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# Chemical and elemental composition of big bluestem as affected by ecotype and planting location along the precipitation gradient of the Great Plains

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**ABSTRACT.** Three big bluestem ecotypes from central Kansas (Cedar Bluffs and Webster populations), eastern Kansas (Konza and Top of the World populations), and Illinois (12Mile and Fults populations), as well as the Kaw cultivar, were harvested from four reciprocal garden planting locations (Colby, Hays, and Manhattan, KS; and Carbondale, IL) and evaluated for their chemical (glucan, xylan, arabinan, lignin and ash) and elemental (carbon, oxygen, hydrogen, nitrogen and sulfur) compositions. The objective of this research was to study the effects of ecotype and planting location on the chemical and elemental compositions of big bluestem along the Great Plains precipitation gradient (~1200 to 400 mm mean annual precipitation). All the populations revealed a large variation in cellulose (31.8–36.5%), hemicellulose (24.96–29.74%), lignin (14.4–18.0%), carbon (47.3–51.3%), and nitrogen (4.91–6.44%). Planting location had significant effects on both chemical and elemental compositions of big bluestem. Ecotype had significant effects on glucan, xylan, lignin, and ash contents as well as on carbon, oxygen, and hydrogen elemental fractions. In addition, the interaction between ecotype and planting location had significant effects on glucan, lignin, and hydrogen. Planting location had a greater effect on chemical and elemental compositions than the ecotype and interaction between location and ecotype. The total sugar content of the big bluestem (regardless of ecotype) increased as the Great Plains precipitation gradient increased from west to east. Annual precipitation, growing degree days and potential evapotranspiration in 2010 explained up to 97%, 88% and 80% of the variation in compositions respectively.

**Keywords:** Big bluestem; chemical composition; elemental composition; ecotype; reciprocal common garden

## 1. INTRODUCTION

With the rapid increase in worldwide consumption of nonrenewable fossil fuels, the production of renewable fuels from biomass is attracting more research attention. Renewable fuels derived from biomass could reduce our dependence on fossil fuel resources and reduce greenhouse gas emissions (Dien et al, 2006). First-generation biofuel, produced from starch-based and sugar-based biomass, is limited because of competition with food crops and other land demands (Tilman et al., 2006). Thus, lignocellulosic biomass, including dedicated energy crops such as switchgrass, big bluestem, forest residues, and agricultural residues, could play an important role in biofuel production because of low production inputs and potentially low competition with food production. A recent analysis indicated that over 25 million hectares of land classified by the USDA as rangeland/grassland within land capability class 3–6 soils (more marginal/less productive soils) could be utilized for bioenergy crop production in select states in the central Great Plains (Kansas, Nebraska, Oklahoma, and South Dakota) (USDA, 2010).

Big bluestem (*Andropogon gerardii*) is a dominant warm-season ($C_4$) perennial native grass that comprises as much as 80% of the plant biomass in prairies in the midwestern grasslands of North America (Gould and Shaw, 1983; Knapp et al., 1998). This research helps lay the foundation for the potential development of big bluestem as a bioenergy feedstock on these range/grasslands. Although big bluestem has been studied extensively for decades in terms of the effect of climate on grass growth; controls on community structure; ecological responses to grazing, burning, and mowing; and restoration effectiveness (He et al., 1992; Epstein et al., 1998; Knapp et al., 2001; Silletti and Knapp, 2003; Fay et al., 2003;

Jackson et al., 2010), the potential use of bluestem for bioenergy has not been evaluated adequately. Ecotypes of *A. gerardii* were originally described nearly 50 years ago (McMillan, 1959), but variables related to biofuel potential across the precipitation gradient of tallgrass prairie have not been broadly characterized. This study will utilize the sharp precipitation gradient across the Great Plains (1200 to 400 mm mean annual precipitation [MAP]) and reciprocal garden research plots to investigate the biofuel potential of *A. gerardii* ecotypes and how such potential is affected by planting location across the Great Plains.

Big bluestem is adaptable in most native prairie ecosystems and can represent as much as three times the biomass as switchgrass in midwestern grasslands (Epstein et al., 1998). Big bluestem productivity is high due to efficient nutrition utilization; it produces twice the biomass per applied nitrogen compared with switchgrass and indiangrass (Johnson and Matchett, 2001), establishes easily from seed, and spreads vigorously by vegetative growth of underground rhizomes with a robust root system (Perry and Baltensperger, 1977). In addition to economic considerations, bluestem prairie serves a range of purposes in the ecosystem because it provides wildlife habitat, cattle grazing, and hay and pasturelands (Fargione et al., 2009).

Previous research has been carried out to evaluate big bluestem for conversion to ethanol. Weimer et al. (2007) studied big bluestem for ethanol production through consolidated bioprocessing. Jung and Vogel (1992) reported that big bluestem leaves contained more neutral detergent fiber and relatively higher levels of cellulose and lignin at the vegetative stage than switchgrass, resulting in a greater *in vitro* fermentability than switchgrass. Bowden (2008) demonstrated that big bluestem produced 39% and 16% more mass than Shawnee and

Cave-in-Rock switchgrass, respectively and big bluestem had larger yields and lower amounts of ash than switchgrass due to the higher nitrogen utilization efficiency.

In this research, three big bluestem ecotypes (Central Kansas [CKS], Eastern Kansas [EKS], and Illinois [IL], with two populations comprising each ecotype) and the widely planted Kaw cultivar (KAW) were harvested from each of four reciprocal garden planting locations (Colby, Hays, and Manhattan, KS; and Carbondale IL). This reciprocal design allows us to study the effect of ecotype and planting location on chemical and elemental composition. Results from this research will provide basic data that will potentially enable more efficient plant breeding for bioenergy production by providing scientific knowledge about the role of the genetic and environmental factors that influence the development of big bluestem varieties for use as a bioenergy crop. The plants analyzed here also were part of a big bluestem ecotype experiment to examine the cline in phenotypic variation (biomass, phenology, canopy characteristics) across the Great Plains precipitation gradient (~1200 to 400 mm mean annual precipitation) and the relative role of environment and ecotype in affecting the phenotype.

# 2. MATERIALS AND METHODS

## 2.1 Materials.

Three big bluestem ecotypes, CKS (Cedar Bluffs [CDB] and Webster [WEB] populations), EKS (Konza [KON] and Top of the World [TOW] populations), and IL (12Mile [12M] and Fults [FUL] populations), and the KAW cultivar, which is widely planted to restore marginal lands, were harvested from reciprocal garden plots in four planting locations (Colby, Hays, and Manhattan, KS; and Carbondale, IL) in 2010. Among the four locations, the Colby planting site was used to test the threshold of drought tolerance and the possibility for planting in the drier locations of the Great Plains. Two populations from each ecotype were evaluated for their chemical and elemental compositions. Glucan, xylan, arabinan, lignin and ash made up a major chemical composition of biomass. Elemental compostion was reported as carbon, oxygen, hydrogen, nitrogen and sulfur. The big bluestem samples were ground into powder using a Retsch cutting mill (Haan, Germany) with a 1 mm sieve. All chemicals used for this research were purchased from Sigma Chemical Co. (St. Louis, MO).

**2.12 Seed Collection.**

Seeds for the populations and ecotypes were collected by hand from pristine ungrazed prairie in the fall of 2008. Figure 1 and Table 1 show the GPS coordinates of seed collection sites (latitude and longitude) for the seeds that were later harvested from grown plants. For each ecotype region (central Kansas, eastern Kansas, and Illinois), four populations were collected within 50 miles of the reciprocal garden planting locations. Two populations per ecotype were analyzed in this paper. Populations were at least 10 miles distant from one another. In fall 2008, a subset of seeds from all populations was germinated and grown in 4 x 4 in pots in the greenhouse using standard greenhouse potting mix (Metro-Mix 510; Sun Gro Horticulture, Vancouver, BC, Canada). For KAW, we obtained seed from the USDA Plant Materials Center, Manhattan, KS. We included KAW because it is widely used for restoration planting in Conservation Reserve Program lands throughout the Great Plains.

**2.13 Planting Locations.**

These plants were later installed at the reciprocal garden sites (Colby, Hays, and Manhattan, KS; and Carbondale, IL) in August 2009. Table 2 shows environmental conditions and short-term and long-term weather patterns at the reciprocal garden planting sites. Mean annual precipitation showed a striking contrast across the four locations. To test the limits of the tolerance of big bluestem, the plants were installed in Colby. At each planting location, all 12 populations (3 ecotypes x 4 populations per ecotype) were replicated in 10 blocks. For this study we used only two of the 4 populations per ecotype. Plants were assigned randomly to blocks, spaced 50 cm apart, and planted into shadecloth to control weeds. The KAW cultivar and sand bluestem (data not included here) were also included, making 14 plants per block.

**2.14 Plant Harvest.**

The plants were part of a large bluestem ecotype variation experiment to examine the phenotypic variation across the Great Plains precipitation gradient (Johnson et al., in preparation) and the role of environment and ecotype in affecting the phenotype. These plants were extensively characterized in terms of canopy area, height, and phenology in the summer of 2010 (Johnson et al., in preparation) and harvested by hand in October 2010. The harvested plant biomass (foliage, inflorescence, stalks) was dried at 60 °C for at least 1 week before being stored at room temperature.

**2.2 Analytical methods.**

**2.2.1 Chemical Composition Analysis.**

Moisture content of ground big bluestem samples was determined by drying about 2 g of each sample in a forced-air oven at 105 °C for 4 h (Sluiter et al., 2008). Extractives and chemical composition of the big bluestem were determined by following NREL laboratory analytical procedures (Sluiter et al., 2008; Sluiter et al., 2005). Structural carbohydrates in biomass were reported as percentages of glucan and xylan. Lignin, the major non-carbohydrate component, is the sum of acid-insoluble and acid-soluble lignin. Glucose, xylose, mannose, and arabinose in acid-hydrolyzed samples were determined by analyzing the supernatant from acid-hydrolysis using an HPLC (Shimadzu, Kyoto, Japan) equipped with an RCM monosaccharide column (300 × 7.8 mm; Phenomenex, Torrance, CA) and a refractive index detector (RID10A, Shimadzu, Kyoto, Japan). The mobile phase was 0.6 mL $min^{-1}$ of double-distilled water, and the oven temperature was 80 °C. The supernatants of acid-hydrolyzed samples were neutralized with $CaCO_3$ to pH 6 before being filtered through 0.2 μm hydrophilic PTFE syringe filters (Millipore, Billerica, MA). The monosaccharide was analyzed by using an HPLC with a Rezex RPM-monosaccharide column (300 × 7.8 mm; Phenomenex, CA) and a refractive index detector (RID-10A, Shimadzu, MD). The column was eluted with double-distilled water at a flow rate of 0.6 mL/min. The temperature of the chromatograph column was maintained at 80 °C.

**2.2.2 Elemental Analysis.**

The elemental composition of the big bluestem samples was measured with CHNS/O Elemental Analyzer (PerkinElmer 2400 Series II, PerkinElmer Inc., Waltham, MA). About 2 to 3 mg (accurate to 0.001mg) of the ground sample with fine uniform particle size was weighed into tin capsules using a PerkinElmer AD-6 Autobalance (PerkinElmer Inc., Waltham, MA). The ground sample was packed with foil, introduced into the combustion chamber through a funnel, and burned under a pure oxygen atmosphere. The gases ($CO_2$, $N_2$, $SO_2$, and $H_2O$) from combustion were separated in a quartz column containing copper wires detected by a thermoconductometer detector. Elemental compositions are reported as a percentage of initial dry weight (w/w, db).

**2.2.3 Statistical Analysis.**

Chemical and elemental compositions of big bluestem samples are reported as the average of duplicates. Analysis of variance (ANOVA) and Tukey's studentized range (HSD) test were analyzed using SAS (SAS Institute, Inc., Cary, NC). In general, fully balanced ANOVA tests were performed following the general linear models (GLM) procedure.

# 3. RESULTS AND DISCUSSION

Both ecotype and planting location had significant effects on chemical and elemental compositions of the big bluestem ($P < 0.05$), except the effect of ecotype on xylan + arabinan, nitrogen, and sulfur contents. The chemical composition of the seven big bluestem populations and 3 ecotypes from four planting locations varied significantly when specific constituents were considered (Table 3). For all of the big bluestem samples, the average and range of the chemical composition across planting locations and ecotypes are 34.5% ±2.4 from 29.6–39.5% for glucan, 23.6% ±2.0 from 19.2–26.8% for xylan, 3.5% ±0.7 from 2.1–4.8% for arabinan, 16.8% ±1.8 from 12.0–19.3% for lignin, and 4.3% ±0.7 from 3.1–5.6% for ash. The range of the chemical constituents in glucan, xylan, and ash contents (Table 3) are similar to those reported by previous research (Jefferson et al., 2004; Wiselogel et al., 1996; Titgemeyer et al., 1996); however, big bluestem had lower lignin content compared with other lignincellulosic biomass (Table 4) such as sorghum biomass (Zhao et al., 2009), corn stover (Zhao et al., 2009; Zeng et al., 2007; Lloyd and Wyman, 2005; Zhu et al., 2007), and wheat straw (Zhu et al., 2007; Sun and Chen, 2008; Saha et al., 2005). This may make pretreatment and enzymatic hydrolysis of structural polysaccharides in the bioconversion processes easier for big bluestem.

The elemental composition analysis is important for calculating biomass heat content, performing mass and heat balances in the bioconversion process, and predicting potential pollution problems during biomass thermal processes. Table 5 shows the elemental carbon (C), hydrogen (H), oxygen (O), sulfur (S), and nitrogen (N) contents in the big bluestem samples. For all of the big bluestem samples, the average and range of the elemental composition across planting locations and ecotypes are 49.1% ±1.4 (range of 47.1–51.4%) for C, 5.9% ±0.3 (range of 4.9–6.5%) for H, 43.3% ±1.6 (range of 40.7–46.1%) for O, 0.84% ±0.2 (range of 0.61–1.27%) for N, and 0.92% ±0.1 (range of 0.78–0.98% for S. Results showed that big bluestems had a desirable molar ratio of H/C, with average of 1.44 and a range of 1.23–1.52, which can result in less smoke and water-vapor formation and thereby reduced energy loss during gasification processes (Bridgeman et al., 2008). The comparison of elemental composition of big bluestem with other lignocellulosic biomass is shown in Table 6. Big bluestem contains relatively higher carbon content than other grasses and crop residues, which potentially translates into a relatively higher heat content for big bluestem. The results show that big bluestem could potentially serve as suitable energy grass in the Midwest with similar or better chemical and elemental compositions compared with other biomass crops and grasses.

**3.1 Effects of Planting Location on Chemical Composition.**

Figure 2 shows the effects of planting location on the chemical composition of big bluestem. Big bluestem populations planted in Illinois generally had higher cellulose (glucan) contents, with an average of 36.5% compared with the average of populations planted in Colby, KS (31.8%); Hays KS (33.8%); and Manhattan, KS (36.0%). The average cellulose content of big bluestem planted in Illinois was 4.7% higher than those from Colby in western Kansas, indicating that the same big bluestem populations would yield ≈15% more cellulose if planted in Illinois instead of western Kansas. Table 7 shows the linear regression results between composition and environmental factors associated with the planting locations. The 2010 annual precipitation explained 37–97% of the variation in biomass composition based on coefficients of determination ($R^2$). In addition to the sharp difference in precipitation from the westernmost planting location (Colby) to the easternmost planting location (Illinois), the difference in potential evapotranspiration between east and west is also responsible for composition differences. The 2010 growing degree days explained 17–88% of the variation in chemical concentrations. The potential evapotranspiration explained 55–80% of the variation in biomass composition (Table 7). The higher precipitation gradient in Illinois is almost one and a half times higher than Colby, which provides a better environment for biomass accumulation. A similar tendency was also observed for hemicellulose (xylan and arabinan). The highest and the lowest hemicellulose contents in the four planting locations, respectively, are Illinois with an average of 29.7% and Colby with an average of 25.0% (Figure 2). The difference in hemicellulose content was about 19% among the four locations. The total structural polysaccharides content of big bluestem planted in Illinois was about 15% higher than that planted in Colby; however, this increase was associated with higher lignin content. The average lignin contents of all planting locations exhibited a decreasing trend with the ecotype from east to west. In fact, 2010 growing degree days and 2010 precipitation explained 88% and 74% of the variation in lignin concentrations, respectively (Table 7). Big bluestem in Colby had average of 14.4% lignin, which is significantly lower than samples planted in Illinois, with average of 18.0% (Fig. 2). Taking into account the adverse effects of lignin in hydrolysis, further research is needed to determine the sugar yield and fermentation efficiency of all samples to determine the overall location effects. The range of ash contents among 28 samples was quite different in four locations. Ash contents of big bluestem from Illinois (with an average of 4.8%; data not shown) were higher than those populations in the other three planting locations in Kansas. Results suggest that big bluestem planted in Kansas with lower ash content would be best suited for the thermoconversion of biomass to biofuel (Monti et al., 2008).

**3.2 Effects of Ecotype on Chemical Composition.**

The composition results also showed a significant variation among the different ecotypes at $P < 0.001$ and $F$ values from 3.36 to 28.5, except xylan+arabinan, with $P = 0.935$ and $F = 0.14$ (Table 8). Based on $F$ value, ecotype had more significant effects on glucan and lignin, with $F$ values of 28.5 and 16.2, respectively. Hays ecotype and KAW had significantly higher glucan contents than East KS and Illinois ecotypes. KAW had the highest glucan content among all the ecotypes (Figure 3). This could be explained by the fact that the KAW cultivar, as the native released cultivar, was selected and bred for carbohydrate accumulation. Of these 28 samples, the highest carbohydrates content was found in KAW at the Illinois location, which indicates combined effects of ecotype and planting location. Although xylan content differs significantly among the different ecotypes, the average values of xylan of the different ecotypes are similar (Figure 3), indicating no clear effect of ecotype on the average xylan contents within the ecotypes from west to east. This result is probably because glucan and xylan contents were not solely affected by ecotype. The highest and lowest lignin contents of big bluestem were Central KS ecotype and Illinois ecotype, respectively. Results suggest that the Central KS ecotype showed higher lignin content (17.5%) than the Illinois ecotype (15.7%) because of adaptation to drought necessitated by a dry growing environment. The high lignin content may result in relatively lower efficiency of degradation in bioconversion.

### 3.3 Effects of Interactions between Location and Ecotype on Chemical Composition.

Variations in the glucan, xylan, xylan+arabinan, lignin, and ash contents among the 28 samples were analyzed by two-way ANOVA for examining the genetic and environmental effects on chemical composition of the big bluestem. In general, ANOVA analysis revealed that ecotype and location had significant effects on chemical composition including glucan, xylan, lignin, and ash contents as well as xylan + arabinan content (Table 8). Location had larger $F$ values (7.2–73.6) than ecotype (0.14–28.5) and interactions (1.12–3.59), showing that location effects were always highly significant with larger $F$ values, at times approaching two orders of magnitude larger; however, significant interactions between location and ecotype have been found only for glucan, with $P < 0.002$ and an $F$ value of 3.59, and lignin, with $P = 0.018$ and an $F$ value of 2.64, indicating that the glucan and lignin contents of big bluestem were significantly affected by the combined effects of ecotype and growing locations.

### 3.4 Effects of Ecotype and Planting Location on Elemental Composition.

Table 5 shows the carbon, hydrogen, nitrogen, oxygen, and sulfur fractions and H/C ratio of the big bluestem samples. The range of elemental fractions is 47.1–51.4% for carbon, 4.93–6.45% for hydrogen, 40.7–46.1% for oxygen, 0.61–1.27% for nitrogen and 0.78–0.98% for sulfur. The variations of the elements are 10.1% for carbon, 30.8% for hydrogen, 13.2% for oxygen, 108% for nitrogen, and 25.6% for sulfur. The average ratio of H/C is 1.44 with variation of 23.6%. Two-way ANOVA analysis shows through larger $F$ values that location had more effects than ecotype and ecotype-location interaction on elemental composition of big bluestem (Table 8). Location had significant effects on all of the elemental fractions, with $F$ values from 12.0 to 80.8 and $P < 0.001$. Ecotype had significant effects on carbon, oxygen, and hydrogen with $F$ values from 2.94–11.50 and $P$ values from 0.001–0.044. Ecotype-location interaction had a significant effect only on carbon content. The linear regression results between composition and environmental factors showed that precipitation explained 37–79% of variation in elemental fractions based on coefficients of determination ($R^2$) from 0.37–0.79 in growing year 2010 (Table 7). Growing degree days and the potential evapotranspiration also explained a large variation in the elemental composition of the big bluestem samples.

Because the carbon content is the most important factor related to its bioconversion yield and heat content, the histogram showed a parabolic trend with ecotype from west to east, indicating that the middle-location ecotype (EKS ecotype) had the lowest carbon content of the three ecotypes (Figure 4). In general, the carbon content of the big bluestem (average of 50.8%) planted in Illinois is higher than its counterparts planted in the Kansas locations (average of 49.2% for Manhattan, 47.7% for Hay, and 47.8% for Colby). Decreased longitude of planting location resulted in increased carbon content, which was similar to the trend of environmental effect on chemical composition. Also noteworthy is that the big bluestem in Colby had significantly lower nitrogen content (average of 0.65%) compared with other locations (average of 0.9%) (Figure 5). Low nitrogen fraction in biomass could be an advantage for the combustion process with low $NO_x$ emission (Obernberger and Thek, 2004). However, planting location had no clear effect on hydrogen and sulfur (Figure 6).

## 4. Conclusions

Planting location had significant effects on both chemical and elemental compositions of big bluestem. Ecotype had significant effects on glucan, xylan, lignin, and ash contents, and C, O, and H elemental fractions, whereas planting location significantly affected all measured variables. The ecotype-location interaction had significant effects on glucan, lignin, and hydrogen contents. In general, big bluestem planted in Illinois had higher cellulose, hemicellulose, and lignin contents than the populations planted in the Kansas locations. Besides environmental effects, the Illinois ecotype had the lowest lignin contents for all four locations. Carbon content increased with eastward movement. Carbon content of the big bluestem planted in Illinois was higher than those planted in the Kansas locations. Up to 97%, 88% and 80% of the variation in compositions can be explained by annual precipitation, growing degree days and potential evapotranspiration in 2010 respectively. The results show that big bluestem could potentially serve as suitable energy grass in the Midwest with similar or better chemical and elemental compositions compared with other biomass crops and grasses.

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**Table 1: Collection sites for *A.gerardii* populations**

| Ecotype | Population collection site | County | Latitude (N) | Longitude (W) | Elevation (m) |
|---|---|---|---|---|---|
| CKS, Hays | Webster Reservoir (WEB) | Rooks | 39$^{o}$ 24’ | 99$^{o}$ 32' | 606 |
| | Cedar Bluffs Reservoir (CDB) | Trego | 38$^{o}$ 45’ | 99$^{o}$ 46' | 688 |
| EKS, Manhattan | Konza Prairie (KON) | Riley/Geary | 39$^{o}$ 05’ | 96$^{o}$ 36' | 366 |
| | Top of the World Park (TOW) | Riley | 39$^{o}$ 13’ | 96$^{o}$ 37' | 379 |
| IL, Carbondale | Twelve Mile (12M) | Effingham, Fayette, and Marion | 38°46' | 88°50' | NA |
| | Fults (FUL) | Monroe | 37°58’ | 89°48' | 215 |

**Table 2. The location of the reciprocal garden in the four planting sites**

| Environment conditions | Reciprocal Garden Planting Site | | | |
|---|---|---|---|---|
| | Colby, KS<br>Northwest Kansas Agricultural Research Center | Hays, KS<br>Agricultural Research Center–Hays | Manhattan, KS<br>USDA Plant Materials Center | Carbondale, Illinois<br>Southern Illinois University Agronomy Center |
| Annual precipitation, 2010 (cm) | 44.57 | 50.11 | 67.82 | 66.95 |
| Mean annual precipitation since 1961 (cm) | 50.47 | 58.22 | 87.15 | 116.73 |
| Precipitation of driest year, cm (year) | 28.37<br>(1967) | 36.27<br>(1988) | 39.16<br>(1966) | 66.95<br>(2010) |
| Growing degree days average since 1961 | 3167 | 3799 | 4156 | 4087 |
| Growing degree days, 2010 | 3461 | 4193 | 4105 | 4474 |
| Potential evapotranspiration (cm) | 144 | 139 | 127 | 99 |
| Aridity index (PET[a]-PPT[b]) | 97 | 81 | 41 | -18 |
| Soil type | Silt-loam | Roxbury silt-loam | Sandy-loam | Stoy silt-loam |

[a] PET: Potential evapotranspiration.

[b] PPT: Precipitation.

**Table 3. Chemical composition of big bluestem by population and planting site.**

| Population-location | Chemical composition (%, db) | | | | | |
|---|---|---|---|---|---|---|
| | Glucan | Xylan | Arabinan | Xylan+ Arabinan | Lignin | Ash |
| CDB (CKS)-Colby | 32.5±0.1 | 21.4±0.1 | 3.73±0.01 | 25.1±0.1 | 14.8±0.4 | 3.97±0.32 |
| WEB (CKS)-Colby | 32.8±0.1 | 22.3±0.2 | 3.20±0.01 | 25.5±0.1 | 15.2±0.1 | 3.91±0.14 |
| KON (EKS)-Colby | 29.6±0.1 | 20.7±0.11 | 3.77±0.02 | 24.5±0.1 | 13.3±0.2 | 5.33±0.90 |
| TOW (EKS)-Colby | 30.8±0.2 | 20.7±0.2 | 4.10±0.01 | 24.78±0.2 | 13.9±0.1 | 4.97±0.94 |
| 12M(ILL)-Colby | 29.6±0.2 | 19.2±0.1 | 3.84±0.06 | 23.01±0.2 | 12.0±0.1 | 5.06±0.25 |
| FUL (ILL)-Colby | 32.6±0.2 | 22.0±0.2 | 3.97±0.10 | 26.0±0.01 | 14.9±0.1 | 3.18±0.06 |
| KAW (CULTIVAR)-Colby | 34.9±0.1 | 23.7±0.3 | 2.12±0.01 | 25.8±0.3 | 16.3±0.1 | 3.89±0.55 |
| CDB (CKS)-Hays | 36.1±0.1 | 22.2±0.6 | 3.15±0.70 | 25.4±1.3 | 18.6±0.2 | 3.79±0.03 |
| WEB (CKS)-Hays | 35.2±0.5 | 23.2±0.3 | 2.66±0.42 | 25.9±0.7 | 17.7±0.1 | 3.41±0.36 |
| KON (EKS)-Hays | 33.3±0.2 | 21.9±0.2 | 3.02±0.29 | 24.9±0.1 | 17.4±0.1 | 4.25±0.26 |
| TOW (EKS)-Hays | 32.7±0.2 | 21.3±0.5 | 3.48±0.41 | 24.8±0.9 | 17.2±0.1 | 5.60±0.24 |
| 12M (ILL)-Hays | 31.8±0.5 | 22.6±0.5 | 3.97±0.06 | 26.6±0.5 | 14.2±0.3 | 3.12±0.24 |
| FUL (ILL)-Hays | 32.8±0.4 | 21.7±0.7 | 3.02±0.43 | 24.8±1.1 | 16.8±0.1 | 3.62±0.31 |
| KAW (CULTIVAR)-Hays | 34. 9±0.1 | 22.5±0.1 | 2.54±0.23 | 25.1±0.4 | 16.9±0.2 | 3.60±0.23 |
| CDB (CKS)-Manhattan | 36.7±0.6 | 23.9±0.3 | 3.28±0.21 | 27.2±0.1 | 18.7±0.2 | 4.66±0.40 |
| WEB (CKS)-Manhattan | 34.6±0.4 | 25.3±0.3 | 3.10±0.01 | 28.4±0.3 | 18.8±0.1 | 3.92±0.26 |
| KON (EKS)-Manhattan | 35.9±0.4 | 25.1±0.5 | 3.33±0.50 | 28.4±0.1 | 17.6±0.4 | 4.52±0.55 |
| TOW (EKS)-Manhattan | 35.1±0.3 | 25.5±0.8 | 3.02±0.16 | 28.5±0.7 | 18.2±0.6 | 4.77±0.30 |
| 12M (ILL)-Manhattan | 34.0±0.2 | 23.4±0.2 | 3.87±0.17 | 27.3±0.4 | 15.2±0.2 | 4.93±0.49 |
| FUL (ILL)-Manhattan | 37.1±0.2 | 26.6±0.1 | 2.90±0.32 | 29.5±0.4 | 17.3±0.3 | 3.18±0.19 |
| KAW (CULTIVAR)–Manhattan | 38.3±0.6 | 24.7±0.4 | 2.28±0.03 | 27.0±0.4 | 17.6±0.1 | 4.51±0.01 |
| CDB (CKS)-Carbondale | 35.6±0.1 | 24.6±0.4 | 3.86±0.11 | 28.5±0.3 | 17.7±0.2 | 4.83±0.18 |
| WEB (CKS)-Carbondale | 36.3±0.4 | 26.1±0.2 | 4.73±0.05 | 30.8±0.2 | 18.2±0.1 | 4.29±0.02 |
| KON (EKS)-Carbondale | 36.2±0.2 | 25.4±0.1 | 4.74±0.15 | 30.1±0.2 | 17.6±0.2 | 5.58±0.34 |
| TOW (EKS)-Carbondale | 36.2±0.1 | 25.8±0.3 | 4.75±0.03 | 30.5±0.3 | 18.5±0.5 | 4.90±0.03 |
| 12M (ILL)-Carbondale | 35.1±0.4 | 24.3±1.2 | 3.78±0.41 | 28.1±1.6 | 16.7±0.1 | 4.82±0.16 |
| FUL (ILL)-Carbondale | 36.6±0.5 | 26.4±0.2 | 4.25±0.04 | 30.7±0.2 | 18.2±0.2 | 4.64±0.10 |
| KAW (CULTIVAR)-Carbondale | 39.5±0.4 | 26.8±0.1 | 2.70±0.14 | 29.5±0.1 | 19.4±0.2 | 4.30±0.04 |
| Average | 34.5 ±2.4 | 23.6 ±2.0 | 3.5 ±0.7 | 27.0 ±2.1 | 16.9 ±1.8 | 4.3±0.7 |

**Table 4. Comparison of the chemical composition of different types of biomass[a]**

| Type of biomass | Chemical composition (%, db) | | | |
|---|---|---|---|---|
| | Glucan | Xylan+Arabinan | Lignin | Ash |
| Big bluestem-this study | 34.5 | 27.0 | 16.8 | 4.3 |
| Corn stover | 38 | 26 | 19 | 6 |
| Soybean | 33 | 14 | - | 6 |
| Wheat straw | 38 | 29 | 15 | 6 |
| Rye straw | 31 | 25 | - | 6 |
| Barley straw | 42 | 28 | - | 11 |
| Switchgrass | 37 | 29 | 19 | 6 |
| Indiangrass | 39 | 29 | - | 8 |
| Little bluestem | 35 | 31 | - | 7 |
| Prairie cordgrass | 41 | 33 | - | 6 |
| Miscanthus | 43 | 24 | 19 | 2 |
| Intermediate wheatgrass | 35 | 29 | - | 6 |
| Reed canarygrass | 24 | 36 | - | 8 |
| Smooth bromegrass | 32 | 36 | - | 8 |
| Timothy | 28 | 30 | - | 6 |
| Tall fescue | 25 | 25 | 14 | 11 |
| Alfalfa | 27 | 12 | - | 9 |
| Forage sorghum | 34 | 17 | 16 | 5 |
| Sweet sorghum | 23 | 14 | 11 | 5 |
| Pearl millet | 25 | 35 | - | 9 |
| Sudangrass | 33 | 27 | - | 12 |

[a] Data source: Lee et al., 2007 (Lee et al, 2007)

**Table 5. Elemental composition of big bluestem and ratio of hydrogen to oxygen (H/C) as affected by population and planting site.**

| Population-location | Elemental composition (%) | | | | | H/C[a] |
|---|---|---|---|---|---|---|
| | C | H | O | N | S | |
| CDB (CKS)-Colby | 48.6±0.1 | 5.56±0.05 | 44.3±0.1 | 0.64±0.01 | 0.85±0.01 | 1.37 |
| WEB (CKS)-Colby | 47.2±0.1 | 5.75±0.06 | 45.5±0.1 | 0.69±0.01 | 0.88±0.04 | 1.47 |
| KON (EKS)-Colby | 47.4±0.1 | 5.57±0.01 | 45.6±0.1 | 0.69±0.01 | 0.83±0.04 | 1.41 |
| TOW (EKS)-Colby | 47.9±0.1 | 5.62±0.01 | 45.1±0.1 | 0.61±0.01 | 0.83±0.03 | 1.40 |
| 12M (ILL)-Colby | 47.6±0.1 | 4.93±0.03 | 46.1±0.1 | 0.57±0.01 | 0.78±0.02 | 1.23 |
| FUL (ILL)-Colby | 48.5±0.1 | 5.66±0.03 | 44.2±0.1 | 0.72±0.01 | 0.89±0.01 | 1.40 |
| KAW (CULTIVAR)-Colby | 49.8±0.1 | 5.36±0.01 | 43.4±0.1 | 0.52±0.01 | 0.83±0.02 | 1.28 |
| CDB (CKS)-Hays | 47.8±0.1 | 6.01±0.02 | 44.3±0.1 | 1.02±0.02 | 0.97±0.02 | 1.51 |
| WEB (CKS)-Hays | 47.5±0.1 | 5.75±0.04 | 44.9±0.1 | 0.92±0.01 | 0.88±0.01 | 1.45 |
| KON (EKS)-Hays | 47.4±0.0 | 5.88±0.02 | 44.7±0.1 | 1.12±0.02 | 0.93±0.01 | 1.49 |
| TOW (EKS)-Hays | 47.7±0.1 | 5.88±0.02 | 44.5±0.1 | 1.00±0.01 | 0.93±0.01 | 1.47 |
| 12M (ILL)-Hays | 47.1±0.1 | 5.76±0.02 | 45.6±0.1 | 0.69±0.02 | 0.87±0.02 | 1.46 |
| FUL (ILL)-Hays | 48.8±0.1 | 6.11±0.01 | 43.1±0.1 | 1.07±0.03 | 0.98±0.01 | 1.50 |
| KAW (CULTIVAR)-Hays | 49.0±0.1 | 6.13±0.04 | 43.2±0.1 | 0.73±0.03 | 0.98±0.01 | 1.49 |
| CDB (CKS)-Manhattan | 49.3±0.1 | 6.03±0.03 | 42.7±0.1 | 1.15±0.07 | 0.92±0.01 | 1.47 |
| WEB (CKS)-Manhattan | 49.8±0.1 | 5.95±0.01 | 42.6±0.1 | 0.77±0.04 | 0.93±0.01 | 1.43 |
| KON (EKS)-Manhattan | 47.9±0.1 | 5.86±0.01 | 44.4±0.1 | 0.86±0.01 | 0.93±0.03 | 1.46 |
| TOW (EKS)-Manhattan | 49.47±0.1 | 5.97±0.02 | 43.0±0.1 | 0.73±0.02 | 0.93±0.02 | 1.45 |
| 12M (ILL)-Manhattan | 49.3±0.1 | 5.94±0.03 | 42.6±0.1 | 1.14±0.03 | 0.96±0.01 | 1.43 |
| FUL (ILL)-Manhattan | 50.0±0.1 | 6.23±0.01 | 42.1±0.1 | 0.66±0.01 | 0.96±0.01 | 1.49 |
| KAW (CULTIVAR)-Manhattan | 49.6±0.1 | 5.90±0.02 | 42.8±0.1 | 0.75±0.01 | 0.94±0.04 | 1.43 |
| CDB (CKS)-Carbondale | 50.7±0.1 | 6.30±0.02 | 41.1±0.1 | 0.93±0.01 | 0.97±0.02 | 1.49 |
| WEB (CKS)-Carbondale | 50.8±0.1 | 6.45±0.01 | 41.1±0.1 | 0.76±0.02 | 0.97±0.02 | 1.52 |
| KON (EKS)-Carbondale | 50.1±0.1 | 6.16±0.01 | 41.5±0.1 | 1.27±0.03 | 0.95±0.01 | 1.47 |
| TOW (EKS)-Carbondale | 51.4±0.1 | 6.11±0.01 | 40.8±0.1 | 0.84±0.01 | 0.94±0.02 | 1.42 |
| 12M (ILL)-Carbondale | 50.5±0.1 | 5.85±0.01 | 41.8±0.1 | 0.88±0.04 | 0.94±0.03 | 1.38 |
| FUL (ILL)-Carbondale | 51.3±0.1 | 6.03±0.01 | 40.8±0.1 | 0.95±0.01 | 0.94±0.01 | 1.41 |
| KAW (CULTIVAR)-Carbondale | 51.3±0.1 | 6.15±0.01 | 40.7±0.1 | 0.87±0.01 | 0.96±0.01 | 1.43 |
| Average | 49.1±1.4 | 5.9 ±0.3 | 43.3 ±1.6 | 0.84 ±0.2 | 0.92 ±0.1 | 1.44±0.1 |

[a] H/C= $\frac{H\%/1}{C\%/12}$

**Table 6. Comparison of the elemental composition of different types of biomass[a]**

| Type of biomass | Elemental composition (%) | | | | | H/C [b] |
|---|---|---|---|---|---|---|
| | C | H | O | N | S | |
| Big bluestem—this study | 49.1 | 5.9 | 43.3 | 0.84 | 0.92 | 1.44 |
| Bagasse (sugarcane) | 44.8 | 5.3 | 39.6 | 0.38 | 0.01 | 1.42 |
| Barley straw | 45.7 | 6.1 | 38.3 | 0.4 | 0.1 | 1.60 |
| Cotton stalk | 13.6 | 5.8 | 43.9 | - | - | 5.12 |
| Corn stover | 43.7 | 5.6 | 43.3 | 0.61 | 0.01 | 1.54 |
| Pine (bark) | 52.3 | 5.8 | 38.8 | 0.2 | - | 1.33 |
| Popular (hybrids) | 48.5 | 5.9 | 43.7 | 0.47 | 0.01 | 1.46 |
| Redwood | 53.5 | 5.9 | 40.3 | 0.1 | - | 1.32 |
| Rice straw | 41.8 | 4.6 | 36.6 | 0.7 | 0.08 | 1.32 |
| Switchgrass | 47.5 | 5.8 | 42.4 | 0.74 | 0.08 | 1.47 |
| Wheat straw | 43.2 | 5.0 | 39.4 | 0.61 | 0.11 | 1.39 |

[a] Data source: Jammel et al., 2010

[b] H/C= $\frac{H\%/1}{C\%/12}$

**Table 7. Effects of environmental conditions on chemical composition and elemental fractions of big bluestem analyzed by linear regression models.**

| Composition (% db) | PPT[a] 2010 (cm) | PPT[a] since 1961 (cm) | GDD[b] avg. (cm) | GDD[b] 2010 (cm) | PET[c] (cm) | Aridity index |
|---|---|---|---|---|---|---|
| Glucan | 0.94 | 0.84 | 0.93 | 0.8 | 0.72 | 0.81 |
| Xylan | 0.97 | 0.93 | 0.78 | 0.66 | 0.8 | 0.88 |
| Xylan+ Arabinan | 0.88 | 0.99 | 0.63 | 0.67 | 0.91 | 0.96 |
| Lignin | 0.74 | 0.63 | 0.96 | 0.88 | 0.55 | 0.63 |
| Ash | 0.37 | 0.65 | 0.08 | 0.17 | 0.67 | 0.64 |
| Carbon | 0.7 | 0.96 | 0.42 | 0.57 | 0.95 | 0.96 |
| Hydrogen | 0.69 | 0.69 | 0.9 | 0.96 | 0.64 | 0.69 |
| Oxygen | 0.79 | 0.99 | 0.61 | 0.76 | 0.98 | 0.99 |
| Nitrogen | 0.37 | 0.34 | 0.74 | 0.82 | 0.32 | 0.36 |
| Sulfur | 0.61 | 0.52 | 0.91 | 0.87 | 0.46 | 0.52 |

[a] PPT: Precipitation

[b] GOD: Growing degree days

[c] PET: Potential evapotranspiration

**Table 8. Effects of ecotype (E), location (L), and interaction between ecotype and planting location on the chemical and elemental composition of big bluestem.**

| Composition /elements (%) | Source of variation | Location | Ecotype | L×E |
|---|---|---|---|---|
| Glucan | F | 73.56 | 28.51 | 3.59 |
| | P | <0.001 | <0.001 | 0.002 |
| Xylan | F | 58.98 | 3.36 | 1.811 |
| | P | <0.001 | 0.028 | 0.096 |
| Xylan+ Arabinan | F | 63.70 | 0.14 | 1.12 |
| | P | <0.001 | 0.935 | 0.369 |
| Lignin | F | 48.98 | 16.23 | 2.61 |
| | P | <0.001 | <0.001 | 0.018 |
| Ash | F | 7.23 | 9.62 | 1.39 |
| | P | <0.001 | <0.001 | 0.224 |
| Carbon | F | 80.77 | 11.50 | 1.69 |
| | P | <0.001 | <0.001 | 0.123 |
| Oxygen | F | 86.66 | 5.98 | 1.67 |
| | P | <0.001 | 0.002 | 0.129 |
| Hydrogen | F | 45.27 | 2.94 | 3.24 |
| | P | <0.001 | 0.044 | 0.005 |
| Nitrogen | F | 12.02 | 2.60 | 1.13 |
| | P | <0.001 | 0.065 | 0.359 |
| Sulfur | F | 29.52 | 0.46 | 1.15 |
| | P | <0.001 | 0.706 | 0.347 |

## Figure captions

**Fig. 1. Reciprocal gardens across the precipitation gradient. Yellow dot is Colby satellite site. Seeds were collected from native prairie with 50 miles of each planting site. The isoclines represent the precipitation gradient in terms of mean annual precipitation (modified from Burke) across the central grasslands of the United States.**

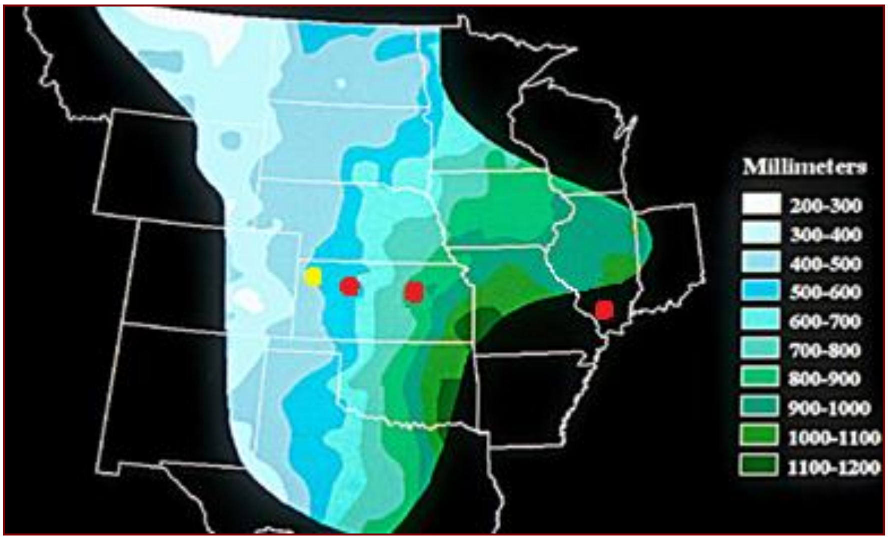
**Fig. 2. Effects of planting location on chemical composition of big bluestem.**

**Fig. 3. Effects of ecotype on chemical composition of big bluestem.**

**Fig. 4. Effects of planting location on carbon content of big bluestem.**

**Fig. 5. Effects of planting location on nitrogen content of big bluestem.**

**Fig. 6. Effect of ecotype on carbon, oxygen contents hydrogen, nitrogen, and sulfur.**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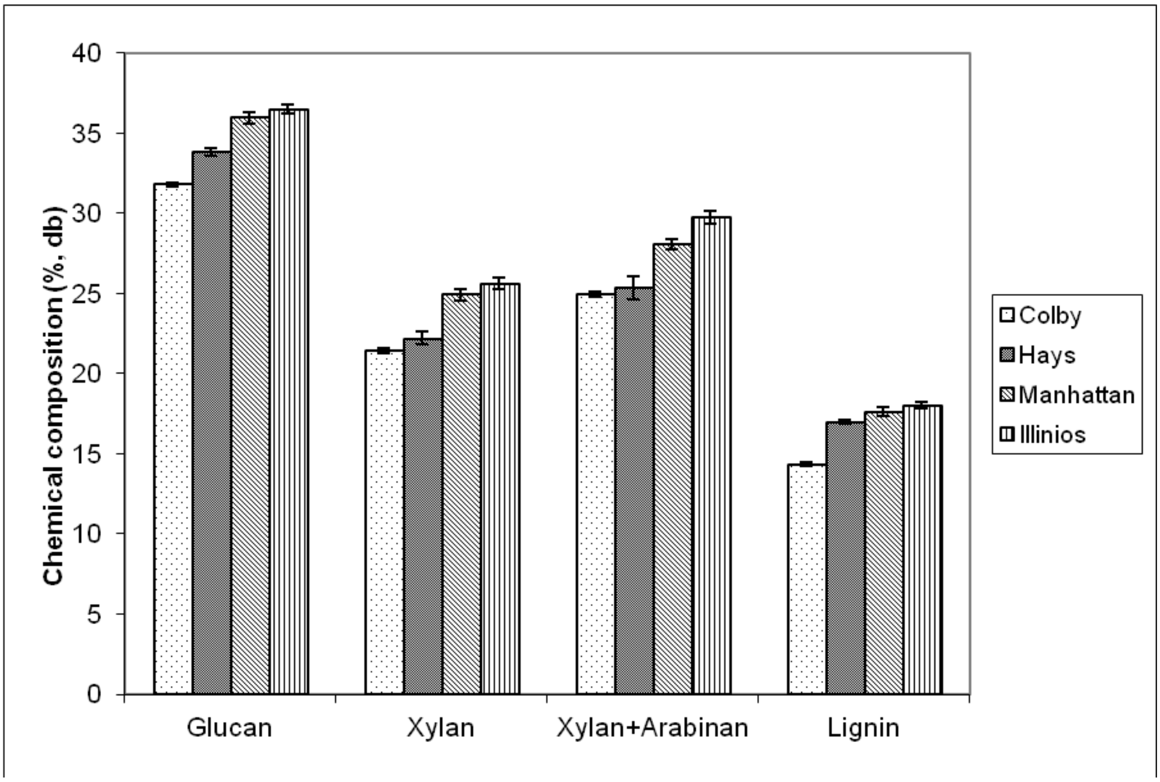
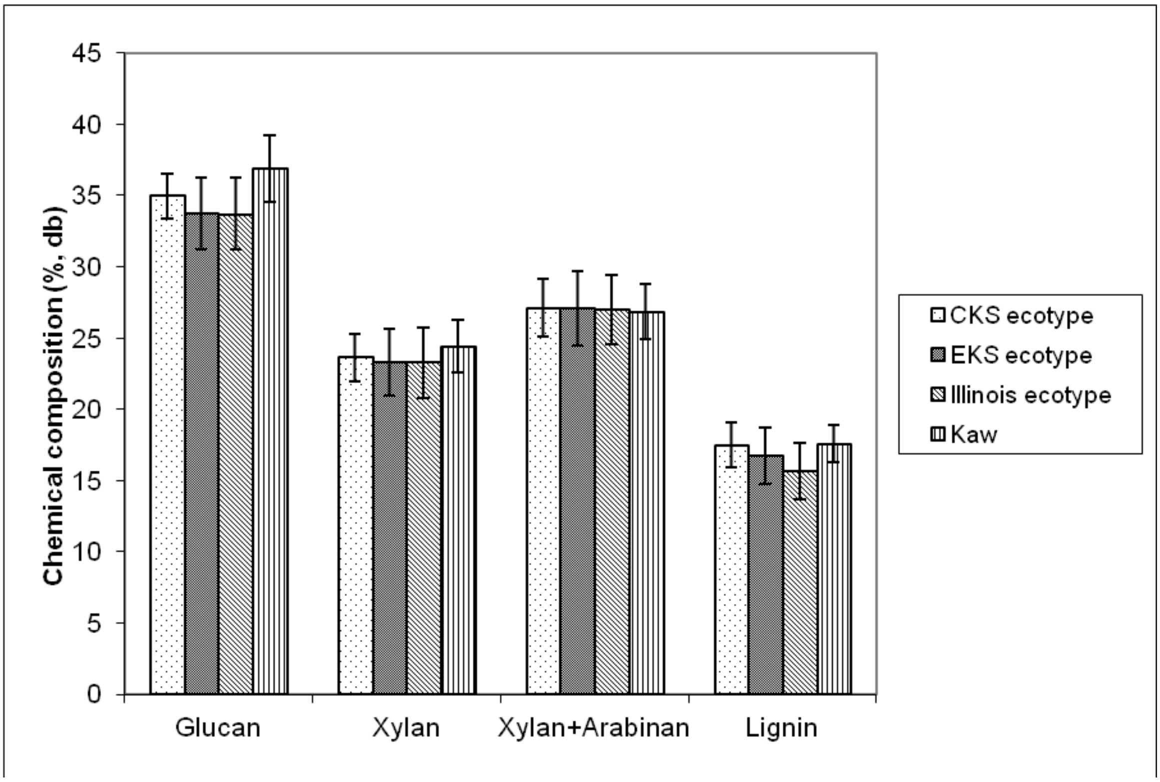
400-500
500-600
600-700
700-800
800-900
900-1000
1000-1100
1100-1200

**Fig. 1.**



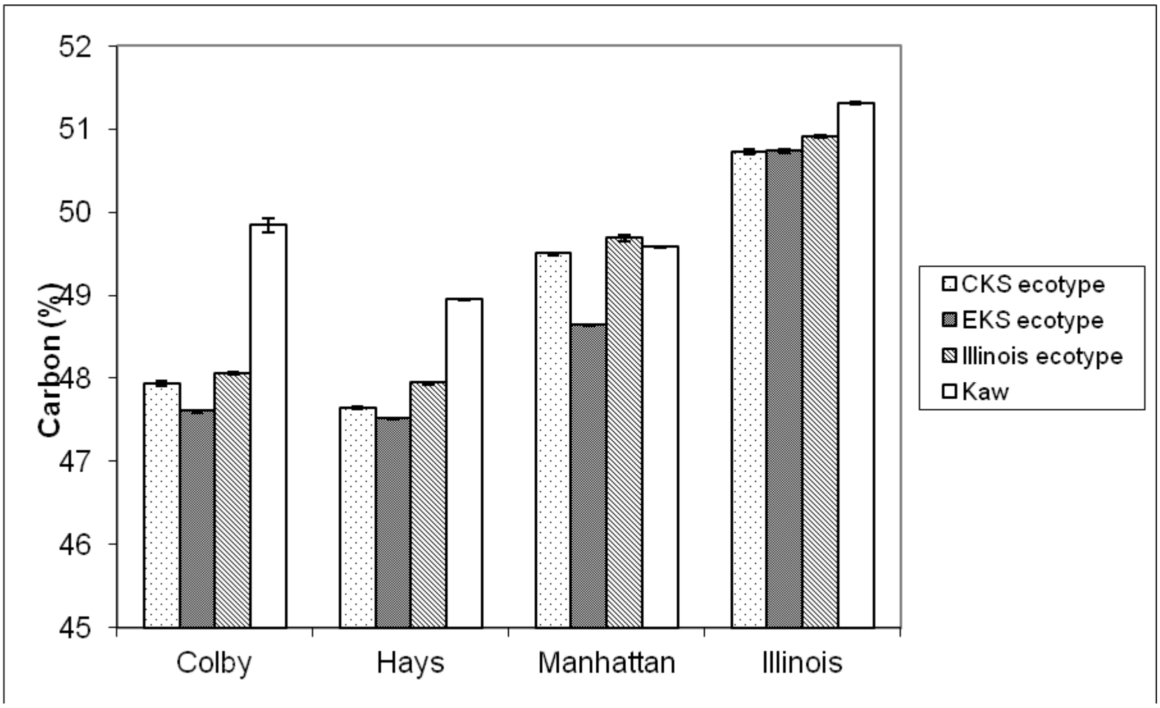
Chemical composition (%, db)
0
5
10
15
20
25
30
35
40
Glucan
Xylan
Xylan+Arabinan
Lignin
Colby
Hays
Manhattan
Illinios


**Fig. 2.**



Chemical composition (%, db)
45
40
35
30
25
20
15
10
5
0
Glucan
Xylan
Xylan+Arabinan
Lignin
CKS ecotype
EKS ecotype
Illinois ecotype
Kaw


**Fig. 3.**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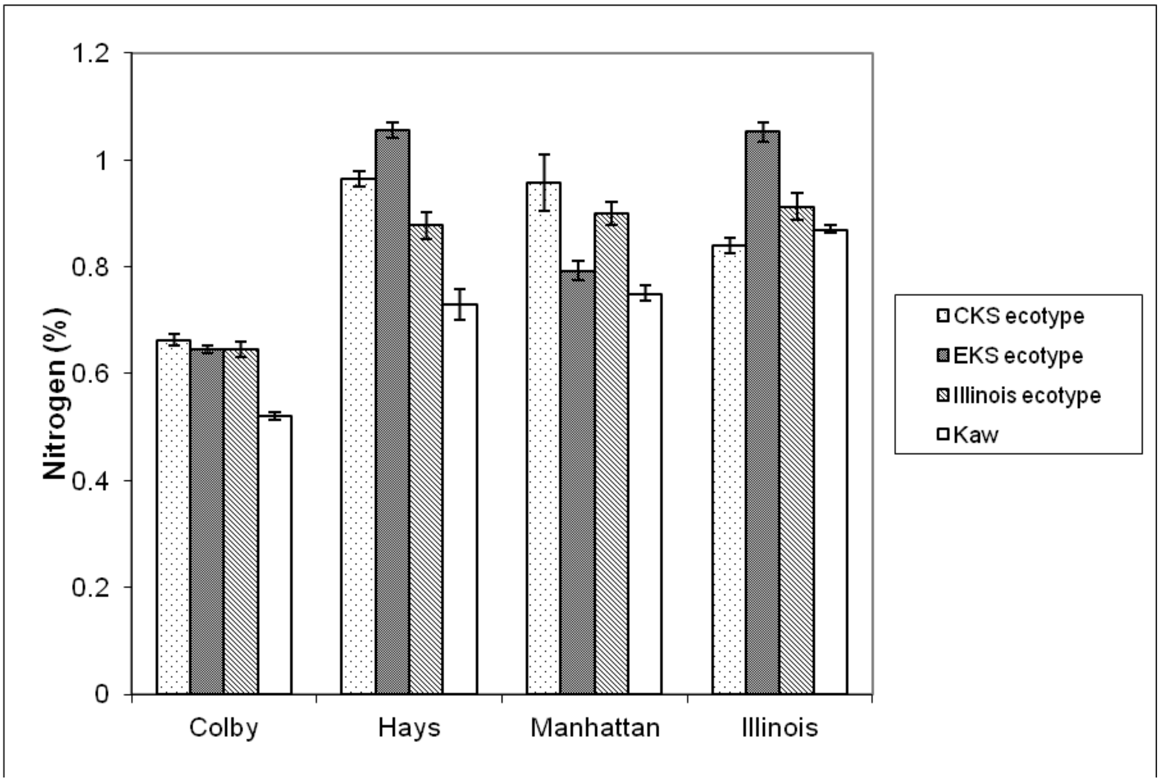
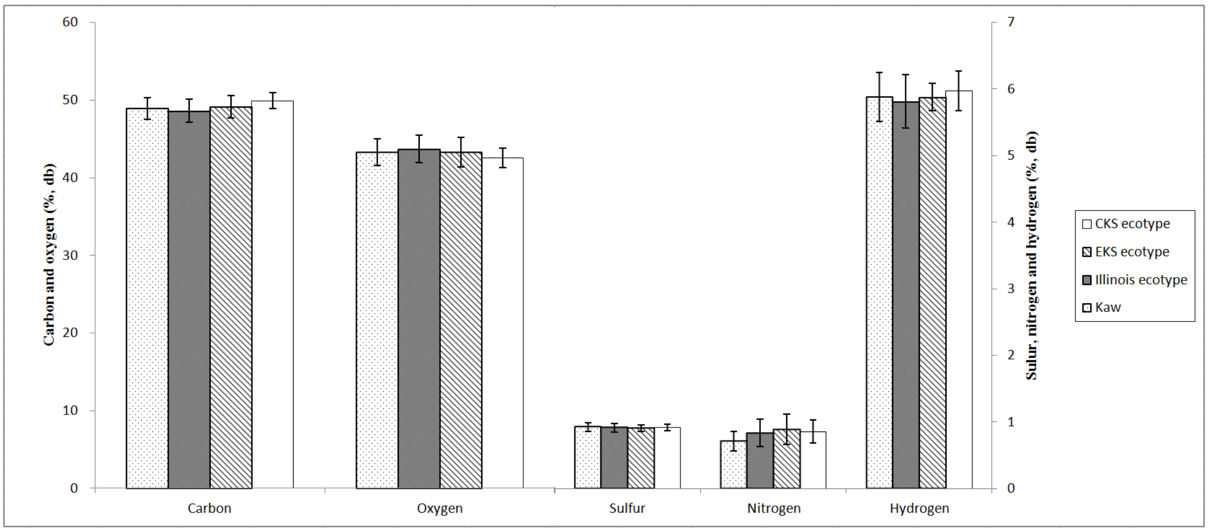
51
50
49
48
47
46
45
Carbon (%)
Colby
Hays
Manhattan
Illinois
CKS ecotype
EKS ecotype
Illinois ecotype
Kaw

**Fig. 4.**



1.2
1
0.8
0.6
0.4
0.2
0
Nitrogen (%)
Colby
Hays
Manhattan
Illinois
CKS ecotype
EKS ecotype
Illinois ecotype
Kaw


**Fig. 5.**



60
50
40
30
20
10
0
Carbon and oxygen (%, db)
7
6
5
4
3
2
1
0
Sulur, nitrogen and hydrogen (%, db)
CKS ecotype
EKS ecotype
Illinois ecotype
Kaw
Carbon
Oxygen
Sulfur
Nitrogen
Hydrogen


**Fig. 6.**