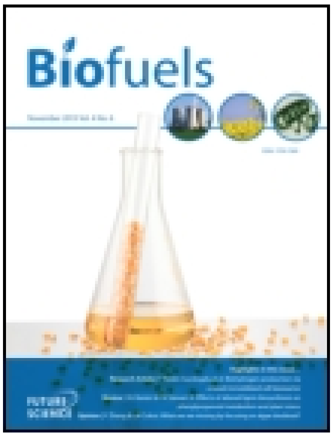

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Drought effects on composition and yield for corn stover, mixed grasses, and Miscanthus as bioenergy feedstocks

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Drought effects on composition and yield for corn stover, mixed grasses, and *Miscanthus* as bioenergy feedstocks

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Drought conditions in 2012 were some of the most severe in recent history. The purpose of this study is to examine the impact of drought on quality, quantity, and theoretical ethanol yield (TEY) of three bioenergy feedstocks, corn stover, mixed grasses from Conservation Reserve Program lands, and *Miscanthus* × *giganteus*. To assess drought effects on these feedstocks, samples from 2010 (minimal to no drought) and 2012 (severe drought) were compared from multiple locations in the US. In all feedstocks, drought significantly increased extractives and reduced structural sugars and lignin; subsequently, TEYs were reduced 10–15%. Biomass yields were significantly reduced for *M.* × *giganteus* and mixed grasses. When reduction in quality and quantity were combined, TEYs decreased 26–59%. Drought negatively affected biomass quality and quantity that resulted in significant TEY reductions. Such fluctuations in biomass quality and yield may have significant consequences for developing lignocellulosic biorefineries.

A consistent supply of high-quality feedstocks for lignocellulosic biorefineries is critical to achieving national biofuel goals [1]. Under the US Energy Independence and Security Act of 2007, the Renewable Fuels Standard (RFS) mandated the use of 36 × 10^9 gallons per year of renewable fuels by 2022, of which 16 × 10^9 gallons are to be derived from cellulosic ethanol [2]. One of the most critical aspects for profitable lignocellulosic bioenergy facilities is having access to biomass feedstocks of predictable composition and quality. In the US, corn stover, perennial mixed grasses, and *Miscanthus* are a few of the potential feedstock materials available for conversion to bioenergy. Quantifying the effects of environmental factors, such as drought, on biomass feedstock quality is critical to establishing a sustainable supply of feedstock for meeting biofuel production goals established under the RFS.

Currently, most biorefineries rely on corn stover as the primary feedstock for ethanol production [102], because of the vast area upon which corn is grown throughout the US and the pre-existing infrastructure for harvest, storage and transport of feedstock in corn growing regions. For example, from 2010 to 2012, corn was planted on an average of 37.4 × 10^6 ha (92,428,667 acres) throughout the US and produced an average of 11.9 × 10^9 bushels of grain [3]. Assuming a harvest index of 0.5 (the ratio of grain compared with combined grain and stover) and an average bushel weight of 25.4 kg (56 lbs) [4],

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Key terms

Drought: A soil moisture deficit low enough to have social, environmental, and/or economic impacts. The severity of drought was categorized into five classes based on multiple parameters (Palmer Drought Index, Climate Prediction Center Soil Moisture Model, US Geological Survey Weekly Streamflow, Standardized Precipitation Index, and other indicators based on the specific location): D0 – abnormally dry; D1 – moderate drought; D2 – severe drought; D3 – extreme drought; and D4 – exceptional drought [101].

Bioconversion: Microbially-mediated conversion of organic materials into one or more sources of energy (e.g., ethanol, butanol, or other advanced biofuels).

Compositional analysis: Series of quantitative analytical procedures to determine ash, protein, total extractives, structural carbohydrates (glucan, xylan, arabinan, galactan and mannan), lignin and organic acids within biological materials.

Compatible (osmotic) solutes: Small, soluble organic molecules capable of moving through the partially permeable membrane of cell walls in order to adjust water potential and maintain solute equilibrium between the cell surrounding and cytosol.

Theoretical ethanol yield: Estimated ethanol yield from biochemical conversion via fermentation based on the sugar content of a biomass material assuming 100% conversion efficiency.

the total quantity of corn stover produced averaged 301 × 10^6 Mg for those three years. To meet projected demands, other feedstocks need to be considered for increased ethanol production.

The hybrid grass *Miscanthus* × *giganteus* is a promising bioenergy crop. It has been primarily investigated in Europe as part of the Miscanthus Productivity Network [5]. *M.* × *giganteus* is a perennial grass that recycles nutrients via a rhizome system, and therefore, requires lower nutrient inputs [6] compared with corn [7]. *M.* × *giganteus* also has the potential to generate substantially higher yields of biomass than corn. For example, *M.* × *giganteus* was reported to have a 29.6 Mg ha^{-1} yield with an estimated production at 1.0% conversion efficiency of 782 L ha^{-1} of ethanol while corn stover yielded 7.4 Mg ha^{-1} and 196 L ha^{-1} of ethanol [7].

Perennial grass crops such as those currently grown on land enrolled in the US Conservation Reserve Program (CRP) have also been suggested for use as a bioenergy feedstock. As with *M.* × *giganteus*, perennial grasses grown on marginal lands do not compete with current food crops, and have ancillary environmental benefits including preventing soil erosion, carbon sequestration and wildlife habitat preservation [8,9]. Perlack *et al.* reported that the 10.3 × 10^6 ha of CRP dedicated land could provide annual biomass yields of up to 4.25 Mg ha^{-1} [10]. Perennial grasses, similar to *M.* × *giganteus*, have also been reported to efficiently use water and nutrients [9]. Low-input high-diversity mixtures of perennial, native prairie grasses grown on agriculturally degraded lands have shown promise for increased bioenergy yields (as much as 238% higher yields than for monocultures) without the requirement for nitrogen fertilization and while providing net ecosystem CO_2 sequestration [11].

The large-scale production of cellulosic biofuels requires a sustainable supply of biomass feedstocks; however, environmental factors that vary from one year to the next may affect the quality and yield of biomass resources. It is necessary to understand how these environmental factors affect the yield and quality of promising feedstocks. Annual weather fluctuations associated with erratic climatic patterns have the potential to adversely impact both yield and quality of bioenergy feedstocks. During the past 30 years, there has been a significant increase in weather extremes, including the incidence of local and regional drought [12]. In 2012 severe drought conditions covered 39% of the US. Six states experienced record drought levels [13] and decreased crop yields. The most severe drought conditions were in the Midwest, including many of the corn producing states (i.e., Iowa, Illinois, Indiana, Minnesota, etc.) [103]. As a result, the US Department of Agriculture (USDA) reported a 13% decrease in corn yields from 2011 to 2012 and the lowest total corn production since 2006 [14]. Decreased yields due to drought pose a serious threat to the biomass supply chain for biofuel production facilities.

Quantifying potential impacts of drought on crop production is important because it is predicted that to meet the projected growth in energy demand [15], total corn grain production will need to exceed 140 × 10^9 bushels by 2017 with more than 30% of the crop being required for biofuel production. The economics of sustaining the biomass supply chain rely heavily on the consistency of crop yield and biomass quality [16]. A reduction in either of these metrics will require additional biomass feedstocks to meet the required biofuel production levels. To compensate for corn yield fluctuations due to drought, biomass supply systems for lignocellulosic industries will require a diverse portfolio of feedstocks including potential bioenergy crops such as *Miscanthus* and perennial grasses. Quantifying drought effects on yield and quality is an essential component for assessing the resource availability for bioenergy production systems.

Many studies have evaluated bioenergy crop yields in response to water stress [17]. Physiologically drought stress causes decreased turgor pressure in plant cells and inhibits cells from elongating in addition to causing wilting, leaf curling, and other physical changes to the plant [18] that negatively impact dry biomass yield. Drought can also alter plant cells in ways that change the overall chemical composition of the plant [19]. The quality of the plant is directly related to a plant's chemical composition. Calculations of changes in ethanol yield are often based on differences in dry biomass yield without consideration of changes in chemical composition of the plant due to abiotic stresses such as drought. To supply sufficient quantities of feedstocks to biorefineries in order to meet biofuel demand during times of drought, it is necessary to understand the impacts of drought on both the quality and the quantity of feedstocks with respect to production of ethanol and other bio-based products.

Al-Hakimi *et al.* reported that drought significantly reduced cellulose, lignin, and pectin content, but increased hemicellulose content in the shoots of soybean plants [19]. In response to drought, plants have been shown to accumulate compatible solutes; a group of small, soluble organic molecules capable of adjusting the water potential in cells without interfering with cellular metabolism. Examples of compatible solutes include monomeric sugars, proline, glycine betaine, and proline betaine [20–22]. Iraki *et al.* proposed that during drought stress cellulose is sacrificed in favor of metabolites that facilitate osmotic adjustment and that hemicellulose is then utilized as the cell wall 'backbone' [23]. Accumulation of compatible solutes contributes to compositional changes in the plant, decreasing the quantity of structural sugars available for biochemical conversion. As structural sugars are one of the most important components in cellulosic ethanol bioconversion processes, a decrease in these constituents would greatly impact the total ethanol yield. Furthermore, compatible solutes in biomass feedstocks are lost during necessary pretreatment processes at cellulosic biorefineries, and thus are not utilized in biochemical conversion pathways. An increase in soluble components results in a decrease in dry biomass yield available for a biochemical conversion; overall increasing the total biomass required to meet ethanol production demands. It is therefore necessary to determine the effect of drought on the structural carbohydrates and other components such as lignin in feedstocks intended for biochemical conversion. The objective of this study is to quantify drought effects on corn stover, mixed perennial grasses, and *M.* × *giganteus* quality and quantity by comparing chemical composition, theoretical ethanol yields, and dry matter yields for samples collected at a field scale relevant to the biofuels industry. To the authors' knowledge, this is the first study to explore the effects of drought on plant chemical composition and ethanol yields at this large of a spatial and temporal scale. Samples were analyzed from multiple US locations in 2010, which experienced minimal to no drought, and 2012, which experienced severe widespread drought.

Material and methods

■ Drought-based sample selection

Sample sets for inclusion in this study were selected based on the University of Nebraska-Lincoln US Drought Monitor [103, 104]. The severity of drought reported by the Drought Monitor was determined based on multiple parameters: the Palmer Drought Index that takes into account both recent precipitation and temperature data, the Climate Prediction Center Soil Moisture Model, the US Geological Survey Weekly Streamflow, the Standardized Precipitation Index, drought duration, and other indicators based on the specific region, including snow water content, river basin precipitation, and the Surface Water Supply Index [101]. Corn stover, mixed perennial grasses, and *M.* × *giganteus* were selected for comparison of feedstock quality from samples harvested in 2010 and 2012, as the growing seasons during these years experienced minimal or no drought (Figure 1) [104] and severe to exceptional drought conditions, respectively (Figure 2) [103]. All three feedstocks had at least one fall harvest; therefore, the end of October was chosen for observation of drought conditions for each year of interest. Perennial grasses and *M.* × *giganteus* samples from two states were chosen for analysis. Although the US experienced widespread drought in 2012, each state had local variations and, as a result, one of the locations from which samples were collected experienced little or no drought in 2010 and severe to exceptional drought in 2012. The second location from which perennial grasses and *M.* × *giganteus* samples were collected experienced little or no drought for both 2010 and 2012. Corn stover samples were only analyzed from one location, which had no drought in 2010 and severe drought in 2012.

■ Sample information

Corn stover (Pioneer 36v75 in 2010 and Pioneer P0461xr in 2012) was harvested in Boone County, IA from the Field 70/71 study on the Iowa State University Agronomy and Agricultural & Biosystems Engineering Research farm in October 2010 and September 2012. Representative samples from 11 of 84 plots were analyzed. For the 11 plots there were four different treatment variables used, which included charcoal additives, varying nitrogen rates, multiple tillage methods, and varying plant population densities. The treatments for each of the plots remained consistent between the two years, and the results from each year, regardless of treatment, were averaged together for reported means. All plots analyzed in this study had a 50% residue removal of stover. Dry biomass for the stover residue was calculated by collecting a subsample from a dual-stream grain and stover combine. The subsample was transferred to a weighed paper bag and dried at 70°C until a constant weight was reached. One subsample was taken for every plot analyzed and the calculated dry weight was extrapolated to the entire plot.

Mixed perennial grasses were harvested in Boone County, MO and Oconee County, GA in 2010 and 2012 from established Conservation Reserve Program grasslands. Eighteen plots were harvested in Boone County, MO in either May or June (for spring harvest) and again in November (for fall harvest) of 2010. The same plots were harvested in either May or June and then again in October of 2012. Compositional values for spring and fall harvests were averaged for each plot prior to statistical analysis for 18 replicates in total. Dry biomass yield was determined from each plot by

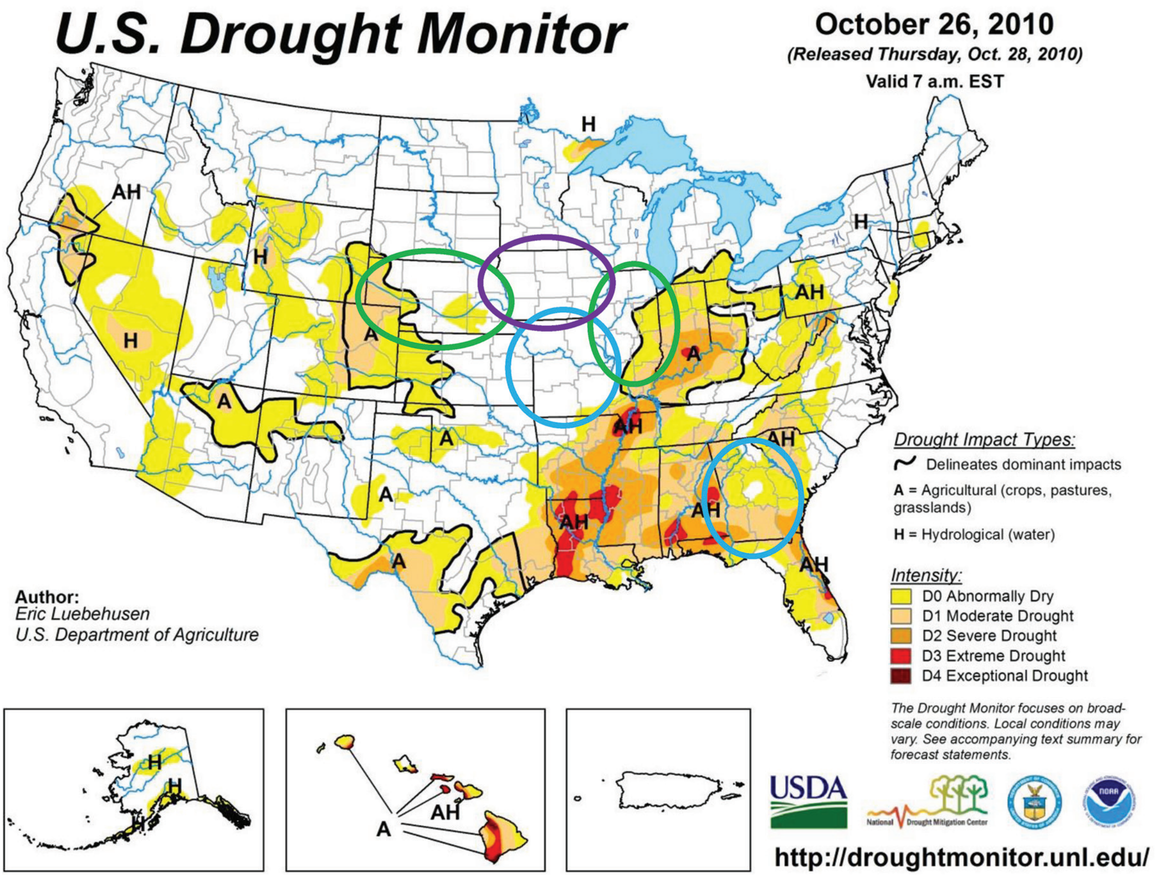

Figure 1. Map of drought conditions in 2010. The single study location for corn stover is in purple. Study locations for mixed perennial grasses are in blue and for *Miscanthus* × *giganteus* are in green. Map courtesy of the National Drought Mitigation Center at the University of Nebraska-Lincoln.

harvesting the biomass at a height of 10–15 cm. The cut biomass from each plot was then baled, weighed, and subsampled. The subsamples were collected with a 5 cm diameter and 50 cm long electric corer. The subsamples were dried at 60°C for 48 h in a forced air oven. The calculated dry matter was extrapolated to the entire plot. Twenty-four plots were harvested in Oconee County, GA in May and October in 2010 and 2012. Samples from May and October for each crop year and plot were physically combined prior to analysis giving a total of 24 replicates. For both locations and analysis years nitrogen rates were controlled at 0, 50, and 100 lbs ac^{-1}. The results from each year, regardless of treatment, were averaged together for reported means. The predominant species composition varied by location. In 2009, samples from Boone County, MO had an average of 55% tall fescue (*Schedonorus phoenix*), 20% red clover (*Trifolium praetense*), 8% yellow sweet clover (*Melilotus officinalis*), and 7% white clover (*Trifolium repens*). The perennial grasses harvested in Oconee County, GA had an average of 50% tall fescue and 15% orchardgrass (*Dactylis glomerata*) in 2010 and 48% tall fescue and 20% orchardgrass in 2012. Additional plant species were present at each location, but species representing small fractions of the biomass were not identified or reported by the collaborating institutions.

Miscanthus × *giganteus* was harvested in Saunders County, NE and Champaign County, IL. Samples from Saunders County, NE were harvested in December 2010 and November 2012, while *M.* × *giganteus* samples from Champaign County, IL were harvested in November 2010 and January 2013. At both locations, there were four replicates of three nitrogen fertilizer treatments (0, 60, and 120 kg ha^{-1}) that remained consistent for each year, thus providing a total of 12 plots for each location year. The dry biomass yields for each plot were calculated for the samples collected from Saunders County, NE by harvesting one 10 m^2 row of each plot. This subsample was weighed and dried in an oven at 60°C for 48 h and then weighed to determine dry matter weight. The dry weight of each subsample was extrapolated to the entire plot.

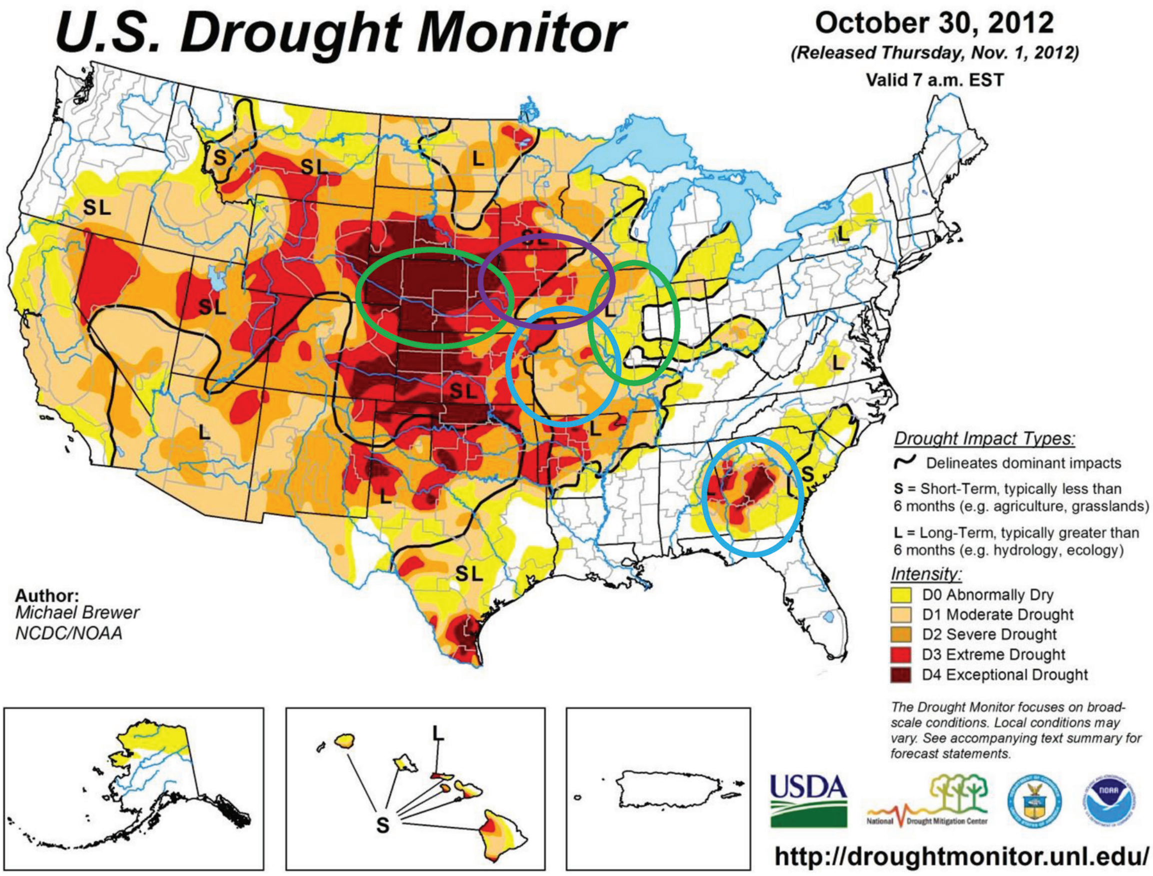

Figure 2. Map of drought conditions in 2012. The single study location for corn stover is in purple. Study locations for mixed perennial grasses are in blue and for *Miscanthus* × *giganteus* are in green. Map courtesy of the National Drought Mitigation Center at the University of Nebraska-Lincoln.

■ Precipitation and temperature records

Two factors that contribute to the determination of drought conditions are precipitation levels and temperature. Total monthly precipitation and average maximum temperature for each location were obtained for 2010 and 2012 using the nearest weather station data available from the Midwestern Regional Climate Center (MRCC) [105]. Monthly precipitation and temperature data for 2010 and 2012 were compared to site-specific, 30-year average (1981–2010) records from the National Oceanic and Atmospheric Association (NOAA), which compiled historical data from the MRCC [106]. The weather stations used for each location were as follows: Boone County, IA used data from AMES 5 SE Station USC00130203; Boone County, MO used data from Columbia Regional Airport Station USW00003945; Oconee County, GA used data from Athens Ben Epps Airport Station USW00013873; Saunders County, NE used data from Mead 6 S Station USC00255362; and Urbana, IL used data from Urbana (IL) Station USC00118740.

■ FT-NIR compositional analysis model

The chemical composition of corn stover, mixed perennial grasses, and *M.* × *giganteus* was predicted using Fourier transform near-infrared (FT-NIR) spectroscopy partial-least-squares (PLS) multivariate calibration models developed by the National Renewable Energy Laboratory (NREL). NREL's calibration models [24] were developed using multivariate statistical analyses to correlate NIR spectroscopic data to compositional data produced using standard wet chemical analysis techniques developed at NREL [25]. Predicting sample composition from NIR spectroscopic data is well established in the literature [26–29].

Samples were ground to pass a 2 mm screen and dried in a dessicator at room temperature to a moisture content of less than 5% (w.b.). Duplicate preparations of each sample were scanned at NREL using a Thermo Anataris II FT-NIR with auto-sampler attachment and Omnic software (Thermo Scientific, Waltham, MA, USA). For each sample, 128 scans were averaged resulting in a single spectrum. Duplicate samples were analyzed and the spectra were averaged prior to prediction.

Predictions for the corn stover samples were generated using a corn stover feedstock PLS2 model built using Unscrambler X 10.3 software (Camo Software Inc., Woodbridge, NJ, USA). The corn stover model included 107 calibration samples. Predictions for mixed perennial grasses and *M.* × *giganteus* samples were produced using a mixed herbaceous feedstock PLS2 model also built using the Unscrambler X 10.3 software. The mixed herbaceous feedstock model included 183 samples of corn stover, sorghum, *Miscanthus*, cool season grasses, switchgrass, and rice straw. Both calibration sets were comprised of a robust set of samples from a variety of years, geographic locations, growing conditions, cultivars, and anatomical fractions. NIR calibration models consisting of a variety of feedstocks or plant types have been well established in the literature [30–34].

$$\text{C6 or C5 Ethanol Yield (L Mg}^{-1}\text{)} = \frac{1.11\text{ g C6 or }1.136\text{ g C5}}{1\text{ g polymeric sugar}} \times \frac{X\text{ g polymeric sugar}}{100\text{ g biomass}} \times \frac{0.51\text{ g ethanol}}{1\text{ g C6 or C5}} \times \frac{3.79\text{ L ethanol}}{2971\text{ g ethanol}} \times \frac{1\times10^{6}\text{ g biomass}}{1\text{ Mg biomass}} \quad (1)$$

$$\text{TEY(L Mg}^{-1}\text{)} = \text{C6 Ethanol Yield(L Mg}^{-1}\text{)} + \text{C5 Ethanol Yield(L Mg}^{-1}\text{)} \quad (2)$$

$$\text{Total TEY (L ha}^{-1}\text{)} = \text{TEY(L Mg}^{-1}\text{)} \times \text{Dry Biomass(Mg ha}^{-1}\text{)} \quad (3)$$

A summary of model statistics for each calibration set is provided in Appendix Tables 1 and 2 for the corn stover and herbaceous feedstock models, respectively. (see Appendix, Tables 1 and 2, online supplemental material, which is available from the article's Taylor & Francis Online page at http://dx.doi.org/10.1080/17597269.2014.913904). Root mean square error of calibration (RMSEC) values for these models were similar to NREL published wet chemical analysis uncertainties, which indicate a robust prediction method [35]. The "Extractives" prediction combines values for ethanol extractives (oils, waxes, and fats), sucrose, and other water extractable components. The mixed herbaceous "Cellulose" prediction provides values for structural glucan, while "Total Glucan" provides values based on cellulose and starch contributions. These models were not summative mass closure models, and values were on a percent dry weight basis. Samples that fell outside the calibration sample population of two times the RMSEC were not used in this study.

■ Theoretical ethanol yield (TEY)/dry biomass yield

Theoretical ethanol yields (TEY) were calculated using the Department of Energy's Theoretical Ethanol Yield Calculator for *L* of ethanol per Mg of biomass [107]. The TEY calculations were done based on equations (1) and (2) [36]. The calculations give a theoretical yield assuming that 100% of the available carbohydrates are converted to ethanol. Only glucan (C6) and xylan (C5) content were used to calculate TEY for the samples in this study, as the other carbohydrates (arabinan, galactan, and mannan) were not measured (equation (2)) because they contribute less to the total amount of carbohydrates in the sample. Total TEY in this study is calculated for an area basis defined as the TEY per hectare of harvested biomass (equation (3)).

The PLS2 model used to predict composition of mixed perennial grasses and *M.* × *giganteus* reports "Total Glucan" (glucan from cellulose and starch) and "Cellulose" (glucan from cellulose). "Total Glucan" values were used in the TEY calculations for mixed perennial grasses and *M.* × *giganteus*. The PLS2 model used to determine corn stover only reports "Cellulose". Only samples that had both glucan and xylan values were used for the TEY calculations.

■ Statistical analysis

Statistical analysis was completed using R 2.14.0 [37]. The effect of year on compositional components, TEY and dry biomass yield was conducted with a one-way ANOVA using the 'anova' function. Data transformations were done if datasets did not meet the assumptions related to homogeneity or normality of residuals. To meet the ANOVA assumptions, IA corn stover and MO mixed perennial grasses xylan data were reciprocal transformed, and IL *M.* × *giganteus* extractives data were raised to the fourth power. The combined effects of harvest year and nitrogen treatment on *M.* × *giganteus* from IL and NE were analyzed with a two-way ANOVA using the 'aov' (analysis of variance) function. The IL *M.* × *giganteus* extractives data were $\log_e$ transformed to meet ANOVA assumptions. For all analyses, differences were considered statistically significant if $p < 0.05$.

Results and discussion

■ Corn stover

Precipitation and temperature

Ames, IA, according to the US Drought Monitor, had no signs of drought in 2010 and extreme drought in 2012 (Figure 1 and 2) in October of both years.

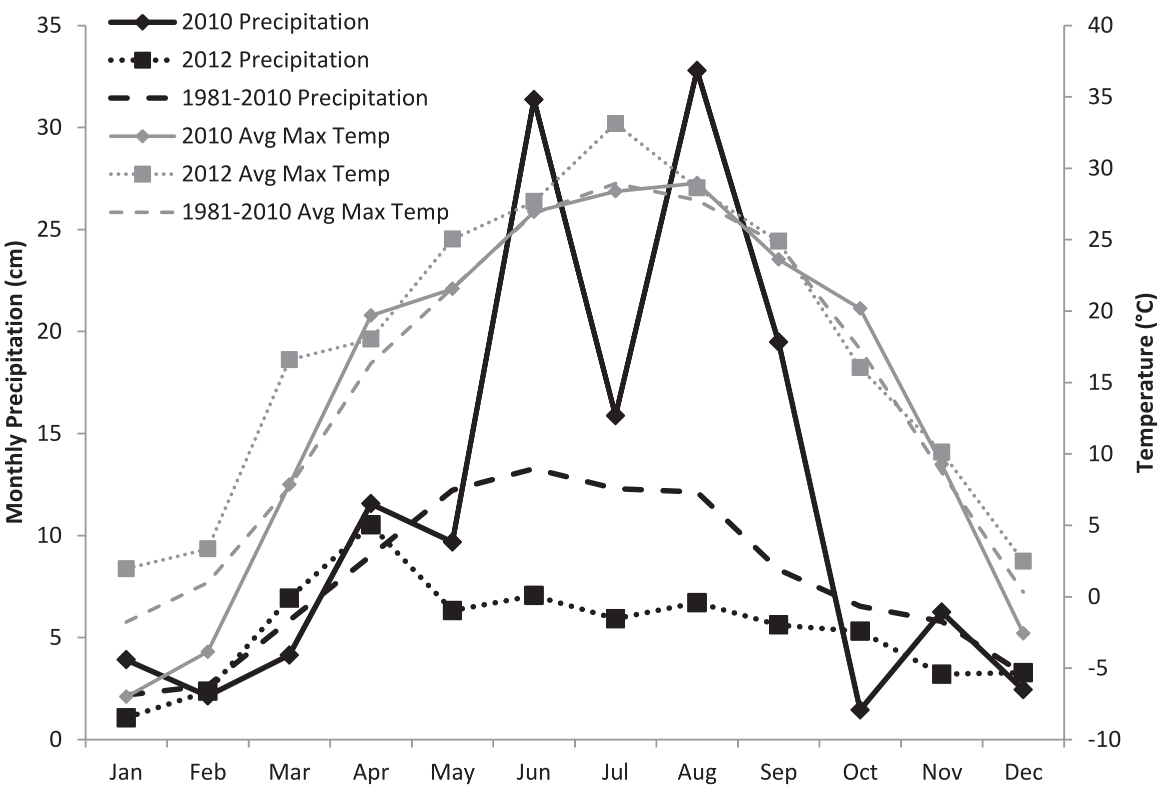

Figure 3. Ames, IA monthly precipitation and average maximum temperature in 2010, 2012, and averaged over a 30-year period (1981–2010).

Contributing to these drought determinations were the precipitation and temperature trends throughout each of the years. Ames experienced greater than average precipitation from June to September 2010 with levels in June and August being three times greater than the 30-year average (Figure 3). The only months in 2010 that had less rainfall than the 30-year average were March and October. In contrast, precipitation levels in 2012 were below the 30-year average for the majority of the year. In 2010 a total of 141 cm of rainfall was reported, while in 2012 only 64 cm was reported, compared with the historical average of 93 cm. The average maximum temperatures in 2010 were very similar to the historical average; only slightly higher in April, August, and October. The average maximum temperature for 2012 from March to August was greater than the 30-year average by as much as 9°C in one instance. The decline in precipitation and elevated temperatures in 2012 correspond to the 'severe' drought measurements for 2012 depicted in Figure 2.

Composition

The chemical composition of corn stover from Ames, IA for 2010 and 2012 is presented in Table 1. The extractives increased significantly, while cellulose (glucan), xylan and lignin were significantly lower in samples from 2012 than those from 2010. The corn stover samples experienced a large increase in extractives (14% to 23%) between 2010 and 2012. This increase can be attributed in large part to the effects of drought rather than hybrid differences, as relative maturity ratings of the hybrids are similar and both hybrids are considered to have high total fermentable (HTF) characteristics [38]. The accumulation of osmolytes, such as soluble sugars and proline, is a common plant response to drought that has been reported previously [20,22,39,40]. These osmolytic solutes are included as components of the reported extractives values in this analysis. Another possible contribution to the increased extractives is an accumulation of fats, waxes, and oil, which are also components included in the extractives values. Previous work on longleaf pine seedlings demonstrated that plants stressed for water had elevated fats, waxes, and oil concentrations [41]. As the precipitation was much lower and temperatures were elevated in 2012 compared with 2010, these compositional trends are most likely associated with the drought rather than the differences in hybrid (Figure 3).

There were significant decreases in the cellulose (glucan) and xylan contents of corn stover from

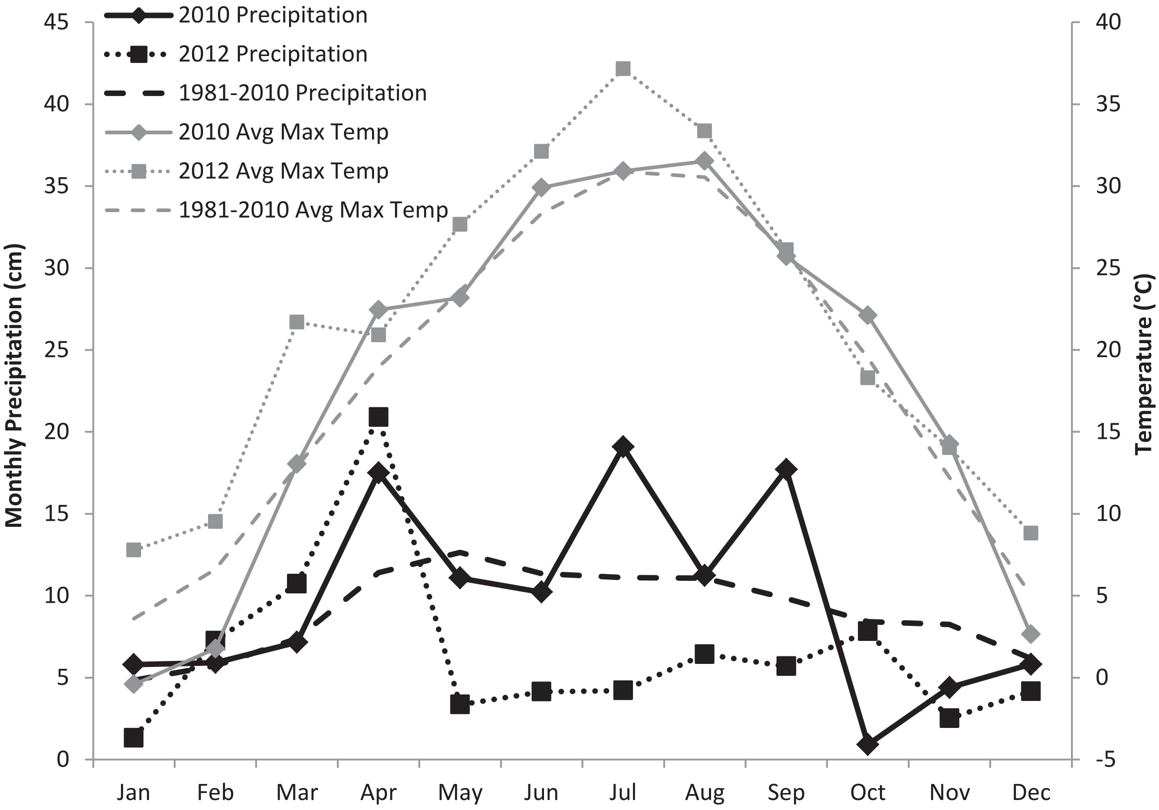

Figure 4. Columbia, MO monthly precipitation and average maximum temperature in 2010, 2012, and averaged over a 30-year period (1981–2010).

2010 to 2012. Al-Hakimi *et al.* reported that soybean (*Glycine max*) shoots had reduced cellulose (primarily glucan content) and increased hemicellulose (primarily xylan content) when subjected to a 30-day drought [19]. Although the xylan decreased for the corn stover discussed here, it should be noted that responses to drought stress can vary among species. For example, a study comparing the effects of drought on wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), and rice (*Oryza sativa*) reported that leaf growth inhibition differed between the three similar species [42].

Like structural sugars, lignin content was also lower in the 2012 corn stover samples. Drought induced changes in cell wall components of plants, including lignin, have been reported. A proteomic study by Vincent *et al.* found that lignin levels were lower in leaves of corn that were subjected to water stress [43]. Another study regarding the xylem sap of maize found that during drought there were increases in certain phenylpropanoids (monomeric units of lignin) and an increase in peroxidases, which could result in decreases of structural lignin [44]. Overall, drought conditions in 2012 within this field-scale study appear to have had an effect on all of the primary structural components within corn stover, which is in agreement with previous small-scale studies quantifying effects of water stress and heat stress on plant physiology [5].

Mixed perennial grasses: Missouri

Precipitation and temperature

In 2010, with the exception of October, precipitation in Columbia, MO between May and December was greater than in 2012 (Figure 4). Total yearly rainfall for 2010 at this location was also greater than for 2012; 116 versus 78 cm. For 2012, precipitation levels were above the 30-year monthly average between February and April but dropped below 30-year monthly averages for the remainder of the year. The maximum average temperature for Columbia, MO for 2012 from January until September was consistently higher than the recorded 30-year average while the temperature for 2010 more closely matched the 30-year average. As the harvest dates for the crops in Missouri were May/June and October, the lack of precipitation and elevated temperatures prior to and during these harvest dates in 2012 would most likely result in evidence of drought stress in these plants. These results are also consistent with the drought conditions observed in Missouri in Figure 2 for 2012.

Table 1. Chemical composition for three feedstocks at one or more locations in 2010 and 2012; mean (1SD). Different letters indicate significant differences from ANOVA for samples from 2010 compared with 2012 for each location separately; $p < 0.05$.

				Chemical composition (%)				
Feedstock	Location	Year	*n*	Extractives	Cellulose§	Total Glucan§	Xylan	Lignin
Corn Stover	Iowa	2010	11	13.8 (1.3)a	35.5 (0.7)a	na	20.3 (0.7)a	15.1 (0.5)a
Corn Stover	Iowa	2012	11	23.3 (2.2)b	32.6 (0.7)b	na	17.6 (0.8)b	12.3 (0.6)b
Mixed grasses	Missouri	2010	18	26.9 (1.7)a	23.6 (1.3)a	24.6 (1.2)a	17.1 (1.1)a	12.0 (0.8)a
Mixed grasses	Missouri	2012	16*	29.8 (1.6)b	19.0 (1.8)b	19.8 (1.7)b	15.9 (1.3)b	11.1 (1.0)b
Mixed grasses	Georgia	2010	24†	22.7 (1.4)a	23.1 (1.8)a	23.5 (1.9)a	17.6 (0.9)a	12.3 (0.7)a
Mixed grasses	Georgia	2012	24	29.1 (1.3)b	22.9 (2.2)a	23.0 (2.3)a	18.5 (1.1)b	10.6 (0.7)b
Miscanthus	Nebraska	2010	12	18.6 (0.8)a	37.7 (0.7)a	36.9 (0.6)a	20.3 (0.4)a	20.0 (0.4)a
Miscanthus	Nebraska	2012	12	26.4 (0.8)b	28.9 (0.8)b	29.0 (0.9)b	19.7 (0.4)b	14.7 (0.8)b
Miscanthus	Illinois	2010	12	19.3 (1.3)a	36.0 (1.5)a	35.6 (1.5)a	21.2 (0.8)a	19.1 (1.0)a
Miscanthus	Illinois	2012	10‡	16.6 (2.9)b	38.5 (0.8)b	37.7 (1.0)b	22.0 (0.6)b	20.8 (0.5)b

*only 14 samples were within 2 root mean square error of calibration (RMSEC) values for xylan.
†only 23 samples were within 2 RMSECs for xylan.
‡only 9 samples were within 2 RMSECs for xylan.
§Corn stover composition was predicted using a *corn stover PLS2* multivariate calibration model that predicts "Cellulose" based on values for structural glucan. The mixed grasses and *Miscanthus* samples were predicted using the *mixed herbaceous PLS2* model that predicts "Cellulose" based on values for structural glucan, and "Total Glucan" based on values for structural glucan and glucan from starch.

Composition

In 2012, mixed perennial grasses harvested in MO had significantly higher concentrations of extractives and significantly lower concentrations of cellulose, total glucan, xylan, and lignin (Table 1). The trends in composition correspond to the precipitation and temperature data, which were indicative of drought, for the area throughout most of 2012. As mentioned previously, increases in soluble carbohydrates and other extractable components and decreases in structural components, such as cellulose and lignin, have been linked to water stress. Starch, similar to cellulose, has been shown to decrease with prolonged drought [40]. In contrast to corn stover, mixed perennial grasses are typically harvested with seed, which contains more starch than other anatomical fractions of the plant; hence, both cellulose and starch contribute to the glucan value for mixed perennial grass samples. The decrease in total glucan is likely due to a combination of decreased cellulose and starch.

■ Mixed perennial grasses: Georgia

Precipitation and temperature

The mixed perennial grasses collected at the University of Georgia were chosen because of the unique isolated drought (Figure 2). According to the US Drought Monitor, both 2010 and 2012 experienced some level of drought. The drought in 2012 was slightly more severe than the drought experienced in 2010 (Figure 1 and 2). Although total precipitation was only slightly lower in 2012 (95 cm) compared with 2010 (121 cm), precipitation in 2010 was notably higher during the time of the spring harvest (May) than in 2012. Overall, monthly precipitation in both years fluctuated around the 30-year average (Figure 5). The average max temperatures in 2010 were higher than the 30-year average from April until October while the average maximum temperatures for 2012 were higher than the 30-year average from January until May. From this monthly data it can be observed that the spring harvest of these samples (May) had higher precipitation in 2010 compared with 2012 and the temperatures prior to this harvest date were higher in 2012. The precipitation and temperature data were similar between the two years during the fall harvest (October).

Composition

Mixed perennial grasses from GA had significantly greater extractives and xylan concentrations and significantly lower lignin concentrations in 2012 than in 2010 (Table 1), possibly due to the differences in precipitation levels prior to and during the time of the May harvest. Total glucan and cellulose both decreased, but not significantly. The similar drought conditions that were experienced in both 2010 and 2012 at different times throughout the year in GA resulted in different compositional trends than in the MO mixed perennial

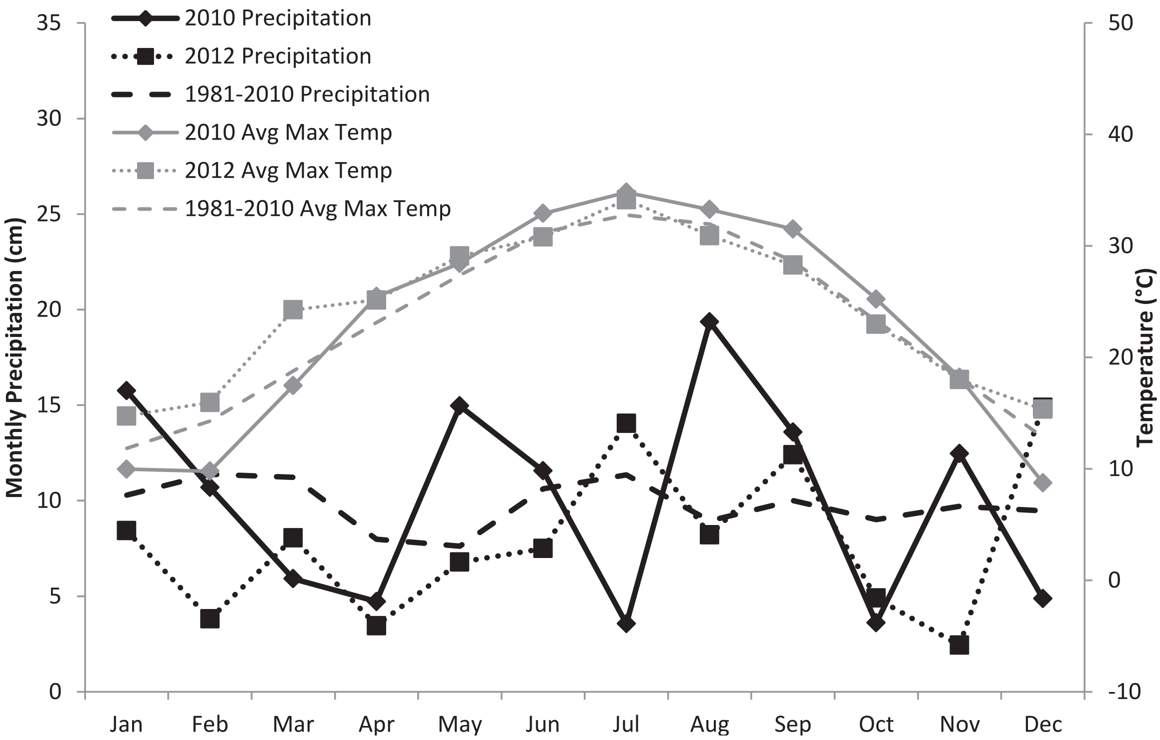

Figure 5. Athens, GA monthly precipitation and average maximum temperature in 2010, 2012, and averaged over a 30-year period (1981–2010).

grasses, which experienced a large difference in drought conditions: severe drought in 2012 compared with 2010. This contrast is important because it exemplifies the effects of drought severity. Corn stover and MO mixed perennial grasses experienced much more severe drought conditions in one year compared with the other, and therefore, had a significant decrease in all cell-structure components and an increase in extractives. The mixed perennial grasses in GA had similar drought conditions between the two years, although 2012 still experienced more drought than 2010, and had an increase in hemicellulose (xylan) and extractives and a decrease in lignin. As one of the first plant responses to drought stress is solute accumulation to maintain turgor pressure, the measured increase in extractives is consistent with literature [45]. This accumulation of osmolytes has been observed in plants even when water stress in minimal or short term. Martinez *et al.* showed that there was an increase in water soluble components (soluble sugars and glycine-betaine) after only 15 days of water stress conditions [39]. There are also many studies that support the increase (enrichment) of hemicellulose with a decrease in cellulose in response to short-term water stress [19]. Although cellulose was not significantly decreased in GA mixed perennial grasses, there was a trend for decreased cellulose. The authors theorize that an increase in hemicellulose may occur following short term or mild drought, but when drought conditions become more severe or prolonged the hemicellulose also decreases; however, further studies are needed to determine the mechanisms behind the observed trends. To the authors' knowledge, there is no literature regarding the effects of drought on lignin within mixed grasses, but the decrease could be attributed to alterations in phenylpropanoid compounds as reported previously for maize [44].

■ *Miscanthus × giganteus*: Nebraska

Precipitation

Miscanthus × giganteus harvested in Saunders County, NE experienced extreme to exceptional drought in 2012 (Figure 2) with total precipitation of only 47 cm compared with 96 cm in 2010. The 2010 monthly precipitation was also greater than or similar to the 30-year average (Figure 6). In 2012 there was notably less precipitation than the 30-year average from May until November. As the harvest dates for these samples were in November and December, the difference in precipitation levels from May until October would have significantly contributed to the drought effects on the plants leading up to the harvest in 2012. Elevated temperatures in 2012 also contributed to the drought conditions.

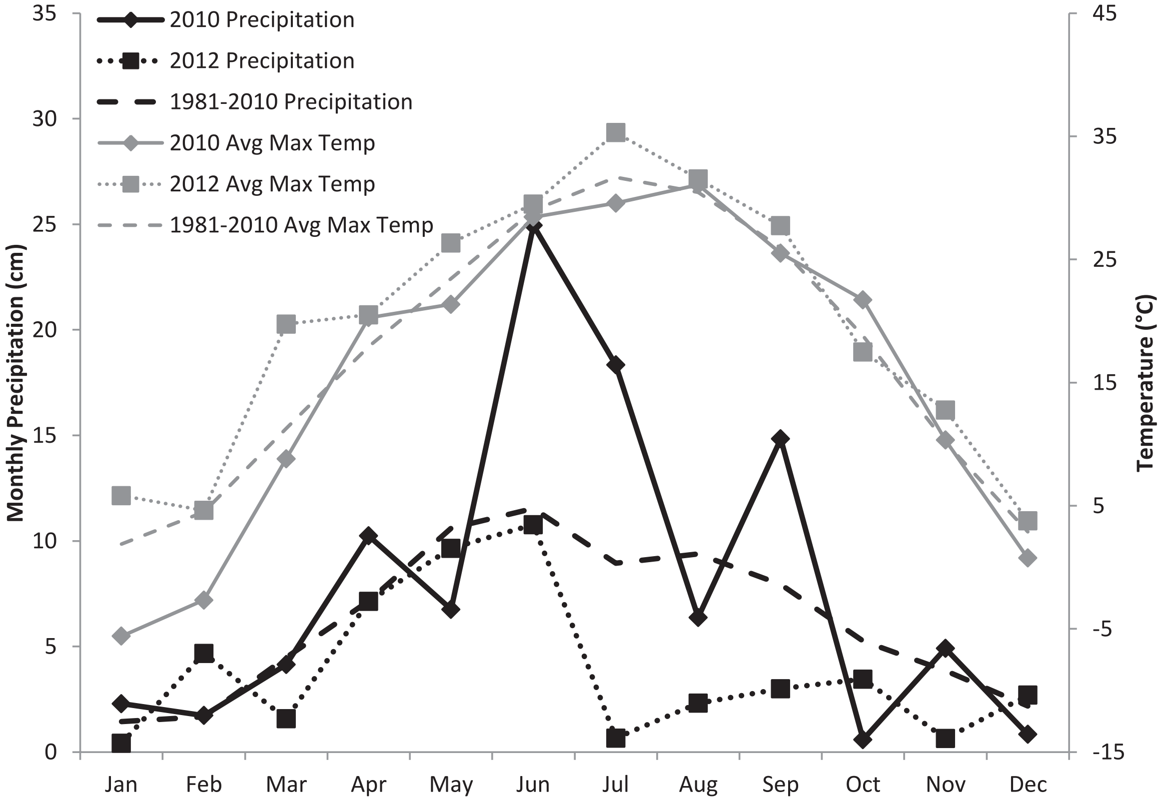

Figure 6. Mead, NE monthly precipitation and average maximum temperature in 2010, 2012, and averaged over a 30-year period (1981–2010).

Overall, July to November in 2012 had higher temperatures than the historical average maximum.

Composition

The compositional results reflect the precipitation and temperature drought related trends for *M.* × *giganteus* harvested in NE. Extractives were significantly greater, while cellulose, total glucan, xylan, and lignin concentrations were significantly lower in 2012 compared with 2010 (Table 1). These compositional trends are consistent with the corn stover samples and MO mixed perennial grasses that were reported to have experienced similar extreme drought conditions. The *M.* × *giganteus* from NE had a large decrease in total glucan (37% to 29%) from 2010 to 2012. Biomass yields of *M.* × *giganteus* have been reported to be strongly influenced by water availability [5], but the authors did not find any literature regarding cell wall composition response of *M.* × *giganteus* in relation to water stress.

■ Miscanthus × giganteus: Illinois

Precipitation and temperature

The levels of precipitation in Urbana, IL for 2012 were an exception to the nationwide drought, as Champaign County was one of the few counties in IL to have received higher than average rainfall during the months prior to harvest (August to October) in 2012 (Figure 7). Precipitation for both 2010 and 2012 was slightly less than the historical average from February until May. Although levels of rainfall for both years were similar (90 cm in 2010 and 86 cm in 2012), a substantial portion of the precipitation for 2012 fell immediately before harvest, while in 2010 this same time period experienced less than average precipitation. Both 2010 and 2012 had higher than average maximum temperatures from April to July. From July to October the temperatures for 2010 were higher than both the 30-year average and the average maximum temperatures for 2012.

Composition

The compositional results for *M.* × *giganteus* samples from IL had opposite trends when compared with those from NE. Extractives were significantly lower and structural components significantly higher in 2012 compared with 2010 (Table 1). This trend can probably be explained by timing of the precipitation levels within the area. *M.* × *giganteus* harvest occurred in IL during November 2010 and January 2013. During the five and three months prior to the harvest, 2012 precipitation

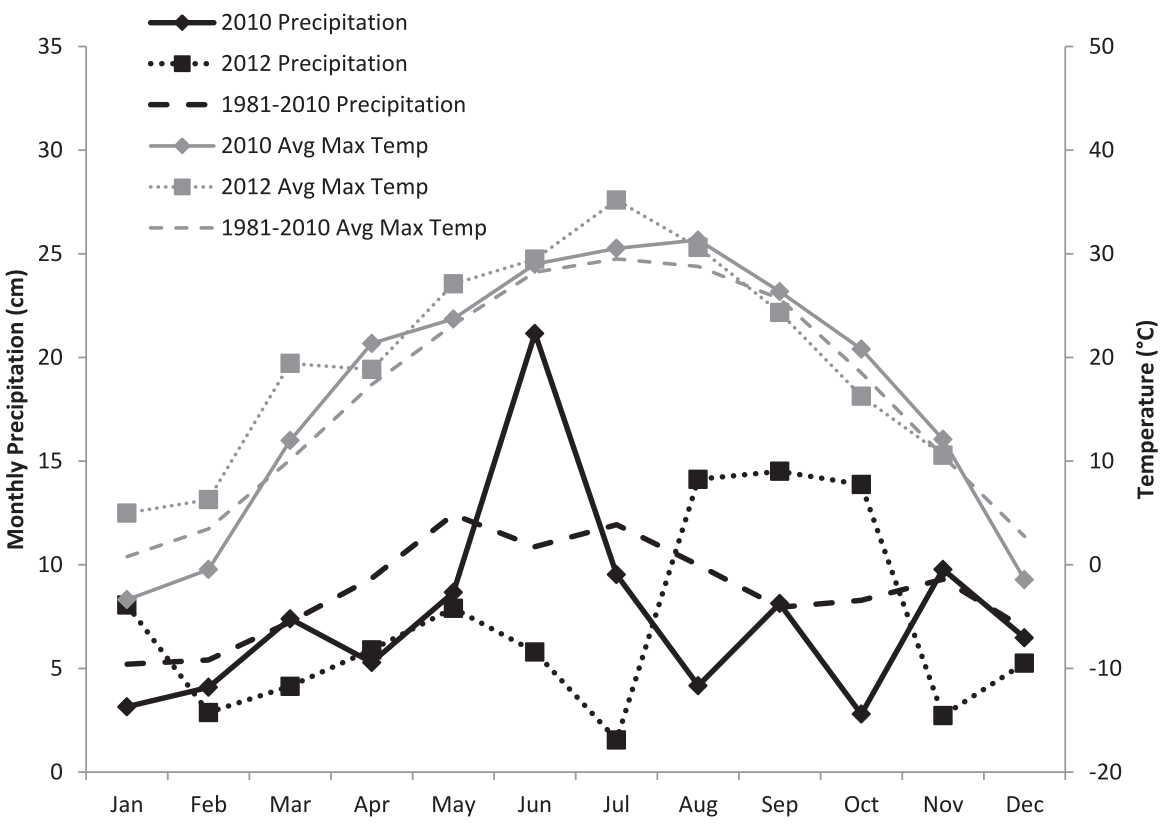

Figure 7. Champaign, IL monthly precipitation and average maximum temperature in 2010, 2012, and averaged over a 30-year period (1981–2010).

levels exceeded the 30-year average while 2010 received less than average precipitation and had higher than normal temperatures. This difference in conditions likely contributed to lower extractives and greater structural components' concentrations. These compositional trends are consistent with the other datasets regardless of whether a drought occurred in 2012; higher rainfall and lower temperatures are associated with lower extractives and greater structural components.

As *M.* × *giganteus* has been identified as a promising dedicated bioenergy feedstock [46], a case study was performed to determine whether the nitrogen (N) treatments applied at both locations resulted in significant compositional differences, or if drought was the main source of compositional variation (Table 2 and 3). Year, and therefore drought, significantly affected all compositional components; however, N fertilizer rate did not significantly affect any of the compositional components for either location. This indicates that even with nutrient amendments, such as N fertilization, environmental factors such as drought are more predominant in determining feedstock quality.

■ Theoretical ethanol yield/dry biomass yield

Table 4 shows the calculated theoretical ethanol yield (TEY) for each of the feedstock sample sets that experienced a severe to exceptional drought in 2012; IA corn stover, MO mixed perennial grasses, and NE *M.* × *giganteus*. As the TEY is based solely on glucan and xylan concentrations, significant decreases in TEY between 2010 and 2012 were expected as those components were significantly lower in 2012 (Table 1). All three feedstocks had a decrease in TEY (10–15%). As each of these feedstocks was grown in different soil conditions and different treatments were applied, comparisons can only be made between the two harvest years of each feedstock and not across locations. The purpose of calculating TEY is to show a theoretical calculation of the potential ethanol production assuming a 100% conversion efficiency.

Dry biomass yield and TEY were both significantly lower for mixed perennial grasses and *M.* × *giganteus* for the drought year. The decrease in dry biomass was an expected result based on previous work [20,22]. A study comparing *M.* × *giganteus* harvest yields in

Table 2. Chemical composition of *Miscanthus × giganteus* grown in Nebraska with three different nitrogen rates; mean (1SD). Results of two-way ANOVA – only year was significant for all components at $p < 0.05$, nitrogen rate and the interaction between nitrogen rate and year were not significant for any of the components.

			Chemical Composition (%)				
Nitrogen Rate (kg ha^{-1})	Year	*n*	Extractives	Cellulose	Total Glucan	Xylan	Lignin
0	2010	4	18.5 (0.3)	37.8 (0.3)	37.1 (0.4)	20.2 (0.3)	20.2 (0.4)
0	2012	4	26.8 (0.9)	28.8 (1.2)	28.9 (1.4)	19.8 (0.3)	14.2 (0.9)
60	2010	4	18.5 (0.8)	37.9 (0.1)	37.1 (0.2)	20.4 (0.6)	19.8 (0.3)
60	2012	4	26.3 (0.2)	28.8 (0.5)	28.9 (0.6)	19.7 (0.4)	14.8 (0.6)
120	2010	4	18.8 (1.3)	37.3 (1.2)	36.6 (1.0)	20.2 (0.4)	20.0 (0.7)
120	2012	4	26.1 (1.1)	29.2 (0.6)	29.2 (0.5)	19.6 (0.4)	14.9 (0.8)

Table 3. Chemical composition of *Miscanthus × giganteus* grown in Illinois with three different nitrogen rates; mean (1SD). Results of two-way ANOVA – only year was significant for all components at $p < 0.05$, nitrogen rate and the interaction between nitrogen rate and year were not significant for any of the components.

			Chemical Composition (%)				
Nitrogen Rate (kg ha^{-1})	Year	*n*	Extractives	Cellulose	Total Glucan	Xylan	Lignin
0	2010	4	19.2 (1.7)	36.1 (1.3)	35.8 (1.2)	21.5 (0.8)	18.7 (1.3)
0	2012	3*	15.2 (5.7)	38.7 (1.6)	38.1 (1.9)	22.4 (0.3)	20.2 (0.1)
60	2010	4	18.9 (1.5)	37.1 (1.5)	36.6 (1.4)	20.6 (0.7)	19.9 (0.8)
60	2012	3	17.5 (0.5)	38.1 (0.5)	37.2 (0.5)	22.1 (0.6)	21.1 (0.5)
120	2010	4	19.7 (0.9)	34.7 (0.9)	34.3 (0.9)	21.5 (0.8)	18.8 (0.7)
120	2012	4	16.9 (1.5)	38.6 (0.2)	37.6 (0.2)	21.7 (0.5)	21.1 (0.4)

*Only two xylan values were available for fertilizer rates of 0 in 2012.

relation to the influence of N, temperature, and water found that yields for *Miscanthus* were most strongly influenced by water availability [5]. However, dry biomass yields were not decreased in the corn stover in this study. Although the USDA estimated a 13% yield loss for bushels of corn between 2011 and 2012 showing that corn dry matter yield is reduced by drought [14], this loss included both grain and stover yields. Corn grain yield has been shown to be more sensitive to environmental stress than corn stover yield [47]. For the corn stover samples analyzed in this study, an analogous grain yield was also measured for each of the plots. The grain yield significantly decreased by 46% between 2010 and 2012. However, the focus of this study was to determine the effects of drought on corn stover. As the grain is not removed prior to harvest for mixed perennial grasses and *M. × giganteus,* this could help to explain why a decrease in yield is seen in these feedstocks but not corn stover. Another possible explanation for the dry biomass yields not decreasing in the corn stover samples could be the large variability caused by the multiple treatments for the corn stover. Table 4 shows a large standard deviation for the dry biomass yields for the corn stover specifically. The decrease in TEY between 2010 and 2012 for corn stover was still significant even with this high variability possibly caused by the treatments. To demonstrate the total effect of the drought on these samples, the TEY was calculated on an area basis including both the differences in structural sugars (glucan and xylan) and differences in dry biomass yields between 2010 and 2012 (Table 4), giving a total TEY (equation (3)). Corn stover total TEY was 36% higher in 2012 than 2010 due mostly to the increase in dry biomass yield, although this increase was not significant. *Miscanthus × giganteus* had 26% lower total TEY in 2012 compared with 2010 ($p < 0.05$), and mixed perennial grasses had a 59% lower total TEY in 2012 compared with 2010 ($p < 0.05$). The majority of the total TEY loss for the mixed perennial grasses was due to lower dry biomass yields in 2012, which decreased 50% relative to 2010. If the dry biomass yield was not factored in for the mixed perennial grasses, the TEY would have only been 14% lower in 2012.

Table 4. Theoretical ethanol yields (TEY) per Mg of dry biomass, dry biomass yields (Mg ha–1), and total TEY per hectare of dry biomass for 2010 and 2012 for three different feedstocks; mean (1SD). Superscript letters denote statistical differences between 2010 and 2012 within each feedstock based on ANOVA; *p* < 0.05.

Feedstock	Location	Year	*n*	TEY (L Mg^{-1})[†]	Dry biomass (Mg ha^{-1})	Total TEY (L ha^{-1})[†]
Corn stover	Iowa	2010	11	334 (7)[a]	3.0 (1.4)[a]	990 (471)[a]
Corn stover	Iowa	2012	11	300 (8)[b]	3.7 (1.1)[a]	1125 (325)[a]
Mixed grasses	Missouri	2010	18	250 (12)[a]	2.5 (0.6)[a]	635 (146)[a]
Mixed grasses	Missouri	2012	14[*]	216 (17)[b]	1.2 (0.6)[b]	259 (119)[b]
Miscanthus	Nebraska	2010	12	342 (5)[a]	27.7 (3.2)[a]	9495 (1159)[a]
Miscanthus	Nebraska	2012	12	292 (5)[b]	23.7 (1.8)[b]	6912 (545)[b]

**n* of 18 for dry biomass yield calculations.
[†]TEY was based on glucan and xylan only at assumption of 100% conversion efficiency; only samples that had both glucan and xylan values were used for calculations.

Even though the corn stover from this study did not have a decrease in dry biomass yield in response to drought, there was a decrease in TEY due to a decrease in quality as the structural sugars necessary for lignocellulosic conversion to ethanol were decreased. Losses of TEY as a result of drought of the magnitudes reported here could have a significant economic impact on biorefineries. A 10% decrease in TEY for corn stover could result in a loss of \$283 per Mg of biomass assuming a minimum ethanol selling price (MESP) of \$8.37 per *L* [48]. As stated earlier, the total quantity of corn stover produced averaged 301 × 10^6 Mg between 2010 and 2012. If all of this corn stover were used to produce cellulosic ethanol and all experienced a 10% decrease in TEY then the economic loss would extrapolate to \$85 × 10^9.

Conclusions

Drought was shown to significantly affect the chemical composition, and hence quality, of all three feedstock types by increasing extractable components and decreasing cellulose, hemicellulose, and lignin. Regarding the *Miscanthus* × *giganteuas* samples, the effects of drought were still predominant even when fertilizer treatments were considered.

Drought had a negative impact on total dry biomass yield for mixed perennial grasses and *M.* × *giganteus,* as reported in previous studies. However the corn stover biomass yield was not affected possibly because of the high variability in the treatments analyzed and the drought primarily affected grain yields which were not considered in this analysis. Overall, drought induced an average decrease of 10–15% in TEY per Mg of dry biomass. The TEY calculated with both reductions in structural carbohydrate content and dry matter yield exemplified the potential impact of drought on ethanol production for biorefineries with decreases in TEY ranging from 26–59% per ha for mixed perennial grasses and *M.* × *giganteus*. This magnitude of decrease in potential ethanol production could result in significant revenue losses for biorefineries. To sustain a lignocellulosic biorefinery, a larger amount of dry biomass would be required to produce the same amount of ethanol.

Although *Miscanthus* × *giganteus* in this study experienced a decrease in dry biomass yield in 2012 relative to 2010, a 15% decrease in yield for *M.* × *giganteus* could theoretically still have a much higher biomass yield per ha than corn stover [7]. Because of the high biomass yields, even during a drought year, *M.* × *giganteus* has the potential to be a viable biomass feedstock. Mixed perennial grasses, were strongly affected by the drought, experiencing both lower dry biomass yields and lower quality; however, perennial grasses also have significant environmental benefits when grown on marginal land and should still be considered a potential feedstock for bioenergy conversion [9, 11].

Future perspective

The economic viability of the cellulosic bioenergy industry relies on a consistent supply of feedstock in terms of both quantity and quality. Understanding sources of feedstock variability is important for developing robust bioenergy business models. A key source of variation in feedstock supply will continue to be environmental conditions, and there has been a significant increase in weather extremes over the past few decades. This study investigated the variability in feedstock quality and quantity as a result of drought. The results could serve to inform future estimates of biofuels production as weather extremes, including drought, may remain commonplace. Potential solutions to losses in dry biomass and sugar yields from drought include improved storage of biomass from prior non-drought years, use of more drought tolerant plants, or development of drought tolerant cultivars.

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Supplemental data

Supplemental data for this article can be accessed here.

Executive summary

Composition

- Drought significantly increased extractives and decreased structural components of the plant cell wall including cellulose (primarily glucan), hemicellulose (primarily xylan), and lignin.
- Severity of drought conditions influenced the chemical composition of biomass; mixed perennial grasses that experienced smaller differences in drought conditions had no significant change in glucan and increased xylan.
- Nitrogen fertilization did not influence chemical composition of *Miscanthus* x *giganteus* biomass significantly as compared with drought influences.

Theoretical ethanol yield/dry biomass yield

- Theoretical ethanol yield was significantly decreased by 10–15% for drought affected corn stover, mixed perennial grasses, and *Miscanthus* x *giganteus*.
- Dry biomass yields for corn stover were not affected by drought but were lowered for the mixed perennial grass and *Miscanthus* x *giganteus*.
- Theoretical ethanol yield on an area basis factoring in both loss in structural sugars and dry biomass yield decreased by 26–59% for mixed perennial grasses and *M. x giganteus*.

Conclusion

- Drought had significant impacts on compositional quality of all three bioenergy feedstocks.
- Corn stover dry biomass yield was not negatively affected by the drought as the other feedstocks were; however, drought-affected *M. x giganteus* still had overall high dry biomass yields.
- Drought appears to be a significant risk factor affecting cellulosic bioenergy feedstock supply.

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