

Article

Nitrogen Fertilization and Harvest Timing Affect Switchgrass Quality

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Abstract: Early season switchgrass (*Panicum virgatum* L.) can be used as animal feed and mature late-season biomass as a biofuel feedstock. However, nitrogen (N) application and harvest timing effects on the quality of both end-use need further evaluation. This study evaluated the changes in nutritive quality for animal feed and biofuel feedstock, under different N application rates (0 to 235 kg N ha⁻¹ yr⁻¹) and different harvest times at a fixed N rate. Plant N removal increased with increasing N application rate ($P < 0.05$). The largest single difference (27%) was found between 0 and 33.6 kg N ha⁻¹ application rates. Nitrogen removal decreased during subsequent harvests at a fixed N rate ($P \leq 0.0001$). Forage quality was affected by N rates, although it was especially 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 ($P \leq 0.0001$). 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 grazing, and late-season harvests are better for biofuel production.

Keywords: switchgrass; biomass quality; forage quality; biofuel biomass quality; harvest timing; nitrogen fertilization

1. 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. There have been calls for the development of renewable transportation fuels for many years, in order to help mitigate environmental degradation and lessen greenhouse gas emissions [1–3]. Switchgrass (*Panicum virgatum*) has been identified as a viable source of sustainable biomass for fuel conversion [4,5], especially as a potential for liquid transportation fuel. Other potential uses that can be used for products like bioplastics and bioactive compounds prior to conversion for energy use also make it an attractive source of material, and potential for phytoremediation. [6–11]. Given an alternative fuel infrastructure and market for bio-based products, the development of practices for switchgrass management are desirable. A focus on a dual-use harvest management program, in which switchgrass biomass is harvested as forage in the early growing season, and harvested for biofuel feedstock later in the season, has been studied by Richner et al. [12], to develop production options for producers.

Managing switchgrass as a dual-use crop could be ideal for many areas of North America [13]. 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. [14]

demonstrated that the latitude of origin of a switchgrass cultivar affects the yield potential and nutrient content of harvested biomass. Desirable quality required is dependent upon its intended end use [15,16]. 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 [17–19]. 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 [20–23]. Harvest timing and number of harvests per year can affect stand longevity and total yield of the stand [12,24–27].

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 by multiplying N content by 6.25), mineral contents, acid detergent fiber (ADF), neutral detergent fiber (NDF), and total digestible nutrients (TDN). 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 [12,28]. 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 [19,29]. On the other hand, higher nutrient concentrations in the forage would be desirable for livestock consumption. The desired qualities for the two different uses are in opposition to one another. This makes harvest timing critical, as concentrations of quality parameters change with morphological and phenological growth stages during the growing season.

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, NDF and TDN contents, and to monitor selected quality parameters during the growing season, by harvesting switchgrass at different accumulated growing degree days (AGDD) to aid the decision on final use.

2. Materials and Methods

2.1. Experiment Design and Treatment Description

A nitrogen (N) 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 N 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) with 4 replications. Each plot measured 6.1 × 6.1 m and was fertilized each year after soil sampling in the spring. Nitrogen was applied manually as urea (46-0-0). 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 the remaining N of other treatments was applied in early May to reach total N needed. Triple superphosphate (TSP; 0-46-0) was applied only to individual plots where soil test P was deficient, in order for mitigate any N × P interaction. The soil at the site is a Norge loam (fine silty, mixed, active, and thermic Udic Paleustoll) [30].

2.2. Soil Analysis

Soil samples were collected from a 0 to 15 cm soil layer from each plot before fertilization each year (Table 1). Samples were oven-dried at 65 °C 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 analyzer [31]. Plant available phosphorus (P), potassium

(K), calcium (Ca), and magnesium (Mg) were extracted using a Mehlich-3 solution [32]. Sulfate-S was extracted by 0.008M calcium phosphate. Micronutrients iron (Fe), zinc (Zn), boron (B), and copper (Cu) were extracted by DPTA-Sorbitol [33]. All nutrients in the extracts were quantified by a Spectro inductively coupled plasma (ICP) spectrometer [34]. The soil pH and nutrient contents were consistent and slightly or not different across years, as per the standard deviation (Table 1). These ensured treatments were different and affected only by the nitrogen rates and harvest timing.

Table 1. Soil pH and plant available nutrients tested by year (2008, 2009, and 2010). Samples prior to fertilizer application (pre-season soil sampling).

Year	pH	NO ₃ -N -N	P	K	SO ₄ -S	Ca	Mg	Fe	Zn	B	Cu
mg kg ⁻¹											
2008	6.3	2.7	16.8	120	8.8	1614	326	48.5	0.7	0.34	1.38
Std. dev.	0.19	0.4	2.9	17	0.9	171	34	10.7	0.07	0.01	0.11
2009	6.2	7.1	15	115	5.5	1612	324	60.3	0.82	0.36	1.5
Std. dev.	0.19	8.6	2.7	20	0.0	172	33	9.3	0.09	0.03	0.04
2010	6.4	2.5	16	114.6	6.3	1587	315	58.4	0.72	0.27	1.5
Std. dev.	0.11	1.0	3.0	13.2	1.8	158	29	8.0	0.18	0.01	0.28

Averaged data was calculated from samples taken on each plot. Std. dev. = Standard deviation of the mean (n = 32).

2.3. Switchgrass Biomass Harvest Sampling

Research plots (6.1 × 6.1 m) were divided into two subplots, each 3.0 × 6.1 m for separate sampling (harvest) regimes. One subplot was harvested using a flail harvester 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 × 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. Therefore, the average N rate of 134.4 kg N ha⁻¹ for 2008, 2009, and 2010 has been 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, and growth stages [35,36] are listed in Table 2. Growing degree days were calculated as shown in Equation (1) [21].

$$\text{GDD} = [(\text{maximum daily temperature} + \text{minimum daily temperature})/2] - 10 \text{ } ^\circ\text{C}, \quad (1)$$

Table 2. Dates of periodic plant biomass harvests from one of the nitrogen treatments (134.4 kg N ha⁻¹) and associated accumulated growing degree days (AGDD).

Year	Harvest	Date	Julian DOY	AGDD	Growth Stage
2008	1	12 June 2008	164	755	E1
2008	2	24 July 2008	206	1452	R2
2008	3	5 September 2008	249	2175	S3
2008	4	30 October 2008	304	2611	Senescence
2008	5	4 December 2008	339	2672	Senescence
2009	6	28 February 2009	425	2748	V0
2009	1	3 July 2009	184	1180	E4
2009	2	9 August 2009	221	1822	S0
2009	3	25 September 2009	268	2426	S5
2009	4	19 November 2009	323	2627	Senescence
2010	5	26 January 2010	391	2642	G5
2010	6	2 March 2010	426	2642	G5

Table 2. Cont.

Year	Harvest	Date	Julian DOY	AGDD	Growth Stage
2010	1	15 July 2010	196	1323	R1
2010	2	3 September 2010	246	2240	S4
2010	3	28 October 2010	301	2803	Senescence
2010	4	2 December 2010	336	2878 288	Senescence
2011	5	7 January 2011	372	2888	G5
2011	6	25 March 2011	449	3031	V1

Growth stages [35], using estimated mean stand count (MSC) calculated from AGDD by calendar year as $MSC = 0.875 + (0.0017 \times AGDD)$ [36]. Julian DOY: Julian day of the year.

Daily maximum and minimum temperatures in Stillwater (2008, 2009 and 2010) were obtained from the Oklahoma Mesonet [37]. Accumulated growing degree days was calculated by summing positive GDD ($GDD > 0$), beginning January 1 of each year [6,9]. The mean growth stage count (MSC) in Table 2 was calculated using Equation (2) [36].

$$MSC = [0.875 + (0.0017 \times AGDD)], \quad (2)$$

Switchgrass growth stages and descriptions are found in Moore et al. [36], and MSC values of 0.0 to 4.9 were used to describe switchgrass growth stages (Table 2). Estimated $MSC > 4.9$ were listed as “post-ripening/senescence” in our study, since some harvests took place after physiological maturity and senescence.

2.4. 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) and neutral detergent fiber (NDF) were determined by the filter bag technique [15]. Total digestible nutrient (TDN) is a measure of forage energy and was calculated from ADF values: $TDN = [98.625 - (1.048 \times ADF)]$, as per Ashworth et al. [16] 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 digestion products were then heated to 115 °C for 2 h and diluted with deionized water to 50 mL [38]. Digested samples were analyzed by an 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.

2.5. Statistical Analysis

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 the SAS program, and the statistical analyses were performed with $n = 3$ for those specific treatments [39]. To evaluate biomass quality as affected by N rates and harvest date, regression analyses were performed for all replicated data by fertilizer rates, and by harvest date in AGDD. Nitrogen and mineral concentrations were evaluated for all study years, but ADF, NDF and TDN were evaluated for 2009 and 2010. 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. Equation coefficients of each model were tested for significance as well (Tables S1–S4, Supplementary Material).

3. Results

3.1. Biomass Quality Parameters as Affected by Nitrogen Fertilization Rates

The relationships between N, ADF, NDF and TDN in plant biomass harvested at maturity and N application rates are shown in Figures 1 and 2. Regression analysis indicated increases in N ($P = 0.002$ in 2008 and 2009, and $P = 0.03$ in 2010) (Figure 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) (Figure 2) as N application rates increased. Trend analysis showed decreases in TDN ($P < 0.0001$ in 2009, and $P = 0.01$ in 2010) by N application rate (Figure 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 $235.2 \text{ kg N ha}^{-1}$ of input. A numerical increase in NDF was seen with increased N rates, ranging from 829 g kg^{-1} for $33.6 \text{ kg N ha}^{-1}$ to 890 g kg^{-1} for $168.0 \text{ kg N ha}^{-1}$ applied. Total digestible nutrients tended to decrease as N rate increased from 394 g kg^{-1} at $235.2 \text{ kg N ha}^{-1}$ to 472 g kg^{-1} at 0 kg N ha^{-1} (Figure 2).

In 2010, no forage analysis was significantly affected by N application rates using ANOVA and PROC GLM. This could be a lack of need for N fertilizer applications in native prairie grasses, as they evolved without additional fertilization [40,41]. 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 $134.4 \text{ kg N ha}^{-1}$. The highest individual value for NDF was 824 g kg^{-1} at $168.0 \text{ kg N ha}^{-1}$. Total digestible nutrients decreased slowly with an increased N rate to an average of 435 g kg^{-1} at $100.8 \text{ kg N ha}^{-1}$. 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 $134.4 \text{ kg N ha}^{-1}$. 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 ($P = 0.076$).

3.2. Mineral Concentration as Affected by Nitrogen Fertilization Rates

Mineral concentrations of harvested biomass at maturity had no significant differences with respect to N rates, with a few exceptions (Table 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 $201.6 \text{ kg N ha}^{-1}$. The largest single increase in N occurred between the control plot and the lowest N rate, $33.6 \text{ kg N ha}^{-1}$, from 3.02 g kg^{-1} to 3.78 g kg^{-1} (Table 3). Phosphorus and potassium concentrations did not change significantly by N rates ($P > 0.05$). Other differences in nutrient concentrations due to N rate were insignificant ($P > 0.05$). There were significant differences according to Duncan's multiple range test in Mg between the 67.2 and $134.4 \text{ kg N ha}^{-1}$ rates, and in Cu between the 33.6 and $67.2 \text{ kg N ha}^{-1}$ rates in 2008.

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 $201.6 \text{ kg N ha}^{-1}$ rate, then decreasing at the $235.2 \text{ kg N ha}^{-1}$ 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 2009, more significant differences were seen in K, Ca, Mg, S, Cu, Fe, Zn and Mn; often with one rate being significantly different from others according to Duncan's multiple range test. P -values of all micronutrients except Cu were insignificant (Table 3). In 2010, no significant differences in macronutrient concentration between N rates were found. Significant differences ($P < 0.05$) 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. In similar studies focused on switchgrass nutrient concentration, response to N fertilization was shown to be insignificant for most parameters [42].

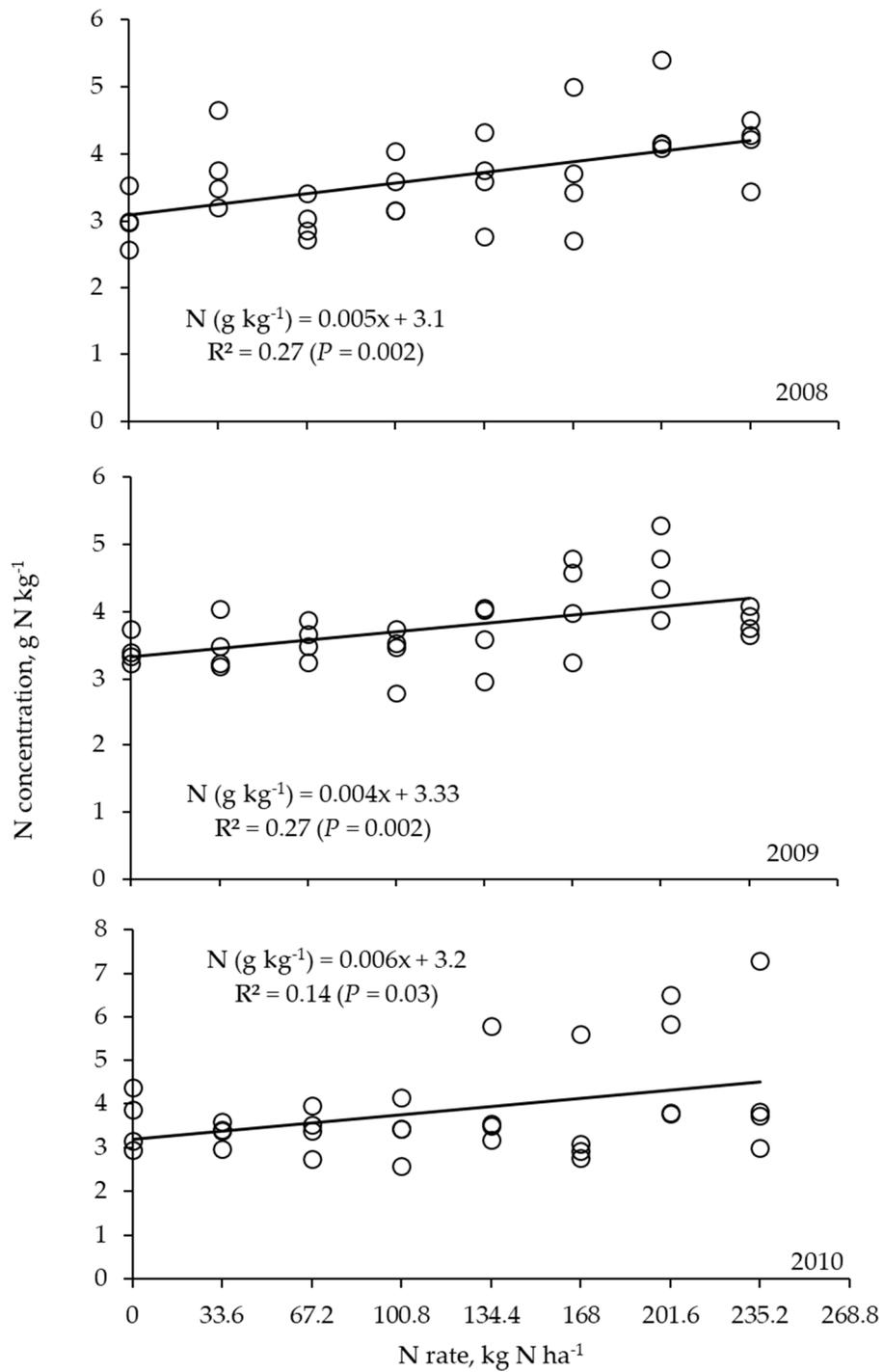


Figure 1. Changes in nitrogen (N) concentrations in switchgrass biomass as a function of the amount of N applied.

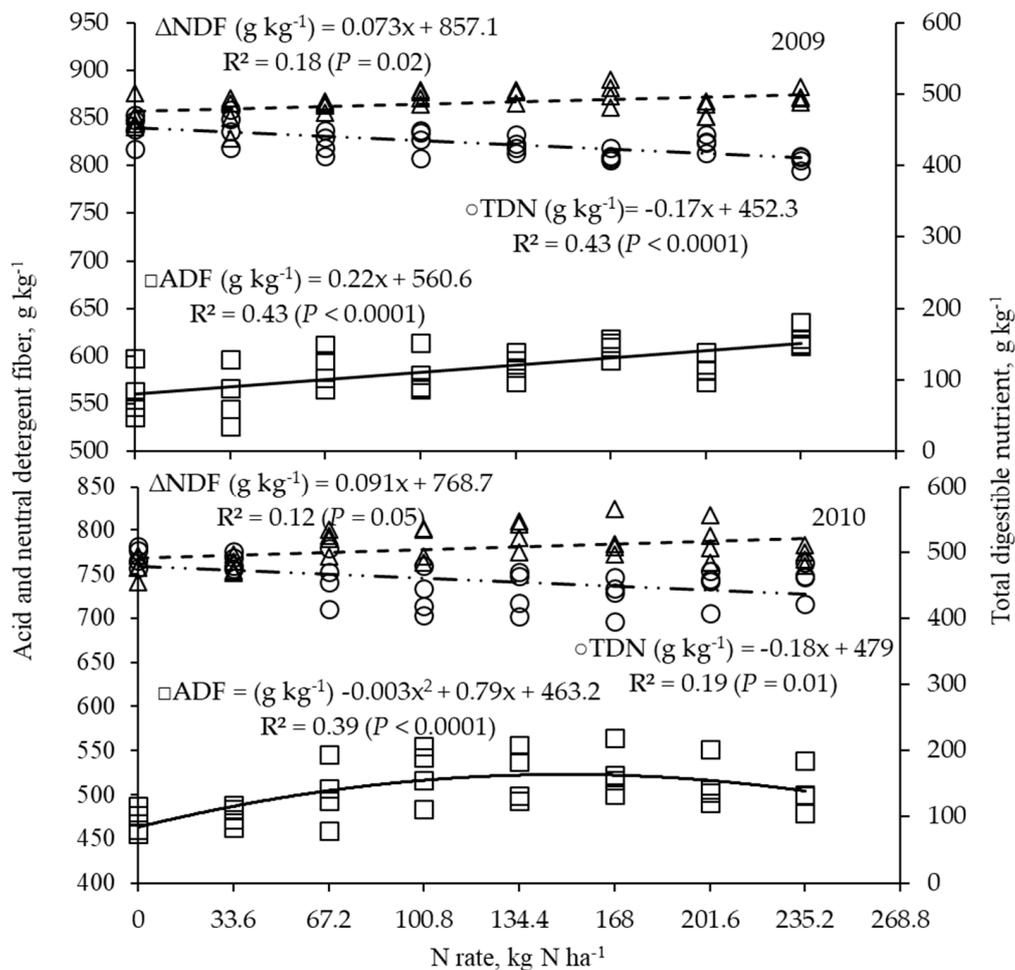


Figure 2. Relationship of acid detergent fiber (ADF), neutral detergent fiber (NDF), and total digestible nutrients (TDN) and nitrogen application rates.

Table 3. Mineral concentrations of harvested biomass, as affected by nitrogen (N) fertilization rate.

Year	N Rate kg ha ⁻¹	g kg ⁻¹									
		N	P	K	Ca	Mg	S	Cu	Fe	Zn	Mn
2008	0	3.02c	0.90	3.95	1.83	1.35ab	0.50	7.7ab	58.0	19.0	51.8
	33.6	3.78abc	0.88	3.35	2.18	1.50ab	0.48	8.1a	57.0	15.6	59.1
	67.2	3.01c	0.83	3.28	1.65	1.30b	0.43	6.4b	63.9	13.0	44.7
	100.8	3.48bc	0.85	3.25	1.95	1.48ab	0.45	7.2ab	61.8	14.2	47.9
	134.4	3.61abc	1.00	4.25	2.00	1.78a	0.53	8.0ab	62.4	17.9	48.4
	168.0	3.71abc	0.78	3.63	1.93	1.58ab	0.45	7.0ab	57.0	15.3	57.2
	201.6	4.44a	0.80	3.80	1.90	1.58ab	0.48	7.1ab	55.7	15.5	46.2
	235.2	4.12ab	0.75	3.23	1.93	1.60ab	0.48	7.4ab	56.9	15.6	44.4
	Avg.	3.64	0.85	3.59	1.92	1.52	0.47	7.4	59.1	15.7	49.9
	Std. dev.	0.70	0.16	0.83	0.34	0.29	0.08	1.1	11.7	3.8	13.5
P	0.013	0.479	0.441	0.633	0.351	0.802	0.315	0.977	0.486	0.515	
F test	3.43	0.98	0.81	0.72	1.10	0.53	1.1	0.2	1.0	0.6	
CV%	19.13	18.97	23.07	17.63	19.03	17.22	14.7	19.7	24.2	26.9	

Table 3. Cont.

Year	N Rate	N	P	K	Ca	Mg	S	Cu	Fe	Zn	Mn
	kg ha ⁻¹	g kg ⁻¹						mg kg ⁻¹			
2009	0	3.43bc	0.80a	2.32a	1.62ab	1.04ab	0.36bc	2.0c	32.5ab	15.4ab	70.7a
	33.6	3.48bc	0.77a	2.34a	1.92a	1.19a	0.41ab	2.4bc	32.0ab	15.3ab	66.5ab
	67.2	3.57bc	0.64ab	2.37a	1.60ab	1.05ab	0.37abc	2.5b	45.6a	12.9ab	64.5ab
	100.8	3.38c	0.76a	2.42a	1.67ab	1.09ab	0.39abc	2.8b	34.0ab	15.9ab	52.7ab
	134.4	3.66bc	0.66ab	2.41a	1.57ab	1.13a	0.38abc	2.7b	33.4ab	14.4ab	58.2ab
	168.0	4.15ab	0.53bc	2.01ab	1.48ab	1.08ab	0.39abc	2.9b	33.4ab	12.0ab	53.6ab
	201.6	4.57a	0.68ab	2.14ab	1.65ab	1.16a	0.44a	3.6a	37.5ab	16.4a	46.3ab
	235.2	3.86bc	0.41c	1.70b	1.24b	0.93b	0.33c	2.5b	20.6b	11.3b	39.7b
	Avg.	3.76	0.66	2.21	1.59	1.08	0.38	2.7	33.6	14.2	56.5
	Std. dev.	0.55	0.16	0.39	0.32	0.13	0.05	0.5	15.6	3.2	19.5
	P	0.012	0.0003	0.102	0.216	0.126	0.091	<0.0001	0.527	0.145	0.292
	F test	3.49	6.83	2.01	1.52	1.87	2.09	8.9	0.9	1.8	1.3
CV%	14.74	23.99	17.51	20.01	12.39	13.23	19.0	46.3	22.6	34.6	
2010	0	3.59	0.73a	1.84	2.80	1.46	0.50a	2.4	44.5	25.2a	85.1
	33.6	3.34	0.56b	2.06	2.41	1.23	0.41ab	1.9	43.8	17.8b	77.6
	67.2	3.41	0.56b	2.05	2.19	1.34	0.38ab	2.4	43.7	16.0b	78.0
	100.8	3.40	0.58b	2.22	1.99	1.24	0.35b	2.7	39.4	15.1b	67.8
	134.4	4.00	0.57b	1.68	2.09	1.43	0.37b	1.8	40.0	15.0b	71.3
	168.0	3.60	0.55b	1.82	2.10	1.41	0.38ab	2.2	40.5	14.4b	64.6
	201.6	4.98	0.62ab	1.66	2.36	1.62	0.46ab	3.0	41.3	17.4b	62.3
	235.2	4.46	0.54b	1.52	2.56	1.54	0.42ab	2.3	41.3	15.0b	70.2
	Avg.	3.85	0.59	1.86	2.31	1.41	0.41	2.3	41.9	17.0	72.1
	Std. dev.	1.14	0.11	0.45	0.50	0.24	0.08	0.8	7.4	4.5	18.1
	P	0.450	0.205	0.368	0.323	0.305	0.136	0.416	0.985	0.005	0.678
	F test	1.01	1.55	1.16	1.25	1.29	1.82	1.1	0.2	4.3	0.7
CV%	29.58	18.60	24.46	21.77	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 ($P > 0.05$) whereas 'a' means higher than 'b' and not different from 'ab' or 'abc' based on Duncan's Multiple Range Test.

3.3. 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 and 4. Parameters ADF and NDF tended to increase with maturity as the harvesting date got later and AGDD increased ($P < 0.0001$). These results were similar to Sena et al. [15], 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.

3.4. Mineral Concentration as Affected by Accumulated Growing Degree Days

Significant differences in mineral concentrations existed among biomass harvested with various AGDD (Table 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. Significant differences were not detected in Ca and Fe in 2010. In 2010, phosphorus, sulfur and manganese were not analyzed due to errors in laboratory analysis and lack of data. Significant variation in annual and field-to-field production has been shown in other studies [43].

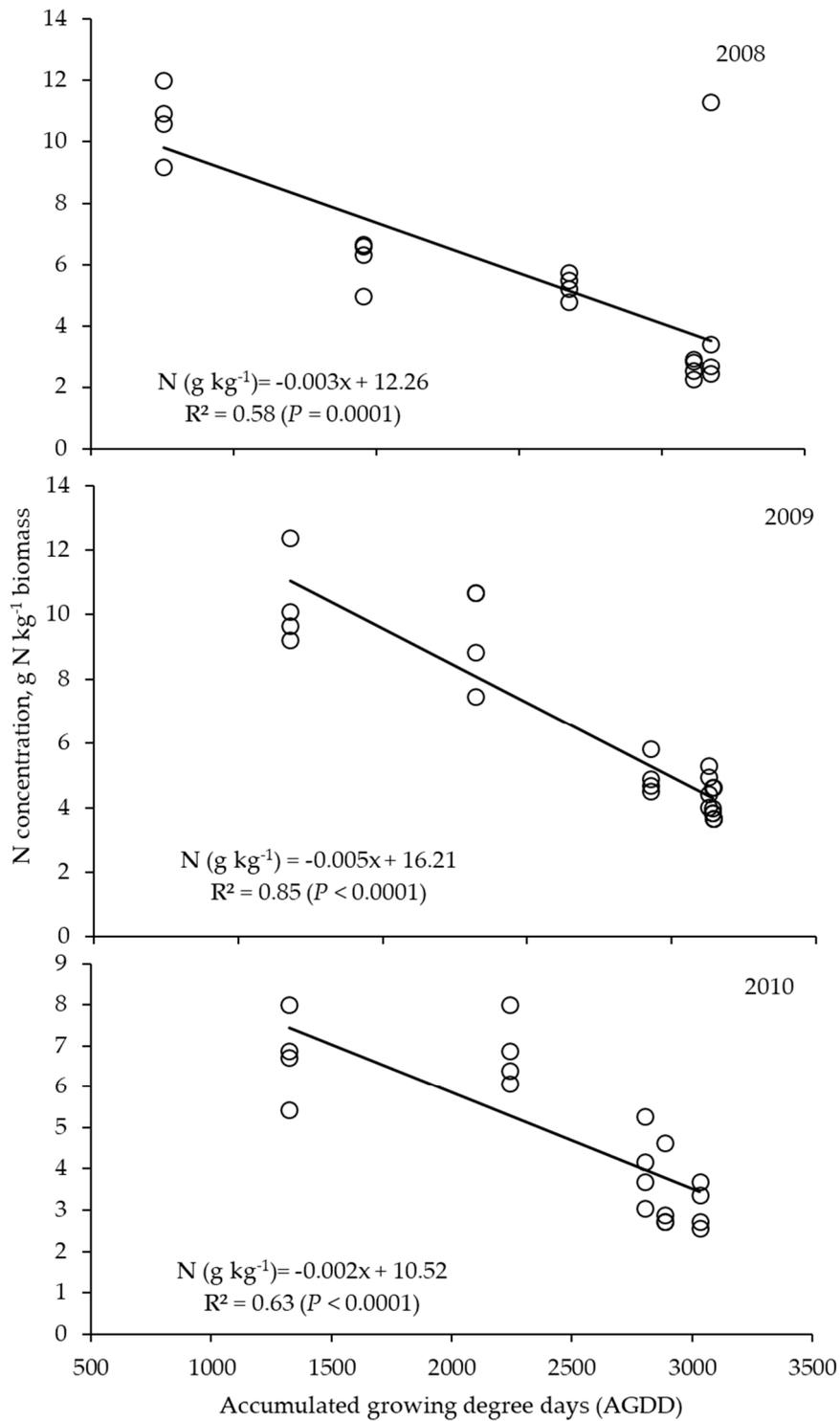


Figure 3. Nitrogen concentration as affected by accumulated growing degree days.

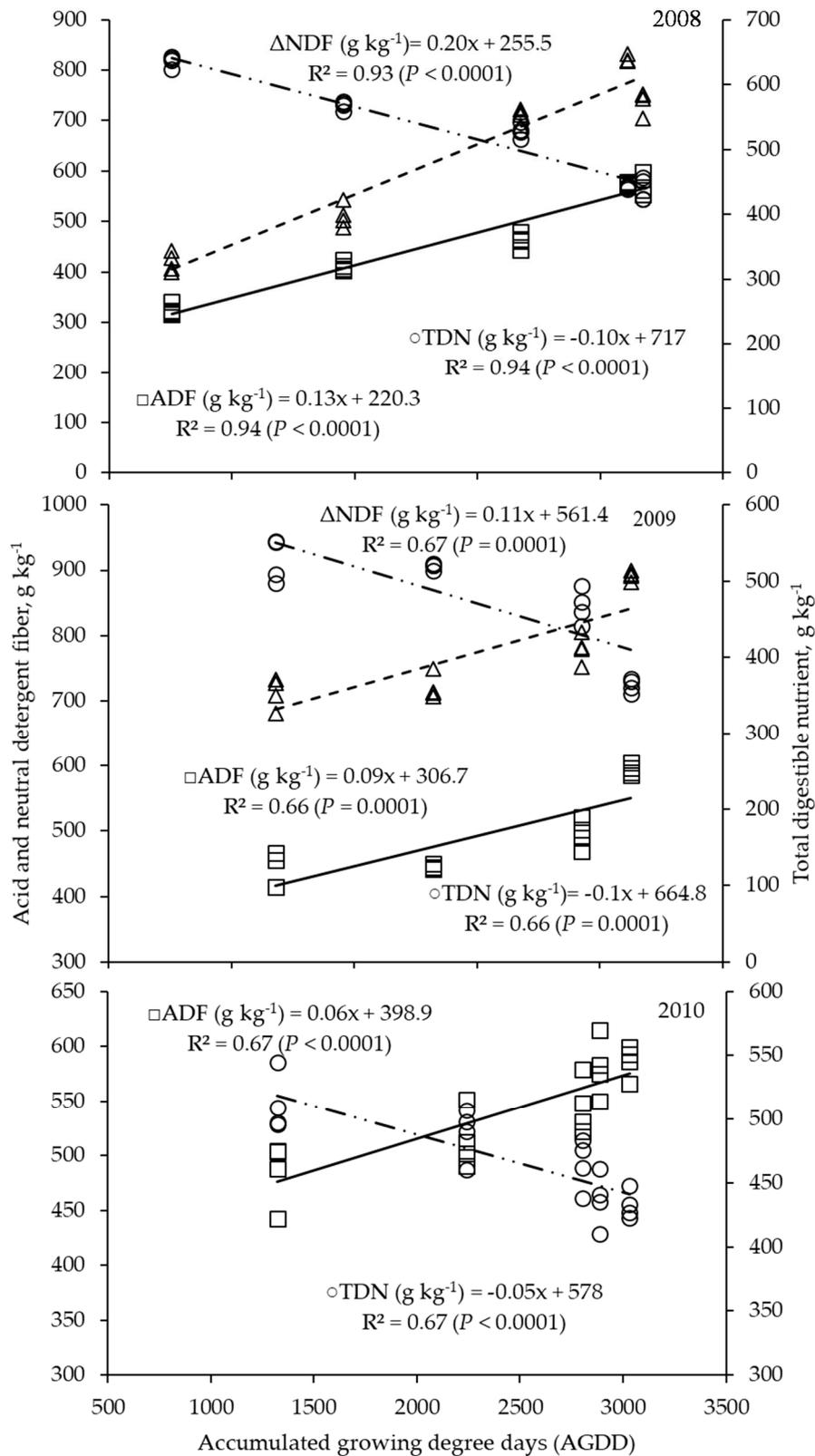


Figure 4. Acid detergent fiber (ADF), neutral detergent fiber (NDF), and total digestible nutrients (TDN), as affected by accumulated growing degree days.

Table 4. Mineral concentrations in harvested biomass as affected by harvest timing (AGDD, accumulated growing degree days).

Year	AGDD	N	P	K	Ca	Mg	S	Cu	Fe	Zn	Mn
						g kg ⁻¹					
						mg kg ⁻¹					
2008	754.7	7.10a	1.78a	15.13a	2.30a	1.80a	0.83a	25.7b	106.8ab	26.2a	77.7a
	1452	4.51b	1.15b	7.93b	2.03ab	1.50ab	0.60b	23.3b	88.7ab	22.7ab	82.0a
	2175	3.44bc	1.10b	6.38c	1.68b	1.38bc	0.50bc	5.9b	54.8ab	19.3bc	54.8b
	2611	3.26bc	0.78c	3.58d	1.68b	1.43b	0.40cd	12.2b	145.6a	15.5c	47.0b
	2672	2.95c	0.75c	2.93d	1.45b	1.08cd	0.35d	6.2b	134.2a	15.2c	41.4b
	2748	3.04c	0.50d	1.50e	1.48b	1.01d	0.07e	220.5a	16.0b	8.5d	43.6b
	Avg.	4.05	1.01	6.44	1.77	1.36	0.47	50.6	94.4	17.9	57.7
	Std. dev.	1.63	0.44	4.64	0.49	0.34	0.24	89.1	64.6	6.5	19.7
	P	<0.0001	<0.0001	<0.0001	0.0272	0.0013	<0.0001	<0.0001	0.0440	<0.0001	0.0010
	F Test	15.59	31.17	204.59	3.49	7.23	49.7	14.3	2.8	15.3	7.6
CV%	40.29	43.96	10.47	27.50	24.60	14.31	87.9	56.4	36.4	34.2	
2009	1180	10.34a	1.64a	10.1a	2.17ab	1.95a	0.77a	9.4a	60.2	25.9a	77.6a
	1822	9.42a	1.55a	7.87b	2.69a	2.07a	0.81a	9.8a	75.1	29.4a	68.7ab
	2426	4.99b	1.08b	4.22c	1.24c	1.05b	0.39b	5.2b	81.2	16.2b	50.6bc
	2627	4.68b	0.76c	2.29d	1.29c	1.05b	0.36b	1.5c	43.0	14.1b	42.1c
	2642	4.00b	1.00b	1.75d	2.00b	—	—	—	—	—	—
	2642	4.00b	1.00b	1.00e	2.00b	—	—	—	—	—	—
	Avg.	6.24	1.17	4.13	1.90	1.53	0.58	6.5	64.9	21.4	59.7
	Std. dev.	2.80	0.35	3.28	0.63	0.55	0.22	3.7	27.7	7.2	21.3
	P	<0.0001	<0.0001	<0.0001	0.0007	<0.0001	<0.0001	<0.0001	0.2616	0.0006	0.0148
	F Test	41.97	33.45	327.32	8.63	26.21	52.04	40.0	1.6	16.3	6.1
CV%	44.85	29.58	8.955	33.22	36.22	38.57	19.1	42.8	33.8	35.7	
2010	1323	6.76a	1.00	7.75a	2.25	1.75a	1.00	5.5a	70.1	23.1a	—
	2240	6.84a	1.00	6.25b	2.25	1.75a	1.00	4.6b	92.1	20.4ab	—
	2803	4.04b	1.00	3.00c	1.75	1.50ab	1.00	4.2b	110.5	19.3ab	—
	2888	3.24bc	1.00	1.75d	1.75	1.00b	—	3.0c	55.7	18.2bc	—
	3031	3.08c	1.00	1.25d	1.50	1.00b	—	3.1c	97.8	14.5c	—
	Avg.	4.79	1.00	4.00	1.90	1.40	1.00	4.1	85.2	19.1	—
	Std. dev.	1.88	0.00	2.70	0.55	0.50	0.00	1.3	42.1	4.6	—
	P	<0.0001	—	<0.0001	0.2859	0.0489	—	<0.0001	0.2569	0.0056	—
	F Test	39.65	—	70.18	1.42	3.29	—	16.6	1.5	6.3	—
	CV%	39.32	0.00	17.08	29.08	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 ($P > 0.05$) whereas 'a' means higher than 'b' and not different from 'ab' or 'abc' based on Duncan's Multiple Range Test.

4. Discussion

Acid detergent fiber and neutral detergent fiber 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, therefore more vegetative growth with N fertilization would be expected [40].

The concentration of N in the biomass generally increased with increasing fertilizer N rates, regardless of the study year. In each year, the highest mean N concentration was 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 [17], thus lignin and cellulose with low nutrient content is needed for biofuel conversion. Nutrients and protein are desirable for livestock consumption.

Therefore, harvest for biofuel feedstock should take place after senescence to avoid high N in the biomass [19,44].

Those trends in NDF and ADF in switchgrass in our study were also similar to NDF and ADF trends reported by Aurangzaib et al. [45], and Kering et al. [27]. Nitrogen and TDN, on the other hand, decreased with increasing AGDD and later harvest date ($P < 0.001$) (Figures 3 and 4). Commonly, nitrogen and nonstructural carbohydrates decline, and lignin and cellulose increase through the growing season of switchgrass [45]. These changes are gradually taking place with plant growth as it moves to maturity and completes its cycle of producing seed.

The ADF and NDF in biomass harvested after flowering and senescence are 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 [46].

Biomass harvested after a killing frost would be mostly fibrous with low nutritive value. 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 [12,16]. The high ADF and NDF of late-harvested biomass, however, is more desirable in lignocellulosic biofuel conversion [47]. 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 [48]. 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 a greater amount consumed and digested [12,49]. A high-quality livestock forage should have ADF $< 300 \text{ g kg}^{-1}$, NDF $< 400 \text{ g kg}^{-1}$, and TDN $> 600 \text{ g kg}^{-1}$ and N $> 30 \text{ g kg}^{-1}$ [16], but the late-harvested, i.e., post senescence, switchgrass is far from considered a quality forage. Therefore, earlier harvest, prior to reproductive growth stages, perhaps early July, should be considered if feeding switchgrass to livestock is desired.

Similar to Sena et al. [15] and Ashworth et al. [44], N concentration in switchgrass was high if harvested in the early season (May–June), and declined throughout the rest of the growing season. Generally, mineral concentration in the plant decreases as the season progresses, as would be expected in native perennial grasses. All mineral analyses indicated decreases in mineral concentration with increasing AGDD. Not all decreases are significant based on Duncan's multiple range test; such as P, Ca, and S in 2010 and Fe in 2009 and 2010. As previously mentioned, year-to-year variation is not uncommon in switchgrass [41,43]. These types of decreases have been shown in another study regarding harvest timing as well [50]. Since mineral concentrations decreased as AGDD increased, harvesting at earlier growing season with lower AGDD would preserve more nutrients and favor forage use, while a later season harvest with higher AGDD would offer more desired quality for biofuel production.

5. Conclusions

Acid detergent fiber, neutral detergent fiber, total digestible nutrient, and nitrogen (or crude protein) and mineral concentrations were affected by N application rates for switchgrass production. Those quality parameters were especially impacted by harvesting time. Overall, 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 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.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2079-9276/9/6/61/s1>, Table S1: Equation parameters of linear regression models between nitrogen (N) concentrations in switchgrass biomass as a function of the amount of N applied in 2008, 2009, and 2010, Table S2: Equation parameters between acid detergent fiber (ADF), neutral detergent fiber (NDF), and total digestible nutrients (TDN) and nitrogen application rates in 2009 and 2010, Table S3: Equation parameters of linear regression models between nitrogen (N) concentrations in switchgrass biomass as a function of accumulated growing degree days (AGDD) in 2008, 2009, and 2010, Table S4: Equation parameters between acid detergent fiber (ADF), neutral detergent fiber (NDF), and total digestible nutrients (TDN) and accumulated growing degree days (AGDD) in 2008, 2009, and 2010.

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