Corn Sustainability for Cellulosic Biofuel and Grain Production in the Southeastern US

by

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Abstract

Biofuel production from plant biomass has been proposed as a solution to mitigate fossil fuel use. Corn (*Zea mays* L.) is an important crop in the Southeast. Its abundance and high yield potential makes it attractive as bioenergy feedstock for the biofuel industry. The objectives of this study were: 1. develop prediction models that could estimate corn grain and stover yield at harvest using simple measurements at the first reproductive growth stage (R1); 2. determine whether the Neutral Detergent Fiber method (NDF) for extraction and determination of structural carbohydrates can be used as an alternative to the National Renewable Energy Laboratory (NREL) extraction procedure; 3. evaluate the effect of in-season weather conditions, the use of rye (*Secale cereale*) as a winter cover crop, and the corn residue management on grain yield and biomass yields (total and partial) on two soil types; 4. investigate variations in the distribution of structural carbohydrates and lignin in total biomass and among four plant fractions: above the first ear excluding cobs (top), below the first ear (bottom), cobs alone (cob), and above the first ear including cobs (above-ear); 5. investigate differences in carbohydrates, theoretical ethanol yield (TEY), high heating value (HHV), and mineral content in the total biomass and among the four plant fractions; 6. develop models that predict the total and partial corn stover TEY per unit of area at harvest using only weather conditions in May, June , and July; and 7. investigate the effect of using rye as a cover crop, and corn residue harvest on carbon (C) and nitrogen (N) dynamics on two major soil types of the southeastern US.

The experiment was established at two locations, one in central and one in north Alabama, in 2009. It consisted of a 3x4x2 complete factorial design arranged in a split-split-plot. Factors were: winter rye cover crop (main plot), nitrogen (N) fertilization rates (sub-plot) and stover residue harvest (sub-sub-plot) replicated three times at each location. A study incorporating stover removal management practices (0 and 100% removal) was also established in South Carolina. Plots in both states were representative of major soil types in their respective region: Alabama plots were Compass and Decatur soils; South Carolina plots were Coxville/Rains-Goldsboro-Lynchburg association.

For the development of grain and biomass yield prediction models, the regression was significant with the amount of explainable variability maximized at R1 stage. For the grain yield model, the maximum R^2 was 0.7705 and for the stover model maximum R^2 reached 0.8473. It seems that total precipitation from planting until R1 growth stage, the amount of N fertilization and simple plant morphological measurements at R1-silking can be used to predict corn grain and stover yield at harvest with some success.

A simplified method for carbohydrate analysis was developed. It included the NDF extraction instead of the two-stage extraction proposed by the NREL. There were statistical differences between the two methods in carbohydrate concentrations and TEYs (l kg^{-1}). However, on average the TEYs varied only by 2% which seemed to be practically insignificant. Furthermore, the TEY (l ha^{-1}) prediction derived by the simplified method did not vary from the NREL method.

Grain yield ranged from 5,328-9,251 kg ha^{-1} for the loamy sand and 4,488-6,423 kg ha^{-1} for the silt loam. Total stover dry weight ranged from 3,486-5,482 kg ha^{-1} and 3,100-5,528 kg ha^{-1} for the same soils. Significant differences in grain and biomass yields were observed between

individual years and locations, with yields generally greater in central Alabama. For the three years of the experiment, the use of a rye cover crop increased yields in both locations while the average effect of three years of stover harvest was not significant.

The use of rye and stover harvest did not affect the concentrations of lignin and structural carbohydrates across plant fractions and soil types. However, their distribution varied greatly among corn plant fractions. Data from this study suggests that in every location the cob, top and above-ear plant portions have the highest holocellulose contents and the lowest lignin contents, which are the most desirable characteristics for bioethanol production.

The distribution of glucan, xylan, arabinan, TEYs, HHV, and mineral contents varied significantly among the corn stover portions in every location. However, the use of a rye cover crop and stover harvest had little effect on these variables. Results from this study suggest that harvesting the above-ear portion of the stover would result in a low lignin feedstock with high bioethanol potential and significantly lower nutrient removal rates than removing the total stover. Furthermore, models were successfully developed to predict the total and partial corn stover TEY (l ha^{-1}) at harvest using only the monthly cumulative precipitation and monthly average temperatures in May, June, and July. The R^2 values of the models were small to moderate; however, there were not significant differences between the actual and fitted TEYs.

A laboratory incubation experiment was performed, in which soil samples were analyzed for total C, N, inorganic N during a 60 days period (0, 30 and 60 days), and CO_2-C evolved (30 and 60 days). Carbon and N content in the northern site (1.3% and 0.1%, respectively) were significantly higher than the central site (0.6% and 0.05%, respectively). The use of rye as a winter cover crop did not affect C and N dynamics at either location. For the silt loam in plots where the stover was harvested, C content (1.2%) was significantly lower than plots that stover was retained (1.4%). In both soil types, N mineralized increased significantly during the 60 day period of the experiment. However, C mineralization did not vary between 30 and 60 days of incubation at either location. Nevertheless, C turnover seemed to be higher in the loamy sand than the silt loam. Results from this study suggest that differences in C and N dynamics due to the use of a rye cover crop and corn stover management are soil dependent.

Results from this study indicate that when the objective of an agricultural system is the simultaneous production of biofuel and grain, harvesting only the above-ear portion of the stover can result in high amount of bioethanol across the southeastern US. This would also lead to significantly lower removal rates of C, N, and nutrients when compared to harvesting the whole plant biomass. Furthermore, this study shows that the use of rye as winter cover crop can increase both corn biomass and grain yields. Despite the high C:N ratio of the rye, in this study, the plant available N in the soil did not appear limited and therefore it is recommended that cultivation of winter rye should be incorporated as a management practice in Alabama.

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List of Abbreviations

AL	Alabama
EVS	E.V. Smith Research Center
SC	South Carolina
PDREC	Pee Dee Research and Education Center
TVS	Tennessee Valley Research and Extension Center
SOM	Soil Organic Matter
SOC	Soil Organic Carbon
TEY	Theoretical Ethanol Yield
DOE	Department of Energy
NDF	Neutral Detergent Fiber
NREL	National Renewable Energy Laboratory
HHV	High Heating Value
NDVI	Normalized Difference Vegetation Index
ACES	Alabama Cooperative Extension Service
NIRS	Near-Infrared Spectroscopy
PCA	Principal Component Analysis
SEC	Standard Error of Calibration
SECV	Standard Error of Cross Validation

I. Introduction

In the last decade, several factors have encouraged countries to show interest in biofuel production. Increasing oil prices, rising greenhouse gas emissions, and energy security issues are only a few reasons that make alternative energy an attractive idea. Biofuels are expected to play an important role in energy security in the future.

According to the Energy Independence and Security Act in 2007, almost 136 billion liters of ethanol should be produced per year by 2022, of which 61 billion liters should be produced from cellulosic biomass (EIA, 2008). In 2009, almost 95% of the total renewable fuel produced in the US was constituted by corn grain ethanol. Biodiesel made from soybean oil, vegetable oils, rendered fats, greases, and corn oil from ethanol production accounted for almost all the remaining biofuel consumed (FAPRI, 2010; EIA, 2010). It is expected however, that more advanced cellulosic feedstocks will be used to produce biofuels, such as agricultural residues (e.g., corn stover), forestry biomass, municipal solid waste (MSW), and dedicated energy crops (e.g., switchgrass) (USEPA, 2010).

Corn (*Zea mays* L.) is an abundant crop in the southeastern US with high biomass yield potential. It can be used as animal feed and by the biofuel industry (Kadam and McMillan, 2003). Cultivation of corn for stover harvest would not compete with the use of land for food production since grain and biomass would be produced simultaneously. Additionally, using corn biomass as a bioenergy feedstock can result in greater greenhouse gas reductions than using dedicated energy crops since there is no need for land use change (Searchinger et al., 2008). Due to these factors, corn is considered to be a desirable bioenergy feedstock.

When the goal of modern corn production system is grain and/or biomass production, timing and supply management of the raw product is very important. Therefore, an estimate early in the growing season of the amount of corn grain, biomass, and biofuel potential can be of great interest to farmers and related industries. Additionally, determining the bioethanol potential from corn stover feedstock requires expensive and time-consuming plant tissue analysis. The need for simplification of already established biomass analysis method has been recognized (Sluiter et al., 2010) and could be of great importance for bioenergy research and related industries.

The importance of a well-established cropping system for corn production is also of importance. Several factors can affect corn grain and biomass yield. Planting date, nitrogen fertilization, tillage, cover crop use, crop rotation, weather conditions, nutrient availability, residue harvest and weed control are just a few practices that can maximize grain and biomass yields and farmer's profit. Rye (*Secale cereale*) as a winter cover crop is well-known for its high biomass production; however its impact on corn productivity appears to vary with geographic location. Studies in the Southeast indicated that cover crops can improve soil productivity, especially when combined with conservation tillage practices (Bruce et al., 1995; Sainju et al., 2002). However, the effect of winter cover crops incorporated into a potential cellulosic biofuel crop production system in the southeastern US is not well-examined.

Corn biomass left in the field after the growing season is very important for erosion control, carbon sequestration, and nutrient cycling, all of which affect soil productivity (Johnson et al., 2007; Johnson et al., 2009; Lindstrom, 1986; Wilhelm et al., 2004). In order to balance these multiple soil demands, a portion of the biomass should be harvested for biofuel production, while the rest should be left in the field to enhance formation of soil organic matter. Partial biomass harvesting is possible by using combines that simultaneously harvest grain and part of the stover (Hoskinson et al., 2007). Stover that remains in the field to sustain soil organic carbon will depend on the amount of biomass that will be removed. However, the stover yield and composition is not distributed uniformly in the plant. Therefore, identification of the most appropriate stover portion for biofuel production is essential to serve as a feedstock and for maintaining soil organic carbon stocks.

Carbon dioxide is one of the major factors causing climate change. Agriculture can play an important role in the mitigation of greenhouse gas emissions. Soils contain the largest amount of carbon on land. Plants also store carbon in their tissue through photosynthesis. As plant material decomposes carbon is stored in the soil in the form of soil organic carbon. Conservation tillage practices (no till, cover crops, and others) can promote and enhance the carbon storage in the soil. It is expected that crop residues left in the field can promote soil organic carbon formation. Crop residues are also of importance for the N cycle in soils because they can create N immobilization issues that affect microbial growth, enzyme synthesis and other nutrient mineralization (Cayuela et al., 2009). Additionally, incorporation of crop residues can stimulate a growth in microbial population and activity. However, removing plant residues for biofuel production can be detrimental to soil organic carbon stocks and affect soil C and N dynamics. Nevertheless, the use of a winter cover crop has the potential to mitigate the impacts of crop residue harvest and this system needs to be further examined in the low C soils of the southeastern US.

The objectives of this study were: 1. develop prediction models that could estimate corn grain and stover yield at harvest using simple measurements during the growing season; 2.

determine whether the Neutral Detergent Fiber method (NDF) for extraction and determination of structural carbohydrates can be used as an alternative to the National Renewable Energy Laboratory (NREL) extraction procedure; 3. evaluate the effect of in-season weather conditions, the use of rye as winter cover crop, and corn residue management on grain yield and biomass yields (total and partial) on two soil types; 4. investigate variations in the distribution of structural carbohydrates and lignin in total biomass and among four plant fractions: above the first ear excluding cobs (top), below the first ear (bottom), cobs alone (cob), and above the first ear including cobs (above-ear); 5. investigate differences in carbohydrates, theoretical ethanol yield (TEY), high heating value (HHV), and mineral content in the total biomass and among the four plant fractions; 6. develop models that predict total and partial corn stover TEY per unit of area at harvest using only weather conditions in May, June , and July; and 7. investigate the effect of using rye as a cover crop, and corn residue harvest on carbon (C) and nitrogen (N) dynamics on two major soil types of the southeastern US.

II. Corn Grain and Stover Yield Prediction at R1 Growth Stage

Abstract

Estimating corn (*Zea mays* L.) grain and stover yield during the growing season is an appealing idea. An accurate yield estimation could benefit farmers, as well as corn related industries. The objective of this study was to develop regression equations to estimate corn grain and stover yield using easily accessible information (nitrogen fertilization rate and cumulative precipitation), and simple plant morphological measurements such as height, stem diameter at various heights, height of the first ear, and plants per hectare. Measurements made at silking (R1) were used since the maximum explainable variability would not exceed 58% at early stages of plant development (V2-V10). The experiment was conducted from 2009 until 2011 in two locations, in north and central Alabama, under no-tillage and non-irrigated conditions. Treatments were assigned to a 3x4x2 complete factorial design arranged in a split-split-plot with three replications. Factors were; winter rye (*Secale cereale*) cover crop (main plot), nitrogen (N) fertilization rates (sub-plot) and stover residue harvest (sub-sub-plot). All measurements from this study, across years and locations, were used in the final regression equations in order to create robust prediction models. Equations, with and without intercept, were developed and compared according to several statistical criteria. The final grain yield equation at R1 growth stage included an intercept with a R^2 of 0.7705. The final stover equation also included an intercept ($R^2 = 0.8473$). This study suggests that N rate, total precipitation amount from planting until silking, and simple plant morphological measurements can be used to predict corn yield.

Introduction

The importance of a well-established cropping system for corn production is well recognized. Several factors can affect corn grain and biomass yield. Planting date, nitrogen fertilization, tillage, cover crop use, crop rotation and weed control are just a few practices that can maximize yield and farmer's profit. Planting corn in a timely manner helps the crop take advantage of optimum air and soil temperatures, as well as available water from precipitation. Additionally, N is an important nutrient for crop biomass production. Insufficient N supply during the growing season reduces grain and biomass yield (Dev and Bhardwaj, 1995).

Maximum profit is obviously the ultimate goal of a crop production system. Maximizing yield is usually a way that farmers attempt to increase profit. However, in modern farming and related industries, the timing and supply management of the raw product is very important. The use of corn extends from food products for human consumption, to animal feed, to a constituent of drugs and construction materials. Additionally, the biofuel industry considers corn grain and stover as important feedstocks. These types of industries require precision in timing of the feedstock availability and delivery logistics. Therefore, knowing the quantity of the product available is also important to determine prices and costs. Consequently, estimation of the amount of corn grain and stover could be beneficial for both, farmers and industry. For example, farmers could contract their corn, prior to harvest, at a more competitive price compared to waiting until harvest when prices may be depressed due to oversupply and have more confidence on what they can deliver. The industry, being aware of an estimate of corn product available ahead of time, could plan logistics and other factory functions enhancing the overall operating efficiency.

Crop growth simulation models have been used to estimate crop yields. Remote sensing data have also been utilized to calibrate the simulation models (Maas, 1988). However, these models utilize processes in the soil, in the plant, and in the atmosphere to describe the development of the plant and require large volume of data for their calibration (Kantanantha, 2007). Another more simple approach to predict crop yields is the use of regression (statistical modeling).

Several statistical models in the past and recent years have used plant height as a key variable to assess corn grain yield. Shrestha et al. (2002) used plant height at V10 growth stage to determine spatial variability of corn response to N. According to Ritchie et al. (1993), the correlation of plant height and corn grain yield was significant at V12 growth stage in a dry season. However, the same relationship was not observed in a wet year (Machado et al., 2002). Other researchers showed inconsistent correlation between plant height at early stages and grain yield, among different sites (Mallarino et al., 1999), while others have reported inconsistent correlations of plant height and grain yield in dry years (Katsvairo et al., 2003). It seems that the use of plant height as the only explanatory variable cannot contribute towards a large amount of the yield variability consistently.

There have also been attempts to use the normalized difference vegetation index (NDVI) to predict crop yields. In 2006, Teal et al. reported that prediction of corn grain yield using the normalized NDVI for growing degree days resulted in 73% explained variability. Furthermore, in the same study they showed that NDVI measurements at V8 corn growth stage resulted in 77% explained variability in biomass yield. In another corn forage yield prediction study, when data were averaged across three years and three locations in Oklahoma, NDVI and plant height at V11-R1 growth stages accounted for 37 and 43% of the explainable variability, respectively (Freeman et al., 2007). The relationship between cotton yield and NDVI was found to be linear with R^2=0.70 (Mkhabela and Mkhabela, 2000). It appears that there are studies that report large

R^2 values by the use of NDVI, and other experiments that the use of that index resulted in poor explained variability. It seems that more work is needed to improve the use of NDVI as predictor variable and to explain more consistently a large amount of variability. Furthermore, NDVI measurements require the use of sophisticated tools that cannot be considered simple.

Most of the corn grain yield estimation attempts have used linear regression to develop prediction models. However, in a more recent study, a model that positively correlates plant height at V6, V10 and V12 growth stages with corn yield was developed using non-linear regression (Yin et al., 2011). In this study, plant height was the only predictor. The maximum explainable variation was 87%, 69% and 81% for 2008 to 2010, respectively, when fitting a non-intercept exponential model. However, separate response functions were used for each individual year, since corn grain yield and plant height had different relationships in different years. Similarly to the previous reported studies, the plant height alone, at early vegetative growth stages failed to capture consistently large amount of variability. It seems that other plant morphological characteristics, cultivation practices, and environmental conditions need to be evaluated as predictor variables.

Precipitation is undoubtedly an important factor that impacts corn yield. Yield can vary significantly depending on the amount and timing of the precipitation received in a given year (Norwood, 2001). According to a study in the Midwest, soil water content was not strongly correlated with corn yield (Lyon et al., 1995). However, according to Nielsen et al. (2009), soil water content at planting (0–180 cm profile), could be a useful predictor of corn grain yield when combined with in-season precipitation data until R1 growth stage. That shows the high impact of in-season precipitation on corn grain yield and implies the need to be evaluated as explanatory variable in future yield prediction attempts.

When trying to predict corn grain yield using statistical modeling, the plant height, N fertilization rate, and precipitation appear to be the most commonly used variables. Further, limited information exists in the literature related to the development of statistical models to predict corn stover yield. In order to improve yield predictions, other morphological measurements could also be used as explanatory variables for these types of predictions. Simple measurements during the growing season, such as stalk diameter at various heights, number of ears, and height up to the first forming ear could contribute towards explainable corn grain yield and/or stover yield variability. The objective of this study was to develop regression equations to estimate corn grain and stover yield at harvest using crop information that is relatively easy to obtain, such as nitrogen fertilization rate, cumulative precipitation, and simple plant morphological measurements (plant height, stem diameter at various heights, height of the first ear, and plants per hectare).

Materials and Methods

Site description

Data used to develop the statistical regression equations came from an experiment that was conducted in two locations from 2009 to 2011. The first location was the E.V Smith Research Center (EVS) near Shorter in central Alabama (32.42884 N, -85.890235 W). The second location was in the north part of the state at the Tennessee Valley Research and Extension Center (TVS) in Belle Mina (34.687953 N, -86.886763 W). These sites were selected because they have different soil types and climate patterns. For all three years of the experiment, the mean annual precipitation at EVS ranged from 87.8-156.8 mm. At TVS for the same period, the range of mean annual precipitation was 86.5-121.6 mm. The mean annual temperature, similar to precipitation levels, was higher in EVS than in TVS by 1.2 °C. Similar trend and magnitude of difference was observed on mean annual minimum and maximum temperatures. The soil at EVS was a Compass loamy sand (Coarse-loamy, siliceous, subactive, thermic Plinthic Paleudults) while at TVS was a Decatur silt loam (Fine, kaolinitic, thermic Rhodic Paleudults).

The experiment consisted of a 3x4x2 complete factorial design arranged in a split-split-plot. The factors were; winter rye (*Secale cereale*) cover crop (main plot), N fertilization (sub-plot) and stover residue harvest (sub-sub-plot). There were three cover crop levels; rye present, rye harvested and plots without cover crop. The four levels of N fertilization rate were 0, 84, 168 and 252 kg ha^{-1}, while the two levels of corn stover harvest were stover harvested or retained on the field. Each treatment combination was replicated three times for a total of 72 plots at each location. Each plot consisted of four rows (91-cm row spacing) and measured 6.1m long by 2.7m wide.

Both locations were under continuous no-tillage corn production with no supplemental irrigation. The rye, on plots receiving a cover crop, was planted in the fall with a grain drill. Corn was sowed in late-March to early-April, as per recommendations from the Alabama Cooperative Extension Service (ACES). Pest management was performed following ACES recommendations.

Data collection

Before data collection, a representative area consisting of a 1-m length of row from both of the two middle rows of every plot was flagged. The same three plants from each of the marked rows were used for data collection throughout the entire growing season to assure consistency. Plant morphological measurements were taken every seven days starting at V2 until R1 growth stage. Morphological measurements collected were plant height and stem diameter at the base of the plant and vertically every 20-cm up to 60 cm. At the R1 growth stage, the number of forming ears and height to the first ear from the soil surface were measured. Plant height and height up to the first ear were measured using a meter stick from the same plants of the two middle rows. For stem diameter, digital calipers in mm scale with two decimal places precision were used. Since the stalk of the corn plant is not completely cylindrical, the narrowest part was used to measure the diameter to maintain consistency. The total precipitation from the planting date until final data collection (R1 stage) was recorded and used as a predictor variable. All predictor variables used for model development, as well as their symbols, are summarized in Table 1. At the end of the growing season, when grain moisture content was less than 18%, the entire plot was machine harvested with a combine, and corn yields were used for the development of the corn grain model. For the stover yield model, stover biomass between the flagged areas was manually harvested, partitioned from the grain, dried at 55 ^{0}C for seven days and weighed, prior to combine harvest.

Statistical analysis

Multiple linear regression techniques were used to develop two models. The dependent variable in the first model was grain yield, while biomass yield was the response variable in the second model. The REG procedure in SAS (SAS for Windows v. 9.3, SAS Institute Inc., Cary, NC) with the STEPWISE selection technique was employed to develop regression equations to predict grain and stover yield. Several regression equations, with and without intercepts, were developed for both grain and biomass yield. The performance of these equations was evaluated using several statistical criteria such as the R^2, adjusted R^2, mean square error (MSE), coefficient of variation (CV), residual sum of squares (RSS), predicted residual sum of squares (PRESS), and Mallow's criterion (C(p)). An independent variable significant at alpha ≤ 0.01 was incorporated into the model and was retained at alpha ≤ 0.001.

Results and Discussion

Only using the coefficient of determination, or R^2, to determine the best fit between two regression models can be misleading. A non-intercept model typically inflates the coefficient of determination (Regression through the origin, UCLA) given that this type of model forces the equation through the origin, or the (0, 0) point. This implies that when x=0, then the expected value of y also equals zero, inferring that at this point the model fits perfectly. However, including this point when it might not actually exist in the observed experimental data can introduce bias. Moreover, the sum of squares used for the calculation of the R^2 values are not corrected in non-intercept regression (e.g. most non-linear models), meaning that the coefficient of determination of a non-intercept model indicates the proportion of explained variability around the origin (zero). This creates artificially large values for the coefficient of determination (Nonlinear Regression in SAS, UCLA). In contrast, the R^2 value of an intercept model indicates the proportion of variability around the dependent variable explained by the regression.

Given these issues listed above, several criteria should be compared for choosing between a non-intercept and an intercept model and determining the final model. The mean square error (MSE) accounts for the variance and bias of the difference between the actual and predicted value. A small MSE value is desirable. This is a useful criterion when the goal of the developed model is to assess how well the predictions match the reference values (Sheiner and Beal, 1981). The coefficient of variation (CV) is another useful criterion to assist model selection since it shows the amount of variability in relation to the population mean. A small CV value is also desirable. Residual sum of squares (RSS) is another measure of how close estimated values are to the actual data. Models with small RSS indicate better fit than models with large RSS. Predicted residual sums of squares (PRESS) is a statistic used in regression analysis to provide a measure of the model fit to a portion of the total data set. This portion of the data, the residuals, was not used to develop the model. As in the case of RSS, small PRESS values are preferred. Finally, Mallow's C(p) can be used in order to find the most appropriate predictors without over-fitting the model. The model with C(p) value close to the number of parameters is desirable. These criteria were used to determine the most appropriate model for the data presented here.

Precipitation levels varied between years and locations (Figure 1). At EVS, the location in central Alabama, total precipitation during the three growing seasons was 570.2, 230.4, and 118.1mm in 2009, 2010 and 2011, respectively. For the same period at TVS, the location in the northern Alabama, total precipitation was 396.5, 222.3, and 216.2 mm. This variation in weather, as well as the difference in soil type between locations, is highly desirable in order to expand the boundaries of the prediction ability of the models. Some variability between years and locations is desired as it would add robustness to confidence intervals of the predictive values generated by the models.

The majority of independent variables used were significantly correlated to each other (Table 2). As a matter of fact, many variables were strongly correlated ($r > 0.80$). This is an indication that these interactions should be considered as possible explanatory variables.

Multiple attempts were performed to develop grain and stover models at very early stages of plant development (V2-V10). However, the maximum explainable variability would not exceed 58% for both, grain and stover yield, during the vegetative growth stages. Similar findings were reported by Yin et al. (2011). In their study, corn yield regression was weak at early growth stages and became stronger at later vegetative growth stages (Yin et al., 2011). During early vegetative growth stages in our study, fewer factors were included in the equation since height of the first ear and number of ears was not available. Due to this low explainable variability for early growth stages, measurements from the first reproductive stage (R1) were used.

Initially, individual prediction equations were developed for every location and year for both, grain and stover yield using measurements at R1 stage (equations in appendix A and B). All models were significant for every location and year. The R^2 and p-value of every grain and stover yield model were significant and large for each individual location-year (Tables 3 and 4). Yin et al. (2011) reported lower correlation coefficients using only plant height in several non-intercept exponential models that predicted grain yield. Correlation coefficients of models that they developed from V6-V12 growth stages ranged between 0.32-0.87. However, an equation that describes the data of an individual location in one year would have poor predictive performance and limited practical application. A model intended to be used for prediction purposes should be more robust. To achieve the desirable robustness, the statistical models should be developed using data that is replicated in time and space. Therefore, all measurements from this study, three years and two locations, were used for the creation of the final models.

After combining the data from all six site-years, two candidate models were developed for grain yield prediction. The first equation had an intercept, while the other had no intercept and a larger R^2. The statistical criteria mentioned previously were calculated and used to compare the regression models (Table 5). A better model fit to the actual data is usually associated with lower criteria values, with the exceptions of R^2 and adjusted R^2. Both regression models exhibited significance of regression ($p < 0.0001$). As expected, R^2 and adjusted R^2 values for the non-intercept model were larger than those of the model that includes the constant. As previously mentioned, R^2 should not be the only criterion used to select a model, thus other criteria were also used to determine the appropriate model (Sheiner and Beal, 1981). The mean squared error, coefficient of variation, residual sum of squares, predicted residual sum of squares and C(p) were lower for the model with a constant than the one without an intercept. Similar to the grain yield, both models (with and without intercept) were developed for corn stover yield estimation. When evaluating both stover models, the above criteria were lower for the intercept model than the non-intercept (Table 6). It appears that the most appropriate statistical models are those that include an intercept for both grain and stover yield prediction.

The final regression models for corn grain and stover yield, the significant predictors, and their associated estimates, are summarized in Tables 7 and 8, respectively. All predictors were significant for the grain yield model except the plant height and the stem diameter at the base of the plant. Almost 80% of the total variation was explained with the final regression model (R^2=0.7705). The adjusted coefficient of determination was almost equal to the R^2, which implies that there were no parameters in the model that should be removed. This is also an indicator that the model is not over-parameterized. The variance inflation factor (VIF) was used to detect possible multicollinearity issues. It seems that for the grain model, all predictors exhibited VIF<10 which indicates no multicollinearity (Table 7). Figure 2 shows the scatter plot of predicted grain yields against actual values. Almost every observation falls inside the 95% prediction limits (dotted lines). The corn grain model overestimated actual mean yield by 1.8%. Also the plant height was not included in the final grain regression equation as a significant variable. However, this does not mean that it is not a valuable morphological measurement. It possible that in this experiment other variables had a greater impact on the final regression model.

For the stover yield model, plant height, precipitation, N rate, stem diameter at 20 and 40 cm of height, height to the first ear, and number of ears per hectare at R1 stage were the significant factors. The maximum explainable variability was 85% (R^2=0.8473), with the adjusted R^2 values being almost identical. Similarly to the grain yield model, no multicollinearity was detected (Table 8). Figure 3 shows the scatter plot of predicted versus actual stover yields. Similar to the grain model, almost all observations fall inside the 95% prediction limits (dotted lines). The stover model overestimated the actual mean biomass yield by 0.3%.

Every statistical model has boundaries and limitations. The robustness of every model depends on how wide these boundaries are. The high robustness of the developed regression equations can be justified for the following reasons: Four N levels (0-252 kg ha^{-1}) were included which provides a good range of N fertilization. It should be noted that the recommended N rate in Alabama is 150 kg ha^{-1}. Also, six different levels of precipitation from planting to R1 stage (118-570 mm) were included in the model development. Further, three different cover crop management practices and two stover residue management treatments were included, making the dataset more robust to possible cultivation techniques. Finally, the two sites were established in two major soil types of the Southeast. For these reasons, the regression models should perform well (interpolate) for corn grown at any level of cumulative precipitation (planting – R1) between 118-570mm, in similar soil type to those in this study, for any N fertilization rate between 0-252 kg ha^{-1}, for any of the three cover crop managements, and whether the farmer removes the corn residues after harvest or not.

Conclusions

The capability of predicting corn grain and stover yield at harvest using information at early growth stages (V2-V10) is an appealing and useful goal. However, according to our study, excessive variability during the vegetative growth stages did not allow for the construction of an acceptable robust prediction equation. Climate variability that includes droughts or large amounts of precipitation, as well as extreme environmental phenomena, can impact the growth and the corn yield. The first reproductive stage was an appropriate time to collect measurements, which can result in accurate corn grain and stover yield estimations. Results from this experiment suggest that total precipitation from planting until R1 growth stage, as well as simple morphological measurements at R1 growth stage, such as plant height, height of the first ear, stem diameter at various heights, and number of forming ears per hectare can be used to assess corn grain and stover yield. According to the results presented, the overall performance of both regression models was acceptable and it is expected that their predictive ability is reliable between the specified growth conditions.

Table 1. Predictor variables and assigned symbols used for corn grain and stover predictions.

Effect	**Symbol**
Precipitation (mm)	Pr
Corn nitrogen rate (kg ha^{-1})	N
Plant height (cm)	Ph
Stem diameter at base of the plant (mm)	S0
Stem diameter at 20 cm height (mm)	S20
Stem diameter at 40 cm height (mm)	S40
Height of the 1st ear (cm)	Eh
Plants ha^{-1}	P
Corn ears ha^{-1}	C

Table 2. Correlation between predictor variables for corn grown at two locations in Alabama between 2009 and 2011.

	Precipitation	N	Plant height	Stem0	Stem20	Stem40	Plants ha^{-1}	Ear height	Ears ha^{-1}
Precipitation	1.000	0.000	-0.156*	-0.105*	-0.113*	-0.045	0.382***	-0.244***	0.081*
N		1.000	0.415***	0.294***	0.347***	0.369***	0.631	0.348***	0.283***
Plant height			1.000	0.507***	0.500***	0.476***	0.163	0.828***	0.436***
Stem0				1.000	0.945***	0.860***	0.178*	0.477***	0.198***
Stem20					1.000	0.899***	0.195***	0.479***	0.204***
Stem40						1.000	0.315***	0.447***	0.210***
Plants ha^{-1}							1.000	0.405***	0.249***
Ear height								1.000	0.315***
Ears ha^{-1}									1.000

*** Significant correlation at Pr≤0.0001

* Significant correlation at Pr≤0.05

Table 3. Coefficient of determination and probability values for corn grain prediction models for individual locations and years.

Location	**Year**	**R^2**	**Pr>F**
†EVS	2009	0.7837	≤0.0001
EVS	2010	0.8119	≤0.0001
EVS	2011	0.8974	≤0.0001
‡TVS	2009	0.8037	≤0.0001
TVS	2010	0.7804	≤0.0001
TVS	2011	0.9000	≤0.0001

†EVS - E.V. Smith Research Center near Shorter in Central Alabama;

‡TVS - Tennessee Valley Research and Extension Center near Belle Mina in Northern Alabama.

Table 4. Coefficient of determination and probability values for corn stover prediction models for individual locations and years.

Location	**Year**	**R^2**	**Pr>F**
†EVS	2009	0.7873	≤0.0001
EVS	2010	0.7493	≤0.0001
EVS	2011	0.8968	≤0.0001
‡TVS	2009	0.7046	≤0.0001
TVS	2010	0.8885	≤0.0001
TVS	2011	0.8644	≤0.0001

†EVS - E.V. Smith Research Center near Shorter in Central Alabama;

‡TVS - Tennessee Valley Research and Extension Center near Belle Mina in Northern Alabama.

Table 5. Comparison of a corn grain yield model with an intercept to a non-intercept model.

Fit Statistic	Model with Intercept	Model without Intercept
Model Pr>F	<0.0001	<0.0001
R^2	0.7705	0.9173
Adjusted R^2	0.7678	0.9116
Mean Square Error (MSE)	1294.56	1766.5
Coefficient of Variation (CV)	23.55	32.14
Residual Sum of Squares (RSS)	703,874,115	1,316,868,208
Predicted Residual Sum of Squares (PRESS)	726,546,994	1,343,802,556
Mallow's C(p)	54.13	61.05

Table 6. Comparison of a corn stover biomass yield model with an intercept to a non-intercept model.

Fit Statistic	**Model with Intercept**	**Model without Intercept**
Model Pr>F	<0.0001	<0.0001
R^2	0.8473	0.9753
Adjusted R^2	0.8455	0.9751
Mean Square Error (MSE)	583.44	688.54
Coefficient of Variation (CV)	14.21	16.77
Residual Sum of Squares (RSS)	142,972,694	200,540,175
Predicted Residual Sum of Squares (PRESS)	147,217,120	203,847,095
Mallow’s C(p)	13.47	119.6

Table 7. Final model for corn grain yield prediction (kg ha^{-1}) using parameters and information collected at R1 growth stage.

†Variable	Coefficient	Pr>F	‡VIF
Intercept	-5665.74434	<0.0001	0
Pr	5.25842	<0.0001	1.03
C	0.08444	<0.0001	1.3
S20*S40	13.70115	<0.0001	1.2
N*P	0.00027691	<0.0001	5.81
N^3	-0.00008037	<0.0001	4.8

† C - corn ears ha^{-1}; N - fertilizer N rate in kg ha^{-1}; Pr - precipitation in mm; S0 - stem diameter at the base of the plant in mm; S20 - stem diameter at 20cm of height in mm; P – Plants ha^{-1}; Ph - plant height in cm; Eh - height of the first ear in cm.

‡ Variance Inflation Factor

Table 8. Final model for corn stover biomass yield prediction (kg ha^{-1}) using parameters and information collected at R1 growth stage.

†Variable	Coefficient	Pr>F	‡VIF
Intercept	71.71855	<0.0001	0
Pr	1.20204	<0.0001	1.08
N	1.94348	<0.0001	1.37
Ph^2	0.03859	<0.0001	2.95
C*Eh	0.00066956	<0.0001	2.7
(Ph*Eh)/(S20*S40)	-18.76462	<0.0001	2.9

† C - corn ears ha^{-1}; N - fertilizer N rate in kg ha^{-1}; Pr - precipitation in mm; S0 - stem diameter at the base of the plant in mm; S20 - stem diameter at 20cm of height in mm; S40 - stem diameter at 40cm of height in mm; P – Plants ha^{-1}; Ph - plant height in cm; Eh - height of the first ear in cm.

‡ Variance Inflation Factor

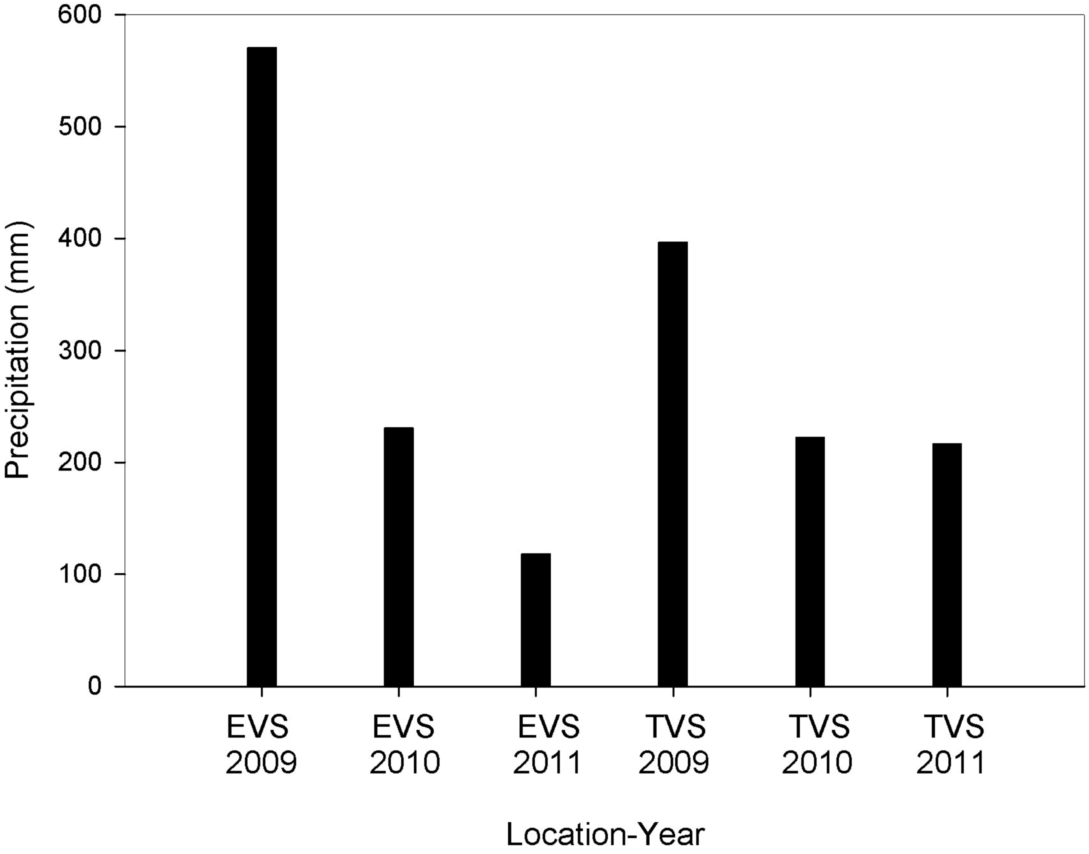

Figure 1. Precipitation during the 2009-2011 growing seasons at the EVS - E.V. Smith Research Center near Shorter in Central Alabama and TVS - Tennessee Valley Research and Extension Center near Belle Mina in Northern Alabama.

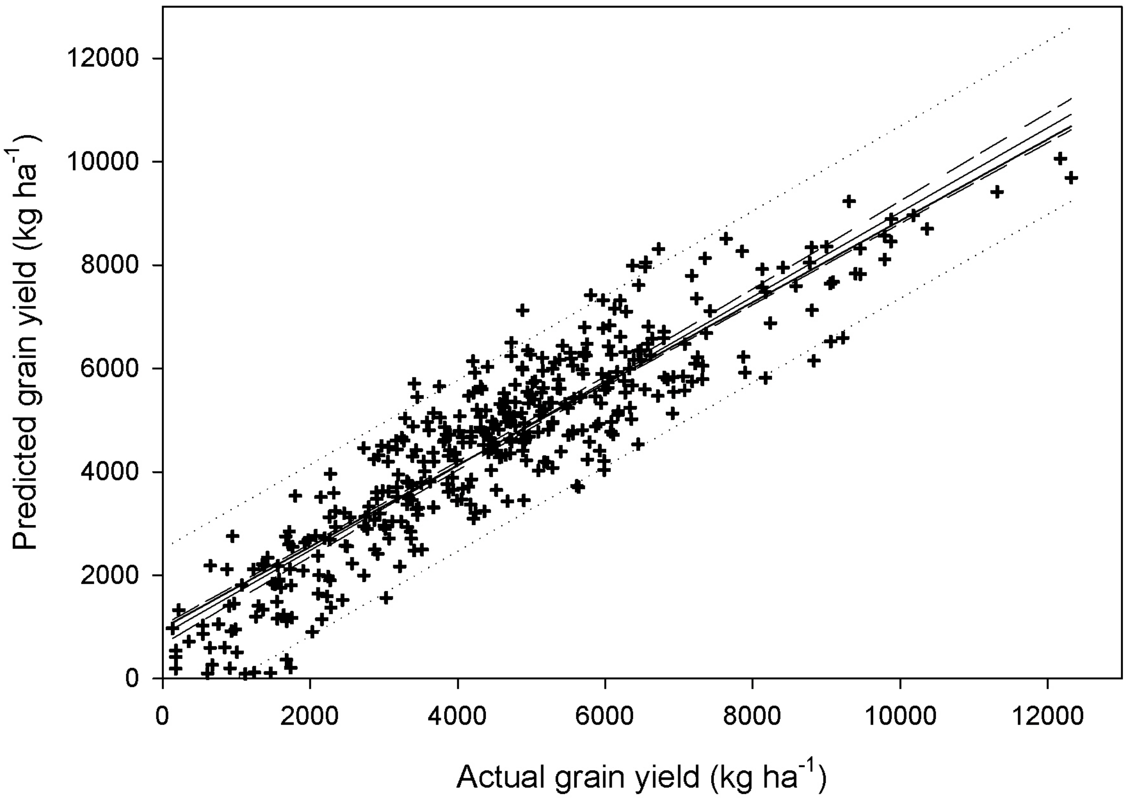

Figure 2. Predicted versus actual corn grain yields for six sites years. The predicted mean grain yield was 5545 and the actual mean yield was 5447 kg ha^{-1}.The dash lines denote the 95% grain yield confidence intervals (5376, 5724) and the dotted lines show the 95% prediction intervals (5107, 6010).

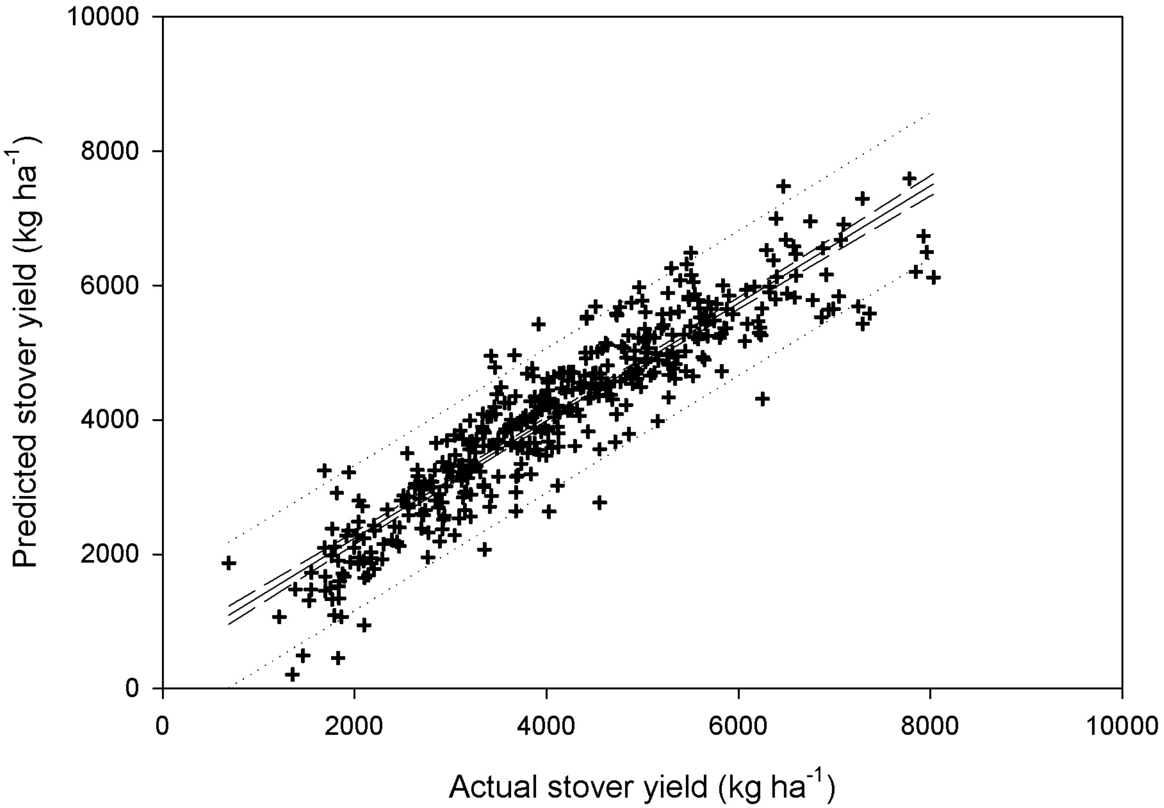

Figure 3. Predicted versus actual corn stover yields for six sites years. The predicted mean stover yield was 4104 and the actual mean yield was 4090 kg ha^{-1}.The dash lines denote the 95% stover yield confidence intervals (3974, 4234) and the dotted lines show the 95% prediction intervals (3775, 4446).

III. A Simplified Method for Monomeric Carbohydrate Analysis of Corn Stover Biomass

Abstract

Biofuel production from plant biomass has been proposed as a solution to mitigate fossil fuel use. Constituent determination of biomass, for theoretical ethanol yield (TEY) estimation, requires the removal of non-structural carbohydrates prior to analysis to prevent interference with the analytical procedure. The objective of this work was to determine whether the Neutral Detergent Fiber method (NDF) for extraction and determination of structural carbohydrates can be used as an alternative to the National Renewable Energy Laboratory (NREL) extraction procedure. According to the accepted U.S. Dept. of Energy-NREL method, biomass extractives in corn stover should be removed by a two-step extraction process. Alternatively, NDF is a fast and cost-effective method used to determine the structural carbohydrate portion of forage biomass. There were statistical differences between the two methods in carbohydrate concentrations and TEY per unit of mass. However, the practical differences in TEY per unit of mass (2%) seemed to be insignificant. Furthermore, the TEY (l ha^{-1}) derived by the proposed method was similar to the NREL method.

Introduction

In the last decade, several factors have encouraged countries to show interest in biofuel production. Increasing oil prices, rising greenhouse gas emissions, and energy security issues are only a few reasons that make alternative energy an attractive idea. Cellulosic biomass from several plant species could be converted to liquid fuels like ethanol (Lynd et al., 1999). Corn (*Zea mays* L.) has the potential to be an important feedstock for biofuel production from lignocellulosic material (McAllon et al., 2000). However, the composition of the biomass affects the final ethanol yield from the conversion. The main components of the plant material are cellulose, hemicellulose, lignin, ash, protein, lipid, pectin, soluble sugars, and phenolic compounds (Cone et al., 1996). Cellulose and hemicellulose are structural polysaccharides contained in plant cell walls (Hatfield, 1989). Cellulose, which is a primary plant component and the most abundant carbohydrate on earth (Chandrakant and Bisaria, 1998), is the most desirable plant component. The least desirable component is lignin which is known to inhibit biomass hydrolysis (Chang and Holtzapple, 2000; Kim and Holtzapple, 2006). Structural carbohydrates are composed of five and six-carbon sugars. The 5-C sugars are xylan and mannan and the 6-C sugars are glucan, arabinan and galactan. The amount of these components in biomass affects the amount of ethanol that can be produced. Feedstock with higher proportions of the 6-C sugars is more desirable due to higher conversion efficiency.

Compositional analysis allows for a close estimation of the proportion of 5-C and 6-C sugars present in the biomass. Knowledge of the quantities of these carbohydrates can be used to calculate the TEY. It seems that there have been attempts to release and quantify carbohydrates from biomass using acid for more than 100 years (Brauns, 1952). Almost 60 years ago, Saeman et al. (1954), developed a paper chromatography detection method which became the standard carbohydrate separation and quantification procedure for several years until 1984 when Pettersen et al. (1984) introduced the HPLC method for quantification of biomass carbohydrates. Despite the statistically significant differences in the results between paper chromatography and HPLC, the authors stated that the method can be useful due to the relatively small variations. Another more recent accepted method, which has been used for biofuel feedstocks and food samples, is the Uppsala method (Theander, 1991).

The most recently commonly accepted method for compositional analysis of biomass for bioenergy research purposes was developed by the U.S. Department of Energy-National Renewable Energy Laboratory (NREL). This method has two stages. The first stage involves the removal of non-structural material to prevent interference with the analysis (Technical Report NREL/TP-510-42619, 2005). A two-step extraction is performed on the biomass samples in order to remove first the water-soluble and then the ethanol-soluble materials. The second stage involves a two-step acid hydrolysis on the extractive-free sample in order to fractionate the polymeric carbohydrates of biomass into monomeric forms. These monomeric forms (glucose, xylose, arabinose, galactose, and mannose) can then be quantified using HPLC (Technical Report NREL/TP-510-42618, 2008).

However, quantifying carbohydrates in biomass is a costly and time-consuming procedure, in particular the first stage of the process (removal of non-structural materials in biomass). During this first stage, corn stover biomass extractives have to be removed. A Soxhlet apparatus is commonly used for such purpose. The samples have to reflux for 6-24 hours in HPLC grade water followed by 16-24 hours reflux with 190 proof ethyl alcohol. A usual Soxhlet apparatus can extract up to 6 samples simultaneously. A cycle of extraction can last from 22-48 hours for up to 6 samples when using a typical Soxhlet apparatus. Even with the newer ANKOM fiber analyzer apparatus, which utilizes filter bags it can batch process up to 24 samples simultaneously (ANKOM 2000).

An older procedure, which was developed for quantifying cell wall constituents mainly for ruminant nutrition purposes, uses a neutral detergent fiber (NDF) to quantify the insoluble fibers of biomass (Van Soest and Wine, 1967). The idea of this method is based on the concept that insoluble fibers cannot be utilized by photolytic enzymes but they are fermentable by microbes like those existing in the digestive system of animals. Since the insoluble fibers quantified by the NDF method are fermentable, this procedure could also be used for bioenergy research purposes. The NDF procedure could be utilized to remove non-structural material from biomass and leave only the fermentable fibers. Quantifying this fermentable portion of the biomass could allow for TEY estimation.

The goal of every laboratory procedure is to produce accurate results. However, other characteristics are also important. Modern scientific research requires a large volume of information to be derived that is accurate in a cost-effective and fast manner. The cost of the analysis is usually the limiting factor in any type of research. An acknowledgement for the NREL procedure is the need to increase efficiency, speed, and cost-effectiveness (Sluiter et al., 2010). Therefore, the objective of this study was to develop a simple, accurate, fast, and cost-effective method for compositional analysis of corn stover biomass. More specifically, we evaluated the effectiveness of the NDF extraction method developed by Van Soest and Wine (1967) as an alternative to the NREL extraction stage. The NDF extraction followed by the two-step acid hydrolysis was examined to determine whether results had comparable accuracy to the accepted two-stage NREL method (Technical Report NREL/TP-510-42618, 2008). Furthermore, this procedure was evaluated for its cost-effectiveness and speed. Since the proposed method is a combination of two already existing methods, hereafter it will be referred to as ‘combined’.

Materials and Methods

Sample collection and preparation

Corn biomass from Pioneer 31G65R hybrid was collected randomly from two studies in Alabama in 2009. The first site was at the E.V. Smith Research Center (EVS) near Shorter in central Alabama (32.42884 N, -85.890235 W). The second location was in the north part of the state at the Tennessee Valley Research and Extension Center (TVS) in Belle Mina (34.687953 N, -86.886763 W). The soil at EVS was a Compass loamy sand (Coarse-loamy, siliceous, subactive, thermic Plinthic Paleudults) while at TVS it was a Decatur silt loam (Fine, kaolinitic, thermic Rhodic Paleudults). Both locations were under continuous no-tillage and non-irrigated corn production. The carbohydrate content in corn stover can vary among different plant portions (Decker et al., 2007). Therefore, various plant fractions consisting of 20-cm stalk segments from the base of the plant to the top and cobs alone were chosen to provide a wide range of sample composition for this type of material. Samples were dried for 7 days at 55 $^{\circ}$C in a forced air oven and then ground through a 2 mm screen in a Wiley mill grinder. Sixty-seven stover samples were analyzed using the standard NREL method (Technical Report NREL/TP-510-42619, 2005; Technical Report NREL/TP-510-42618, 2008) and the proposed combined procedure that uses NDF extraction (Van Soest and Wine, 1967) followed by the second stage of the NREL method (the two-step acid hydrolysis).

NREL method

For the analysis of the samples via the two stage NREL method, the laboratory analytical procedure (LAP) was used which was published by the NREL as technical reports (Technical Report NREL/TP-510-42619, 2005; Technical Report NREL/TP-510-42618, 2008). Briefly, 3 g of biomass were weighed and added to a flask with 190 ml HPLC grade water. The flask was placed on a Soxhlet apparatus and refluxed for 18 h. As directed by the LAP, the water reflux duration should be from 6-24 h. Extracting the samples for 18 h seemed to be a safe choice for complete extraction since it is closer to the upper limit of the proposed extraction duration. Furthermore, due to the large number of samples (n=67), decreasing the extraction time by 6 hours from the maximum made a significant difference in the extraction duration. At the end of the 18 h, 190 ml of ethyl alcohol was added to the flask and 20 h of extraction followed. The duration of ethanol extraction should be 16-24h as directed by the LAP (Technical Report NREL/TP-510-42619, 2005). At the end of the extraction (first stage), the samples had to dry for 24 hours. A day later, when the samples were dry, 300 mg of the extractive-free sample and 3 ml of 72% sulfuric acid were added in a pressure tube to perform the two-step acid hydrolysis. The tube was incubated for 60 min in a water bath at 30 °C. At the end of the 60 minute hydrolysis, 84 ml of de-ionized water was added. Finally, the samples were placed in an autoclave at 121 °C for 1 hour (Technical Report NREL/TP-510-42618, 2008). For the separation and quantification of the sugars in corn stover samples, a Shimadzu (LC-20A) HPLC system was used which consisted of a degasser, autosampler, LC-20AD pump, and RID-10A detector. The detector was equipped with a 300 mm × 7.8mm i.d., 9 µm, Aminex HPX-87P column and a 30 mm × 4.6 mm i.d.guard column of the same material (Bio-Rad, Hercules, CA). The mobile phase consisted of water at a 0.6 mL/min flow rate. During the elution, the temperature of the column was maintained at 85 °C.

NDF method

For the analysis of the samples via the combined method, the procedure which was published by Van Soest and Wine (1967) was used. Concisely, 1 g of the ground dry sample was placed in a refluxing apparatus. The neutral detergent solution along with the decahydronaphthalene and sodium sulfate were added and the samples heated to boiling for 60 minutes. At the end of the reflux, samples were transferred in vacuum to crucibles and left to dry at 100 °C for 8 hours. The dry sample in the crucible was the extractive-free portion. Then the two-step acid hydrolysis of the NREL method was performed to the extractive-free sample (Technical Report NREL/TP-510-42618, 2008). Finally, the samples were analyzed for monomeric carbohydrates via HPLC using the same instrumentation and column used with the samples processed via the NREL method.

Statistical analysis

The NREL method has been certified as an official method for biomass compositional analysis. To measure the accuracy of the proposed method, results obtained with the NREL method were considered as the reference value (i.e. assumed that this was the true sugar concentrations in corn stover). Therefore, a comparison of means was used to detect differences in the composition of the samples extracted by the two methods. The variables of interest were the polymeric carbohydrates (glucan, xylan, arabinan, galactan, and mannan) and the calculated TEYs per unit of mass ($l\ Mg^{-1}$) and per unit of area ($l\ ha^{-1}$). However, only glucose, xylose and arabinose were above the detectable limits, so this report focuses on glucan, xylan, and arabinan. The TTEST procedure in SAS (SAS for Windows v. 9.3, SAS Institute Inc., Cary, NC) was used to compare mean concentrations of the polymeric carbohydrates in the two groups and the theoretical ethanol yields. A 10% level of significance was used for all comparisons (alpha=0.1).

Results and Discussion

Accuracy comparison

Comparisons of the concentrations of glucan, xylan and arabinan (%) measured by the two procedures showed statistically significant differences (Table 9). Side by side box-plots with the percentages of the polymeric carbohydrates show the differences between the two procedures (Figure 4). The first two box-plots show the glucan concentrations in the 67 samples from the NREL and the combined method, respectively. The median and mean values for glucan and xylan were lower for the combined procedure. The ranges, however, were similar for both methods. The mean and median values for arabinan content in the 67 samples were larger with the combined procedure than with the NREL. It is interesting that outliers were observed with both methods of analysis. However, it was not possible to detect specific samples that contributed consistently towards the outliers for both methods and all three polymeric carbohydrates.

Additionally, the TEY ($\mathrm{l\,Mg^{-1}}$) was calculated using carbohydrate values obtained from both methods of sample analysis and the U.S. Department of Energy TEY calculator (DOE). A 100% conversion efficiency was assumed for all calculations for comparison consistency. The median and mean values for the TEY ($\mathrm{l\,Mg^{-1}}$) were lower for the combined procedure (Figure 5). The average theoretical ethanol yields for the 67 samples were 492.9 and 483.9 $\mathrm{l\,Mg^{-1}}$ of biomass for the NREL and combined method respectively (Table 10). The TEY ($\mathrm{l\,Mg^{-1}}$) difference between both methods (NREL vs. combined) was statistically significant (p-value = 0.0124) and the combined procedure underestimated the ‘true’ ethanol yield in corn biomass (Table 10). However, despite the statistical differences, the TEY between both methods varied only by 2%. Also, standard deviations and ranges of calculated ethanol yields were similar for both methods. The magnitudes of these differences were small and practically could be insignificant. Furthermore, in applied agricultural research it is common that results are reported in l ha^{-1} rather than l Mg^{-1} of biomass. When the TEYs per unit of area were calculated using the results from both methods and dry biomass yields, TEYs (l ha^{-1}) were very similar and there were no significant differences (Table 11, Figure 6). Therefore, the combined procedure, which involves the NDF extraction followed by the two-step acid hydrolysis, could be used to estimate the TEY from corn biomass.

Cost and time comparison

The cost-effectiveness and speed of the NDF extraction were evaluated by comparing them with the NREL. There was no reason to estimate the cost and speed of the second stage of the procedure (two-step acid hydrolysis) since it is identical in both methods. The cost of the chemicals used per sample was $0.10 for the NREL method (Table 12). However, the labor cost was more difficult to determine. The duration of the extraction procedure proposed by the NREL is approximately three days. Nevertheless, there are many idle hours between every extraction cycle (18 h in water, 20 h in ethanol and 24 hours drying). Nonetheless, it is difficult to hire skilled labor for just 1 or 2 hours of work per day. Instead of calculating the cost of 3 days (3x8=24 hours), the cost of 16 h was used in order to make a fair comparison. Assuming the use of a typical Soxhlet apparatus (six samples per cycle) and $10/h the cost of labor per hour, total cost per sample would be $26.76. Even using the ANKOM apparatus, which has the capacity to batch-process up to 24 samples, the total cost per sample would be $6.76.

Cost estimation of the NDF procedure was more straightforward. The chemical cost was \$0.65 per sample and the extraction duration was approximately 1 hour per sample. A typical reflux apparatus can batch-process up to 12 samples. For consistency, we assumed \$10 to be the cost of the labor hour, which makes the total cost per sample to be \$2.38.

For the extraction of one sample using the NREL methodology, it required approximately 62 hours. However, using the combined procedure, the extraction stage was completed in 16 hours. Although a direct comparison was difficult, the combined procedure was faster than the NREL. Also the combined method was more cost-effective than the NREL method; however, this was mainly due to the fact that it was faster and less labor hours were needed for its completion.

Conclusions

Fast and accurate methods that are cost-effective can be of great help for research purposes and industry. The NDF extraction is a simple, rapid, and inexpensive procedure. According to this study, the proposed procedure, which involves the NDF extraction, resulted in statistically different carbohydrate concentrations and TEYs ($\mathrm{l\ Mg^{-1}}$) estimations compared to the NREL method. However, these differences were small (2%) and could be considered not significant for practical purposes. Furthermore, when carbohydrate concentrations of both methods were used to calculate the TEY ($\mathrm{l\ ha^{-1}}$) there were no differences. Results from this study suggest that when utilization of the NREL extraction is not possible due to high cost, time constrains or apparatus availability, the NDF extraction can be used as an alternative procedure with similar results to the NREL method.

Table 9. Polymeric carbohydrates in corn biomass for the NREL and the combined method.

Carbohydrate	**Extraction Method**	**Mean (%)**	**Standard Deviation**	**Minimum**	**Maximum**	**†Pr>F**
Glucan	NREL	48.5	3.9	39.45	55.1	0.0904
	combined	47.46	3.1	40.45	54.5	
Xylan	NREL	25.4	3.45	18.1	33	0.0443
	combined	24.73	3.17	17.55	32	
Arabinan	NREL	0. 93	0.59	0.13	2.42	0.0005
	combined	1.26	0.49	0.38	2.6	

† Probability of a larger F by chance between extraction methods

Table 10. Theoretical ethanol yields per unit of mass (l Mg^{-1} of biomass) for the NREL and the combined method.

Extraction Method	**Mean**	**†Pr>F**	**Standard Deviation**	**Minimum**	**Maximum**
NREL	492.9	0.0124	19.1	440.9	538.4
Combined	483.9		22	420.8	538.5

† Probability of a larger F by chance between extraction methods

Table 11. Theoretical ethanol yields per unit of area (l ha^{-1} of biomass) for the NREL and the combined method.

Extraction Method	Mean	†Pr>F	Standard Deviation	Minimum	Maximum
NREL	439.4	0.8885	341	68.2	1653
Combined	431.2		332.6	67.9	1582.5

† Probability of a larger F by chance between extraction methods

Table 12. Extraction cost and duration per sample for the NREL and the combined method.

Extraction Method	**Cost ($)/sample**	**Time (hours)/ sample**
NREL	$26.76	62
Combined	$2.38	16

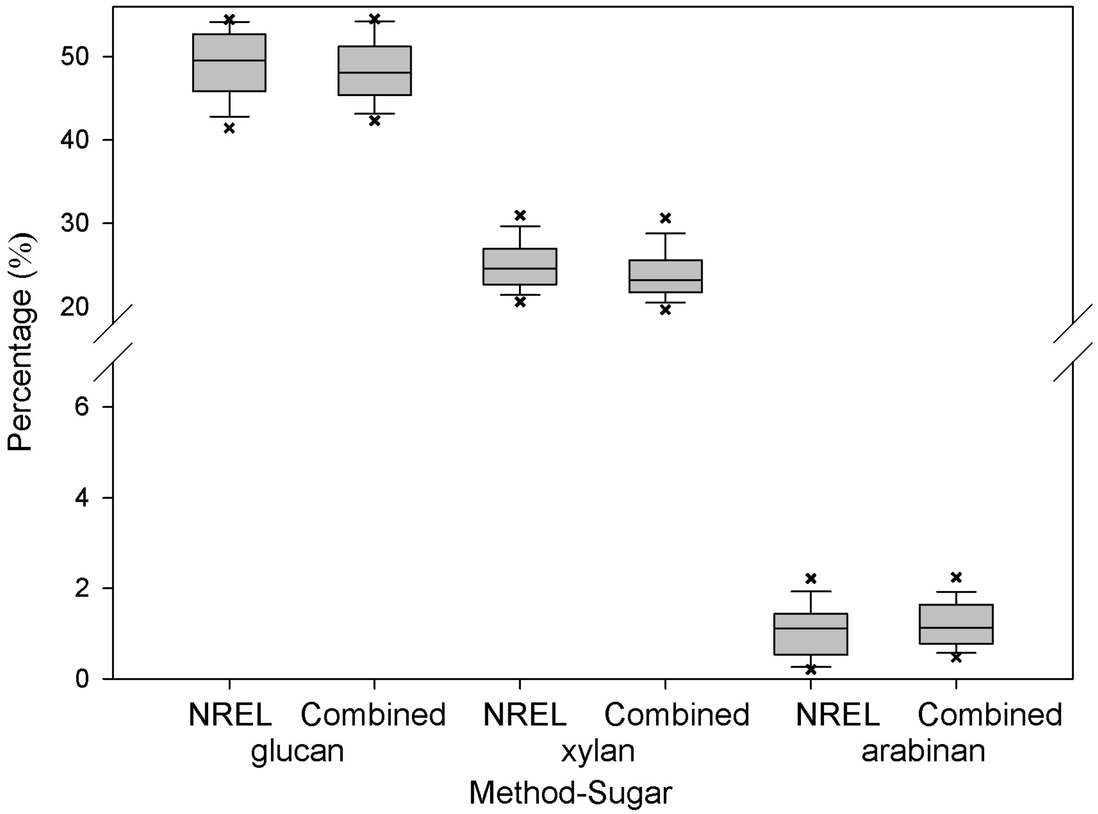

Figure 4. Comparison of variability of the monomeric carbohydrates (%) between the combined and Natural Renewable Energy Laboratory procedures.

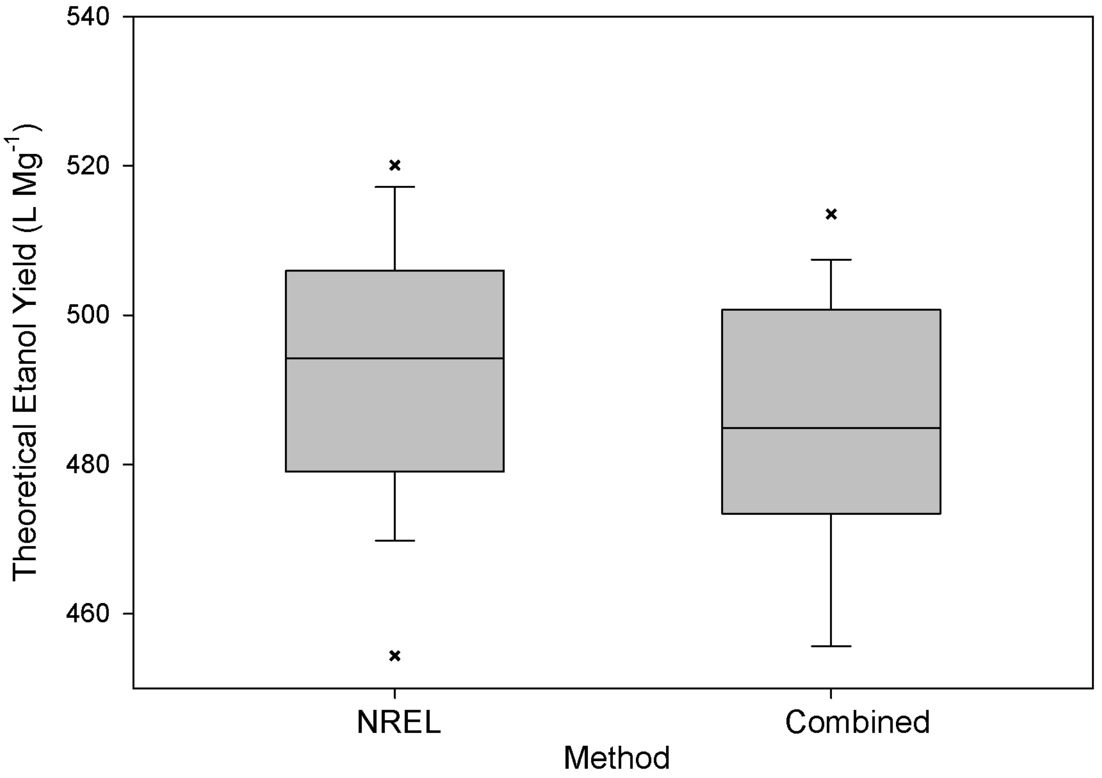

Figure 5. Comparison of variability of the theoretical ethanol yields (l Mg^{-1}) between the combined and Natural Renewable Energy Laboratory procedures.

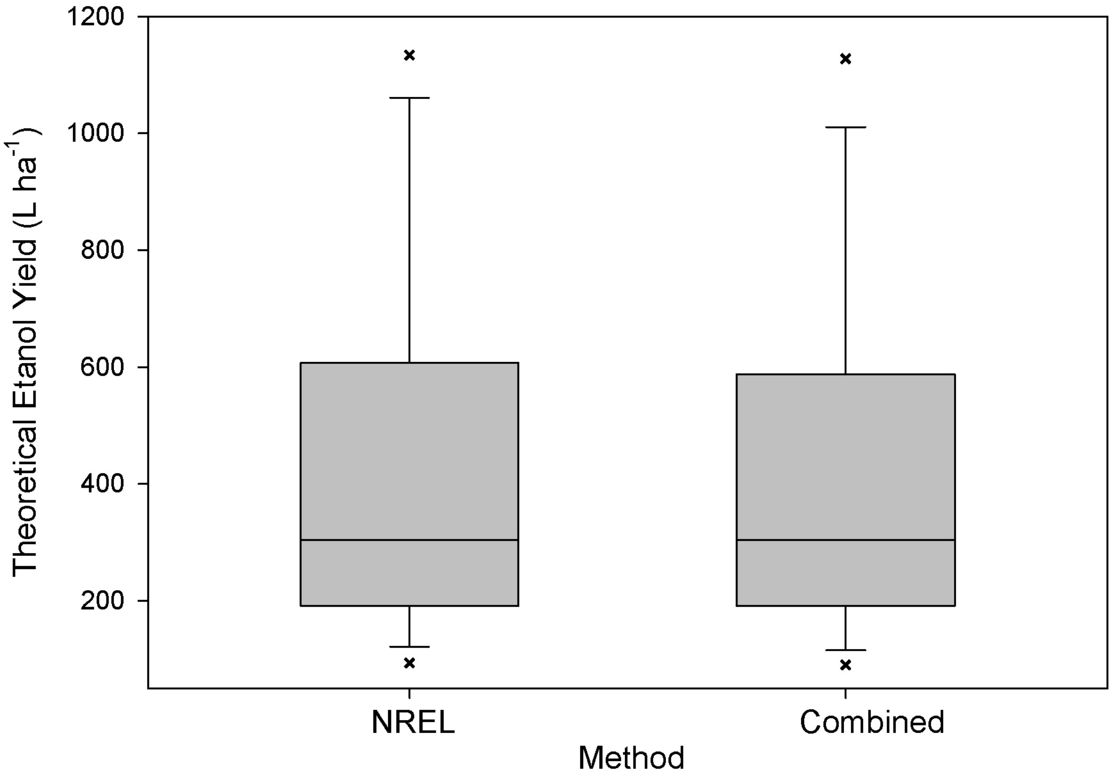

Figure 6. Comparison of variability of the theoretical ethanol yields (l ha^{-1}) between the combined and Natural Renewable Energy Laboratory procedures.

IV. Corn Grain Yield and Vertical Distribution of Stover Affected by Weather, Cover Crop and Residue Removal on Two Soil Types in the Southeastern US

Abstract

Corn (*Zea mays* L.) is an important crop in the Southeast. Its abundance and high biomass yield potential makes it attractive as bioenergy feedstock for the biofuel industry. The objective of this study was to evaluate the effect of in-season weather conditions, rye (*Secale cereale*) as a winter cover crop, and harvest of corn residue on grain yield and vertical biomass distribution across two soil types. Grain yield, as well as, total and partial stover yield were measured from 2009-2011 at two sites with different soil types: loamy sand and silt loam, in central and north Alabama, respectively. Grain yield ranged from 5,328-9,251 kg ha^{-1} for the loamy sand and 4,488-6,423 kg ha^{-1} for the silt loam. Total stover dry weight ranged from 3,486-5,482 kg ha^{-1} and 3,100-5,528 kg ha^{-1} for the same soils. Corn biomass was partitioned into four groups: below the ear (bottom), above the ear excluding cobs (top), cobs, and above the ear including top and cobs (above-ear). Significant differences in grain and biomass yields were observed between individual years and locations, with yields generally greater in central Alabama. Grain yields were positively correlated with seasonal cumulative precipitation and negatively with seasonal average temperature at both locations. For the three years of the experiment, the use of a rye cover crop increased yields at both locations, while the average effect of three years' residue management was not significant. Data from this study suggest that

when the objective is to maximize corn grain and biomass, the use of rye as a winter cover crop can increase yields in central and north Alabama.

Introduction

Biofuel production from biomass seems to be an alternative solution to mitigate fossil fuel use and to reduce greenhouse gas emissions. However, growing food crops like corn grain, soybean [*Glysine max* (L.) Merr.] and cereals for biofuel production would compete with land use for food production. Cellulosic biomass derived from crop residues would not compete with food use since both can be produced simultaneously; therefore, it seems to be a promising alternative renewable source of energy.

Corn is a highly promising crop for biomass production. Biomass and grain yield could be affected by factors like weather conditions, nutrient availability, winter cover crop rotation, and residue harvest. Rye is a well-known winter cover crop for its superior winter hardiness, its sensitivity to herbicide kill, and its consistent large residue production (Moschler et al., 1967; Odhiambo and Bomke, 2001). The impact of rye as a winter cover crop on corn productivity appears to vary with geographic location. A study in Canada indicated that a rye cover crop resulted in significantly lower corn grain and biomass yield (Raimbault et al., 1990). Another study conducted in the northern USA by Bundy and Andraski, (2005) reported that whole corn plant biomass was not significantly affected by the use of rye as a winter cover crop. However, other studies in the Southeast indicated that cover crops can improve soil productivity, especially when combined with conservation tillage practices (Bruce et al., 1995; Sainju et al., 2002).

Corn stover is composed of the stalk, leaves, cobs and husks. The stalk, which accounts for more than 50% of the total biomass, is the largest fraction of the stover. The remaining portion is composed of leaves, cobs and husk (Atchison and Hettenhaus, 2003; Masoero et al., 2006). Shinner and Binversie (2007) found that the stalk accounts for 56% of the stover dry weight, the cob for 15%, the husk for 8%, and the remaining 21% is leaves. An older study reported that stalks, leaves and tassels account for 70% of total corn stover biomass, with the remaining 30% being husks, shanks, silks and cobs (Hanway, 1963). Similar results have been found in more recent studies. Pordesimo et al. (2005) found that the highest corn stover biomass occurred at the time of grain physiological maturity, around 118 days after planting. The aboveground biomass distribution, including grain, was 46% grain, 28% stalk, 11% leaf, 8% cob, and 7% husk (grain:stover= 0.85:1). Without considering grain, the biomass distribution in stover was 51% stalk, 21% leaf, 15% cob, and 13% husk.

Corn biomass left in the field after the growing season is very important for erosion control, C sequestration, and nutrient cycling, which all affect soil productivity (Johnson et al., 2007; Johnson et al., 2009; Lindstrom, 1986; Wilhelm et al., 2004). In order to balance these multiple soil demands, a portion of the biomass could be harvested for biofuel production, while the rest should be left in the field to enhance formation of organic C. Combines that harvest grain, as well as, stover or part of the stover have already been developed (Hoskinson et al., 2007). Stover that remains in the field to sustain SOC will depend on the amount of biomass that will be removed. In large scale biomass production, it is difficult to harvest specific parts of the plant like husks alone or leaves without the stalk. It is more feasible to harvest a specific portion, e.g. the bottom; cobs; top part of the plant alone; or top and cobs together. However, long-term partial corn biomass harvesting could negatively impact soil productivity. The use of rye as a winter cover crop could mitigate these impacts. However, the response of total and partial biomass yield could vary between locations with different climates, soil types, and under different cultivation techniques (e.g., use of winter cover crops and corn residue harvest).

The first objective of this experiment was to determine the impact of selected cropping practices, including stover harvest and use of cover crops, on grain, total and partial biomass yields at two locations in Alabama. The second objective was to determine the vertical distribution of corn biomass under different cultivation techniques and soil types. This information can be useful in the creation of stover harvest recommendations that could be used as a decision making tool for the biofuel industry and farmers who wish to harvest the corn stover sustainably.

Materials and Methods

Site description

Vertical distribution of corn biomass was assessed at two locations in Alabama. The first location was the E.V. Smith Research Center near Shorter in central Alabama (32.42884 N, -85.890235 W). The second location was in the north part of the state, the Tennessee Valley Research and Extension Center in Belle Mina (34.687953 N, -86.886763 W). Each location has different soil types and climate. The soil at the E.V. Smith Research Center (EVS) was a Compass loamy sand (Coarse-loamy, siliceous, subactive, thermic Plinthic Paleudults). At the Tennessee Valley Research and Extension Center (TVS), the soil was a Decatur silt loam (Fine, kaolinitic, thermic Rhodic Paleudults).

Plots were arranged in a split-plot design with three replications. Treatments included: three levels of rye as a winter cover as the main plot (no cover, rye as a cover crop removed in spring and rye retained), and two corn residue removal treatments (removed or retained) as the sub-plot. This experiment also included corn N fertilizer rates as a sub-sub plot factor, but for simplicity the data presented is for the recommended N rate in Alabama according to the Alabama Cooperative Extension Service (ACES) of 168 kg $ha^{-1}$for corn planted into small grain stubble (ACES, 1994). Plots consisted of four rows (91-cm row spacing) and were 6.1m long by 2.7m wide. Urea ammonium nitrate (UAN 28-0-0) was used as the N fertilizer source with the total amount applied split in two equal portions early in the growing season, approximately two and five weeks after planting. Both locations were non-irrigated, continuous, no-tillage corn production. Corn was planted in late-March to early-April, and cultural practices were performed according to ACES recommendations to maximize corn yield.

Data collection

Square steel frames measuring 0.25 m^2 were used to sample the rye. Eight frames/samples from each main plot were taken every spring for rye biomass determination. Plots where there was no rye (no rye treatment) were sampled to determine the biomass of winter weeds.

Whole corn plant samples were taken at harvest for biomass determination. Corn grain was partitioned from the cobs and the rest of the stalk. Plants were further separated into four fractions: below the ear (bottom); above the ear excluding cobs (top); and the cobs alone. An additional plant portion (above-ear) was calculated by summing the top and cob dry yields to determine the dry biomass of this crop portion. Samples were oven dried for seven days at 55^{o}C and weighed to determine dry weight. Grain yield was adjusted to 15.5% moisture content, while biomass is reported on a dry matter basis.

Weather data were obtained from the Alabama Mesonet Weather network. Cumulative seasonal and monthly precipitation (mm) and average seasonal and monthly temperature (^{o}C) from May-August were calculated and used as independent variables for statistical analysis.

Statistical analysis

The CORR procedure in SAS 9.3 (SAS for Windows v. 9.3, SAS Institute Inc., Cary, NC) was used to detect any correlations of weather conditions during the growing season with corn grain and biomass yields. Analysis of variance was performed using the MIXED procedure in SAS 9.3 in order to detect differences in grain and biomass yields between locations as affected by rye cover crop and residue removal treatments. The six variables of interest included: grain yield (grain), total biomass (stover), bottom portion of the plant biomass (bottom), top portion of the plant biomass excluding cobs (top), cobs alone (cob), and the above the first ear portion of the plant biomass including cobs (above-ear). A factor was considered significant at level lower than 0.10 (alpha < 0.10).

Results and Discussion

Rye biomass yield

Rye yields varied between both two locations every year. Yields were consistently greater at EVS compared to TVS every year the experiment was conducted. Overall, rye yield at EVS ranged between 1503-6275 kg ha^{-1} with an average of 3343 kg ha^{-1}. At TVS, the corresponding yield range was 1937-3281 kg ha^{-1} with an average of 2475 kg ha^{-1}. These yields were similar to those which have been reported by Duiker and Curran (2005) in a silt loam.

Winter weeds grew in plots assigned to no rye treatment. This weed biomass was measured at the same time rye biomass was determined since it can have an effect on corn productivity. At EVS, weed biomass ranged between 900-1200 kg ha^{-1} with an average yield of 1030 kg ha^{-1}, while at TVS yields ranged between 370-880 kg ha^{-1} with an average yield of 625 kg ha^{-1}.

Effect of in-season weather

In Alabama, one of the most limiting factors in corn production is the lack of adequate water (ACES, 1994). However, crop residues that cover 31% or more of the soil surface can reduce water losses due to evaporation. Therefore, correlation of corn yields with in-season weather conditions can vary in plots with a cover crop compared to plots without a cover crop.

Cumulative precipitation levels and average temperatures varied over the six location-years (Table 13). At EVS, total precipitation and average temperatures were consistently higher than those in TVS during all three growing seasons. At EVS, the effect of weather conditions on corn yields varied among rye treatments (Table 14). Correlations between precipitation and temperature with grain and stover yields were significant and stronger in no rye and rye removed plots than plots where rye was retained. This highlights that using a cover crop might reduce the impact of weather on corn productivity. Nevertheless, the direction of the correlations was consistent between rye treatments, regardless of whether rye was retained, removed or not used at all. The strongest negative correlations between corn yields and air temperatures were detected in plots where rye was removed in spring and in plots without a cover crop. This negative correlation between air temperature and yield underscores the impact of heat stress on corn productivity in the Southeast. Furthermore, this is an indication that when rye is retained in the field it has the potential to lower the daily maximum soil temperature, especially in June and July that corresponds to critical period of development of corn. Similar results have also been reported by Teasdale and Mohler, (1993).

Different strength of correlations among rye treatment levels was also observed in TVS (Table 15). Grain yields exhibited similar correlations with weather conditions to EVS. However, stronger correlations between grain yields and weather conditions were detected where rye was retained in the field. Stover yields were positively correlated with cumulative precipitation in June and negatively correlated with cumulative seasonal precipitation and precipitation in July and August. This response varied from what was observed at EVS. Differences in soil types between the two locations could explain these variations. The soil at EVS was a loamy sand and has lower water retention capacity than the silt loam at the TVS location. Given the texture of the soil at EVS, the frequency of rainfall events needs to be more timely during the growing season in order for corn plants to have an adequate water supply. This could partially explain the positive correlation between yields and precipitation. However, the silt loam soil at TVS has a greater ability to retain water than the loamy sand. Large precipitation events during the growing season could result in flooding conditions and negatively impact corn production. Flooding conditions were observed at the tasseling growth stage (VT) in 2009 and 2011, and could explain the negative correlations.

Variations in plant population could also explain these correlation differences between the two locations. Plant stand varied among locations and years for different rye treatments (Figure C1). At EVS, the plant population was greater than in TVS which could be explained by the differences in seasonal precipitation between the two locations. At TVS, the plant population varied among years and rye treatments. There was no consistent trend in plant population among rye treatments for the three years of the experiment which could be the reason for the opposite correlations from EVS.

A rye cover crop retained in the field could reduce moisture losses due to less evaporation and cooler soil temperatures (Teasdale and Mohler, 1993). Stronger correlations between corn yields and weather conditions without a rye cover crop could indicate a higher vulnerability of yields to in-season climate. For example, corn yields increased with increasing precipitation and decreased with increasing temperatures in the no cover treatment at EVS. Similar strong correlations were observed where rye was removed in spring. However, there was no significant correlation where rye was present. In such scenarios, rye could reduce heat stress and plant water stress due to decreased evaporation, both of which could impact corn yields. Thus, it seems that rye could be used as a management practice to reduce the impact of year-to-year weather variability on corn productivity in Coastal Plain soils.

Corn grain yield

Grain yields varied significantly between locations and years (Table 16). At EVS, there were no effects on grain yields due to the use of rye and stover management in 2009 (Table 17). The second year of the experiment, grain yield where rye was retained was the greatest (p = 0.0613). The same response was observed where stover was retained in the field (p = 0.0014). The same trend was also observed in 2011; however, the use of a rye cover crop was the only significant factor (p = 0.0228). At TVS, the only significant effect on grain yield was observed during the third year of the experiment due to the use of a rye cover crop (Table 18). This effect at both locations might be due to a cumulative effect of cover crop use on soil properties. Similarly, Duiker and Curran (2005) reported that the benefits of a cover crop use can take several years to be observed. At EVS, the three-year average effect of rye retention in the field resulted in the highest corn grain yield (Table 19, Figure 7). However, 100% corn residue removal did not have an impact on the amount of grain produced (p = 0.6506). This result is in agreement with a 12-year study conducted in a silt loam in Indiana (Barber, 1979). When examining the three-year average effect of the independent variables on grain yield at TVS, neither the use of a rye cover crop nor stover management had a significant effect on grain. (Table 19, Figure 7). Despite the lack of statistical significance, numerically the grain yield was greater when rye was retained and where no stover removal was performed. These results are in agreement with a previous study which was conducted in a silt loam that involved no-till and rye management (Duiker and Curran, 2005). In their study, the use of a cover crop either increased or had no effect on corn yield.

Corn grain is the most valuable part of the plant. Calculation of the harvest index can be used by farmers and biofuel industry as a simple way to estimate the amount of stover left in the field after grain harvest. At EVS the harvest index was 0.60-0.61 for all rye treatments while at TVS it was 0.56-0.58 (Table C1). The practical differences of these indexes between locations are small and therefore, direct comparison of corn yields would be more informative.

Corn biomass yields

All corn biomass yields, varied significantly among the six location-years of this experiment (Table 16). This was not surprising due to the variations in climates between the central and the northern part of the state. Despite climate variations, there was no distinct pattern in total and partial stover yields across years (Figure 8). For the first year at EVS and the first two years of the experiment at TVS, there were no indications that use of a rye cover crop and that of stover residue removal had a significant effect on corn biomass yields (Tables 17, 18). However, in 2011, which was the third year of the study, the cover crop effect was significant on all stover yields at EVS and on the total stover and the above-ear portion at TVS.

The three-year average total biomass yield at EVS was maximized where the cover crop was retained (5083 kg ha^{-1}). That yield was significantly greater from where rye was removed by 20% and no rye plots by 11% (Table 19). At TVS, where rye was retained also produced the largest amount of total biomass; however, differences from the other rye treatments were small. Retention of rye in the field increased total corn stover yields at both locations. For all three years of this study, total stover biomass in the loamy sand was 8% higher than the silt loam where rye was retained (Table 19). This contradicts results reported by Raimbault et al. (1990) that rye in combination with no-till in a loam soil decreased corn biomass yield and retarded crop growth. However, their study was conducted in Ontario, Canada, and the colder climate in that region could cause a delay in spring soil warming, which might explain the adverse response they reported.

The bottom and top fractions of the plant, exhibited a similar yield response to the total stover yield. At both locations, mean maximum yields were observed where rye was retained in the field (Table 19). Corn grown at EVS produced a higher bottom yield compared to TVS by 5% and almost identical top portion yields. Despite numerical differences, the three-year average effects of rye and stover management were not significant at both locations.

Cobs alone have been recognized as an attractive bioenergy feedstock. They contain approximately 19.18 MJ kg^{-1}, when a kilogram of total stover biomass contains up to 17 MJ (Zych, 2008). Cobs can be harvested by existing equipment and they are sufficiently dense that they do not require densification (Zych, 2008). Intuitively, cobs are a highly desirable portion of corn residue as a feedstock for bioenergy production. At EVS, retention of rye in the field resulted in 14% higher cob yield than plots without the use of a cover crop and plots where rye was removed in spring. A similar trend was also observed at TVS, however the differences were smaller. The three-year average maximum yield of cobs at EVS was 1144 kg ha^{-1} and at TVS was 957 kg ha^{-1} when rye was retained in the field, which represents a difference of 16.5% between the two locations.

Despite the attractive characteristics of cobs as a bioenergy feedstock, the relative low yields per unit area can be a disadvantage for biofuel production. However, when the top fraction was combined with the cobs, the result was greater yield per unit area, while including the desirable compositional characteristics of cobs. During the three years of this experiment, the above-ear biomass at EVS was higher in plots where rye was retained (Table 17). A similar trend was also observed at TVS (Table 18). At both locations, the three-year average maximum above-ear yields where observed in the rye retained treatment, with corn grown at EVS exhibiting slightly higher yields than TVS (Table 19, Figure 10). However, the three-year average effect of rye was significant only at EVS ($p = 0.0754$), while the effect of stover management was significant only at TVS, with yields lower where 100% removal was performed ($p = 0.0882$).

The average yield of cobs during all three years of this study accounted for about 20-22% of total biomass, while the above-ear fraction was the highest portion of the stover at both locations. The above-ear biomass accounted for 66% and 69% of the total stover, with the remaining 34% and 31% consisting of the bottom portion at EVS and TVS, respectively. Averaged over all three years of the experiment, the stover-to-grain ratios were 0.69 and 0.83 at EVS and TVS, respectively. These results vary from those which have been reported by Wilhelm et al. (2010) in a multi-location study where the above-ear biomass and cobs alone accounted for 50% and 18% of total stover, respectively. However, the stover-to-grain ratios at grain maturity were similar to the range reported among the locations of their study.

Conclusions

Weather conditions during the six location-years of the experiment varied significantly. However, significant correlations were detected between precipitation and temperatures for grain and biomass yields. Differences in soil type probably also contributed to the variability in corn yields due to rainfall and air temperature differences between locations. It appears a rye cover crop could be used as a management practice to reduce the impact of year-to-year weather variability on corn productivity across Coastal Plain soils.

Three-year average grain yields were greater at EVS than TVS by 19%. Total and partial stover yield differences between both locations varied slightly and, in general, the greatest were observed in the loamy sand. It is expected that in non-irrigated cropping systems, the greatest yield would be produced in finer textured soils. In this study, the opposite was observed, which can be explained by the differences in seasonal and monthly amounts of precipitation between the two locations. At EVS, the seasonal and monthly cumulative precipitation was consistently greater than TVS by 18-34%. Therefore, despite the coarser soil texture of the loamy sand, it seems that moisture was not a factor that could significantly reduce corn yields at EVS when compared to TVS.

Three years of corn residue removal did not affect almost any of the plant yield parameters studied at either location. Additionally, the use of rye as a winter cover crop, when retained in the field, was an effective way to increase corn biomass yields in both loamy sand and silt loam soils in Alabama. It is interesting though, that corn grown where rye was removed in spring resulted in the lowest yields at both locations. As reported in previous studies, high rye yield can deplete the moisture in the soil surface (Ebelhar et al., 1984; Raimbault et al., 1991). Then, removing the rye could result in unfavorable initial conditions for corn production, such as unprotected soil surface and reduced soil moisture content. Nevertheless, the contribution of rye on soil organic matter, even when harvested in spring, should be considered. According to Barber et al., (1979), the roots of crops that have been harvested can assist to maintain SOC levels.

Results from this three year study suggest that the use of a winter rye as a cover crop can increase corn yields in two major soil types in Alabama. Vertical fractionation of biomass could result in significant amounts of biomass that could be harvested as biofuel feedstock while leaving a portion of the plant residue in the field for erosion control and SOM maintenance. According to this experiment, harvest of the above-ear corn plant fraction could be an attractive option for partial biomass harvesting. Nevertheless, it is important that other impacts, not discussed in this paper, should be evaluated. The effect of partial biomass harvesting on corn yields and soil properties should be monitored and assessed in long-term studies.

Table 13. Seasonal and monthly cumulative precipitation, and seasonal and monthly average temperature during the three growing seasons (May-August) at EVS - E.V. Smith Research Center near Shorter in Central Alabama, and at TVS - Tennessee Valley Research and Extension Center near Belle Mina in Northern Alabama.

location	year	Precipitation					Temperature				
		Season	May	June	July	August	Season	May	June	July	August
		-------------------- mm --------------------					------------------ ºC ------------------				
EVS	2009	629	262	100	75	192	24.5	23.0	21.7	26.6	26.7
	2010	483	176	56	128	122	27.6	24.1	28.0	29.1	29.3
	2011	333	56	57	204	16	26.7	21.9	28.3	28.3	28.4
TVS	2009	516	242	28	140	106	24.2	20.7	25.9	24.9	25.1
	2010	320	138	57	94	31	26.3	21.9	26.7	27.9	28.6
	2011	259	42	79	109	29	25.7	21.7	26.8	27.6	26.6

Table 14. Three-year average Pearson correlations (r-values) of corn grain and stover yields with seasonal cumulative precipitation, seasonal average air temperature, monthly cumulative precipitation, and monthly average air temperature during the growing season (May-August) at E.V. Smith Research Center near Shorter in Central Alabama (EVS) when rye was retained as a cover crop and plots without rye.

Cover crop	Rye removed					
Precipitation	Grain	Stover	Bottom	Top	Cob	Above-ear
Season	0.730***	0.610***	-0.045	0.755***	0.628***	0.773***
May	0.700***	0.561**	-0.104	0.710***	0.636***	0.739***
June	0.792***	0.806***	0.317	0.900***	0.478**	0.850***
July	-0.696***	-0.554**	0.111	-0.704***	-0.637***	-0.735***
August	0.689***	0.543**	-0.124	0.693***	0.638***	0.727***
Temperature						
Season	-0.736***	-0.819***	-0.473**	-0.875***	-0.344	-0.795***
May	0.097	-0.160	-0.617***	-0.036	0.424*	0.083
June	-0.796***	-0.798***	-0.290	-0.897***	-0.496**	-0.853***
July	-0.720***	-0.814***	-0.497**	-0.864***	-0.318	-0.779***
August	-0.719***	-0.814***	-0.499**	-0.863***	-0.316	-0.778***
Cover crop	Rye retained					
Precipitation	Grain	Stover	Bottom	Top	Cob	Above-ear
Season	0.311	-0.065	-0.473**	0.265	0.247	0.272
May	0.256	-0.126	-0.532**	0.206	0.227	0.222
June	0.594***	0.313	-0.039	0.571**	0.328	0.526**
July	-0.249	0.134	0.539**	-0.199	-0.224	-0.216
August	0.236	-0.147	-0.551**	0.186	0.220	0.205
Temperature						
Season	-0.676***	-0.478**	-0.195	-0.669***	-0.334	-0.601***
May	-0.386	-0.650***	-0.855***	-0.440*	-0.067	-0.350
June	-0.576**	-0.284	0.076	-0.551**	-0.325	-0.510**
July	-0.685***	-0.503**	-0.235	-0.681***	-0.332	-0.610***
August	-0.686***	-0.505**	-0.238	-0.682***	-0.332	-0.611***
Cover crop	No rye					
Precipitation	Grain	Stover	Bottom	Top	Cob	Above-ear
Season	0.426*	0.364	-0.041	0.575**	0.694***	0.640***
May	0.369	0.297	-0.116	0.524**	0.679***	0.596***
June	0.699***	0.705***	0.417*	0.792***	0.675***	0.797***
July	-0.362	-0.289	0.126	-0.517**	-0.676***	-0.590***
August	0.349	0.274	-0.141	0.505**	0.672***	0.580**
Temperature						
Season	-0.764***	-0.805***	-0.612***	-0.817***	-0.588**	-0.790***
May	-0.339	-0.469**	-0.776***	-0.204	0.223	-0.087
June	-0.683***	-0.683***	-0.382	-0.782***	-0.684***	-0.792***
July	-0.770***	-0.817***	-0.642***	-0.815***	-0.568**	-0.782***
August	-0.770***	-0.818***	-0.644***	-0.814***	-0.567**	-0.782***

*, **, *** denote significance at 0.10, 0.05, and 0.01 probability levels respectively

Table 15. Three-year average Pearson correlations (r-values) of corn grain and stover yields with seasonal cumulative precipitation, seasonal average air temperature, monthly cumulative precipitation, and monthly average air temperature during the growing season (May-August) at Tennessee Valley Research and Extension Center near Belle Mina in Northern Alabama (TVS) when rye was retained as a cover crop and plots without rye.

Cover crop	Rye removed					
Precipitation	Grain	Stover	Bottom	Top	Cob	Above-ear
Season	0.166	0.573***	-0.626***	-0.485**	-0.262	-0.413*
May	0.018	-0.360	-0.414*	-0.354	-0.179	-0.252
June	0.351	0.404*	0.457**	0.382*	0.197	0.286
July	-0.317	-0.832***	-0.884***	-0.618***	-0.355	-0.614***
August	-0.214	-0.701***	-0.757***	-0.560***	-0.310	-0.511**
Temperature						
Season	0.194	0.825***	0.878***	0.617***	0.353	0.608***
May	0.126	0.793***	0.848***	0.606***	0.343	0.583***
June	-0.026	0.695***	0.751***	0.557***	0.308	0.507**
July	0.058	0.754***	0.810***	0.588***	0.329	0.552**
August	0.384*	0.866***	0.897***	0.602***	0.360	0.644***
Cover crop	Rye retained					
Precipitation	Grain	Stover	Bottom	Top	Cob	Above-ear
Season	0.640***	-0.691***	-0.744***	-0.478*	-0.347	-0.573**
May	0.674***	-0.566**	-0.578**	-0.370	-0.407	-0.525**
June	-0.670***	0.593**	0.614**	0.394	0.397	0.537**
July	0.449*	-0.823***	-0.935***	-0.603**	-0.155	-0.565**
August	0.588**	-0.762***	-0.840***	-0.541**	-0.285	-0.588**
Temperature						
Season	-0.465*	0.821***	0.930***	0.599**	0.169	0.570**
May	-0.512**	0.809***	0.907***	0.584**	0.211	0.582**
June	-0.592**	0.759***	0.836***	0.538**	0.289	0.588**
July	-0.552**	0.790***	0.879***	0.566**	0.248	0.588**
August	-0.276	0.800***	0.948***	0.612**	0.019	0.489*
Cover crop	No rye					
Precipitation	Grain	Stover	Bottom	Top	Cob	Above-ear
Season	0.456**	-0.821***	-0.766***	-0.701***	-0.362	-0.668***
May	0.482*	-0.743***	-0.612***	-0.645***	-0.446*	-0.681***
June	-0.480**	0.762***	0.646***	0.660***	0.432*	0.682***
July	0.307	-0.795***	-0.898***	-0.656***	-0.120	-0.497**
August	0.414*	-0.841***	-0.846***	-0.709***	-0.280	-0.625***
Temperature						
Season	-0.319	0.803***	0.898***	0.664***	0.135	0.511**
May	-0.355	0.824***	0.890***	0.686***	0.185	0.555**
June	-0.417*	0.841***	0.843***	0.709***	0.284	0.628***
July	-0.385	0.837***	0.874***	0.701***	0.232	0.592***
August	-0.188	0.682***	0.861***	0.550**	-0.028	0.342

*, **, *** denote significance at 0.10, 0.05, and 0.01 probability levels respectively

Table 16. Corn grain and biomass yields across years (Y) and locations (L). EVS - E.V. Smith Research Center near Shorter in Central Alabama; TVS - Tennessee Valley Research and Extension Center near Belle Mina in Northern Alabama.

Year	2009		2010		2011		Y x L
Location	EVS	TVS	EVS	TVS	EVS	TVS	†Pr > F
Grain	9251	6423	5328	6279	6545	4488	≤0.0001
Stover	5482	3100	3486	5528	4713	4740	≤0.0001
Bottom	1696	593	954	2270	1955	1517	≤0.0001
Top	2595	1714	1522	2405	1842	2213	≤0.0001
Cob	1186	793	1010	853	916	1048	≤0.0001
Above-ear	3786	2507	2532	3258	2758	3261	≤0.0001

†Probability of a larger F by chance among years and locations.

Table 17. Mean grain and biomass yields at E.V. Smith Research Center near Shorter in Central Alabama (EVS) from 2009-2011 for all levels of cover crop and stover management.

Factor	Rye removed	Rye retained	No rye	Stover removed	Stover retained	Cover crop	Stover management
Year			2009			†Pr>F	‡Pr>F
			kg ha^{-1}				
Grain	8732 a	9602 a	9420 a	9560	8942	0.3545	0.6689
Stover	5133 a	5600 a	5714 a	5524	5440	0.6113	0.869
Bottom	1460 a	1684 a	1944 a	1653	1740	0.145	0.6368
Top	2517 a	2668 a	2582 a	2644	2534	0.879	0.6567
Cob	1122 a	1248 a	1188 a	1206	1166	0.7959	0.6844
Above-ear	3672 a	3916 a	3770 a	3871	3701	0.8473	0.6351
			2010				
Grain	5289 a b	5675 a	5021 b	4894	5762	0.0613	0.0014
Stover	3343 a b	3870 a	3245 b	3198	3775	0.0574	0.0021
Bottom	971 a	1117 a	775 b	917	991	0.0068	0.302
Top	1352 a	1680 a	1531 a	1346	1698	0.0015	0.8578
Cob	1020 a	1073 a	938 a	935	1086	0.3633	0.0041
Above-ear	2371 a	2752 a	2470 a	2280	2783	0.2534	0.0039
			2011				
Grain	4805 a	7850 b	6979 a b	6232	6858	0.0228	0.2198
Stover	3725 a	5778 b	4636 a b	4555	4871	0.0163	0.3255
Bottom	1495 a	2371 b	2001 b	1849	2062	0.0051	0.2465
Top	1417 a	2296 b	1812 c	1794	1889	0.0007	≤0.0001
Cob	814 a	1111 b	823 a	912	920	0.0167	0.9206
Above-ear	2230 a	3407 b	2635 a	2706	2809	0.0042	0.6658

†Probability of a larger F by chance among levels of rye cover crop within individual years.

‡ Probability of a larger F by chance between levels of corn residue removal within individual years.

Note. Means within row followed by the same letter are not significantly different at the 0.10 level among the three levels of rye cover crop. Separation of means was achieved using the Tukey adjustment for multiple comparisons.

Table 18. Mean grain and biomass yields at Tennessee Valley Research and Extension Center near Belle Mina in Northern Alabama (TVS) from 2009-2011 for all levels of cover crop and stover management.

Factor	Rye removed	Rye retained	No rye	Stover removed	Stover retained	Cover crop	Stover management
Year			2009			†Pr>F	‡Pr>F
			kg ha^{-1}				
Grain	5462 a	7112 a	6695 a	6058	6757	0.3644	0.7745
Stover	2852 a	3454 a	2995 a	2701	3495	0.5896	0.0077
Bottom	513 a	686 a	581 a	527	659	0.5944	0.3003
Top	1607 a	1899 a	1636 a	1466	1963	0.6753	0.0012
Cob	731 a	869 a	778 a	712	874	0.4297	0.0514
Above-ear	2338 a	2768 a	2415 a	2178	2836	0.6061	0.002
			2010				
Grain	6597 a	6051 a	5906 a	5932	6437	0.2738	0.6564
Stover	5556 a	6045 a	5120 a	5368	5780	0.2048	0.3003
Bottom	2316 a	2530 a	2026 a	2188	2393	0.1742	0.3261
Top	2365 a	2645 a	2298 a	2367	2504	0.4576	0.4832
Cob	881 a	873 a	796 a	811	889	0.5354	0.2883
Above-ear	3247 a	3514 a	3094 a	3178	3392	0.4786	0.4079
			2011				
Grain	3816 a	4634 b	5015 b	4377	4600	0.0924	0.6724
Stover	4004 a	5068 b	5147 b	4701	4778	0.0102	0.7907
Bottom	1442 a	1608 a	1558 a	1533	1539	0.7341	0.9772
Top	2041 a	2268 a	2354 a	2201	2241	0.406	0.7927
Cob	809 a	1100 a	1235 a	1008	1088	0.1441	0.6366
Above-ear	2702 a	3491 b	3589 b	3224	3297	0.0467	0.6461

†Probability of a larger F by chance among levels of rye cover crop within individual years.

‡ Probability of a larger F by chance between levels of corn residue removal within individual years.

Note. Means within row followed by the same letter are not significantly different at the 0.10 level among the three levels of rye cover crop. Separation of means was achieved using the Tukey adjustment for multiple comparisons.

Table 19. Three year average corn yields affected by the use of rye as a winter cover crop from 2009 until 2011 at E.V. Smith Research and Extension Center (EVS) in central Alabama and the Tennessee Valley Research and Extension Center (TVS) in northern Alabama.

Factor	Rye removed	Rye retained	No rye	Stover removed	Stover retained	Cover crop	Stover management
Location			EVS			†Pr>F	‡Pr>F
			kg ha^{-1}				
Grain	6275 a	7709 b	7140 a b	6895	7188	0.0946	0.6506
Stover	4067 a	5083 b	4532 a b	4426	4695	0.0415	0.4013
Bottom	1309 a	1724 b	1573 a b	1473	1598	0.0978	0.4277
Top	1769 a	2215 a	1975 a	1933	2040	0.1927	0.5168
Cob	985 a	1144 a	983 a	1017	1057	0.2475	0.4904
Above-ear	2758 a	3359 b	2958 a b	2953	3098	0.0754	0.5008
Location			TVS				
Grain	5452 a	5917 a	5872 a	5515	5979	0.6361	0.3069
Stover	4287 a	4707 a	4421 a	4265	4684	0.6340	0.2573
Bottom	1509 a	1476 a	1388 a	1395	1521	0.8900	0.5715
Top	2055 a	2217 a	2096 a	2012	2234	0.6550	0.1087
Cob	816 a	957 a	936 a	851	955	0.2982	0.2037
Above-ear	2813 a	3226 a	3033 a	2869	3179	0.1745	0.0882

†Probability of a larger F by chance among levels of rye cover crop within individual years.

‡ Probability of a larger F by chance between levels of corn residue removal within individual years.

Note. Means within row followed by the same letter are not significantly different at the 0.10 level among the three levels of rye cover crop. Separation of means was achieved using the Tukey adjustment for multiple comparisons.

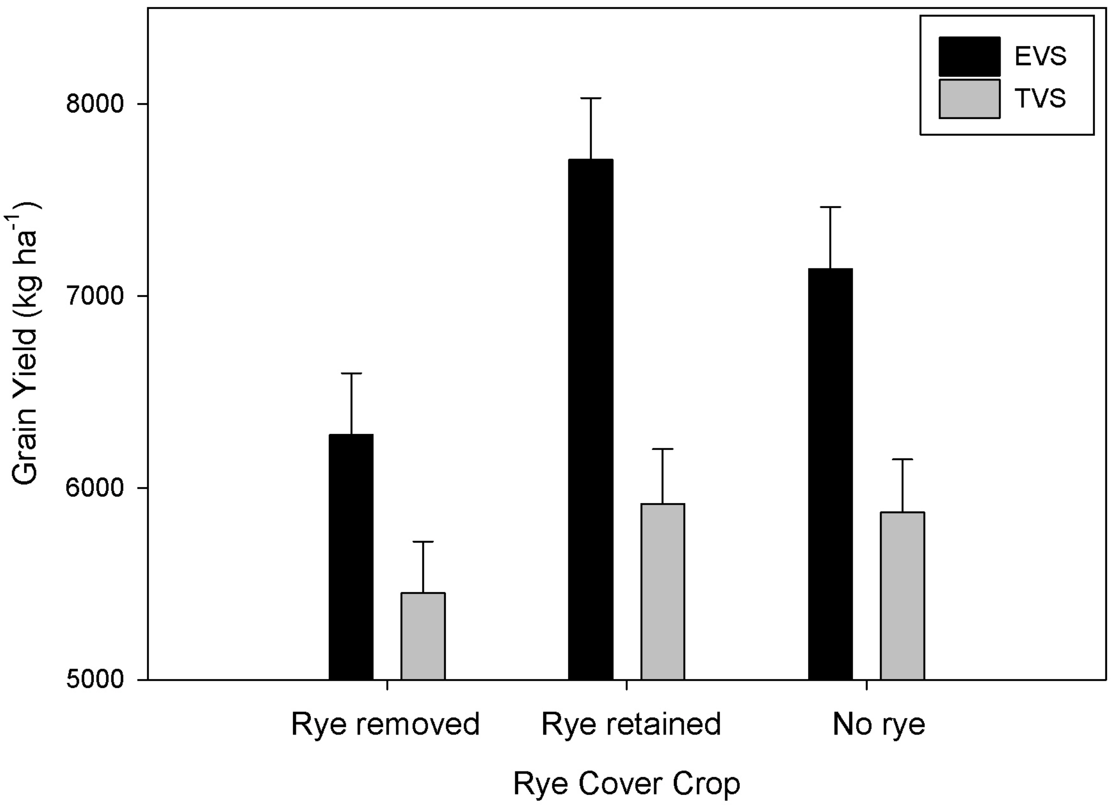

Figure 7. Three-year average effect of rye management on grain yield (kg ha^{-1}). EVS - E.V. Smith Research Center near Shorter in Central Alabama; TVS - Tennessee Valley Research and Extension Center near Belle Mina in Northern Alabama. The error bars represent the standard error of the mean.

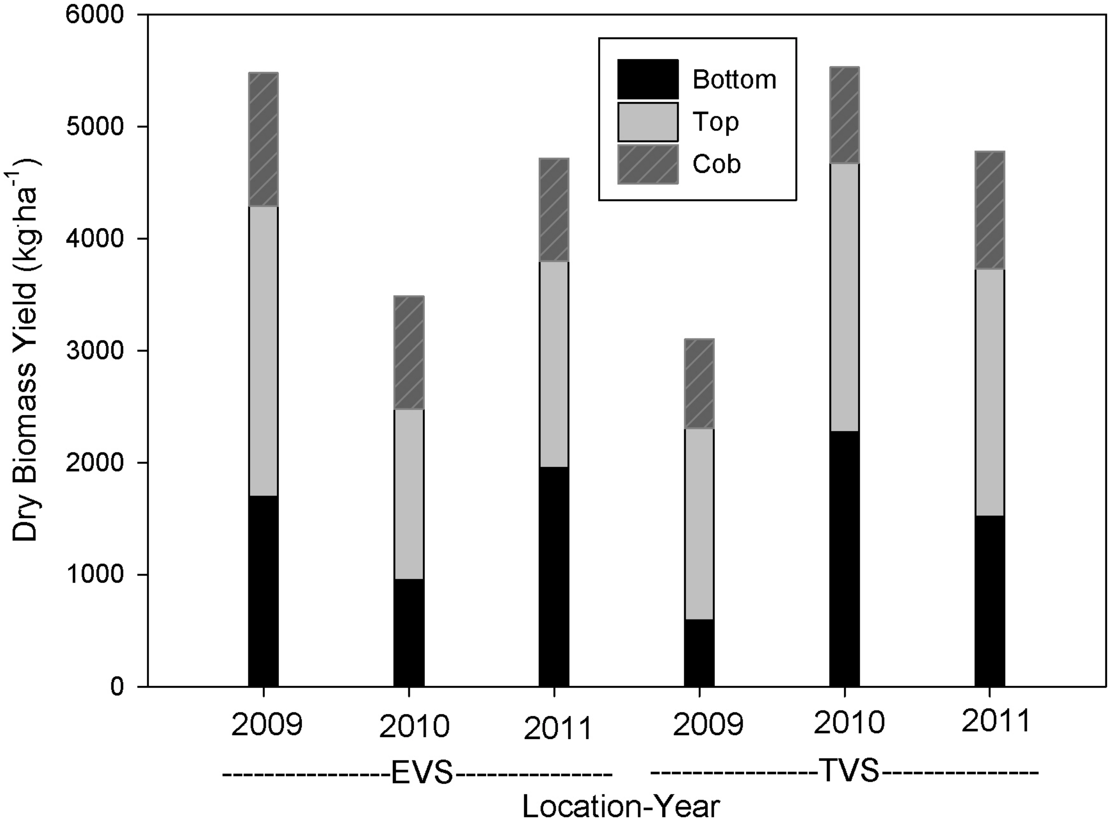

Figure 8. Three year average incremental biomass yields (kg ha^{-1}). EVS - E.V. Smith Research Center near Shorter in Central Alabama; TVS - Tennessee Valley Research and Extension Center near Belle Mina in Northern Alabama.

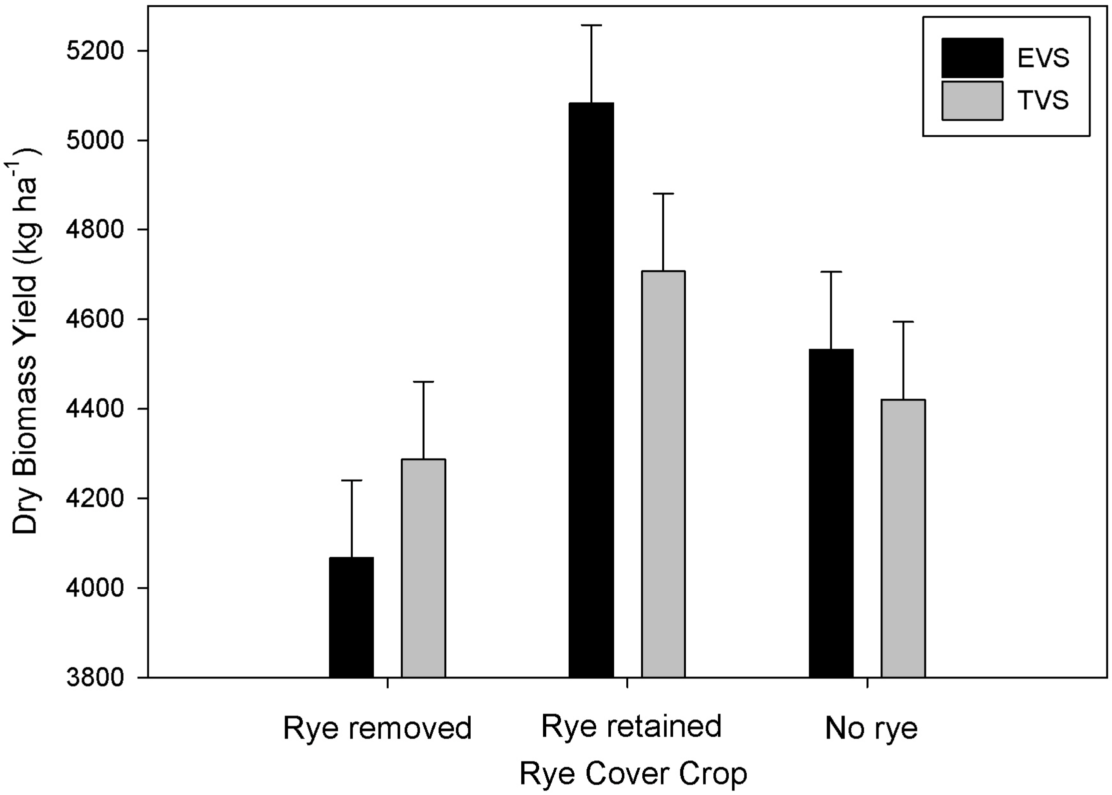

Figure 9. Three-year average effect of rye management on total stover yield (kg ha^{-1}). The error bars represent the standard error of the mean. EVS - E.V. Smith Research Center near Shorter in Central Alabama; TVS - Tennessee Valley Research and Extension Center near Belle Mina in Northern Alabama.

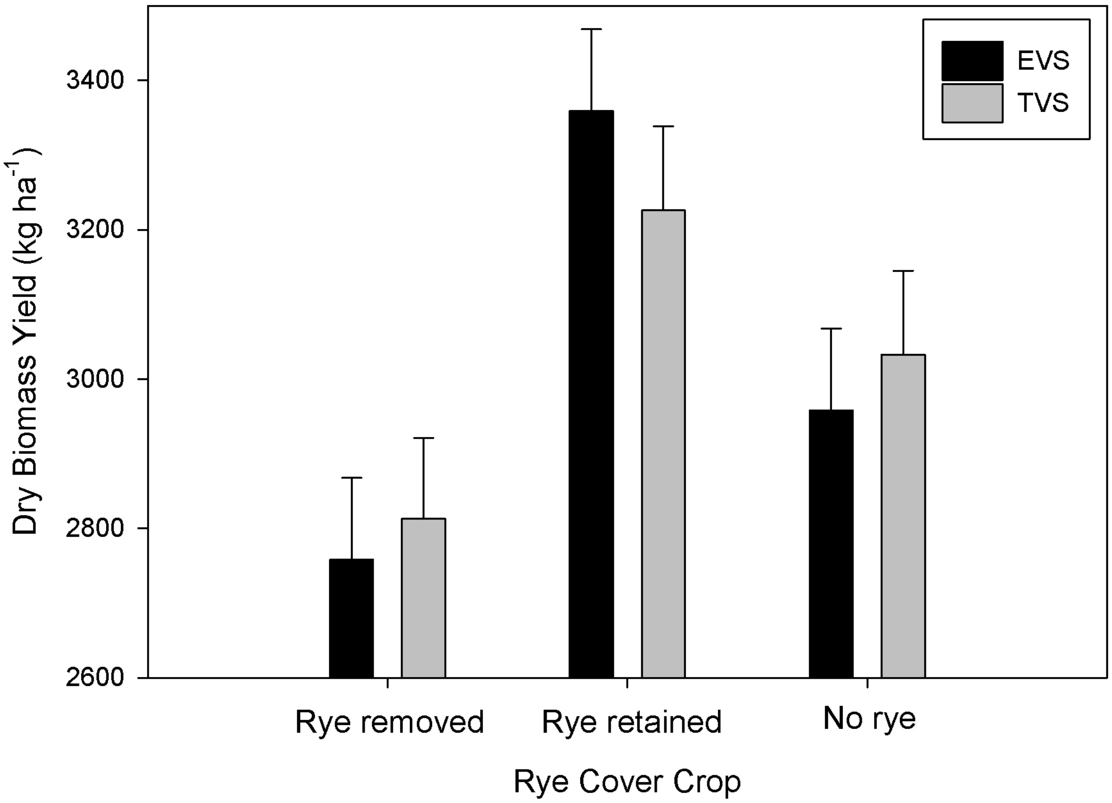

Figure 10. Three-year average effect of rye management on above-ear biomass yield (kg ha^{-1}). The error bars represent the standard error of the mean. EVS - E.V. Smith Research Center near Shorter in Central Alabama; TVS - Tennessee Valley Research and Extension Center near Belle Mina in Northern Alabama.

V. Distribution of Structural Carbohydrates in Corn Plants Across the Southeastern US

Abstract

Continuous corn (*Zea mays* L.) field studies incorporating stover removal management practices (0 and 100% removal) were established in both Alabama and South Carolina as part of the Sun Grant Regional Partnership corn stover project. In Alabama, the use of rye (*Secale cereale*) as a winter cover crop was included as an additional factor. Plots in both states were representative of major soil types in their respective region: Alabama plots were Compass and Decatur soils; South Carolina plots were Coxville/Rains-Goldsboro-Lynchburg association. Both sites were investigated for variations in the distribution of lignin and structural carbohydrates among five plant fractions: whole plant; above the first ear excluding cobs (top); below the first ear (bottom); cob; and above the first ear including cobs (above-ear). Using a combination of wet chemistry methods and near infrared spectroscopy (NIRS), the distribution of lignin, ash, and structural carbohydrates varied between sites. The use of rye and stover management did not affect carbohydrate concentrations across plant fractions or soil types. Total precipitation and average air temperature during the growing season were strongly correlated with total and partial stover carbohydrate and ash composition. When compared to the above-ear fractions, bottom plant partitions contained greater lignin and cellulose concentrations. However, holocellulose concentration was consistently greater in cobs, tops and above-ear fractions at every location. Data from this study suggest that the cob, top and above-ear plant portions have the most desirable characteristics for bioethanol production at every location.

Introduction

Several crops have been proposed as potential biomass feedstocks for biofuel production. Corn has high biomass yield potential and abundance so it is considered to be a desirable bioenergy feedstock. Furthermore, cultivation of corn for biomass production would not compete with the use of land for food production since grain can be produced simultaneously.

Corn is an important crop that is used as animal feed as well as by the biofuel industry (Kadam and McMillan, 2003). The benefits to both industries can greatly increase by improving biomass yield and the conversion efficiency process (Lorenz et al., 2009). The biofuel yield of a conversion process can be significantly affected by the biomass composition (Philip Ye et al., 2008). The main components of the plant tissue are cellulose, hemicellulose, lignin, and ash (Cone et al., 1996). Cellulose and hemicellulose are structural polysaccharides of plant cell walls (Hatfield, 1989). Cellulose, a polymer of glucose and a major plant component, is the most abundant carbohydrate on earth (Chandrakant and Bisaria, 1998). For bioethanol production via fermentation, cellulose is the most desirable plant component. The least desirable component is lignin, which is known to inhibit biomass hydrolysis (Chang and Holtzapple, 2000; Kim and Holtzapple, 2006).

Literature related to the composition of the total stover and of the various parts of the plant dates back to the late 1920's. According to a study by Waksman and Tenney (1928), the composition of corn stalks (% of dry weight) is 28.7% cellulose, 21.9% hemicellulose, 9.5% lignin, and 7.5% ash materials. In a more recent study, Kim and Dale (2004) measured lignin in corn stover and found it to be 18.7%. In the same study, carbohydrates were 58.3% of total biomass. Several studies have indicated the heterogeneity of corn biomass composition and the impact on cellulose enzymatic hydrolysis (Akin et al., 2006; Bootsma and Shanks, 2005; Duguid et al., 2009). Genetic differences (Thomas et al., 2001) and yearly environmental variations (Templeton et al., 2009) have been identified as factors that can affect corn stover composition.

A major concern when harvesting crop residues is the impact on chemical, physical and microbial properties of soil. Crop residue is an important energy source for soil microbial processes (Franzluebbers, 2002). The below-ground biomass alone is not enough to maintain soil organic matter content (Wilts et al., 2004). Soil erosion is another issue that is strongly associated with crop residue removal (Wilhelm et al., 2010). The amount of residue required to control soil erosion is not the same for every soil type. It depends upon soil erodibility, rainfall erosivity, land use, tillage methods and other management practices (Lindstrom, 1986; Johnson et al., 2010). Cover crops capable of high residue production can mitigate the stover removal impact. Cereal rye has been identified as a winter cover crop with large biomass production potential (Moschler et al., 1967). Crofcheck and Montross (2004) proposed another way to minimize the effect of residue removal on soil chemical properties by collecting only the fraction of corn stover with the greatest glucose content. The remaining stover would remain in the field for soil erosion control and to sustain soil organic matter contents.

Harvesting crop residues can change the chemistry of the soil. By altering the soil composition, there could be an effect on crop biomass compositional characteristics. There is information in the literature on how drought stress (Schittenhelm, 2010), planting densities (Hansey and De Leon, 2011), and crop development stage (Pordesimo et al., 2005) affect biomass composition. However, there is a lack of information on how cover crops and corn residue management affect corn stover composition. The goal of this study was to assess impacts of southeastern US corn management practices averaged over multiple (3) years on stover composition by accomplishing the following objectives: 1) evaluate the correlation between weather conditions during the growing season and biomass composition; 2) evaluate how the use of rye as a winter cover crop and corn residue management affect corn stover composition; and 3) determine the vertical distribution of lignin, ash, and structural carbohydrates in corn biomass grown at three locations across the southeastern US.

Materials and Methods

Site description

This study was conducted from 2009-2011 at two locations in Alabama and one location in South Carolina. The first location in Alabama (AL) was the E.V. Smith Research Center (EVS) in central Alabama (32.42884 N, -85.890235 W). The second location was at the Tennessee Valley Research and Extension Center in Belle Mina (34.687953 N, -86.886763 W) in the northern part of the state. The soil at EVS was a Compass loamy sand (Coarse-loamy, siliceous, subactive, thermic Plinthic Paleudults). At the Tennessee Valley Research and Extension Center (TVS), the soil was a Decatur silt loam (Fine, kaolinitic, thermic Rhodic Paleudults). The location in South Carolina (SC) was at the Clemson University Pee Dee Research and Education Center (PDREC) at Florence (34.283767 N, -79.7415 W). The soil type was a Coxville/Rains-Goldsboro-Lynchburg association.

In SC, corn was sampled from plots arranged in a randomized complete block design. Treatments included two levels of corn residue management (0 and 100% removal). At both sites in Alabama, plots were arranged in a split-plot design. Main plots consisted of rye as a winter cover with three levels (no cover, rye as a cover crop removed in spring and rye retained); and sub-plots were consisting of two corn residue removal levels (0 and 100% removal). In SC, DeKalb C69-71 corn hybrid was grown, while Pioneer 31G65R was grown at both locations in

Alabama. Urea ammonium nitrate (UAN 28-0-0) at a rate of 168 kg N ha^{-1} was used as the nitrogen source at all three sites. In SC, the plots were under continuous corn production with strip-till and sub-soiling of the E soil horizon performed annually to 30 to 40 cm deep. Alabama plots were non-irrigated, continuous, and no-tillage corn production.

Weather data and sample collection

Daily precipitation and air temperature data were collected from weather stations located at each experimental site. Cumulative precipitation (mm) and average air temperature (^{o}C) during the entire growing seasons were calculated and used as independent variables.

Before sample collection, a representative area consisting of a 1-m length row from both of the two middle rows of every plot was flagged. All corn plants from that area were taken at harvest at each site. All plants from the same plot were combined to create a plot sample. Corn grain and cobs were separated from the stalks. The grain was separated from the cobs using a shelling machine. Stalks were further separated into four increments: below the ear (bottom); above the ear excluding cobs (top); and cobs alone. Stover samples were oven dried for approximately seven days at 55^{o}C, and ground in a Willey mill to pass through a 2 mm sieve.

NIR preprocessing

Due to the large number of samples (~2500), near-infrared spectroscopy (NIRS) techniques were employed for data acquisition. All ground samples were scanned in a FOSS 5000 NIRS instrumentation (© FOSS Analytical AB 2004) with the ISIscan^{TM} and WinISI 4 software installed. After scanning all samples, the Standard Normal Variate (SNV) and Detrend (Detrend) scatter correction was used to reduce particle size effects and remove the linear and quadratic curvatures from the spectra. Then, the spectra were ranked according to the global

Mahalanobis distance (GH) and 300 representative samples were chosen for wet chemistry analysis.

Chemical analysis

Chemical procedures were developed by Van Soest and Wine (1967) and Van Soest (1963) to determine Neutral Detergent Fiber (NDF), Acid-Detergent Fiber (ADF), Acid-Detergent Lignin (ADL), and ash content in the selected 300 corn stover samples. Cellulose content was calculated by the difference of ADF-ADL. Hemicellulose content was calculated by the difference of NDF-ADF. Lignin content was calculated by the difference of ADL-ash. Holocellulose was defined as the summation of cellulose and hemicellulose.

NIR calibration

For the calibration of the NIRs, the most appropriate regression method was the modified partial least squared (modified PLS). The math treatment used for the calibration was the (1, 4, 4, 1). This math treatment involved the 1st derivative, a 4 nm gap with 4 initial smoothing points, and no further smoothing. The standard error of calibration (SEC) and the standard error of cross validation (SECV) were the lowest achieved concurrently with the highest possible R^2 values (Table 20). To further evaluate the accuracy of the developed models, an additional dataset (n=160) of known carbohydrate content values, was included and scanned in the NIR with the stover samples. The actual compositional values in these samples were compared to the NIR-derived values. There was no significant difference between the actual and NIR predicted values, which was an additional indication of the acceptable performance of the NIR models.

An additional stover portion was calculated (above-ear) by using the chemical analysis results of the cobs and top portions of the plant. Due to differences in dry biomass yields between the two partitions, a weighted average was calculated taking into account the dry biomass yield and the concentration of the component of interest in each stover portion.

Statistical Analysis

The CORR procedure in SAS 9.3 (SAS for Windows v. 9.3, SAS Institute Inc., Cary, NC) was used to detect correlations between weather conditions during the growing season and individual components across total and partitions of corn biomass pooling treatments. Repeated measures analysis of variance, utilizing the MIXED procedure, was used to detect differences in the partial biomass composition due to the three-year average effect of rye cultivation, and of corn residue management at every location. Five plant portions were of interest: the total biomass (Stover); bottom portion (Bottom); top portion excluding cobs (Top); cobs alone (Cob); and the plant portion above the first ear, which included the top portion of the plant and the cobs (Above-ear). A factor was considered to be significant at level lower than 0.1 (alpha < 0.1).

Results and Discussion

In-season weather effects on biomass composition

Cumulative precipitation levels and average air temperatures varied over the nine location-years of the experiment (Table 21). Significant correlations between biomass components and seasonal climate conditions were observed (Table 22).

In SC, significant negative correlations were detected between cellulose and the weather variables (seasonal cumulative precipitation and average air temperature). Holocellulose content exhibited correlations similar in strength and direction to cellulose. Ash content in biomass was positively correlated with seasonal precipitation and air temperature. However, apart from the cob plant portion, no significant correlations were detected between climate conditions and lignin content.

At both AL sites, correlations were not as strong as in SC and varied between positive and negative. Cellulose, lignin, and holocellulose contents in total stover and partitions were positively correlated with cumulative seasonal precipitation and negatively correlated with seasonal average air temperatures (Table 22).

The biomass compositional response to climate conditions varied significantly between the two corn hybrids. As precipitation and temperatures increased during each growing season, the holocellulose content decreased in the Dekalb hybrid (SC location). Conversely, as precipitation and temperatures increased in AL, holocellulose content increased in the Pioneer hybrid. These variations could be attributed to genetic differences between corn hybrids (Thomas et al., 2001) and differences in environmental conditions (Templeton et al., 2009). As a result, the differences in composition of the biomass can affect the amount of biofuel produced due to in-season climate variations.

Rye and corn stover management effect on biomass composition

A rye winter cover crop was used only at the AL sites; however, corn stover management was performed at all three sites at 0% or 100% removal. For all three sites, the three-year average effect of both, corn residue removal and use of a rye winter cover crop was minimal on biomass composition (Table 23). For example, 100% corn residue removal in SC had a marginal impact only on ash content in the whole plant biomass ($p = 0.099$). At both AL locations, no effect of corn residue removal was observed in whole plant composition. The use of rye had no effect on structural carbohydrate content in whole plant biomass in EVS and a marginal effect on lignin ($p = 0.09$). At TVS, a more significant effect was observed on holocellulose content in total stover ($p = 0.001$) and a marginal effect on hemicellulose ($p = 0.08$). Limited and variable effects were observed across different plant portions due to rye cultivation and due to residue removal; however, there was not a distinct pattern that would allow for decisive conclusions.

The lack of compositional variation due to residue management implies that 100% corn residue removal would not have an impact on the downstream bioenergy production practices. However, the duration of the experiment should be considered before recommending corn stover harvesting practices. A long-term study could reveal significant variations in the chemistry of corn stover tissue as a result of cultivation practices. Furthermore, despite the limited effect of rye on stover composition, the benefits on soil productivity of cover crops under conservation tillage practices should always be considered (Bruce et al., 1995; Sainju et al., 2002). These results refer to the specific corn hybrids that were used at each location and generalizations to other hybrids grown elsewhere should be avoided.

Vertical biomass composition

Total and partial biomass composition was highly variable at all three locations (Table 24). The DeKalb hybrid in SC exhibited greater amounts of cellulose, holocellulose, lignin, and ash when compared to the AL Pioneer hybrid. These variations, in addition to the compositional responses to in-season climate, further indicate the possible differences between the two experimental corn varieties. However, there were similarities in the way that the components of interest were distributed among different plant portions across locations: The greatest holocellulose content was observed in the cobs and above-ear fractions; alternatively, the least amount of holocellulose was detected in the bottom plant fractions (Figure 11); and lastly, the bottom portions of the stover exhibited the greatest amount of lignin, while the cobs and above-ear fractions contained the lowest amounts (Figure 12). The ash portion was the only component distributed differently among the plant parts between state locations. In SC, the least amount of ash was detected in the cobs; the greatest amount of ash was measured in the bottom stover portion. Even though both locations in AL produced cobs with the least amount of ash, the greatest ash content was observed in the top fractions (Figure 13).

It is known that corn biomass can be used by the bioenergy industry for biofuel production (Kadam and McMillan, 2003). Biomass feedstock with large amounts of cellulose and hemicellulose and low lignin is the most desirable for bioethanol production via fermentation (Chang and Holtzapple, 2000; Kim and Holtzapple, 2006). During the three years of the experiment across all three locations, the cobs, tops, and above-ear portions exhibited the greatest holocellulose contents and lowest amounts of ash and lignin. Therefore, regardless of corn hybrid, climate, or soil type that the corn was grown in, the top, cob, and above-ear fractions had the most suitable compositional characteristics for bioethanol production via fermentation. Nevertheless, quantification of pentoses (glucan, and galactan) and hexoses (xylan, arabinan, and mannan) in corn biomass would allow for a close estimation of the TEY and further evaluate the appropriateness of different plant portions as biofuel feedstocks.

Conclusions

Cellulosic corn biomass can be used as feedstock for bioethanol production. In this multi-location study, the three-year average effect of both 100% corn residue removal and of rye cultivation was minimal and inconsistent on biomass composition. The DeKalb hybrid in SC resulted in biomass with higher amounts of structural carbohydrates and lignin than the Pioneer hybrid grown in Alabama. There was a difference between corn hybrid in the quantitative vertical distribution of structural carbohydrates, lignin, and ash content. However, at all three sites, the relative distribution of cellulose, hemicellulose, and lignin was similar among different plant portions. Due to the strong correlations between biomass composition and in-season weather conditions, it is necessary to further investigate the climate impacts on biofuel production. Results from this study indicate the tops, cobs, and above-ear fractions of both corn hybrids grown in major soil types of the southeastern US compared to the below-ear portion have the more desirable composition for bioethanol production via fermentation.

Table 20. NIR calibration statistics.

Compositional attributes	†SEC	R^2	‡SECV
Cellulose	1.086	0.9103	1.528
Hemicellulose	1.68	0.909	1.812
Lignin	0.591	0.8653	0.678
Ash	0.362	0.8818	0.505

† Standard error of calibration

‡ Standard error of cross validation

Table 21. Seasonal cumulative precipitation (mm) and seasonal average temperature (°C) during the three growing seasons (May-August) at Pee Dee Research and Education Center in South Carolina (SC), at E.V. Smith Research and Extension Center (EVS) in central Alabama, and the Tennessee Valley Research and Extension Center (TVS) in northern Alabama.

Location	Year	Cumulative precipitation (mm)	Average temperature (°C)
SC	2009	648.46	23.96
	2010	922.27	25.89
	2011	959.83	25.99
EVS	2009	976.12	24.30
	2010	514.35	25.81
	2011	426.72	26.42
TVS	2009	808.22	22.60
	2010	367.28	24.78
	2011	329.18	25.54

Table 22. Pearson correlations (r-values) between total precipitation and average temperature during the growing season (May-August) with major plant components in total stover and partitions of corn stover biomass at Pee Dee Research and Education Center in South Carolina (SC), the E.V. Smith Research and Extension Center (EVS) in central Alabama and the Tennessee Valley Research and Extension Center (TVS) in northern Alabama.

Location			SC		
	Above-ear	Bottom	Top	Cob	Stover
			cellulose (%)		
Total Precipitation	-0.753***	-0.658***	-0.744***	-0.584***	-0.778***
Average Temperature	-0.741***	-0.647***	-0.732***	-0.576***	-0.759***
			hemicellulose (%)		
Total Precipitation	-0.352*	-0.411**	0.509**	-0.021	-0.138
Average Temperature	-0.304	-0.355*	0.458**	-0.043	-0.089
			holocellulose (%)		
Total Precipitation	-0.718***	-0.611***	-0.685***	-0.671***	-0.633***
Average Temperature	-0.688***	-0.564***	-0.643***	-0.640***	-0.585***
			lignin (%)		
Total Precipitation	-0.131	0.291	0.307	-0.829***	-0.086
Average Temperature	-0.186	0.235	0.250	-0.841***	-0.144
			ash (%)		
Total Precipitation	0.659***	0.401*	0.612***	0.608**	0.715***
Average Temperature	0.625***	0.360*	0.587***	0.569**	0.678***
Location			EVS		
			cellulose (%)		
Total Precipitation	0.409***	0.311**	0.075	0.546***	0.393***
Average Temperature	-0.488***	-0.428***	-0.189	-0.568***	-0.494***
			hemicellulose (%)		
Total Precipitation	-0.246*	-0.375***	-0.217	-0.234*	-0.321**
Average Temperature	0.243*	0.412***	0.221	0.202	0.342**
			holocellulose (%)		
Total Precipitation	0.278**	0.103	-0.076	0.478***	0.240*
Average Temperature	-0.362***	-0.206	-0.021	-0.519***	-0.338**
			lignin (%)		
Total Precipitation	0.736***	0.546***	0.579***	0.672***	0.709***
Average Temperature	-0.723***	-0.636***	-0.585***	-0.636***	-0.755***
			ash (%)		
Total Precipitation	-0.626***	0.581***	-0.292**	-0.525***	0.214
Average Temperature	0.581***	-0.534***	0.334**	0.450***	-0.203
Location			TVS		
			cellulose (%)		
Total Precipitation	0.291**	0.001	0.159	0.542***	0.217
Average Temperature	-0.339**	-0.152	-0.222	-0.498***	-0.318**

hemicellulose (%)					
Total Precipitation	-0.441***	0.540***	-0.135	-0.452***	-0.023
Average Temperature	0.477***	-0.429***	0.262*	0.356***	0.120
holocellulose (%)					
Total Precipitation	-0.046	0.651***	0.071	-0.245*	0.265*
Average Temperature	0.025	-0.701***	-0.004	0.149	-0.309**
lignin (%)					
Total Precipitation	0.743***	0.505***	0.717***	0.306**	0.777***
Average Temperature	-0.698***	-0.599***	-0.749***	-0.169	-0.788***
ash (%)					
Total Precipitation	-0.297**	0.066	-0.356***	0.043	-0.174
Average Temperature	0.212	-0.217	0.311**	-0.142	0.041

*, **, *** denote significance at 0.1, 0.05, and 0.01 probability levels respectively.

Table 23. Three-year average effect of corn residue management and use of a rye cover crop on corn biomass composition at the Pee Dee Research and Education Center in South Carolina (SC), the E.V. Smith Research and Extension Center (EVS) in central Alabama and the Tennessee Valley Research and Extension Center (TVS) in northern Alabama.

Location	SC									
Plant Fraction	Whole plant		Bottom		Top		Cob		Above-ear	
Factor	Stover removal	Rye cover crop	Stover removal	Rye cover crop	Stover removal	Rye cover crop	Stover removal	Rye cover crop	Stover removal	Rye cover crop
	†Pr > F									
Cellulose	0.943	N/A	0.623	N/A	0.589	N/A	0.113	N/A	0.172	N/A
Hemicellulose	0.635	N/A	0.775	N/A	0.285	N/A	0.210	N/A	0.832	N/A
Lignin	0.373	N/A	0.589	N/A	0.731	N/A	0.980	N/A	0.334	N/A
Holocellulose	0.819	N/A	0.614	N/A	0.620	N/A	0.350	N/A	0.405	N/A
Ash	0.099	N/A	0.128	N/A	0.195	N/A	0.884	N/A	0.420	N/A
Location	EVS									
Cellulose	0.187	0.363	0.439	0.004	0.551	0.046	0.694	0.586	0.230	0.934
Hemicellulose	0.962	0.699	0.177	0.626	0.24	0.248	0.019	0.642	0.135	0.830
Lignin	0.866	0.090	0.353	0.008	0.244	0.052	0.937	0.427	0.141	0.348
Holocellulose	0.297	0.822	0.510	0.168	0.261	0.879	0.187	0.718	0.019	0.919
Ash	0.156	0.004	0.490	<.001	0.370	0.008	0.441	0.039	0.245	0.064
Location	TVS									
Cellulose	0.352	0.682	0.634	0.211	0.982	0.112	0.097	0.215	0.421	0.453
Hemicellulose	0.318	0.080	0.100	0.243	0.888	0.307	0.887	0.271	0.699	0.110
Lignin	0.757	0.151	0.785	0.523	0.860	0.899	0.372	0.208	0.778	0.180
Holocellulose	0.857	0.001	0.201	0.023	0.873	0.008	0.329	0.330	0.680	0.018
Ash	0.708	0.543	0.644	0.579	0.625	0.200	0.726	0.869	0.755	0.150

† The Pr > F values represent the probability of a larger F by chance between residue retained and residue removed and between plots where rye was removed, retained, and plots without the use of cover crop within locations.

§ Not available in this location.

Table 24. Vertical distribution of major plant components at the Pee Dee Research and Education Center in South Carolina (SC), the E.V. Smith Research and Extension Center (EVS) in central Alabama and the Tennessee Valley Research and Extension Center (TVS) in northern Alabama.

Location			SC			
Plant Fraction	Stover	Bottom	Top	Cob	Above-ear	†Pr > F
			%			
Cellulose	41.25	43.17	37.20	39.20	38.20	0.05
Hemicellulose	31.37	25.66	36.11	40.37	38.24	<.0001
Lignin	6.62	9.22	5.46	4.54	5.00	0.0015
Holocellulose	71.41	68.40	72.95	79.4	76.17	0.1127
Ash	3.99	4.16	3.6	2.19	2.89	0.0004
Location			EVS			
			%			
Cellulose	41.21	43.18	40.80	39.46	40.21	0.065
Hemicellulose	22.02	15.92	22.06	28.43	25.15	<.0001
Lignin	6.45	8.22	5.65	5.43	5.53	<.0001
Holocellulose	63.32	59.22	62.92	67.93	65.42	<.0001
Ash	2.87	3.12	3.35	2.14	2.74	<.0001
Location			TVS			
			%			
Cellulose	41.07	43.08	40.33	39.79	40.06	0.0002
Hemicellulose	23.02	15.44	23.57	30.04	26.81	<.0001
Lignin	6.52	8.81	5.34	5.40	5.37	<.0001
Holocellulose	63.97	58.38	63.72	69.81	66.77	<.0001
Ash	3.37	3.88	4.16	2.17	3.12	<.0001

†The Pr > F values represent the probability of a larger F by chance between plant fractions within locations.

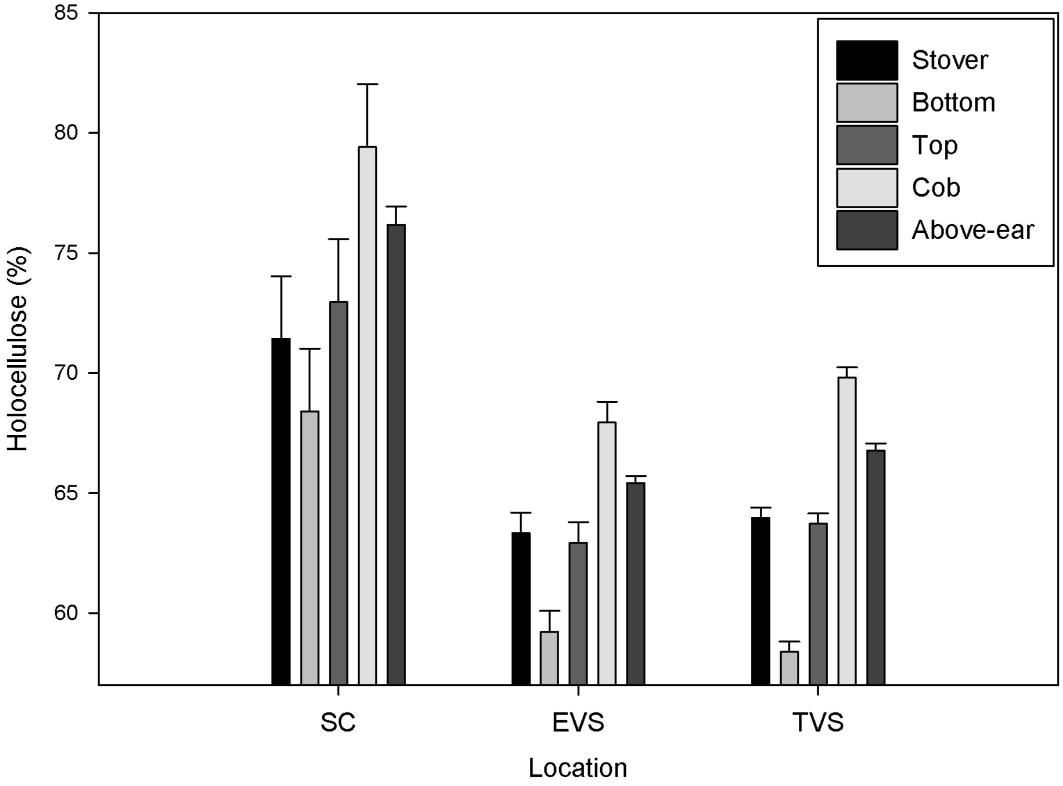

Figure 11. Distribution of holocellulose content (Cellulose + Hemicellulose) in total and partial corn biomass at Pee Dee Research and Education Center in South Carolina (SC), at E.V. Smith Research and Extension Center (EVS) in central Alabama and at Tennessee Valley Research and Extension Center (TVS) in northern Alabama. The error bars represent the standard error of the mean.

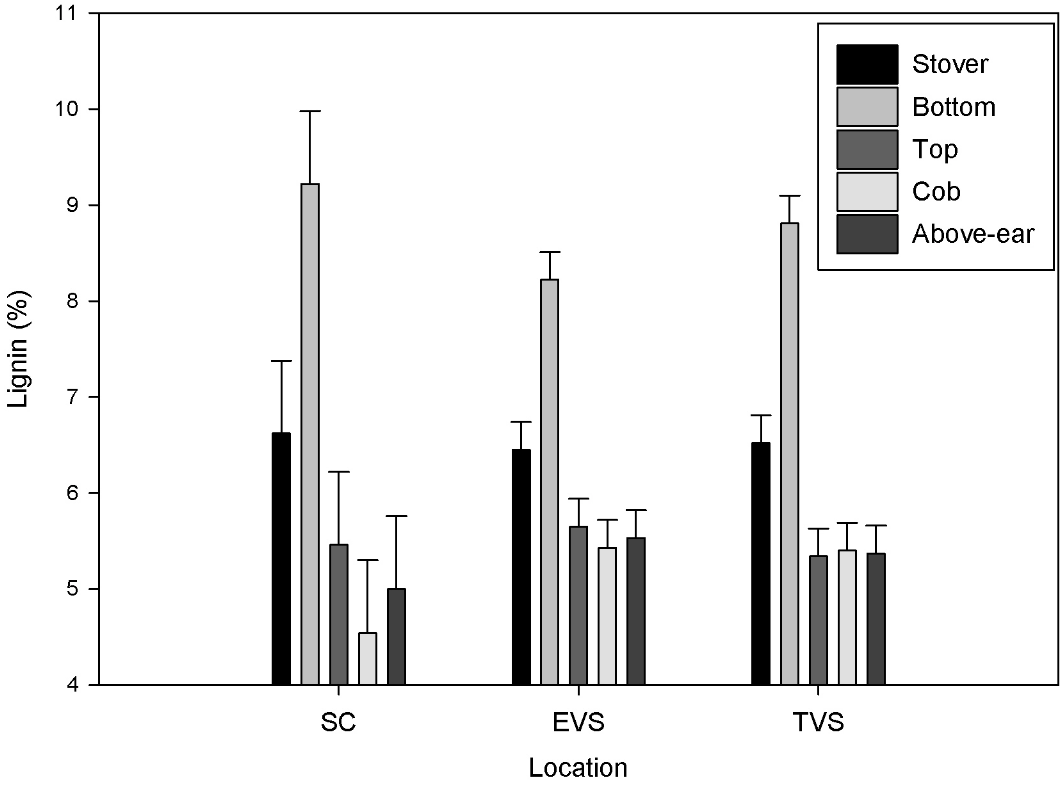

Figure 12. Distribution of lignin content in total and partial corn biomass at Pee Dee Research and Education Center in South Carolina (SC), at E.V. Smith Research and Extension Center (EVS) in central Alabama and at Tennessee Valley Research and Extension Center (TVS) in northern Alabama. The error bars represent the standard error of the mean.

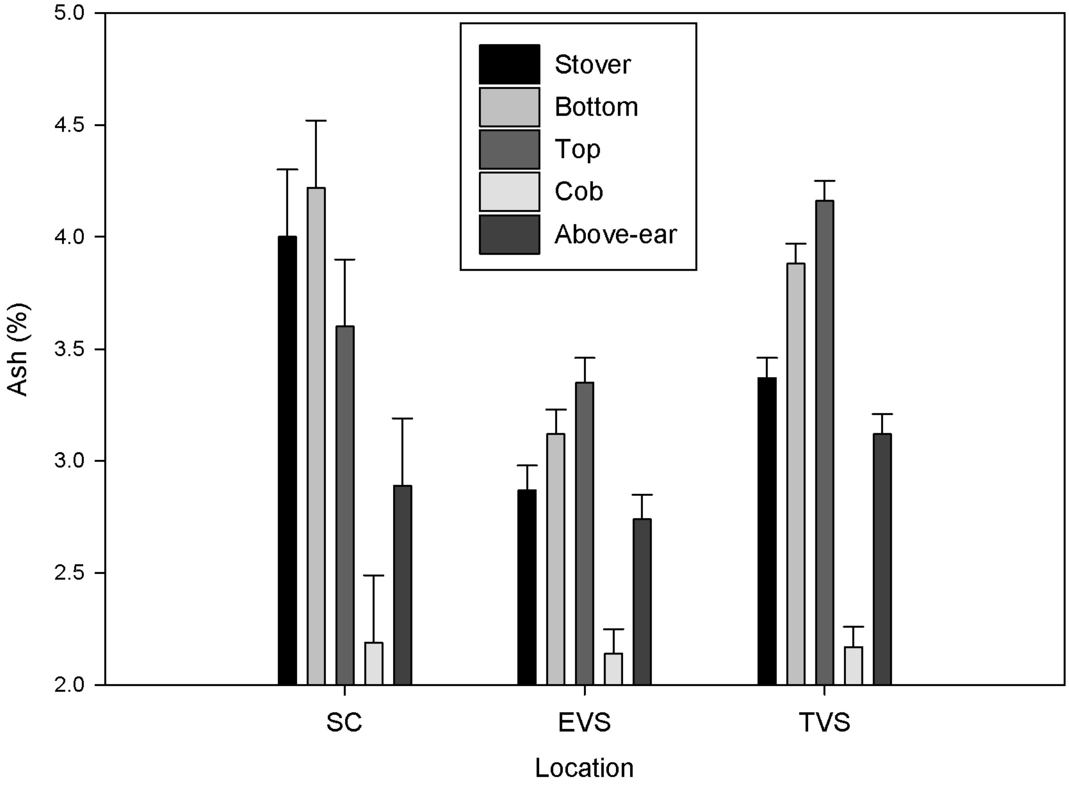

Figure 13. Distribution of ash content in total and partial corn biomass at Pee Dee Research and Education Center in South Carolina (SC), at E.V. Smith Research and Extension Center (EVS) in central Alabama and at Tennessee Valley Research and Extension Center (TVS) in northern Alabama. The error bars represent the standard error of the mean.

VI. Corn Stover Biofuel Potential, Nutrient Removal and Theoretical Ethanol Yield Modeling Across the Southeastern US

Abstract

Corn (*Zea mays* L.) biomass yield and composition vary with cultivar, plant portion and management practices. It is essential to understand the theoretical ethanol potential of the whole and portions of the corn biomass to determine which parts of the plant should be harvested as biofuel feedstock. Two continuous corn field studies were established in Alabama and one in South Carolina. Stover removal management practices (0 and 100% removal) were performed at all three locations. In Alabama the use of rye (*Secale cereale*) as a winter cover crop was also incorporated as a management practice. The soil types in Alabama were Compass and Decatur; in South Carolina the soil was a Coxville/Rains-Goldsboro-Lynchburg association. These three sites were investigated for differences in carbohydrate content, theoretical ethanol yield (TEY), energy content in the form of high heating value (HHV), and nutrient composition among five plant fractions: whole plant (stover); above the first ear excluding cobs (top); below the first ear (bottom); cobs alone (cob); and above the first ear including cobs (above-ear). The distribution of carbohydrates, nutrients, TEY, and HHV varied significantly among corn stover portions in every location. The use of a rye cover crop and stover harvest had minimal impact on TEY, HHV, and plant composition. Removing the above-ear portion of the stover only would result in lower removal of carbon (C) by 32-46%, lower nitrogen (N) removal by 32-43%, and lower potassium (K), sulfur (S), phosphorus (P), magnesium (M)g, and calcium (Ca) removal by 24-

61% when compared to harvesting the whole plant biomass. Models were developed to predict the total and partial corn stover TEY (l ha^{-1}) in late July using only the in-season weather conditions. The R^2 values of the models were small to moderate; nevertheless, there were not significant differences between the actual and fitted TEYs. Data from this study suggests that the plant portions with the highest biomass yield potential (e.g., above-ear portion) are the most desirable for bioethanol production at every location.

Introduction

Bioethanol is expected to play an important role in energy security the following years. The ethanol that is produced currently in the US is mainly derived from corn grain (Karlen, 2010). According to the Energy Independence and Security Act in 2007, almost 136 billion liters of ethanol should be produced per year by 2022, of which 61 billion liters should be produced from cellulosic biomass (EIA, 2008).

Corn is an abundant crop in the southeastern US with high biomass yield potential. It can be used as animal feed and by the biofuel industry (Kadam and McMillan, 2003). Cultivation of corn for stover harvest would not compete with the use of land for food production since grain and biomass would be produced simultaneously. Additionally, using corn biomass as a bioenergy feedstock can result in greater greenhouse gas reductions than using dedicated energy crops since there is no need for land use change (Searchinger et al., 2008). Due to these factors, corn is considered to be a desirable bioenergy feedstock.

Corn stover, as any form of plant biomass, is composed of cellulose, hemicellulose, lignin, ash, and extractives (Cone et al., 1996). Cellulose and hemicellulose are the most desirable portions of biomass for bioethanol conversion (Chang and Holtzapple, 2000; Kim and Holtzapple, 2006). Cellulose is homogenous and composed of glucose monomers linked by glycosidic bonds. However, hemicellulose is composed of 5-C (xylose and arabinose) and 6-C monomers (glucose, galactose, and mannose). The 6-C carbohydrates are more desirable for bioethanol production due to their higher conversion efficiency. The proportions of carbohydrates in the plant seem to vary among different plant portions (Reed et al., 2007). Corn biomass yield and composition also vary with cultivar, and management practices (Monono et al., 2013). Drought stress (Schittenhelm, 2010), planting densities (Hansey and De Leon, 2011), and crop development stage (Pordesimo et al., 2005) can also affect biomass yield and composition. Furthermore, specifically in the southeastern USA, cultivation of corn can be affected by temporal weather variability (Hansen et al., 1998), as well as by spatial soil and climate variability (Persson et al., 2009).

It seems that corn residue removal for biofuel production will become a standard management practice. A major concern when harvesting crop residues is the impact on soil properties. Corn residue left in the field after the growing season is very important for microbial processes (Franzluebbers, 2002), erosion control, carbon sequestration, and nutrient cycling, which all affect soil productivity (Johnson et al., 2007; Johnson et al., 2009; Lindstrom, 1986; Wilhelm et al., 2004). A way to mitigate the negative impacts on soil properties, would be the partial biomass harvesting. Furthermore, conservation tillage practices could be used towards the same goal since they are well-known for their numerous environmental and soil quality benefits, such as soil erosion reduction, soil organic matter (SOM) content increase, limitation of P runoff, improvement of soil infiltration (Uri, 1999) and soil aggregate stability (Riley et al., 2008). Studies in the Southeast have shown that cover crops can improve soil productivity, especially when combined with conservation tillage practices (Bruce et al., 1995; Sainju et al., 2002). The use of winter rye as a cover crop is common in the southeastern US (Ashford and Reeves, 2003).

Due to its superior winter hardiness, sensitivity to herbicide kill, and its high residue production (Moschler et al., 1967; Odhiambo and Bomke, 2001) rye is considered an important cover crop.

Obviously, the development of the corn plant varies due to cultivation practices and the environmental conditions during the growing season. While stover harvest and the combination of conservation tillage with the use of rye as a cover crop are well-examined agricultural systems, there is a lack of information in the literature on their effects on plant nutrient composition and on carbohydrate content, which is important for bioethanol production. Therefore, it is essential to understand how the use of cover crops and stover harvest affect total and partial biomass composition.

In the biofuel industry, as in all types of industry, the timing and supply management of the biomass feedstock is very important. Having some idea of the estimated amount of bioethanol that could be produced from a feedstock grown in a specific region can also assist the operation of biofuel plants. Such an estimate could be helpful in determining prices of both feedstock and the produced fuel. Consequently, estimation of the amount of corn stover ethanol early in the growing season (e.g. in late July) could be beneficial for both farmers and industry. Farmers could contract their biomass early in the growing season at a more competitive price compared to waiting until harvest, and the industry, being aware of an estimate of the ethanol that can be produced ahead of time, could enhance overall plant operating efficiency.

The first objective of this study was to evaluate the total and partial corn biomass TEY potential and HHV across the southeastern US. The second objective was to evaluate the C, N, and nutrient content in the total and partial corn biomass at the three locations of this study. The third objective of this experiment was to assess the three-year average effect of the use of both rye as a winter cover crop and corn residue management on carbohydrate content, TEY, HHV, and C, N and nutrient content in the total and partial corn biomass. The fourth objective of this study was to develop regression models that predict whole and partial stover TEY (l ha^{-1}) at harvest using only readily available weather data in May, June and July.

Materials and Methods

Site description

This study was conducted for three years (2009-2011) at three locations across the southeastern US. The first site was at the E.V. Smith Research Center near Shorter in central Alabama (32.42884 N, -85.890235 W). The second was at the Tennessee Valley Research and Extension Center in Belle Mina (34.687953 N, -86.886763 W) which was in the northern part of the state. The third site was at the Pee Dee Research and Education Center in South Carolina (SC) (34.283767 N, -79.7415 W). The soil at E.V. Smith Research Center (EVS) was a Compass loamy sand (Coarse-loamy, siliceous, subactive, thermic Plinthic Paleudults) and at Tennessee Valley Research and Extension Center (TVS) the soil was a Decatur silt loam (Fine, kaolinitic, thermic Rhodic Paleudults). The soil type in SC was a Coxville/Rains-Goldsboro-Lynchburg association.

Plots at both sites in Alabama were arranged in a split-plot design. Two treatments were included: rye as a winter cover (no cover, rye as a cover crop removed in spring and rye retained) being the main plot; and corn residue removal the sub-plot (0 and 100% removal). Plots in SC were arranged in a randomized complete block design. The treatment included two levels of corn residue management (0 and 100% removal). In SC the corn hybrid used was the DeKalb C69-71 and in both locations in Alabama the Pioneer 31G65R. Urea ammonium nitrate (UAN 28-0-0) at rate of 168 kg ha^{-1} was used as nitrogen fertilizer at all three sites. It was applied in two applications early in the growing season. The plots in Alabama were under non-irrigated, continuous, no-tillage corn production. South Carolina plots were also under non-irrigated, continuous corn production, and strip-till with sub-soiling of the E soil horizon performed annually to 30-40 cm depth.

Data and sample collection

Precipitation (mm) and temperature (^{o}C) data during the growing season (May-July) were gathered from the weather stations located at each experimental site (Table 25). Monthly cumulative precipitation and monthly average air temperature were calculated and used as variables in the analysis that follows.

At every location corn plant samples were taken by hand at harvest. Before harvesting, a representative area consisting of a 1-m length row from both of the two middle rows of every plot was flagged and all the corn plants from that area were taken at harvest at each site. All the plants from the same plot were combined to create a whole plot sample. Corn grain and cobs were separated from the stalks. The grain was separated from the cobs using a shelling machine. The plants were further separated in four increments: below the ear (bottom); above the ear excluding cobs (top); and cobs alone (cobs). Stover samples were oven dried at 55^{o}C for seven days, and then ground in a Willey mill to pass through a 2 mm sieve.

NIR preprocessing

Near-infrared spectroscopy (NIRS) techniques were employed for data acquisition due to the large number of samples (n~2500). All ground samples were scanned in a FOSS 5000 NIRS (© FOSS Analytical AB 2004) instrumentation using the ISIscan^{TM} software. After scanning all the samples, the WinISI 4 software was utilized to perform the Standard Normal Variate (SNV) and Detrend scatter correction (Detrend) to reduce particle size effects and to remove the linear and the quadratic curvatures from the spectra. Then, the spectra were ranked according to the global Mahalanobis distance (GH) and 300 representative samples were chosen for wet chemistry analysis.

Chemical analysis

The C and N content of every sample (n~2500) was determined via dry combustion in a LECOR TruSpec C/N analyzer (Leco Corp., St. Joseph, MI). The 300 NIR selected samples were analyzed for carbohydrate composition, mineral composition and energy content. To quantify the polymeric carbohydrates (glucan, xylan, mannan, galactan, and arabinan) in the selected 300 corn stover samples, the procedure which was described in Chapter 2 was performed, which was a neutral detergent fiber (NDF) extraction (Van Soest and Wine, 1967) followed by a two-step acid hydrolysis of the extractive-free sample (Technical Report NREL/TP-510-42618, 2008). Then the samples were analyzed for monomeric carbohydrates via high pressure liquid chromatography (HPLC) using a Shimadzu (LC-20A) system (Shimadzu Corp. Kyoto, Japan). The system was equipped with a 300 mm × 7.8mm i.d., 9 µm detector, an Aminex HPX-87P column and a 30 mm × 4.6 mm i.d.guard column of the same material (Bio-Rad, Hercules, CA). The mobile phase consisted of water at 0.6 mL/min flow rate and the temperature of the column was maintained at 85 °C during elution. Only glucose, xylose and arabinose were above the detectable limits, so this report focuses on these three sugars. Samples were extracted following a microwave digestion procedure to determine aluminum (Al), manganese (Mn), calcium (Ca), zinc (Zn), sulfur (S), phosphorus (P), potassium (K), copper (Cu), and magnesium (Mg) concentrations for the 300 samples using concentrated HNO_3 acid in a Mars Xpress Microwave Digester (CEM Corp., Mathews, NC, USA). The digestion procedure was based on the USEPA 3051A method. All extracts were analyzed using an inductively coupled plasma-atomic emission spectrograph (ICP-AES). The energy content of the samples (MJ kg^{-1}) was quantified in the form of high heating value (HHV) via dry combustion in a Calorimeter System (IKA® C 2000 basic C 2000 control; IKA® Works, Inc NC, USA).

NIR calibration

The modified partial least squared (modified PLS) was found to be the most appropriate for all the chemical components of interest for the calibration of the NIR data. The math treatment used for the calibration was the (1, 4, 4, 1). That involves the 1st derivative, a 4 nm gap and 4 initial smoothing points, and no further smoothing. The standard error of calibration (SEC), the standard error of cross validation (SECV), and the coefficient of determination were used to evaluate the fit of the model and the accuracy of the obtained data (Table 26). For nutrients, only K, P, S, Mg, and Ca concentrations were successfully predicted and therefore, this report focuses only on these minerals.

An additional validation dataset (n=160), of known composition (carbohydrate and nutrient content values) was also scanned in the NIR along with the rest stover samples. The actual wet chemistry values of these samples were compared to the estimated values that were obtained from the NIR. There was no significant difference between the actual and predicted values which was an additional indication of the acceptable performance of the NIR models.

An additional stover portion (above-ear) was calculated using the chemical analysis results of the cobs and top portions of the plant (above-ear). Due to differences in dry biomass yields between the two partitions, a weighted average was calculated taking into account the dry biomass yield and the concentration of the component of interest in each stover portion.

The TEY was calculated using the carbohydrate concentrations in corn stover samples and the U.S. Department of Energy TEY calculator (DOE). The calculator reports the TEY yield in gal Mg^{-1} of biomass. Therefore, the yields were multiplied with 3.785 and converted to l Mg^{-1} of corn biomass.

Statistical analysis

The compositional characteristics of five plant portions of interest were: stover; bottom portion; top portion; cobs; and above-ear plant portion (tops + cobs). Repeated measures analysis of variance, utilizing the MIXED procedure in SAS 9.3 (SAS for Windows v. 9.3, SAS Institute Inc., Cary, NC), was used to detect differences in the total and partial biomass carbohydrate composition, nutrient content, TEY, and HHV. The same procedure was used to detect differences in the same variables due to the three-year average effect of both rye cultivation and corn stover management. A factor was considered to be significant at a level less than 0.10 (alpha < 0.10).

Significant and strong correlations were detected between carbohydrates in corn biomass and the in-season weather conditions (Table 27). In chapter 3, significant and strong correlations were detected between in-season weather conditions and corn biomass yields. Therefore, considering these correlations, the development of regression models that predict the whole plant and partial biomass TEY (l ha^{-1}) at harvest utilizing the monthly in-season weather data (May-July) as predictor variables (Table 25) was attempted. However, significant correlations were observed among weather variables (Tables 28, 29, and 30) which caused severe multicollinearity issues. To overcome this problem the methodology followed was similar to that reported by Fekedulegn et al. (2002). Initially, data from both locations in Alabama were combined since the same corn variety was used. Then, principal component analysis (PCA) was performed on the weather variables for the SC plots and for the Alabama plots separately using the PRINCOMP procedure in SAS 9.3 (Table 31). A useful property of the PCA procedure is the development of principal components (PC) which they are uncorrelated to each other. Therefore they can be used as independent variables in regression analysis minimizing the multicollinearity issues. The number of PCs that resulted in cumulative variability ≥ 0.9 were used as predictor variables in the model development. The REG procedure was utilized in SAS 9.3 to develop the TEY (l ha^{-1}) regression models.

Results and Discussion

Distribution of carbohydrates, theoretical ethanol yield, and energy content in corn biomass

The carbohydrate content in corn biomass was highly variable among the different plant portions at all three locations (Table 32). Biomass grown in SC had higher glucan, xylan and arabinan content than biomass grown in both locations in Alabama. These differences could be a result of the differences between the two hybrids (Pioneer vs. DeKalb). However, at all three locations the relative distributions of glucan, xylan, and arabinan among the different corn portions were similar. The highest glucan content was observed in the bottom portion of the plants while the above-ear and cob portions had the highest xylan content. The cobs had the highest TEY (l Mg^{-1}) in biomass grown at every location; however the difference among the other plant portions was significant only in TVS ($p < 0.0001$). In the same location, some differences existed in the partial TEY with the bottom portion of the stover exhibiting the lowest bioethanol potential (l Mg^{-1}). Due to the high variability in biomass yields in every location (Table 32), a comparison of TEY per unit area (l ha^{-1}) is more appropriate. Among the different plant portions, the above-ear biomass resulted in the highest TEY, this was 2115, 1326, and 1280 l ha^{-1} in SC, EVS, and TVS respectively (Figure 14). The bottom plant portion resulted in the second highest bioethanol yield potential in SC, while in both sites in Alabama it was the top portion that exhibited the second highest TEY (l ha^{-1}).

The data suggest that the effect of biomass yield on TEY per unit of area (l ha^{-1}) is far more important than the effect of carbohydrate concentration. A similar conclusion was reported in an experiment which was investigating the ethanol potential of herbaceous biomass in North Dakota (Monono et al., 2013). Furthermore, the results reported are in agreement with the results of a similar study which was conducted in Iowa (Reed et al., 2007). In that study the highest glucan content (37.6%) was also observed in the bottom plant portion and the lowest glucan concentration (33.7%) was detected in the above-ear biomass. In the same study, the TEY per unit of area ranged from 757 to 3002 l ha^{-1} with the above-ear portion yielding the highest bioethanol yield potential (2135 l ha^{-1}).

Despite the similarities between the two studies in the relative distribution of carbohydrates and the TEY among the different plant fractions, the carbohydrate concentrations which were reported by Reed et al. (2007) are lower than those reported in this study. Therefore, it should be noted that they harvested the corn samples from a site near Ames, IA where the climate is considerably different than that of the southeastern US. Furthermore, it is known that the compositional characteristics of the plant vary with the growth stage of the plant (Pordesimo et al., 2005). In their study, the researchers collected the corn samples at R6 growth stage from a site where the previous year crop was soybean [*Glycine max* (L.) Merr.]. Also, they used a Fontenell 5393 corn hybrid which could result in different compositional characteristics from the DeKalb and Pioneer used in SC and Alabama sites respectively.

Distribution of carbon, nitrogen, and nutrients in corn biomass

When the goal of an agricultural system is sustainable corn stover harvest for biofuel and grain production, it is desirable to harvest a high amount of biomass without impacting soil quality. Intuitively then, a portion of the stover with high TEY (l ha^{-1}) and low amounts of C, N and nutrients would qualify as a desirable biofuel feedstock. In all three locations of this study, there were differences in the relative distributions of C, N and nutrients in corn biomass (Table 33). In every location, the highest C content was observed in the cobs and above-ear portions while the tops exhibited the highest N concentrations. The bottom plant portions had the highest K and P contents. The highest S content was detected in the top and above-ear portions while Mg and Ca were evenly distributed between the bottom and above-ear fractions of the corn plants. These results are in agreement with a similar multi-location nutrient removal study (Johnson et al., 2010)

As mentioned in the previous section, the biomass yield seems to be the most important factor in the maximization of bioethanol production. Therefore, estimation of the amount of C, N, and nutrients that would be removed in different biomass harvesting scenarios is essential. Such an assessment would allow for an estimation of the component quantities that would have to be replaced in the soil to maintain long-term productivity.

Cobs have been recognized as a desirable feedstock for biofuel conversion (Zych, 2008). In all three locations, harvesting of cobs alone would result in the lowest removal rates of C, N and other elements (Table 34). However, as mentioned in Chapter 3, this would be a result of their low yield. The top portion of the stover would result in a high partial biomass harvest; however, it does not appear sensible to exclude the cobs due to their low lignin and ash content and their high holocellulose content (Chapter 4). As reported in the previous section, of all the portions of the stover, the bottoms and above-ears have the highest biomass yields and could be attractive for sustainable bioethanol production.

In all three locations, harvesting the above-ear biomass, which has the highest partial biomass yield (Chapter 3) and the highest TEY (previous section), would result in the largest removal of C, N, and S. It would also lead to the highest removal rate of K, P, Mg, and Ca. However, to put the numbers into perspective, the above-ear portion, when compared to the total stover, would result in lower removal of C by 32-46%, N by 32-43%, K by 41-61%, S by 24-32%, P by 41-53%, Mg by 37-39%, and Ca by 31-40%, depending on the location (Table 34). These removal rates, although slightly higher than those reported by Johnson et al., (2010), follow the same pattern. The bottom portion of the plant, when compared to the total stover, would result in lower removal of C by 54-67%, N by 56-67%, K by 34-58%, S by 53-75%, P by 40-60%, Mg by 48-65%, and Ca by 48-67%, depending on the location.

It is obvious that the most appropriate stover portion for sustainable biofuel-grain production is a function of several factors. A feedstock that would result in the lowest possible removal of C, N and other nutrients in combination with a high TEY would be highly attractive. Other important desirable attributes are low lignin and high energy content. In this experiment, harvesting the above-ear portion would result in up to 50% higher energy content and up to 51% higher TEY than the bottom portion, depending on the location (Table 35). The significantly lower lignin content (49-84%) of the above-ear fraction when compared to the bottom stover is also a highly desirable characteristic (Chapter 4). However, choosing to harvest the above-ear biomass would result in significantly higher C, N, and S removal rates than harvesting the bottom part. Nevertheless, the combination of high yield, high TEY, and low lignin content, significantly reduced C and nutrients removal rates of the above-ear portion when compared to the whole biomass, indicate its superiority as a possible sustainable biofuel feedstock. This conclusion is also in agreement with other previous studies (Johnson et al., 2010; Duguid et al., 2009).

It should be noted that estimating the removal rates using only plant composition data can lead to incorrect projections on soil quality impacts. Consistent stover removal has been reported to decrease soil N mineralization rates (Kapkiyai et al., 1999; Salinas-Garcia et al., 2001) and eventually to decrease the soil organic N content (Blanco-Canqui and Lal, 2009; Dolan et al., 2006). Therefore, monitoring and evaluating changes of soil nutrient status as a function of stover harvest should be an essential part of biofuel sustainability research.

Effect of rye and corn stover management on theoretical ethanol yield, energy content, and nutrient composition

The three-year average effect of corn residue removal and the effect of a rye winter cover crop on glucan, xylan, and arabinan was minimal at all locations (Table 36). In SC, corn stover harvest had an effect only on the TEY (l ha^{-1}) of the bottom plant portion (*p* = 0.029). At EVS, significant effects of corn residue management were only detected on arabinan content in whole (*p* = 0.058) and above-ear (*p* = 0.013) plant biomass and on xylan content in the bottom plant portion (*p* = 0.046). At TVS, 100% corn residue removal affected only the TEY (l ha^{-1}) of the top (*p* = 0.044) and above-ear plant (*p* = 0.076) portions (Figure 15).

Previous studies in the southeastern USA have indicated that cover crops under conservation tillage practices can improve soil productivity (Bruce et al., 1995; Sainju et al., 2002). The results presented in Chapter 3 indicate that the three-year average effect of 100% corn stover harvest on biomass yield was not significant while the use of rye increased stover biomass yields. Additionally, in the previous section, the conclusion drawn was that biomass yield was the most important factor in maximizing TEY (l ha^{-1}). It is surprising therefore, that the use of rye had only a marginal effect on whole plant TEY (l ha^{-1}) at EVS ($p = 0.098$) and no significant effect at TVS (Table 36). It is possible that the positive effect of the use of rye on biomass yield was not capable to increase significantly the TEY (l ha^{-1}). However, despite the lack of statistical significance, the three-year average TEY (l ha^{-1}) in plots where rye was retained in the field were higher than plots without a cover crop at both EVS and TVS (Figure 16 and 17). Furthermore, the effect of a cover crop on biomass yield is cumulative (Duiker and Curran, 2005) and long-term cultivation could significantly increase biomass yield which would cause a significant and more obvious increase on TEY (l ha^{-1}).

The effect of the cultivation practices on HHV was also minimal at most. Corn stover management had an effect on the HHV (GJ ha^{-1}) of the bottom portion in SC ($p = 0.008$), and on the top ($p = 0.068$) and above-ear ($p = 0.063$) plant portions in TVS. No other significant effect was observed. As with TEY and HHV, the three-year average effect of the cultivation practices on the total and partial biomass C, N, and nutrient contents was minimal and inconsistent (Table 37). Due to the lack of a distinct pattern effect, it was not possible to draw decisive conclusions.

As mentioned in previous chapters and earlier in this section, despite the small and inconsistent impact of both rye use and stover management on biomass composition, their benefits on long-term soil productivity should always be considered.

Theoretical ethanol yield prediction

The polymeric carbohydrate concentrations in whole plant biomass were found to be significantly correlated with the in-season weather conditions at every location (Table 27). In SC, there were significant correlations between glucan and arabinan, and the amount of precipitation and air temperature in June and July, while xylan content was correlated with precipitation in June and air temperature in May, June, and July. At EVS, the correlations between glucan content and weather conditions were significant but weaker than those in SC. At TVS, the strongest correlations were detected between weather conditions in May and glucan content. In the same location, xylan and arabinan were correlated significantly with precipitation and air temperature in May, June, and July. Similar correlations between polymeric carbohydrates and weather conditions were detected in all corn fractions and locations (data not shown due to space constrains). Genetic characteristics between the two corn varieties and climate differences among the three locations could cause the variations in the observed correlations.

Regional regression models that predict TEY in whole and partial corn biomass, using only in-season weather conditions (May-July) were attempted to develop due to the differences between the SC and Alabama sites. However, it was not possible to construct models for SC due to insufficient yearly variation since only one site was included.

The three PCs that account for 93% of the in-season weather variability were used as independent variables (Table 31). Significant regression models predicting TEY in Alabama were successfully developed for whole plant and partial biomass portions (Table 38). The coefficients of determination were used as initial criterion to assess the fit of the model. The practical performance was evaluated by comparing the actual TEY (l ha^{-1}) and fitted for individual location-years and for the three years combined at both EVS and TVS. The whole plant model resulted in a small amount of explained variability (R^2=0.5333). However, a comparison of the whole plant TEY per unit of area (actual vs. fitted) showed no significant difference for the three-years combined at both locations. Satisfactory model performance was also observed when the individual years TEYs were compared. Similar model performance was also observed for the partial stover regression equations. Despite the low R^2 values, actual and fitted TEY differences were not statistically or practically different. However, the cob was the only portion that the regression was not significant. This could probably be due to the combination of low sample size and low biomass yield of cobs.

Despite the low and moderate R^2 values, the practical differences between actual TEYs and the fitted were not significant (Table 38). This could be due to the small effect of biomass carbohydrate content on TEY in combination with the strong impact of biomass yield. This could be further justified due to the strong correlations between the in-season weather conditions and biomass yields which reported in Chapter 3.

Conclusions

The first objective of this study was to evaluate the variability of the carbohydrates content, and biofuel potential in total and partial corn biomass in the southeastern US. There were significant differences in carbohydrate concentrations among different corn stover fractions. However, these variations had little to no effect on TEY (l Mg^{-1} of biomass). Similar results were reported for herbaceous biomass grown in North Dakota (Monono et al., 2013). Results from this study suggest that the amount of biomass yield is the most significant factor that influences TEY in AL and SC. The above-ear portion resulted in the highest TEY (l ha^{-1}) in every location of the study. This was in agreement with results reported in a similar study conducted in Iowa (Reed et al., 2007). This was attributed to the higher partial biomass yield when compared to the rest plant portions.

The second objective was to evaluate the C, N, and nutrient content in total and partial corn biomass in the three locations of this study. According to the results of this experiment, harvesting the above-ear stover portion would result in a low lignin feedstock with the highest bioethanol potential. Also, it would lead to significantly lower C and nutrient removal rates compared to removing the total stover in both Alabama and SC.

The third goal of this study was to evaluate the effects of management practices on corn biomass composition and biofuel potential. Total corn stover removal did not affect the energy content and bioethanol potential in SC. The same result was detected in Alabama sites whether rye was used as winter cover crop or not. Furthermore, the cultivation practices did not affect the carbohydrates, C, N, and other nutrient concentrations in total and partial biomass. However, the duration of the experiment should be considered before recommending corn stover harvesting practices for maximizing TEY. The effects of residue management and of the use of rye are cumulative and usually observed after several years. Also, despite that the cover crop did not increase TEY, other desirable effects of rye, such as large residue production, weed suppression, erosion control etc. should not be ignored. These characteristics of the cover crop are of great importance for the sustainability of the agricultural biofuel-food system. Furthermore, the results reported in this section concern the Pioneer and DeKalb corn hybrids which were grown in the specific locations. Generalizations and comparisons to other corn hybrids grown in different climates and soil types should be avoided.

The fourth objective of this multi-location experiment was to develop models that would allow for modeling TEY (l ha^{-1}) at harvest using only weather data from May through July. Due to the strong correlations among biomass carbohydrates and climates, regression models that predict the total and partial biomass TEY were successfully developed. The results indicate that using the monthly cumulative precipitations, and monthly average air temperatures in May, June, and July, an early season TEY (l ha^{-1}) estimation is possible. However, it should be noted that these models can assist in estimating the TEY that can be produced from specific corn hybrids grown in specific locations and climates. Further data collection is important to capture greater amount of climate variability and develop robust and reliable models.

Table 25. Seasonal cumulative precipitation (mm) and seasonal average temperature (°C) during the three growing seasons (May-July) at the Pee Dee Research and Education Center in South Carolina (SC), the E.V. Smith Research and Extension Center (EVS) in central Alabama and the Tennessee Valley Research and Extension Center (TVS) in northern Alabama.

Location	Year	Precipitation			Temperature		
		May	June	July	May	June	July
SC	2009	176.27	121.66	232.15	22.17	26.26	26.25
	2010	317.75	212.85	225.04	23.70	27.97	28.21
	2011	22.86	229.86	601.44	22.31	27.82	28.24
EVS	2009	262.38	99.57	75.18	22.99	21.72	26.61
	2010	175.76	56.38	128.01	24.05	28.00	29.13
	2011	56.39	56.89	203.71	21.90	28.25	28.29
TVS	2009	242.06	27.94	139.70	20.69	25.92	24.94
	2010	137.67	56.89	93.98	21.91	26.72	27.93
	2011	41.91	78.99	108.71	21.67	26.75	27.64

Table 26. Near-infrared calibration statistics for glucan, xylan, arabinan, high heating value (HHV), potassium, phosphorus, sulfur, magnesium, and calcium.

Compositional attributes	†SEC	R^2	‡SECV
Glucan	2.07	0.713	2.28
Xylan	1.31	0.896	1.48
Arabinan	0.44	0.827	0.513
HHV	0.262	0.791	0.343
K	642.77	0.9621	798.65
P	181.16	0.8173	203.59
S	59.14	0.911	69.7
Mg	149.9	0.9633	169.71
Ca	220.76	0.9521	269.81

† Standard error of calibration

‡ Standard error of cross validation

Table 27. Pearson correlation values between carbohydrates in whole plant biomass and weather variables at the Pee Dee Research and Education Center in South Carolina (SC), the E.V. Smith Research and Extension Center (EVS) in central Alabama and the Tennessee Valley Research and Extension Center (TVS) in northern Alabama for three growing seasons (2009-2011). The weather variables include: monthly cumulative precipitation (May-July) and monthly average air temperature (May-July).

Location	Carbohydrate	Precipitation			Temperature			
		May	June	July	May	June	July	
SC	Glucan	0.409	-0.925	-0.766	-0.181	-0.844	-0.883	
		0.047	<.0001	<.0001	0.398	<.0001	<.0001	†Pr>|r|
EVS		-0.032	0.065	-0.481	0.422	0.311	0.374	
		0.647	0.356	<.0001	<.0001	<.0001	<.0001	Pr>|r|
TVS		0.613	0.116	-0.620	0.867	-0.156	0.180	
		<.0001	0.423	<.0001	<.0001	0.280	0.211	Pr>|r|
SC	Xylan	0.230	0.866	0.224	0.727	0.930	0.909	
		0.280	<.0001	0.294	<.0001	<.0001	<.0001	Pr>|r|
EVS		0.613	0.116	-0.620	0.867	-0.156	0.180	
		<.0001	0.423	<.0001	<.0001	0.280	0.211	Pr>|r|
TVS		0.457	-0.498	0.887	-0.853	-0.764	-0.818	
		0.001	0.0001	<.0001	<.0001	<.0001	<.0001	Pr>|r|
SC	Arabinan	-0.317	0.927	0.692	0.266	0.867	0.897	
		0.132	<.0001	0.001	0.209	<.0001	<.0001	Pr>|r|
EVS		-0.604	-0.196	0.609	-0.746	0.230	-0.064	
		<.0001	0.173	<.0001	<.0001	0.108	0.657	Pr>|r|
TVS		0.564	-0.587	0.711	-0.721	-0.707	-0.719	
		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	Pr>|r|

† The Pr>|r| values represent the probability of a larger r by chance between two variables.

Table 28. Pearson correlation values between weather variables at the Pee Dee Research and Education Center in South Carolina (SC) for three growing seasons (2009-2011). The predictor variables include: Season total precipitation; season average air temperature; monthly cumulative precipitation (May-July); and monthly average air temperature (May-August).

		Precipitation			Temperature		
		May	June	July	May	June	July
Precipitation	May	1.000	-0.169	-0.885	0.808	0.056	-0.037
	†Pr>|r|		0.065	<.0001	<.0001	0.544	0.692
	June		1.000	0.608	0.444	0.975	0.991
	Pr>|r|			<.0001	<.0001	<.0001	<.0001
	July			1.000	-0.441	0.415	0.497
	Pr>|r|				<.0001	<.0001	<.0001
Temperature	May				1.000	0.633	0.559
	Pr>|r|					<.0001	<.0001
	June					1.000	0.996
	Pr>|r|						<.0001
	July						1.000

† The Pr>|r| values represent the probability of a larger r by chance between two variables

Table 29. Pearson correlation values between weather variables at E.V. Smith Research and Extension Center (EVS) in central Alabama for three growing seasons (2009-2011). The weather variables include: Season total precipitation; season average air temperature; monthly cumulative precipitation (May-July); and monthly average air temperature (May-August).

		Precipitation			Temperature		
		May	June	July	May	June	July
Precipitation	May	1.000	0.811	-1.000	0.584	-0.836	-0.583
	†Pr>lrl		<.0001	<.0001	<.0001	<.0001	<.0001
	June		1.000	-0.804	-0.002	-0.999	-0.948
	Pr>lrl			<.0001	0.983	<.0001	<.0001
	July			1.000	-0.592	0.830	0.574
	Pr>lrl				<.0001	<.0001	<.0001
Temperature	May				1.000	-0.042	0.320
	Pr>lrl					0.695	0.002
	June					1.000	0.933
	Pr>lrl						<.0001
	July						1.000

† The Pr>lrl values represent the probability of a larger r by chance between two variables

Table 30. Pearson correlation values between weather variables at Tennessee Valley Research and Extension Center (TVS) in northern Alabama for three growing seasons (2009-2011). The predictor variables include: Season total precipitation; season average air temperature; monthly cumulative precipitation (May-July); and monthly average air temperature (May-August).

		Precipitation			Temperature		
		May	June	July	May	June	July
Precipitation	May	1.000	-0.999	0.682	-0.774	-0.893	-0.833
	†Pr>|r|		<.0001	<.0001	<.0001	<.0001	<.0001
	June		1.000	-0.720	0.806	0.915	0.861
	Pr>|r|			<.0001	<.0001	<.0001	<.0001
	July			1.000	-0.991	-0.938	-0.973
	Pr>|r|				<.0001	<.0001	<.0001
Temperature	May				1.000	0.976	0.995
	Pr>|r|					<.0001	<.0001
	June					1.000	0.993
	Pr>|r|						<.0001
	July						1.000

† The Pr>|r| values represent the probability of a larger r by chance between two variables

Table 31. Principal components for three-year in-season weather data (2009-2011) at the Pee Dee Research and Education Center in South Carolina (SC), and at two combined locations in Alabama (E.V. Smith Research and Extension Center (EVS) in central Alabama and Tennessee Valley Research and Extension Center (TVS) in northern Alabama).

		Alabama			South Carolina	
		†PC1	±PC2	§PC3	PC 1	PC 2
Cumulative precipitation	May	-0.463	-0.174	0.641	-0.074	0.615
	June	-0.299	0.489	-0.511	0.541	-0.043
	July	0.486	-0.194	0.099	0.284	-0.529
Air temperature	May	-0.060	0.600	0.531	0.218	0.568
	June	0.588	0.008	0.157	0.531	0.122
	July	0.332	0.577	0.106	0.540	0.055
	Proportion of Variability	0.4469	0.338	0.1448	0.5672	0.4328
	Cumulative Variability		0.9298		1	

†PC1 represents the first principal component.

±PC2 represents the second principal component.

§PC3 represents the third principal component.

Table 32. Three-year average carbohydrate content, theoretical ethanol yield (TEY), and high heating value (HHV) per unit of mass and per unit of area in total and several corn biomass fractions at the Pee Dee Research and Education Center in South Carolina (SC), the E.V. Smith Research and Extension Center (EVS) in central Alabama and the Tennessee Valley Research and Extension Center (TVS) in northern Alabama.

Location				SC				
	Glucan	Xylan	Arabinan	Biomass Yield	TEY	TEY	HHV	HHV
		%		kg ha^{-1}	l Mg^{-1} of biomass	l ha^{-1}	MJ Kg^{-1} of biomass	GJ ha^{-1}
Stover	45.43	23.11	1.9	7852.15	463.44	3639	18.77	147.3
Bottom	47.64	21.35	1.45	4210.3	464.1	1954	18.27	77.13
Top	41.04	25.82	2.85	3298.54	459.9	1517	18.61	60.50
Cob	37.87	29.62	2.80	1406.72	464.2	653	19.18	27.00
Above-ear	39.45	27.32	3.02	4638.54	460.49	2136	18.42	86.70
§Pr>F	<0.0001	<0.0001	0.0003	<0.0001	0.7663	<0.0001	0.044	<0.0001
Location				EVS				
Stover	42.52	22.46	2.29	4570.75	443.21	2025.8	17.78	81.45
Bottom	45.2	20.28	1.25	1562.83	439.14	686.3	17.44	27.29
Top	42.9	22.18	2.58	2003.59	445.8	893.2	17.69	35.39
Cob	37.09	27.61	2.86	1073.75	446.1	479	19.68	21.05
Above-ear	41.23	23.59	2.78	3020.2	445.6	1345.8	17.99	54.46
Pr>F	<0.0001	<0.0001	<0.0001	<0.0001	0.1747	<0.0001	<0.0001	<0.0001
Location				TVS				
Stover	41.55	23.07	2.33	4286.17	441.14	1890.8	17.62	75.64
Bottom	44.57	20.31	1.16	1403.49	434.63	610	17.45	24.50
Top	42.86	22.2	2.57	2061.65	445.76	919	17.68	36.49
Cob	37.05	27.59	2.87	913.64	445.8	407.3	19.67	17.84
Above-ear	40.19	24.36	2.84	2929.26	444.31	1301.5	17.72	52.01
Pr>F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

§The Pr > F values represent the probability of a larger F by chance among the plant fractions.

Table 33. Three-year average carbon, nitrogen, potassium, sulfur, phosphorus, magnesium, and calcium in total and partial corn biomass at the Pee Dee Research and Education Center in South Carolina (SC), the E.V. Smith Research and Extension Center (EVS) in central Alabama and the Tennessee Valley Research and Extension Center (TVS) in northern Alabama.

Location				SC			
Element	C	N	K	S	P	Mg	Ca
	g kg^{-1}		mg kg^{-1}				
Stover	472.8	5.8	14140	670.6	813.74	1826.49	1918.6
Bottom	473.4	5.7	17806	583.5	874.73	1770.6	1926.9
Top	471.6	6.8	7982.8	648	737.11	1402.72	1460
Cob	487.2	6.1	9482.4	590.1	808.27	575.81	468.2
Above-ear	479.4	6.4	9138.5	799.5	883.64	1847.84	1911.2
†Pr>F	0.0017	0.6382	0.0128	0.1546	0.6646	<0.0001	<0.0001
Location				EVS			
Stover	460.2	10.4	7184.9	959.05	776.6	1145.71	1668.67
Bottom	458.3	11	8368.03	879.77	871.9	1290.3	1711.04
Top	457.8	11.9	6006.32	1236.86	631.5	1140.4	1829.79
Cob	464.5	8.4	8407.87	669.69	812.4	1079.89	1429.47
Above-ear	461.1	10.2	6579.97	999.41	714.5	1066.57	1637.26
Pr>F	0.0063	0.0019	0.2233	0.0028	0.0642	0.422	0.0044
Location				TVS			
Stover	464	8.4	7997.63	836.46	727.78	1182.07	1974.84
Bottom	464.7	8.4	9895.99	644.66	978.49	1281.12	1925.1
Top	458.6	8.9	7121.92	1051.41	693.36	1115.39	2208.8
Cob	468.9	8	6799.07	635.12	477.36	1060.76	1415.6
Above-ear	463.7	8.5	7134.93	935.95	631.91	1114.98	1989.53
Pr>F	<0.0001	<0.0001	0.0014	<0.0001	<0.0001	0.8888	0.0029

†The Pr > F values represent the probability of a larger F by chance among the plant fractions.

Table 34. Three-year average carbon, nitrogen, potassium, sulfur, phosphorus, magnesium, and calcium in total and partial corn biomass at the Pee Dee Research and Education Center in South Carolina (SC), the E.V. Smith Research and Extension Center (EVS) in central Alabama and the Tennessee Valley Research and Extension Center (TVS) in northern Alabama.

Location				SC			
Element	C	N	K	S	P	Mg	Ca
				kg ha^{-1}			
Stover	4891.1	63.6	102.7	5.0	6.0	14.2	14.6
Bottom	2267.9	27.7	68.1	2.3	3.6	7.3	7.6
Top	1773.9	26.4	24.0	2.0	2.1	4.5	4.5
Cob	832.7	10.4	11.8	0.7	1.0	0.7	0.5
Above-ear	2623.2	35.8	40.3	3.6	3.5	8.7	8.8
†Pr>F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Location				EVS			
Stover	2127.5	46.3	33.8	4.3	3.3	5.2	7.7
Bottom	723.2	16.9	14.4	1.4	1.7	2.0	2.7
Top	917.8	22.7	12.2	2.4	1.2	2.2	3.6
Cob	488.8	8.4	8.1	0.7	0.6	1.2	1.5
Above-ear	1409.1	29.3	19.2	3.0	1.6	3.3	5.0
Pr>F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Location				TVS			
Stover	2042.7	37.4	35.8	3.6	3.4	5.5	8.6
Bottom	677.1	12.3	15.0	0.9	1.3	1.9	2.9
Top	964.7	18.7	15.2	2.2	1.5	2.4	4.5
Cob	436.3	7.5	6.3	0.6	0.5	1.0	1.2
Above-ear	1389.1	25.3	21.3	2.8	2.0	3.4	5.8
Pr>F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0028	<0.0001

†The Pr > F values represent the probability of a larger F by chance among the plant fractions.

Table 35. Percent differences in carbon, nitrogen, potassium, sulfur, phosphorus, magnesium, calcium, lignin, theoretical ethanol yield (TEY), and high heating value (HHV) between the above-ear and the bottom plant fractions at the Pee Dee Research and Education Center in South Carolina (SC), the E.V. Smith Research and Extension Center (EVS) in central Alabama and the Tennessee Valley Research and Extension Center (TVS) in northern Alabama.

	C	N	K	S	P	Mg	Ca	Lignin	TEY	HHV
	% (Above-ear - Bottom)								l ha^{-1}	GJ ha^{-1}
SC	13.5	22.6	-68.9	34.5	-3.8	15.3	13.6	-84.4	8.5	11
EVS	48.7	42.3	25.1	53.5	-8.5	39.9	46.5	-48.6	51	50
TVS	51.3	51.4	29.5	67.4	32.3	43.8	50.5	-65	46.9	47.1

Table 36. Three-year average effect of corn residue management and use of a rye cover crop on corn stover carbohydrate content (%),Theoretical Ethanol Yield (TEY), and High Heating Value (HHV) per unit of mass and per unit of area at the Pee Dee Research and Education Center in South Carolina (SC), the E.V. Smith Research and Extension Center (EVS) in central Alabama and the Tennessee Valley Research and Extension Center (TVS) in northern Alabama.

Location	SC									
Plant Fraction	Stover		Bottom		Top		Cob		Above-ear	
Factor	Stover harvest	Rye cover crop	Stover harvest	Rye cover crop	Stover harvest	Rye cover crop	Stover harvest	Rye cover crop	Stover harvest	Rye cover crop
	†Pr > F									
Glucan	0.62	§N/A	0.515	N/A	0.638	N/A	0.695	N/A	0.936	N/A
Xylan	0.879	N/A	0.732	N/A	0.705	N/A	0.541	N/A	0.498	N/A
Arabinan	0.328	N/A	0.816	N/A	0.941	N/A	0.291	N/A	0.647	N/A
TEY (l Mg^{-1})	0.709	N/A	0.375	N/A	0. 893	N/A	0.489	N/A	0.667	N/A
TEY (l ha^{-1})	0.216	N/A	0.029	N/A	0.511	N/A	0.485	N/A	0.69	N/A
HHV (MJ kg^{-1})	0.675	N/A	0.072	N/A	0. 979	N/A	0.237	N/A	0.166	N/A
HHV (GJ ha^{-1})	0.16	N/A	0.008	N/A	0.939	N/A	0.384	N/A	0.811	N/A
Location	EVS									
Glucan	0.463	0.058	0.134	0.377	0.346	0.309	0.157	0.264	0.341	0.004
Xylan	0.242	0.521	0.046	0.254	0.2	0.228	0.694	0.586	0.333	0.703
Arabinan	0.058	0.584	0.433	0.925	0.923	0.62	0.219	0.642	0.013	0.314
TEY (l Mg^{-1})	0.459	0.013	0.568	0.011	0.983	0.036	0.937	0.027	0.61	0.038
TEY (l ha^{-1})	0.389	0.098	0.176	0.126	0.24	0.184	0.579	0.341	0.523	0.293
HHV (MJ kg^{-1})	0.807	0.383	0.306	0.556	0.413	0.475	0.937	0.427	0.631	0.331
HHV (GJ ha^{-1})	0.374	0.116	0.161	0.178	0.247	0.151	0.503	0.4	0.516	0.287
Location	TVS									
Glucan	0.59	0.587	0.869	0.919	0.338	0.267	0.247	0.376	0.658	0.607
Xylan	0.347	0.378	0.222	0.739	0.795	0.342	0.297	0.215	0.391	0.426
Arabinan	0.355	0.268	0.149	0.667	0.944	0.869	0.86	0.379	0.457	0.211
TEY (l Mg^{-1})	0.126	0.773	0.276	0.806	0.436	0.038	0.372	0.208	0.226	0.914
TEY (l ha^{-1})	0.414	0.312	0.311	0.479	0.044	0.177	0.254	0.463	0.076	0.133

HHV (MJ kg^{-1})	0.968	0.281	0.753	0.566	0.445	0.34	0.361	0.975	0.207	0.349
HHV (GJ ha^{-1})	0.357	0.273	0.291	0.432	0.068	0.159	0.198	0.47	0.063	0.146

† The Pr > F values represent the probability of a larger F by chance between residue retained and residue removed and between plots where rye was removed, retained, and plots without the use of cover crop within locations.

§ Not available in this location.

Table 37. Three-year average effect of corn residue management and use of a rye cover crop on corn stover nutrient composition at the Pee Dee Research and Education Center in South Carolina (SC), the E.V. Smith Research and Extension Center (EVS) in central Alabama and the Tennessee Valley Research and Extension Center (TVS) in northern Alabama.

Location	SC									
Plant Fraction	Stover		Bottom		Top		Cob		Above-ear	
Factor	Stover harvest	Rye cover crop	Stover harvest	Rye cover crop	Stover harvest	Rye cover crop	Stover harvest	Rye cover crop	Stover harvest	Rye cover crop
	†Pr > F									
C (g kg^{-1})	0.6350	§N/A	0.6020	N/A	0.7410	N/A	0.8890	N/A	0.7880	N/A
N (g kg^{-1})	0.7119	N/A	0.8013	N/A	0.5422	N/A	0.9892	N/A	0.6599	N/A
K (mg kg^{-1})	0.1807	N/A	0.2308	N/A	0.3742	N/A	0.6349	N/A	0.7659	N/A
S (mg kg^{-1})	0.9469	N/A	0.5459	N/A	0.4035	N/A	0.6733	N/A	0.3901	N/A
P (mg kg^{-1})	0.1240	N/A	0.1012	N/A	0.3244	N/A	0.1775	N/A	0.6217	N/A
Mg (mg kg^{-1})	0.3592	N/A	0.2092	N/A	0.5501	N/A	0.9247	N/A	0.9682	N/A
Ca (mg kg^{-1})	0.7579	N/A	0.5415	N/A	0.6691	N/A	0.3287	N/A	0.9924	N/A
Location	EVS									
C (g kg^{-1})	0.1730	0.2780	0.6760	0.1410	0.3460	0.1910	0.1570	0.264	0.1190	0.3200
N (g kg^{-1})	0.0678	0.9354	0.3775	0.7785	0.5292	0.8237	0.0301	0.6394	0.0832	0.8335
K (mg kg^{-1})	0.9130	0.3503	0.2215	0.2086	0.5465	0.3777	0.5730	0.2992	0.3250	0.5892
S (mg kg^{-1})	0.6179	0.0573	0.3741	0.8835	0.0520	0.0813	0.1263	0.3931	0.4835	0.0193
P (mg kg^{-1})	0.1433	0.4892	0.0028	0.2572	0.3625	0.7686	0.7005	0.7950	0.5479	0.5442
Mg (mg kg^{-1})	0.6577	0.9017	0.8114	0.7199	0.9802	0.5117	0.6289	0.6450	0.5213	0.8397
Ca (mg kg^{-1})	0.3751	0.1875	0.5422	0.9140	0.8876	0.8894	0.5644	0.8114	0.4417	0.2426
Location	TVS									
C (g kg^{-1})	0.1000	0.2960	0.0180	0.1850	0.7650	0.7940	0.2470	0.3760	0.2980	0.2300
N (g kg^{-1})	0.5336	0.0924	0.2803	0.5976	0.4220	0.7362	0.6200	0.1453	0.7864	0.1132
K (mg kg^{-1})	0.9253	0.9304	0.5384	0.8167	0.5632	0.8006	0.4525	0.5641	0.8332	0.8874
S (mg kg^{-1})	0.8288	0.1804	0.8647	0.1331	0.5163	0.5089	0.4675	0.6029	0.6614	0.9161
P (mg kg^{-1})	0.9683	0.4556	0.8432	0.1864	0.6706	0.7457	0.4456	0.8224	0.9482	0.6069
Mg (mg kg^{-1})	0.5076	0.3975	0.6096	0.7532	0.0161	0.6907	0.1578	0.0833	0.4986	0.4090

Ca (mg kg^{-1})	0.9293	0.9811	0.9041	0.6762	0.7331	0.8245	0.2333	0.7401	0.9982	0.8727

† The Pr > F values represent the probability of a larger F by chance between residue retained and residue removed and between plots where rye was removed, retained, and plots without the use of cover crop within locations.

§ Not available in this location.

Table 38. Theoretical Ethanol Yield (TEY) regression models and performance for whole plant and partial corn biomass at the E.V. Smith Research and Extension Center (EVS) in central Alabama and the Tennessee Valley Research and Extension Center (TVS) in northern Alabama.

Increment	Model	R^2	Location	Year	†Actual TEY (l ha^{-1})	±Fitted TEY (l ha^{-1})	§Difference (l ha^{-1})	£Pr>F
Stover	1935.08094-69.20965*PC1+140.35309*PC2-319.43043*PC3	0.5333	EVS	2009	2423.3	2341.2	82.1	0.7306
				2010	1480.2	1615.3	-135.1	0.1679
				2011	2009.6	1871.25	138.4	0.3116
				3 years	1914.5	1892.8	21.7	0.7851
			TVS	2009	1262.9	1367.3	-104.4	0.6158
				2010	2244.1	1984	260.1	0.0078
				2011	2217.8	2448.9	-231.1	0.1084
				3 years	1946.2	1966.7	-20.5	0.8163
Top	912.36711-57.95672*PC1+43.24936*PC2-136.14459*PC3	0.5280	EVS	2009	1165.4	1164.6	0.8	0.9929
				2010	695.4	729.4	-34.0	0.6461
				2011	837.6	822.5	15.1	0.8347
				3 years	899.5	905.5	-6.0	0.8849
			TVS	2009	711.5	735.3	-23.8	0.8566
				2010	1029.3	932	97.3	0.0041
				2011	1058.4	1113.9	-55.5	0.3914
				3 years	933	927	6.0	0.8976
Bottom	644.92812-10.22463*PC1+72.36335*PC2-165.21015*PC3	0.4061	EVS	2009	856.5	770.2	86.3	0.1784
				2010	354.5	497.9	-143.4	0.0007
				2011	859.7	669.8	189.9	0.0765
				3 years	690.2	646	44.2	0.3573
			TVS	2009	232.2	238.1	-5.9	0.1459
				2010	876.6	670.1	206.5	0.0310
				2011	668.4	919.4	-251.0	0.0042
				3 years	613.6	660.5	-46.9	0.4377
Cob	¥NS	¢NA	EVS	2009	NA			
				2010				

				2011				
				3 years				
			TVS	2009				
				2010				
				2011				
				3 years				
Above-ear	1310.49678-76.62314*PC1+62.84778*PC2-151.56088*PC3	0.4904	EVS	2009	1615.3	1644.6	-29.3	0.8645
				2010	1125.8	1120.1	5.7	0.9480
				2011	1149.8	1181.5	-31.7	0.6084
				3 years	1257.2	1274.5	-17.3	0.7229
			TVS	2009	1076.5	1076	0.5	0.9965
				2010	1367.4	1333.6	33.8	0.0979
				2011	1549.4	1537.5	11.9	0.9242
				3 years	1331.1	1315.7	15.4	0.7780

†Actual data TEY (l ha^{-1}).

±Fitted TEY (l ha^{-1}).

§Difference=Actual TEY-Fitted TEY.

£The Pr > F values represent the probability of a larger F by chance between actual and fitted TEY.

¥ Not Significant model.

¢ Not Available.

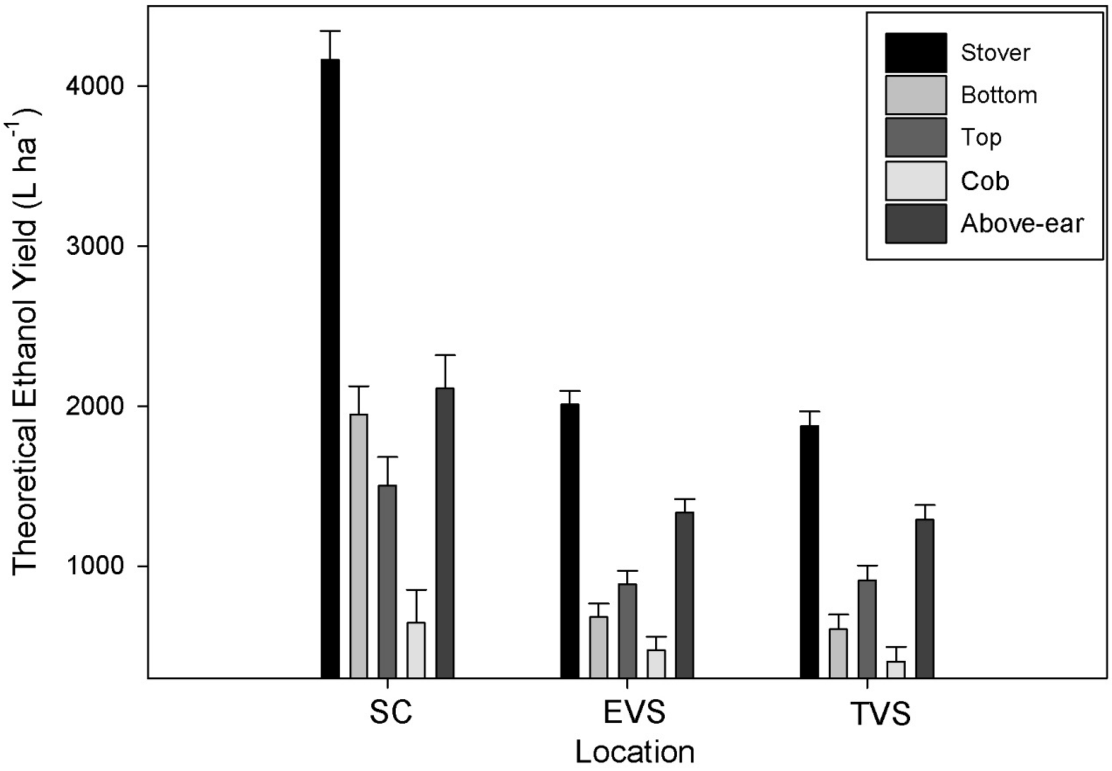

Figure 14. Three-year average theoretical ethanol yield from different corn plant fractions at the Pee Dee Research and Education Center in South Carolina (SC), the E.V. Smith Research and Extension Center (EVS) in central Alabama and the Tennessee Valley Research and Extension Center (TVS) in northern Alabama. The error bars represent the standard error of the mean.

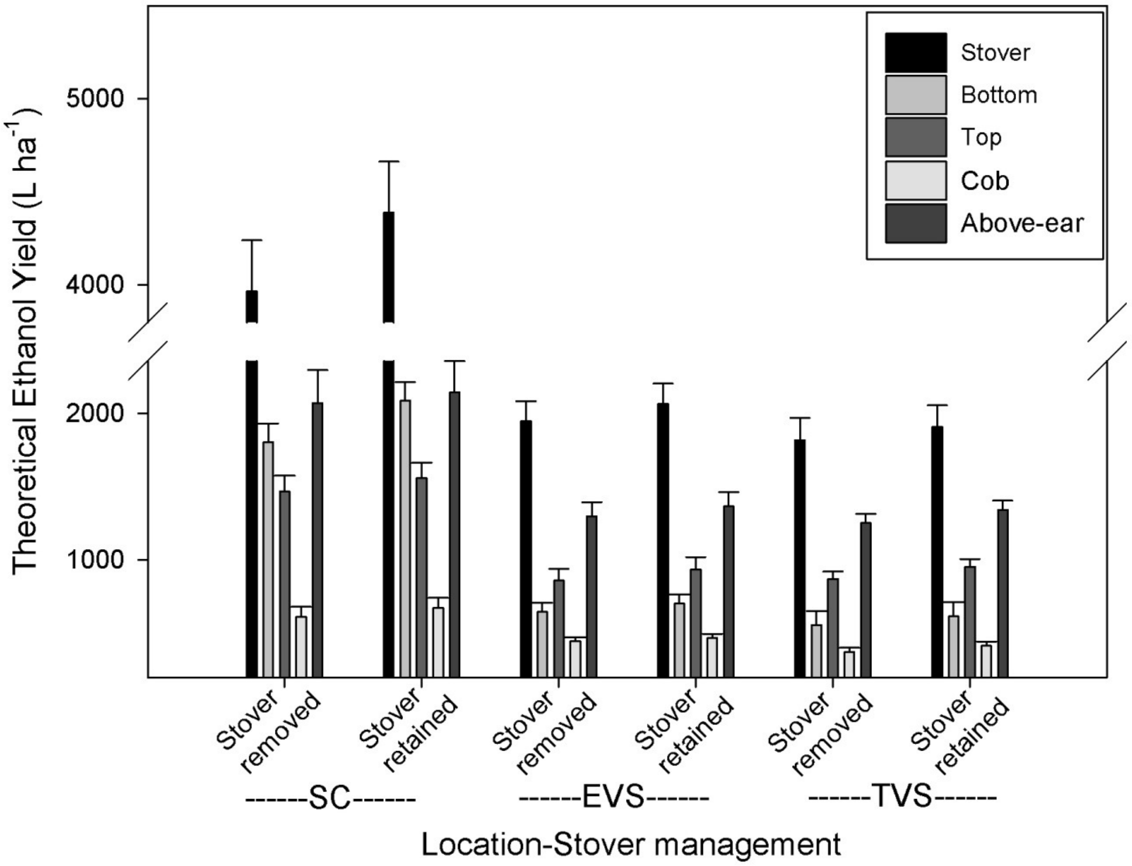

Figure 15. Average theoretical ethanol yield from different corn plant fractions as affected by three years of corn stover management at the Pee Dee Research and Education Center in South Carolina (SC), the E.V. Smith Research and Extension Center (EVS) in central Alabama and the Tennessee Valley Research and Extension Center (TVS) in northern Alabama. The error bars represent the standard error of the mean.

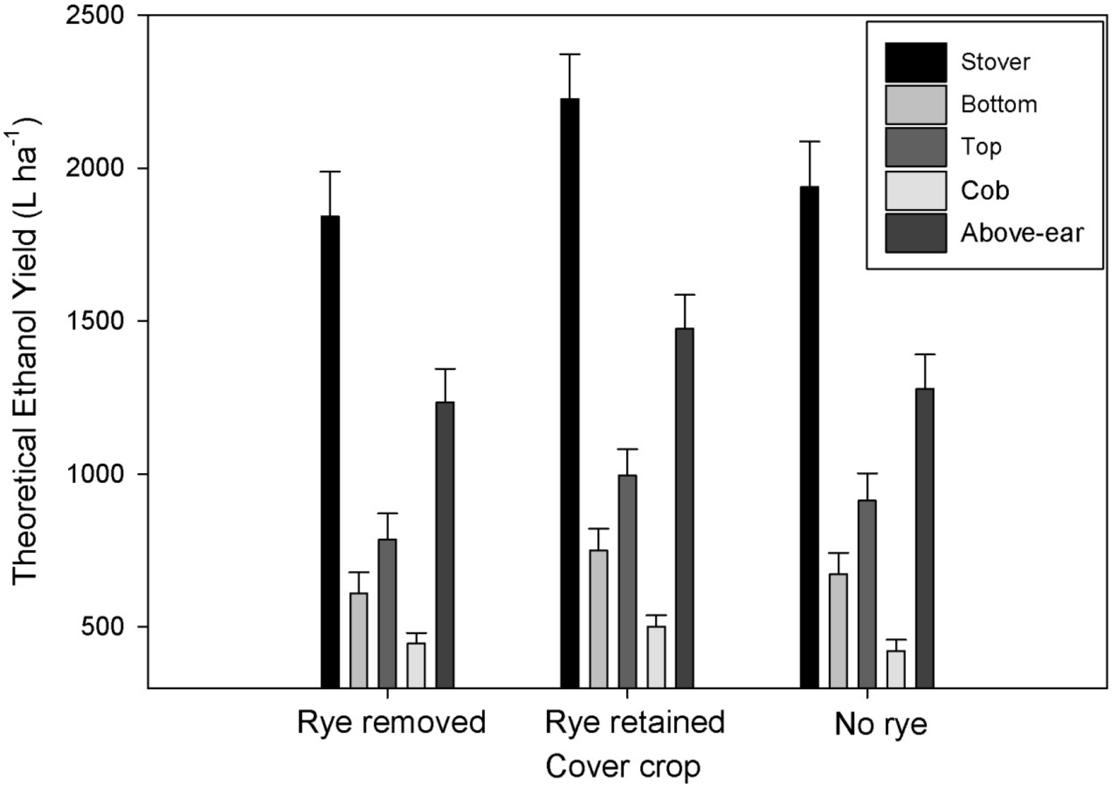

Figure 16. Average theoretical ethanol yield from different corn plant fractions as affected by three years of rye management at EVS - E.V. Smith Research Center near Shorter in Central Alabama. The error bars represent the standard error of the mean.

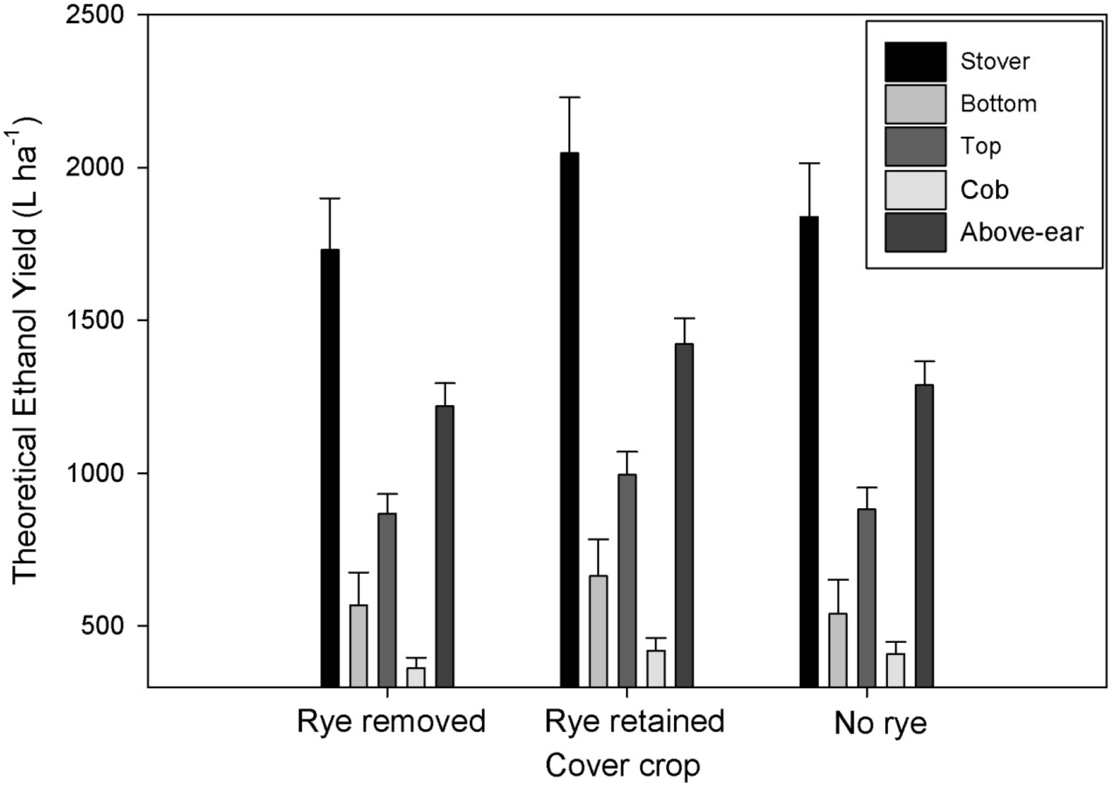

Figure 17. Average theoretical ethanol yield from different corn plant fractions as affected by three years of rye management at TVS - Tennessee Valley Research and Extension Center near Belle Mina in Northern Alabama. The error bars represent the standard error of the mean.

VII. Soil Carbon and Nitrogen Dynamics as Affected by Rye Cover Crop and Corn Stover Management on Two Soil Types in the Southeastern US

Abstract

Biofuel production from plant biomass is a solution being pursued to mitigate fossil fuel use and reduce greenhouse gas emissions. Corn (*Zea mays* L.) is a highly promising crop for biomass production. However, stover harvest could negatively impact soil properties since it would change the quantity of residue returned to the soil, and affect soil C (carbon) and N (nitrogen). The objective of this study was to investigate the three-year cumulative effect of rye (*Secale cereale*) as winter cover crop (no rye, residue removed and residue retained) and corn residue management (0 and 100% harvested) on soil C and N dynamics for two soil types. Soil samples were collected from two locations with different soil types, a loamy sand and a silt loam in central and northern Alabama respectively. A laboratory incubation experiment was performed in which soil samples were analyzed for total C, N, inorganic N, and CO_2-C evolved during a 60 day period. Carbon and N content in the silt loam (1.3% and 0.1%, respectively) were significantly higher than in the loamy sand (0.6% and 0.05%, respectively). Rye as winter cover crop did not affect C and N dynamics at either location. Soil C content (1.2%) was significantly lower with stover harvest than without (1.4%). For both soil types, N mineralization increased significantly during the 60 day incubation period. However, C mineralization did not vary between 30 and 60 days of incubation at either location. Nevertheless, C turnover seemed to be higher in the loamy sand than the silt loam. Results from this study suggest that differences in

C and N dynamics due to the use of a rye cover crop and corn stover management are soil dependent.

Introduction

Carbon is known to be the major constituent for life on earth. It can be found in various forms in the environment, including atmospheric CO_2, geological deposits and plant biomass. Carbon dioxide is one of the major factors causing climate change. Agriculture can play an important role in mitigation of greenhouse gas emissions. Soils contain the largest amount of carbon on land. Plants also store carbon in their tissue through photosynthesis. As plant material decomposes carbon is stored in the soil in the form of soil organic carbon (SOC). Conservation tillage practices (no plow, cover crops, and others) can promote and enhance carbon storage in the soil. However, removing plant residues for biofuel production can be detrimental to SOC stocks and affect soil C dynamics.

Crop residues are of importance for the N cycle in soils because they can create N immobilization issues that affect microbial growth, enzyme synthesis and other nutrient mineralization (Cayuela et al., 2009). Additionally, incorporation of crop residues can stimulate a growth in microbial population and activity. It is expected that crop residues left in the field can promote SOC formation. When plant material with a C:N ratio greater than 20:1 is added to the soil, then N immobilization will likely occur during the first weeks of decomposition (Green and Blackmer, 1995). In a study conducted in a clay loam, Gregorich et al. (1996) stated that up to 20% of corn residues can be converted to organic carbon. A more recent study conducted in a silt loam soil by Blanco-Canqui and Lal (2007b), reported that almost one third of the carbon from wheat residues was converted to SOC during the 10 years of the experiment. The same authors in another study reported that stover removal from long-term no tillage corn cultivation had a negative impact on near surface soil properties like SOC sequestration and compaction levels over a period of 2.5 years (Blanco-Canqui and Lal, 2007a). They also concluded that low stover removal rates of just 25% have been shown to reduce SOC, plant available water, increased soil strength and decreased crop yields. Banziger et al. (1999) suggested that approximately one-third of the total plant N sequestered in stover would have to be replenished by fertilizer application if the stover is harvested, but this would depend on the amount of stover removed. In a long term study conducted in sandy soils, Whalen et al. (2010) showed that a 16 year corn residue management had marginal effect on soil organic carbon and in total N. Results from another study indicated that complete harvest of crop residues could decrease SOM content and increase soil erosion (Mann et al., 2002).

Several studies have concluded that up to two-thirds of corn stover could be removed in some corn growing regions of the U.S. without a significant impact on SOM content, if sustainable practices, such as no-till, were used (Wilhelm et al., 2004; Perlack et al., 2005; Johnson et al., 2006; Graham et al., 2007). However, there are several studies that support that even limited removal of crop residues can lead to a reduction of SOM content (Buyanovsky and Wagner, 1997; Clapp et al., 2000).

There is significant information in the literature on soil C and N mineralization. However, it appears that different studies report various results on soil C and N dynamics even when examining the effect of similar factors. This variability could be a result of the differences in soil types as well as the locations of the studies. The objective of this study was to determine soil C and N dynamics, as well as C turnover, on two soil types (silt loam vs. loamy sand) after three years of using rye as winter cover crop and corn stover harvest.

Material and Methods

Site description

The experiment was conducted at two locations in Alabama with different soil types. The first location was the E.V. Smith Research Center near Shorter in central Alabama (32.42884 N, -85.890235 W). The second location was in the northern part of the state, the Tennessee Valley Research and Extension Center in Belle Mina (34.687953 N, -86.886763 W). The soil at E.V. Smith Research Center (EVS) was a Compass loamy sand (Coarse-loamy, siliceous, subactive, thermic Plinthic Paleudults) on a 1-3% slope. The range of water content at field capacity for this soil was 0.07-0.10 g g^{-1}. At Tennessee Valley Research and Extension Center (TVS) the soil was a Decatur silt loam (Fine, kaolinitic, thermic Rhodic Paleudults).The slope at this site was 1-2% and the water content range at field capacity was 0.17-0.24 g g^{-1}. The range in water content at field capacity for each soil type was estimated using the Soil Water Characteristics calculator version 6.02.70 (Saxton and Rawls, 2006).

Soil samples were collected from an already established experiment that had a three year history of winter cover crop and corn stover residue management. Treatments included the use of cereal rye as a winter cover as the main plot (no cover, rye removed in spring and rye retained), and corn stover residue removal as the sub-plot (0 and 100% removal). Treatments were arranged in a split-plot design with three replications at each location. The plots consisted of four rows (91-cm row spacing) and they were 6.1m long by 2.7m wide. Both locations were under non-irrigated continuous no-tillage corn production. The nitrogen fertilizer used was urea ammonium nitrate (UAN 28-0-0) at rate of 168 kg N ha^{-1}, with the total amount applied in two equal portions early in the growing season, approximately two and five weeks after planting. Corn residue removal was performed every year after grain harvest early in the fall. Corn was planted in late-March to early-April, and cultural practices were performed according to Alabama Cooperative Extension Service recommendations (ACES, 1994).

Soil sampling and analysis

Soil was sampled in order to investigate the cumulative effect of three years' (2008-2010) rye use as a cover crop and corn residue removal on two soil types in Alabama. The sampling took place in late spring of 2011, approximately seven months after the last corn residue removal. The samples were collected at 0-15 cm depth from the two middle rows of each plot with a stainless steel soil core sampler (15 cm long, 3.8 cm diameter). Three soil cores were taken between the two middle rows and three cores were taken from the two middle rows from each plot. These six cores were mixed together to form a composite soil sample from each plot. All the samples were immediately refrigerated at 2 °C until the initiation of the laboratory incubation.

In order to determine the potential C and N mineralization, similar methods described by Prior et al. (2008) were used. Before the incubation, the samples were ground through a 2 mm sieve and analyzed for total C and N with a LECO^{R} TruSpec C/N analyzer (Leco Corp., St. Joseph, MI). A 2 M KCl extraction was performed on the soil samples to determine soil inorganic N (NO_3^- and NH_4^+). The extract was analyzed colorimetrically with an Autoanalyzer 3 Bran+Luebbe (SPX Flow Technology, Delavan, WI) segment and flow injection analyzer.

Incubation procedure

The gravimetric water content (GWC) of every sample was determined prior to adjusting the moisture content of each sample Samples from the two soil types had differences in moisture content so adjustments were made by soil type. At EVS (Compass loamy sand) the highest GWC was 4.3% so the water content of every sample was adjusted to 10% GWC, which was the upper limit of the FC for this soil. At TVS (Decatur silt loam), the highest GWC was 22.9% and the water content of every sample was adjusted to the upper limit of the FC of 24% GWC. Subsequently, a 25 g soil sub-sample was placed in a plastic specimen cup (125 mL size) and the moisture adjusted to the predetermined FC level for each soil type by adding de-ionized water. Plastic cups with soil were placed individually in 1 L sealed glass Mason jars. Each soil sample in the Mason jars was done in duplicates to allow for sampling after 30 and 60 days of incubation. A small quantity of water was added at the bottom of each jar to maintain adequate humidity in the head space of sealed Mason jar and reduce soil desiccation during the incubation period. Finally, a vial containing 10 ml of 1 M NaOH was placed in every jar to serve as a CO_2 trap. The jars were placed in an incubator at 25 °C and incubated in the dark. Twelve jars that contained only CO_2 traps without soil in the plastic cups were also included during the incubation to serve as blanks. The jars were placed in the incubator in a completely randomized manner. The first set of jars was removed from the incubator after 30 days (day 30) and the second set after 60 days (day 60). The CO_2-C evolved was determined by titrating the NaOH traps with 0.25 M HCl in the presence of 1 M $BaCl_2$. The potential C mineralization was calculated by subtracting the CO_2-C in the blanks from the soil samples. The potential N mineralization was calculated by subtracting the initial inorganic N (day 0) from the 30 and 60 days values, respectively. Carbon turnover was calculated by dividing the potential C mineralization at every incubation period by the initial total organic C in the soil.

Statistical analysis

Analysis of variance was used in order to detect differences in soil C and N content as affected by the rye cover crop and corn residue removal treatments. Repeated measures analysis of variance with auto-correlated errors was used to detect differences in C and N mineralization rates during the incubation period. The MIXED procedure was used in SAS 9.3 (SAS for Windows v. 9.3, SAS Institute Inc., Cary, NC) to perform these analyses and a factor was considered significant at level lower than 0.10 (alpha < 0.10).

Results and Discussion

C and N dynamics between locations

The overall cumulative impact of three-years corn residue removal on C and N content and mineralization rates are shown in Table 39. The C content in EVS was 0.6%, which was significantly lower than the 1.3% in TVS ($p = 0.0003$). Analogous results were observed in N contents between the two soil types (Table 39 and Figure 18). The C:N ratio was greater in the silt loam plots than the loamy sand (Table 39; $p = 0.0063$).

Net N mineralization occurred during the 30 and 60 days of incubation in both EVS and TVS. The amount of inorganic N before the incubation varied significantly between the two locations (Table 39; $p = 0.0051$). After 30 days of incubation, the silt loam soil exhibited higher N mineralization rates than the loamy sand ($p = 0.0347$); however, the difference between the two locations after 60 days was not significant ($p = 0.7855$). The amount of N mineralized from day 0 to day 60 increased significantly at EVS. At TVS, a significant increase was observed for the first 30 days of incubation only (Table 39 and Figure 19).

Carbon mineralization rates did not vary between the two soils (Table 39). Although the differences between locations were not significant, there was a trend of greater C mineralization rates in TVS than EVS. This was expected due to the higher C content of the silt loam compared to the loamy sand. Furthermore, no differences were observed between 30 and 60 days of incubation in both loamy sand and silt loam. At EVS, 3.4 and 3.7% of the initial C was mineralized after 30 and 60 days of incubation respectively. At TVS, 2.0 and 2.1% of the initial C was mineralized after 30 and 60 days of incubation respectively. However, the loamy sand in EVS had significantly lower C content than that of the silt loam in TVS. Intuitively, the amount of CO_2 evolved will be different between the two locations due to different total organic carbon (TOC) content. Therefore, standardization of C losses was performed by dividing the C mineralization rate by the TOC for each soil type to make a C dynamics comparison between these soils more meaningful. The C turnover was significantly different between EVS and TVS at both incubation periods (Table 39). For the first 30 days of incubation, the C turnover in EVS was 36.3 mg kg^{-1} while in TVS it was 20.6 mg kg^{-1} of soil ($p = 0.0506$). Thirty days later, or 60 days after the initiation of the incubation, the loamy sand also exhibited greater C losses than the silt loam (Figure 20). These differences indicate the higher vulnerability of the loamy sand to C losses compared to the silt loam due to the lower clay content.

The differences in C and N dynamics between the two locations were attributed to differences in soil texture. The higher clay content of the silt loam in TVS assists in higher C retention and subsequently lower C losses compared to the loamy sand in EVS.

Rye and corn stover management effect on C and N dynamics

Rye yields were consistently greater at EVS compared to TVS every year the experiment was conducted. Overall, rye yield at EVS ranged between 1503-6275 kg ha^{-1} with a three-year average of 3343 kg ha^{-1}. At TVS, the corresponding yield range was 1937-3281 kg ha^{-1} with an average of 2475 kg ha^{-1}.

Winter weeds grew in plots assigned to no rye treatment. This weed biomass was measured at the same time rye biomass was determined since it can have an effect on corn productivity. At EVS, weed biomass ranged between 900-1200 kg ha^{-1} with a three-year average yield of 1030 kg ha^{-1}, while at TVS yields ranged between 370-880 kg ha^{-1} with an average yield of 625 kg ha^{-1}.

The effect of rye was not significant on C and N content, and C:N ratio at either location (Table 40 and 41). At EVS, corn stover management did not have a significant effect on C and N contents (Table 40). However, at TVS, where corn stover was retained, the C content was greater compared to 100% removal (Table 41). These variable responses to cultivation practices between the two locations could be explained due to the differences in texture between the two soil types. At EVS, the soil is a loamy sand while at TVS is a silt loam. Sandy soils are known to have limited ability to accumulate and store SOC compared to clay soils (Laganière et al., 2009). Similar results were reported in a long-term study in which 16 years of corn residue management in a sandy loam had only a marginal effect on SOC (Whalen et al., 2010).

At EVS none of the treatments had a significant effect on C:N ratio (Table 40). The low clay content in the loamy sand could be the reason for the lack of significance. However, corn stover harvest had a significant effect on C:N ratio at TVS (Table 41; $p < 0.0007$). Plots where corn residue was not harvested exhibited higher C:N than plots with 100% stover removal. An explanation for the observed response at TVS could be the high C:N (~60:1) of the corn residue that decomposed in the soil for three years. However, it is surprising that three years decomposition of rye residue, which has also a high C:N ratio, did not alter significantly the C:N ratio at either soil type. It is possible that three years of rye cultivation were not enough to increase significantly the low C content of the soil types in this study.

At EVS, the use of rye had a significant effect on inorganic N before the incubation was initiated at 0 days (Table 40; $p = 0.0644$). Plots without a rye cover crop had the highest amount of inorganic N. This was somewhat expected due to the relatively high C:N ratio of the rye residue. N mineralization increased significantly with increasing incubation time under all treatments at EVS. However, no other significant effect was observed on inorganic N (0 days) or N mineralization at 30 and 60 days of incubation (Table 40). At TVS, neither the use of rye nor stover management had a significant effect on N mineralization (Table 41). Similarly, incubation time did not have a significant effect on N mineralization for any of the treatments, except rye removed and stover retained after 30 days of incubation.

The effects of cover crop and stover management on C mineralization were not significant at either location (Tables 40 and 41). However, a trend of higher C mineralization was observed after 60 days of incubation where corn stover was retained at both locations (Tables 40 and 41). Three years of corn residue decomposition in the soil seemed to increase microbial activity due to the higher amounts of CO_2 that evolved from plots where no residue removal was performed. This trend is in agreement with the results reported by Whalen et al. (2010). Likewise, Tian et al. (2011) reported that the retention of residue in the field increased the potential C mineralization compared to when residue was harvested. However, C turnover did not vary significantly among the levels of rye treatment, nor between the two levels of corn stover removal for either soil type (Table 40 and 41). It appears that three years of rye cultivation and corn stover management is not enough to alter C losses rates significantly in the examined soils. A possible explanation could be the low C content of these soils that makes it more difficult to detect changes in the short term. Overall, the relatively short duration of the experiment, in combination with low rye biomass yields that result in low C inputs to the soil, were not adequate to alter significantly C and N dynamics of both soil types.

Conclusions

Carbon and nitrogen are basic and important components in agricultural systems, and their role in soil and crop productivity is essential. It is conceivable that in the near future corn stover will be harvested as a feedstock for biofuel production. However, removing corn stover for biofuel production can affect soil C turnover and stocks. Therefore, development of sustainable stover removal practices is necessary. Results from this study indicate that C mineralization was greater in a silt loam than a loamy sand soil. However, C losses in term of C turnover over a short period of time were greater in the loamy sand than the silt loam soil. The silt loam exhibited a greater amount of inorganic N and mineralization rates when compared to the loamy sand. The use of rye as winter cover crop in this study did not affect C and N dynamics at either soil type. Three years of 100% corn residue removal caused significant differences on C content only in the silt loam soil. This can be attributed to greater initial C and N contents in the silt loam relative to the low contents in the loamy sand, which made the changes more apparent over a short period of time. According to the results from this experiment, a generalized assessment of the corn stover harvest effect on soil productivity can lead to incorrect conclusions if the soil type is not considered. Results from this study suggest that recommendations for the management of a rye as winter cover crop and corn stover removal should be location-specific. Additionally, it should be noted that despite the lack of significant effects from the different rye management schemes and stover harvest on soil C and N dynamics, changes in soil properties can occur over long period. Long-term studies can reveal impacts of greater magnitude than those that were detected in this three year experiment.

Table 39. Overall mean soil carbon (C) and nitrogen (N) contents, mineralization rates, and C turnover differences between E.V. Smith Research Center near Shorter in Central Alabama (EVS) and Tennessee Valley Research and Extension Center near Belle Mina in Northern Alabama (TVS).

Incubation Period	Factor	EVS	TVS	†Pr > F
-- day --		%		
0	Total C	0.6	1.3	0.0003
0	Total N	0.05	0.1	≤ 0.0001
0	C:N	13.0:1	10.4:1	0.0063
		mg kg^{-1}		
0	Initial inorganic N	6.1 a‡	13.2 a	0.0051
30	Inorganic N mineralized	12.1 b	17.9 b	0.0347
60	Inorganic N mineralized	18.8 c	19.7 b	0.7855
30	C mineralization	214.9 a	266.9 a	0.4045
60	C mineralization	233.8 a	278.1 a	0.2475
30	C turnover	36.3 a	20.6 a	0.0506
60	C turnover	38.3 a	21.1 a	0.0356

† Probability of a greater F by chance between locations for a particular factor.

‡ Means of the same factor within a column between incubation days that are followed by the same letter are not significantly different at the 0.10 level. Separation of means was achieved using the Tukey adjustment for multiple comparisons.

Table 40. Mean soil carbon (C) and nitrogen (N) contents, mineralization rates, and C turnover as affected by three years use of rye as a cover crop and corn stover removal in E.V. Smith Research Center near Shorter in Central Alabama.

Incubation Period	Factor	Cover crop				Stover management		
		Rye removed	Rye retained	No rye	†Pr > F	Stover removed	Stover retained	‡Pr > F
-- day --			%			%		
0	Total C	0.6	0.6	0.7	0.7423	0.6	0.7	0.2495
0	Total N	0.05	0.05	0.05	0.7127	0.05	0.05	0.2240
0	C:N	12.1:1	13.2:1	13.7:1	0.3831	13.0:1	13.1:1	0.8843
			$mg\ kg^{-1}$			$mg\ kg^{-1}$		
0	Inorganic N	5.4 a§	4.9 a	8.1 a	0.0644	5.5 a	6.7 a	0.1860
30	N mineralization	13.5 b	10.8 b	12.2 b	0.2267	11.9 b	12.4 b	0.5934
60	N mineralization	18.8 c	18.1 c	19.6 c	0.9244	19.1 c	18.6 c	0.7913
30	C mineralization	172.8 a	265.3 a	206.4 a	0.4199	230.8 a	199.0 a	0.5592
60	C mineralization	276.9 a	228.1 a	196.2 a	0.4427	217.0 a	250.5 a	0.4944
30	C turnover	31.9 a	41.1 a	31.9 a	0.5457	42.3 a	30.3 a	0.2871
60	C turnover	45.8 a	38.0 a	31.2 a	0.5743	39.2 a	37.4 a	0.6485

† Probability of a greater F by chance among cover crop levels.

‡ Probability of a greater F by chance between 0 and 100% corn stover removal.

§ Means of the same factor within a column between incubation days that are followed by the same letter are not significantly different at the 0.10 level. Separation of means was achieved using the Tukey adjustment for multiple comparisons.

Table 41. Mean soil carbon (C) and nitrogen (N) contents, mineralization rates, and C turnover as affected by three years use of rye as a cover crop and corn stover removal in Tennessee Valley Research and Extension Center near Belle Mina in Northern Alabama.

Incubation days	Factor	Cover crop				Stover management		
	Level	Rye removed	Rye retained	No rye	†Pr > F	Stover removed	Stover retained	±Pr > F
		——— % ———				——— % ———		
0	Total C	1.4	1.3	1.3	0.9083	1.2	1.4	0.0283
0	Total N	0.1	0.1	0.1	0.8929	0.1	0.1	0.6166
0	C:N	10.4:1	10.3:1	10.4:1	0.9924	9.8:1	10.9:1	0.0007
		——— mg kg^{-1} ———				——— mg kg^{-1} ———		
0	Inorganic N	12.5 a§	14.3 a	12.8 a	0.8028	11.4 a	15.0 a	0.1734
30	N mineralization	18.3 a b	16.5 a	18.9 a	0.8524	16.4 a	19.9 a b	0.2575
60	N mineralization	24.0 b	16.0 a	19.8 a	0.6368	16.5 a	23.0 b	0.2700
30	C mineralization	246.7 a	232.8 a	321.2 a	0.2882	249.3 a	284.5 a	0.4288
60	C mineralization	303.3 a	281.7 a	249.4 a	0.6885	252.2 a	304.0 a	0.1893
30	C turnover	18.2 a	17.7 a	25.8 a	0.2679	20.02 a	21.1 a	0.7800
60	C turnover	22.4 a	22.0 a	19.0 a	0.6454	20.32 a	21.9 a	0.5837

† Probability of a greater F by chance among cover crop levels.

± Probability of a greater F by chance between 0 and 100% corn stover removal.

§ Means of the same factor within a column between incubation days that are followed by the same letter are not significantly different at the 0.10 level. Separation of means was achieved using the Tukey adjustment for multiple comparisons.

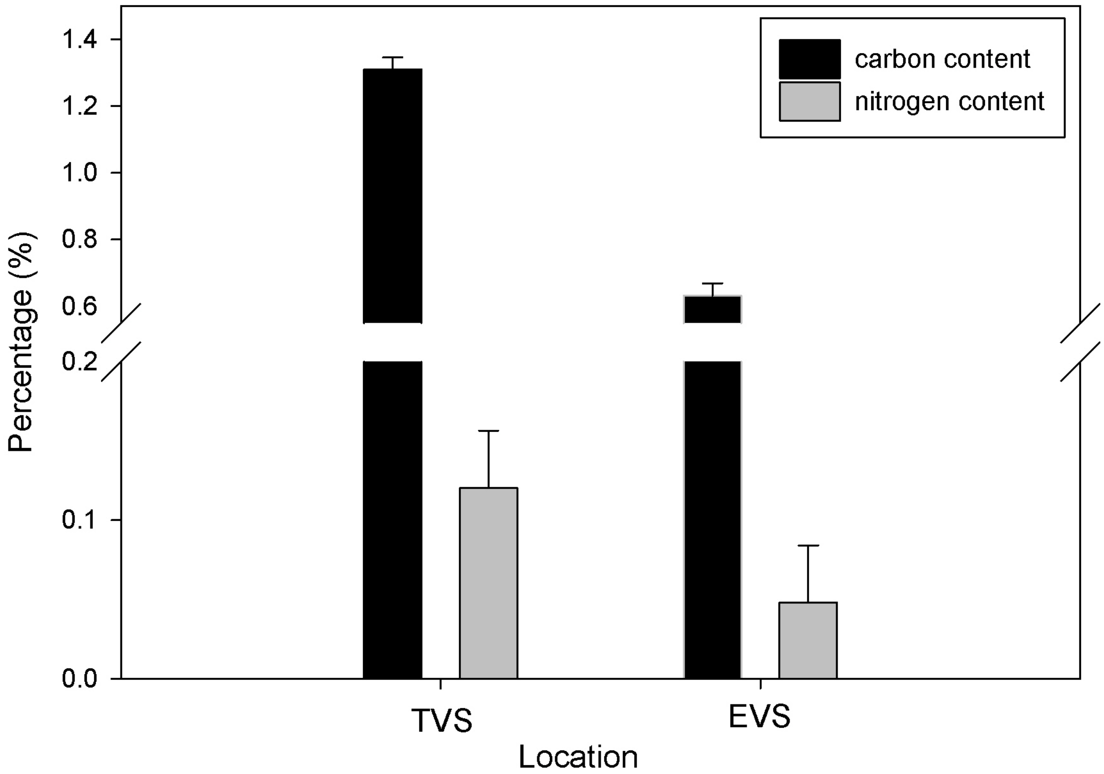

Figure 18. Soil carbon and nitrogen content at the central and northern Alabama sites. The error bars represent the standard error of the mean. EVS - E.V. Smith Research Center near Shorter in Central Alabama (Compass loamy sand); TVS - Tennessee Valley Research and Extension Center near Belle Mina in Northern Alabama (Decatur silt loam).

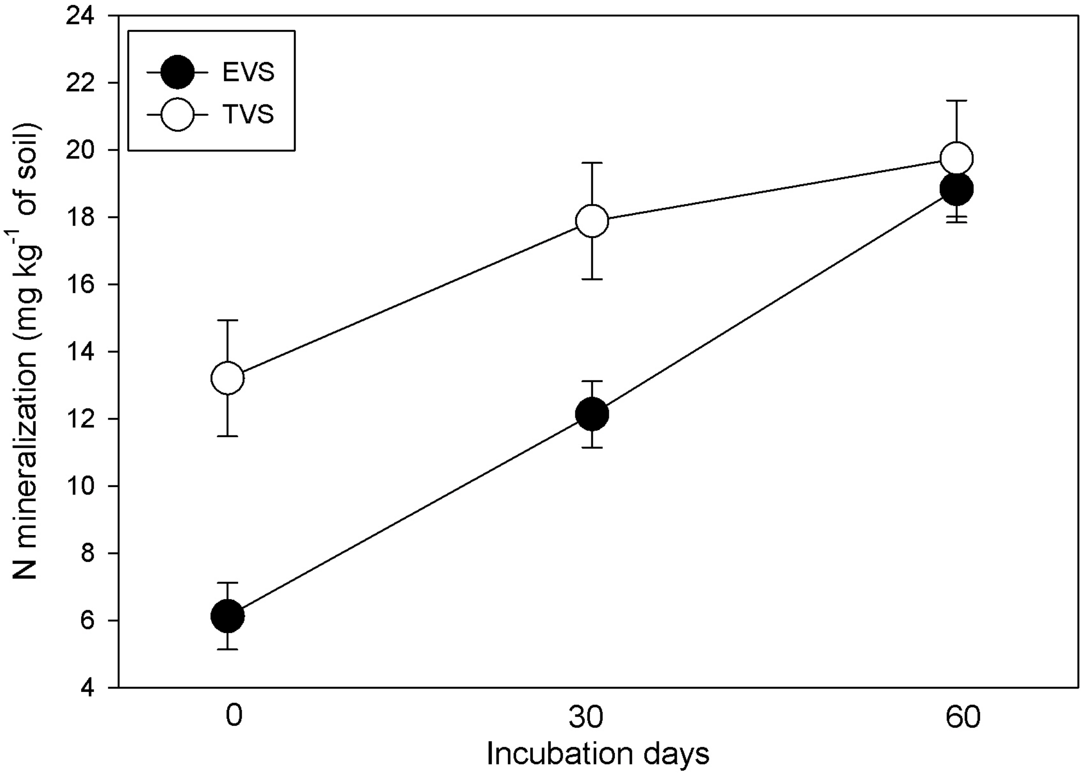

Figure 19. Soil nitrogen mineralization at the central and northern Alabama sites. The error bars represent the standard error of the mean. EVS - E.V. Smith Research Center near Shorter in Central Alabama (Compass loamy sand); TVS - Tennessee Valley Research and Extension Center near Belle Mina in Northern Alabama (Decatur silt loam).

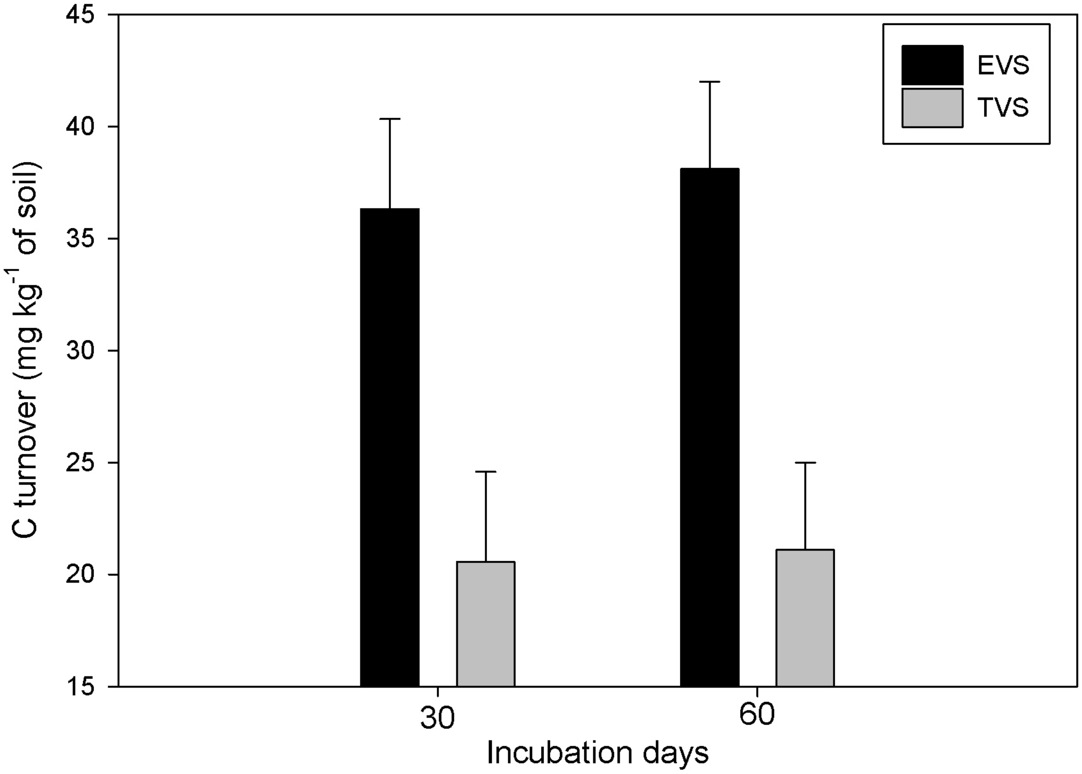

Figure 20. Soil carbon turnover at the central and northern Alabama sites. The error bars represent the standard error of the mean. EVS - E.V. Smith Research Center near Shorter in Central Alabama (Compass loamy sand); TVS - Tennessee Valley Research and Extension Center near Belle Mina in Northern Alabama (Decatur silt loam).

VIII. Conclusions

Part of this study involved research that attempted to develop statistical models that provide estimates of corn grain and biomass yield, as well as bioethanol potential, early in the growing season. These successful attempts resulted in the development of models that can be used and further expanded by farmers and related industries to the level that serve their interests. Additionally, a simplified, fast, and inexpensive procedure for carbohydrates and theoretical ethanol yield estimation was developed which could be used as an alternative to the NREL method.

The major part of this work involved optimization of grain and biomass yield, and biofuel potential in the southeastern US. It seems that when the goal is grain production, the use of rye as winter cover crop has the potential to increase yields in Alabama. When the goal is bioethanol production from corn stover, results from this study indicate that high biomass yield is the most important factor. Similarly to the grain yield, a rye cover crop can increase biomass yield and the biofuel potential.

Despite the increasing interest of biofuels, significant challenges remain before widespread production will occur. One of the most important issues is the deterioration of soil quality due to stover harvest and the impacts on corn grain yield. Results from this work suggest that apart from using rye as winter cover crop, there may be advantages in harvesting the above-ear biomass when compared to total stover removal in that:

1. The above-ear portion can lead to high bioethanol potential in the southeastern US (60-69% of the total stover).

2. It can significantly limit the removal rates of C, N and macronutrients when compared to total stover harvest.

3. It can result in biofuel feedstock with the most desirable characteristics for bioethanol production (e.g., lowest lignin concentration) when compared to other stover partitions.

Furthermore, these data suggest that there is no need to change N fertilization rates in Alabama when stover harvest is performed, even when retaining a rye cover crop in the field. Nevertheless, long-term monitoring of the N dynamics in the soil is necessary to ensure that appropriate amount of N will be available to the cash crop.

As in any type of research there are limitations to the extent that the results apply to the real world. The main limitation of this study is the relatively short duration. It lasted for three years and therefore, the results presented should be assumed as short-term. Generalizations of the results reported in this dissertation to corn grown in locations with different soil types, weather conditions, and under different cultivation practices should be avoided. Due to the high variability of the agricultural systems, long-term multi-location studies are necessary to further evaluate the sustainability of corn for simultaneous cellulosic biofuel and grain production.

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Appendices

Appendix A: Grain prediction models for individual locations and years

Table A1. Model for corn grain yield prediction (kg ha^{-1}) using parameters and information collected at R1 growth stage at EVS in 2009.

†Variable	Coefficient	Pr>F	‡VIF
Intercept	-6017.614	0.0006	0
N	11.33	<0.0001	1.56
Ph	36.53	0.0008	2.34
Eh*C	0.00085	0.0002	3.03

† C - corn ears ha^{-1}; N - fertilizer N rate in kg ha^{-1}; Ph - plant height in cm; Eh - height of the first ear in cm.

‡ Variance Inflation Factor

Table A2. Model for corn grain yield prediction (kg ha^{-1}) using parameters and information collected at R1 growth stage at EVS in 2010.

†Variable	Coefficient	Pr>F	‡VIF
Intercept	-589.547	0.1440	0
N	6.644	<0.0001	1.32
Eh*C	0.00085	<0.0001	1.32

† C - corn ears ha^{-1}; N - fertilizer N rate in kg ha^{-1}; Eh - height of the first ear in cm.

‡ Variance Inflation Factor

Table A3. Model for corn grain yield prediction (kg ha^{-1}) using parameters and information collected at R1 growth stage at EVS in 2011.

†Variable	**Coefficient**	**Pr>F**	**‡VIF**
Intercept	-121293	<0.0001	0
S20*S40	16.408	<0.0001	1.94
Ph/Eh	2953.148	0.0001	1.24
N*C	0.000152	<0.0001	1.47
Eh*C	0.00108	<0.0001	1.91

† C - corn ears ha^{-1}; N - fertilizer N rate in kg ha^{-1}; S20 - stem diameter at 20cm of height in mm; Ph - plant height in cm; Eh - height of the first ear in cm.

‡ Variance Inflation Factor

Table A4. Model for corn grain yield prediction (kg ha^{-1}) using parameters and information collected at R1 growth stage at TVS in 2009.

†Variable	Coefficient	Pr>F	‡VIF
Intercept	-2633.39	0.0014	0
Eh	123.339	<0.0001	1.57
N*C	0.000249	<0.0001	1.57

† C - corn ears ha^{-1}; N - fertilizer N rate in kg ha^{-1}; Eh - height of the first ear in cm.

‡ Variance Inflation Factor

Table A5. Model for corn grain yield prediction (kg ha^{-1}) using parameters and information collected at R1 growth stage at TVS in 2010.

†Variable	Coefficient	Pr>F	‡VIF
Intercept	-4291.4815	0.0003	0
Ph	31.465	<0.0001	1.85
N*C	0.0001805	<0.0001	1.85

† C - corn ears ha^{-1}; N - fertilizer N rate in kg ha^{-1}Ph - plant height in cm;
‡ Variance Inflation Factor

Table A6. Model for corn grain yield prediction (kg ha^{-1}) using parameters and information collected at R1 growth stage at TVS in 2011.

†Variable	Coefficient	Pr>F	‡VIF
Intercept	-1154.452	<0.0001	0
Ph*Eh	0.1478	<0.0001	1.37
N*C	0.000228	<0.0001	1.37

† C - corn ears ha^{-1}; N - fertilizer N rate in kg ha^{-1}; Ph - plant height in cm; Eh - height of the first ear in cm.

‡ Variance Inflation Factor

Appendix B: Stover prediction models for individual locations and years

Table B1. Model for corn stover yield prediction (kg ha^{-1}) using parameters and information collected at R1 growth stage at EVS in 2009.

†Variable	Coefficient	Pr>F	‡VIF
Intercept	-1506.837	0.0025	0
Eh*C	0.000833	<0.0001	1.05
S0/(Eh*Ph)	2.81	<0.0001	1.05

† C - corn ears ha^{-1}; S0 - stem diameter at the base of the plant in mm; Ph - plant height in cm; Eh - height of the first ear in cm.

‡ Variance Inflation Factor

Table B2. Model for corn stover yield prediction (kg ha^{-1}) using parameters and information collected at R1 growth stage at EVS in 2010.

†Variable	**Coefficient**	**Pr>F**	**‡VIF**
Intercept	-141.942	0.5833	0
Eh*C	0.000858	<0.0001	1.00

† C - corn ears ha^{-1}; Eh - height of the first ear in cm.

‡ Variance Inflation Factor

Table B3. Model for corn stover yield prediction (kg ha^{-1}) using parameters and information collected at R1 growth stage at EVS in 2011.

†Variable	Coefficient	Pr>F	‡VIF
Intercept	-1709.766	0.0184	0
C	0.072	<0.0001	1.01
S20*S40	19.914	<0.0001	1.25
S20/Ph	-41029	0.0001	1.26

† C - corn ears ha^{-1}; S0 - stem diameter at the base of the plant in mm; S20 - stem diameter at 20cm of height in mm; S40 - stem diameter at 40cm of height in mm; Ph - plant height in cm;
‡ Variance Inflation Factor

Table B4. Model for corn stover yield prediction (kg ha^{-1}) using parameters and information collected at R1 growth stage at TVS in 2009.

†Variable	Coefficient	Pr>F	‡VIF
Intercept	-1380.207	0.0053	0
Ph	14.4359	0.0001	1.43
Ph*C	0.000763	<0.0001	1.43

† C - corn ears ha^{-1}; Ph - plant height in cm;

‡ Variance Inflation Factor

Table B5. Model for corn stover yield prediction (kg ha^{-1}) using parameters and information collected at R1 growth stage at TVS in 2010.

†Variable	Coefficient	Pr>F	‡VIF
Intercept	-2792.5828	<0.0001	0
C	0.05931	<0.0001	1.25
$[(S0*S20)/Eh]^2$	85.905	<0.0001	1.08
Ph*Eh	0.1358	<0.0001	1.19

† C - corn ears ha^{-1}; S0 - stem diameter at the base of the plant in mm; S20 - stem diameter at 20cm of height in mm; Ph - plant height in cm; Eh - height of the first ear in cm.

‡ Variance Inflation Factor

Table B6. Model for corn stover yield prediction (kg ha^{-1}) using parameters and information collected at R1 growth stage at TVS in 2011.

†Variable	Coefficient	Pr>F	‡VIF
Intercept	-86.635	0.0714	0
Eh^2	0.1801	0.0002	3.04
Eh*C	0.000532	<0.0001	3.38
N^3	0.00008537	<0.0001	1.25

† C - corn ears ha^{-1}; N - fertilizer N rate in kg ha^{-1}; Eh - height of the first ear in cm.

‡ Variance Inflation Factor

Appendix C: Plant populations and harvest indexes at the central and northern Alabama sites for the winter cover crop treatments

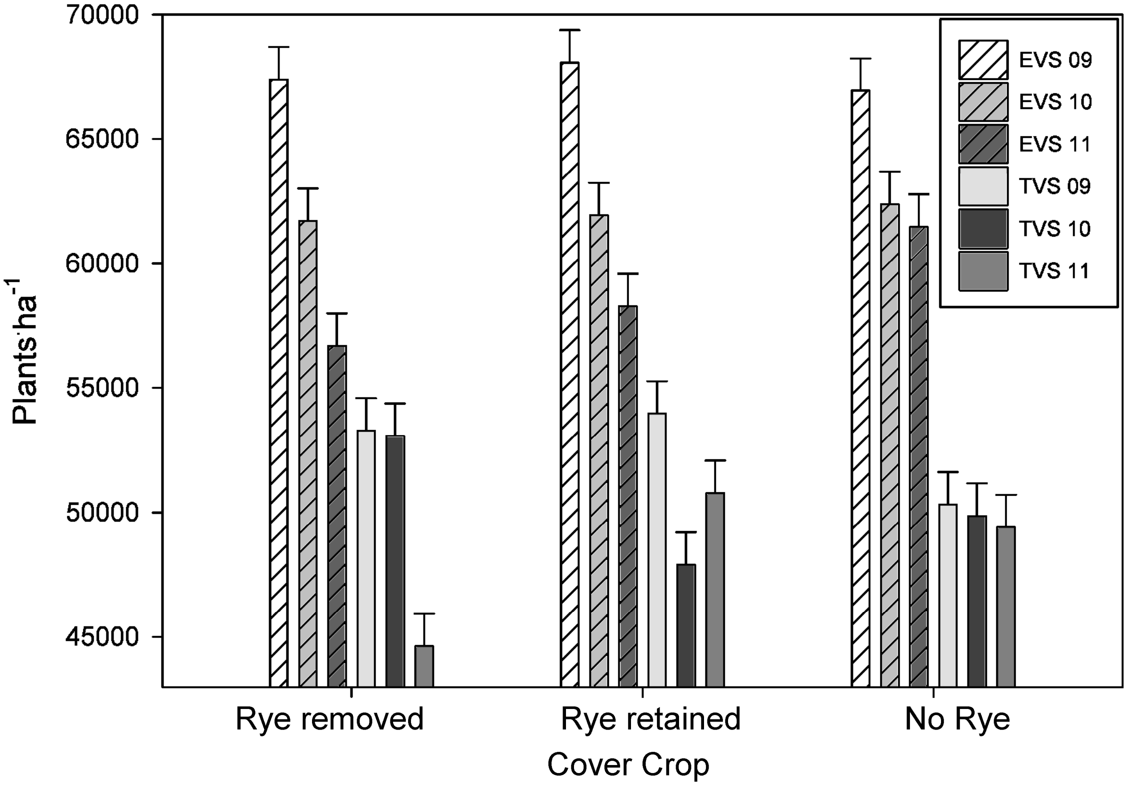

Figure C1. Plant populations (plants ha^{-1}) at the central and northern Alabama sites for the winter cover crop treatments. The error bars represent the standard error of the mean. EVS - E.V. Smith Research Center near Shorter in Central Alabama (Compass loamy sand); TVS - Tennessee Valley Research and Extension Center near Belle Mina in Northern Alabama (Decatur silt loam).

Table C1. Three-year average (2009-2011) harvest indexes at the E.V. Smith Research Center near Shorter in Central Alabama (EVS) and the Tennessee Valley Research and Extension Center near Belle Mina in Northern Alabama (TVS).

Location	Rye Removed	Rye Retained	No Rye
	Harvest Index		
EVS	0.60 a†	0.60 a	0.61 a
TVS	0.56 b	0.56 b	0.58 b

† Means that are followed by the same letter are not significantly different at the 0.10 level.