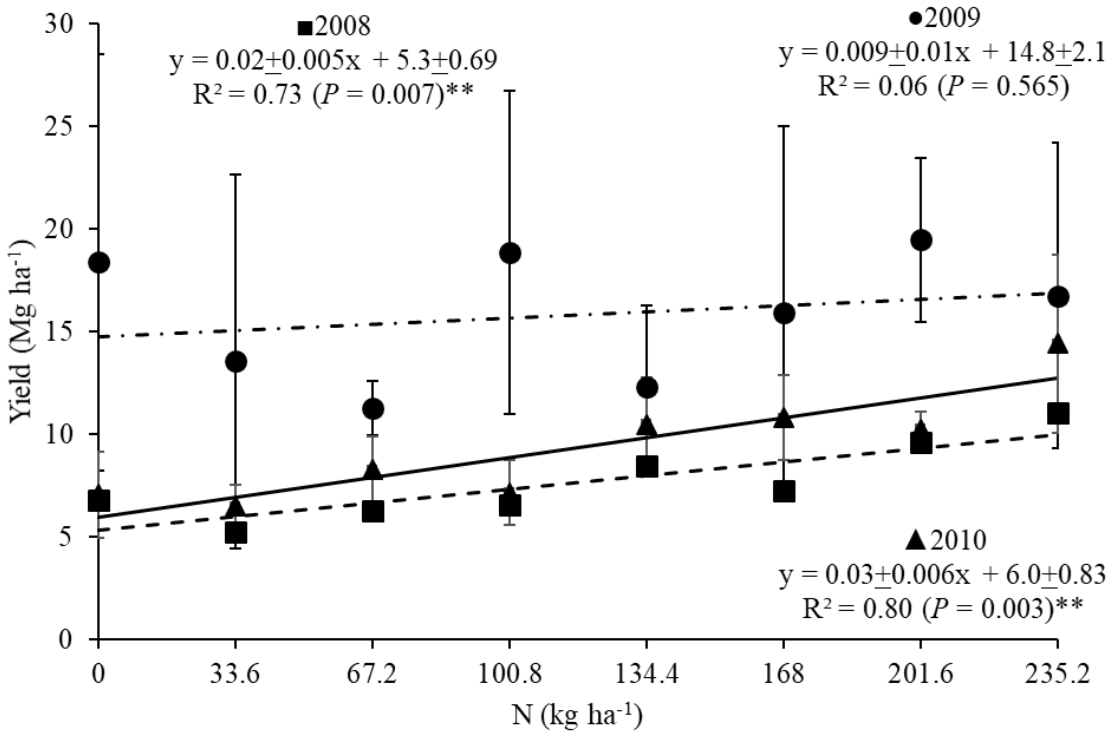

Fig. 1.1. Relationship between N fertilizer rates and switchgrass yield in the seasons 2008, 2009, and 2010 (average values from replicate data, n=4). **: Significant at $P < 0.01$.

No significant relationship between switchgrass yield and P fertilization was found in trend analysis ($P = 0.186$) (Fig. 1.2). Since the soil test P was close to the 100% of sufficiency level for native grasses, differences in yield due to P fertilization were minimal. Mycorrhizal associations enhancing P use efficiency may also contribute to this lack of yield response (Brejda et al., 1993).

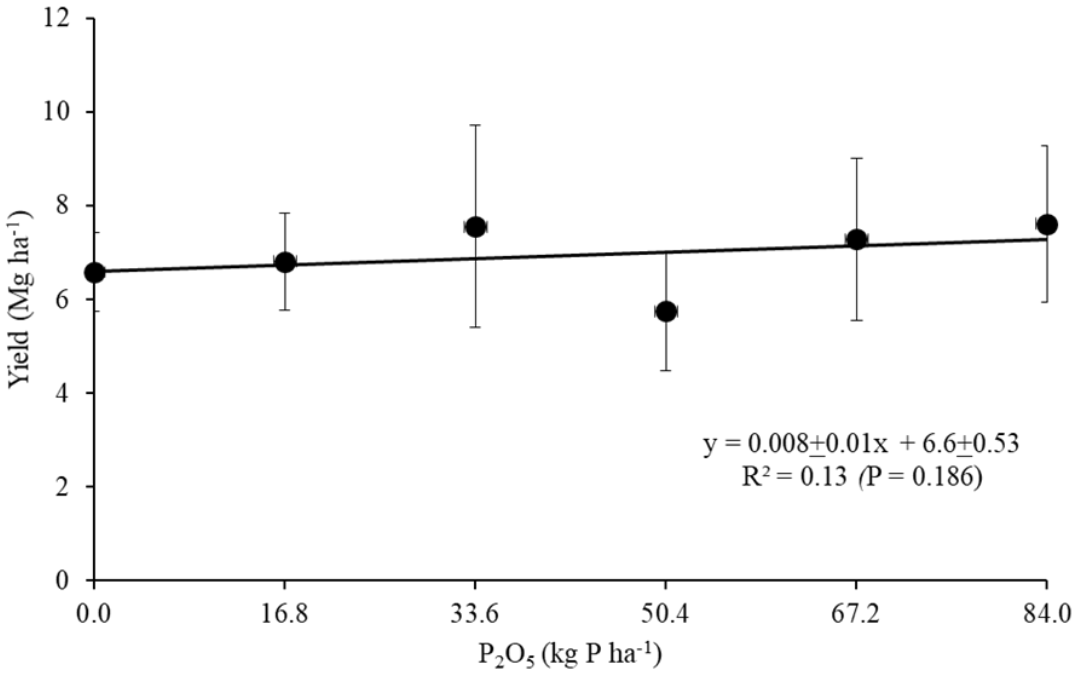

Fig. 1.2. Relationship between P fertilizer rates and switchgrass yield in 2008 (average values from replicate data; n = 4).

Applied N did not result in consistent response of biomass yields to standardize N fertilizer recommendation. Variation in biomass yields among years has been reported in other N studies (Reynolds et al., 2000; Muir et al., 2001; Lemus et al., 2008; Seepaul et al., 2016). In this study, the 2010 yield was the most affected by N fertilization, having the largest yield per kg N applied (24 to 36 kg ha^{-1} per kg N ha^{-1}) compared to prior years. This could be a cumulative effect due to prior years' fertilization, as suggested by Lemus et al. (2008), where N fertilization effects were not shown in the first two years. It has also been shown previously that little yield response to applied N occurs in established older stands. Nutrient management plans must include historic N application in prior years and age of the stand. Mature switchgrass stands have developed under areas of low fertility, often on land considered marginal for crop production. Switchgrass

growth has not historically relied on anthropogenic fertilization. Its root system allows for deep mining of the soil profile and mycorrhizal associations that enhance nutrient uptake in many natural systems.

Nutrient removal with harvest

The amount of nutrients removed is a function of biomass yield and the concentration of a particular nutrient in the plant sample. Nitrogen and P removal was not statistically different at 0 kg N ha^{-1} than in some of the other treatments that received nutrient inputs (Table 1.2). This was also true for K removal in 2008 and 2009. For N removal, significant differences ($P < 0.05$) were seen in 2008 between 235.2 and the 0, 33.6, 67.2 kg N ha^{-1} treatments (Table 1.2). In 2010, significant differences ($P < 0.01$) were seen between 235.2 and 0, 33.6, 67.2, 100.8, 134.4, and 168.0 kg N ha^{-1} treatments. Phosphorus removal in 2010 had significant differences ($P < 0.05$) between 235.2 and 0, 33.6, 67.2, and 100.8 kg N ha^{-1} treatments (Table 1.2).

When averaged across all years, N removal from control plots was 38 kg N ha^{-1}, demonstrating the ability of switchgrass to use available N by mining it from the subsurface in the soil profile and cycling N throughout the plant. With few exceptions, N removal in unfertilized plots was equivalent to removal in plots receiving 33.6 to 201.6 kg N ha^{-1}. This suggests an ability of the grass to use N sources other than anthropogenic inputs. As annual harvests are completed, a critical amount of nutrients will be eventually removed in harvested biomass and will need to be replenished by fertilization. Otherwise, long term high yield production cannot be sustained.

Table 1.2. Yield and nutrient removal by year as a function of nitrogen application rates.

kg N ha^{-1}	Yield	N*	P	K	Yield	N	P	K	Yield**	N**	P*	K
	Mg ha^{-1}	kg ha^{-1}			Mg ha^{-1}	kg ha^{-1}			Mg ha^{-1}	kg ha^{-1}		
	……2008……				……2009……				……2010……			
0	6.8abc	20.0bc	6.1ab	26.6ab	18.4a	62.5ab	15.3a	42.6a	7.1bc	26.5c	4.0b	12.3b
33.6	5.3c	18.1c	4.5b	17.7b	8.3a	29.3b	6.3a	21.0a	6.5c	21.5c	3.6b	13.5ab
67.2	6.3bc	19.0bc	5.2b	20.3b	11.3a	40.3b	7.0a	26.8a	9.2bc	28.3c	4.6b	16.7ab
100.8	6.6abc	22.7abc	5.6ab	18.8b	18.8a	65.1ab	14.3a	44.2a	7.2bc	24.7c	4.1b	15.9ab
134.4	9.2a	30.1ab	8.7a	36.9a	12.3a	43.5b	8.1a	23.8a	10.5abc	32.4bc	6.2ab	17.1ab
168	7.2abc	27.2abc	5.7ab	27.1a	10.9a	41.4b	8.3a	22.4a	10.8ab	29.9c	5.9ab	17.8ab
201.6	6.6abc	30.4ab	5.1b	23.1ab	19.5a	88.2a	13.4a	47.6a	10.3bc	51.7ab	6.3ab	17.1ab
235.2	8.3ab	33.9a	5.7ab	23.5ab	16.7a	65.0ab	7.2a	28.7a	14.4a	61.0a	7.9a	19.4a
Average	7.0	25.2	5.823	24.24	14.5	54.4	10.0	32.1	9.5	34.5	5.3	16.2
Std. Dev.	1.2	6.0	1.244	6.126	4.3	19.1	3.7	10.9	2.6	14.1	1.5	2.3
F test	2.12	2.66	1.59	1.76	1.41	2.23	1.67	1.79	4.26	4.91	2.53	1.49
MSD	3.0	12.3	4.0	18.4	14.4	45.6	10.9	30.6	4.0	19.9	3.2	7.6
CV%	22	27	33	37	46	44	52	46	27	37	34	23

Std. Dev.: Standard deviation of the mean.

CV%: Coefficient of variation as percentage.

MSD: Minimum significant difference.

*: Significant $p<0.05$, **: Significant $p<0.01$ (Duncan's Multiple Range Test)

Nitrogen use efficiency (NUE)

Regression analyses of averaged data showed significant trends of NUE and N rates in each year. Levels of significance were $P = 0.04$ in 2008, $P = 0.03$ for 2009, and $P = 0.02$ for 2010 (Fig. 1.3). Although these trends are significant, standard deviations are wide ranging and NUE is negative up to a rate of 134.4 kg N ha^{-1} for 2008 and 2010. In 2009, NUE is only positive at the 100.8 and 201.6 kg N ha^{-1} rate. Average positive NUE values ranged from 2.7 to 18.3 kg DM (Dry Matter) kg^{-1} N in 2008 and 12.1 to 28.5 kg DM kg^{-1} N in 2010. As stated earlier, many yield values were higher in the control plots than in those plots receiving fertilizer. In a single harvest system, harvest was conducted after senescence. Therefore, the tendency of forage plants to have high plant N loss at flowering and maturity is reflected as harvest was conducted in this study. Variation within experimental plots and between years may be due to precipitation rather than N, as suggested by Zemenchik and Albrecht (2002) (Fig. 1.4). Total rainfall was similar in all years, but the majority of rainfall distribution (> 180 mm) occurred late in the year (August and October) for 2009 (Fig. 1.4). This may have contributed to late season growth, mainly stem growth, near timing of flowering, seed set and senescence. As switchgrass has a bunch-type growth habit, stand uniformity can be marginal and can contribute to stand variation as well.

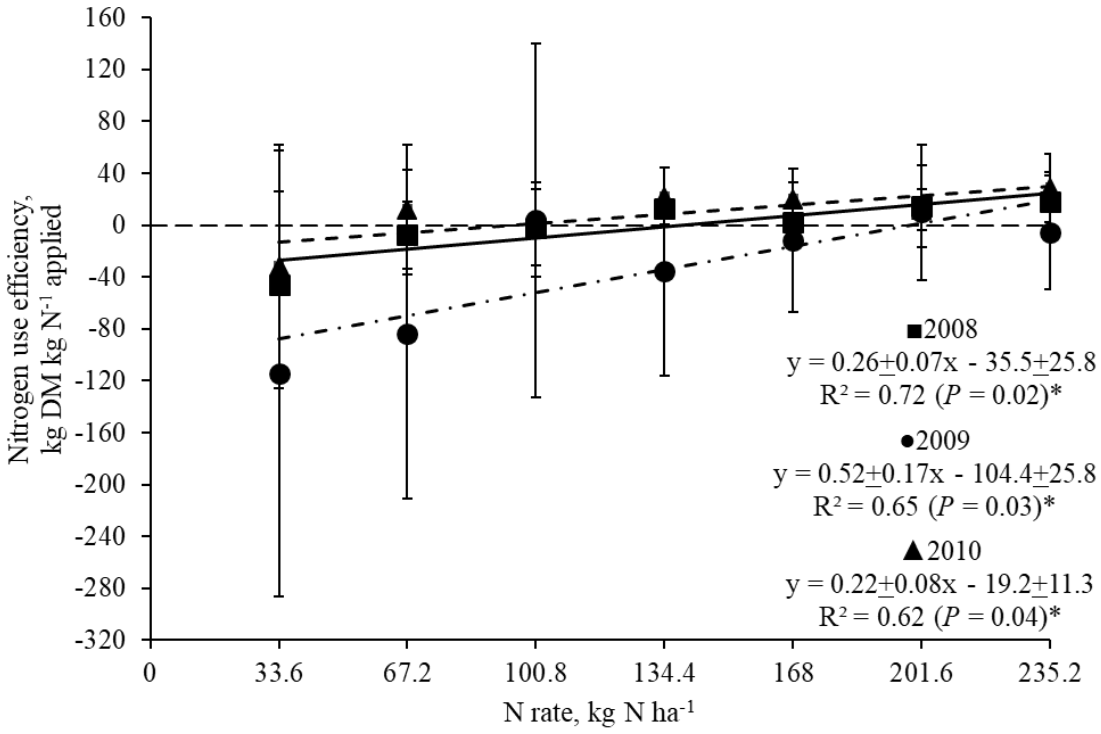

Fig. 1.3. Relationship between N fertilizer rates and nitrogen use efficiency (kg yield ha^{-1} per kg N ha^{-1} applied) in the seasons 2008, 2009, and 2010 (average values from all replicate data, n=4). *: Significant at $P < 0.05$.

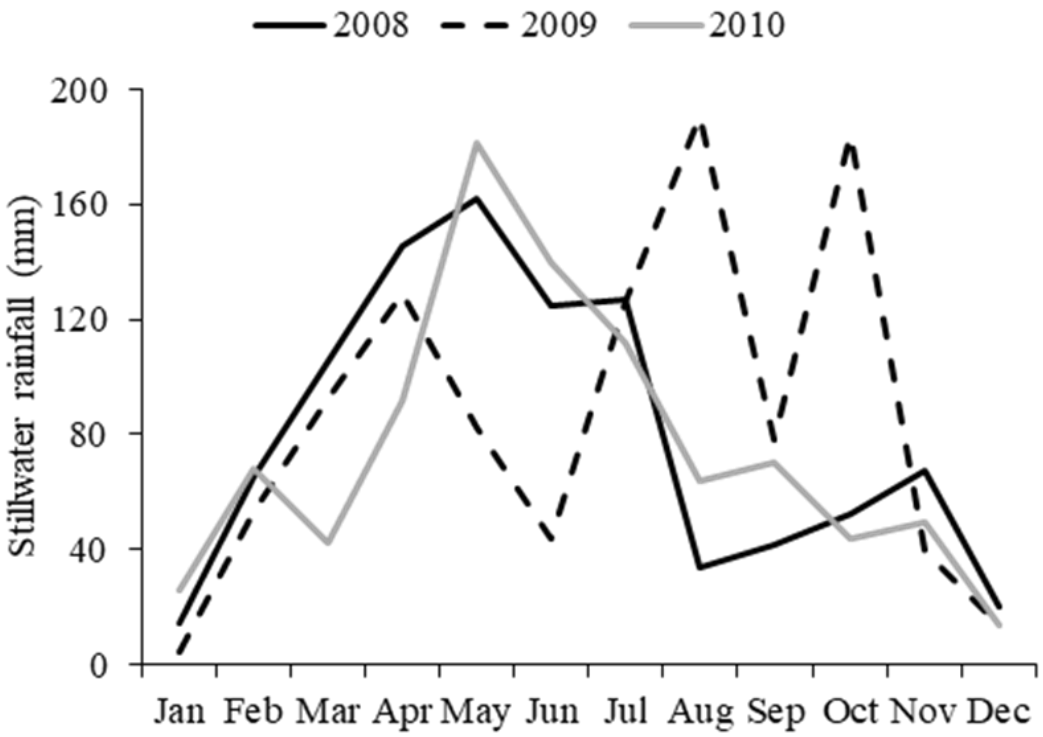

Fig. 1.4. Stillwater rainfall distribution in 2008, 2009 and 2010.

CONCLUSIONS

Switchgrass yield and nitrogen use efficiency is less responsive at lower fertilization rates than improved grass varieties. Understanding dynamics of stand age, nutrient uptake, removal and cycling within the plant is essential to nutrient management for switchgrass. Other strategies for obtaining nutrient sources available to the plant, different than those in managed systems, need to be better elucidated for accurate fertilizer recommendation. More work involving fertilization, nutrient translocation and partitioning within the plant, and harvest timing is needed in developing switchgrass as a forage and biofuel crop.

CHAPTER II

NUTRIENT DYNAMICS IN SWITCHGRASS (*PANICUM VIRGATUM* L.) AS A FUNCTION OF TIME

ABSTRACT: There is a wide variation in the fertilizer recommendations for switchgrass (*Panicum virgatum* L.) as biofuel feedstock or forage. As with other native grasses, low and inconsistent yield response to fertilization is common for switchgrass. Nutrient translocation is a likely contributor to this lack of fertilizer response. A field study was initiated to evaluate how major nutrients are cycled within switchgrass during the growing season for 3 years. Aboveground (AG) biomass (leaves and stems) and belowground (BG) (roots) were separated and analyzed for yield (AG) and nutrient concentrations (AG and BG). Maximum yields of aboveground biomass ranged from 13.7±2.9 to 19.8±1.2 Mg ha^{-1} across years until the post-ripening/senescence growth stage. Nitrogen (N) concentration of AG biomass decreased from 12.4 to 2.1 g N kg^{-1} as the season progressed ($P < 0.0001$). Belowground biomass N concentration increased over time from 3.3 to 13.9 g N kg^{-1} in two of three study years ($P < 0.05$). Similar trends were observed for phosphorus (P) and potassium (K). However, trends of decreases with time in AG biomass and increases with time in BG biomass were not consistent for secondary nutrients and micronutrients. Our

findings indicate macronutrients movement from AG to BG. Nutrients stored in roots can be used for regrowth in the following growing season, which may reduce the grass's response to fertilizers. Greater understanding of nutrient cycling as it relates to the timing of harvest is needed to better manage the switchgrass production systems for different purposes of use.

INTRODUCTION

Switchgrass (*Panicum virgatum* L.) yield response to applied nitrogen (N) fertilizer has often been inconsistent in fertilization studies (Fike et al., 2006b; Guretzky et al., 2010; Kering et al., 2011; Muir et al., 2001). Some work has suggested nutrient transfer from aboveground portions of the plant (AG) to belowground (BG) as the reason for the poor response to applied nutrients (Jach-Smith and Jackson, 2020). A transfer such as this would likely be important for regrowth during the following growing season (Parrish et al., 2003; Ashworth et al., 2017a and 2017b). Nutrient dynamics within switchgrass plants during the growing season offers insight to the need and use of fertilizer in biomass production.

Native switchgrass was developed in areas under low N input, typically without anthropogenic N sources (Clark, 1977; Chapin, 1980; Parrish and Fike, 2005). Without the application of N, nutrient needs are met by inputs from natural N sources, such as N deposition from lightning and rainfall, microbial decomposition and mineralization from plant and animal residues. The nutrient translocation within the plant is also an important source of nutrients for the subsequent season. Nitrogen and other nutrients have been reported to translocate from the AG to the BG portions as the plant matures towards senescence (Heggenstaller et al., 2009; Parrish and Fike, 2005). This translocation may be

one of the reasons why switchgrass yield has often shown no or inconsistent responses to applied N when cultivated as a forage crop. Seasonal nutrient movement within the plant has been observed in other prairie grasses, such as big bluestem, little bluestem and indiangrass (Clark, 1977; Heggenstaller et al., 2009). Heckatorn and Delucia (1994 and 1996) who researched nutrient translocation in prairie grass response to fire and drought conditions and found tallgrass prairie species translocated 30% of N held in AG shoots to BG rhizomes to conserve N, and to limit N losses by fire and grazing. Observations like these indicate that switchgrass and other native prairie species are very efficient in their use of nutrients (Parrish and Fike, 2005) and they should be managed accordingly.

Phosphorus and potassium needs of switchgrass are often met by reserved nutrients in the soil profile through chemical release and mycorrhizal associations (Clark, 2002; Petipas et al., 2020). Studies involving P have found little to no yield response to P fertilization, although increased yield and P-use efficiency with an N x P interaction have been shown (Brejda, 2000). Mycorrhizal activity cannot be overlooked when considering P requirements by switchgrass, but the amount of P received in the plant contributed by mycorrhizae associations is unknown (Parrish and Fike, 2005). Similarly, K fertilization requirements are considered to be little to none; and studies have often reported no response to K fertilization (Parrish and Fike, 2005). Seasonal translocation within the plant may have a role in the cycling of P, K, secondary and micronutrients. Little research has been conducted for switchgrass concerning P, K, and other nutrients, as N is considered to be the most limiting nutrient. Those that have considered other nutrients (Makaju, 2013) have not shown different concentrations of secondary and micronutrients in AG and BG to be a significant contributor to switchgrass growth.

A better understanding of nutrient concentration in AG and BG as it relates to nutrient translocation in switchgrass is needed to more efficiently manage the crop for production. During harvest, nutrient removal can become a sustainability issue in nutrient management. Nitrogen removal rates have been recorded as 18 to 39 kg N ha^{-1} (Vogel et al., 2002; Heaton et al., 2009; Heggenstaller et al., 2009; Propheter et al., 2010; Wilson et al., 2013a). Depending upon harvest systems, Reynolds et al. (2000) reported total N removal could range from 31 to 63 kg N ha^{-1} yr^{-1} in a single harvest system and from 90 to 144 kg N ha^{-1} yr^{-1} in a two-harvest system. Nutrient removal due to harvesting would eventually lead to the need for additional fertilization. Changes on the removal rates of nutrients are dependent on the time of year in which the harvest occurs (Mislevy and Martin, 2006), along with nutrient concentration in the harvested portions of the plant.

The objective of this study was to evaluate the nutrient concentrations within switchgrass parts throughout the growing season to determine the role of nutrient cycling in the plant life cycle. Understanding these intra-seasonal changes will assist in determining the role of anthropogenic fertilization in switchgrass management. Investigating seasonal changes in yield and tissue nutrient concentration, and nutrient cycling and dynamics in switchgrass as it relates to the nutrient translocation will add to the knowledge of both managed and natural systems.

MATERIALS AND METHODS

Experimental area and treatment description

The study was initiated in 2008 in an established Kanlow switchgrass stand in Stillwater, OK (36°08’01.54” N; 97°06’17.16” W), which was first established in 1998.

To evaluate nutrient concentration, whole switchgrass plants were sampled periodically throughout the growing season each year (2008, 2009, and 2010). The plots measured 6.1 x 6.1 m with four replications (n=4) (Fig. 2.1). Although eight N fertilization rates were applied in the entire experimental area, temporal (subsequent) aboveground (AG) harvests were hand-harvested from each plot under a single N fertilization rate, in a 0.9 m by 3.0 m swath, to estimate AG switchgrass yield (Fig. 2.1). The yield was measured at harvest with a hanging scale. With these temporal AG harvests, a whole plant sample (AG: shoots + BG: roots) was additionally harvested at the same intervals, in an adjacent 0.9 m x 1.5 m area, to estimate temporal nutrient concentration in above and belowground portions of the plant (Fig. 2.1). The N rates chosen for this evaluation were slightly different among the 3 years (134.4 kg N ha^{-1} in 2008, 100.8 kg N ha^{-1} in 2009, and 168 kg N ha^{-1} in 2010, as urea) because early harvests did not allow plants to grow back in the same area the following year, probably due to injury to the stand or lack of accumulated nutrients for the subsequent year (Casler and Boe, 2003; Adler et al., 2006; Wilson et al., 2013b). This prevented using the same plots so sampling in plots with similar but different N rates (>100 kg ha^{-1}) were used in the subsequent years. Four replications were sampled at each harvest. Aboveground production in switchgrass is responsive to N fertilization, however, fertilizer rates for maximum production are >100 kg N ha^{-1} (Garten Jr. et al., 2010). Moreover, critical rates of N application, as related to switchgrass biomass yield response, was found to be between 49 to 170 kg ha^{-1}, according to Anderson et al. (2013) when studying nitrogen fertility and harvest management of switchgrass in several locations of Illinois. Therefore, the chosen N rates used in this study (100.8, 134.4, and 168 kg N ha^{-1}) are within that critical range found by Anderson et al. (2013). Other studies have also reported the use and/or the critical

rates of 100 (Fike et al., 2006a), 134 (Kering et al., 2011; Sanderson and Moore, 1999a), and 168 kg N ha^{-1} (Kering et al., 2011; Garten Jr. et al., 2010) for switchgrass biomass yield response. Phosphorus and potassium were applied if needed according to Oklahoma State University soil test recommendations.

Soil sampling and analysis

The soil at the site is a Norge Loam (fine silty, mixed, active, and thermic Udic Paleustoll) (Soil Survey Staff, USDA). Before fertilization each year, soil samples were taken from 0 to 15 cm. Samples were oven-dried at 65 °C for 24 hours and ground to pass in a 2-mm sieve. Soil pH was measured with an electrode in a 1:1 soil to water suspension (Thomas, 1996). Plant available N was extracted by 1 M KCl and analyzed by a flow injection auto-analyzer (LACHAT QuickChem 8000, Milwaukee, WI; Kachurina et al., 2000). Plant available phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) were extracted by a Mehlich-3 solution (Mehlich, 1984). Sulfate-S was extracted by 0.008 M calcium phosphate (Zhang and Henderson, 2018). Micronutrients iron (Fe), zinc (Zn), boron (B), and copper (Cu) were extracted using DPTA-Sorbitol (Hanson et al., 1998). Extracts were properly filtered and analyzed for nutrient concentrations using inductively coupled plasma – atomic emission spectroscopy (ICP-AES) (Soltanpour et al., 1996). The results of soil testing from samples taken at the beginning of each growing season are shown in Table 2.1.

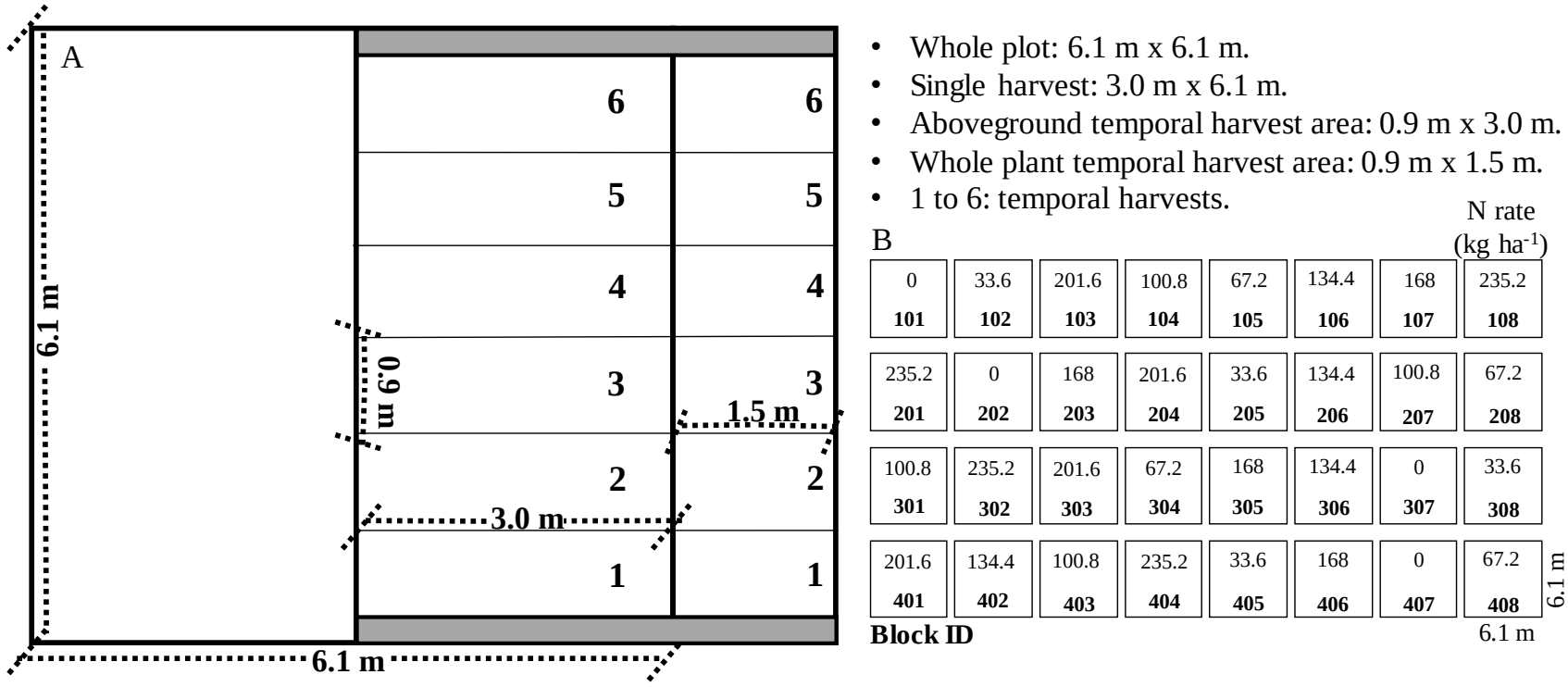

Fig. 2.1. Plot design (A) and experimental area (B). Gray color reflects a total area of 0.7 m x 4.5 m extra space in interval (temporal) harvests.

Table 2.1. Soil pH and plant available nutrients tested by year (2008, 2009 and 2010). Samples taken prior to fertilization.

Year	pH	NO_3-N	P	K	SO_4-S	Ca	Mg	Fe	Zn	B	Cu
			----------kg ha^{-1}----------						----------mg kg^{-1}----------		
2008	6.3±0.2	3±0.8	16±1	118±16	8.4±0.2	1563±125	317±32	48.5±11	0.7±0.1	0.34±0.0	1.4±0.1
2009	6.2±0.2	6.4±2.7	17±2	109±14	5.7±0.3	1538±172	309±31	60.3±9	0.8±0.1	0.36±0.0	1.5±0.0
2010	6.4±0.1	2.5±1.5	14.9±3	110±8	5.9±0.5	1560±107	310.7±16	58.4±8	0.7±0.2	0.27±0.0	1.5±0.3

±: Standard deviation of the mean (n=4).

Switchgrass sampling and analyses

Switchgrass plants were harvested periodically from June to January, February or March of the following year. The temporal harvests were designated as accumulated growing degree days (AGDD) starting from January 1 of each year (Table 2.2). Switchgrass AG biomass yield and nutrient removal data were all replicated within year (2008, 2009 and 2010) and standardized by AGDD (Temperature base = 10 °C) (Sena et al., 2019). Growing degree days (GDD) were calculated as Sanderson and Moore (1999a), where GDD = [(maximum daily temperature + minimum daily temperature)/2]-10. Daily maximum and minimum temperatures in Stillwater (2008, 2009 and 2010) were acquired from the Oklahoma Mesonet website (www.mesonet.org). Accumulated growing degree days (AGDD) was obtained by summing up positive GDD (GDD > 0) beginning on January 1 of each year (Dhillon et al., 2019; Sanderson and Moore, 1999a; Sena et al., 2019). The mean growth stage count of switchgrass (MSC) was calculated as per Mitchell et al. (1997), where MSC = [0.875 + (0.0017 × AGDD)] (Table 2.2). The switchgrass growth stages and their descriptions can be found in Moore et al. (1991) (and Moore and Moser, 1995), who considered MSC values from 0.0 to 4.9 to describe the growth stages of switchgrass (Table 2.2). Estimated MSC > 4.9 were listed as "post-ripening/senescence" for our study because some harvests took place after physiological maturity and senescence (Table 2.2).

A whole plant from the adjacent area was harvested to estimate temporal nutrient concentration in above- and belowground portions of the plant (AG and BG, respectively). One plant was randomly selected and harvested. Switchgrass is a bunchgrass with large AG and BG biomass, so a single plant was adequate to represent the whole plot, and more

practical than a fixed area to harvest for this study. Four plants were sampled at each AGDD harvest, one from each replicated plot. The single plant from each plot was excavated to a depth of approximately 0.9 m to obtain the root biomass. The diameter of the excavated area was approximately 0.5 m. Whole plant samples were separated into AG and BG plant portions and analyzed for nutrient concentrations separately. Aboveground portions were chopped and ground to pass through a 1-mm sieve. For BG portions, the soil was washed from roots and samples were dried intact. Roots were chopped and ground to pass through a 1-mm sieve to prepare samples for analysis. Both AG and BG plant samples were digested with nitric acid (HNO_3), in which 0.5 g of ground plant materials were predigested for 1 h with 10 ml of trace metal grade HNO_3 in the HotBlock^{TM} Environmental Express block digester. The digests were then heated to 115 °C for 2 h and diluted with deionized water to 50 mL (Jones and Case, 1990). Digested samples were analyzed by ICP-AES for mineral nutrients (P, K, Ca, Mg, S, Na, Fe, Cu, Zn, Mn, and Ni). Total N was determined with a carbon/nitrogen (C/N) dry combustion analyzer (Undersander et al., 1993).

Table 2.2. Dates of periodic whole plant biomass harvests from one of the nitrogen treatments (134.4 kg N ha^{-1} for 2008, 100.8 kg N ha^{-1} for 2009, and 168 kg N ha^{-1} for 2010), and the accumulated growing degree days (AGDD) according to the Julian Day of the Year (DOY).

Year	Harvest	Date	Julian DOY	AGDD§§	MSC	Growth Stage	Description
2008	1	6/12/2008	164	755	2.2	E1: Elongation-Stem elongation	First node palpable/visible
2008	2	7/24/2008	206	1452	3.3	R2: Reproductive-Floral development	Spikelets fully emerged/peduncle not emerged
2008	3	9/5/2008	249	2175	4.6	S3: Seed development and ripening	Hard dough
2008	4	10/30/2008	304	2611	5.3	-- Post-ripening/senescence	--
2008	5	12/4/2008	339	2672	5.4	-- Post-ripening/senescence	--
2009	6	2/28/2009	425†	2748	1.0	V0: Vegetative-Leaf development	Emergence of 1st leaf
2009	1	7/3/2009	184	1180	2.9	E4: Elongation-Stem elongation	4th node palpable/visible
2009	2	8/9/2009	221	1822	4.0	S0: Seed development and ripening	Caryopsis visible
2009	3	9/25/2009	268	2426	5.0	S5: Seed development and ripening	Endosperm dry/seed ripe
2009	4	11/19/2009	323	2627	5.3	-- Post-ripening/senescence	--
2010	5	1/26/2010	391¶	2642	0.9	G5: Germination	Coleoptile emergence from soil
2010	6	3/2/2010	426§	2642	0.9	G5: Germination	Coleoptile emergence from soil
2010	1	7/15/2010	196	1323	3.1	R1: Reproductive-Floral development	Inflorescence emergence/ 1st spikelet visible
2010	2	9/3/2010	246	2240	4.7	S4: Seed development and ripening	Endosperm hard/physiological maturity
2010	3	10/28/2010	301	2803	5.6	-- Post-ripening/senescence	--
2010	4	12/2/2010	336	2878	5.8	-- Post-ripening/senescence	--
2011	5	1/7/2011	372††	2888	0.9	G5: Germination	Coleoptile emergence from soil
2011	6	3/25/2011	449¶¶	3031	1.1	V1: Vegetative-Leaf development	First leaf collared

Julian DOY: Julian Day of the Year starting from January 1.
†: 366 days of 2008 + 59 days of 2009. ¶: 365 days of 2009 + 26 days of 2010. §: 365 days of 2009 + 61 days of 2010.
††: 365 days of 2010 + 7 days of 2011. ¶¶: 365 days of 2010 + 84 days of 2011. §§: Accumulated Growing Degree Days.
MSC: Mean Stage Count (MSC considers AGDD from January 1 of the current year, without taking into account the previous year).

Statistical analysis

Regression analyses between AGDD and AG yield, AG and BG nutrient concentration were conducted. Trend analysis was conducted using best-fit models. Best-fit models were determined from linear and quadratic regression models by the level of significance using $P \leq 0.05$. The higher level of significance for each model (lower *p-value*), higher coefficient of determination (R^2), and the lowest root mean square error (RMSE) were used to determine a best-fit model, using the PROC REG procedure in SAS ver. 9.4 (SAS Institute, 2011). Equation coefficients (parameters) of each model and the best evidence of no lack-of-fit ($P \geq 0.05$) were also tested for significance using PROC REG.

RESULTS

Switchgrass yield

The switchgrass biomass yield from periodic harvests throughout the growing season increased through the summer as the season progressed, and a significant quadratic relationship between biomass yield and AGDD was observed ($P < 0.0001$) in 2008 (Fig. 2.2). However, the yield started to decrease after setting seed with inflorescence and crop senescence (AGDD 2611 in Fig. 2.2, and growth-stage description in Table 2.2). Generally, yield increased up to the December harvest (which was after the first killing frost), and then started to decline for the first year of the study. The best-fit polynomial trendline ($P < 0.0001$) produced a R^2 of 0.71, this trendline estimating a maximum yield of 19.8±1.2 Mg ha^{-1} at AGDD 2672 (DOY 339, December 4).

A significant trend in yield at $P < 0.05$ was seen in 2009, with plots receiving 100.8 kg N ha^{-1} being harvested (Fig. 2.2). For this year, only four sampling dates were recorded because of the physical loss of data and samples. Therefore, AGDD 2627, DOY 323, November 19th, was the last harvest date with yield data recorded. An expected plateau or decrease in harvested yield was not indicated here because of a lack of harvest data for the rest of the growing season. Yield at AGDD 1822, DOY 221, August 9th, was much higher (13.7±2.9 Mg ha^{-1}) than the first harvest date (4.8±3.8 Mg ha^{-1}) and also higher than the other two harvest dates (8.0±2.3 and 9.5±3.1 Mg ha^{-1}, respectively).

In 2010, plots received 168 kg N ha^{-1} were harvested. No significant differences were found ($P > 0.05$) in yield throughout the season and the data were widely scattered (Fig. 2.2). Probably, yields would start to decrease between the October 28th and December 2nd harvest dates. Likely, there was a killing frost during that time, when plants began senescence, produced seed and started to die back. The polynomial trendline ($P > 0.05$) produced a R^2 of 0.19, not a good fit. If the trendline was significant, the predicted maximum yield would be 17.0 Mg ha^{-1} at AGDD 2240 (DOY 246, September 3rd). As illustrated in Fig. 2.2, yields reached a peak between 2000 and 2500 AGDD across years.

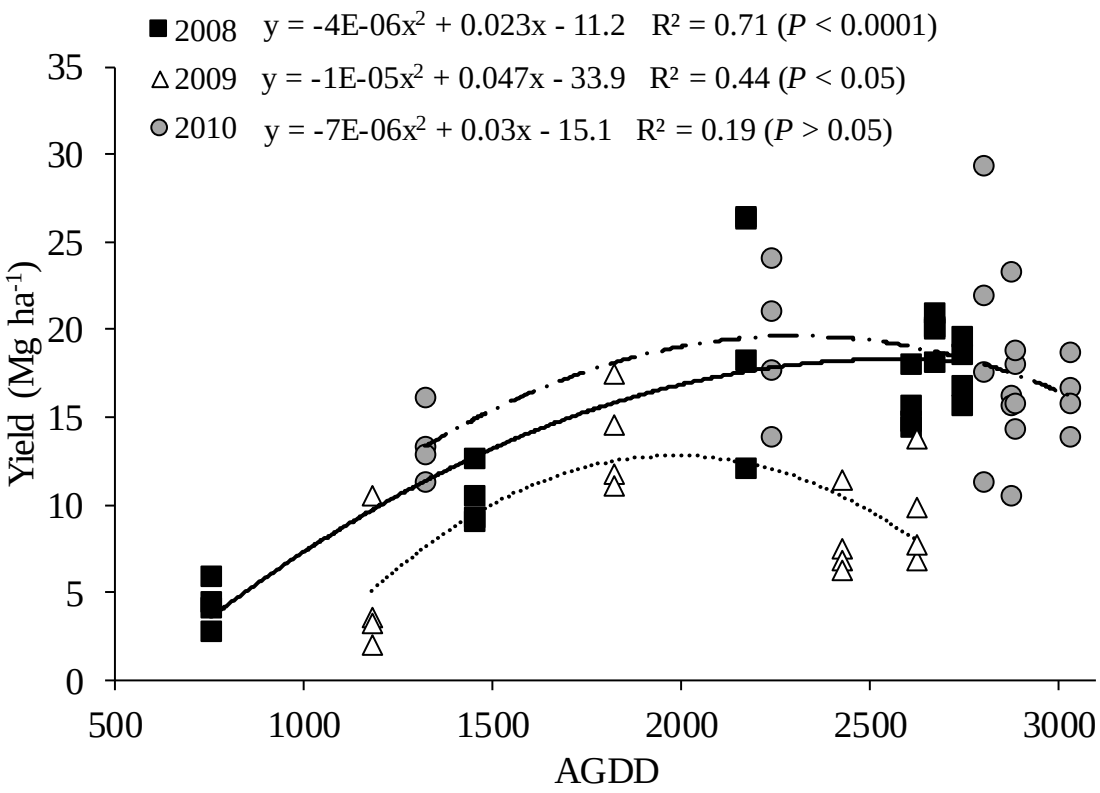

Fig. 2.2. Switchgrass yield as a function of AGDD (accumulated growing degree days) harvests in 2008 (■—), 2009 (Δ····), and 2010 (●—•—).

Nitrogen dynamics

Generally, concentrations of N decreased in AG harvested biomass but increased with time in BG biomass as AGDD increased for all 3 years (Fig. 2.3), suggesting N was moving from the AG to BG starting at ~1500 to 2000 AGDD (Elongation-Stem elongation to Seed development and ripening) (Fig. 2.3 and Table 2.2). Changes in N concentrations ranged from 12.4 to 2.1 g N kg^{-1} in AG biomass over all study years and from 2.9 to 13.9 g kg^{-1} in BG biomass. There seems to be an equal N concentration between the AG and BG portions of the plant at approximately the same time each year, which is between AGDD 1500 and 2000, (late July, early August), ranging from 4.0 to 9.0 g kg^{-1}. Quadratic

models were significant for each year in describing nitrogen dynamics, except for N concentration in AG and BG biomass in 2009 and 2010, respectively (Fig. 2.3).

As the growing season progresses, the plant is maturing and moving towards flowering and producing seed, and then to senescence (Table 2.2). Ashworth et al. (2017b) found a peak of N uptake in AG yields in August 2009 at 80 kg N ha^{-1} and 141 kg N ha^{-1} in 2010. Makaju et al. (2013) studied changes in nutrient concentration monthly and found changes in N concentrations in winter months insignificant. At this time, insignificant changes in AG and BG N concentration would be expected since physiologically the plant has met its reproductive goals in producing seed. Decreasing N in AG portions as increasing N in BG portions are taking place within the plant, which might indicate N translocation from the AG portion to BG parts. Moreover, high N requirements for optimum AG production linked to reports of relatively low fertilizer N use efficiency by switchgrass (Garten Jr. et al., 2010; Staley et al., 1991; Stout and Jung, 1995) could be also explained by N translocation within the plant. Hence, it is reasonable to assume that N increases in the BG would serve the purpose of aiding regrowth the following spring in perennial plants such as switchgrass (Parrish and Fike, 2005; Richner et al., 2014). On the other hand, although most of the root biomass resides in the surface soil, deeper roots could have a slower decomposition rate due to their higher carbon to nitrogen ratio (C/N) (Garten Jr. et al., 2010; Silver and Miya, 2001), and there could be differences in the proportion of fine to coarse root biomass throughout the switchgrass growing season (Garten Jr. et al., 2010; Ma et al., 2000). As harvest frequency also affects N use by switchgrass (Garten Jr. et al., 2010), further research should emphasize N cycle monitoring in switchgrass at different stand ages during a growing season.

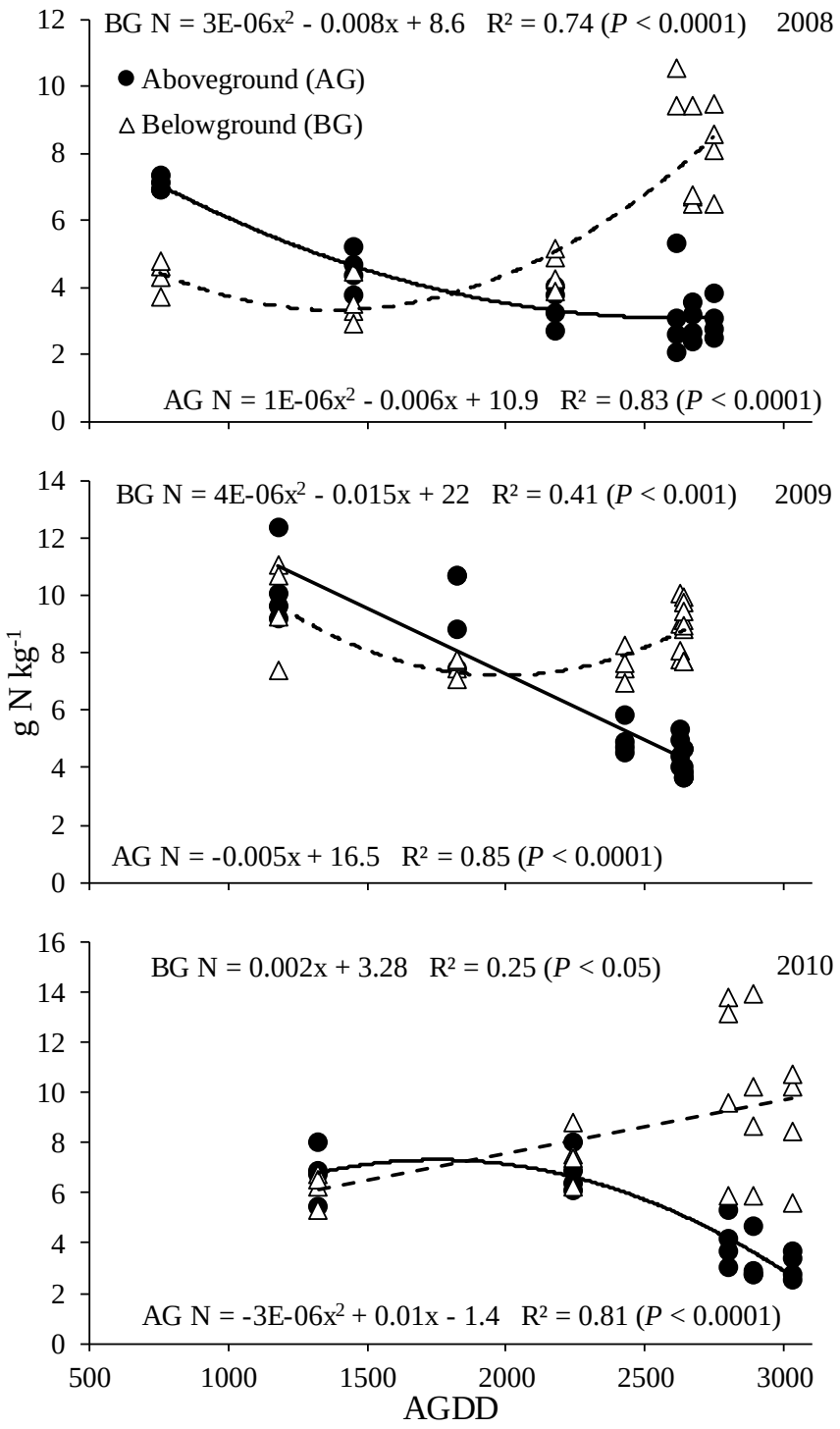

Fig. 2.3. Switchgrass above (●—) and belowground (Δ---) nitrogen concentration (g N kg^{-1}) as a function of AGDD (accumulated growing degree days) harvests in 2008, 2009, and 2010.

Phosphorus and potassium dynamics

Overall, phosphorus (P) concentrations ranged from 1.8 to 0.4 g kg^{-1} in AG biomass and 0.5 to 1.3 g kg^{-1} in BG biomass for 2008 and 2009. Aboveground and BG P concentration for 2008 and 2009 were described with significant linear trends with AGDD (Fig. 2.4), and it sharply decreased in AG P and slightly increased in BG P as AGDD increased. Increases in BG P were more prominent in 2008 than in 2009. The P data for 2010 are not available due to an analytical problem. Potassium concentrations ranged from 15.7 to 1.0 g kg^{-1} in AG biomass and 1.0 to 7.0 g kg^{-1} in BG biomass over all study years (Fig. 2.5). All years AG and BG were described with a significant linear or quadratic model except the 2008 BG K concentration ($P > 0.05$).

As previously mentioned, a general pattern of nutrient concentrations of the AG portions decreasing over time as BG concentrations increase is seen, especially with the primary macronutrients (N, P, and K). Ashworth et al. (2017b) found peaks of P uptake in July and August at 15.7 kg P ha^{-1} (2009) and 16.8 kg P ha^{-1} (2010). Ashworth et al. (2017b) also observed peak K removal of 136 kg K ha^{-1} in early July 2009 (DOY 184, AGDD 1180) and 185 kg K ha^{-1} in June 2010 (DOY 165, AGDD 788). In another study, Ashworth et al. (2017a) found peaks in AG concentrations in mid-September at approximately 1.75 kg Mg^{-1} on a dry matter basis (DM) for both P and K.

Peaks in concentrations of P and K in BG portions were found in late winter or early spring of the following year (for P, 1.3 g kg^{-1} in 2008 and 1.2 g kg^{-1} in 2009; for K, 2.2 g kg^{-1} in 2008, 7.0 g kg^{-1} in 2009, and 6.0 g kg^{-1} in 2010), at the last harvests in February (2010 DOY 449, AGDD 3031) and March (2008 DOY 425, AGDD 2748, and 2009 DOY

426, AGDD 2642). Makaju et al. (2013) found changes in P and K concentrations of AG biomass was significant ($P = 0.001$) even through winter months in monthly harvests.

The timing of nutrient translocation taking place can affect decisions on harvest timing for the plant regrowth of the following year and the sustainability for the longevity of the switchgrass stand. Harvests need to be timed to take place after an adequate amount of nutrient translocation has occurred so that the subsequent regrowth is benefited. Statistical parameters of switchgrass yield, N, P, and K concentrations AG and BG as a function of AGDD can be found in Appendices, Tables A2-1, A2-2, A2-3 and A2-4.

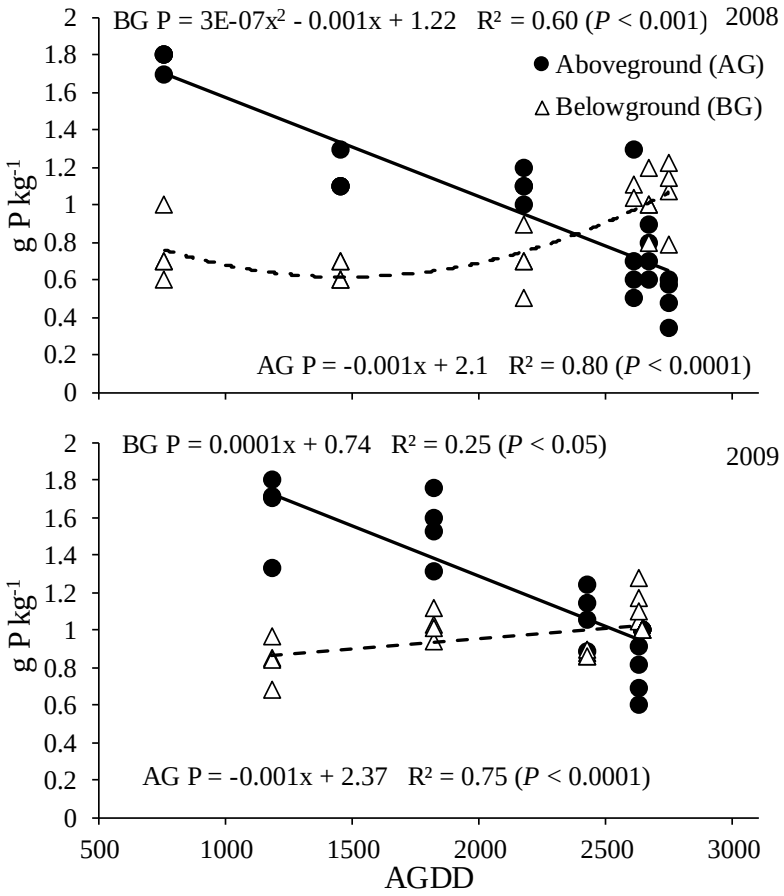

Fig. 2.4. Switchgrass above (●—) and belowground (Δ---) phosphorus concentration (g P kg^{-1}) as a function of AGDD (accumulated growing degree days) harvests in 2008, 2009, and 2010.

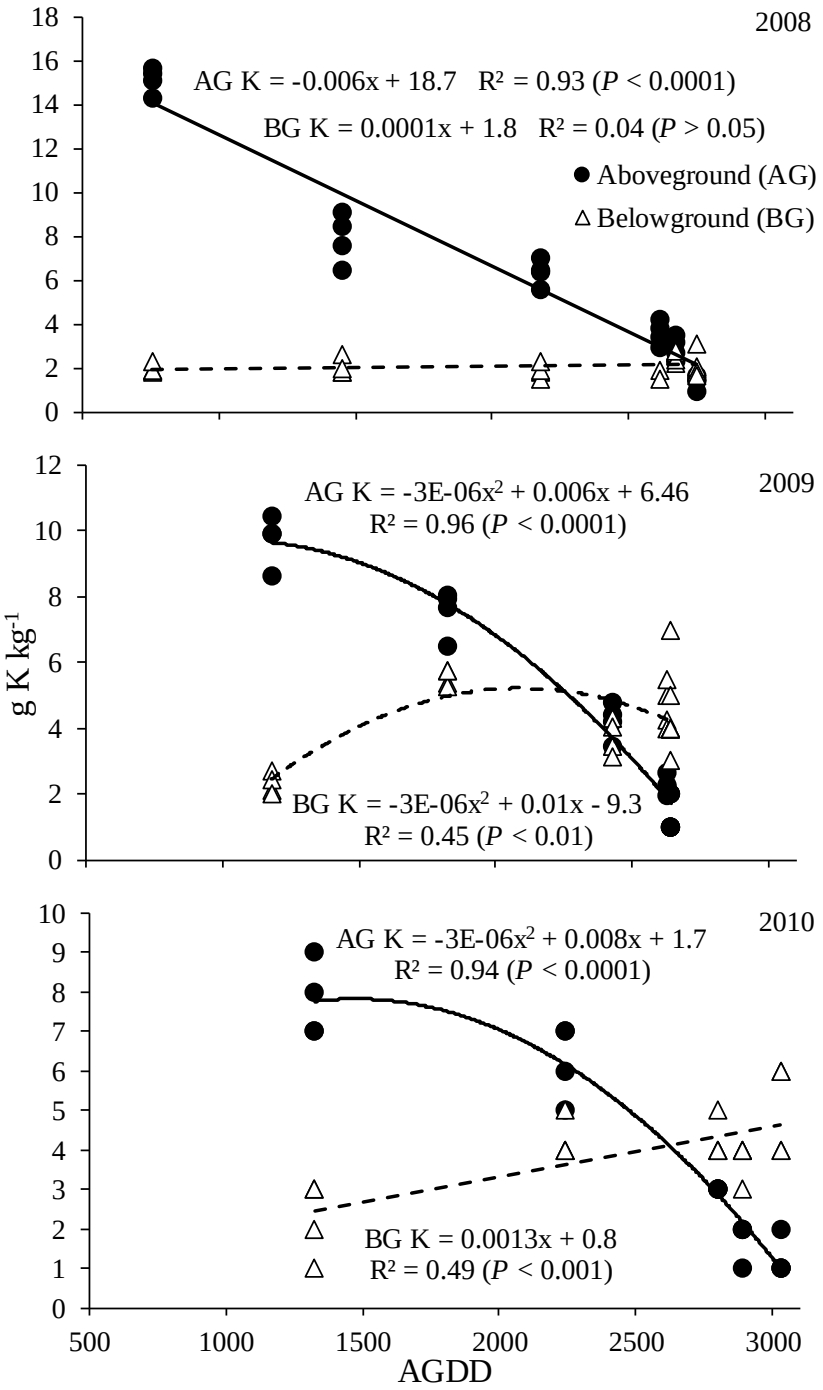

Fig. 2.5. Switchgrass above (●—) and belowground (Δ---) potassium concentration (g K kg^{-1}) as a function of AGDD (accumulated growing degree days) harvests in 2008, 2009, and 2010.

Secondaries and micronutrient dynamics

Secondary and micronutrients did not exhibit the same patterns of increasing concentrations in BG biomass and decrease in AG biomass with time as definitively as macronutrients (Table 2.3). Calcium (Ca), magnesium (Mg), and sulfur (S) displayed decreases in AG and increases in BG in 2008 and 2009. For both years, Ca ranged from 3.2 to 1.0 g kg^{-1} AG and 1.0 to 4.7 g kg^{-1} in BG biomass. Magnesium showed a decrease in both AG and BG ranging from 2.4 to 0.8 g kg^{-1} AG and 0.8 to 1.5 g kg^{-1} BG. Micronutrients were not consistent from year to year in AG and BG concentration changes. Sodium (Na) had slight increases in AG and BG for 2008 but slight decreases in 2009, ranging from 0.1 to 0.5 g kg^{-1} AG, and 0.3 to 1.2 g kg^{-1} BG in 2008, and 0.5 to 0.1 g kg^{-1} AG and 1.4 to 0.6 g kg^{-1} BG in 2009. Copper (Cu) and nickel (Ni) displayed similar trends as Na. Copper ranged from 1.42 to 325 mg kg^{-1} in AG and 13.7 to 2055 mg kg^{-1} in BG biomass in 2008, and 12 to 1.0 mg kg^{-1} AG and 35 to 15 mg kg^{-1} BG in 2009. While Ni ranged from 0.13/4.26 to 83.7/97.6 mg kg^{-1} in AG/BG in 2008, and 75.5/179 to 0.13/2.3 mg kg^{-1} in AG/BG biomass in 2009. Since the soil nutrient availability was similar between 2008 and 2009 (Table 2.1), a reason behind such increase in one year followed by a decrease the next year could be attributed to a dilution effect of increased AG and BG biomasses in 2009 lowering the concentration of micronutrients in plants tissue.

Iron (Fe) displayed no significant trends in AG in any year (ranging from 34.6 to 194 mg kg^{-1}). For BG portions, Fe decreased for both 2008 and 2009 and had no significant increase in 2010 (range from 52.6 to 6456 mg kg^{-1} across all years). Manganese (Mn) decreased in AG and BG for 2008 and 2009 (98.5 to 24.7 mg kg^{-1} AG and 417 to 69.0 mg

kg^{-1} BG). Zinc (Zn) displayed decreases in AG and BG, except for 2010, with an increase in BG, ranging from 6.76 to 34.5 mg kg^{-1} AG and 23.8 to 113.7 mg kg^{-1} BG (Table 2.3).

Secondary and micronutrients did not display a consistent pattern of decreasing AG concentrations while increasing BG concentrations because they varied from year to year depending on the nutrient, except for S and perhaps Ca. More field research is warranted for secondary and micronutrient dynamics with time during the switchgrass growth cycle.

Table 2.3. Significance levels of regression models on nutrient movement through the plant (aboveground (AG) and belowground portions (BG)) as a function of different stand ages (AGDD: accumulated growing degree days). When indicated as significant, AG nutrients are decreasing with increasing AGDD and BG nutrients are increasing with increasing AGDD.

Regression Model	Plant Portion	Ca	Mg	S	Na	Cu	Fe	Zn	Mn	Ni
		……*P*-value……								
2008 Linear	AG	0.001	<0.0001	<0.0001	NS	NS	NS	<0.0001	<0.0001	0.008
	BG	NS	0.0013	<0.001	NS	0.001	<0.001	NS	0.0095	0.0003
2008 Quadratic	AG	0.006	<0.001	<0.0001	NS	NS	NS	<0.0001	<0.0001	0.015
	BG	NS	0.002	<0.0001	NS	NS	<0.0001	NS	<0.0001	<0.0001
2009 Linear	AG	0.032	<0.001	<0.0001	NS	<0.0001	NS	<0.001	0.005	NS
	BG	0.002	0.032	0.012	NS	0.0005	0.0037	NS	0.022	<0.0001
2009 Quadratic	AG	NS	<0.001	<0.0001	NS	<0.0001	NS	<0.0001	0.017	NS
	BG	0.001	NS	0.004	NS	0.0027	0.0049	NS	0.037	<0.001
2010 Linear	AG	0.03	0.0126	---	---	0.001	NS	0.012	---	---
	BG	NS	---	---	NS	NS	NS	NS	---	---
2010 Quadratic	AG	0.044	0.0107	---	---	0.0036	NS	0.032	---	---
	BG	NS	---	---	NS	NS	NS	NS	---	---

NS: Non-significant ($P > 0.05$).

DISCUSSION

Even with some yield responses shown by temporal harvests in some of the studied years, there is no indication of a need for N fertilization. The lack of yield response to N is common in many fertilization studies with switchgrass. However, fertilization will be necessary as more nutrient is removed from the system annually. Additional nitrogen will eventually need to be applied to preserve the longevity of the switchgrass stand and to sustain the yield.

It is evident that nutrient movement likely from AG to BG is occurring as plant senescence since nutrient concentrations are decreasing in AG and increasing in BG biomasses. It was also observed that plants harvested in the early season did not regrow; hence, it was necessary to use plots receiving different fertilizer rates each year. Early harvest may limit the ability of switchgrass to regrow for a second harvest because nutrients are not left in the root system to replenish growth. This study does not address timing for forage hay, June is generally thought of as an ideal grazing time because of forage quality. Earlier grazing or harvest, and overgrazing, could hinder regrowth.

Several studies have supported switchgrass harvesting once a year after frost to allow for maximal translocation of nutrients and building of storage reserves in the roots (Guretzky et al., 2010; Heaton et al., 2009; Mitchell et al., 2008; Muir et al. 2001; Sanderson et al. 1999b; Vogel et al. 2002). Indeed, a seasonal nutrient translocation would seem to contribute to the next season's growth as nutrients increase in the root system allowing the switchgrass to overwinter and is a key component in its perennial plant growth. However, mineral

nutrients may not only be translocated to BG during the fall since they can also be leached out from the leaves during rainfall (Anderson et al., 2013). Nutrients stored in BG parts in the early growing season make the plant less reliant on anthropogenic nutrient sources. This type of translocation and BG storing of certain nutrients have been observed in other native grasses (Anderson et al., 2013; Yang et al., 2016). Native C4 grasses developed these nutrient-cycling characteristics over many years during their evolution. This could partially explain why switchgrass has low response to fertilization.

When evaluating the concentration of nutrients through the plant, it is important to realize what processes are occurring within the plant physiologically. Avila-Ospina et al. (2014) observed changes in source-sink relationships in different plant parts, and that nutrient recycling by translocation was a part of leaf senescence physiological process. Hence, harvesting after plant senescence reduces mineral concentrations in biomass due to changes in source-sink, which is beneficial to direct combustion and thermochemical conversion systems when the biomass is used as the feedstock for biofuel (Adler et al., 2006; Heaton et al., 2009; Kering et al., 2011; Sanderson et al., 2007). These types of source-sink relationships were also observed in some of the data from this study, e.g., N, P, and K changes in AG and BG over time. Perennial grasses, such as switchgrass, remobilize nutrients from AG to BG across the growing season, which affect the levels of N, P, and K in harvested material depending on harvest time (Adler et al., 2006; Heaton et al., 2009; Kering et al., 2011). Thomas (2013) reported the importance of the senescence process in the plants' life cycle and that time is a stress factor

triggering responses leading to senescence, nutrient remobilization, and recycling. Blagosklonny and Hall (2009) suggested senescence should be seen as a nutrient driven process in cells. Particularly, the transition and relocation of nutrients and remobilization are occurring after nutrient uptake and assimilation (Masclaux et al., 2001). Other studies have noticed that translocation occurred as a response to drought or fire or other stresses (Clark, 1977; Heckathorn and Delucia, 1994; Heaton et al., 2009) although it is an integral part of the life cycle of many prairie grasses and other plants. In addition to these naturally occurring environmental stresses, time needs to be included as a stress factor (Thomas, 2013). Gregersen et al. (2013) noted that leaf senescence should be viewed more as recycling for nutrient management. As observed in our study, nutrient concentrations change in AG and BG occurred with the aging of the plant and senescence. However, changes in the nutrients concentration in various plant tissues can occur for many reasons and it should not be assumed that there was a direct transfer of nutrients from one tissue to another simply because the concentrations changed as observed. For example, in the case of nitrogen (N), switchgrass stems have lower N concentration compared to leaves, thus as the plants develop and the ratio of stems to leaves increases, so will the overall concentration of N in total AG biomass (Kering et al., 2011).

Nutrient translocation has an important impact on nutrient management and harvest timing for different purposes. If switchgrass is to be cultivated as a commercially viable crop, it will likely be as a dual-purpose switchgrass, that is either grazed or harvested as hay for livestock and then again as biofuel feedstock

post-senescence. Some switchgrass cultivars appear better suited to biomass production in the upper southeastern US (Fike et al., 2006a) due to their greater productivity and tendency to remain vegetative longer (Casler et al., 2004), while plants of northern origin are less productive than southern cultivars (Sanderson et al., 1999c).

Generally, harvests for hay occurring in the early season, e.g., June, have higher nutrient concentration than later harvests. Therefore, the quality of early harvested forage will be higher than late-harvested forage for hay (Aravindhakshan et al., 2011; Wilson et al., 2013b; Kering et al., 2013). On the other hand, late-season switchgrass harvests will have lower N and mineral concentration than early season harvests, which are more suitable for biofuel conversion. Switchgrass can be used for biofuel feedstock or forage production, but some management decisions can differ. Harvest timing may be the most important management difference between biofuel feedstock and forage production systems (Madakze et al., 1998; Sanderson and Wolf, 1995; Casler and Boe, 2003; Ashworth et al., 2017a; Miesel et al., 2017) because the former requires high nutrients and the latter proffers low nutrients. The late-season harvest of biomass should happen after senescence in order to allow nutrients to be translocated from AG to BG and provide root nutritional reserves for regrowth in the following spring. Generally, lower nutrient concentration has been seen in a single season harvest after switchgrass senescence and is suited to biofuel feedstock, as it allows for greater quality fuel and less fouling of conversion equipment (Guretzky et al., 2010; Kering et al., 2013; Richner et al., 2014). In summary, dual-use purpose can work if allowing for no

more than one harvest after grazing. Multiple harvests may have produced greater yields overall, but the quality of switchgrass for producing biofuels would generally lower because of higher N concentrations.

CONCLUSIONS

Maximum yields of switchgrass were found to be 19.8±1.2, 13.7±2.9, and 16.7±2.1 Mg ha^{-1} in 2008, 2009 and 2010, respectively. Significant decrease of N, P, and K concentrations in AG and an increase in BG plant portions occurred as switchgrass moved to senescence in its life cycle. However, no micronutrients displayed consistent trends in AG and BG concentrations. Understanding the dynamics of nutrient uptake and cycling within the plant is essential to nutrient management, and harvesting time for different purposes. Harvesting for hay should take place in the early growing season for high nutritive values but not too early to hinder regrowth of the stand and harvesting for biofuel feedstock should occur after senescence with low N and minerals.

CHAPTER III

NITROGEN FERTILIZATION AND HARVEST TIMING AFFECT SWITCHGRASS QUALITY

ABSTRACT: Switchgrass (*Panicum virgatum* L.) can be used as animal feed during the early growth season or biofuel feedstock when harvested at maturity. However, the impacts of nitrogen (N) application and harvest timing on switchgrass quality for both end uses need further evaluation. This study evaluated the changes of switchgrass nutritive quality for animal feed and nutrient quality as biofuel feedstock under different N application rates (0 to 235 kg N ha^{-1} yr^{-1}) and when harvested at different times at a fixed N rate throughout the growing seasons in 2008, 2009 and 2010. Tissue-N removal gradually increased from the 0 to the 235 kg N ha^{-1} application rate ($P < 0.05$). However, the largest single difference (27 %) was found between the non-treated control (0 kg ha^{-1}) and the lowest N rate applied (33.6 kg ha^{-1}). Conversely, N removal decreased during subsequent harvests at a fixed N rate ($P \leq 0.0001$). Forage quality varied, and was in general affected by N rates, but quality parameters were especially impacted by harvesting time. In general, fibers and most minerals in the biomass increased as accumulated growing degree days (AGDD) increased ($P \leq 0.0001$), but N and total

digestible nutrients (TDN) decreased as AGDD increased ($P \leq 0.0001$). Since high crude protein and minerals with low fiber are desired forage qualities and the opposite is true for biofuel feedstock, earlier harvests are beneficial for hay production or livestock forage grazing, and late-season harvests are better for biofuel production.

INTRODUCTION

Public interests in plant-based renewable fuels within the United States have been varied over the last decade or so, gaining or declining often based on oil production, fuel costs, and economic and political changes. Switchgrass *(Panicum virgatum*) has been identified as a viable source of sustainable biomass for fuel conversion (McLaughlin and Kszos, 2004, 2005), given an alternative fuel infrastructure and market. Development of practices for dual-use switchgrass management, in which biomass is harvested as forage in the early growing season, and harvested for biofuel feedstock later in the season, have been researched by Richner et al. (2014) to develop production options for producers.

Managing switchgrass as a dual-use crop could be ideal for many areas of North America (Mosali et al., 2013). Switchgrass as biofuel biomass and forage hay would be attractive to growers desiring to use land unsuitable for other crops. Considering its wide adaptability across North America, Casler et al. (2004) demonstrated that the latitude of origin of a switchgrass cultivar affects yield potential and nutrient content of harvested biomass. Desirable quality required is dependent upon its intended end use (Sena et al., 2018; Ashworth et al., 2020). Management decisions, such as fertilization and harvest timing, can affect biomass quality. High concentrations of nutrients in harvested biomass can cause fouling of processing equipment and produce a lower-quality fuel. However,

more nutrients, such as protein and minerals, and less fiber are preferred as feed for livestock consumption. Many studies have shown harvest timing had a greater effect on biomass quality and mineral content than nitrogen (N) fertilization (Madakadze et al., 1998; Sanderson and Moore, 1999; Vogel et al., 2002; Casler and Boe, 2003). Harvest timing and number of harvests per year can affect stand longevity and total yield of the stand (Parrish and Fike, 2005; Aravindhakshan et al., 2011; Guretzky et al., 2011; Kering et al., 2013; Richner et al., 2014).

Several parameters of plant biomass are often used to determine the forage quality for livestock uses. They can also be used in accessing quality for biofuel production. Common parameters used are crude protein (calculated from N content), mineral contents, acid detergent fiber (ADF), neutral detergent fiber (NDF), and total digestible nutrients (TDN) calculated from ADF. Neutral detergent fiber measures the fibrous fraction of the forage, comprised of cellulose, hemicellulose, and lignin. These components are the slowly digestible and indigestible parts of the plant so it is directly linked to animal intake of the forage. Acid detergent fiber measures the cellulose and lignin component of the plant, and determines forage digestibility for livestock. As ADF increases, total digestibility will decrease. Total digestible nutrient is a measure of energy that can be derived from the forage and is calculated using ADF values (Richner et al., 2014; Moore, 2015). Nutrient contents of harvested biomass also affect the quality and its use. Nutrient concentration within the plant can greatly affect the quality of biofuel produced and the conversion process. A large amount of nutrients in the biomass, especially N, is known to cause fouling of equipment (McKendry, 2002; Ibrahim et al., 2017). On the other hand, higher nutrient concentrations in the forage would be desirable

for livestock consumption. The desired quality for the two different uses are in opposition to one another. This makes harvest timing is critical, as quality parameters change during the growing season (more details in Chapter 2).

Fertilization and harvest timing can affect switchgrass biomass quality. These two management practices can be used in producing quality switchgrass for biofuel production or as forage. The objectives of this study were to evaluate how N rates affect N, ADF and NDF contents, and to monitor selected quality parameters during the growing season by harvesting switchgrass at different growing degree days (GDD) to aid the decision on final use.

MATERIALS AND METHODS

Experiment design and treatment description

A nitrogen fertilization study was initiated in 2008 in Stillwater, OK in a previously established Kanlow switchgrass stand (36°08'01.54" N: 97°06'17.16" W) using eight rates (0, 33.6, 67.2, 100.8, 134.4, 168.0, 201.6, and 235 kg N ha^{-1}) in a randomized complete block design (RCBD). Each plot measured 6.1 m x 6.1 m and was fertilized each year after soil sampling in the spring. Nitrogen was applied as urea or as a combination of urea and Diammonium phosphate (DAP; 18-46-0) to supply P when necessary. The N input was a single application when rates were ≤ 67.2 kg N ha^{-1}, and a split-application when rates were ≥ 100.8 kg N ha^{-1}. The 67.2 kg N ha^{-1} or less rate was applied in mid-March and remaining N of other treatments in early May to reach total N needed. The soil at the site is a Norge loam (fine silty, mixed, active, and thermic Udic Paleustoll) (Soil survey staff, NRCS-USDA).

Soil analysis

Soil samples were collected from a 0 to 15 cm soil layer from each plot before fertilization each year (Table 3.1). Samples were oven-dried at 65 °C for 24 hours and ground to pass through a 2-mm sieve. Soil pH was measured with an electrode in a 1:1 soil:water suspension. Plant available N was extracted by 1M KCl and analyzed by a flow-injection analyzer (LACHAT QuickChem 8000, Milwaukee, WI) (Kachurina et al., 2000). Plant available phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) were extracted using a Mehlich-3 solution (Mehlich, 1984). Sulfate-S was extracted by 0.008M calcium phosphate. Micronutrients iron (Fe), zinc (Zn), boron (B), and copper (Cu) were extracted by DPTA-Sorbitol (Hanson et al., 1998) and quantified by an inductively coupled plasma (ICP) spectrometer (Soltanpour, 1996).

Table 3.1. Soil pH and plant available nutrients tested by year (2008, 2009, and 2010). Samples taken before fertilization.

Year	pH	NO_3-N	P	K	SO_4-S	Ca	Mg	Fe	Zn	B	Cu
			----------mg kg^{-1}----------								
2008	6.3	1.5	8.0	64	4.2	782	160	48.5	0.7	0.34	1.38
Std. dev.	0.19	0.79	0.65	16	0.22	125	32.4	10.7	0.07	0.01	0.11
2009	6.2	3.2	9.0	55	3.0	769	155	60.3	0.82	0.36	1.5
Std. dev.	0.19	2.7	2.3	14.2	0.34	172	31.4	9.3	0.09	0.03	0.04
2010	6.4	1.3	7.5	55	3.0	780	156	58.4	0.72	0.27	1.5
Std. dev.	0.11	1.5	2.7	8.4	0.53	107	16	8.0	0.18	0.01	0.28

Std. dev. = Standard deviation of the mean (n = 4).

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Switchgrass biomass harvest sampling

Research plots (6.1 x 6.1 m) were divided into two subplots, each 3.0 m x 6.1 m for separate sampling (harvest) regimes. One subplot was harvested using a flail harvester (Carter Manufacturing Co., Inc., Brookston, IN) as a single harvest after senescence and killing frost in November of each year and analyzed for biomass nutrient composition. The other subplot was further divided into 0.9 m x 3.0 m sections to harvest at pre-determined intervals during the year. These temporal harvests were hand-harvested with garden shears and prepared for nutrient analysis.

In four replications of one N rate each year, harvest date was used as a treatment to evaluate biomass quality throughout the growing season. Lack of regrowth in early season harvests made using a different N rate each year necessary. Therefore, 134.4 kg N ha^{-1} for 2008, 100.8 kg N ha^{-1} for 2009, and 168 kg N ha^{-1} for 2010 were chosen for the temporal sampling from June to February or March of the following year. The harvest dates, accumulated growing degree days (AGDD) since Jan. 1 of each year (more discussion on AGDD can be found in Chapter 2) and growth stages (Mitchell et al., 1997; Moore et al., 1991) are listed in Table 3.2.

Table 3.2. Dates of periodic whole plant biomass harvests from one of the nitrogen treatments (134.4 kg N ha^{-1} for 2008, 100.8 kg N ha^{-1} for 2009, and 168 kg N ha^{-1} for 2010) and associated accumulated growing degree days.

Year	Harvest	Date	Julian DOY	AGDD	Growth Stage
2008	1	6/12/2008	164	755	E1
2008	2	7/24/2008	206	1452	R2
2008	3	9/5/2008	249	2175	S3
2008	4	10/30/2008	304	2611	Senescence
2008	5	12/4/2008	339	2672	Senescence
2009	6	2/28/2009	425	2748	V0
2009	1	7/3/2009	184	1180	E4
2009	2	8/9/2009	221	1822	S0
2009	3	9/25/2009	268	2426	S5
2009	4	11/19/2009	323	2627	Senescence
2010	5	1/26/2010	391	2642	G5
2010	6	3/2/2010	426	2642	G5
2010	1	7/15/2010	196	1323	R1
2010	2	9/3/2010	246	2240	S4
2010	3	10/28/2010	301	2803	Senescence
2010	4	12/2/2010	336	2878	Senescence
2011	5	1/7/2011	372	2888	G5
2011	6	3/25/2011	449	3031	V1

Growth stages were based upon Moore et al., 1991, using estimated mean stand count (MSC) calculated from AGDD by calendar year as MSC = 0.875 + (0.0017 * AGDD) (Mitchell et al., 1997).

Sample preparation for nutrient analysis and biomass quality

Switchgrass plant samples were chopped and ground to pass through a 1.0-mm sieve. Acid detergent fiber (ADF), neutral detergent fiber (NDF) were determined by the filter bag technique (Sena et al., 2018). Total digestible nutrient (TDN) is a measure of forage energy and was calculated from ADF values: TDN = [98.625 - (1.048 × ADF)], as per SGS (Société Générale de Surveillance) Agrifood Laboratories and Ashworth et al.

(2020). Plant samples were digested with nitric acid (HNO_3) for mineral nutrients, in which 0.5 g of ground plant materials were predigested for 1 h with 10 ml of trace metal grade HNO_3 in the HotBlock^{TM} Environmental Express block digester. The digestion products were then heated to 115 °C for 2 h and diluted with deionized water to 50 mL (Jones and Case, 2018). Digested samples were analyzed by inductively coupled plasma (ICP) for P, K, Ca, Mg, S, Cu, Fe, Zn, and Mn. Total N was determined with a carbon/nitrogen (C/N) dry combustion analyzer.

Statistical analysis

To evaluate biomass quality as affected by N rates and harvest date, regression analyses were performed for all replicated data by fertilizer rates, and AGDD by harvest date. Nitrogen and mineral concentrations were evaluated for all study years; ADF, NDF, and TDN were evaluated for 2009 and 2010. Accumulated GDD was calculated from January 1st of each study year, using the equation [(maximum daily temperature $^{\circ}$C + minimum daily temperature $^{\circ}$C) / 2] – 10 $^{\circ}$C (Mitchell et al., 1997; Sanderson and Moore, 1999). Forage analysis parameters were the dependent variables (y-axis). Nitrogen fertilization rate (kg N ha^{-1}) and AGDD were independent variables (x-axis). Trend analysis was conducted using best-fit models determined from linear and quadratic regression by level of significance using $P \leq 0.05$. The higher level of significance for each model (lower *p-value*) was used to determine a best-fit model, using the PROC REG procedure in SAS ver. 9.4 (SAS Institute). Equation coefficients (parameters) of each model were also tested for significance using PROC REG. Differences in biomass quality indices and nutrient content were determined using analysis of variance (ANOVA) and

Duncan's Multiple Range test. Only a few outliers were removed from the replication dataset of treatments by using IML and UNIVARIATE (ROBUSTSCALE) procedures of SAS program, and the statistical analyses were performed with n = 3 for those specific treatments (Antonangelo et al., 2019).

RESULTS AND DISCUSSION

Biomass quality parameters as affected by nitrogen fertilization rates

Regression analysis indicated increases in N (P = 0.002 in 2008 and 2009, and P = 0.03 in 2010) (Fig. 3.1), ADF (P < 0.0001 in 2009, and P < 0.0001 in 2010), and an increase or no change in NDF (P = 0.02 in 2009, and P = 0.05 in 2010) (Fig. 3.2) as N application rates increased. Trend analysis showed decreases or no change in TDN (P < 0.0001 in 2009, and P = 0.01 in 2010) by N application rate (Fig. 2). In the 2009 study year, ADF tended to increase with increasing N fertilization, from a low value of 536 g kg^{-1} in the control to a high value of 636 g kg^{-1} with 235.2 kg N ha^{-1} of input. A numerical increase in NDF was seen with increased N rates, ranging from 829 g kg^{-1} for 33.6 kg N ha^{-1} to 890 g kg^{-1} for 168.0 kg N ha^{-1} applied. Total digestible nutrients tended to decrease as N rate increased from 394 g kg^{-1} at 235.2 kg N ha^{-1} to 472 g kg^{-1} at 0 kg N ha^{-1} (Fig. 3.2).

In the 2010 study year, no forage analysis was significantly affected by N application rates using ANOVA and PROC GLM. Acid detergent fiber tended to increase to a high of 564 g kg^{-1} at 168 kg N ha^{-1} applied, then decreased. The data appeared to be widely scattered. Neutral detergent fiber followed a similar pattern to ADF, reaching a maximum average of 796 g kg^{-1} at 134.4 kg N ha^{-1}. The highest individual value for NDF

was 824 g kg^{-1} at 168.0 kg N ha^{-1}. Total digestible nutrients decreased slowly with an increased N rate to an average of 435 g kg^{-1} at 100.8 kg N ha^{-1}. Total digestible nutrients reached a high of 509 g kg^{-1} at 0 kg N ha^{-1}, and an individual low value of 403 g kg^{-1} at 134.4 kg N ha^{-1}. Significant differences were not identified in N concentration and other forage quality parameters by nitrogen rate, although significant linear trends were found in all regression analyses, except for NDF in 2009 (P = 0.076).

Acid detergent fiber and NDF increased with increasing N rates. Total digestible nutrients generally decreased with increasing fertilizer N rates. These trends of decreases in TDN and increases in ADF and NDF with increased N fertilization may be due more to the late single harvest rather than N fertilization rates. It would be expected that N applications would encourage a delayed growth response because of adequate N provided. With lower stress to the plant due to fertilization maturity and seed production was delayed. More vegetative growth with N fertilization would be expected.

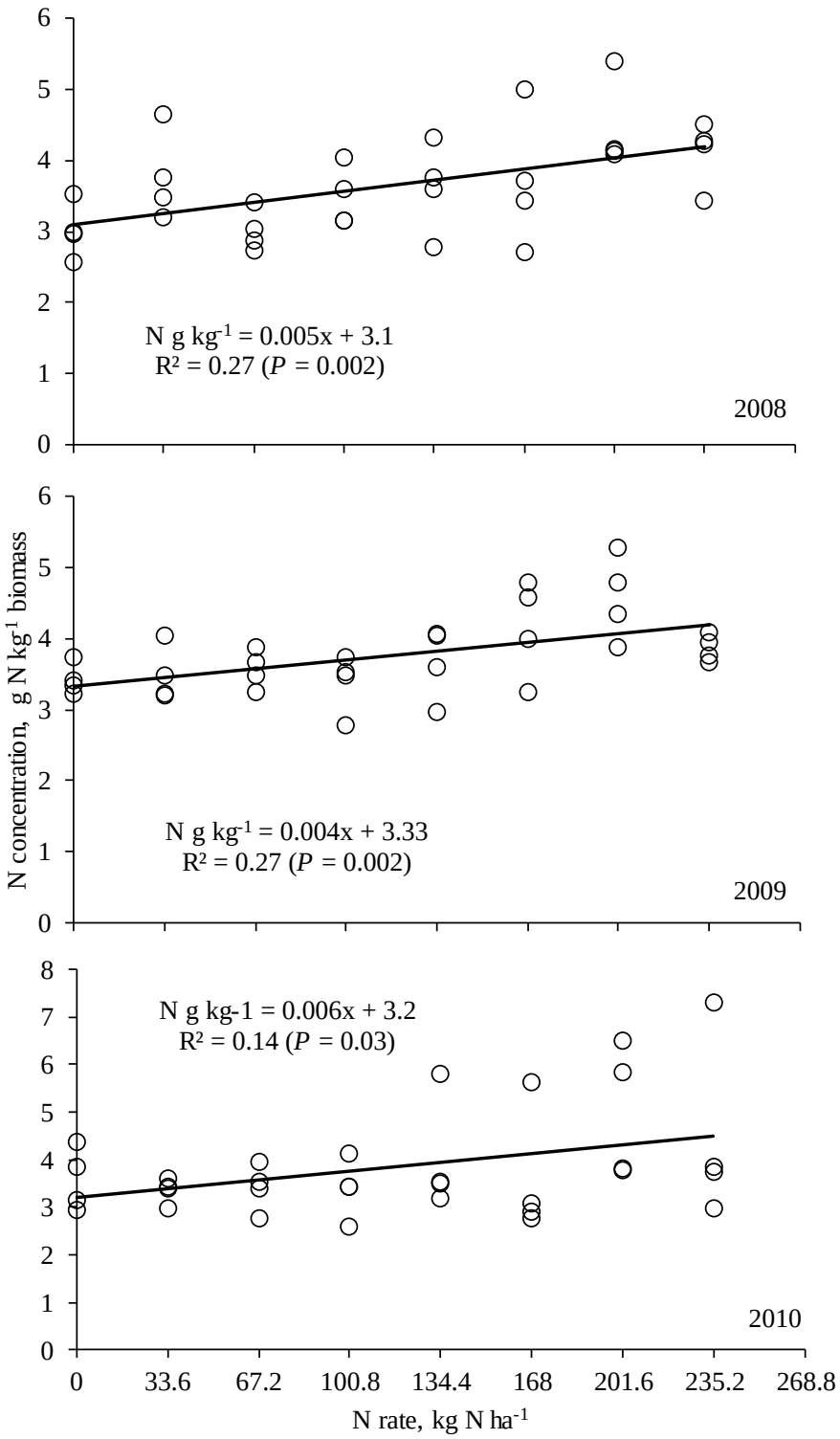

Fig. 3.1. Changes in nitrogen (N) concentrations in switchgrass biomass as a function of the amount of N applied.

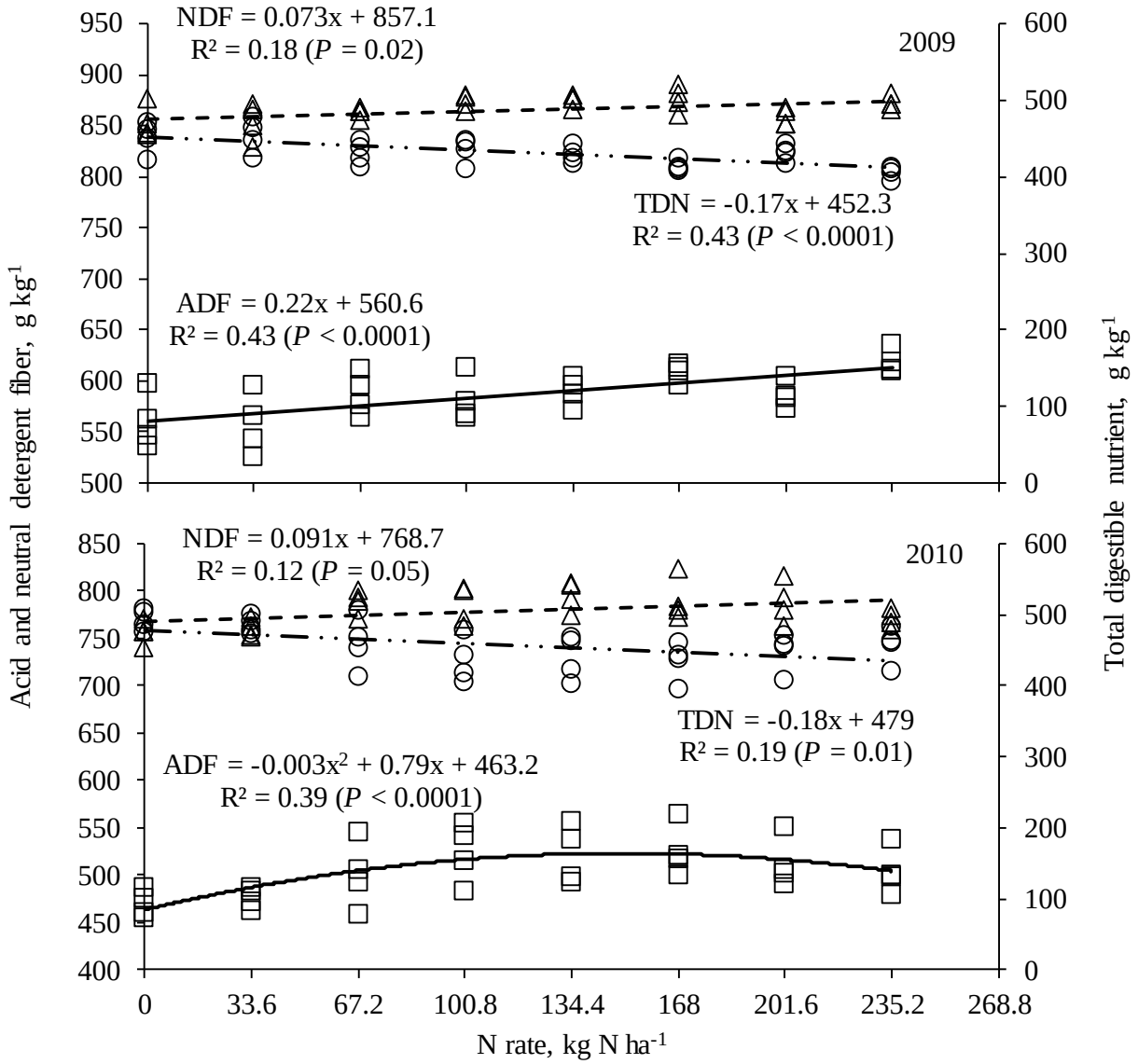

Fig. 3.2. Relationship of acid detergent fiber (ADF), neutral detergent fiber (NDF), and total digestible nutrients (TDN) and nitrogen application rates.

Mineral concentration as affected by nitrogen fertilization rates

Mineral concentrations of harvested biomass had no significant differences with respect to N rates, with a few exceptions (Table 3.3). In 2008, nitrogen concentration

within harvested biomass increased significantly ($P = 0.013$) with increasing N rates, from an average of 3.02 g kg^{-1} in the control to 4.44 g kg^{-1} with the application of 201.6 kg N ha^{-1}. The largest single increase in N occurred between the control plot and the lowest N rate, 33.6 kg N ha^{-1}, from 3.02 g kg^{-1} to 3.78 g kg^{-1} (Table 3.3). Other differences in nutrient concentrations were insignificant ($P > 0.05$). Phosphorus and K concentrations did not change significantly by N rates ($P > 0.05$).

In 2009, significant differences were shown in N and P ($P = 0.012$ and 0.0003, respectively). Nitrogen concentration (g kg^{-1}) tended to increase with increasing N rate, from an average of 3.43 g kg^{-1} for the control to 4.57 g kg^{-1} for the 201.6 kg N ha^{-1} rate, then decreasing at the 235.2 kg N ha^{-1} rate to 3.86 g kg^{-1}. Phosphorus concentration decreased with increasing N rate ranging from 0.41 to 0.80 g kg^{-1}. In 2010, no significant differences in macronutrient concentration between N rates were found.

In analysis of micronutrients, there were significant differences according to Duncan's Multiple Range Test in Mg between the 67.2 and 134.4 kg N ha^{-1} rates, and in Cu between the 33.6 and 67.2 kg N ha^{-1} rates in 2008. In 2009, more significant differences were seen in Ca, K, Mg, S, Cu, Fe, Zn and Mn; often with one rate being significantly different from others according to Duncan's Multiple Range Test. Type 1 *P*-values of all micronutrients except Cu were insignificant (Table 3.3). Significant differences from Type 1 tests ($P < 0.05$) in 2010 were only shown in Zn. Differences in concentration by Duncan's Multiple Range Tests in secondary and micronutrient concentrations were shown in P, S, and Zn among N treatments.

The concentration of N in the biomass generally increased with increasing fertilizer N rates, regardless of the study year. This could indicate some luxury

consumption/uptake of N, the plant assimilating more N as more N is available. In each year, the highest mean N concentrations were found in plots receiving the 201.6 kg N ha^{-1}. Nitrogen concentration in harvested biomass can significantly affect the quality to meet the final use of the crop. High concentrations of nutrients, especially N, can cause fouling of equipment in the biofuel conversion process, thus lignin and cellulose with low nutrient content is needed for biofuel conversion. Nutrients and protein are desirable for livestock consumption. Therefore, the N application rate should be minimized if the biomass is intended for biofuel conversion since excess N resulted in high N in the biomass.

Table 3.3. Mineral concentrations of harvested biomass as affected by nitrogen (N) fertilization rate.

Year	N rate	N	P	Ca	K	Mg	S	Cu	Fe	Zn	Mn
	kg ha^{-1}	g kg^{-1}						mg kg^{-1}			
2008	0	3.02c	0.90a	1.83a	3.95a	1.35ab	0.50a	7.7ab	58.0a	19.0a	51.8a
	33.6	3.78abc	0.88a	2.18a	3.35a	1.50ab	0.48a	8.1a	57.0a	15.6a	59.1a
	67.2	3.01c	0.83a	1.65a	3.28a	1.30b	0.43a	6.4b	63.9a	13.0a	44.7a
	100.8	3.48bc	0.85a	1.95a	3.25a	1.48ab	0.45a	7.2ab	61.8a	14.2a	47.9a
	134.4	3.61abc	1.00a	2.00a	4.25a	1.78a	0.53a	8.0ab	62.4a	17.9a	48.4a
	168.0	3.71abc	0.78a	1.93a	3.63a	1.58ab	0.45a	7.0ab	57.0a	15.3a	57.2a
	201.6	4.44a	0.80a	1.90a	3.80a	1.58ab	0.48a	7.1ab	55.7a	15.5a	46.2a
	235.2	4.12ab	0.75a	1.93a	3.23a	1.60ab	0.48a	7.4ab	56.9a	15.6a	44.4a
	Avg	3.64	0.85	1.92	3.59	1.52	0.47	7.4	59.1	15.7	49.9
	Std dev	0.70	0.16	0.34	0.83	0.29	0.08	1.1	11.7	3.8	13.5
	P	0.013	0.479	0.633	0.441	0.351	0.802	0.315	0.977	0.486	0.515
	F test	3.43	0.98	0.72	0.81	1.10	0.53	1.1	0.2	1.0	0.6
	CV%	19.13	18.97	17.63	23.07	19.03	17.22	14.7	19.7	24.2	26.9
2009	0	3.43bc	0.80a	1.62ab	2.32a	1.04ab	0.36bc	2.0c	32.5ab	15.4ab	70.7a
	33.6	3.48bc	0.77a	1.92a	2.34a	1.19a	0.41ab	2.4bc	32.0ab	15.3ab	66.5ab
	67.2	3.57bc	0.64ab	1.60ab	2.37a	1.05ab	0.37abc	2.5b	45.6a	12.9ab	64.5ab
	100.8	3.38c	0.76a	1.67ab	2.42a	1.09ab	0.39abc	2.8b	34.0ab	15.9ab	52.7ab
	134.4	3.66bc	0.66ab	1.57ab	2.41a	1.13a	0.38abc	2.7b	33.4ab	14.4ab	58.2ab
	168.0	4.15ab	0.53bc	1.48ab	2.01ab	1.08ab	0.39abc	2.9b	33.4ab	12.0ab	53.6ab
	201.6	4.57a	0.68ab	1.65ab	2.14ab	1.16a	0.44a	3.6a	37.5ab	16.4a	46.3ab
	235.2	3.86bc	0.41c	1.24b	1.70b	0.93b	0.33c	2.5b	20.6b	11.3b	39.7b
	Avg	3.76	0.66	1.59	2.21	1.08	0.38	2.7	33.6	14.2	56.5
	Std dev	0.55	0.16	0.32	0.39	0.13	0.05	0.5	15.6	3.2	19.5
	P	0.012	0.0003	0.216	0.102	0.126	0.091	<0.0001	0.527	0.145	0.292
	F test	3.49	6.83	1.52	2.01	1.87	2.09	8.9	0.9	1.8	1.3
	CV%	14.74	23.99	20.01	17.51	12.39	13.23	19.0	46.3	22.6	34.6
2010	0	3.59a	0.73a	2.80a	1.84a	1.46a	0.50a	2.4a	44.5a	25.2a	85.1a
	33.6	3.34a	0.56b	2.41a	2.06a	1.23a	0.41ab	1.9a	43.8a	17.8b	77.6a
	67.2	3.41a	0.56b	2.19a	2.05a	1.34a	0.38ab	2.4a	43.7a	16.0b	78.0a

100.8	3.40a	0.58b	1.99a	2.22a	1.24a	0.35b	2.7a	39..4a	15.1b	67.8a
134.4	4.00a	0.57b	2.09a	1.68a	1.43a	0.37b	1.8a	40.0a	15.0b	71.3a
168.0	3.60[a]	0.55b	2.10a	1.82a	1.41a	0.38ab	2.2a	40.5a	14.4b	64.6a
201.6	4.98a	0.62ab	2.36a	1.66a	1.62a	0.46ab	3.0a	41.3a	17.4b	62.3a
235.2	4.46a	0.54b	2.56a	1.52a	1.54a	0.42ab	2.3a	41.3a	15.0b	70.2a
Avg	3.85	0.59	2.31	1.86	1.41	0.41	2.3	41.9	17.0	72.1
Std dev	1.14	0.11	0.50	0.45	0.24	0.08	0.8	7.4	4.5	18.1
P	0.450	0.205	0.323	0.368	0.305	0.136	0.416	0.985	0.005	0.678
F test	1.01	1.55	1.25	1.16	1.29	1.82	1.1	0.2	4.3	0.7
CV%	29.58	18.60	21.77	24.46	17.25	19.33	32.2	20.0	26.4	25.1

Avg: Average. Std. dev.: Standard deviation of the mean (n=4). CV%: Coefficient of variation as a percentage. Numbers in a column followed by the same letter exhibited no significant differences ($P < 0.05$) based on Duncan's Multiple Range Test.

Biomass quality parameters as affected by AGDD

The relationship between tested quality parameters (N, ADF, NDF, and TDN) and AGDD are shown in Figures 3.3 and 3.4. Parameters ADF and NDF tended to increase with maturity as the harvesting date got later and AGDD increased ($P < 0.0001$). Those results were similar to Sena et al. (2018), who found that the NDF exhibited a consistent positive trend through the end of the growing season, and that the ADF increased consistently through the season in two switchgrass cultivars. Those trends in NDF and ADF in switchgrass in our study were also similar to NDF and ADF trends reported by Aurangzaib et al. (2016), and Kering et al. (2013). Nitrogen and TDN, on the other hand, decreased with increasing AGDD and later harvest date ($P < 0.001$) (Figs 3.3 and 3.4). Commonly, nitrogen and nonstructural carbohydrates decline and lignin and cellulose increase through the growing season of switchgrass (Aurangzaib et al., 2016). These changes are gradually taking place with plant growth as it moves to maturity and completes its cycle of producing seed.

In later harvests, after flowering and senescence, ADF and NDF were high, whereas N and TDN have all decreased to low levels. These high ADF and NDF provide lignocellulosic material needed for biofuel conversion. The low N and other nutrients are considered favorable because large concentrations of nutrients can foul conversion equipment used in biofuel production. Therefore, monitoring the quality before harvest may be beneficial to determine the appropriate use, grazing and hay for livestock use or biomass feedstock for biofuel production.

In late-season harvests, after a killing frost, biomass would be mostly fibrous, low nutrient material. Switchgrass harvested at this late time of maturity makes a very low

quality hay with low crude protein and high fiber contents and is likely unpalatable to livestock. The high ADF and NDF of late-harvested biomass, however, is more desirable in lignocellulosic biofuel conversion. If the end-use is for biofuel feedstock, this would suggest higher fertilization rates can increase biomass yields with high lignin and cellulose content if harvested late. Low ADF and NDF and high N (crude protein) and TDN would be better for livestock feeding. Total digestible nutrient is a measure of the energy value of the hay and is inversely related to ADF. Lower ADF and NDF would indicate more digestibility and forage intake by animals, with less plant cell wall fibrous materials, making forage more palatable and greater amount consumed and digested. A high-quality livestock forages should have ADF < 300 g kg^{-1}, NDF < 400 g kg^{-1}, and TDN > 600 g kg^{-1} and N > 30 g kg^{-1} (Moore, 2015), but the late-harvested switchgrass is far from considered a quality forage. Earlier harvest should be considered if feeding switchgrass to livestock is desired.

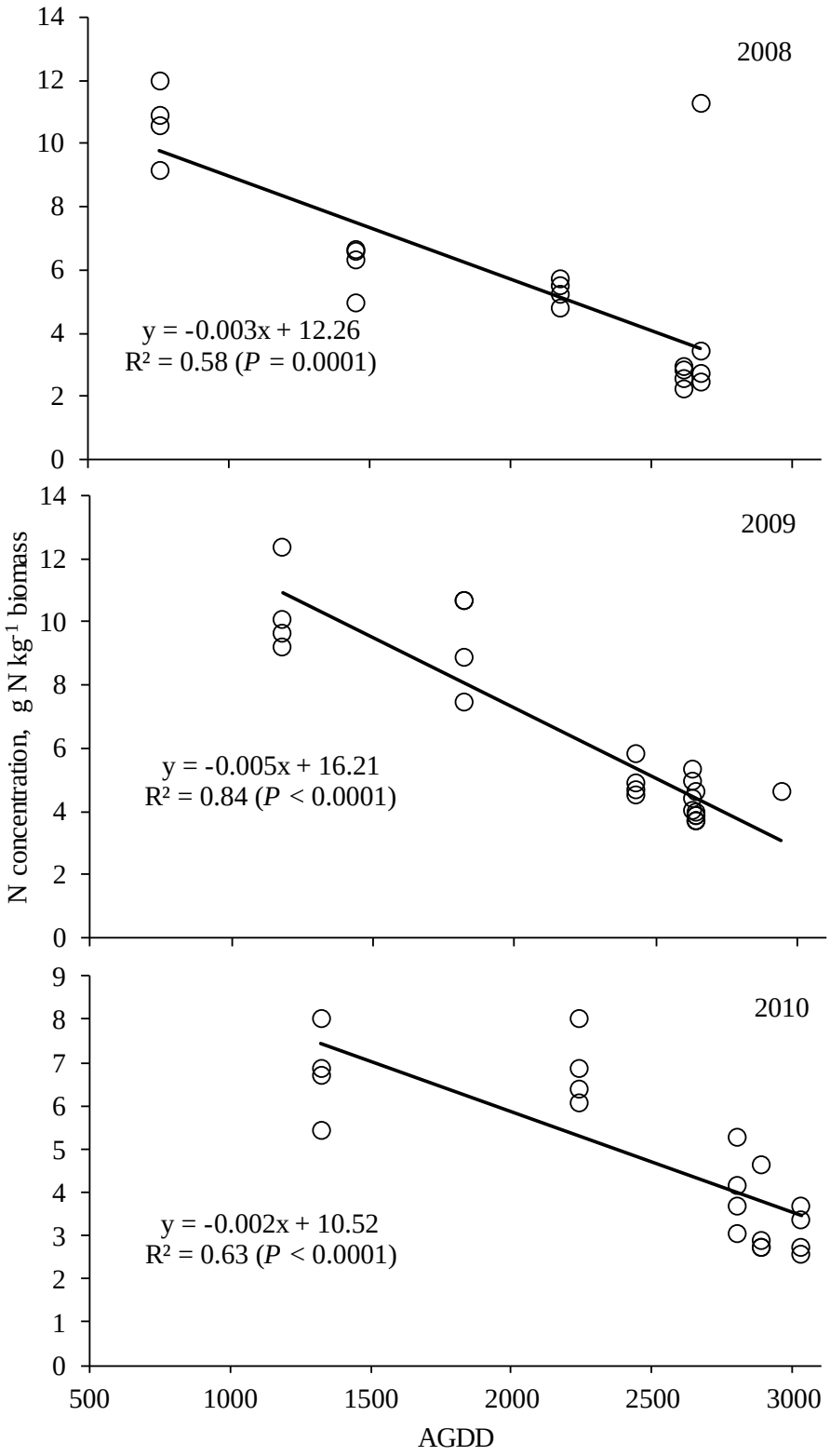

Fig. 3.3. Nitrogen concentration as affected by accumulated growing degree days.

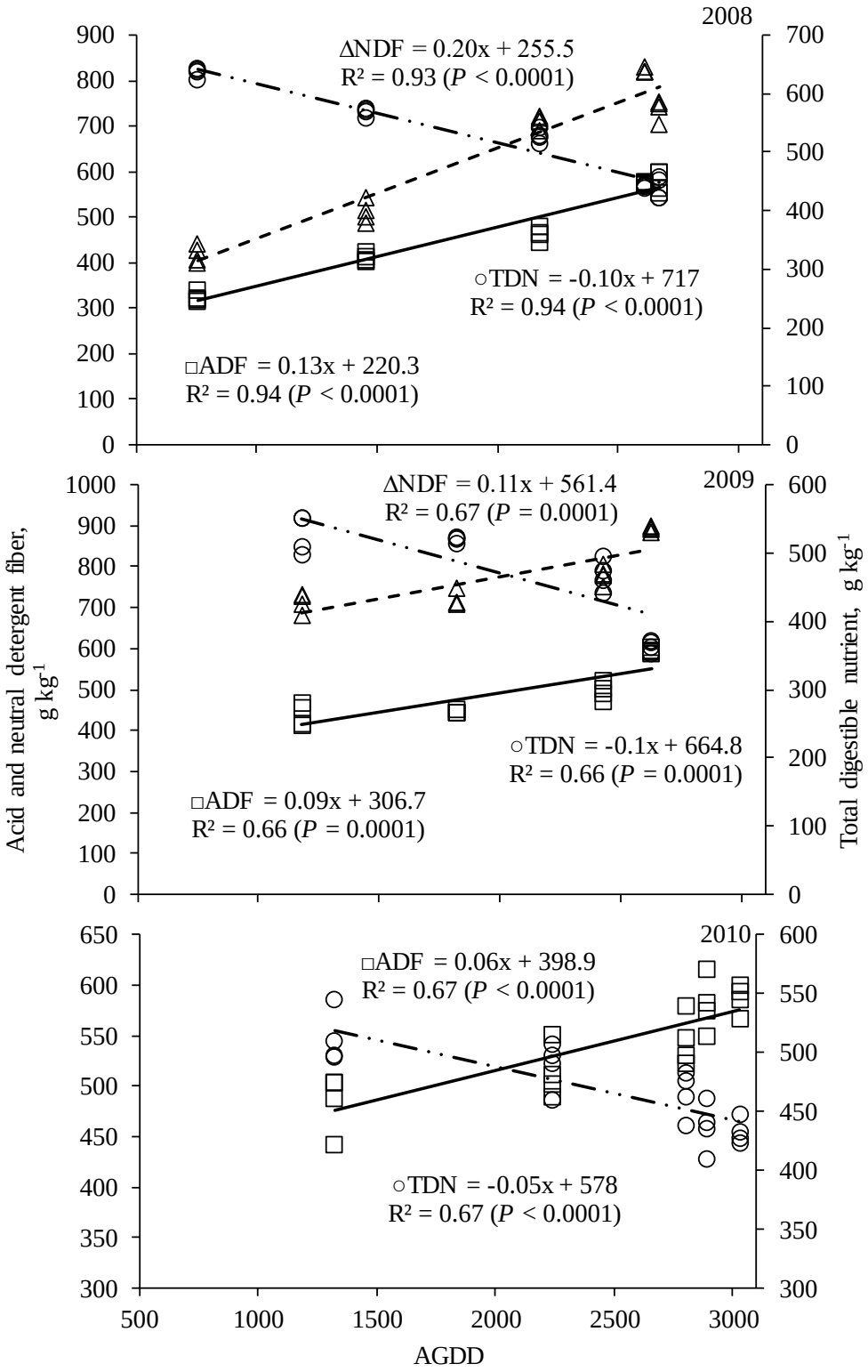

Fig. 3.4 Acid detergent fiber (ADF), neutral detergent fiber (NDF), and total digestible nutrients (TDN) as affected by accumulated growing degree days (AGDD).

Mineral concentration as affected by accumulated growing degree day

Significant differences in mineral concentrations existed among biomass harvested with various AGDD (Table 3.4). In 2008, all mineral concentrations analyzed showed significant differences ($P < 0.05$) by AGDD. In most cases, mineral concentrations of nutrients decreased with increasing AGDD. Significant differences were shown in all nutrients except Fe in 2009. For the 2010 season, significant differences were seen in all minerals except in Ca and Fe. Phosphorus, S and Mn were not analyzed due to errors in analysis and lack of data. Similar to Ashworth et al. (2017) and Sena et al. (2018), N concentration in switchgrass was highest in the early season harvest (May-June), and declined throughout the rest of the growing season. Generally, mineral concentration is decreasing as the season progresses, as would be expected in native perennial grasses. With few exceptions, all mineral analyses indicated decreases in mineral concentration with increasing AGDD. Not all decreases are significant based on Duncan's Multiple Range Test. These types of decreases have been shown in other studies regarding harvest timing as well (Makaju et al., 2013). Since mineral concentrations decreased as AGDD increased, harvesting at earlier growing season with lower AGDD would preserve more nutrients and favorable forage use, while a later season harvest with higher AGDD would offer more desired quality for biofuel production.

Table 3.4. Mineral concentrations in harvested biomass as affected by harvest timing (AGDD, accumulated growing degree days).

Year	AGDD	N	P	Ca	K	Mg	S	Cu	Fe	Zn	Mn
		----------g kg^{-1}----------						----------mg kg^{-1}----------			
2008	754.7	7.10a	1.78a	2.30a	15.13a	1.80a	0.83a	25.7b	106.8ab	26.2a	77.7a
	1452	4.51b	1.15b	2.03ab	7.93b	1.50ab	0.60b	23.3b	88.7ab	22.7ab	82.0a
	2175	3.44bc	1.10b	1.68b	6.38c	1.38bc	0.50bc	5.9b	54.8ab	19.3bc	54.8b
	2611	3.26bc	0.78c	1.68b	3.58d	1.43b	0.40cd	12.2b	145.6a	15.5c	47.0b
	2672	2.95c	0.75c	1.45b	2.93d	1.08cd	0.35d	6.2b	134.2a	15.2c	41.4b
	2748	3.04c	0.50d	1.48b	1.50e	1.01d	0.07e	220.5a	16.0b	8.5d	43.6b
	Avg	4.05	1.01	1.77	6.44	1.36	0.47	50.6	94.4	17.9	57.7
	Std dev	1.63	0.44	0.49	4.64	0.34	0.24	89.1	64.6	6.5	19.7
	P	<0.0001	<0.0001	0.0272	<0.0001	0.0013	<0.0001	<0.0001	0.0440	<0.0001	0.0010
	F Test	15.59	31.17	3.49	204.59	7.23	49.7	14.3	2.8	15.3	7.6
	CV%	40.29	43.96	27.50	10.47	24.60	14.31	87.9	56.4	36.4	34.2
2009	1180	10.34a	1.64a	2.17ab	10.1a	1.95a	0.77a	9.4a	60.2a	25.9a	77.6a
	1822	9.42a	1.55a	2.69a	7.87b	2.07a	0.81a	9.8a	75.1a	29.4a	68.7ab
	2426	4.99b	1.08b	1.24c	4.22c	1.05b	0.39b	5.2b	81.2a	16.2b	50.6bc
	2627	4.68b	0.76c	1.29c	2.29d	1.05b	0.36b	1.5c	43.0a	14.1b	42.1c
	2642	4.00b	1.00b	2.00b	1.75d	---	---	---	---	---	---
	2642	4.00b	1.00b	2.00b	1.00e	---	---	---	---	---	---
	Avg	6.24	1.17	1.90	4.13	1.53	0.58	6.5	64.9	21.4	59.7
	Std dev	2.80	0.35	0.63	3.28	0.55	0.22	3.7	27.7	7.2	21.3
	P	<0.0001	<0.0001	0.0007	<0.0001	<0.0001	<0.0001	<0.0001	0.2616	0.0006	0.0148
	F Test	41.97	33.45	8.63	327.32	26.21	52.04	40.0	1.6	16.3	6.1
	CV%	44.85	29.58	33.22	8.955	36.22	38.57	19.1	42.8	33.8	35.7
2010	1323	6.76a	1.00a	2.25a	7.75a	1.75a	1.00a	5.5a	70.1a	23.1a	---
	2240	6.84a	1.00a	2.25a	6.25b	1.75a	1.00a	4.6b	92.1a	20.4ab	---
	2803	4.04b	1.00a	1.75a	3.00c	1.50ab	1.00a	4.2b	110.5a	19.3ab	---
	2888	3.24bc	1.00a	1.75a	1.75d	1.00b	---	3.0c	55.7a	18.2bc	---
	3031	3.08c	1.00a	1.50a	1.25d	1.00b	---	3.1c	97.8a	14.5c	---
	Avg	4.79	1.00	1.90	4.00	1.40	1.00	4.1	85.2	19.1	---
	Std dev	1.88	0.00	0.55	2.70	0.50	0.00	1.3	42.1	4.6	---
	P	<0.0001	---	0.2859	<0.0001	0.0489	---	<0.0001	0.2569	0.0056	

F Test	39.65	---	1.42	70.18	3.29	---	16.6	1.5	6.3	---
CV%	39.32	0.00	29.08	17.08	35.90	0.00	32.8	49.4	24.0	---

Avg: Average. CV%: Coefficient of variation as a percentage. Std. dev.: Standard deviation of the mean (n=4).

Numbers in a column followed by the same letter exhibited no significant differences ($P < 0.05$) based on Duncan's Multiple Range Test.

CONCLUSIONS

Acid detergent fiber, NDF, TDN and N (or crude protein) and mineral concentrations were affected by N rates. Those quality parameters were especially impacted by harvesting time. In general, fibers and most minerals in the biomass increased as AGDD increased, but N and TDN decreased as AGDD increased. Since high crude protein and minerals, and low fiber are desired forage quality and the opposite is true for biofuel feedstock, earlier harvests are beneficial for hay production or for livestock forage grazing, and late-season harvests are better for biofuel production. Our results are consistent with the literature concerning forage quality patterns throughout the growing season, therefore, these trends will help in the decision-making for switchgrass management for biomass and/or forage.

CHAPTER IV

VEGETABLE PRODUCTION AND SOIL HEALTH AS AFFECTED BY COVER CROPS

ABSTRACT: Vegetable production is intensive and growers face challenges of soil quality degradation with conventional tillage practices. Continuous tillage and fallow ground can be detrimental to many soil properties necessary for sustainable and profitable vegetable production due to erosion, loss of soil organic matter (SOM) and aggregate breakdown. A field study was initiated to assess the ability of cover crops to increase SOM and their effects on soil health parameters and vegetable crop production. Four cover crop treatments were used, alternating between cool season and warm season crops. Cool season treatments used were: 1. cereal rye and crimson clover, 2. winter wheat and crimson clover, 3. cereal rye, Austrian winter pea, tillage radish, and 4. clean fallow. Warm season treatments were: 1. sorghum x sudan and cowpea, 2. forage cowpea, 3. pearl millet and forage cowpea, and 4. clean fallow. Clean fallow will serve as the control treatment. Soil pH was significantly higher ($P \leq 0.05$) in a cereal rye-crimson clover/sorghum x sudan-cowpea cover and soil nitrate-N was significantly higher in a winter wheat-crimson clover/ forage cowpea cover than those of other cover treatments and the fallow control treatment. Gravitational water content, water stable aggregates,

and CO_2 emission were significantly lower on the fallow treatment when compared to some other cover treatments. When cool season cover crop treatments were compared, cereal rye-Austrian winter pea, tillage radish cover had the greatest yield and lowest P concentration. The winter wheat-crimson clover and cereal rye-Austrian winter pea-tillage radish treatments had the greatest N concentration. No significant differences in biomass yield of cover crops were seen among warm season cover crops, pearl millet-cowpea had the lowest N concentration and the sorghum x sudan cowpea mixture had the highest P concentration. In assessments of vegetable crops, spinach had the greatest stand count within the fallow treatment, but no yield differences were seen in yields of cowpea and sweet potato among any cover treatments. Protein and P concentrations of cowpea cash crop were both the lowest in the winter wheat-crimson clover/forage cowpea treatment. Few significant differences in cash crop yields with different cover crop combinations were seen in this study. It may be because the treatments had not been in place long enough to significantly improve soil health parameters such as SOM.

INTRODUCTION

Cover crops have been promoted to improve many parameters of soil health and have resulted in improved soil quality (Butler et al., 2016) and crop production (Delaney et al., 2014; Alvarez et al., 2017; Chu et al., 2017). These crops are planted to cover the soil between cash crops; and the "green manure" adds organic matter (OM) to soils and scavenges available nutrients in the soil. Many of the benefits are seen in changes in soil chemical, physical and biological properties due to cover crops. Increases in OM aid in nutrient retention, enhance nitrogen (N) fixation (when legumes are used as a part of

cover crops), improve water infiltration and aggregate stability, reduce bulk density, erosion and soil compaction (Kaspar and Singer, 2011), which result in overall soil improvement. Organic matter content is typically very low in Oklahoma soils and averages about 0.5 to 0.7% (Brandenberger et al., 2018). Poor soil quality due to low OM is limiting vegetable yield and quality. Conversely, increases in SOM can improve nutrient stabilization and availability, and enhance microbial diversity in the soil (Obi, 1999; Liesch et al., 2011; Khan et al., 2016; Williams and Weil, 2004). Soil fertility has been shown to be improved by increasing SOM content with cover crops (Sainju et al., 2007; Krueger et al., 2010) as well.

Plant available N can be increased by incorporation of a cover crop with a low carbon to nitrogen ratio (C:N) into the soil (Handayanto et al., 1997; Brennan et al., 2013). For example, when legumes are used as cover crops, N availability within the soil can be further increased as a consequence of the low C:N attributed to the cover crop. Brennan and Boyd (2012) compared cover crop varieties and seeding rate for shoot N accumulation and found a legume–rye mixture had greater N accumulation than mustard or rye alone. The soil C:N ratio can be reduced as well, consequently increasing N availability to the cash crop during residue mineralization. Therefore, cover crops capture soil available N, and release it back into the soil later. This allows N to be used by the crop rather than being lost by leaching (Owens et al., 2000) or erosion from bare soils (Kaspar et al., 2001), and reduces the need for commercial N fertilizers.

Soil physical properties are also improved with cover crops. Decreased bulk density, increased water infiltration and water holding capacity, and improved aggregate stability have been demonstrated by using cover crops. Williams and Weil (2004)

demonstrated improved yields of soybeans as a consequence of the root penetration provided by the prior winter *Brassica* cover crop on a compacted soil. Liesch et al. (2011) found that rye double-cropping systems improved soil structure by increased hydraulic conductivity, decreased bulk density, smaller aggregate sizes, and increased porosity.

Soil microbial activity has also been shown to increase in cover cropping systems. Microbial populations beneficial to soil and crop health are enhanced with improved soil chemical and physical properties which in turn increased the activity of fungal hyphae and mycorrhizae on nutrient uptake. Caban et al. (2018) found hairy vetch favored more increase of microbial populations in antibiotic contaminated soils compared to compost or chemical fertilizer. Nair and Ngouajio (2012) concluded that rye and rye-vetch mixtures affected soil microbial communities, but the communities were not significantly different from one another.

More focus on measurements to maintain and improve soil health, rather than only the crop yield and quality have begun to be seen in commercial and university soil testing laboratories. Measurements for assessing soil health and quality have begun to be used in developing soil health management plans. Methods currently in use to better define and quantify soil health move beyond standard soil chemical (fertility) testing and incorporate soil physical and biological properties. Haney (2014) and Haney et al. (2017) developed a tool for soil health evaluation incorporating soil physical and biological parameters to be used in conjunction with standard soil fertility testing. This process allows for an estimation of nutrient mineralization as they become inorganic and made plant-available by microbial action. The Haney method includes CO_2 respiration for an

estimate of microbial activity, C:N ratio of soil, organic C and organic N to develop a soil health score. Other laboratories have developed similar tools for soil health measurements. The Cornell method, known as the Comprehensive Assessment of Soil Health, or CASH, is another popular soil health assessment tool to which others have made comparisons (Fine et al., 2017; McGowen et al., 2018). Some commercial laboratories have used the Solvita method for quantifying soil microbial activity with a 24-hour soil incubation. Carbon dioxide (CO_2) emitted from this incubation is measured using color paddles for a quick turnaround of results (Solvita, 2017; Woods End Laboratories, 2016). McGowen et al. (2018) developed a method to measure CO_2 concentration by gas chromatograph analysis for assessment of microbial respiration. Microbial respiration is directly related to soil nutrient cycling and nutrient mineralization, and can estimate nutrient availability to the cash crop through decomposition of residues from a cover crop.

Auburn University's Soil, Forage and Water Testing Lab offers a test that consists of a routine soil test, estimated cation exchange capacity (CEC), percent base saturation, SOM content, soil respiration, and aggregate stability; and an Alabama Soil Health Index is calculated with management practice suggestions (Gamble, 2018; Alabama Coop. Ext. Sys. Staff, 2019).

In addition, research working groups like the Soil Health Institute have been established in order to encourage and enhance research focusing on soil health and education. It seeks to collaborate with researchers, producers, and others to research, educate, and implement management practices that promote, improve, and sustain soil health (soilhealthinstitute.org).

Each change to soil chemical, physical, or biological properties from cover crop practices can improve soil health, and theoretically improve crop production. The increase in SOM has aided in vegetable crop production with improved stand establishment, as it can be difficult to achieve by direct seeding because of low SOM.

A better understanding of cover crops and how they affect soil quality relating to soil chemical, physical, and biological properties could assist in sustaining and improving soil health in vegetable production. This study evaluates selected chemical, physical and biological parameters of soil health as affected by various cover crop and fallow treatments during the off-season of vegetable crop production. Vegetable crops used to evaluate production are spinach, cowpea (above ground) and sweet potato (below ground). The objectives of this study are i) to assess the impact of cover crops on important soil health parameters, ii) to evaluate yield and quality of species used as cover crops and iii) to evaluate the effects of various cover crops on subsequent cash crops spinach (*Spinacia oleracea* var. Avon), cowpea (*Vigna unguiculata* var. Empire) and sweet potato (*Ipomoea batatas* var.Covington) production.

MATERIALS AND METHODS

Experimental design and treatment description

A cover crop field experiment was established consisting of four 27.4 m x 100.6 m strips in the fall of 2016. The area had been fallow bermudagrass for several years prior to cover crop and vegetable plot establishment. Cover crops have been rotated between cool and warm seasons since 2016. Cool season cover crop treatments were initiated in fall of 2016 and warm season cover crops were started in the spring of 2017.

Cool season cover treatments were: 1. Rye (*Secale cereale*) and crimson clover (*Trifolium incarnatum*); 2. Wheat (*Triticum*) and crimson clover; 3. Rye and Austrian winter pea (*Pisum sativum*); and 4. Fallow. Warm season cover crop treatments were: 1. Sorghum x sudan (*Sorghum* x *drummondii*) and cowpea (*Vigna unguiculata*); 2. Forage cowpea; 3. Pearl millet (*Pennisetum glaucum*) and forage cowpea; and 4. Fallow. Cool season treatments for 2017 were the same except for the addition of tillage radish (*Raphanus sativus*) to rye and Austrian winter pea in treatment 3. Three out of four areas have and will continue to follow a specific cover crop regime. The fourth strip will be maintained as fallow and serve as a control plot. Treatments are listed in Table 4.1.

Table 4.1. Cover crop treatments used in study

Treatment	Warm season	Cool season
1	Sorghum x Sudan and cowpeas	Cereal rye and Crimson clover
Abbreviation	SS-CP	CR-CC
2	Forage cowpea	Winter wheat and Crimson clover
Abbreviation	FCP	WW-CC
3	Pearl millet and cowpea	Cereal rye, Austrian winter pea, tillage radish
Abbreviation	PM-CW	CR-AWP-TR
4	Fallow	Fallow
Abbreviation	FW	FW

Tillage was conducted in a 1.8 m wide strip within each treatment area in order to prepare for vegetable crop planting and will serve as plots. Each tillage strip will be divided into five 15.2 m sections in order to leave a 1.5 m space as alleys between sampled areas. Vegetable crops were planted into cover crops in a split-plot design; spinach and cowpea planted on the west side of each cover crop treatment area and sweet potato planted to the

east side of each area (Fig. 4.1). This design is to ensure that vegetable crops would be cultivated along the entire strip, from south to north. The study is considered a randomized complete block design (RCB) with five replications. The arrangement did not allow for proper replication and sampling for all studied parameters were pseudo-replicated within each cover crop treatment. Cover crops were planted using a Hege 1000 plot planter (Hege Equipment Inc., Colwich, KS). Cool season cover crops were planted October 23, 2018 and disked into soil June 3, 2019. Sampling of cover treatments for biomass yield and mineral concentration was conducted April 12, 2019. Warm season cover crops were planted July 2, 2019 and disked into soil September 12, 2019. Biomass yield and sampling was conducted September 5. Cool season cover crops were planted October 4, 2019 for the following year. Cover crops did not receive irrigation.

The soil in the study area is classified as a Teller loam (fine-loamy, mixed, active, and thermic Udic Agriustolls) (USDA Soil Survey Staff, NRCS-USDA).

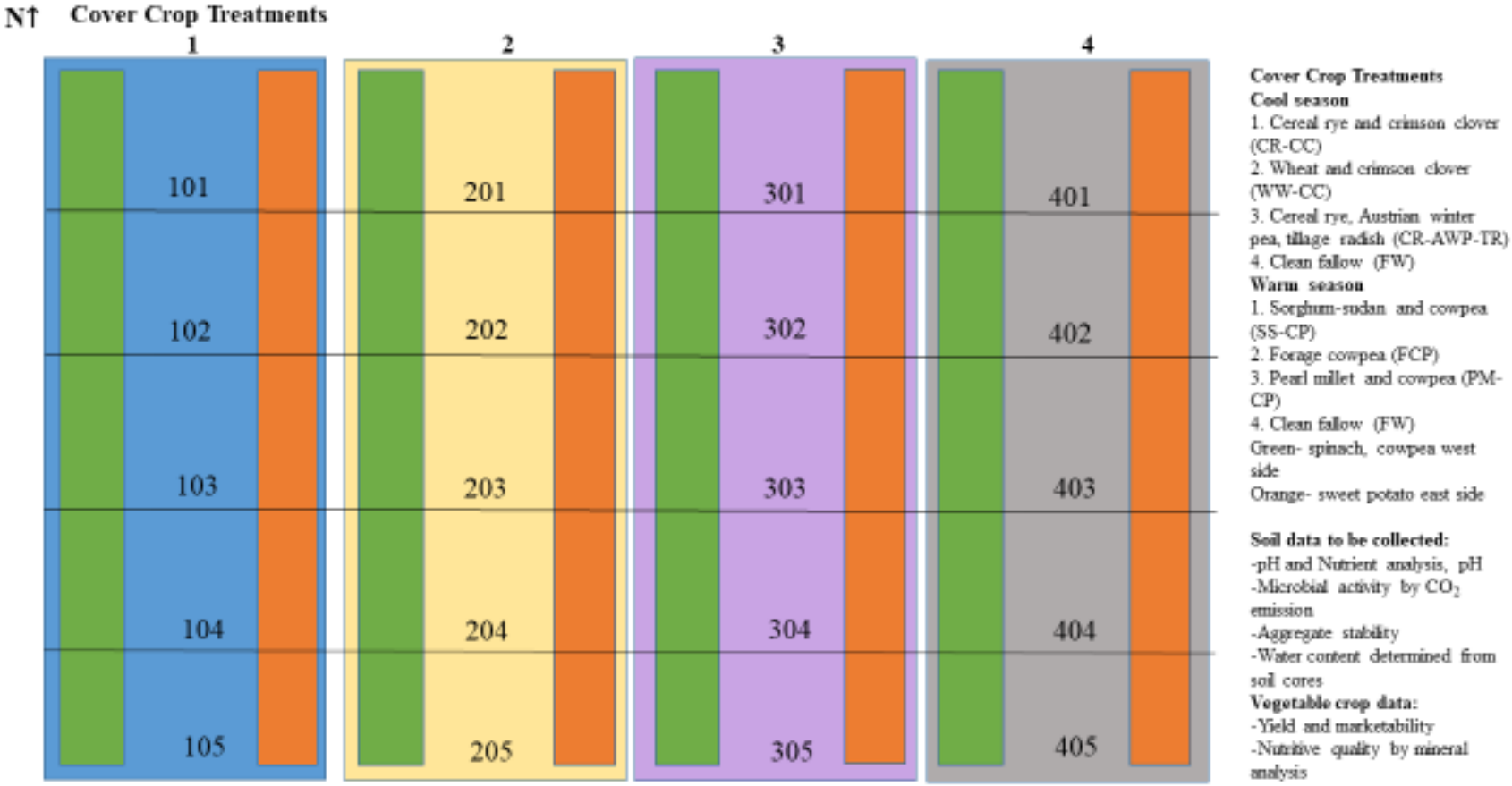

Fig. 4.1. Cover crop study design

Soil analysis

Three (3) replicated soil samples were taken at a depth of 0 to 6" (~15 cm) from each treatment area to obtain a baseline information on soil properties in the fall of 2017 and 2018 (Table 4.2). In 2019, each treatment strip was divided into five (5) pseudo-replications. Soil samples were collected from each individual plot, making a total of 20 soil samples (5 per treatment). Soil sampling will continue throughout the study using five pseudo-replicates per treatment strip. Samples will be taken at intervals over the growing season as soils are prepared for planting either to cover crop or vegetable research. Soil samples were oven-dried at 65°C for 24h and ground to pass a 2-mm sieve. Soil pH was measured using a 1:1 soil/water suspension and measured with a combination electrode, as described by Thomas (1996). Plant available N was extracted by 1M KCl and analyzed by a flow injection auto-analyzer (Kachurina et al., 2000; Lachat QuickChem 8000, Loveland, CO). Plant available P and K were extracted from soil using Mehlich-3 extractant (Mehlich, 1984). Extracts were analyzed using an inductively coupled plasma emission spectrometer (Zhang and Henderson, 2018).

Table 4.2. Background soil test results by cover crop treatment, 2017 and 2018 Cimarron Valley Research Station, Perkins, OK. Data from the 2017 study was summarized from previously published 2017 Vegetable Trial Report (MP-164, Brandenberger and Carrier, January 2018).

Year	Treatment	pH		N		P		K		SOM	
				----------mg kg^{-1}----------						g kg^{-1}	
2017	1	6.7	a[z]	11	b	11	b	187	c	18.4	b
	2	6.5	b	12	b	15	a	216	b	21.7	a
	3	6.4	b	10	b	11	b	197	bc	18.3	b
	4	6.2	c	16	a	17	a	244	a	23.7	a
	Avg	6.5		12		14		211		20.5	
	Std dev	0.21		2.5		3.4		24.7		2.59	
	P	0.0002		0.034		0.020		0.003		0.005	
	F	44.3		5.75		7.36		16.5		12.9	
	CV%	3.18		20.83		25.12		11.69		12.62	
2018	1	6.6	a	4.5	c	14	a	237	bc	19.9	ab
	2	6.4	b	12	a	11	b	247	ab	18.7	bc
	3	6.2	c	6.0	b	11	b	214	c	16.7	c
	4	6.1	c	11	a	16	a	267	a	21.8	a
	Avg	6.3		8.3		12.6		241		19.3	
	Std dev	0.22		3.35		2.64		24.8		2.19	
	P	0.001		<0.0001		0.002		0.021		0.012	
	F	22.8		95.6		20.6		7.16		8.99	
	CV%	3.48		40.15		20.90		10.29		11.37	

Treatments: 1. Winter-cereal rye and crimson clover, summer-sorghum x sudan and cowpea; 2. Winter-winter wheat and crimson clover, summer-forage cowpea; 3. Winter-cereal rye, Austrian winter pea, tillage radish, summer-pearl millet and cowpea; 4. Winter-fallow, summer-fallow.

Avg: Average. Std. dev.: Standard deviation of the mean (n = 3).CV%: Coefficient of variation as a percentage. Numbers in a column followed by the same letter exhibited no significant differences ($P < 0.05$) based on Duncan's Multiple Range Test.

Soil Physical Properties

Soil physical properties measured included aggregate stability using a single sieve analysis (Warren, J., personal communication; Almajmaie et al., 2017) on dry soil samples. Soil used for aggregate stability was from prepared soil samples for soil fertility analysis as described previously. In addition, soil cores were sampled from the field, 3 cores per cover crop treatment, using a 3-point tractor-mounted Giddings 39.7 mm diameter soil probe (Giddings Machine Company). Each core was separated into 15.2 cm sections by depth for analysis. Gravimetric and volumetric soil water content was calculated from these cores. After sampling, cores were weighed then oven-dried at 105 °C for 24 h. Cores were weighed again and water contents were calculated using:

$$\text{Gravimetric water content} = [(\text{wet weight}_{\text{soil core}} - \text{dry weight}_{\text{soil core}}) / (\text{dry weight}_{\text{soil core}})]$$

$$\text{Volumetric water content} = [(\text{wet weight}_{\text{soil core}} - \text{dry weight}_{\text{soil core}}) / (\text{volume}_{\text{soil core}})]$$

Soil Biological Properties

Estimates of soil biological properties were made among cover crop treatments by assessing microbial activity, using methods described by McGowen et al. (2018), of gas chromatography to determine CO_2 respiration after a 24 h soil incubation period. This method was developed in order to shorten the length of time required for microbial activity assessment using the Solvita burst method (Woods End Laboratories, 2016; Solvita, 2017) and instead uses direct analysis of headspace by gas chromatography. This measurement of activity may be used to estimate nutrient mineralization, especially of N, for crop use (Haney et al., 2017).

Cover crop sampling

Samples of each cover crop were taken to estimate biomass of the cover crop from a 0.6 x 0.6 m square area. Three (3) random sections were harvested from each 15.2 m plot. Biomass was weighed and then dried for 72 h at 48.9 °C. Samples were ground to pass through a 1.0-mm sieve for nutrient analysis. Plant samples were digested with nitric acid (HNO_3) for mineral nutrients, in which 0.5 g of ground plant materials were predigested for 1 h with 10 ml of trace metal grade HNO_3 in the HotBlock™ Environmental Express block digester. The digests were then heated to 115 °C for 2 h and diluted with deionized water to 50 mL (Jones and Case, 2018). Digested samples were analyzed by inductively coupled plasma (ICP) for P, K, Ca, Mg, S, Cu, Fe, Zn, and Mn. Total N was determined with a carbon/nitrogen (C/N) dry combustion analyzer.

Vegetable crop planting and harvest

In preparation for spring spinach planting, single strips with a width of 1.5 m in the cover crop treatments were tilled using a Priefert roto-tiller. Strips were tilled on March 7, 21, and immediately before planting on March 28, 2018. Avon cultivar spinach was planted in five 15.2 m long plots separated by 1.5 m alleys in a strip-tillage setup within each cover crop treatment using a Hege plot planter. Plots were 4 rows wide, 0.3 m row spacing, 1 seed per 2.54 cm in-row spacing. Heavy rainfall immediately followed planting, resulting in a poor stand. Spinach stand counts were taken on April 26 using 3.0 m in each of rows. Spinach was harvested by hand on June 3.

Sweet potato slips were planted by hand into raised beds within cover crop treatments on June 6, 2019. Beds used Netafilm drip irrigation tape (1.05 l h^{-1} flow rate at

1.0 bar pressure, with 3.75 m emitter spacing) for irrigation. Sweet potatoes were harvested October 10 and 14, using a 3-point tractor-mounted potato digger and gathered by hand. Total weight was taken for each plot. Potatoes were sorted into marketable categories outlined by USDA grades (https://www.ams.usda.gov/grades-standards/sweetpotatoes-grades-and-standards). Weight and number of marketable potatoes from each plot were recorded.

Cowpea was planted on June 27, 2019 using a Hege plot planter and harvested September 27, using a Wintersteiger Delta plot combine (Wintersteiger). Peas were dried, ground and analyzed for nutrient concentration as described previously for cover crop biomass.

Statistical Analysis

The study area is planted in strips of cover crops divided into pseudo-replicates in this design. Five replicated samples were taken from each strip, each sample taken from one of the five sections of each treatment strip. Data was analyzed as a completely randomized design using analysis of variance using SAS ver. 9.4 (SAS Institute). Significant differences in sampled data by treatment will be determined at alpha = 0.05 based on Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Soil fertility as affected by cover crop treatment

In 2019, significant differences in soil pH and soil nitrate-N among cover crop treatments were seen (Table 4.3). Soil pH in the first treatment (CR-CC/SS-CP) was 0.5 unit higher than in other treatments (pH 6.5). This trend was also seen in 2017 and 2018 and perhaps contributed to poor spinach emergence from direct seeding. Soil N was significantly higher in treatment 2 (WW-CC/FCP) than other treatments. Numerical differences were shown in SOM, especially between fallow and other cover crops. In 2019, treatment 4 (fallow) numerically displayed the lowest SOM, which has not been the case in prior years. It would be expected that the fallow treatment would have lower SOM than treatments with cover crops. However, SOM was the highest in the fallow treatment for 2017 and 2018 (Table 4.2), and it is thought to be affected by prior bermudagrass ground cover of the study area. With continuous additions of cover crop residues, testing results of SOM would be expected to continue to increase in cover crop treatments, resulting in greater differences between fallow and cover crop treatments.

Table 4.3. Basic soil properties as affected by cover crop treatment, 2019.

Treatment	pH	N	P	K	SOM
		----------mg kg^{-1}----------			g kg^{-1}
1	7.0a	4.2c	15a	244a	20.1a
2	6.5b	8.1a	13a	245a	21.2a
3	6.5b	6.0b	10a	211a	20.7a
4	6.5b	5.2bc	15a	224a	16.9a
Avg	6.6	6.6	13	231	19.7
Std dev	0.26	1.7	4.1	36.7	3.1
P	<0.0001	<0.0001	0.174	0.467	0.187
F Test	43.1	20.1	1.96	0.91	2.06
CV%	3.95	28.01	30.63	15.90	15.75

Treatments: 1. Cool-cereal rye and crimson clover, warm-sorghum x sudan and cowpea; 2. Cool-winter wheat and crimson clover, warm-forage cowpea; 3. Cool-cereal rye, Austrian winter pea, tillage radish, warm-pearl millet and cowpea; 4. Cool-fallow, warm-fallow.

Avg: Average. Std. dev.: Standard deviation of the mean (n=5).CV%: Coefficient of variation as a percentage.

[z]Numbers in a column followed by the same letter exhibited no significant differences based on Duncan's Multiple Range Test where P=0.05.

Soil physical and biological properties as affected by cover crop treatment

Gravimetric and volumetric water contents were calculated from soil cores. No significant differences were found with the exception of gravimetric water content at a depth of 0 to 15.2 cm (Table 4.4). Gravimetric water content was significantly lower in the fallow treatment than treatments 2 (WW-CC/FCP) and 3 (CR-AWP-TR/PM-CP) at 0 to 15.2 cm depth, and numerically lower in the fallow treatment compared to cover treatments at all other depths. Volumetric water content was numerically lower in the fallow treatment than cover treatments at all depths. Greater water contents in cover crop

treatments suggest the increased water holding capacity cover crops can provide the soil compared to bare soil (Chu et al., 2017; Fuentes et al., 2004). Increased water availability to cash vegetable crops is important especially in times of drought.

Table 4.4. Gravimetric and volumetric water content by depth as affected by cover crop treatments. Samples collected October 1, 2019.

Treatment	Gravimetric water content g g soil^{-1}				Volumetric water content g cm^{-3}			
Depth (cm)	0-15.2	15.2-30.5	30.5-45.7	45.7-61.0	0-15.2	15.2-30.5	30.5-45.7	45.7-61.0
1	0.147ab	0.134a	0.126a	0.128a	0.208a	0.212a	0.190a	0.198a
2	0.161a	0.124a	0.120a	0.129a	0.212a	0.199a	0.184a	0.198a
3	0.159a	0.127a	0.115a	0.127a	0.227a	0.197a	0.174a	0.196a
4	0.133b	0.116a	0.113a	0.122a	0.194a	0.183a	0.181a	0.179a
Avg	0.150	0.130	0.120	0.130	0.210	0.200	0.180	0.190
Std dev	0.01	0.02	0.01	0.01	0.02	0.03	0.02	0.02
P	0.040	0.912	0.712	0.356	0.316	0.877	0.840	0.428
F	5.31	0.17	0.47	1.31	1.46	0.22	0.28	1.07
CV%	9.39	18.60	9.86	4.92	9.07	17.38	9.97	9.05

Treatments: 1. Winter-cereal rye and crimson clover, summer-sorghum x sudan and cowpea; 2. Winter-winter wheat and crimson clover, summer-forage cowpea; 3. Winter-cereal rye, Austrian winter pea, tillage radish, summer-pearl millet and cowpea; 4. Winter-fallow, summer-fallow.

Avg: Average. Std. dev.: Standard deviation of the mean (n = 5).CV%: Coefficient of variation as a percentage. Numbers in a column followed by the same letter exhibited no significant differences ($P < 0.05$) based on Duncan's Multiple Range Test.

Water stable aggregates were analyzed using prepared soil samples from soil fertility testing. Significant differences were seen between the fallow treatment (462 g kg^{-1}) and two of the three other cover treatments. Treatments 1 (CR-CC/SS-CP) and 3 (CR-AWP-TR/PM-CP) displayed significantly higher water stable aggregates, at 558 and 566 g kg^{-1}, respectively (Table 4.5). Water stable aggregates were significantly lower in fallow treatment than treatments 1 and 3, and numerically lower than treatment 2 (WW-CC/FCP). It should be noted there has been observation of wind erosion occurring in treatment 4 (FW) while cover treatments had minimization of soil loss by wind erosion. Cover crop treatments are enhancing soil aggregate stability when compared to the fallow treatment by protection of the soil surface and root action of the cover crops (Williams and Weil, 2004).

The amounts of CO_2-C determined to estimate microbial activity were significantly different among some treatments. Microbial activity in treatments 1 (CR-CC/SS-CP) and 2 (WW-CC/FCP) was higher than that in treatment 4 (FW) at 45.8 and 51.4 mg CO_2-C kg $soil^{-1}$, compared to 32.2 mg CO_2-C kg $soil^{-1}$ in the fallow treatment (Table 5). Treatment 4 was also numerically lower than treatment 3 (CR-AWP-TR/PM-CP) although no significant differences were observed. It has been cited in other studies (Caban et al., 2018; Chavarria et al., 2018) that microbial activity is greater in areas under cover crops than fallow ground. This activity leads to greater soil stabilization (Cobb and Wilson, 2018) and enhanced nutrient cycling (Gonzalez-Chavez et al., 2010).

Table 4.5. Water stable aggregates and microbial respiration as affected by cover crop treatments.

Treatment	Water Stable Aggregates g kg^{-1}	CO_2-C mg CO_2-C kg $soil^{-1}$
1	558a	46.0ab
2	508ab	51.4a
3	566a	38.6bc
4	462b	32.1c
Avg	524	42.0
Std dev	61.3	11.9
P	0.015	0.005
F Test	5.28	7.16
CV%	11.70	28.31

Treatments: 1. Winter-cereal rye and crimson clover, summer-sorghum x sudan and cowpea; 2. Winter-winter wheat and crimson clover, summer-forage cowpea; 3. Winter-cereal rye, Austrian winter pea, tillage radish, summer-pearl millet and cowpea; 4. Winter-fallow, summer-fallow.

Avg: Average. Std. dev.: Standard deviation of the mean (n = 5). CV%: Coefficient of variation as a percentage. Numbers in a column followed by the same letter exhibited no significant differences ($P < 0.05$) based on Duncan's Multiple Range Test.

Comparisons of yield and mineral concentrations of cover crop treatments

For cool season cover crop treatments, biomass yield of the cover crop in treatment 3 (CR-AWP-TR) was significantly greater than those of treatments 1 (CR-CC) and 2 (WW-CC). Nitrogen and P concentrations were significantly different from one another as well. Concentrations of N of treatment 2 (WW-CC) and 3 (CR-AWP-TR) were significantly greater than treatment 1 (CR-CC), while P concentration was significantly less in treatment 3 (CR-AWP-TR) than in other cover treatments. No significant differences were shown in K concentrations (Table 4.6).

In warm season cover crop treatments, yields and K concentrations were not significantly different from one another. Nitrogen concentration in treatment 3 (PM-CP) was significantly less than the other two cover treatments, and P concentration of treatment 1 (SS-CP) was significantly higher than the other two cover treatments (Table 6). Greater biomass yield would increase OM into the soil, and lower nitrogen would produce greater C:N ratios of the biomass, providing lower N mineralization when compared to crops with a low C:N ratio (Brennan et al., 2011). An additional use for these cover crops could be as hay, provided harvest timing allowed for regrowth to till into the soil. In evaluating nutrient quality from these results, cool and warm season treatment 1 (CR-CC/SS-CP) may be the best choice as hay.

Table 4.6. Yields and nitrogen (N), phosphorus (P) and potassium (K) concentrations of cover crop biomass. Cool season samples collected April 12, 2019 and warm season samples collected on September 5, 2019.

Treatment	Cool season cover crops				Warm season cover crops			
	Yield	N	P	K	Yield	N	P	K
	Mg ha^{-1}	----------g kg^{-1}----------			Mg ha^{-1}	----------g kg^{-1}----------		
1	2.5b	7.62b	1.80a	13.6a	4.2a	24.3a	3.22a	32.7a
2	2.4b	10.6a	1.44a	15.3a	3.7a	24.9a	2.20b	38.0a
3	4.7a	10.6a	0.67b	13.7a	3.8a	17.4b	2.28b	34.8a
4	---	---	---	---	---	---	---	---
Avg	3.2	9.63	1.30	14.2	3.9	22.2	2.57	35.2
Std dev	1.36	1.88	0.58	1.63	0.83	4.83	0.69	4.53
P	0.001	0.003	0.005	0.306	0.715	0.006	0.043	0.199
F Test	21.0	13.5	11.0	1.38	0.35	10.3	4.79	1.99
CV%	42.19	19.53	44.55	11.50	21.25	21.75	26.86	12.89

Treatments: 1. Winter-cereal rye and crimson clover, summer-sorghum x sudan and cowpea; 2. Winter-winter wheat and crimson clover, summer-forage cowpea; 3. Winter-cereal rye, Austrian winter pea, tillage radish, summer-pearl millet and cowpea; 4. Winter-fallow, summer-fallow.

Avg: Average. Std. dev.: Standard deviation of the mean (n = 3).CV%: Coefficient of variation as a percentage. Numbers in a column followed by the same letter exhibited no significant differences ($P <$ 0.05) based on Duncan's Multiple Range Test.

Vegetable crop yields and nutrient concentrations as affected by cover crop treatments

Spinach stand counts were taken due to poor stand emergence. Treatments 1 (CR-CC/SS-CW) and 3 (CR-AWP-TR/PM-CW) were significantly different from treatment 4 (FW). Stand counts of the fallow treatment was significantly greater than treatments 1 and 3 (Table 4.7). Two events may have contributed to this poor stand emergence. Immediately after planting, a heavy rainfall event occurred. It is likely spinach seed was pushed with rainfall to a too great a depth to allow for good stand establishment. Another factor may be the cover crop species used with spinach. There are studies showing cover crops may compete with cash crops for resources or even have allelopathic effects on the cash crop, depending upon the species used as cover. For example, cereal rye being detrimental to corn yields, as found by Dhima et al. (2006).

Table 4.7. Stand counts of spinach planted in 4 different cover crop treated plots in 2019.

Treatment	Counts
1	16.6 b
2	30.2 ab
3	19.0 b
4	41.4 a
Avg	26.8
Std. dev.	15.1
P	0.021
F	4.75
CV%	56.31

Treatments: 1. Winter-cereal rye and crimson clover, summer-sorghum x sudan and cowpea; 2. Winter-winter wheat and crimson clover, summer-forage cowpea; 3. Winter-cereal rye, Austrian winter pea, tillage radish, summer-pe arl millet and cowpea; 4. Winter-fallow, summer-fallow.

Avg: Average. Std. dev.: Standard deviation of the mean (n = 5). CV%: Coefficient of variation as a percentage. Numbers in a column followed by the same letter exhibited no significant differences ($P < 0.05$) based on Duncan's Multiple Range Test.

Cowpea yields showed no significant differences among cover crop treatments. However, numerically, yield of treatment 4 (FW) was the highest at 390 kg ha^{-1}. Significantly higher protein content of the fallow treatment than treatment 2 (WW-CC/FCP) was found. Protein of the fallow treatment was also numerically greater than in treatments with cover crops. Phosphorus concentration of treatment 2 (WW-CC/FCP) was significantly lower, at 4.66 g kg^{-1}, than other treatments (Table 8). No significant differences in K concentrations were seen among treatments.

Table 4.8. Yields and nutrient concentrations of cowpea as affected by cover crop treatments in 2019.

Treatment	Yield kg ha^{-1}	Protein g kg^{-1}	P g kg^{-1}	K g kg^{-1}
1	363 a	264 ab	5.20 a	15.8 a
2	271 a	260 b	4.66 b	15.4 a
3	324 a	272 ab	5.08 a	16.1 a
4	390 a	276 a	4.98 a	15.5 a
Avg	337	268	4.98	15.7
Std.dev.	83.7	10.4	0.32	0.64
P	0.163	0.058	0.018	0.289
F	2.04	3.29	5.02	1.41
CV%	24.82	3.90	6.40	4.09

Treatments: 1. Winter-cereal rye and crimson clover, summer-sorghum x sudan and cowpea; 2. Winter-winter wheat and crimson clover, summer-forage cowpea; 3. Winter-cereal rye, Austrian winter pea, tillage radish, summer-pearl millet and cowpea; 4. Winter-fallow, summer-fallow.

Avg: Average. Std. dev.: Standard deviation of the mean (n = 5). CV%: Coefficient of variation as a percentage. Numbers in a column followed by the same letter exhibited no significant differences ($P < 0.05$) based on Duncan's Multiple Range Test.

No differences were seen in yield, marketable weights and marketable numbers of sweet potato due to cover crop treatments (Table 4.9).

Table 4.9. Total and marketable yields of sweet potato as affected by cover crop treatments in 2019

Treatment	Yield Mg ha^{-1}	Market weight Kg	Marketable number
1	177.3 a	82.1 a	208.8 a
2	148.9 a	69.0 a	174.0 a
3	158.1 a	73.2 a	177.8 a
4	149.4 a	69.2 a	190.2 a
Avg	158.4	73.4	187.7
Std. dev.	32.2	14.9	31.8
P	0.430	0.430	0.298
F	0.99	0.99	1.37
CV%	20.29	20.29	16.95

Avg: Average. Std. dev.: Standard deviation of the mean (n = 5). CV%: Coefficient of variation as a percentage. Numbers in a column followed by the same letter exhibited no significant differences ($P < 0.05$) based on Duncan's Multiple Range Test.

CONCLUSIONS

Although significant differences due to cover crop treatments were observed among soil physical, chemical and microbial parameters, differences between cash vegetable crop yields and nutrient concentrations in this study were few, some numerical differences are trending towards indicating some benefits in the use of cover crops, especially to soil health improvement. Cover crops improved soil OM numerically and gravimetric water content significantly when compared with the no cover crop fallow treatment. Changes in soil OM and improvement and other soil health attributes may become more evident with time and cash crop yield improvement will follow.

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APPENDICES

Table A2-1. Statistical parameters of switchgrass yield (Mg ha^{-1}) in 2008, 2009, and 2010 as a function of accumulated growing degree days (AGDD) harvests.

Year/Model	Param.	Value	Param. Prob>|t|	Model Prob>|t|	RMSE	LOF Prob>|t|
2008	Intercept	0.32924	0.89281	2.20E-06	3.95899	1.53E-02
Linear	Slope	0.00698	2.20E-06			
2008	Intercept	-11.21795	0.06408	2.11E-06	3.65804	4.16E-02
Quadratic	B1	0.02297	0.00534			
(Polynomial)	B2	-4.47E-06	0.04047			
2009	Intercept	5.02272	0.22759	0.31725	4.30172	0.00747
Linear	Slope	0.00197	0.31725			
2009	Intercept	-33.93458	0.02813	0.02345	3.4708	0.05764
Quadratic	B1	0.04708	0.00968			
(Polynomial)	B2	-1.19E-05	0.01202			
2010	Intercept	12.36996	0.00494	0.24196	4.43689	0.39058
Linear	Slope	0.00183	0.24196			
2010	Intercept	-15.05674	0.33548	0.10459	4.21029	0.74927
Quadratic	B1	0.03007	0.0629			
(Polynomial)	B2	-6.52E-06	0.07806			

Param.: Parameter. Prob>|t|: Probability. RMSE: Root Mean Square Error. LOF: Lack of Fit.

Table A2-2. Statistical parameters of nitrogen (N) concentration (g kg^{-1}) (aboveground, AG, and belowground, BG) in 2008, 2009, and 2010 as a function of accumulated growing degree days (AGDD) harvests.

Year/Model	Param.	Value	Param. Prob>|t|	Model Prob>|t|	RMSE	LOF Prob>|t|
	----------Aboveground (AG)----------					
2008 Linear	Intercept	7.96836	1.42331E-13	3.04E-08	0.81898	1.42E-01
	Slope	-0.00189	3.04E-08			
2008 Quadratic (Polynomial)	Intercept	10.90801	2.8761E-09	1.03E-08	0.71115	8.86E-01
	B1	-0.00597	0.000453766			
	B2	1.14E-06	0.00938			
2009 Linear	Intercept	16.47628	2.24265E-14	1.54655E-10	1.10724	0.0494
	Slope	-0.00461	1.54655E-10			
2009 Quadratic (Polynomial)	Intercept	6.99864	0.06753	1.00069E-10	0.97808	0.27862
	B1	0.00616	0.14127			
	B2	-2.76E-06	0.01395			
2010 Linear	Intercept	10.52159	9.73366E-09	2.64402E-05	1.17049	0.00847
	Slope	-0.00233	2.64402E-05			
2010 Quadratic (Polynomial)	Intercept	-1.40142	0.66599	9.18268E-07	0.87935	0.38259
	B1	0.00995	0.00635			
	B2	-2.84E-06	0.00126			
	----------Belowground (BG)----------					
2008 Linear	Intercept	1.47889	0.15807	1.14E-04	1.64273	1.55E-04
	Slope	0.00224	1.14E-04			
2008 Quadratic (Polynomial)	Intercept	8.60899	0.000309835	2.37E-06	1.24788	6.96E-03
	B1	-0.00764	0.00689			
	B2	2.77E-06	0.000845029			
2009 Linear	Intercept	9.12743	8.08913E-09	0.55831	1.19117	0.01761
	Slope	-0.00026358	0.55831			
2009	Intercept	21.97315	3.04965E-06	0.00363	0.94044	0.55368

Quadratic	B1	-0.01486	0.000966837			
(Polynomial)	B2	3.74E-06	0.0011			
2010	Intercept	3.2811	0.15213	0.02347	2.42722	0.57916
Linear	Slope	0.00214	0.02347			
2010	Intercept	2.62028	0.77591			
Quadratic	B1	0.00282	0.75976	0.08261	2.49717	0.38647
(Polynomial)	B2	-1.57E-07	0.94084			

Param.: Parameter. Prob>|t|: Probability. RMSE: Root Mean Square Error. LOF: Lack of Fit.

Table A2-3. Statistical parameters of phosphorus (P) concentration (g kg^{-1}) (aboveground, AG, and belowground, BG) in 2008 and 2009 as a function of accumulated growing degree days (AGDD) harvests.

Year/Model	Param.	Value	Param. Prob>|t|	Model Prob>|t|	RMSE	LOF Prob>|t|
----------Aboveground (AG)----------						
2008 Linear	Intercept	2.10282	3.44169E-14	3.38E-09	0.20159	4.48E-02
	Slope	-0.000529052	3.38E-09			
2008 Quadratic (Polynomial)	Intercept	2.18052	1.12831E-06	3.96E-08	0.206	2.38E-02
	B1	-0.000636673	0.14122			
	B2	3.01E-08	0.7967			
2009 Linear	Intercept	2.36659	1.72751E-13	3.80909E-08	0.17576	0.01654
	Slope	-0.000537357	3.80909E-08			
2009 Quadratic (Polynomial)	Intercept	1.04521	0.09533	4.02197E-08	0.16122	0.05091
	B1	0.000963821	0.16166			
	B2	-3.85E-07	0.03395			
----------Belowground (BG)----------						
2008 Linear	Intercept	0.50416	0.000201639	3.16E-03	0.18109	4.62E-02
	Slope	0.000173123	3.16E-03			
2008 Quadratic (Polynomial)	Intercept	1.22385	3.85317E-05	1.57E-04	0.14644	6.64E-01
	B1	-0.000824557	0.0118			
	B2	2.79E-07	0.00298			
2009 Linear	Intercept	0.73962	5.86035E-08	0.01338	0.10841	0.00048376
	Slope	0.000108552	0.01338			
2009 Quadratic	Intercept	0.52918	0.20995	0.04398	0.11023	0.000230547
	B1	0.000347636	0.45264			

(Polynomial)	B2	-6.12E-08	0.60273

Param.: Parameter. Prob>|t|: Probability. RMSE: Root Mean Square Error. LOF: Lack of Fit.

Table A2-4. Statistical parameters of potassium (K) concentration (g kg^{-1}) (aboveground, AG, and belowground, BG) in 2008, 2009 and 2010 as a function of accumulated growing degree days (AGDD) harvests.

Year/Model	Param.	Value	Param. Prob>|t|	Model Prob>|t|	RMSE	LOF Prob>|t|
			----------Aboveground (AG)----------			
2008 Linear	Intercept	18.64792	0	3.16E-14	1.25985	1.33E-05
	Slope	-0.00601	3.18E-14			
2008 Quadratic (Polynomial)	Intercept	21.71536	1.58912E-10	1.62E-13	1.20348	1.79E-05
	B1	-0.01026	0.000387051			
	B2	1.19E-06	0.09241			
2009 Linear	Intercept	17.0318	2.22045E-16	4.13003E-14	0.8995	0.000111368
	Slope	-0.00567	4.13003E-14			
2009 Quadratic (Polynomial)	Intercept	6.45791	0.01587	8.88178E-16	0.66311	0.01373
	B1	0.00634	0.03053			
	B2	-3.08E-06	0.000241565			
2010 Linear	Intercept	13.53421	2.59641E-11	4.46762E-09	1.04094	0.00123
	Slope	-0.00388	4.46762E-09			
2010 Quadratic (Polynomial)	Intercept	1.69747	0.50841	3.61389E-11	0.69259	0.3182
	B1	0.00832	0.00422			
	B2	-2.82E-06	0.000145734			
			----------Belowground (BG)----------			
2008 Linear	Intercept	1.84346	8.6569E-07	3.47E-01	0.42919	1.81E-01
	Slope	0.000117844	3.47E-01			
2008 Quadratic (Polynomial)	Intercept	2.33859	0.00274	4.78E-01	0.43325	1.36E-01
	B1	-0.000568539	0.52408			
	B2	1.92E-07	0.43844			
2009 Linear	Intercept	2.10751	0.04446	0.04318	1.15987	0.000262702
	Slope	0.00092654	0.04318			
2009	Intercept	-9.27789	0.01735	0.00186	0.96769	0.00388

Quadratic	B1	0.01386	0.00226			
(Polynomial)	B2	-3.31E-06	0.00377			
2010	Intercept	0.8	0.31637	0.000640426	0.8587	0.11112
Linear	Slope	0.00126	0.000640426			
2010	Intercept	-2.35508	0.4588			
Quadratic	B1	0.00451	0.16564	0.00207	0.85634	0.08685
(Polynomial)	B2	-7.51E-07	0.30903			

Param.: Parameter. Prob>|t|: Probability. RMSE: Root Mean Square Error. LOF: Lack of Fit.

VITA

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Publications:

Nutrient dynamics in switchgrass (*Panicum virgatum* L.) as a function of time. Submitted to Agronomy Journal

Switchgrass nutrient removal and yield response to nitrogen and phosphorus fertilization. Submitted to Agrosystems, Geosciences & Environment

Professional Memberships:
American Society of Agronomy