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### Research paper

# Compositional differences among upland and lowland switchgrass ecotypes grown as a bioenergy feedstock crop



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#### 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<sup>-1</sup> DM respectively), and Cave-in-Rock had the highest lignin concentration (33 g kg<sup>-1</sup> DM). The lowest nitrogen and ash concentrations were observed in Kanlow (14 and 95 g kg<sup>-1</sup> 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.

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#### 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

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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<sub>4</sub> grass native to United States, has been identified as a strong candidate for a 2nd 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].

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

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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 [16]. 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 abovementioned 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 (42°0′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 (Cavein-Rock, Blackwell and Trailblazer) and Iowland (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<sup>-1</sup> and 0.56 kg a.i. ha<sup>-1</sup> respectively. Nitrogen, P and K fertilizer were applied every year in early May at 78, 67 and 90 kg ha<sup>-1</sup>, 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 lowa Environmental Mesonet (2014) (Figs. 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<sup>2</sup> 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 h 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 h 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<sup>-1</sup> sample dry matter. Nitrogen (N) and carbon (C) were determined using a LECO True Spec<sup>TM</sup> 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.

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