4-2016

Compositional Differences among Upland and Lowland Switchgrass Ecotypes Grown as a Bioenergy Feedstock Crop

Muhammad Aurangzaib
Iowa State University, zaib@iastate.edu

Kenneth J. Moore
Iowa State University, kjmoore@iastate.edu

Sotirios V. Archontoulis
Iowa State University, sarchont@iastate.edu

Emily A. Heaton
Iowa State University, heaton@iastate.edu

Andrew W. Lenssen
Iowa State University, alenssen@iastate.edu

See next page for additional authors

Authors
Muhammad Aurangzaib, Kenneth J. Moore, Sotirios V. Archontoulis, Emily A. Heaton, Andrew W. Lenssen, and Shuizhang Fei

Compositional Differences among Upland and Lowland Switchgrass Ecotypes Grown as a Bioenergy Feedstock Crop

Muhammad Aurangzaib[1*], Kenneth J. Moore[1], Sotirios V. Archontoulis[1], Emily A. Heaton[1], Andrew W. Lenssen[1], Shuizhang Fei[2]

[1] Department of Agronomy, Iowa State University, Ames, IA. 50011, USA
[2] Department of Horticulture, Iowa State University, Ames, IA. 50011, USA
[*]Corresponding Author**,** zaib@iastate.edu

Abstract

Feedstock quality mainly depends upon the biomass composition and bioenergy conversion system being used. Higher cellulose and hemicellulose concentrations are desirable for biochemical conversion, whereas higher lignin is favored for thermochemical conversion. The efficiency of these conversion systems is influenced by the presence of high nitrogen and ash concentrations. Switchgrass (*Panicum virgatum* L.) varieties are classified into two ecotypes based on their habitat preferences, i.e., upland and lowland. The objectives of this study were to quantify the chemical composition of switchgrass varieties as influenced by harvest management, and to determine if ecotypic differences exist among them. A field study was conducted near Ames, IA during 2012 and 2013. Upland ('Cave-in-Rock', 'Trailblazer' and 'Blackwell') and lowland switchgrass varieties ('Kanlow' and 'Alamo') were grown in a randomized block design with six replications. Six biomass harvests were collected at approximately 2-week intervals each year. In both years, delaying harvest increased cellulose, hemicellulose and lignin concentrations while decreasing nitrogen and ash concentrations in all varieties. On average, Kanlow had the highest cellulose and hemicellulose concentration (354 and 321 g kg^{-1} DM respectively), and Cave-in-Rock had the highest lignin concentration (33 g kg^{-1} DM). The lowest nitrogen and ash concentrations were observed in Kanlow (14 and 95 g kg^{-1} DM respectively). In general, our results indicate that delaying harvest until fall improves feedstock quality, and ecotypic differences do exist between varieties for important feedstock quality traits. These findings also demonstrate potential for developing improved switchgrass cultivars as bioenergy feedstock by intermating lowland and upland ecotypes.

Keywords

Switchgrass; Ecotypes; Biomass yield; Biomass quality; Harvest management; Feedstock composition

Abbreviations

ADF: Acid detergent fiber; *ADL*: Acid detergent lignin; *NDF*: Neutral detergent fiber; *TNC*: Total nonstructural carbohydrates; *N*: Nitrogen; *DM*: Dry matter

This is the accepted manuscript of an article published in *Biomass and Bioenergy* 87 (2016): 169–177, doi:10.1016/j.biombioe.2016.02.017.

1. **Introduction**

Interest in replacing fossil fuels with alternative biofuels has increased due to instability in oil producing countries, uncertainty of crude oil prices in international markets and environmental concerns. One of the solutions to these problems is the use of alternative energy resources, including biofuels [1]. The United States produces ethanol mainly from grain crops [2, 3], but grain based ethanol has been reported to produce an equal amount of greenhouse gas emissions as production of petroleum based fuels [3]. One way to overcome this problem is to produce biofuels from lignocellulosic feedstocks [2, 3]; therefore, maximizing biomass production of dedicated bioenergy crops through improved genetics and agricultural practices is a necessity.

Recently, warm-season grasses have gained increased attention by the scientific community as a reliable source for lignocellulosic feedstock supply. Switchgrass (*Panicum virgatum* L.), a C_4 grass native to United States, has been identified as a strong candidate for a 2^{nd} generation bioenergy feedstock in Central and North America [4]. Some of its desirable traits include perenniality, adaptability to marginal land and high biomass yield potential [5].

There are two ecotypes of switchgrass classified by their habitat preference i.e., upland and lowland. Upland ecotypes are frequently found in dry climates and at higher latitudes while lowland ecotypes are mostly found in wet climates at lower latitudes [6-8]. Biomass yields of these ecotypes mainly depend on the origin of cultivar and cultural practices like fertilizer rate and harvest management. Usually, when both ecotypes are grown at the same latitude, cultivars selected from northern latitude tend to have lower biomass yields, early flowering and longer winter dormancy than cultivars that originated in more southern latitudes; whereas, southern ecotypes have delayed flowering, produce thicker stems and more biomass than upland ecotypes [9, 10]. Generally, establishment of a switchgrass stand is slow and two to three years are required to reach peak biomass yields. However, once established it can be maintained for more than 10 years [11-13].

High biomass yield is an important attribute of an ideal bioenergy crop, but the quality of a feedstock for conversion is also important. Lignocellulose is the most important and the largest constituent of biomass dry matter from dedicated bioenergy crops, which is primarily made up of cellulose, hemicellulose, lignin and mineral elements. Feedstock quality, however, depends upon the bioenergy conversion system used to convert the biomass to fuel (e.g. thermochemical, biochemical or direct combustion system [14, 15]. High mineral concentration, notably nitrogen and ash concentrations, decrease the efficiency of direct combustion and thermochemical conversion systems [17]. Lignin, on the other hand, is important for thermochemical conversion processes, but since it also binds with cellulose and hemicellulose, higher concentrations of lignin also limits the availability of cellulose and hemicellulose during biochemical conversion processes, resulting in reduced biofuel yields [14, 15, 17]. In addition to the above-mentioned structural carbohydrates (i.e. cellulose and hemicellulose), switchgrass also contains nonstructural carbohydrates including sucrose, glucose, fructose and starch. These sugars are not present in very high concentrations compared to the structural carbohydrates, but can be used as a source of fermentable sugars for liquid fuel production [18, 19].

Concentrations of these important feedstock components can vary significantly due to geographic location, genetic factors, plant maturity and agronomic practices [14, 20, 21]. While many switchgrass compositional and harvest management studies address variability in forage quality, understanding of ecotypic variation in switchgrass quality for bioenergy applications is more limited. To fill this void we conducted an experiment to: (i) quantify the chemical composition of switchgrass cultivars as influenced by harvest date, and (ii) to determine if ecotypic differences exist between upland and lowland switchgrass ecotypes for their chemical composition. This information will help biomass producers choose the best suited variety for biomass production, and also allow researchers to select and improve current ecotypes for increased adaptability and maximum biomass and biofuel production.

2. Materials and Methods

2.1. Experimental Site

To evaluate the dry matter (DM) composition of upland and lowland ecotypes of switchgrass, a study was conducted during 2012 and 2013 on a pre-existing switchgrass variety trial established in 2007 at Iowa State University, Sorenson Research Farm, near Ames, IA (42º0'41" N, 93'44'34" W). The experiment was arranged as a randomized complete block design with six replications of five switchgrass varieties of two distinct origins, upland (Cave-in-Rock, Blackwell and Trailblazer) and lowland (Kanlow and Alamo). Every year before spring growth initiation, standing dead material was mowed to a stubble height of 5 cm and the plant residue removed from the field. To control weeds each year atrazine [6-Chloro-N-ethyl-N'-(1-methylethyl)-1, 3, 5-triazine-2, 4-diamine] and quinclorac [3, 7-Dichloro-8-quinolinecarboxylic acid] were applied before switchgrass emergence at 2.23 kg a.i. ha^{-1} and 0.56 kg a.i. ha^{-1} respectively. Nitrogen, P and K fertilizer were applied every year in early May at 78, 67 and 90 kg ha^{-1}, respectively. Mean monthly air temperature and total precipitation were measured during 2012 and 2013 at a site located less than eight kilometers from the experimental field, and the data were compiled from the Iowa Environmental Mesonet (2014) (Fig.1 and 2).

2.2. Biomass Harvest

Biomass samples were collected from three randomly selected blocks in 2012 and the remaining three blocks were harvested the following year. Each plot was divided into six subplots corresponding to six biomass harvests. Each year depending on the spring growth, the first harvest occurred at early vegetative growth (approximately two weeks after emergence). In 2012 and 2013, the first harvest occurred on the 136th and 149th day of the year (DOY), respectively, and the remaining five harvests were collected at approximately 2-week intervals. At each harvest date, two-0.1 m^2 samples were harvested within each subplot by using a hand clipper at ground level. Each subplot was harvested only once during the growing season.

2.3. Sample Processing

Harvested samples were dried at 60°C in a forced-air oven for 72 h or until a constant sample dry weight was observed. Dried samples were ground to 1 mm with a shear mill (Thomas Scientific, Philadelphia, PA, USA). Ground samples were then mixed thoroughly to obtain subsamples for compositional analysis. These subsamples were kept at ambient humidity for about 48 hr and then stored in plastic vials at room temperature.

2.4. Biomass Composition

All chemical analyses were performed in duplicate. Biomass samples were mixed again before weighing samples for chemical analysis. Sequential fiber analysis was used to determine neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) using an ANKOM 200 Fiber Analyzer (ANKON Technology Corp. Fairport, NY). All values were corrected for moisture concentration. The moisture concentration of each sample was determined by drying another subsample in the oven at

105°C for 4 h. Hemicellulose was calculated as the difference between NDF and ADF concentration, cellulose as the difference between ADF and ADL, and lignin as ADL corrected for ash concentration.

Total nonstructural carbohydrates (TNC) were determined using methods described by Murphy et al. (2012) and Guiragossian et al. (1979). In this procedure, a 0.125 g sample was weighed and placed in a test tube to be refluxed for 1 hr in 25 mL of 0.2-N sulfuric acid. After cooling, the mixture was filtered with Whatman #42 filter paper. A 1-mL subsample was drawn from the filtrate and diluted by a factor of 20 with distilled water. Then, 1 mL of 5% phenol solution and 5 mL of 18-M sulfuric acid were added to the diluted mixture, and the solution's absorbance measured at 490 nm. The absorbance values were then used to determine TNC concentration by using a glucose reference calibration and calculated on the basis of g of glucose kg^{-1} sample dry matter. Nitrogen (N) and carbon (C) were determined using a LECO True Spec™ CN Analyzer (LECO Corp. St. Joseph, MI). Total ash concentration was determined by the method of Undersander et al., (1993) by ashing a 0.5 g subsample in pre-weighed crucibles at 500°C for 4 h..

2.5. Statistical Analysis

Statistical analysis was performed by analysis of variance using the GLIMMIX procedure of SAS 9.4 (SAS Institute Cary, NC USA). Switchgrass variety, year and harvest date were considered fixed effects, while blocks and the interactions with blocks were considered as random effects. Means were compared using *t*-tests and linear contrasts. Differences were considered significant at $P<0.05$.

3. Results

In both years, lowland varieties of switchgrass (Kanlow and Alamo) performed better than the upland varieties (Cave-in-Rock, Blackwell, and Trailblazer) in terms of feedstock quality traits. Climatic conditions were different in both years. The first half of 2012 was warmer compared to 2013 and the 20-year average monthly temperature, while the remaining of the growing season temperatures were close to the 20-year average (Fig. 1). Precipitation in both years was lower than the 20-year average except in April and May of 2013, which received about 49 % and 69 % more rainfall, respectively, than the 20-year average (Fig. 2).

3.1. Cellulose

Cellulose concentration for all switchgrass varieties increased continuously with advanced maturity in both years (Fig. 3). Year, variety and harvest date significantly affected the cellulose concentration and there was an interaction between them (Table 1, Fig. 3). Averaged over both years, Kanlow had the highest cellulose concentration, which was 354.2 g kg^{-1} DM, and was significantly different from all other varieties. Cave-in-Rock had the lowest cellulose concentration, which averaged 330.2 g kg^{-1} DM.

Because there was a significant variety × year interaction, each year was evaluated separately. In 2012, variety and harvest date significantly affected cellulose concentration (Fig. 3). However, in 2013 only harvest date was found to have an effect on cellulose concentration (Fig. 3), indicating that the cellulose concentration among switchgrass varieties changed differently in 2013 as compared to 2012.

In both years, maximum cellulose concentration for all switchgrass varieties was observed at the late season harvests. Cellulose concentration in 2012 ranged from 225 g kg^{-1} DM for Cave-in-Rock at the first harvest date to 394.1 g kg^{-1} DM and 351.5 g kg^{-1} DM for Kanlow and Cave-in-Rock, respectively, at the last harvest date (Fig. 3). Similarly in 2013, cellulose concentration ranged from 238.2 g kg^{-1} DM for

Cave-in-Rock at the first harvest date to 431.3 g kg^{-1} DM and 398.4 g kg^{-1} DM for Kanlow and Cave-in-Rock respectively, at the final harvest date (Fig. 3).

3.2. Hemicellulose

Variety and harvest date affected hemicellulose concentration, but the response to harvest date varied among varieties (Table 1, Fig. 4). Averaged over both years, Kanlow had the highest hemicellulose concentration of 321 g kg^{-1} DM, which was significantly different from all other varieties, and Cave-in-Rock had the lowest hemicellulose concentration of 306.5 g kg^{-1} DM.

Differences in hemicellulose concentrations among the varieties varied between the two years. In 2012, variety and harvest date affected hemicellulose concentration and there was a significant interaction between them. However, in 2013 variety and harvest date were found to have an effect on hemicellulose concentration, but there was no interaction. This reflects that switchgrass varieties in 2012 had higher variability for hemicellulose concentration between harvests than in 2013.

Generally, in both years, earlier harvest dates typically had lower hemicellulose concentrations than later harvests (Fig. 4). In 2012, hemicellulose concentration among switchgrass varieties ranged from 275 g kg^{-1} DM for Blackwell to 335 g kg^{-1} DM for Kanlow for first and third harvest, respectively. Whereas in 2013, hemicellulose concentration for switchgrass varieties did not change greatly between harvests and ranged from 286.3 g kg^{-1} DM for Blackwell and 322.4 g kg^{-1} DM for Trailblazer for the first and third harvest, respectively. This indicates that mid-season harvests usually contain higher concentrations of hemicellulose as compared to early and late season harvests.

3.3. Lignin

Lignin concentration increased with plant maturity in both years. Year, variety and harvest date affected lignin concentration in switchgrass varieties and there was an interaction between year and harvest date. Averaged over both years, Trailblazer and Kanlow had the lowest lignin concentration of 30.3 and 31.1 g kg^{-1} DM, respectively, and Cave-in-Rock had the highest concentration of 33.3 g kg^{-1} DM (Table 1, Fig. 5).

In 2012, variety and harvest date affected lignin concentration, but their respective two-way interaction was not significant. Lignin concentration in 2012 ranged from 14.1 g kg^{-1} DM for Alamo at the first harvest date to 52.2 g kg^{-1} DM and 52.7 g kg^{-1} DM for Alamo and Cave-in-Rock, respectively, at the last harvest date. In 2013, the only factor that affected the lignin concentration among switchgrass varieties was harvest date. Average lignin concentration ranged from 15.2 g kg^{-1} DM for Kanlow to 49.1 g kg^{-1} DM for Cave-in-Rock between the first and last harvest date respectively. This suggests that lignin concentration of each variety vary from one harvest date to another and typically later harvests will have a higher lignin concentration than earlier harvest dates, but differences in lignin concentrations among varieties at a given day of the year will not be significant.

3.4. Total Nonstructural Carbohydrates (TNC)

Total nonstructural carbohydrate (TNC) concentration for all switchgrass varieties decreased with maturity in both years (Fig. 6). The occurrence of a variety and harvest date interaction for TNC concentration (Table 1) indicated that changes in amount of TNC concentration were inconsistent among varieties throughout the growing season. Averaged over both years, Cave-in-Rock had the highest TNC concentration of 189.3 g kg^{-1} DM, and Blackwell had the lowest of 175.2 g kg^{-1} DM.

There were no significant differences among varieties for their TNC concentration in either year. However, in 2012, an interaction between harvest date and variety was observed for TNC concentration, indicating that differences in TNC concentrations between varieties depended on harvest date. In 2012, average TNC concentration ranged from 157.9 g kg^{-1} DM for Alamo to 142 g kg^{-1} DM for Blackwell. In 2013, variety, harvest date and their interaction were all non-significant, suggesting that TNC concentration of varieties did not change a lot over the growing season. The average amount of TNC in 2013 ranged from 208.5 g kg^{-1} DM for Blackwell to 223.4 g kg^{-1} DM for Cave-in-Rock.

3.5. Nitrogen

The nitrogen concentration of all switchgrass varieties decreased curvilinearly with delayed harvest, and this trend was consistent in both years (Fig. 7). Varieties differed significantly with harvest date (Table 1); earlier harvest dates had higher nitrogen concentrations than later harvest dates. Averaged over both years, the nitrogen concentration of switchgrass varieties between first and last harvest date ranged from 32.5 g kg^{-1} DM for Cave-in-Rock to 5.5 g kg^{-1} DM for Kanlow.

In 2012, an interaction between variety and harvest date occurred for nitrogen concentration. The decrease in nitrogen concentration for switchgrass varieties between first and last harvest ranged from 33 g kg^{-1} DM for Cave-in-Rock to 6.9 and 6.3 g kg^{-1} DM for Alamo and Kanlow, respectively (Fig. 7). However in 2013, harvest date was the only factor that affected nitrogen concentration, indicating that nitrogen concentration among switchgrass varieties changed differently in 2013 compared to 2012. In 2013, the decrease in nitrogen concentration between first and final harvest ranged from 32.1 g kg^{-1} DM for Cave-in-Rock to 4.5 and 4.6 g kg^{-1} DM for Kanlow and Alamo, respectively (Fig. 7).

3.6. Carbon-to-Nitrogen Ratio (C-N ratio)

Carbon-to-nitrogen ratio (C-N ratio) for all switchgrass varieties increased continuously with advancing maturity in both years (Fig. 8). Year, variety and harvest date had significant effects on the C-N ratio (Table 1, Fig. 8). Averaged over both years, Kanlow had the highest C-N ratio of 44, which was significantly different from all other varieties except Blackwell and Alamo, and Trailblazer had the lowest C-N ratio of 35.

In 2012, varieties differed in C-N ratio across harvest dates; earlier harvest dates in 2012 ranged from 13 for Alamo at first harvest date to 68 for Kanlow at the last harvest date (Fig. 8). However in 2013, the only factor that affected the C-N ratio among switchgrass varieties was harvest date, and the average C-N ratio ranged from 13 for both Kanlow and Cave-in-Rock at the first harvest date to 96 for Kanlow at the last harvest date (Fig. 8). This indicates that C-N ratio of switchgrass varieties vary from one harvest date to another and typically later harvests will have higher C-N ratio than earlier harvest dates.

3.7. Ash

The ash concentration of all switchgrass varieties decreased continuously with advancing maturity in both years (Fig.9). Varieties differed significantly with harvest date (Table 1); earlier harvest dates had higher ash concentrations compared to the later harvest dates. Averaged over both years, the lowest ash concentration was recorded at the sixth harvest for Alamo and Kanlow, 65 g kg^{-1} DM and 65.5 g kg^{-1} DM, respectively.

In 2012, variety and harvest date affected ash concentration, but there was no interaction between them. Ash concentration in 2012 ranged from 163 g kg^{-1} DM for Blackwell at the first harvest to 66.6 g kg-

^{1} DM and 68.8 g kg^{-1} DM for Alamo and Kanlow, respectively, at the last harvest (Fig. 9). However in 2013, the only factor that affected the ash concentration among switchgrass varieties was harvest date. Average ash concentration ranged from 132.6 g kg^{-1} DM for Alamo at the first harvest date to 62.2 g kg^{-1} DM and 63.3 g kg^{-1} DM for Alamo and Kanlow, respectively, at the last harvest date (Fig. 9). This suggests that ash concentration of varieties vary from one harvest date to another and typically later harvests will have lower ash concentrations than earlier harvest dates, but there will be no significant differences among varieties for their ash concentration at a given day of the year.

4. Discussion

This study provided new data on chemical composition of five switchgrass cultivars and revealed the magnitude of ecotypic differences between upland and lowland cultivars. This information is critical, fills knowledge gaps and has the potential to assist decision making about the proper time of harvest of switchgrass for further industrial processing.

An efficient lignocellulosic feedstock-based bioenergy system requires optimization of desired compositional traits of the feedstock [9]. Optimization of compositional traits depends on the conversion process used, since thermochemical and biochemical conversion systems contrast in their requirements of feedstock composition. Generally, for an ideal bioenergy feedstock production a higher concentration of fiber and reduced levels of nitrogen and ash are the goals [15, 16].

Results from this study demonstrated a significant variation between switchgrass ecotypes for important quality traits. Harvest time was also observed to play an important role in influencing the biomass feedstock composition over the growing season. Delayed harvest to later maturity stages increased the cellulose, hemicellulose and lignin concentration, but a curvilinear decrease was observed for nitrogen and ash concentration in all switchgrass cultivars. These findings are consistent with past studies, which also found that warm-season grasses increase in cell wall constituents [14, 22-25], and decrease their nitrogen and ash concentration [26-28] with advancing maturity. Griffin et al. [29] and Jung et al. [30] reported that in grasses with advanced maturity stems contain higher fiber concentrations than the leaves [29, 30]. Switchgrass stems contain less nitrogen compared to the leaves [27]. This indicates that variation in these chemical constituents with maturity is likely due to the differences in stem to leaf ratio at later developmental stages in switchgrass [23, 24, 26, 31]. However, results from this study have also revealed that ecotypes differentially vary in their fiber concentration with advanced maturity and cultivar. Lowland ecotypes had higher cellulose and hemicellulose concentrations compared to their upland counterparts, and these results are consistent with the earlier findings [23, 32]. For biochemical conversion systems, higher cellulose and hemicellulose concentrations are desirable by contributing more fermentable sugars for liquid fuel synthesis [33, 34].

Lignin concentrations were higher in Cave-in-Rock, which is an upland ecotype that tended to maintain higher lignin levels in both years. Lemus et al. [23] and Bhandari et al. [32] reported similar findings that upland ecotypes contain higher lignin concentration than lowland ecotypes. However, in our results it was also noteworthy that lowland types did not have the lowest levels of lignin, and the concentrations were fairly comparable to the upland ecotypes. Higher lignin concentrations are desirable for thermochemical conversion systems [35]; however, because lignin is recalcitrant to degradation, it also reduces biochemical conversion efficiency by reducing the cellulose and hemicellulose availability for fermentation, thus reducing the ethanol yields [18, 37, 38].

The occurrence of high nitrogen concentration in herbaceous bioenergy feedstock has significant implications of reducing the conversion efficiency of biomass to biofuel [16, 39-41]. Our results demonstrate that nitrogen concentration in all switchgrass cultivars decreased with advanced maturity. Lowland ecotypes had lower nitrogen concentration compared to the upland ecotypes at the last harvest. This curvilinear decrease in nitrogen concentration as the growing season progressed is similar to that reported in prior studies of nitrogen cycling in grasses [26, 27, 42, 43]. In addition to the increase in stem to leaf ratio, this decrease in nitrogen concentration might also be partially attributed to the nutrient translocation from aboveground plant parts to the belowground storage organs [21, 27].

In concert with nitrogen concentration, the TNC and C-N ratio changed dramatically over the growing seasons. In both years TNC concentrations decreased early during the growing season, and then a slight increase was observed at the last harvest in all switchgrass cultivars. In contrast to TNC, a continuous increase was observed for C-N ratio in all switchgrass cultivars in both years. At the end of each growing season, lowland ecotypes had the highest C-N ratio compared to the upland ecotypes. This might be the result of reduction in nitrogen concentration and increase in carbohydrates before the onset of winter. Past studies have reported similar increases in carbohydrate levels in cold acclimated *Arabidopsis* plants than in non-acclimated [44].

The presence of higher ash concentrations in herbaceous feedstock negatively interfere with conversion processes by reducing hydrocarbon yields [16], and creating fusible slag that fouls boilers and machinery and also increases maintenance costs [5, 45]. In our results, a continuous decrease in ash concentration in all switchgrass ecotypes occurred with advancing maturity and delayed harvest, and this is consistent with past findings [24, 25, 28]. However, this study also found that lowland ecotypes contained less ash as compared to the upland ecotypes, and these results are consistent with previous studies, as well [9, 46].

5. Conclusion

Based on results presented here and elsewhere, we conclude that ecotypic differences do exist between switchgrass cultivars for their fiber, nitrogen and ash concentrations. Lowland ecotypes usually contain higher cellulose, hemicellulose and lower nitrogen and ash contents; whereas, upland ecotypes produce higher lignin, nitrogen and ash concentrations. Moreover, delaying biomass harvest to later maturity stages or until fall will ensure greater fiber and lower nitrogen and ash concentrations.

This study was designed to understand the feedstock compositional differences between upland and lowland ecotypes and their harvest management. Results from this study will also aid in future switchgrass breeding for improved cultivar development as a bioenergy feedstock.

Acknowledgements

The authors thank the CenUSA Bioenergy project for financial support.

References

1. A.J. Ragauskas, C.K. Williams, B.H. Davison, G. Britovsek, J. Cairney, C. A. Eckert, W.J. Frederick, J.P. Hallett Jr, D.J. Leak, C.L. Liotta, J.R. Mielenz, R. Murphy, R. Templer, T. Tschaplinski. 2006. The path forward for biofuels and biomaterials. Science (2006) 311:484–487

2. CAST. Convergence of agriculture and energy: II. Producing cellulosic biomass for biofuels. Council for Agricultural Science and Technology, Ames, Iowa (2007)

3. A.E. Farrell, J.P. Richard, T.T. Brian, D.J. Andrew, O'Hare Michael, and M.K. Daniel. Ethanol can contribute to energy and environmental goals. Science (2006) 311(5760): 506-508

4. J.H. Bouton, Molecular breeding of switchgrass for use as a biofuel crop. Current opinion in genetics and development (2007)17:553–558.

5. S.B. McLaughlin, R. Samson, D. Bransby, and A. Wiselogel. Evaluating physical, chemical, and energetic properties of perennial grasses as biofuels. p. 1–8. Proc. BioEnergy' 96-the Seventh National Bioenergy Conf.: Partnerships to Develop and Apply Biomass Technologies, Nashville, TN. 15–20 Sept. 1996. Dep. of Energy, Oak Ridge Lab., Oak Ridge, TN.

6. J.N. Brunken, and J.R. Estes. 1975. Cytological and Morphological Variation in *Panicum virgatum L*. The Southwestern Naturalist (1975)19:379-385

7. M.A. Sanderson, R.L. Reed, S.B. McLaughlin, S.D. Wullschleger, B.V. Conger, D.J. Parrish, D.D. Wolf, C. Taliaferro, A.A. Hopkins, W.R. Ocumpaugh, M.A. Hussey, J.C. Read, and C.R. Tischler. Switchgrass as a sustainable bioenergy crop. Bioresour. Technol. (1996) 56:83–93

8. M.D. Casler, K.P. Vogel, C.M. Taliaferro, and R.L. Wynia. Latitudinal adaptation of switchgrass populations. Crop Science (2004) 44:293–303

9. K.A. Cassida, J.P. Muir, M.A. Hussey, J.C. Read, B.C. Venuto, W.R. Ocumpaugh. Biofuel component concentrations and yields of switchgrass in south central US environments. Crop Science (2005) 45:682–692

10. D.J. Parrish, J.H. Fike, D.I. Bransby and R. Samson. Establishing and managing switchgrass as an energy crop. Forage and Grazinglands (2008) doi:10.1094/FG-2008-0220-01-RV

11. G.K. Evanylo, A.O. Abaye, C. Dundas, C.E. Zipper, R. Lemus, B. Sukkariyah, and J. Rockett. Herbaceous vegetation productivity, persistence, and metals uptake on a biosolids-amended mine soil. J. Environ. Qual. (2005) 34:1811-1819

12. J.H. Fike, D.J. Parrish, D.D. Wolf, J.A. Balasko, J.J.T. Green, M. Rasnake, and J.H. Reynolds. Long term yield potential of switchgrass for biofuel systems. Biomass Bioenergy (2006) 30:198-206

13. R.D. Perlack, L.L. Wright, A.F. Turhollow, R.L. Graham, B.J. Stokes, and D.C. Erbach. U.S. billion-ton update: Biomass supply for a bioenergy and bioproducts industry. Oak Ridge National Laboratory. Oak Ridge, TN. (2011) pp. 227

14. P. R. Adler, M.A. Sanderson, A.A. Boateng, P.J. Weimer and H.-J.G. Jung. Biomass yield and biofuel quality of switchgrass harvested in fall or spring. Agron. J. (2006) 1518-1525.

15. M.A. Sanderson, N.P. Martin, P. Adler. In Barnes RF et al (eds) Forages. Biomass, energy, and industrial uses of forages. The science of grassland agriculture, Ames, Iowa. Blackwell Publisher. V.II (2007)

16. F.A. Ablevor, B. Rejai, R.J. Evans, and K.D. Johnson. Pyrolytic analysis and catalytic upgrading of lignocellulosic materials by molecular beam mass spectrometry. (1992) pp. 69–75. In D.L. Klass (ed.) Energy from biomass and wastes XVI. Elsevier Applied Sci. Publ., Chicago

17. G. Trebbi. Power-production options from biomass: The vision of a southern European utility. Bioresour. Technol. (1993) 46:23–29

18. B.S. Dien, H.G. Jung, K.P. Vogel, M.D. Casler, J.F.S. Lamb, P.J. Weimer, L. Iten, R.B. Michell, and G. Sarath. Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass. Biomass Bioen. (2006) 30:880–891

19. J.F. Johnson, N. W. Barbour and S. L. Weyers, Soil. Sci. Soc. Am. J., (2007) 71(1), 155–162

20. Royal Society, 2008. Sustainable biofuels: prospects and challenges. *Science Policy Section*: The Royal Society, London. https://royalsociety.org/policy/publications/2008/sustainable-biofuels/

21. K.P. Vogel, J.J. Brejda, D.T. Walters, and D.R. Buxton. Switchgrass biomass production in the Midwest USA: Harvest and nitrogen management. Agron. J. (2002) 94:413–420.

22. R. Mitchell, J. Fritz, K. J. Moore, L. Moser, K. P. Vogel, D. Redfearn, and D. Wester. Predicting forage quality in switchgrass and big bluestem. Agron. J. (2001) 93:118–124

23. R. Lemus, E.C. Brummer, K.J. Moor, N.E. Molstad, C.L. Burras, M.F. Barker. Biomass yield and quality of 20 switchgrass populations in southern Iowa, USA. Biomass and Bioenergy, (2002) 23:433–442

24. V.R. Mulkey, V.N. Owens, and D.K. Lee. Management of switchgrass dominated Conservation Reserve Program lands for biomass production in South Dakota. Crop Sci. (2006) 46:712–720

25. N. Waramit, K.J. Moore, A.H. Heggenstaller. Composition of native warm-season grasses for bioenergy production in response to nitrogen fertilization rate and harvest date. Agron. J. (2011) Vol. 103 no. 3

26. I.C. Madakadze, K. Stewart, P. R. Peterson, B. E. Coulman, and D. L. Smith. Switchgrass biomass and chemical composition for biofuel in Eastern Canada. Agron. J. (1999) 91:696–701

27. D.M. Wilson, E.A. Heaton, M. Liebman, K.J. Moore. Intraseasonal changes in switchgrass nitrogen distribution compared with corn. Agron. J. (2013) Vol. 105 no. 2

28. D.M. Wilson, D.L. Dalluge, M. Rover, E.A. Heaton, R.C. Brown. Crop management impacts biofuel quality: Influence of switchgrass harvest time on yield, nitrogen and ash of fast pyrolysis products. BioEnergy Research (2013) Vol. 6, Issue 1, pp 103-113

29. J.L. Griffin, and G.A. Jung. Leaf and stem forage quality of big bluestem and switchgrass. Agron. J. (1983) 75:723–726

30. H.G. Jung, and K. P. Vogel. Lignification of switchgrass (Panicum virgatum) and big bluestem (*Andropogon gerardii*) plant parts during maturation and its effect on fiber degradability. J. Sci. Food Agric. (1992) 59:169–176

31. E.K. Twidwell, K. D. Johnson, J. H. Cherney, and J. J. Volenec. 1988. Forage quality and digestion kinetics of switchgrass herbage and morphological components. Crop Sci. (1988) 28:778–782

32. H. Bhandari, D.W. Walker, J.H. Bouton, and C.M. Saha. Effects of ecotypes and morphotypes in feedstock composition of switchgrass (Panicum virgatum L.). GCB Bioenergy (2014) 6:26–34

33. M. Kurakake, W. Kisaka, K. Ouchi, T. Komaki. Pretreatment with ammonia water for enzymatic hydrolysis of corn husk, bagasse, and switchgrass. Applied Biochemistry and Biotechnology (2001) Vol. 90, Issue 3, pp 251-259

34. R.P. Datar, R.M. Shenkman, B.G. Cateni, R.L. Huhnke, and R.S. Lewis. Fermentation of biomass-generated producer gas to ethanol. Biotechnol. Bioeng. (2004) 86:587–594

35. M. Hayn, W. Steiner, R. Klinger, H. Steinmueller, M. Sinner, and H. Esterbauer. Basic research and pilot studies on the enzymatic conversion of lignocelluloses. p. 33–70. J.N. Saddler (ed.) Bioconversion of forest and agricultural plant residues. CAB Int., Wallingford, Oxon, UK. Basic research and pilot studies on the enzymatic conversion of lignocelluloses. p. 33–70. In J.N. Saddler (ed.) Bioconversion of forest and agricultural plant residues. CAB Int., Wallingford, Oxon, UK. 1993.

36. K.P. Vogel, and H.J. Jung. Genetic modification of herbaceous plants for feed and fuel. Critical reviews in Plant Sciences (2001) 20: 15-49

37. Y. Sun, and J. Cheng. Hydrolysis of lignocellulosic materials for ethanol production: A review. Bioresour. Technol. (2002) 83:1–11

38. C.X. Fu, J.R. Mielenz, X.R. Xiao, Y. Ge, C.Y. Hamilton, M. Rodriguez Jr., F. Chen, M. Fosten, A. Ragauskas, J. Bouton, R. Dixon, Z. Wang. Genetic manipulation of lignin reduces recalcitrance and improves ethanol production from switchgrass. Proceedings of the National Academy of Sciences of the United States of America, (2011) 108:3803–3808

39. A. V. Bridgwater. Catalysis in thermal biomass conversion. Appl Catal A Gen (1994) 116:5–47

40. A.A. Boateng, K.B. Hicks, K.P. Vogel. Pyrolysis of switchgrass (*Panicum virgatum*) harvested at several stages of maturity. J. Anal. Appl. Pyrolysis (2006) 75:55-64

41. S. Czernik, A.V. Bridgwater. Overview of applications of biomass fast pyrolysis oil. Energy Fuel (2004) 18:590–598

42. M.A. Sanderson, J.C. Read, and R.L. Reed. Harvest management of switchgrass for biomass feedstock and forage production. Agron. J. (1999) 91:5–10

43. C. V. Beale, and S.P. Long. Seasonal dynamics of nutrient accumulation and portioning in the perennial C-4 grasses Miscanthus x giganteus and Spartina Cynosuroides. Biomass and Bioenergy (1997) 12:419-428

44. D. Cook, S. Flower, O. Fiehn, M. Thomashow. A prominent role of the CBF cold response pathway in configuring the low-temperature metablome of Arabidopsis. Proc Natl Acad Sci USA (2004) 101:15243-15248

45. U. Jorgensen. Genotypic variation in dry matter accumulation and content of N, K and Cl in Miscanthus in Denmark. Biomass Bioenergy (1997) 12:155–169

46. C.O. Hong, V. N. Owens, D. Bransby, R. Farris, J. Fike, E. Heaton, S. Kim, H. Mayton, R. Mitchell, D. Viands. Switchgrass Response to Nitrogen Fertilizer Across Diverse Environments in the USA: a Regional Feedstock Partnership Report. Bioenerg. Res. (2014) 7:777–788

Table 1. Analysis of variance for cellulose, hemicellulose, lignin, TNC, nitrogen, C-N ratio and total ash concentrations and significance in response to switchgrass varieties and harvest dates during growing seasons 2012 and 2013 at Ames, IA.

	Cellulose	Hemicellulose	Lignin	TNC	Nitrogen	C-N Ratio	Total Ash
Y	273**	4	25.6**	153**	96.7**	178**	14.43*
H	532.8**	56**	615.1**	16.6**	731.3**	177.8**	176.8**
Y × H	13.8**	3.9**	20.5**	6.2**	20**	17.4**	5.1**
V	18**	22**	5.4**	4.4**	15**	6.0**	5**
Y × V	6.1**	6.4**	1.2	1.2	6.7**	0.27	2.9*
H × V	0.74	2.1**	0.9	1.9*	1.4	0.74	0.91
Y × H × V	1.2	1.3	1.2	1.2	1.7*	0.39	0.95

* Significant at the 0.05 probability level

**	Significant at the 0.01 probability level
Y	Year
V	Variety
H	Harvest date

Fig. 1 Mean monthly air temperature for 2012, 2013 and 20 year average at Ames, IA.

Fig. 2 Mean monthly precipitation for 2012, 2013 and 20 year average at Ames, IA.

Fig. 3 Cellulose concentration of switchgrass varieties during year 2012-2013.

Fig. 4 Hemicellulose concentration of switchgrass varieties during year 2012-2013.

Fig. 5 Lignin concentration of switchgrass varieties during year 2012-2013.

Fig. 6 Total non-structural carbohydrate (TNC) concentration of switchgrass varieties during year 2012-2013.

Fig. 7 Nitrogen concentration of switchgrass varieties during year 2012-2013.

Fig. 8 Carbon to Nitrogen Ratio (C-N Ratio) of switchgrass varieties during year 2012-2013.

Fig. 9 Total ash concentration of switchgrass varieties during year 2012-2013.