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Ramdeo Seepaul

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Genotype, nitrogen and harvest management effects on switchgrass production

By

Ramdeo Seepaul

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Genotype, nitrogen and harvest management effects on switchgrass production

By

Ramdeo Seepaul

Approved:

Bisoondat Macoon
(Co-Major Professor)

K. Raja Reddy
(Co-Major Professor)

William B. Evans
(Committee Member)

John J. Read
(Committee Member)

Michael S. Cox
(Graduate Coordinator)

J. Mike Phillips
(Committee Member/Department Head)

George M. Hopper
Dean of the College of Agriculture and Life Sciences

Name: Ramdeo Seepaul

Date of Degree: May 16, 2014

Institution: Mississippi State University

Major Field: Agronomy

Co-Major Professors: Dr. Bisoondat Macoon and Dr. K. Raja Reddy

Title of Study: Genotype, nitrogen and harvest management effects on switchgrass production

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Candidate for Degree of Doctor of Philosophy

Use of switchgrass (*Panicum virgatum* L.) as a forage and feedstock species requires knowledge of fertilizer application rates and harvest timing to optimize yield and quality. Three experiments were conducted at the Brown Loam Branch Experiment Station, Raymond, MS to quantify nitrogen rates, harvest timing, and genotype effects on biomass, nutrient removal, chemical composition and ethanol yield. Dry matter yield varied with N rate, genotype, harvest frequency and timing. Yields among genotypes were: NF/GA992 = NF/GA001 (13.7 Mg ha^{-1}) > Alamo (11.6 Mg ha^{-1}) > Cave-in-Rock (6.1 Mg ha^{-1}). A single (9.5 Mg ha^{-1}) or two harvests annually (10.3 Mg ha^{-1}) produced the greatest dry matter yield. As harvest frequency increased from three (7.3Mg ha^{-1}) to six (5.9 Mg ha^{-1}) harvests annually, yield decreased. There was an effect of N application on yield, but not at application rates greater than 80 kg ha^{-1}. Nitrogen did not consistently affect tissue nutrient concentrations but more frequent harvests led to increased nutrient concentration. Nutrient removal responses to N application were mostly similar to the yield responses. Nitrogen use efficiency and recovery declined as N rate increased. Estimated ethanol yield averaged 162 L Mg^{-1} for Alamo, NF/GA001 and NF/GA992 . A single (2.4 kL ha^{-1}) or 2 harvests annually (2.3 kL ha^{-1}) produced the greatest ethanol production and was correlated with by biomass yield. Nutrient removal, N use efficiency, N recovery and ethanol production were related to biomass yields rather than chemical composition differences. The findings in this dissertation will enable a database on management effects on ethanol yield and composition, enhance current biomass models, facilitate improved management of feedstock production inputs and improve feasibility of alternative fuel development.

DEDICATION

This dissertation is dedicated to my dad, Jadoo Bance Seepaul, mom, Rajpattie Seepaul and all my family.

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“Knowledge is in the end based on acknowledgement.” Ludwig Wittgenstein

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LIST OF ABBREVIATIONS

ADF	Acid Detergent Fiber
ADG	Average Daily Gain
ADL	Acid Detergent Lignin
ANR	Apparent Nitrogen Recovery
ARA	Arabinose
Cell.	Cellulose
CP	Crude Protein
DMY	Dry Matter Yield
ETOH	Ethanol
FRU	Fructose
FUE	Fertilizer Use Efficiency
GAL	Galactose
GLC	Glucose
GLCS	Soluble Glucose
Hemi.	Hemicellulose
HF	Harvest Frequency
Lig.	Lignin
MAN	Mannose
NDF	Neutral Detergent Fiber

NIRS	Near Infrared Spectroscopy
NUE	Nitrogen Use Efficiency
OPC	Orthogonal Polynomial Contrast
PFP	Partial Factor Productivity
SSF	Simultaneous Saccharification And Fermentation
STA	Starch
SUC	Sucrose
TEP	Theoretical Ethanol Production
TEY	Total Ethanol Production.
TTEP	Total Theoretical Ethanol Production
TTEY	Total Theoretical Ethanol Yield
XYL	Xylose

CHAPTER I

INTRODUCTION

Population increases and associated increased demand for resources, volatile geopolitics, and dependency on dwindling oil resources have fostered the development of renewable energy resources. The world population is currently approximately 7.1 billion (U.S. Census Bureau, 2012) and is projected to reach 10.1 billion by 2100 (U.N. Population Division, 2010). The increases in population will create a greater demand for ecosystem goods and services and simultaneously contribute to global climate change. Current CO_2 level of 380 µmole mole^{-1} is projected to reach between 560 and 970 µmole mole^{-1} by 2100 (Houghton et al., 2001). As a consequence of increased CO_2, global air temperatures are projected to increase by 1.5 to 11 °C by 2100 (Stainforth, Aina et al. 2005). Global geopolitics influences the availability, accessibility and equitable distribution of oil resources. The USA and the world have experienced several oil shortages since the 1970's variously caused by the Organization of the Petroleum Exporting Countries oil export embargo, the Iranian Revolution induced oil crisis and Gulf War related oil price shock (Akins, 1973; Kilian, 2008). The effects of global climate change resulted from increased consumption of fossil fuels and the decline in fuel resources have resulted in intensive research for renewable and "cleaner" sources of energy.

Second-generation biofuels derived from lignocellulosic feedstocks have potential to enhance U.S. national energy security and environmental quality and revitalize rural economies (Perlack et al., 2005). The Energy Policy Act of 2005 and the Energy Independence and Security Act of 2007 are legislations that have advanced the energy independence and security in the USA. The former created the first Renewable Fuel Standard (RFS1) and mandate renewable energy volumes while the latter overhauled the RFS1 and replaced it with RFS2. This new legislation mandated the production of 36 billion gallons of renewable fuel annually by 2022 and placed a cap on corn ethanol at 15 billion gallons per year. The remaining 21 billion gallons must be derived from cellulosic feedstocks. The lignocellulose in native warm season grasses represents a vast and renewable source of feedstock for producing ethanol, via bioconversion techniques, or electricity via co-firing with coal.

The identification of switchgrass (*Panicum virgatum* L.) as a potential biofuel feedstock was initiated in 1978 by the Department of Energy's Bioenergy Feedstock Development Program (BFDP) following the evaluation of yield and agronomic characteristics on 34 candidate species (McLaughlin and Walsh, 1998). In 1991, the BFDP commenced a 10-year research program concentrated on developing dedicated herbaceous bioenergy crops that were compatible with conventional farming practices (Lewandowski et al., 2003). Switchgrass selection was based on superior agronomic and production characteristics including broad adaptation and distribution, high yields on marginal lands, compatibility with conventional farming equipment, high nutrient use efficiency, and high biomass production (Wright and Turnhollow, 2010).

Switchgrass belongs to the Poaceae family and is a perennial warm-season, C4 grass indigenous to the Central and North American tall grass prairie. The plant is widely distributed and covers most of the continental USA east of the Rocky Mountains and extends into Mexico and Canada. This broad ecoedaphic distribution is one reason why switchgrass is adapted to marginal soils including those with less fertile, flooded, and drought stressed conditions. Switchgrass is a highly diverse species with significant genetic (Das et al., 2004) and phenotypic variation (Eberhart and Newell, 1959) resulting from gene migration, random genetic drift, mutation, natural selection combined with environmental variation due to latitude, altitude, soil type, and precipitation (Casler et al., 2007). Productivity, survival, and adaptation traits of switchgrass vary depending on origin and genotypes when growing in a common environment (Casler et al., 2004; Sanderson and Moore, 1999). Information of genotypes that are broadly adapted is required for genotype selection for adaptability in diverse environments and production potential (Sanderson et al., 1999b). Two ecotypes of switchgrass, lowland and upland, have evolved that are genetically and phenotypically distinct from each other. Lowland ecotypes are tall (60 to 305 cm) and erect, coarse-stemmed, glabrous and more robust, adapted to flood plains and found in more southern habitats. Upland ecotypes are short (90 to150 cm), fine-stemmed, semi-decumbent, have varying amounts of pubescence on the leaf blades and found in drier mid and northern latitudes. Successful switchgrass feedstock production would require adapted, persistent and high yielding genotypes.

Cultivar biomass production varies with latitude (Casler, 2005; Casler and Boe, 2003) and environmental conditions (Casler et al., 2004); therefore enhancing feedstock productivity would require region-specific switchgrass cultivars (Fike et al., 2006a;

Parrish and Fike, 2005). Lowland cultivars are more responsive to N application and have greater growth potential than upland cultivars due to mid-season growth vigor and delayed maturity (Stroup et al., 2003). Lowland cultivars have an 8% yield advantage relative to upland cultivars when harvested once annually. Under a two-cut management system, lowland ecotypes produce 36% more dry matter (DM) than upland ecotypes (Fike et al., 2006b). In upper southeastern USA, lowland ecotypes 'Alamo' and 'Kanlow' produced mean yields of 15.8 Mg ha^{-1} compared to 12.6 Mg ha^{-1} produced by upland 'Cave-in-Rock' and 'Shelter' (Fike et al., 2006c). In Oklahoma, Alamo and Kanlow produced 15 -17 Mg ha^{-1} while Cave-in-Rock and Shelter produced 8 to 11 Mg ha^{-1} (Fuentes and Taliaferro, 2002). Therefore lowland cultivars (Alamo and Kanlow) are better suited for biomass production in south central USA due to their greater productivity and yield stability (Fike et al., 2006b; Fuentes and Taliaferro, 2002). Economics and environmental stewardship of production systems will be influenced by management decisions, which include fertilizer application and harvest management.

Nitrogen fertility is an important determinant in crop productivity, quality and profitability (Read et al., 2002). Nitrogen is absorbed by plants as nitrate (NO_3^-) or ammonium (NH_4^+) ions and is used to synthesize amino acids that are incorporated into proteins, DNA and RNA. Amino acids are used in forming protoplasm, the site for cell division and thus are important for plant growth and development. The synthesized proteins become the framework for chloroplasts, mitochondria and other structures and therefore play a major role in almost all enzymatic reactions and plant metabolic processes. Nitrogen promotes rapid growth, increases quantity and quality of dry matter in forages, protein content in grain crops and promotes fruit and seed development.

Nitrogen is considered to be the yield determining nutrient in warm-season grass productivity and in the absence of water stress, it is the major factor controlling the rate of switchgrass biomass accumulation; hence its efficient use in feedstock production is critical.

Many studies have quantified the effects of N on plant growth and development and persistence across many species. There is an exponential increase in yield response to incremental increase in N fertilizer application with optimal rates varying among cultivars. Derived N response curves indicate that the initial response is linear and then plateaus, subsequently leveling off or declining gradually. Switchgrass responds positively to N application (Madakadze et al., 1999; Sanderson and Reed, 2000), therefore understanding and quantifying the response of varying N application rates is important in developing an efficient nutrient management program for feedstock production. Relative to other elements, N is highly mobile in the soil solution and can be easily lost by leaching and/or run-off. Leached forms of N (nitrates) can enter waterways affecting surface and ground-water quality. Modifications of fertilizer application practices to attain optimization of N application, use and efficiency have potential for reducing run-off and leaching. In addition to environmental issues associated with N management, the efficient monitoring of plant N status and appropriate N fertilizer management are essential to balance the factors of increasing cost of N fertilizer and crop demand (Jaynes et al., 2001). Nitrogen management will be influenced by the harvesting system adopted to exploit the seasonal nutrient cycling in switchgrass.

Switchgrass is sensitive to frequent harvesting since its apical meristems are elevated above the ground in early vegetative growth, has a high ratio of reproductive to vegetative tillers and new growth must occur from crown buds or aerial axillary meristems (Haferkamp and Copeland, 1984; Sanderson and Wolf, 1995). The aim in feedstock production is to maximize biomass production with a high lignocellulose concentration and low concentrations of water, N, ash, and lignin. Consequently, in optimizing switchgrass productivity, it is important to adopt an effective harvest management technique that is based on establishing a compromise between yield, quality, and plant persistence. Multiple harvests maximized yields when compared to a single end-of-season harvest with three, two and a single annual harvest producing 16.3, 14.7 and 12.9 Mg DM ha^{-1}, respectively (Thomason et al., 2005). Two annual harvests increased Alamo yields by 13, 38 and 66% compared with a single late fall harvest fertilized with application of 0, 90 and 180 kg N ha^{-1}, respectively (Guretzky et al., 2011). Multiple harvests result in decreased plant persistence and stand reduction, however, frequent harvests (> 2) in a single season reduced switchgrass stands by 58% compared with 39% reduction with a single annual harvest (Anderson and Matches, 1983). Nitrogen and harvest management have implications for nutrient removal, nutritive value and chemical composition of switchgrass feedstock.

Nutrient uptake and loss from production sites are important issues for high-biomass producing crops such as switchgrass. Nutrient removal is a function of biomass yield and nutrient concentration. Nutrient concentration is usually greatest in the summer harvest of a two-harvest system and lowest in the fall harvest of a single-harvest system and consequently nutrient removal is greater with two annual harvests than for a single annual harvest (Reynolds et al., 2000). Guretzky et al. (2011) reported that nutrient concentration and removal were 50% greater with two annual harvests than a single harvest. This is related to the seasonal dynamics in nutrient concentration, which generally decline with maturity and are translocated to the crown and roots and remobilized for spring regrowth. Perturbations in the nutrient cycling process through frequent harvesting may increase plant nutrient concentration and removal rates.

Associated with the nutrient cycling are changes in chemical composition of the biomass (Mulkey et al., 2006). Switchgrass forage nutritive value (protein and digestibility) decline with plant physiological maturity (Kering et al., 2013a; Mosali et al., 2013; Waramit et al., 2012), while fiber and lignin concentration increase. Kering et al. (2013b) found that harvesting once produced biomass with mean acid detergent fiber (ADF) concentration of 465 g kg^{-1}, which is reduced to 388 g kg^{-1} when harvested twice annually. Sanderson et al. (1999a) reported mean single harvest neutral detergent fiber (NDF) values of 741 g kg^{-1}, which decreased to 675 g kg^{-1} when 4 annual harvests are made for Alamo switchgrass. Chemical composition of biomass feedstock is an important factor affecting efficiency of biofuel production and energy outputs.

Switchgrass is a versatile feedstock with several biochemical and thermochemical conversion platforms including simultaneous saccharification and fermentation (SSF), gasification, combustion, and pyrolysis. The SSF conversion to ethanol involves thermochemical pretreatment, enzymatic hydrolysis of cell wall polysaccharides to monosaccharides, and fermentation of the released simple sugars to ethanol. Fertility and harvesting management can affect the mineral and sugar concentration, which may influence switchgrass energy conversion efficiency. Typical theoretical ethanol production ranges from 380 to 430 L Mg^{-} while ethanol production ranges from 1750 to 3700 L ha^{-1} (Schmer et al., 2012).

The objectives of this dissertation research are to determine adaptable and high producing adaptable switchgrass varieties to a central Mississippi location and to quantify the effect of N application rate, harvest frequency and timing on biomass yield, N use and recovery, chemical composition and ethanol yield and production. This dissertation consists of four research papers from three independent multiyear field experiments. The first study was a two-year field experiment that quantified the effects of N application rate and genotypes on biomass production, nutrient removal rates, chemical composition, and ethanol yield. The second study quantified the effects of N application rate and harvest frequency on biomass production and nutrient removal rates, chemical composition and ethanol yield. The third study quantified the effects of harvest timing and N application rates on switchgrass production, however, this study was split into two papers. The first paper of the third study quantified the effects of N application rates on N use and recovery and the changes in plant nutrients, nutrient partitioning, and nutrient removal capacities during the growing season. The second paper of the third study quantified harvest time and N application rate effects on whole plant, leaf, and stem forage nutritive value and feedstock composition. In addition, the effects of changes in chemical composition on ethanol yield and production were determined.

CHAPTER II

LITERATURE REVIEW

Biofuels: A Justification

Man has harnessed energy from biomaterials since antiquity. Therefore, producing biofuels is not a novel concept but one that has gained importance very recently in the USA and the world. The world has experienced several oil crises since the 1970's variously caused by the Organization of the Petroleum Exporting Countries oil export embargo, the Iranian Revolution induced oil shortage and Gulf War related oil price shock (Akins, 1973; Kilian, 2008). These shortages and the desire for improved energy independence have led many countries, including the USA, to launch initiatives aimed at developing a biofuel industry. Recent development of the biofuel sector in the USA is justified by the need for national energy security related to finite supplies of fossil fuel, the need for improved environmental stewardship to reduce green house gas emissions, sustain U.S. economic growth and rural revitalization (Perlack et al., 2005a). Global world population growth was 1.2% from 2010 to 2011 and total population is projected to reach 9 billion by 2050 (Tilman et al., 2001), with a 2.1% annual growth rate. About 84% of the growth is expected to occur in developing countries. The increases in population will create a greater demand on the ecosystem for goods and services (Cohen, 2003; Vörösmarty et al., 2000). A substantial percentage of global arable lands are already under production with limited new lands that can be cultivated;

therefore any increased food supply must be derived from more intensive cultivation of existing arable lands (Foley et al., 2005). Despite the disadvantages biofuels may have on the global food system and the environment, if managed judiciously, biofuels can produce substantial global, regional and state benefits (Tilman et al., 2009). In the USA, the Energy Independence and Security Act of 2007 set mandatory renewable fuel standard requiring the production of 36 billion gallons of cellulosic (plant-derived) liquid transportation fuel annually by 2022 (Sorda et al., 2010). Biofuels can be environmentally friendly, for example switchgrass produced 540% more energy than the non-renewable energy consumed when it is pyrolysed (Schmer et al., 2008). Also, switchgrass ethanol averaged 94% lower greenhouse gas emissions than gasoline (Schmer et al., 2008). Corn based ethanol, which accounts for more than 90% of the ethanol currently produced in the USA requires more units of fossil fuel energy to produce than units of biofuel delivered. A thriving biofuel sector can help create jobs for the 9.2% unemployed in Mississippi and contribute significantly to the revitalization of the state's rural economy.

The effects of global climate change resulted largely from increased consumption of fossil fuels; this and the accompanying decline in fossil fuel resources have resulted in intensive research for renewable sources of energy. Studies have shown that native warm season grasses, including switchgrass, are suitable for use as an energy feedstock, either for producing ethanol, via bioconversion techniques, or electricity via co-firing with coal (Thomason et al., 2004a).

Switchgrass: History, Description and Uses

In 1978, the Department of Energy's Bioenergy Feedstock Development Program (BFDP) identified switchgrass as a potential biofuel feedstock following the evaluation of yield and agronomic characteristics of 34 candidate species (McLaughlin and Walsh, 1998). The BFDP commenced a 10-year research program in 1991 concentrated on developing dedicated herbaceous bioenergy crops that were compatible with conventional farming practices (Lewandowski et al., 2003). The selection of switchgrass was based on its broad adaptation and wide geographic distribution; high yields on marginal and erosive lands; compatibility with conventional farm practices, for example, the ability to be harvested with conventional hay-making equipment; perenniality; high nutrient use efficiency, hence relatively low fertilizer requirements; and high biomass yield production (Wright and Turhollow, 2010). Research efforts were concentrated on developing comprehensive switchgrass agronomic production practices, which included fertilizer timing and applications, harvesting protocols, and establishment techniques (McLaughlin and Walsh, 1998).

Switchgrass is a native tall-growing, warm-season, perennial rhizomatous grass that is widely adapted to many soils including those with infertile, flooded and drought stressed conditions. It is best adapted moist conditions, but it's also considered drought resistant, hardy, and adaptable to a wide range of soil and climatic conditions. Switchgrass is highly heterozygous, self incompatible and an out-crossing species with significant genetic (Das et al., 2004) and phenotypic variation (Eberhart and Newell, 1959). Being an allogamous species, gene migration is accomplished via pollen or seed resulting in highly heterogeneous populations with the potential for natural selection.

Switchgrass is the earliest maturing of the warm-season grasses, growing as tall as 1.8 to 2.2 m high but is typically shorter than big bluestem or indiangrass. Two main ecotypes of switchgrass have evolved: lowland and upland ecotypes. Lowland ecotypes are tetraploids ($2n = 4x = 36$) are erect, coarse-stemmed, glabrous and more robust; found in bunches standing 60 to 305 cm tall while upland ecotypes can be either tetraploids ($2n = 4x = 36$) or octoploids ($2n = 8x = 72$) and are fine-stemmed, have varying amounts of pubescence on the leaf blades, are semi-decumbent, and 90 to 150 cm tall. Lowland switchgrass performs better under flooded conditions (Porter Jr., 1966), is more susceptible to drought, has a lower N requirement, and produces greater biomass yields than upland switchgrass genotypes that are more adapted to drier climates. Upland varieties grow faster with greater photosynthetic rates and a shorter growth cycle as opposed to lowland varieties (Monti et al., 2008), however, upland varieties yielded 12.6 versus 15.8 Mg ha^{-1} for lowland varieties (Fike et al., 2006a). Common lowland varieties include Alamo and Kanlow while upland varieties include: ‘Cave-in-Rock’, ‘Blackwell’, ‘Shelter’, ‘Pathfinder’, ‘Sunburst’, ‘Forestburg’, and ‘Pangburn’. Switchgrass begins growing in late April to early May with floral initiation occurring before the onset of high summer temperatures and moisture stresses. Flowering begins in early June and continues into early August followed by seed dispersal in late August to early September. More than 90% of its dry matter accumulates from June to August.

Switchgrass is important as forage for livestock and is primarily used for warm-season pastures and hay. Switchgrass is palatable and is readily grazed by cattle, horses and sheep during the spring and early summer before the nutritive value declines and cannot support animal gains (Sanderson et al., 2004). For wildlife habitats, switchgrass is an excellent seed source for upland birds and a forage source for game animals, while as a cover crop; it is especially beneficial in winter due to its standing canopy (Roth et al., 2005). Switchgrass is suitable for use as an energy feedstock, either for producing ethanol, via bioconversion techniques, or electricity via co-firing with coal (Larson et al., 2009; Choi et al., 2010).

Nitrogen Management

Nitrogen is the most abundant element in the atmosphere and usually the most important growth limiting factor in crop production. Nitrogen application to switchgrass production fields must be optimized because of the economics and energy costs associated with fertilizer production and application (Sanderson et al., 2006). Switchgrass may translocate significant amounts of N to the stem bases and roots at the end of the growing season and frequent defoliation may reduce quantity of nutrients translocated. In the following spring, the carbohydrates are remobilized to the actively growing shoots. With a one-cut system, minimal N application is required because of this seasonal N translocation and remobilization, however, sustainable production of biomass is not feasible without N fertilizer application (Muir et al., 2001). This internal cycling of N coupled with switchgrass C4 physiology enables an efficient use of N by switchgrass.

Nitrogen is very dynamic with several influx and efflux pathways that affect the N balance in soils, N uptake and N utilization efficiency. Biological fixation, fertilizer application, precipitation, mineralization and nitrification constitute N gains in the N cycle while denitrification, volatilization, leaching and immobilization are N transformation processes presenting N losses.

Nitrogen transformations in the soil include nitrification and mineralization. Nitrification is a two step process of enzymatic oxidation brought about by bacteria, that oxidizes ammonium (NH4+) to nitrite (NO_2^-) by the nitrosomonas organisms and then nitrite (NO_2^-) to nitrate (NO_3^-) by nitrobacter, as follows:

$$2\ NH_4^+ + 3\ O_2 \rightarrow \text{enzymatic oxidation} \rightarrow 2\ NO_2^- + 2\ H_2O + 4\ H^+ + \text{energy} \quad (1.1)$$

$$2\ NO_2^- + O_2 \rightarrow \text{enzymatic oxidation} \rightarrow 2\ NO_3^- + \text{energy} \quad (1.2)$$

Since nitrification requires oxygen, any management factor that improves soil aeration will aid nitrification. Another N gain in the N cycle is mineralization where organic N in the residues is mineralized to ammonium (NH_4^+) by heterotrophic soil bacteria and fungi. Nitrogen gained through nitrification and mineralization can be lost by denitrification, volatilization, leaching or immobilization. Denitrification occurs under saturated soil conditions in fine textured soils and is the stepwise reduction of nitrate (NO_3^-) to N_2O and eventually N_2 and loss to the atmosphere and is mediated by anaerobic bacteria ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$). Soils with high organic matter provide a source of energy for denitrifying microorganisms. Denitrification occurs only in anaerobic conditions. Although the process can occur in aerated soils, it is limited to those microsites that lack oxygen. Nitrogen in the form of ammonia is lost to the atmosphere through volatilization. Volatilization is less in well-buffered soils and is highest in alkaline soils ($pH > .5$) where the ammonium in the soil is converted to ammonia. In addition, soil evaporation and drying beyond field capacity coupled with increases in soil temperatures up to 45 °C enhances volatilization losses. Another source of N loss is leaching where water-soluble, mobile nitrates are displaced from the root zone as excess water moves through the soil profile. Immobilization is a process that converts inorganic N to organic nitrogen (NH_4^+ or NO_3^- → plant or microbial tissue). It is the reverse reaction of mineralization and is constantly occurring simultaneously with mineralization. Residues that have C:N ratios greater than about 30, equivalent to N concentration of about 1.5 g kg^{-1} or less, result in lowering of mineral N reserves because of net immobilization.

N Use Efficiency (NUE)

The definition of NUE varies within the literature with two different perspectives, use efficiency concerned with increasing yield with N and uptake efficiency deals with the internal nutrient requirements of the plants (nutrient uptake per unit root length, surface area or weight). For grasses, NUE can be defined as the DM produced per unit N available in the soil (Lemus et al., 2008b; Zemenchik and Albrecht, 2002). It indicates the relative balance between the amount of fertilizer N taken up and used by the crop versus the amount of fertilizer N "lost". Determination of the NUE of a crop is achieved by N rate field experiments having incremental increases in N rate, followed by harvest measurements and validated through chemical analysis. Parameters to determine N use are calculated using the following three formulae:

1. Total N accumulation (kg ha^{-1}) – calculated as DM yield × total N concentration.
2. Apparent N recovery (ANR) – calculated by the difference method as [(kg N accumulated at Nx – kg N accumulated at N0)/(N fertilizer applied at Nx)] × 100
3. NUE – calculated as (yield at Nx – yield at N0)/N fertilizer applied at Nx

where x is the N application rate.

Nitrogen recovery, accumulation and/or absorption by grasses is affected by factors such as species, growth habit, N rate, precipitation, and soil type and is calculated by [(N uptake at N*x* – N uptake at No)/(applied N at N*x*) * 100%]. Lemus et al. (2008c) reported ANR values ranging from 25 to 80% when the amount of N removed in the control plots was discounted. Muir et al. (2001) reported NUE values of 108, 76, 50, and 37 kg kg^{-1} in Stephenville, Texas and in Beeville, NUE was 30, 33, 23, and 22 kg kg^{-1} at 56, 112, 168, and 224 kg applied N ha^{-1}. These authors noted that NUE is location specific and is affected by the quantity and timing of in–season precipitation. Nitrogen use efficiency was lower for the first harvest (22 kg kg^{-1}) than at subsequent harvests (57 kg kg^{-1} second harvest mean). Lemus et al. (2008) derived fertilizer use efficiency (FUE) values based on biomass production, with higher values being more relatively efficient than lower values, 56 kg N ha^{-1} (FUE = 2) is more efficient than 112 kg N ha^{-1} (FUE = 1.5) and 224 kg N ha^{-1} (FUE = 0.85). Therefore, the application of additional fertilizer is dependent on the value of the biomass relative to the cost of the additional fertilizer. Apparent N recovery (ANR) can either increase or decrease based on environmental factors. Zemenchik and Albrecht (2002) found that the ANR varied between locations resulting from differences in soil type and the extent of precipitation. In their study, switchgrass recovered about 90% of N at 50 kg applied N ha^{-1} with declining recovery with increasing rates.

Harvest management

Harvest management is important to exploit internal N cycling with the switchgrass plant. In addition, harvesting protocols can greatly influence biomass yield and chemical composition (Adler et al., 2006; Ball, 1990; Brejda et al., 1994; Sanderson et al., 1999). Switchgrass is sensitive to frequent or intense defoliation because it elevates the apical meristem above the ground during vegetative development, has a high ratio of reproductive to vegetative tillers, and new growth must occur from crown buds or aerial axillary meristems (Haferkamp and Copeland, 1984; Sanderson and Wolf, 1995). The major costs involved in producing switchgrass feedstock include N fertilizer application, harvesting, and transportation (Keeney and DeLuca, 1992). Therefore, the number of harvests and the yield per harvest affect the economics of harvesting switchgrass (Vogel et al., 2002).

Harvest management of switchgrass for forage has emphasized infrequent harvests at or before the boot stage (Vogel et al., 2002). This species has been used as forage throughout the Midwest and new varieties have been developed (Miller et al., 1995) with improved forage nutritive value. Switchgrass produces more than 60 % of its total growth after June (Anderson and Matches, 1983). When the first harvest occurred at late heading, less than 30% of the total growth was available during late July and August. In contrast, when the first harvest was made at the jointing stage, over 50% of its total growth was available in late summer for grazing (Anderson and Matches, 1983). Anderson and Matches (1983) found the switchgrass IVDMD declined as the plants matured from vegetative to the heading stages. Similarly, Sanderson et al (1999) found that as the plant matures NDF increases while CP decreases irrespective of harvest frequency. A single harvest is most economical when compared to several harvests over the growing season based on the assumption that frequent harvesting does not allow for the storage of adequate photosynthetic assimilates for winter survival and subsequently reduced spring growth. Increased reduction of switchgrass stands associated with frequent harvests has been reported. With two or three annual harvests, stands were reduced 58% compared to a 39% reduction with a single annual harvest (Anderson and Matches 1983). In related studies, it was found that severity of switchgrass stands reduction increased with increasing frequency of harvest when cut one, two or four times each season and stand losses were lesser when harvested at 10 cm stubble height instead of 5 cm (Anderson and Matches 1983). Frequent defoliation produced greater forage nutritive value than a single harvest at the end of the growing season. Based on the flexibility of the species, some farmers prefer to have several harvests before inflorescence initiation as forage and mature growth targeted as biomass.

Several studies have reported the yield responses of switchgrass to timing and frequency of harvest (Sanderson et al., 1999). These authors reported that total seasonal yields decreased as harvest frequency increased from one to four cuts annually. Conversely, Thomason et al., (2005) reported that multiple harvests result in greater yields compared to a single end of season harvest with three, two and a single annual harvest producing 16.3, 14.7 and 12.9 Mg DM ha^{-1}. Two annual harvests increased Alamo yields by 13, 38 and 66% at 0, 90 and 180 kg N ha^{-1} application relative to a single late fall harvest (Guretzky et al., 2011). Other studies have reported decreased total seasonal yields with increased harvest frequency (Sanderson et al., 1999) as a result of stand reduction. Lowland cultivars produced 36% biomass more biomass than upland cultivars under a two-cut management system. When harvested once annually, lowlands had an 8% yield advantage over upland cultivars (Fike et al., 2006b). As a result, lowland cultivars (Alamo and Kanlow) are better suited for biomass production in south central USA due to their greater productivity (Fike et al., 2006b). Harvesting after a killing frost produced higher total yields and improved switchgrass persistence compared with anthesis harvests (Mulkey et al., 2006). The concentration of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) increased between anthesis and killing-frost harvests, while total N and ash decreased. Harvesting established switchgrass stands once per year after a killing frost and applying N at 56 kg ha^{-1} was an effective system for switchgrass biomass production and persistence on land enrolled in or managed similarly to CRP in South Dakota (Mulkey et al., 2006). For maximum biomass yields in the south central USA, switchgrass should be harvested once in the autumn, about mid-September (Fike et al., 2006b; Sanderson et al., 1999).

Nutrient Concentration and Removal

Nutrient uptake and loss from production sites are important issues for high-biomass producing crops such as switchgrass. Producing switchgrass for biomass is vastly different from forage production with respect to nutrient management. The aim in biomass production is to minimize nutrient concentration that may contribute to ash at combustion causing slagging and fouling of processing equipment. An ideal biomass crop should produce high yields with high lignocellulose, low N, low ash and minimal undesirable mineral concentrations (Adler et al., 2006).

Harvest management influences nutrient concentration in switchgrass. Reynolds et al. (2000) reported that Alamo switchgrass had higher N concentration in midsummer (7.1 g kg^{-1}) and fall (5.4 g kg^{-1}) harvests than a single fall harvest (3.2 g kg^{-1}) (). Similarly, Guretzky et al. (2011) found that N concentration in a single harvest after seed set or frost kill (6.5 g kg^{-1}) increased to a mean of 9.5 g kg^{-1} when harvested twice annually. Seasonal N translocation was found to reduce N concentration from 18.9 to 3.7

g kg^{-1} from May to December (Kering et al., 2012). Frequent harvests disrupt the seasonal N translocation and hence next season regrowth, which is partly sustained from stored reserves and in turn are depleted by multiple harvests. In lowland Cave-in-Rock, Lemus et al. (2008d) reported that N concentration increased linearly with N application rate up to 200 kg N ha^{-1}. In addition, Pedroso et al. (2013) reported that N concentration was positively correlated with N rate at four out of five locations in California for upland Trailblazer switchgrass with N concentration ranging from 2.7 g kg^{-1} at 0 N to 15.8 g kg^{-1} at 300 kg N ha^{-1}. Reynolds et al. (2000a) reported a 5-yr mean 137% increase in N removal with two harvests (116 kg N ha^{-1}) vs. a single harvest (49 kg N ha^{-1}) with 78% of N removed in the first harvest of the two harvest system, reflecting the greater N concentration in a summer harvest. Similarly, Guretzky et al. (2011) reported a 2-yr mean 97% increase in N removal in a two harvests system vs. a single harvest system. A 5-yr study by Lemus et al. (2009) reported an 83% increase in N removal with two harvests vs. a single harvest.

Lemus et al. (2009) reported P concentration in Alamo switchgrass ranging from 0.68 to 1.39 g kg^{-1} when harvested once and from 0.79 to 2.02 g kg^{-1} when harvested twice. Guretzky et al. (2011) also found that harvesting twice (1.3 g kg^{-1}) increased P concentration relative to after seedset (1.1 g kg^{-1}) or frostkill (0.8 g kg^{-1}) harvest. Two harvests (23 kg P ha^{-1}) removed 64% more P than a single harvest (14 kg P ha^{-1}) with the first of the two harvests removing 65% of the total P (Lemus et al., 2009). Harvesting twice removed more P than a single midsummer (53%) or late fall harvest (109%) (Guretzky et al., 2011). Although the P concentration was similar between harvesting once or twice annually, the midsummer harvest in two-harvest system generally has greater P concentration (Lemus et al., 2009). Phosphorous concentration decreased by 45% from May to October and by 30% from October to December declining to 0.92 g kg^{-1} at harvest. Guretzky et al. (2011) found that P concentration increased marginally from 0.7 to 0.9 g kg^{-1} as N increased from 0 to 225 kg N ha^{-1} in a single after-frost kill harvest while with two annual harvests, P concentration increase from 1.3 to 1.4 g kg^{-1} as N application increased from 0 to 225 kg N ha^{-1}. With multiple harvests, K concentrations varied and were higher in a midsummer than a late fall harvest, ranging from 5.3 to 10.3 g kg^{-1} when harvested once and from 2.4 to 12.6 g kg^{-1} when harvested twice annually (Lemus et al., 2009). Harvesting after seed set (6.6 g kg^{-1}) or frost kill (4.2 g kg^{-1}) reduced the K concentration relative to two annual harvests (12.2 g kg^{-1}) (Guretzky et al., 2011). Potassium concentration decreased by 65% from May to October and by 1% from October to December reducing to 7.2 g kg^{-1} at harvest (Kering et al., 2012). Guretzky et al. (2011) found that K concentration increased from 3.4 to 4.5 g kg^{-1} as N increased from 0 to 225 kg N ha^{-1} in a single after-frost kill harvest while with two annual harvests, K concentration increased from 11.1 to 12.7 g kg^{-1} as N application increased from 0 to 225 kg N ha^{-1}. Harvesting twice annually (95.5 kg ha^{-1}) was found to increase the K removal by 48% relative to a single midsummer harvest (141.1 kg ha^{-1}) averaged across 5 yrs with the midsummer harvest removing 75% of K in the two annual harvest system (Lemus et al., 2009). Guretzky et al. (2011) found that harvesting twice annually removed 217 kg K ha^{-1} representing139% more K removed in a single midsummer harvest or 295% more K in a single late fall harvest.

Nitrogen removal rates in the biomass are indicative of the N fertilizer requirements of switchgrass (Reynolds et al., 2000). The magnitude of nutrient removal is affected by cultural management practices such as cultivar selection (Fike et al., 2006b; Stroup et al., 2003), N application rate (Lemus et al., 2008b; Madakadze et al., 1999; Muir et al., 2001), harvest frequency (Reynolds et al., 2000; Thomason et al., 2004a), and harvest timing (Fike et al., 2006b; Madakadze et al., 1999; Reynolds et al., 2000). Removal rate of a particular element is a function of tissue concentration of that element and biomass production. Nutrient removal in the harvested biomass is affected by the following factors:

Selection of Cultivar

Lowland cultivars are more responsive to N application and have greater growth potential than upland cultivars as a result of mid-season growth vigor and delayed maturity (Stroup et al., 2003). Cave-in-Rock, a northern upland variety is expected to produce less than a lowland such as Alamo in southern locations (Sanderson et al., 1999; Stroup et al., 2003). Maximum single year yields for Alamo and Cave-in-Rock were reported to be 17.5 and 9.6 Mg ha^{-1}, respectively from a southern Iowa study (Lemus et al., 2002). Lowland cultivars are later maturing producing proportionately greater biomass later in the season than upland cultivars and therefore have increased nutrient removal potential.

Quantity of N Applied

Grasses are responsive to N fertilizer application; however, the magnitude of the increase usually follows the law of diminishing returns with the response to incremental increase being progressively less with each increase in N application (Lemus et al., 2008a). Annual yield averaged across a 3-yr period was 7.6, 12.1, 12.0, and 13.3 Mg ha^{-1}

at N application rates of 0, 90, 180 and 270, respectively for Cave-in-Rock grown in Virginia (Lemus et al., 2008b). In a Tennessee study, maximum yield for Cave-in-Rock was 15.3 Mg ha^{-1} while Alamo was 19.9 Mg ha^{-1} (Reynolds et al., 2000). At the N application rate (168 kg N ha^{-1}) that produced the greatest yield in the study, average annual yield of Alamo switchgrass was 14.5 Mg ha^{-1} in a 7-yr trial at Stephenville, TX and 10.7 Mg ha^{-1} in a 3-yr trial at Beeville TX (Muir et al., 2001). A maximum annual yield of 22.5 Mg ha^{-1} occurred at Stephenville. Madakadze et al. (1999b) reported mean N concentrations of switchgrass under a 4-wk cut system were 12.4, 13.9 and 15.4 g kg^{-1} for 0, 75 and 150 kg N ha^{-1} respectively. At 120 kg N ha^{-1}, the amount of N removed was approximately the amount of N applied and rates above this level resulted in increases in soil nitrate-N levels (Vogel *et al.*, 2002). Fike et al. (2006b) found that N removal was 50 % greater than the amount of N applied and suggested that this may be attributed to the ability of switchgrass to scavenge N and benefit from symbiotic relationships with soil microbes. Atmospheric deposition may contribute substantial N to these systems as well.

Harvest Frequency

The quantity of N removed is also affected by the number of annual harvests. Reynolds et al. (2000c) reported that season-long N removal was greater in a two-cut system (late summer and fall) than a one-cut system(after senescence). In addition, the amount of N removed in the two-cut system exceeded the amount of N applied. Thomason et al. (2004b) findings were similar for a 3-cut harvest system. Vogel et al. (2002) suggested that the previous year harvest management may have implications for the following spring soil nitrate N levels in soils receiving no fertilizer.

Time of Harvesting

Delaying harvest results in reduced N removed in switchgrass because the N concentration is probably diluted by an increase in the cell wall concentration of the biomass as it matures. Comparing 4- and 6-wk harvest intervals at three N application rates, Madakadze et al. (1999b) reported less N concentration in the 6-wk system by 7.0, 7.9, 7.8 g kg^{-1} for 0, 75 and 150 kg N ha^{-1} respectively. Generally, as grasses mature their N concentration reduces while fiber concentration increases. Reynolds et al. (2000c) and Nitrogen removal in the fall is less because of the seasonal N cycling. Vogel et al. (2002) concluded that if substantial amounts of N are translocated to the stem bases and roots, the translocated N could be used in the production of new growth the following spring and could reduce N input requirements in switchgrass stands harvested for biomass after a killing frost.

Plant Lignocellulosics

Chemical composition of biomass feedstock is an important factor affecting efficiency of biofuel production and energy outputs. The lignocellulose in forage crops represents a vast and renewable source of feedstock for producing ethanol, via bioconversion techniques, or electricity via co-firing with coal. Acid detergent fiber represents the cell wall portions of the forage that are made up of cellulose and lignin. As ADF increases, the digestibility of a forage usually decreases. Kering et al. (2013) found that harvesting once produced biomass with mean ADF concentration of 465 g kg^{-1} which is reduced to 388 g kg^{-1} when harvested twice annually. Similar findings were reported by Guretzky et al. (2011) where ADF concentrations were greater in a single after-frost harvest (510 g kg^{-1}) than two annual harvests (440 g kg^{-1}). Neutral detergent fiber represents the total cell wall, which is comprised of the ADF fraction plus hemicellulose. As NDF increases, digestibility decreases. Sanderson et al. (1999) reported mean single harvest NDF values of 741 g kg^{-1} that decreased to 675 g kg^{-1} with four annual harvests of Alamo switchgrass. Waramit et al. (2012) reported season-long NDF ranging from 523 to 756 g kg^{-1} while Allison et al. (2012) reported NDF ranging from 621 to 814 g kg^{-1} in two different years when harvested four times annually. In an Oklahoma study, Alamo switchgrass NDF ranged from 739 to 854 g kg^{-1} in two different years when harvested twice at two different locations (Guretzky et al., 2011). Lemus et al. (2008e), reported that N fertilizer application had no effect on biofuel quality, hence N can be used to substantially increase yield without affecting the quality of feedstock in a one-cut harvest system. At N rates more than 160 kg ha^{-1}, there was no effect on NDF (Allison et al., 2012; Guretzky et al., 2011). Mulkey et al. (2006) found that lignin concentration was related to physiological maturity, being less at anthesis harvests than after a frost-killing harvest. Lemus et al., (2008d) reported lignin concentration increased linearly with N application rate for Cave-in-Rock switchgrass ranging from 66 to 73 g kg^{-1}. Greater cellulose and lignin concentrations were reported at 140 relative to 65 kg N ha^{-1}, however there was no N rate effect on hemicellulose (Waramit et al., 2011). Guretzky et al., (2011) reported an effect of N rate on early season ADF and NDF concentrations but there was no effect with a frost-kill harvest. Acid detergent fiber increased marginally, by 2%, as N rate increased from 0 (471 g kg^{-1}) to 225 (482 g kg^{-1}) kg N ha^{-1} for Alamo switchgrass (Guretzky et al., 2011). Similarly, Kering et al. (2013) reported that boot stage ADF concentration decreased linearly with increasing N application rate from 373 g kg^{-1} at 0 kg N ha^{-1} to 351 g kg^{-1} at 270 kg N ha^{-1}. These authors reported also that NDF concentrations increased linearly with increasing N rate in the first harvest of a two harvest system but there was no effect in the second harvest. Lemus et al. (2008d) reported that hemicellulose concentration decreased linearly from 323 to 308 g kg^{-1} with increasing N rate from 0 to 200 kg N ha^{-1} for Cave-in Rock switchgrass.

CHAPTER III

NITROGEN APPLICATION RATE AND GENOTYPE EFFECTS ON SWITCHGRASS BIOMASS PRODUCTION AND NUTRIENT REMOVAL

Abstract

It is critical to understand how nitrogen (N) fertilizer application and other management factors affect agronomic performance of switchgrass (*Panicum virgatum* L.), an important biofuel species. This study quantified the effects of N application rate and genotype on biomass and ethanol yield and nutrient removal of switchgrass in a 2-yr study with four N rates and four entries. Dry matter yield (DMY) in response to N rate was linear in 2008 and quadratic in 2009. Among entries, DMY averaged across N rate and years ranked NF/GA992 (13.9 Mg ha^{-1}) = NF/GA001 (13.4 Mg ha^{-1}) > Alamo (11.5 Mg ha^{-1}) > Cave-in-Rock (6.1 Mg ha^{-1}). There was no N rate effect on tissue mineral concentrations. There was an N rate effect on Ca, Mg, Fe, Mn and B removal and year × N rate on Zn and Cu removal. N use and recovery declined as N rate increased. Total ethanol yield (TEY) was highest in Alamo (165.8 L Mg^{-1}) and averaged 162 L Mg^{-1} for the other three entries. Total ethanol production (TEP) was related to biomass yield rather than biomass composition differences. Among lowland entries TEP was similar but different from Cave-in-Rock in 2008 (1.7 vs. 0.9 kL ha^{-1}) and 2009 (2.6 vs. 1.1 kL ha^{-1}). Feedstock grown from Alamo, NF/GA001 or NF/GA992, at 80 kg N ha^{-1} maximizes

biomass and ethanol production as well as N use efficiency and recovery. These results indicate that there is opportunity to increase switchgrass biomass yields and productivity through genotype selection and N management.

Introduction

Biofuel crops are expected to contribute substantially towards energy security, environmental stewardship, and economic gains in the future. Environmental benefits to be realized include greenhouse gas reduction, carbon-neutral biofuel production, use of and reclamation of marginal lands and relatively low-input production systems (Perlack et al., 2005). Switchgrass was selected for research and development as a dedicated bioenergy crop for the lignocellulosic biofuel industry in 1992 by the U.S. Department of Energy's Bioenergy Feedstock Development Program (Sanderson et al., 1996). Optimizing and sustaining bioenergy crop production systems requires knowledge of the system itself and factors driving sustainable productivity. Understanding and quantifying nutrient removal rates in switchgrass production systems can aid in cultivar selection, feedstock quality control, optimized agronomic resource management, and reduction in nutrient loss.

Nutrient uptake and loss from production sites are important issues for high biomass producing crops such as switchgrass. Nitrogen affects crop growth, development, and physiological processes and determines crop productivity, chemical characteristics, and profitability. Its dynamism makes it one of the most difficult nutrients to manage effectively, however. With biomass crop production, it is important to minimize feedstock nutrient concentration that may contribute to ash at combustion causing slagging and fouling of processing equipment as well as reduced efficiency of ethanol conversion processes. A high quality biofuel feedstock can be defined as having minimal moisture, low N and ash contents, minimal undesirable mineral concentration with high cellulose (Adler et al., 2006; Lemus et al., 2002).

Nitrogen removal rates in the biomass are indicative of the fertilizer requirements of switchgrass (Reynolds et al., 2000). The quantity of N removed in harvested biomass is affected by the genotype, quantity of N applied, harvest frequency and timing, and temporal weather variability. Lowland cultivars are more responsive to applied N than upland cultivars (Fike et al., 2006; Stroup et al., 2003). Lowland cultivars are also later maturing and thus produce higher biomass later in the season than upland cultivars.

Biomass N removal is a function of biomass yield and tissue N concentration of the biomass (Vogel et al., 2002). Incremental increases in N application invariably result in the increase in switchgrass biomass production and tissue N concentration (Muir et al., 2001). The magnitude of increase usually follows the law of diminishing returns with the response to incremental increase being progressively less with each N application (Lemus et al., 2008). At 120 kg N ha^{-1}, the amount of N removed was approximately the amount of N applied and rates above this level resulted in increased soil nitrate-N levels (Vogel et al., 2002). In Alamo, N removal was found to be 50% greater than the amount of N applied and suggested that this may be attributed to the ability of switchgrass to scavenge N possibly through symbiotic relationships with soil or plant microbes (Fike et al., 2006). Atmospheric deposition may contribute N to these systems as well. Accumulation of P, K, and other plant nutrients in switchgrass are additional factors in understanding sustainable production of quality feedstock. These parameters and their interactions with N application and switchgrass cultivars have not been reported.

Management decisions not only affect the yield and nutrient balance of production systems, but can also the biomass quality and ethanol yield and this issue has not been adequately addressed. Biomass feedstock composition varies with harvest date, location and fertility management (Lemus et al., 2002). Previous reports on biomass quality are restricted mainly to cell wall composition traits with no quantification of ethanol yield (Adler et al., 2006; Lemus et al., 2002; Mulkey et al., 2006). Ethanol production via fermentation can be achieved from direct conversion of sugars and enzyme-mediated hydrolysis of starch or cellulose. Lignocellulose is a complex of cellulose, hemicellulose, and lignin and therefore requires additional processes for ethanol conversion. These steps include delignification to release cellulose and hemicellulose, depolymerisation of cellulose and hemicellulose to produce fermentable sugars, and fermentation to produce ethanol (Olofsson et al., 2008). Simultaneous saccharification and fermentation (SSF) is one process option for the production of ethanol from lignocellulosic biomass and integrates the enzymatic hydrolysis of cellulose to glucose and fermentation of glucose to ethanol processes (Faga et al., 2010). The SSF procedure requires complex fermentation assays and compositional analysis of biomass. With the development of near-infrared spectroscopy (NIRS), however, it has become analytically acceptable to determine biomass composition using equations developed by the USDA-ARS Near–Infrared Spectroscopy consortium (Vogel et al., 2011). Switchgrass has been extensively researched as a renewable bioenergy crop since the mid 1980s, however, to our knowledge, this is the first study to evaluate the influence of different entries and nitrogen application rates on biomass composition and ethanol yield using near infrared spectroscopy (NIRS). This paper quantifies the effects of nitrogen rates and switchgrass entries on nutrient removal rates and also demonstrates the capacity of NIRS to provide rapid, low cost and accurate estimates of biomass composition and ethanol yield. These findings will have direct implications for nutrient management and feedstock quality control in switchgrass production systems in the U.S. Midsouth.

A systematic approach to nutrient management requires an understanding of nutrient removal capacities, biomass production and feedstock quality. Quantitative information required to optimize bioenergy production systems, specifically nutrient dynamics, yield and quality in switchgrass growth is incomplete and inadequate. The objective of this study was to determine the effects of N application rate and genotypes on biomass production and nutrient removal rates, including micro-nutrients, biomass and ethanol yield in switchgrass grown in a site that represents the U.S Midsouth.

Materials and Methods

Study Site

This experiment was conducted in 2008 and 2009 at the Brown Loam Branch Experiment Station, Raymond, MS (32° 15' N, 90° 30'W) on 2-yr old switchgrass stands. The soil at the site is classified as a Loring silt loam (fine-silty, mixed, thermic Typic Fragiudalfs) characterized by 2 to 5% slopes, eroded, moderately well-drained with a fragipan.

Weather

Growing season (April-November) precipitation for 2008 (1023 mm) and 2009 (955 mm) was higher than the 30-yr average (924 mm). March 2008 precipitation (71 mm) was the driest March recorded, while March 2009 (235 mm) precipitation was 230%

above March 2008 and 69% higher than the long-term March average precipitation (139 mm) (Table 3.1). Mean air temperature for 2008 (21.6°C) and 2009 (21.9°C) was very close to the long term average temperature of 21.8°C (Table 3.1).

Table 3.1 Monthly precipitation and mean monthly air temperature for 2008 and 2009 and the 30-yr average (1979-2009) at the Brown Loam Branch Experiment Station, Raymond, MS.

Month	Precipitation (mm)			Air temperature (°C)		
	2008	2009	30-yr avg.	2008	2009	30-yr avg.
January	93	134	136	7.9	7.5	7.4
February	175	96	129	9.7	9.7	9.6
March	71	235	139	14.7	15.3	13.8
April	99	94	125	17.1	16.9	17.7
May	103	89	119	21.0	22.2	22.3
June	50	17	129	26.8	26.6	24.3
July	47	187	104	28.0	26.2	27.4
August	265	98	114	26.1	26.2	27.2
September	260	140	91	23.7	24.7	24.1
October	40	309	109	17.8	17.9	18.4
November	159	22	134	12.4	14.4	13.3
December	227	180	137	9.0	7.3	8.9

Treatments and Experimental Design

The experimental design was a randomized complete block with four replications. Treatments were switchgrass entries (Alamo, Cave-in-Rock, NF/GA001, and NF/GA992) as whole plots and N rates (0, 80, 160, and 240 kg ha^{-1} N) as subplots in a split-plot arrangement.

Plot Management

Following seedbed preparation in May 2006, entries were seeded with a small-plot planter (Kincaid Equipment and Manufacturing, Haven, KS) at a rate of 5.6 kg PLS ha^{-1} in rows 22 cm apart. Whole plots were 5 × 1.5m with 1-m alleyways between plots and 2-m alleyways between blocks. Subplots were 1.5 × 1 m, separated within whole plots by a 0.25-m border. In late fall of each year, (including the year prior to the initiation of this experiment) all plots were harvested at a10-cm stubble height. In early May of both study years, fertilizer was applied to subplots according to treatment in a single broadcast application of urea (46-0-0). No chemical weed and pest control or supplemental irrigation was applied during the experiment period.

Harvest Management, Tissue Sampling and Analysis

In late November of both years, a 1 × 0.5 m area in the center of each subplot was hand-clipped at a 10-cm stubble height. The total harvested material was weighed fresh and an approximately 1-kg subsample was taken and dried in a forced-air oven at 55 to 60ºC for 72 h or until constant weight was achieved in order to determine dry matter (DM) concentration. A second subsample taken from the harvested material was dried similarly and ground to pass a 2-mm stainless steel screen using a Wiley Mill (Model 4; Thomas Scientific, Swedesboro, NJ). The ground samples were submitted to the Mississippi State University Extension Service Soil Testing Laboratory for analysis of tissue concentrations of N (Kjeldahl), P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, (ICP spectrophotometry) and B (Curcumin method) (Crouse, 2001). Subsamples were analyzed for chemical composition by NIRS using the Foss model 6500 spectrophotometer (Foss NIRSystems, Inc., Laurel, Maryland, USA) using grass hay and switchgrass equations (Vogel et al., 2011) that estimated biomass composition and feedstock traits. Equations for ethanol estimation have been derived from simultaneous SSF using commercial cellulases and *Saccharomyces cerevisiae* after pretreatment. The results from these wet chemistry analyses was used to develop NIRS prediction equations, as described by Vogel et al. (2011).

Calculations and Statistical Analysis

Nutrient removal was calculated by multiplying biomass yield by elemental tissue concentration. Nitrogen use efficiency (NUE) and Apparent N Recovery (ANR) was calculated as follows:

$$\text{NUE} = \text{xN yield} - \text{0N yield})/\ \text{xN applied} \tag{3.1}$$

$$\text{ANR} = [(\text{N removed at xN applied} - \text{N removed at 0N})/\ \text{xN applied}]*100 \tag{3.2}$$

where x represents the treatment N rate.

Ethanol yield was calculated as follows:

Total ethanol yield (TEY):

$$(\text{ETOH} \times 1.267) + ((\text{ARA} + \text{XYL}) \times 0.579 \times 1.267) \tag{3.3}$$

Total theoretical ethanol yield (TTEY) (assuming 100% conversion):

$$\begin{aligned}[(((\text{MAN} + \text{GAL} + \text{GLC} + \text{STA}) \times 0.57) + (\text{GLCS} + \text{FRU}) \times 0.51) + \\ (\text{SUC} \times 0.537) \times 1.267)] + [(\text{ARA} + \text{XYL}) \times 0.579 \times 1.267)]\end{aligned} \tag{3.4}$$

where ETOH: ethanol/g dry forage, ARA: arabinose, FRU: fructose, GAL: galactose, GLC: glucose, GLCS: soluble glucose, MAN: mannose, STA: starch, SUC: sucrose, and XYL: xylose.

The data were analyzed by fitting mixed models using PROC MIXED of SAS (SAS Institute Inc., Cary, NC). Entry, N application rate, and year were considered fixed effects while replication was considered random. Year was considered as a repeated measure and responses were considered different at $P < 0.05$.

Results and Discussion

Biomass yield

There was a year × N rate interaction ($P = 0.009$) effect on annual DM yield. Generally, DM yield during 2009 was greater than 2008; however, the responses within year were different (Figure 3.1). In 2008, yield increased linearly with N rate ($P = 0.013$), but in 2009, the yield response was quadratic ($P < 0.001$). There was also a year × entry interaction effect on annual DM yield ($P = 0.019$). The interaction occurred because of differences in the patterns of means separation among entries within years. In 2008, yields of NF/GA001 (9.3 Mg ha^{-1}), NF/GA992 (8.4 Mg ha^{-1}) and Alamo (7.9 Mg ha^{-1}) were similar but in 2009 yields of NF/GA001 (13.8 Mg ha^{-1}) and NF/GA992 (15.0 Mg ha^{-1}) were greater than that of Alamo (12.6 Mg ha^{-1}). Cave-in-Rock yield was always lesser (4.6 and 6.3 Mg ha^{-1} in 2008 and 2009, respectively) than the other entries. Other studies have reported variation in yield responses across years which vary markedly (Lemus et al., 2008b; Muir et al., 2001; Reynolds et al., 2000). Yield increases in the second year of harvest in 1-yr switchgrass stands ranged from 58% (Reynolds et al., 2000) to 85% (Lemus et al., 2008b). Data from these studies indicate that response trends for yield with age of stand are similar across geographic locations although absolute values vary. Differences in absolute values of responses observed across location may be attributable to early-season precipitation variability between years

(Guretzky et al., 2011), inherent soil physical and chemical properties, photoperiodism, harvest frequency, as well as age-related stand productivity potential. Interannual yield variability is highly correlated with growing season rainfall (Muir et al., 2001). Switchgrass yield was reported to increase with stand age up to 3 yr, plateau at around 4 to 5 yr and decline thereafter (Alexopoulou et al., 2008; Muir et al., 2001; Reynolds et al., 2000). The yield differences observed in this study between years may have been influenced, in part, by the greater precipitation in March 2009.

Averaged across entries, yield increased by 29% (2008) and 69% (2009) at 80 kg N ha^{-1} compared to the control, but there was no further benefit to yield with the additional N rates (Figure 3.1). In the Muir et al. (2001) study, yield began to level off at higher N rate (168 kg ha^{-1}). In addition, plant persistence declined in the absence of yearly N application (Muir et al., 2001).

Lowland cultivars are more responsive to N application and have greater growth potential than upland cultivars due to mid-season growth vigor and delayed maturity (Stroup et al., 2003). Cave-in-Rock, a northern upland variety, is expected to produce less than Alamo and the experimental lines (NF/GA001 and NF/GA992) in southern locations such as in this study (Sanderson et al., 1999; Stroup et al., 2003). The breeding lines NF/GA001 and NF/GA992 are selections from parental Alamo populations, possibly explaining why no difference in yield response to N application rate was found among these three related entries.

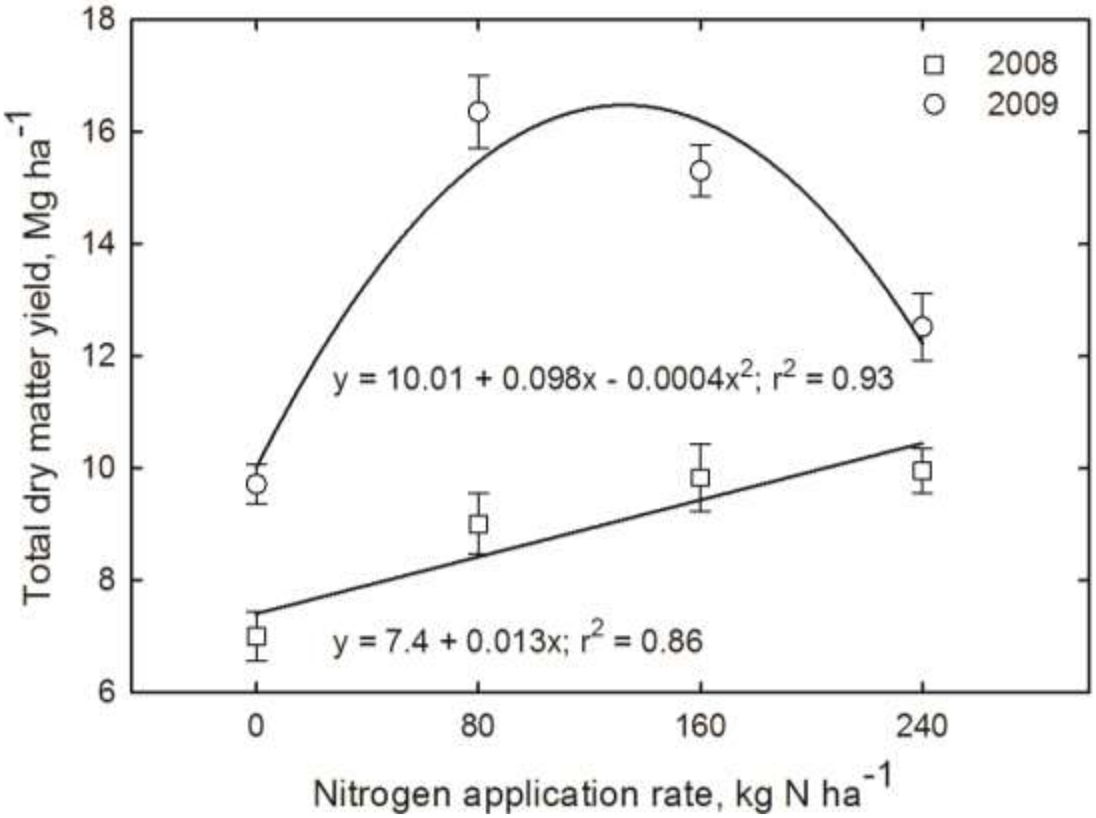

Figure 3.1 Year × N rate interaction effects on dry matter yield of switchgrass grown during 2008 and 2009 at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA**.**

Tissue Nutrient Concentrations

Tissue N concentration was not affected by entry or N rate or their interaction, and ranged from 7.16 to 8.15 g kg^{-1} with a mean of 7.72 g kg^{-1} (data not shown). Other studies have reported tissue N concentration increased with N application rate (Guretzky et al., 2011; Jung et al., 1990; Madakadze et al., 1999). The average tissue N concentration among entries at the zero N rate (7.64 g kg^{-1}) was within range of 8.2 g kg^{-1} (Jung et al., 1990) but more than 4.3 g kg^{-1} (Guretzky et al., 2011) under similar treatment conditions. These differences may result from inherent soil physicochemical properties, the plants ability to sequester N deeply in the soil profile through its extensive root system, and time of harvest. Since N is highly mobile in the plant and is translocated from shoots to roots at the onset of senescence, harvesting after senescence will lower the N concentration in the biomass (Lemus et al., 2008b).

Nitrogen rate also did not affect the tissue concentration of the other elements studied. Tissue P, K, Ca, and Mg concentration were affected by a year × entry interactions ($P < 0.05$). Tissue P concentration of Cave-in-Rock and NF/GA992 decreased from 2008 to 2009 (Table 3.2) but did not change with year in the other two entries. Across years and entries, P concentration ranged from 0.33 to 0.85 g kg^{-1}, compared to the 0.7 to 0.9 g kg^{-1} reported by Guretzky et al. (2011) and 0.90 to 1.50 g kg^{-1} reported by Propheter et al. (2010). Tissue K concentration was greater in 2008 than 2009 and was similar across entries in 2009, but in 2008 Cave-in-Rock had greater K concentration that the other entries (Table 3.2). Observed tissue P and K concentrations were lower than those reported previously (Guretzky et al., 2011; Propheter et al., 2010). Tissue Ca concentration within all entries was greater in 2008 than 2009 and among entries within both years, was greater for Cave-in-Rock than the other entries (Table 3.2). Tissue Mg concentration in 2008 was greater than 2009 for all entries except NF/GA992, which has similar concentration both years. Among entries, Mg concentrations were similar in 2008 but the magnitude of decrease in 2009 was larger for Cave-in-Rock than the other entries, resulting in it being the least among entries (Table 3.2). Year effects were observed for S, Mn, and Cu concentration also. Tissue concentration was greater in 2008 than 2009 for S and Mn but Cu concentration was lesser in 2008 (Table 3.2). Entry effects were observed for Fe ($P = 0.0145$) and B ($P = 0.0168$). Cave-in-Rock tissue Fe and B concentration was greater than the other entries in both years (Table 3.2). Tissue Zn concentration was affected by a year × N rate interaction ($P = 0.026$). In 2008, Zn concentration (mean = 11.30 mg kg^{-1}) did not respond to N rate but in 2009, there was a linear decrease ($P = 0.030$) with increasing N rate (range = 14.56 to 12.38 mg kg^{-1}). At the 0 and 80 kg ha^{-1} N application rate, Zn concentration in 2009 was greater than in 2008 but did not change across years at the higher N rates (data not shown). Therefore, we infer from the results that N fertility did not affect the concentration of tissue macro– and micro– nutrients in switchgrass grown in a fertile soil. Production using a particular genotype will require specific nutrient management in soils with low levels of plant available nutrients. The nutrient concentration differences among entries result from inherent differences in plant growth and physiology and maturation period.

Table 3.2 Mean P, K, Ca, Mg, S, Fe, Mn, B, and Cu tissue concentrations among switchgrass entries during 2008 and 2009 growing seasons at Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Entry	P		K		Ca		Mg		S	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
	g kg^{-1}									
Alamo	0.56 bA†	0.49 aA	1.88 bA	0.27 aB	1.88 bA	1.46 bB	1.69 aA	0.94 aB	0.40	0.25
Cave-in-Rock	0.85 aA	0.52 aB	2.51 aA	0.31 aB	3.05 aA	2.10 aB	1.82 aA	0.76 bB	0.44	0.26
NF/GA001	0.39 cA	0.46 abA	1.69 bA	0.36 aB	1.90 bA	1.38 bB	1.50 aA	0.85 aB	0.41	0.39
NF/GA992	0.61 bA	0.33 bB	1.84 bA	0.27 aB	1.74 bA	1.36 bB	1.38 aA	1.16 aA	0.37	0.27
Mean									0.40 A	0.29 B
SEM	0.08	0.02	0.09	0.01	0.15	0.09	0.05	0.04	0.01	0.02

Entry	Fe		Mn		Cu		B	
	2008	2009	2008	2009	2008	2009	2008	2009
	mg kg^{-1}							
Alamo	12.31 b	9.25 b	160.25 b	120.75 b	3.88 b	6.44 ab	2.69 b	2.25 b
Cave-in-Rock	13.50 a	12.97 a	269.70 a	214.07 a	5.63 a	7.41 a	3.40 a	2.43 a
NF/GA001	10.13 b	8.88 b	131.69 b	110.75 b	2.88 c	6.00 b	2.88 b	2.31 b
NF/GA992	12.82 b	10.81 b	159.87 b	133.81 b	3.51 bc	6.13 b	2.46 b	2.13 b
Mean			180.38 A	144.85 B	3.97 B	6.49 A	2.86 A	2.28 B
SEM	0.36	0.47	15.26	11.78	0.29	0.16	0.1	0.03

†Within column, means followed by the same lowercase letters, and within rows, means followed by the same uppercase letters are not different using the pairwise difference option (PDIFF) in PROC MIXED ($P > 0.05$).

Nutrient Removal

There were N rate ($P = 0.0009$), entry ($P < 0.0001$), and year ($P = 0.0184$) main effects on N removal but no interactions were detected. Among entries, N removal followed the same pattern as that of biomass yield. Nitrogen removal among Alamo, NF/GA001 and NF/GA992 was quadratic in response to N application rate ($y = 74.44 + 0.59x – 0.002 x^2$, $r^2 = 0.80$) and linear for Cave-in-Rock ($y = 32.51 + 0.123x$; $r^2 = 0.99$) (Figure 3.2). Averaged across entry and N rate, 2009 N removal (99.4 kg N ha^{-1}) was 37% greater than 2008 (72.5 kg ha^{-1}). The increase in N removal rates across years relates to the 54% increase in biomass production from 2008 to 2009. There was no effect of tissue N concentration on N removal possibly because of post maturity shoot to root translocation of N (Lemus et al., 2008b). Jung et al., (1990) reported that N removal increased by 69% from 0 (48.8 kg ha^{-1}) to 75 kg N ha^{-1} (81.88 kg ha^{-1}) and this difference was dependent on both biomass production and tissue N concentration. For Alamo and NF/GA001, and NF/GA992 (selections from Alamo), N removal was 112% greater than that of Cave-in-Rock (Figure 3.2). Nutrient removal rates in the biomass are indicative of the fertilizer requirements of switchgrass (Reynolds et al., 2000). Since the magnitude of element removal is affected by management practices such as cultivar selection (Fike et al., 2006a; Stroup et al., 2003), N fertilizer application rate (Madakadze et al., 1999; Muir et al., 2001), harvest frequency (Reynolds et al., 2000; Thomason et al., 2004b), and harvest timing (Fike et al., 2006a; Madakadze et al., 1999; Reynolds et al., 2000), we can minimize nutrient removal from production systems by adopting the optimum combination of management factors. The nutrient removal rate is a function of tissue concentration and biomass production.

Phosphorus removal was affected by a year × entry interaction ($P = 0.0284$) (Table 3.3). Between years, P removal rates were similar for all entries except for NF/GA001, which had 68% greater P removal in 2009 than 2008. In 2008, NF/GA992 (7.6 kg ha^{-1}) recorded the greatest P removal while for 2009, Alamo (6.5 kg ha^{-1}), NF/GA001 (7.4 kg ha^{-1}) and NF/GA992 (5.4 kg ha^{-1}) had greater P removal than Cave-in-Rock (3.3 kg ha^{-1}) (Table 3.3). Phosphorus removal was not affected by N rate; contrary to previous findings where P removal rates tended to increase with N rate (Guretzky et al., 2011). In our study, P removal was found to be related to tissue P concentration ($P < 0.0001$) (Table 3.3).

There were entry and year main effects on K removal but no N rate effect (Table 3), similar to previous findings (Guretzky et al., 2011). In 2009, K removal (17.1 kg ha^{-1}) was 76% less than 2008 (4.0 kg ha^{-1}) for all entries (Table 3.3). Cave-in-Rock K removal rates were less than the other entries in both years. These removal rates are less than those reported by Guretzky et al., (2011). Potassium removal was correlated with tissue K concentration ($P < 0.0001$). Calcium, Mg, Fe, Mn, and B removal responded to N rate main effects (Table 3.3). Calcium removal increased by 51% from 0 (13.8 kg ha^{-1}) to 80 kg applied N ha^{-1} (20.8 kg ha^{-1}), but higher N applications did not increase Ca removal (Table 3.3. Similarly, Mg, Mn, and B removal increased by 60, 86 and 52%, respectively, as N application increased from 0 to 80 kg N ha^{-1}. Quadratic responses to N were observed for Ca ($P = 0.0004$, $r^2 = 0.99$), Mg ($P = 0.0484$, $r^2 = 0.95$), Fe ($P = 0.0014$, $r^2 = 0.86$), Mn ($P < 0.0001$, $r^2 = 0.97$) and B ($P = 0.001$, $r^2 = 0.97$) concentrations. Calcium removal was not correlated with Ca tissue concentration, however, Mg, Fe, Mn, and B removal and respective tissue concentrations were correlated ($P < 0.0001$). There was a main effect of entry on S removal (*P* = 0.0092) with Cave-in-Rock removal lesser by 55% than the average of the other entries. A significant positive correlation was found between S removal rate and tissue S concentration (*P* < 0.0001).

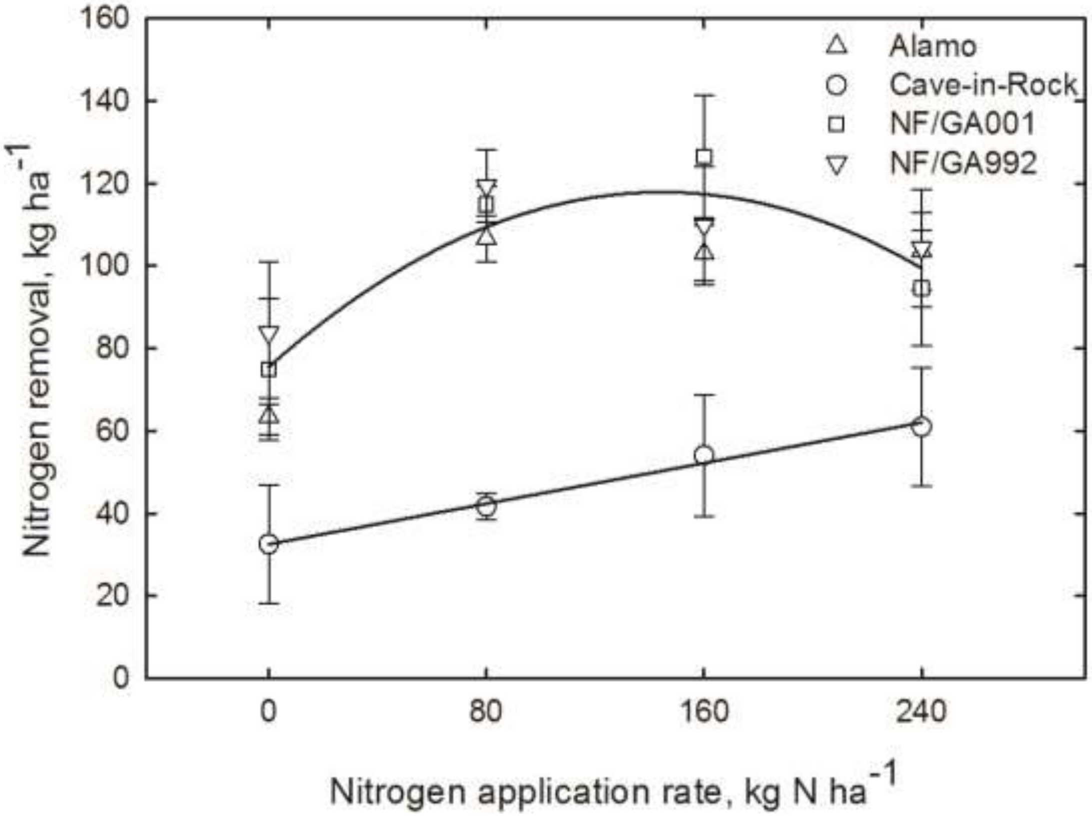

Figure 3.2 Genotype × N rate interaction effects on dry matter yield of four switchgrass genotypes grown during 2008 and 2009 at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Zinc and Cu removal rates were affected by both year × entry (*P* > 0.05) and year × N rate interactions (*P* > 0.05). Both Zn and Cu removal rates were found to be greater in 2009 than 2008 for all entries except Cave-n-Rock (Table 3). Among entries, Cave-in-Rock exhibited the lowest Zn removal rate in both years and lowest Cu removal in 2009, which may be related to the reduced Cave-in-Rock biomass production. Removal rates for these two elements were the same for the remaining entries except Alamo Zn removal in 2009, which was intermediate between Cave-in-Rock and the two experimental lines. In 2008, there was no effect of N rates ($P > 0.05$) but in 2009 both Zn and Cu removal had a quadratic response (Table 3). There was correlation between Cu removal ($P < 0.0001$) and Zn removal ($P < 0.0001$) with tissue concentration.

Nitrogen Use Indices

There was entry × N rate interaction on both NUE ($P = 0.0003$) and ANR ($P = 0.0265$). Nitrogen use efficiency has been used to describe a plant's capacity to acquire and utilize N for producing biomass and is expressed as the DM produced per unit N applied. Nitrogen–use efficiency declined monotonically above 80 kg ha^{-1} N for all entries except Cave-in-Rock. Among entries, NUE varied in response to N application rate ($P < 0.0001$) with a linear response observed for Cave-in-Rock and quadratic responses for Alamo, NF/GA001 and NF/GA992 (Figure 3.3A). Averaged across entries, NUE declined by 39% from 80 to 160 kg N ha^{-1} and further declined by 23% from 160 to 240 kg N ha^{-1}. At 80 kg N ha^{-1}, NUE for Cave-in-Rock was 50% lower than other three entries (Figure 3.3A).

Table 3.3 Mean P, K, Ca, Mg, S, Fe, Mn, B, Zn, and Cu removal rates among switchgrass entries and N rates during 2008 and 2009 growing seasons at Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Treatment	Nutrient Removal													
	P		K		Ca	Mg	S	Fe	Mn	B	Zn		Cu	
	2008	2009	2008	2009							2008	2009	2008	2009
Entry	kg ha^{-1}													
Alamo	5.0 abA†	6.5 aA	17.5 A	4.2 B	18.8 a	14.5 a	3.7 a	0.13 b	1.6 b	0.03 a	0.11 aB	0.17 cA	0.04 aB	0.09 aA
Cave-in-Rock	4.1 bA	3.3 bA	12.8 A	1.7 B	14.5 b	7.1 b	2.0 b	0.075 c	1.5 b	0.02 b	0.07 bA	0.11 bA	0.03 aA	0.05 bA
NF/GA001	4.2 bB	7.4 aA	18.0 A	5.8 B	21.2 a	15.0 a	5.7 a	0.13 b	1.6 b	0.04 a	0.11 aB	0.21 aA	0.03 aB	0.10 aA
NF/GA992	7.0 aA	5.4 abA	20.2 A	4.5 B	20.7 a	16.4 a	4.3 a	0.16 a	2.0 a	0.03 a	0.13 aB	0.24 aA	0.04 aB	0.11 aA
N rate														
0	4.2	5.2	13.1	3.0	13.8	9.1	2.7	0.09	1.1	0.02	0.08	0.14	0.03	0.06
80	5.3	6.5	16.7	4.3	20.8	14.6	5.0	0.12	2.0	0.03	0.10	0.24	0.03	0.11
160	4.8	5.1	17.8	5.1	21.6	14.8	4.1	0.16	2.0	0.03	0.11	0.19	0.04	0.10
240	5.9	4.9	21.1	3.8	19.0	14.5	3.8	0.12	1.7	0.03	0.11	0.17	0.05	0.08
OPC‡	NS	NS	L**	NS	L**, Q***	L*, Q*	Q**	L**, Q**	L,** Q***	L*, Q**	L*	Q***, C**	L*	Q***

†Within column, means followed by the same lowercase letters, and within rows, means followed by the same uppercase letters are not different using the pairwise difference option (PDIFF) in PROC MIXED ($P > 0.05$).

‡ OPC, orthogonal polynomial contrast (L, linear; Q, quadratic; C, cubic); *, **, ***, OPC significant at the 0.05, 0.01, and 0.001 levels, respectively; NS, not significant.

This implies that Alamo, and the two experimental lines derived from Alamo, NF/GA001 and NF/GA992, are more efficient in utilizing N and converting to biomass production. Biomass yield peaked at 80 kg ha^{-1} N with no further benefits to yield at higher N rates. As a result, NUE declined with increasing N application rates. Apparent N recovery responses were similar to NUE, that is, decreased with increasing N rate (Figure 3.3B). Apparent N recovery among entries varied in response to N application rate ($P < 0.0001$) with a linear response observed for Cave-in-Rock and a quadratic response for Alamo NF/GA001, and NF/GA992. Lemus et al. (2008b) reported 80% N recovery at 90 kg ha^{-1} N over a 3-yr period which was greater than Alamo and the experimental lines N recovery rates at 80 kg ha^{-1} N, in part due to the lower biomass production and N removal in this study. Stout et al., (1991) reported lower N recovery rates of 31 and 23% for 90 and 180 kg ha^{-1} N, respectively. At 80 kg ha^{-1} N, Cave-in-Rock recovered 41% less N than the average recovery of the other three entries in this study. Our data implies that applications of N above 80 kg ha^{-1} results in lower recovery rates and potential for N leaching. Much lower biomass production and N recovery rates for Cave-in-Rock compared to the other three entries indicate that it is not suitable as a feedstock in the U.S. Midsouth.

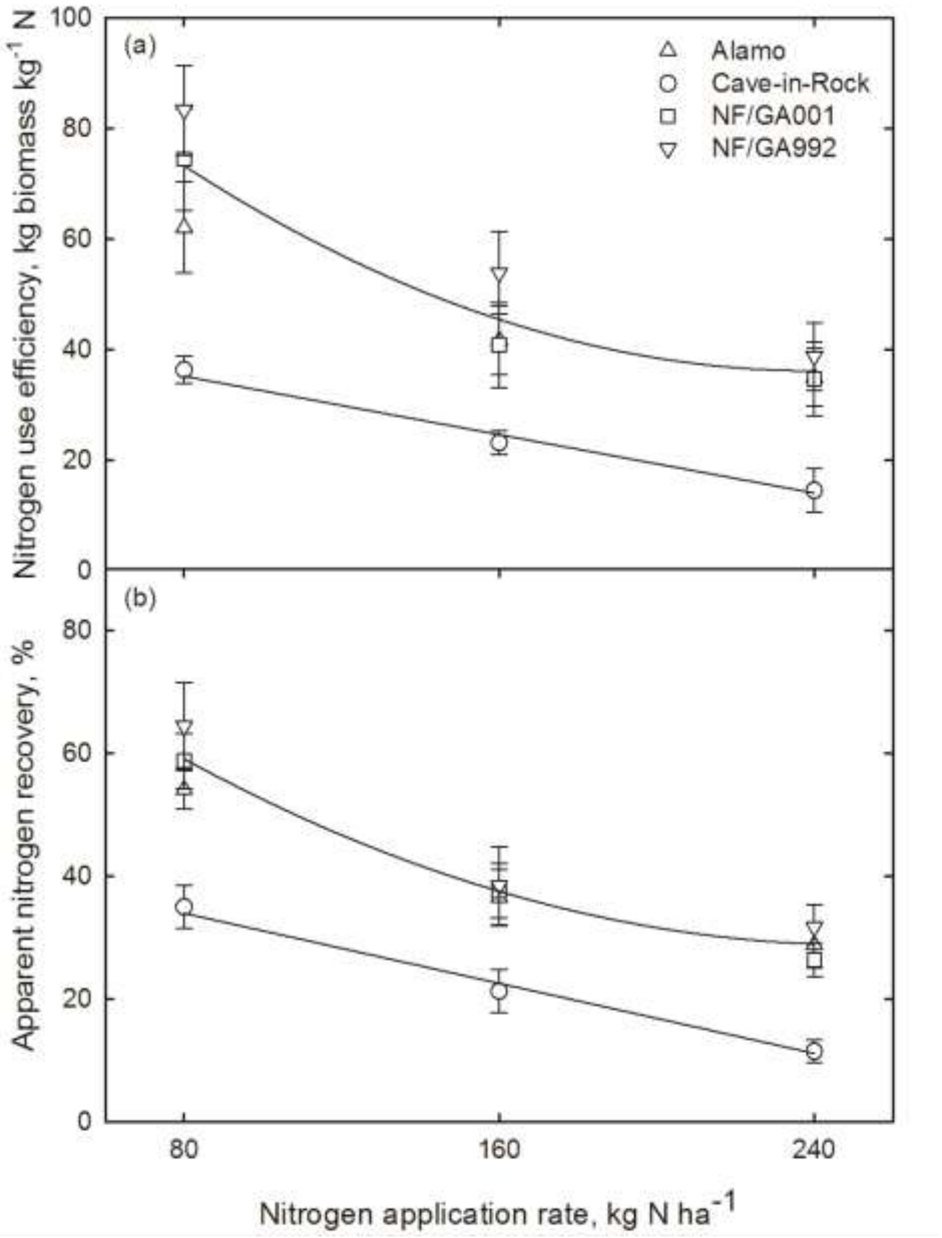

Figure 3.3 The effect of nitrogen application rate on (A) nitrogen use efficiency (NUE) and (B) apparent nitrogen recovery (ANR) of switchgrass, averaged over 2008 and 2009 growing season, at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

The symbols indicate the NUE and ANR across entries and the lines are fitted lines using a quadratic function for lowland entries ($y = 119.57 – 0.694x + 0.0014x^2$, $r^2 = 0.87$) and a linear function for upland Cave-in-Rock ($y = 45.75 – 0.132x$, $r^2 = 0.98$) for NUE. The lines for ANR are fitted lines using a quadratic function for lowland entries ($y = 93.59 – 0.5122x + 0.001x^2$, $r^2 = 0.95$) and a linear function for upland Cave-in-Rock ($y = 45.36 – 0.1426x$, $r^2 = 0.99$). Data are means and ± SE of four replications.

Cell Wall Properties

Feedstock quality parameters acid detergent fiber (ADF), lignin, and hemicellulose differed among entries ($P < 0.05$) but there was no effect of N application rates. There was a year × entry effect on neutral detergent fiber (NDF) ($P = 0.0146$) and cellulose ($P = 0.0266$) concentration. Acid detergent fiber concentration was greatest in Cave-in-Rock, followed by the experimental lines and Alamo. Hemicellulose and ash concentrations were the lowest in Cave-in-Rock and the highest in NF/GA992 and similar in Alamo and NF/GA001 (Table 3.4). This interaction indicates that cell wall properties may change in different years in response to precipitation and temperature with entries responding dissimilarly. Lignin concentration was greatest in Cave-in-Rock (Table 3.4). These lignin results are greater than those reported previously (Lemus et al., 2002) with no entry by year interaction present in our study. Neutral detergent fiber and cellulose increased by 6 and 10% in 2009 over 2008 across all entries (Table 3.4). Harvesting switchgrass after frost produced relatively high ADF and NDF concentrations, both highly desirable biofuel traits. Lowland entries produced the greatest yields and generally had the lowest lignin concentration, indicating that these lowland entries may be more suitable for feedstock production in the U.S. Midsouth. Ash concentration differed among entries. Cave-in-Rock had the lowest ash concentration while lowland NF/GA992 had the greatest ash concentration indicating that high yields increase the ash concentration (Table 3.4). These results are contrary to previous reports (Lemus et al., 2002) where lowland entries had the lowest ash concentration relative to upland entries. Nitrogen application rate did not affect the cell wall composition in our study, supporting findings of Guretzky et al. (2011) of only 1 and

2% increase in ADF and NDF as N rate increased from 0 to 225 kg N ha^{-1} after a frost harvest for Alamo switchgrass. Lignin ($r = 0.69$; $P < 0.0001$ and $r = 0.98$; $P < 0.0001$). and cellulose ($r = 0.60$; $P < 0.0001$ and $r = 0.92$; $P < 0.0001$) concentration was positively correlated with ADF and NDF concentrations, respectively. Hemicellulose was positively correlated with only NDF ($r = 0.53$; $P < 0.0001$).

Table 3.4 Cell wall components and ash of switchgrass biomass harvested during 2008 and 2009 growing seasons at Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Treatment	ADF†	NDF		Lignin	Cellulose		Hemicellulose	Ash
		2008	2009		2008	2009		
Entry				g kg^{-1}				
Alamo	536.4 c	831.9 cB‡	892.4 bA	121.1 b	390.6 bB	439.9 bA	325.8 b	82.3 b
Cave-in-Rock	551.9 a	836.3 cB	905.0 aA	125.4 a	401.6 aB	451.4 aA	318.8 c	77.2 c
NF/GA001	544.9 b	846.8 bB	898.3 aA	124.3 ab	399.3 aB	442.5 bA	327.6 b	80.2 b
NF/GA992	545.0 b	858.7 aB	903.3 aA	122.1 b	402.8 aB	441.8 bA	336.7 a	84.0 a

†ADF = acid detergent fiber; NDF = neutral detergent fiber

‡Within column, means followed by the same lowercase letters, and within rows, means followed by the same uppercase letters are not different using PDIFF option in PROC MIXED ($P > 0.05$).

Sugar composition

Carbohydrate concentrations in switchgrass varied with entry and N rate. Glucose, xylose and pentose represent the largest components of cell-wall carbohydrates. Glucose concentration was similar in the experimental lines and between Alamo and Cave-in-Rock but the experimental lines concentration was greater than the known cultivars (Table 3.5). Genotype NF/GA992 had the greatest arabinose, mannose and xylose concentrations but also had lower starch and sucrose concentrations among all entries (Table 3.5).

Table 3.5 Composition of switchgrass biomass harvested during 2008 and 2009 growing seasons at Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Treatment	Ara.†	ETOH	Fru.	Gal.	Glc.	Glcs.	Man.	Pent.	Sta.	Suc.	Xyl.
Entry	g kg^{-1}										
Alamo	24.3 b‡	53.5 a	2.8 a	9.3 a	300.6 b	3.2 a	8.8 b	189.7 b	5.3 b	2.7 a	205.8 b
Cave-in-Rock	22.9 d	49.1 b	2.6 ab	8.5 b	302.2 b	3.2 a	8.7 b	193.3 a	6.7 a	1.5 b	207.9 b
NF/GA001	23.6 c	49.8 b	1.9 ab	8.8 b	303.6 a	2.7 a	8.6 b	188.9 b	5.0 b	2.3 a	209.0 b
NF/GA992	25.0 a	48.2 b	1.3 b	9.4 a	307.5 a	1.9 b	9.4 a	195.2 a	2.8 c	0.5 c	213.0 a
N rate											
0	24.5 a	50.8	1.8	9.0	307.1	2.6	9	196.1 a	4.3 b	1.8	212.1 a
80	23.9 ab	50.3	2.3	9.0	302.5	3.0	8.8	191.6 b	4.9 ab	1.9	208.8 ab
160	24.0 ab	49.8	2.2	9.1	302.1	2.6	8.9	190.3 b	4.9 ab	1.4	207.7 ab
240	23.4 a	50.0	2.2	8.9	302.3	2.7	8.7	189.1 b	5.7 a	2.0	207.2 b
Mean		50.2	2.1	9.0	303.5	2.7	8.9			1.8	
OPC§	L*, Q*	NS	NS	NS	NS	NS	NS	L**, Q**	L*, Q*	NS	Q*

†Ara. = arabinose, ETOH = ethanol g^{-1} dry forage, Fru. = fructose, Gal. = galactose, Glu. = glucose, Glcs. = soluble glucose, Man. = mannose, Pent. = pentose, Sta. = starch, Suc. = sucrose and Xyl. = xylose.

‡Within column, means followed by the same lowercase letters are not different using PDIFF option in PROC MIXED ($P > 0.05$).

§ OPC, orthogonal polynomial contrast (L, linear; Q, quadratic; C, cubic); *, **, ***, OPC significant at the 0.05, 0.01, and 0.001 levels, respectively; NS, not significant.

Table 3.6 Significant correlation coefficients ($P < 0.05$, Pearson's correlation test) among switchgrass biomass yield, cell wall properties and ethanol yield of switchgrass harvested during the 2008 and 2009 growing seasons at Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

	Yield	ADF†	NDF	Lig	Cell	Hemi	Ara.	ETOH	Fru.	Gal.	Glc.	Pent.	Sta.	Xyl.	Ash	TEY	TEP
ADF	–																
NDF	–	0.93															
Lig	–	0.69	0.60														
Cell	–	0.98	0.92	0.55													
Hem	–	–	0.53	–	–												
Ara.	–	–	–	-0.74	–	–											
ETOH	–	-0.92	-0.89	-0.74	-0.87	–	–										
Fru.	–	-0.88	-0.92	-0.58	-0.87	–	–	0.87									
Gal.	–	-0.87	-0.81	-0.75	-0.82	–	0.61	0.74	0.71								
Glc.	–	0.91	0.97	0.54	0.91	0.50	–	-0.81	-0.91	-0.8							
Pent.	–	0.76	0.86	–	0.81	0.54	–	-0.63	-0.79	-0.59	0.88						
Sta.	-0.51	–	-0.53	–	–	-0.68	-0.61	–	0.52	–	-0.55	-0.56					
Xyl.	–	0.89	0.97	–	0.91	0.55	–	-0.81	-0.91	-0.77	0.98	0.93	-0.57				
Ash	–	-0.82	-0.82	-0.57	-0.81	–	–	0.66	0.7	0.94	-0.82	-0.72	–	-0.83			
TEY	–	–	–	-0.7	–	–	0.58	0.68	–	–	–	–	–	–	–		
TEP	1.0	–	–	–	–	–	–	–	–	–	–	–	-0.5	–	–	–	
TTEY	–	0.82	0.92	–	0.84	0.57	–	-0.68	-0.83	-0.8	0.96	0.9	–	0.96	-0.87	–	–
TTEP	1.0	–	–	–	–	–	–	–	–	–	–	–	-0.52	–	–	–	0.99

†ADF = acid detergent fiber; NDF = neutral detergent fiber; Lig = lignin; Cell = cellulose; Hemi = hemicellulose; Ara.= arabinose; ETOH = ethanol g^{-1} dry forage; Fru. = fructose; Gal. = galactose; Glu. = glucose; Pent. = pentose; Sta. = starch; Xyl.= xylose; TEY is the total ethanol yield from SSF; TEP is the total ethanol production per hectare from SSF; TTEY is the total theoretical ethanol yield (100% conversion) and TTEP is the total theoretical ethanol yield production per hectare (100% conversion).

Arabinose concentration differed with each entry. Arabinose, pentose and xylose were the only sugars that responded to N application with their concentrations decreasing with increasing N rate (Table 3.5). Ethanol concentration, fructose and galactose were inversely correlated to ADF (Table 3.6). As lignin concentration increased, the sugar concentrations tend to decrease except for glucose.

Ethanol Yield

There was an entry (*P* = 0.0197) and N rate (*P* = 0.0161) main effect on total ethanol yield (TEY). Total ethanol yield was highest in Alamo (165.8 L Mg^{-1}) and averaged 162 L Mg^{-1} for the other 3 entries (Table 3.7). The TEY decreased linearly with N application rate. There was a year × entry (*P* = 0465) and year × N rate (*P* = 0.0063) interaction effect on total ethanol production (TEP). The year × entry interaction is partly due to the lesser TEP in Cave-in-Rock in both years. The TEP also decreased linearly with N application rate in both years. The TEP is calculated from the biomass yield and therefore the trends in response to N application and entry are similar to biomass production trends. The TEP increase in 2009 was due to the increase in the second year biomass yields.

Total ethanol yield was inversely related to lignin concentration and positively correlated with arabinose and ethanol concentration (Table 3.6). On the contrary, TEP was not correlated with the cell wall properties but was strongly dependent on the biomass production (Table 3.6). Although, differences in biomass composition exist among the entries, biomass production was found to have a greater influence on ethanol yield than biomass quality. The selection of entries for a specific eco-edaphic zone can be therefore be based primarily on biomass production (Lemus et al., 2002) since differences in cell wall composition are not large enough to affect the biomass quality (Lemus et al., 2008b).

Theoretical ethanol yield

There was a year × entry interaction effect on total ethanol yield from all biomass sugars (TTEY) ($P = 0.0422$). In 2008, TTEY was similar across all entries but in 2009, Alamo had less than CIR, although neither was different from the two breeding lines (Table 3.7). Nitrogen application did not have an effect on TTEY in our study and this supports previous reports (Lemus et al., 2008b). Total theoretical ethanol production (TTEP) from all biomass sugars is calculated from biomass yield and the sugar components concentrations of the biomass. This represents the potential ethanol production if there is 100% conversion of the sugars to ethanol. The TTEP of Alamo and the experimental lines averaged 4.1 kL ha^{-1} in 2008 and 6.8 kL ha^{-1} in 2009 while Cave-in-Rock was 2.0 and 2.9 in 2008 and 2009 respectively (Table 3.7). Across entries, TTEP in 2009 was 61% greater than 2008. These trends are similar to and are correlated with biomass production responses. There was an N rate × year interaction ($P = 0.0031$) on TTEP. During 2008, TTEP response to N application was linear but in 2009, the response was quadratic (Table 3.7). Total theoretical ethanol yield per mega gram was positively correlated with ADF ($r = 0.82$; $P < 0.001$), NDF ($r = 0.92$; $P < 0.001$), cellulose ($r = 0.84$; $P < 0.001$), hemicellulose ($r = 0.57$; $P < 0.001$), glucose ($r = 0.96$; $P < 0.001$), pentose ($r = 0.90$; $P < 0.001$), and xylose ($r = 0.96$; $P < 0.001$) concentration. Similar to TEP, the TTEP was strongly correlated with biomass production but not biomass composition (Table 3.6). Total ethanol yield from SSF and all biomass sugars did not correlate because of the differences in the methods of estimation between the two.

On the other hand, TEP from SSF and TTEP from all biomass sugars were strongly correlated (*r* = 0.99) with each other and both were also strongly correlated with biomass yields (*r* > 0.99). Across all treatment combinations, TEP from SSF and all biomass sugars is 1.9 kL and 4.7 kL, respectively. Total theoretical ethanol production from all biomass sugars therefore increased by factor of 2.5 relative to TEP from SSF.

Our data suggests that there is potential in increasing the ethanol output from lignocellulosic biomass. Current methods of conversion are affected by cell wall recalcitrance and therefore do not fully liberate the cellulose and hemicellulose from the lignin complex for chemical, microbial or enzymatic depolymerization (Jiang et al., 2013; Yang and Wyman, 2007). Moreover, current methods are not cost-effective to facilitate commercial biomass to ethanol conversion (Alvira et al., 2010; Yang and Wyman, 2007). The ethanol yield potential difference in our study is 231 (141%) and 260 l Mg^{-1} (160%) in 2008 and 2009, respectively. Developing novel pretreatment methods to maximize the cellulose accessibility to cellulase enzymes may lead to improved biofuel processing and lower the cost of these biomass-based fuels.

Table 3.7 Estimated and theoretical ethanol yield of switchgrass biomass during 2008 and 2009 growing seasons at Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Treatment	Estimated ethanol yield and production			Theoretical ethanol yield and production			
	TEY†	TEP		TTEY		TTEP	
		2008	2009	2008	2009	2008	2009
Entry	l Mg^{-1}	kL ha^{-1}		l Mg^{-1}		kL ha^{-1}	
Alamo	165.8 a	1.6 aB‡	2.6 aA	393.3 aB	418.5 bA	3.7 aB	6.6 aA
Cave-in-Rock	162.4 b	0.9 bB	1.1 bA	390.2 aB	426.4 aA	2.0 bA	2.9 bA
NF/GA001	160.8 b	1.8 aB	2.5 aA	395.5 aB	421.9 abA	4.2 aB	6.5 aA
NF/GA992	162.0 b	1.8 aB	2.8 aA	398.9 aB	423.5 abA	4.4 aB	7.2 aA
N rate							
0	165.9	1.2 A	1.5 A	399.1	425.8	2.7 A	3.9 A
80	162.7	1.5 B	2.7 A	395.1	422.4	3.6 B	6.9 A
160	161.6	1.6 B	2.5 A	391.6	421.9	3.9 B	6.4 A
240	160.8	1.6 B	2.2 A	392.7	418.7	3.9 B	5.6 A
OPC§	L	L	L, Q	NS	NS	L	L, Q

†TEY is the total ethanol yield from SSF, TEP is the total ethanol production per hectare from SSF, TTEY is the total theoretical ethanol yield (100% conversion) and TTEP is the total theoretical ethanol yield production per hectare (100% conversion).

‡Within column, means followed by the same lowercase letters, and within rows, means followed by the same uppercase letters are not different using PDIFF option in PROC MIXED ($P > 0.05$).

§ OPC, orthogonal polynomial contrast (L, linear; Q, quadratic; C, cubic); *, **, ***, OPC significant at the 0.05, 0.01, and 0.001 levels, respectively; NS, not significant.

Summary and Conclusions

Nitrogen fertilizer application will be necessary to optimize and maintain feedstock yield and persistence in switchgrass production systems. Biomass production and nutrient removal was affected by both cultivar and N rate. Nutrient removal was driven primarily by biomass yield that was, in turn, dependent on cultivar-inherent biomass production capacities and their response to N rather than tissue nutrient concentration. Nitrogen use efficiency and ANR decreased with increased N application.

Application rates above 80 kg N ha^{-1} reduced NUE and ANR and are likely to result in increased N leaching and volatilization. Biomass production, nutrient removal, NUE and ANR for Cave-in-Rock were less than Alamo and the two experimental lines derived from Alamo. Coupled with soil chemical characteristics, nutrient removal rates can be used to develop cultivar-specific fertility recommendations. Although compositional differences exist among entries, nitrogen application had no effect on cell wall properties and several sugars. The differences in yield had greater importance than the differences in biomass quality. Ethanol yield was more dependent on the biomass yield rather than the small differences in compositional traits among the entries. The differences between estimated and theoretical ethanol yield provide opportunities for improving the pretreatment techniques to maximize the ethanol output. Findings from this research can further aid in cultivar selection, feedstock quality control, agronomic management, and policy decisions.

CHAPTER IV

HARVEST FREQUENCY ALTERS SWITCHGRASS YIELD, QUALITY, NUTRIENT REMOVAL, AND ETHANOL PRODUCTION

Abstract

Switchgrass (*Panicum virgatum* L.*)*, a native warm season perennial bunchgrass is a potential lignocellulosic biofuel feedstock. Harvest frequency and fertilizer management can affect biomass production, nutrient removal and ethanol yield in switchgrass production systems but little data is available on harvest frequency effects. This 4-yr study quantified yield and composition responses of 'Alamo' switchgrass to two N rates (80 and 160 kg N ha^{-1}) and four harvest frequencies (HF; harvesting 1, 2, 3 or 6 times annually). The experiment was arranged as a 4 × 2 factorial in a randomized complete block design with three replications. Annual yields decreased linearly with HF in all years. In the 1-cut HF, yield increased from 8.8 Mg ha^{-1} in Year 1 to 14.7 Mg ha^{-1} in Year 2 and plateaued thereafter. The 2-cut HF averaged 10 Mg ha^{-1} across years while in the 3-cut HF and 6-cut HF, yields increased from Year 1 to Year 2 but declined thereafter. Nutrient concentration generally increased with HF while nutrient removal decreased with HF, influenced by reduced biomass production. Acid detergent fiber, neutral detergent fiber, hemicellulose, cellulose and lignin concentrations decreased with HF but there was no effect of N rate. Theoretical ethanol production (TEP) was similar in the 1-cut HF (3.9 kl ha^{-1}) and 2-cut HF (4.2 kl ha^{-1}) in Years 1 and 2, however, as HF increased to 3-cut HF (2.6 – 3.4 kl ha^{-1}) and 6-cut HF (1.4 – 2.5 kl ha^{-1}), TEP decreased. In Years 3 and 4, TEP was greatest in the 1-cut HF (6.3 – 6.7 kl ha^{-1}) and decreased linearly with increasing HF. Harvest frequency had a major effect on feedstock yield, quality, ethanol yield and production, but N application rates >80 kg N ha^{-1} had little effect on these responses.

Introduction

The need for cleaner energy sources, national energy security, and economic growth has resulted in intensive research for renewable sources of energy. The Energy Policy Act of 2005 and its successor, the Energy Independence and Security Act of 2007 are two legislations that have advanced energy independence and security in the USA (Hochman et al., 2008). The federal Renewable Fuel Standard (RFS) mandates the increase of renewable fuel used as liquid transportation fuels from 9 billion gallons in 1998 to 36 billion gallons per year by 2022. The 2010 revised RFS2 limits corn (*Zea mays* L.) based ethanol production to 15 billion gallons per year; therefore, the remaining 21 billion gallons must be produced from other, most likely, cellulosic sources (Dixon et al., 2010). Native perennial warm-season grasses are high potential bioenergy candidates as a result of their high biomass production, potential to proliferate in marginal lands with relatively low inputs and capacity to aid in environmental conservation (McLaughlin and Adams Kszos, 2005).

Switchgrass (*Panicum virgatum* L.), a native warm season C4 perennial bunchgrass, has been identified as a potential feedstock for a lignocellulose-based biofuel industry since 1978 (McLaughlin and Adams Kszos, 2005). Switchgrass is a versatile energy feedstock; it can be fermented to produce ethanol, direct combusted or co-fired to produce electricity (Larson et al., 2009), or thermochemically converted by gasification/pyrolysis to produce syngas (Choi et al., 2010). Earlier uses of switchgrass include alleviation of summer forage deficits as grazed pasture or hay (Anderson and Matches, 1983). With appropriate nutrient and harvest management, switchgrass can be a dual purpose species providing early season forage and late season feedstock (Guretzky et al., 2011; Sanderson et al., 1999). Optimal dual purpose use, however, will depend on the biomass and ethanol yields and quality both as forage and feedstock. In addition, dual purpose switchgrass use requires multiple seasonal harvesting, which can influence the nutrient balance of production systems.

Switchgrass yield and quality is known to be affected by harvest management (Adler et al., 2006; Guretzky et al., 2011; Reynolds et al., 2000; Sanderson et al., 1999; Thomason et al., 2005). Switchgrass is sensitive to frequent defoliation because it elevates the apical meristem above the ground during vegetative development, it has a high ratio of reproductive to vegetative tillers, and new growth must occur from crown buds or aerial axillary meristems (Sanderson et al., 1999). Perennial grasses translocate significant amounts of nutrients to stem bases and roots at the end of the growing season (Vogel et al., 2002), therefore, sustainable switchgrass production will require optimum harvest management to sustain biomass yields and prevent early stand decline. Multiple harvests produced greater yields when compared to a single end-of-season harvest with three, two and a single annual harvest yielding 16.3, 14.7 and 12.9 Mg DM ha^{-1} (Thomason et al., 2005). Two annual harvests increased Alamo yields by 13, 38 and 66% relative to a single late fall harvest with application of N fertilizer at 0, 90 and 180 kg N ha^{-1} (Guretzky et al., 2011). On the contrary, other studies have reported decreased total seasonal yields with increased harvest frequency resulting from stand reduction (Sanderson et al., 1999).

Nitrogen is the primary limiting factor for switchgrass production, accounting for 80% of the variation in yields when water is not a limiting production factor (Stout et al., 1988). Several studies have reported N effects on biomass production and fiber quality in the USA (Heaton et al., 2009; Lemus et al., 2008b; Muir et al., 2001; Thomason et al., 2005; Vogel et al., 2002; Wilson et al., 2013). Although disparities exist among these studies with respect to harvesting schedules, agronomic practices, and environmental conditions, invariably switchgrass is responsive to incremental N application. Nutrient removal by switchgrass is influenced by both harvest management and N fertilizer rate (Vogel et al, 2002). Reynolds et al. (2000) reported that N removal with two annual harvests was greater than that for a single harvest each year after establishment. Also, Guretzky et al. (2011) reported that nutrient concentration and removal were 50% greater with two annual harvests than a single harvest.

Nitrogen concentration is usually greatest in the summer harvest of a two-harvest system and lowest in the fall harvest of a single harvest system (Reynolds et al., 2000). Harvesting after a killing frost produced greater total yields and improved switchgrass persistence compared with anthesis harvests (Mulkey et al., 2006). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) concentrations increased between anthesis and killing-frost harvests, while N and ash concentrations decreased. Harvesting switchgrass stands once annually after a killing frost and applying N at 56 kg ha^{-1} was an effective system for switchgrass biomass production and persistence on land enrolled in or managed similarly to Conservation Reserve Program in South Dakota (Mulkey et al., 2006).

Biomass yield and composition influences ethanol yield and production (Schmer et al., 2012), therefore N rate and harvest management will have an effect on the feedstock quality through compositional and yield modifications (Waramit et al., 2011). Simultaneous saccharification and fermentation (SSF) is one process option for the production of ethanol from lignocellulosic biomass and integrates the enzymatic hydrolysis of cellulose to glucose and fermentation of glucose to ethanol processes (Faga et al., 2010). Theoretical ethanol yield assumes 100% conversion of all biomass sugars to ethanol and ranges from 381 to 430 L Mg^{-1} while ethanol production ranges from 1.8 to 3.7 kl ha^{-1} (Schmer et al., 2012).

An understanding of the effects of HF and N rate across years on switchgrass grown as a feedstock is needed to determine the stability of dry matter yield (DMY) under different management systems. The effects of frequent harvesting and N application on ethanol production and productivity of switchgrass has not been evaluated previously in Mississippi and this dearth of information needs to be addressed. The objective of this study was to determine the effects of HF (harvesting beginning in June each year at 4-, 8-, or 12-wk harvest intervals, or a single end-of-season harvest resulting in 6, 3, 2 or 1 harvests annually; and referred to as 6-cut, 3-cut, 2-cut, and 1-cut HF, respectively) and N rate (80 and 160 kg N ha^{-1} in a single broadcast spring application) on biomass production, nutrient removal rates and ethanol yield and production on Alamo switchgrass grown as a biofuel feedstock under south central Mississippi conditions.

Materials and Methods

Study Site

This 4-yr field study was planted in 2007 and treatment imposition and data collection was conducted from 2008 through 2011 at the Brown Loam Branch Experiment Station, Raymond, MS (32° 15’ N, 90° 30’W). The soil at the site is classified as a Loring silt loam (fine-silty, mixed, thermic Typic Fragiudalfs) characterized by 2 to 5% slopes, eroded, moderately well-drained with a fragipan.

Weather

Weather data were collected from the climate station located at the research site (Figure 4.1). Annual growing season (April-October) precipitation fluctuated among the four production years. Precipitation was 9 and 21% higher than the long-term average in 2008 and 2009; however, in 2010 and 2011, the precipitation was 36 and 26% below the long-term average (Figure 4.1A). Long-term average annual growing season precipitation (30-yr) was 790 mm. Mean air temperature was comparable to the long-term average for all years except May 2010 which was 2 °C above the long-term average (Figure 4.1B).

Treatments and Experimental Design

The experimental design was a randomized complete block with three replications. Treatments were factorial combinations of four HF and two N application rates. Following seedbed preparation, Alamo switchgrass was seeded in May 2007 using a small-plot planter (Kincaid Equipment Manufacturing, Haven, KS) at a rate of 5.6 kg PLS ha^{-1} in rows 22 cm apart. Plots were 5 × 1.5 m with 1-m alleyways between plots and 2-m alleyways between blocks. In early May of all years, a single broadcast application of N as urea (46-0-0) was applied to plots based on the treatment N rate. No herbicide or insecticide was used and no supplemental irrigation was provided throughout the experiment.

Data Collection

Plant height was measured at each harvest from 6 plants per replicate from the soil level to the ligule of the uppermost leaf. Live tiller numbers were counted from two 0.25 m^2 quadrats within each plot and then extrapolated to determine tillers m^2. Canopy light interception was measured using a LI-190 SA Quantum sensor (LICOR, Lincoln, NE) connected to a LI-1000 data logger (LICOR, Lincoln, NE). One above-canopy and three below-canopy measurements were recorded between 1000 and 1300 h during cloud free conditions. Two randomly selected plants from outside the yield sampling area within each plot were collected by hand clipping a 5-cm stubble height and separated into leaf blade and stem (stem plus leaf sheath), then oven-dried at 55 to 60ºC for 72 hours for dry matter determination to estimate leaf:stem ratio. To determine biomass yield, a 2 × 1-m area in the center of each plot was cut to a 5-cm stubble height using a Troy Bilt sickle bar mower (Garden Way Inc., Troy, NY; no longer manufactured). After the yield sample was harvested, the remainder of the plot was mowed similarly and the material was removed and discarded offsite. The total harvested material from the yield sample was weighed fresh and an approximately 1-kg subsample was taken and dried in a forced-air oven at 55 to 60 ºC for 72-h to determine dry matter (DM) concentration. A second subsample was taken and dried similarly then ground to pass a 2-mm stainless steel screen using a Wiley Mill (Model 4; Thomas Scientific, Swedesboro, NJ).

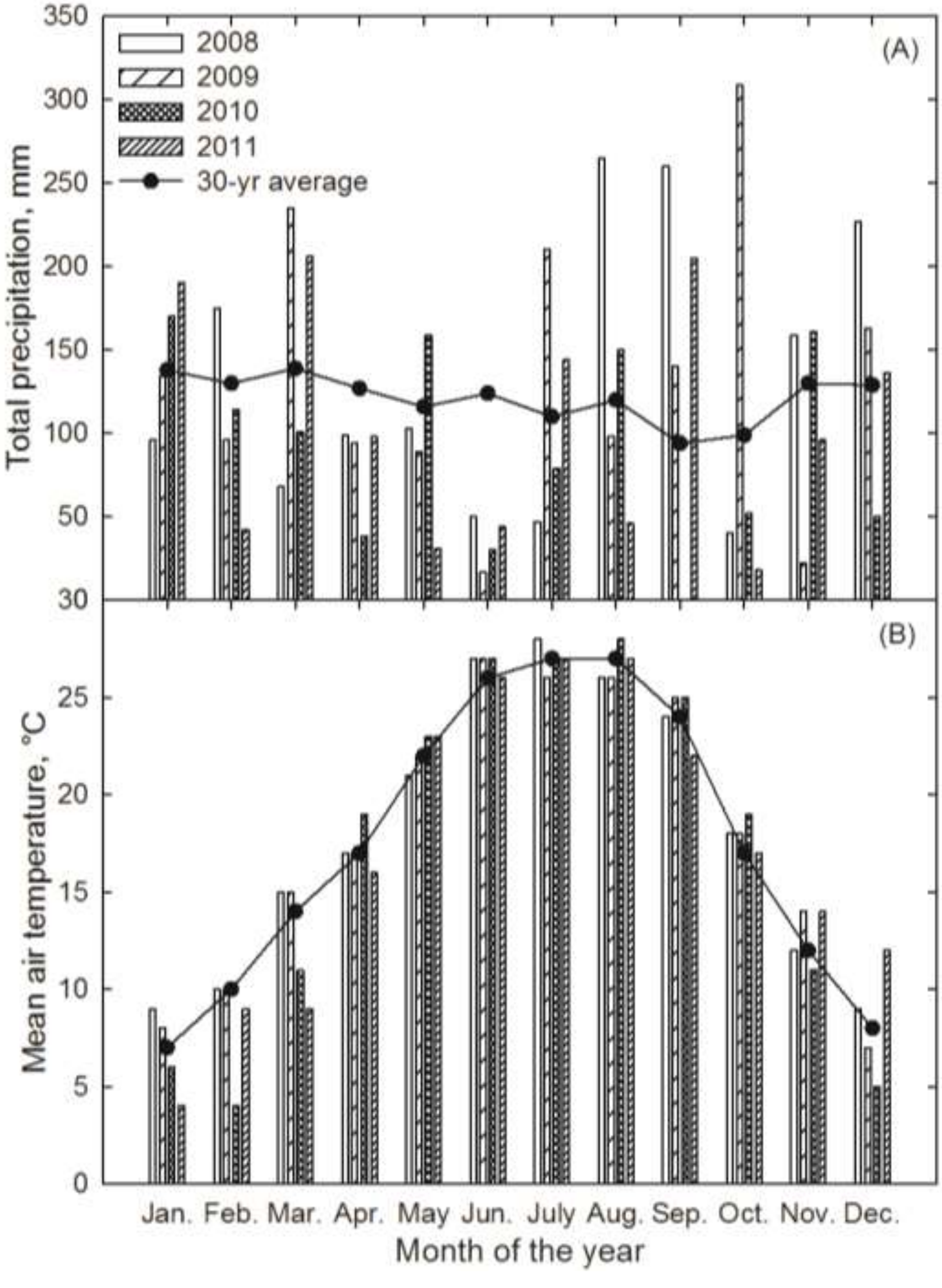

Figure 4.1 Total monthly precipitation (A) and mean monthly air temperature (B) at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA during the four years of this experiment (bars) and the average across 30 years, 1981 to 2011 (line).

Ground samples were analyzed for chemical composition by NIRS using the Foss model 6500 spectrophotometer (Foss NIR Systems, Inc., Laurel, MD.). The samples were scanned using FOSS ISIScan software version 4.4 (Vision, 2007) and prediction equations for grass hay and switchgrass developed by the NIRS Forage and Feed Testing Consortium (Hillsboro, WI) were used to estimate forage and feedstock parameters. Calibration statistics for trait characterization are adapted from Vogel et al. (2011). Hemicellulose and cellulose concentrations were calculated as the difference between NDF and ADF and the difference between ADF and ADL, respectively.

Calculations and Statistical Analysis

For the multiple harvests, season-long chemical composition concentrations were calculated as a weighted mean, which took into account the yield at each of the harvests. For example, weighted mean of season-long ADF concentration in the 3-cut HF system was calculated as ADF = [(ADF1 × DMY1) + (ADF2 × DMY2) + (ADF3 × DMY3)] / (DMY1 + DMY2 + DMY3), where ADF1, ADF2 and ADF3 is the ADF concentration (g kg^{-1}) in harvests 1, 2, and 3, and DMY 1, 2, and 3 is the dry matter yield of each harvest (kg ha^{-1}) respectively. Nutrient removal was calculated by multiplying biomass yield by elemental tissue concentration. Ethanol yields were calculated as per the methods of Vogel et al. (2011) using Eqn. 1 and 2 as described below.

$$\text{Total ethanol yield (TEY)} = [\text{ETOH} \times 1.267) + ((\text{ARA} + \text{XYL}) \times 0.579 \times 1.267] \quad (4.1)$$

$$\text{Total theoretical ethanol yield (TTEY)} = [(((\text{MAN} + \text{GAL} + \text{GLC} + \text{STA}) \times 0.57) + (\text{GLCS} + \text{FRU}) \times 0.51) + (\text{SUC} \times 0.537) \times 1.267)] + [(\text{ARA} + \text{XYL}) \times 0.579 \times 1.267)] \quad (4.2)$$

where: ETOH, ethanol/g dry forage; ARA, arabinose; FRU, fructose; GAL, galactose; GLC, glucose; GLCS, soluble glucose; MAN, mannose; STA, starch; SUC, sucrose; XYL, xylose.

The data were analyzed by fitting mixed models with repeated measures using PROC MIXED in SAS (SAS Institute Inc., Cary, NC). Harvest frequency, N application rate, year and their interactions were considered fixed effects while replication and replication × year were considered random effects. Year was considered as a repeated measure and responses were considered different at the 0.05 probability level. Differences among years were separated using the PDIFF option in SAS and responses to HF were tested using orthogonal polynomial contrasts. When linear and quadratic or linear, quadratic, and cubic functions were detected, the functional form of the regression relation was determined by starting with the linear function then adding successively higher order polynomials in conjunction with plotting the data and making visual observations (Neter et al., 1996). If it was determined that the higher order polynomial did not substantially improve the explanation of the response curve (based on r^2 values), then those higher order polynomials were ignored. Correlation coefficients describing relationships of DMY to nutrient concentration and removal, ethanol yield and production to DMY and fiber properties were determined by correlation analysis.

Results and Discussion

Dry Matter Yield and Agronomic Traits

There were year × HF ($P < 0.0001$) and year × N application rate ($P = 0.0009$) interaction effect on total annual DMY. Dry matter yield decreased linearly with HF in all years but the magnitude of the response differed among years contributing to the interaction effect (Figure 4.2). In the 1-cut HF, yield increased from 8.8 Mg ha^{-1} in Year 1 to 14.7 Mg ha^{-1} in Year 3 and plateaued thereafter. There was no difference in yield across years in the 2-cut HF, with average of 10 Mg ha^{-1} across the 4 years of the study. In the 3- and 6-cut HF, yield increased from Year 1 to Year 2 but declined thereafter (Figure 4.2). For the single harvest, yield increased through the first three growing seasons and stabilized thereafter, however, more than two annual harvests reduced yield in the second growing season. Relative to the 1-cut HF, total seasonal yields decreased by 29, 36, and 50% as HF increased to 2-, 3- and 6-cut HF, respectively, when averaged across all years and N rates. Other studies have reported a reduction in total yield with more than one annual harvest, for example, Madakadze et al. (1999) found that a mean yield decline by 20 and 49 % when upland switchgrass was harvested at 6- and 4- wk intervals relative to a single end-of-season harvest, similar to our observations.

The year × N rate interaction occurred partly because annual DMY was unresponsive to N rate in Year 1 (7.5 Mg ha^{-1}) and Year 4 (9.0 Mg ha^{-1}) but increased in Year 2 (8.0 to 9.5 Mg ha^{-1}) and decreased by 14% in Year 3 (11.0 – 9.5 Mg ha^{-1}) as N rate increased from 80 to 160 kg N ha^{-1} (Figure 4.3). The reason for greater DMY at the lower N rate in Year 3 is unclear but it could be related to the low rainfall during that year causing reduced growth and a lack of response to greater N application.

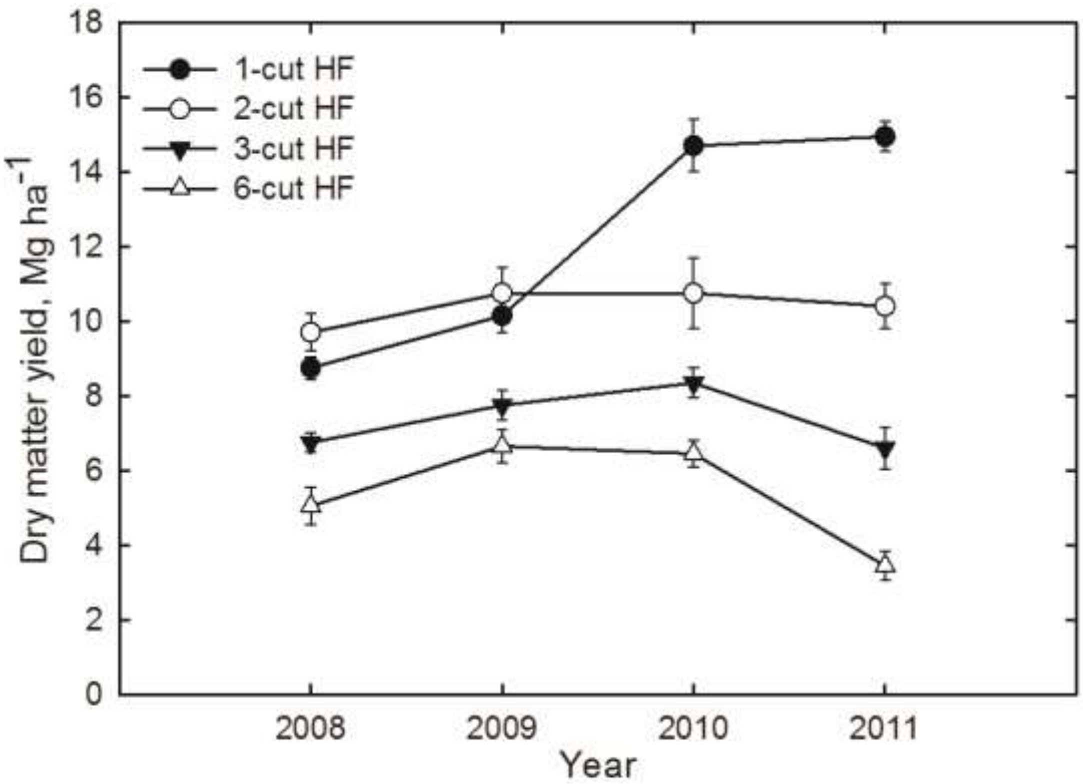

Figure 4.2 Harvest frequency × year interaction effect on switchgrass dry matter yield during four growing seasons (2008 to 2011) at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Error bars denote standard error.

Yield distribution within the season was affected by harvest frequency. The first harvest in multiple harvest treatments accounted for >70% of the total season yield with subsequent yield reduction with each additional harvest (Figure 4.4). An exception was the 6-cut HF, where the yields from the third harvest were greater than the second harvest in 2008 and 2010, which were relatively wet periods, thereby influencing the regrowth potential. Sanderson et al. (1999) reported that distribution and timing of rainfall affects the biomass yields as much as harvest frequency does. Production systems that minimize the number of harvests will be both economically efficient and environmentally benign

(Pedroso et al., 2013), therefore, a single after-frost kill harvest would capitalize on the natural nutrient translocation processes while maximizing economic outputs.

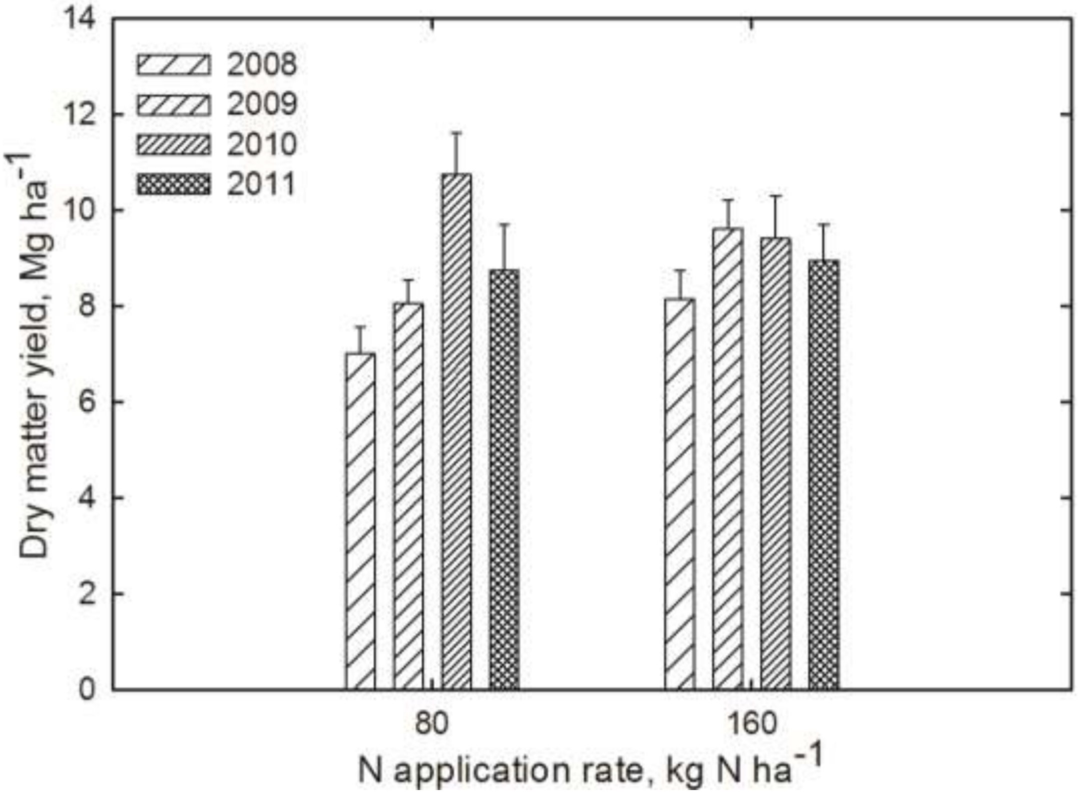

Figure 4.3 Year × N rate interaction effect on switchgrass dry matter yield during four growing seasons (2008 to 2011) at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Error bars denote standard error.

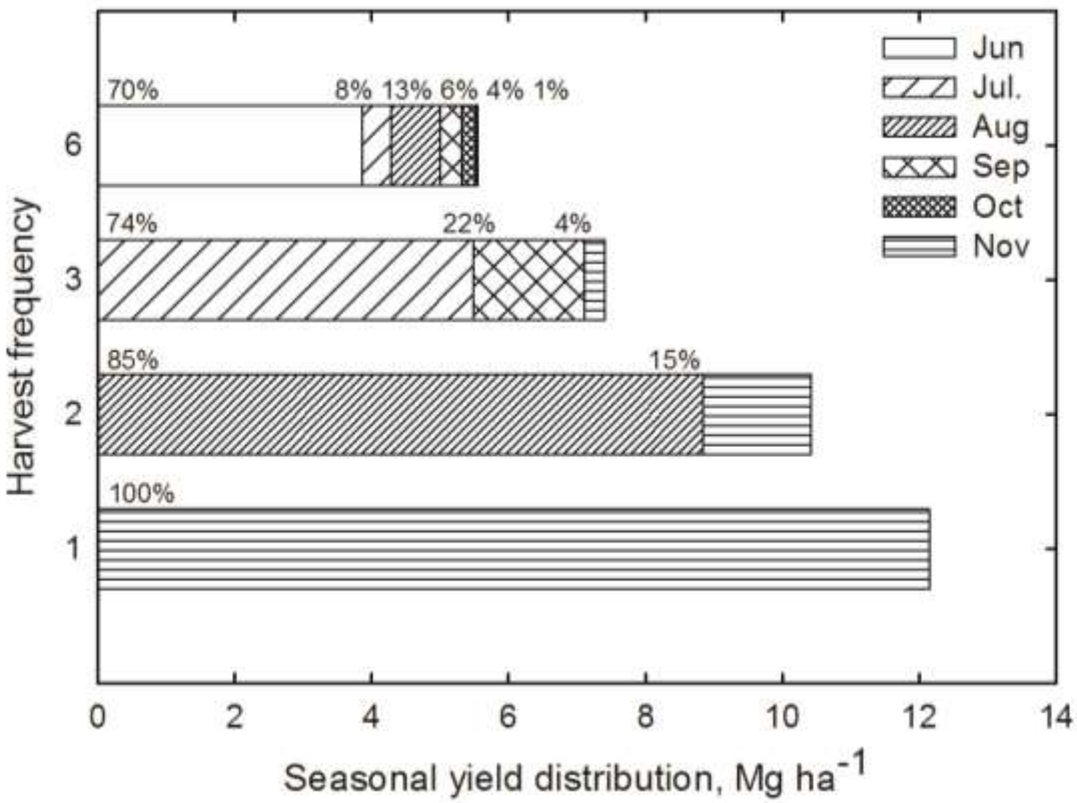

Figure 4.4 Harvest frequency effects on switchgrass seasonal yield distribution averaged over four growing seasons (2008 to 2011) at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

There was a year × HF interaction ($P < 0.0001$;Table 4.1) effect on plant height and tiller density. Across years, plant height was similar in the 6-cut HF but was greater in 2009 than 2008 in the other HF treatments, which partially explains the interaction. In both years, plant height decreased linearly with increased HF. Tiller density showed a linear increase with increasing HF in Year 1 but there was no difference among HF in Year 2 (Table 4.1). Also, tiller density was less in the second year. Light interception decreased linearly with increased HF, attributed to changes in canopy density and architecture (Table 4.1). Leaf:stem ratio also increased linearly with increasing HF.

Leaves have greater mineral concentration than stems, therefore feedstock with a high leaf:stem ratio will produce greater ash content when pyrolysed, an antiquality feedstock parameter that causes slagging and fouling of processing equipment (Qin et al., 2006).

Table 4.1 Harvest frequency effects on switchgrass plant height, tiller density, light interception and leaf:stem ratio during 2008 and 2009 growing seasons at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Harvest frequency	Plant height, (cm)		Tiller density, (no. m^{-2})		Light interception (%)	Leaf:stem ratio
	2008	2009	2008	2009		
1	112.4 b	171.6 a	2181.7 a	1973.9 b	93.3	0.4
2	76.9 b	108.1 a	2489.2 a	1529.7 b	87.9	0.7
3	50.5 b	71.9 a	3426.3 a	1663.2 b	71.1	1.5
6	38.7 a	39.8 a	3871.3 a	1708.3 b	61.6	3.2
OPC†	L^{***}, Q^{**}	L^{***}, Q^{***}	L^{***}	NS	L^{***}, Q^{**}	L^{***}

† *, **, *** Orthogonal polynomial contrasts significant at the 0.05, 0.01, and 0.001 levels, respectively.

‡Means within a row followed by the same lowercase letter are not different ($P > 0.05$) using PDIFF option in PROC MIXED.

Nutrient Concentration

Nitrogen Concentration

There was a year × HF interaction effect on average annual N concentration ($P <$ 0.0001;Table 4.2). Nitrogen concentration increased with HF in all years. Concentrations were similar across the first 2 yrs but decreased in the ensuing years. There was almost a six-fold decrease from Year 1 to Year 4 at the 1-cut HF but only about a two-fold decrease at the 6-cut HF, which contributed to the interaction effect.

Table 4.2 Harvest frequency × year interaction effect on switchgrass tissue N, P, K, Ca, and Mg concentration during four growing seasons (2008 to 2011) at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Harvest frequency	Nutrient concentration			
	2008	2009	2010	2011
	g kg^{-1}			
	Nitrogen concentration			
1	5.3 a	4.7 a	1.3 b	0.9 b
2	7.0 a	8.2 a	5.7 b	1.8 c
3	10.8 a	10.3 a	8.2 b	3.8 c
6	16.5 a	15.8 a	13.9 b	8.6 c
OPC†	L^{***}, Q^{***}	L^{***}, Q^{*}, C^{*}	L^{***}, C^{**}	L^{***}, Q^{***}
	Phosphorus concentration			
1	1.5 a	1.5 a	1.2 b	0.8 c
2	1.7 b	1.8 a	1.6 c	1.2 d
3	1.9 a	2.0 a	1.8 b	1.3 c
6	2.2 a	2.3 a	1.9 b	1.5 c
OPC	L^{***}, Q^{*}	L^{***}	L^{***}, Q^{***}	L^{***}, Q^{**}, C^{**}
	Potassium concentration			
1	11.3 b	12.5 a	11.0 b	6.9 d
2	13.6 ab	13.9 a	13.1 b	11.5 c
3	16.8 a	16.1 b	15.4 b	13.2 c
6	20.2 a	19.3 b	18.3 c	16.4 b
OPC	L^{***}, Q^{*}	L^{***}, Q^{***}	L^{***}	L^{***}, Q^{**}, C^{***}
	Calcium concentration			
1	1.6 b	2.0 a	1.3 c	0.6 d
2	1.8 b	2.5 a	1.9 b	0.6 c
3	3.0 a	3.0 a	2.4 b	1.3 c
6	3.5 a	3.5 a	3.8 a	2.6 b
OPC	L^{***}, C^{*}	L^{***}	L^{***}, C^{**}	L^{***}, C^{***}
	Magnesium concentration			
1	0.2 a	0.2 a	0.1 a	0.2 a
2	2.2 a	2.1 a	1.9 b	1.7 b
3	2.7 a	2.5 b	2.4 b	1.9 c
6	2.9 a	2.9 a	2.8 a	2.1 b
OPC	L^{***}, Q^{***}, C^{***}	L^{***}, Q^{***}, C^{***}	L^{***}, Q^{***}, C^{***}	L^{***}, Q^{***}, C^{***}

† *, **, *** Orthogonal polynomial contrasts significant at the 0.05, 0.01, and 0.001 levels, respectively.

‡Means within a row followed by the same lowercase letter are not different (*P* > 0.05) using PDIFF option in PROC MIXED.

Studies have reported that midsummer and fall harvests in Alamo switchgrass had greater N concentration than a single fall harvest (Guretzky et al., 2011; Reynolds et al., 2000), supporting the theory that N translocation reduces above ground plant tissue N concentration from May to December (Kering et al., 2012). Frequent harvests disrupt N translocation and affects regrowth, which is partly sustained from stored reserves, which in turn are depleted by multiple harvests. There was an N rate effect (*P* = 0.0341) on N concentration but there was no HF × N rate interaction (*P* > 0.05). Averaged across HF and years, N concentration at 80 and 160 kg N ha^{-1} was 7.5 and 7.9 g kg^{-1} (data not shown). Our findings support those of Guretzky et al. (2011) who found that N concentration increases with N rate as high as 225 kg N ha^{-1}, however, the magnitude of N concentration is dependent on the number and timing of harvests.

Phosphorus Concentration

There was a year × HF interaction effect on average annual P concentration (*P* = 0.0005;Table 4.2). In Years 1 and 2, P concentration increased linearly with HF while in Years 3 and 4, the response was quadratic. Phosphorous concentration was similar in the first two years for the 1-, 3- and 6-cut HF, and then decreased in Years 3 and 4 (Table 4.2). In the 2-cut HF system, P concentration was greater in Year 2 than in Year 1. The patterns of differences across years at the different HF contributed to the interaction. Phosphorous concentration declined progressively from Year 2 to Year 4 across all HF. The P concentration in the 1 and 2-cut HF in the current study is comparable to data reported in other studies (Lemus et al., 2009). Similar to N concentration, there was a seasonal decline in biomass P concentration (Kering et al., 2012), therefore two annual harvests have a greater P concentration than a single annual harvest (Guretzky et al., 2011; Lemus et al., 2009). In the current study, P concentration increased with HF across all years indicating that multiple harvests reduce the translocation potential of P to the crown and root region.

Potassium Concentration

There was a year × HF interaction effect on annual average K concentration ($P <$ 0.0001;Table 4.2). The interaction is partly due to a linear increase in K concentration with HF in Years 2 and 3 but in Years 1 and 4 there was a quadratic response (Table 4.2). At the 1-cut HF, K concentration was greatest in Year 2, similar in Years 1 and 3 and decreased in Year 4 (Table 4.2). At the 2-cut HF, K concentration was similar in Years 1 and 2 and declined progressively from Year 3 to Year 4 (Table 4.2). At the 3- and 6-cut HF, K concentration was greatest in Year 1 and decreased progressively from Year 2 to Year 4 for both, except in the 3-cut HF where K concentration was similar in Years 2 and 3. Potassium concentration decreases with plant maturity (Kering et al., 2012), therefore multiple harvests result in greater average annual K concentration than a single annual harvest (Guretzky et al., 2011). In the current study, K concentration increased with HF across all years indicating as the frequency of harvesting events increased, the potential to increase K concentration in the biomass also increases. Regression relationships showed that K concentration decreased linearly with DMY ($y = 2.0809 - 0.0732x$; $R^2 = 0.53$).

Calcium Concentration

There was a year × HF interaction effect on annual average annual Ca concentration ($P = 0.001$;Table 4.2). During the first 3 yr, Ca concentration increased linearly with HF but in Year 4, the response was quadratic (Table 4.2). Calcium concentration in the 1-, 2- and 3-cut HF was greatest in Year 2 and decreased thereafter, while in the 6-cut HF, the Ca concentration in the first 3 yr was similar, contributing to the interaction effect. The Ca concentration was considerably lower than for similar treatments reported by Lemus et al., (2009).

Magnesium Concentration

There was a year × HF interaction on annual average Mg concentration ($P <$ 0.0001;Table 4.2). Across all years, there was a quadratic response to HF (Table 4.2). At the 1-cut HF, Mg concentration was similar across all years but in the 2- and 6-cut HF, Mg concentration was similar across the first 3 yr then declined in Year 4. In the 3-cut HF, Mg concentration was greatest in Year 1, decreased but similar in Years 2 and 3 and further decreased in Year 4 (Table 4.2). The high translocation potential of Mg may be responsible for the relatively lower Mg concentration at the 1-cut HF. Magnesium concentrations in the current study were less than season-long concentrations reported by Kering et al. (2012), which ranged from 2.4 to 4.4 g kg^{-1}.

There was an effect of N rate on tissue N concentration ($P = 0.0341$) but not P, K, Ca and Mg concentrations ($P > 0.05$). Guretzky et al. (2011) reported similar lack of effects of N rate on P and K concentration even at N rates as high as 225 kg N ha^{-1}, while Kering et al. (2013) found N effects on N, P and Mg concentrations at N rates as high as 270 kg N ha^{-1}. The results of our study show that N concentration was similar in Years 1 and 2 and lowest in Year 4 across HF, while P, K, Ca, and Mg concentration generally decreased over time. The increase in nutrient concentration with HF is related to the physiological maturity of the harvested material. Switchgrass nutrient concentration declines as the growing season progresses from anthesis, seed maturation to frost-kill

(Kering et al., 2012). As HF increases, physiological maturity at each harvest decreases and the concentration of N, P, K, Ca and Mg increases in the aboveground tissue (Waramit et al., 2011; Wilson et al., 2013). A negative correlation was found between total yield and nutrient concentration ($r < -0.58$; $P < 0.0001$) (data not shown). Since nutrient concentration decreases across the growing season (Kering et al., 2012; Wilson et al., 2013), multiple harvests will have implications for nutrient management. Less frequent harvests had greater yields with lower nutrient concentration indicating there is greater potential for nutrient translocation with fewer harvests annually. A single post-frost kill harvest resulted in reduced tissue N, P and K concentration. Multiple harvests increased mineral concentration, attributable to a greater leaf:stem ratio and ash content thus increasing the potential for slagging and fouling of processing equipment when switchgrass is thermochemically converted.

Nutrient Removal

Total Nitrogen Removal

There were year × HF ($P < 0.0001$) and year × N rate ($P < 0.0001$) interaction effects on total annual N removal. The year × HF interaction occurred partly because among years there were fluctuations in the shape of the response curves showing increased N removal with increasing HF (Table 4.3). Also, total N removal in the 1-cut HF was similar in Years 1 and 2 (47.3 kg ha^{-1}) then decreased to similar levels in Years 3 and 4 (15.9 kg ha^{-1}), at 2- and 6-cut HF N removal was greater in 2009 than 2008 and 2010, and at 3-cut HF was similar during the first three years. Among all multiple harvests treatments, there was a sharp decrease in N removal in Year 4 (Table 4.3). Increases in N removal in multiple annual harvests have been reported by Guretzky et al.

(2011), Lemus et al. (2009), and Reynolds et al. (2000). In the current study, N removal differed among years and reflected the inter-year N concentration fluctuation rather than the DMY changes. In fact, total N removal was positively correlated with N concentration ($r = 0.83$; $P < 0.0001$) but (perhaps due to the relatively large influence of the single harvest DMY and its lower N concentration) there was no correlation with yield across all HF. Across years and N rate, the 2-, 3- and 6-cut HF removed 99, 119 and 178 % more N than the 1-cut HF. The N removal in multiple harvest systems exceeded the amount of N applied in the first 3 yr of the study while in 2011, N removal across all harvest treatments was comparatively low. Although the 2011 N concentration was lesser in all harvests relative to other years, the lower biomass production in 3- and 6-cut HF contributed to the lower N removal rates. These results support previous findings (Lemus et al., 2009; Reynolds et al., 2000) where N removed is greater in pre-senescence harvests than after frost-kill harvests. This low N concentration in a single annual post frost-kill harvest has been attributed to the seasonal translocation of N from shoots to roots and crown. In multiple harvest systems, frequent biomass removal reduced the stand persistence and influenced the regrowth potential of these treatments.

Table 4.3 Harvest frequency × year interaction effect on switchgrass total N, P, K, Ca, and Mg removal during four growing seasons (2008 to 2011) at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Harvest frequency	Nutrient Removal			
	2008	2009	2010	2011
	kg ha^{-1}			
	Total nitrogen removal			
1	46.9 a	47.6 a	18.9 b	12.8 b
2	68.4 b	86.4 a	61.2 b	18.4 c
3	73.1 a	79.5 a	68.3 a	24.7 b
6	83.1 b	105.8 a	90.6 b	29.6 c
OPC†	L***	L***, C***	L***, Q*, C**	L**
	Total phosphorus removal			
1	12.9 b	15.4 a	17.3 a	11.4 b
2	16.3 b	18.9 a	17.0 ab	12.1 c
3	12.7 b	15.1 a	15.0 a	8.4 c
6	11.3 b	15.3 a	12.4 b	5.1 c
OPC	L*, Q**, C*	Q*, C**	L***	L***, Q*
	Total potassium removal			
1	99.2 c	126.0 b	162.4 a	103.7 c
2	132.4 ab	148.9 a	142.1 a	120.2 b
3	113.8 a	124.0 a	129.3 a	87.6 b
6	102 b	128.7 a	118 ab	55.7 c
OPC	Q**	C*	L***	L***, Q**
	Total calcium removal			
1	13.8 b	20.0 a	19.3 a	8.4 c
2	17.8 b	27.1 a	20.2 b	6.2 c
3	19.9 a	22.9 a	20.2 a	9.1 c
6	17.9 b	23.5 a	24.9 a	9.0 c
OPC	ns	C*	L*	ns
	Total magnesium removal			
1	15.5 a	15.7 a	16.5 a	22.9 b
2	21.1 a	22.6 a	20.9 a	17.2 b
3	18.2 a	19.0 a	19.8 a	12.3 b
6	14.8 b	19.3 a	18.0 ab	7.4 c
OPC	Q**	Q*, C*	Q*	L***

† *, **, *** Orthogonal polynomial contrasts significant at the 0.05, 0.01, and 0.001 levels, respectively.

‡Means within a row followed by the same lowercase letter are not different ($P > 0.05$) using PDIFF option in PROC MIXED.

The year × N rate interaction occurred partly because annual N removal increased with N rate in Years 1 and 2 but there were no differences in Year 3 and 4 (Figure 4.5). Total N removal was positively correlated with N concentration ($r = 0.83$; $P < 0.0001$) but a weak negative correlation ($r = – 0.29$; $P = 0.0047$) was found between N removal and yield, indicating that N removal was probably influenced by N concentration rather than DMY. Nitrogen removal increased linearly with N concentration in the biomass ($y = 16.30 + 53.31$; $R^2 = 0.70$). Pedroso et al. (2013) and Guretzky et al. (2011) reported that N removal is linearly related to N application rate similar to the results of the current study.

Total Phosphorus Removal

There were year × HF ($P < 0.0001$) and year × N rate ($P < 0.0001$) interaction effects on total annual P removal. The year × HF interaction occurred partially because total P removal in the 1-, 3-, and 6-cut HF was similar in Year 1 (12.3 kg ha^{-1}) and Year 2 (15.3 kg ha^{-1}), while in Years 3 and 4, total P removal was different among the multiple harvest systems (Table 4.3). In the first 2 yr, there was a quadratic increase in P removal with HF while in last 2 yr, there was a linear increase. Increases in P removal in multiple annual harvests have been previously reported (Guretzky et al., 2011; Lemus et al., 2009; Reynolds et al., 2000). Phosphorus removal reported in the current study ranged from 11.4 to 18.9 kg P ha^{-1} for single and two annual harvests. These results are more than reported for Alamo switchgrass in other studies (Guretzky et al., 2011; Lemus et al., 2009; Reynolds et al., 2000) and are a reflection of the greater biomass yields reported at this location.

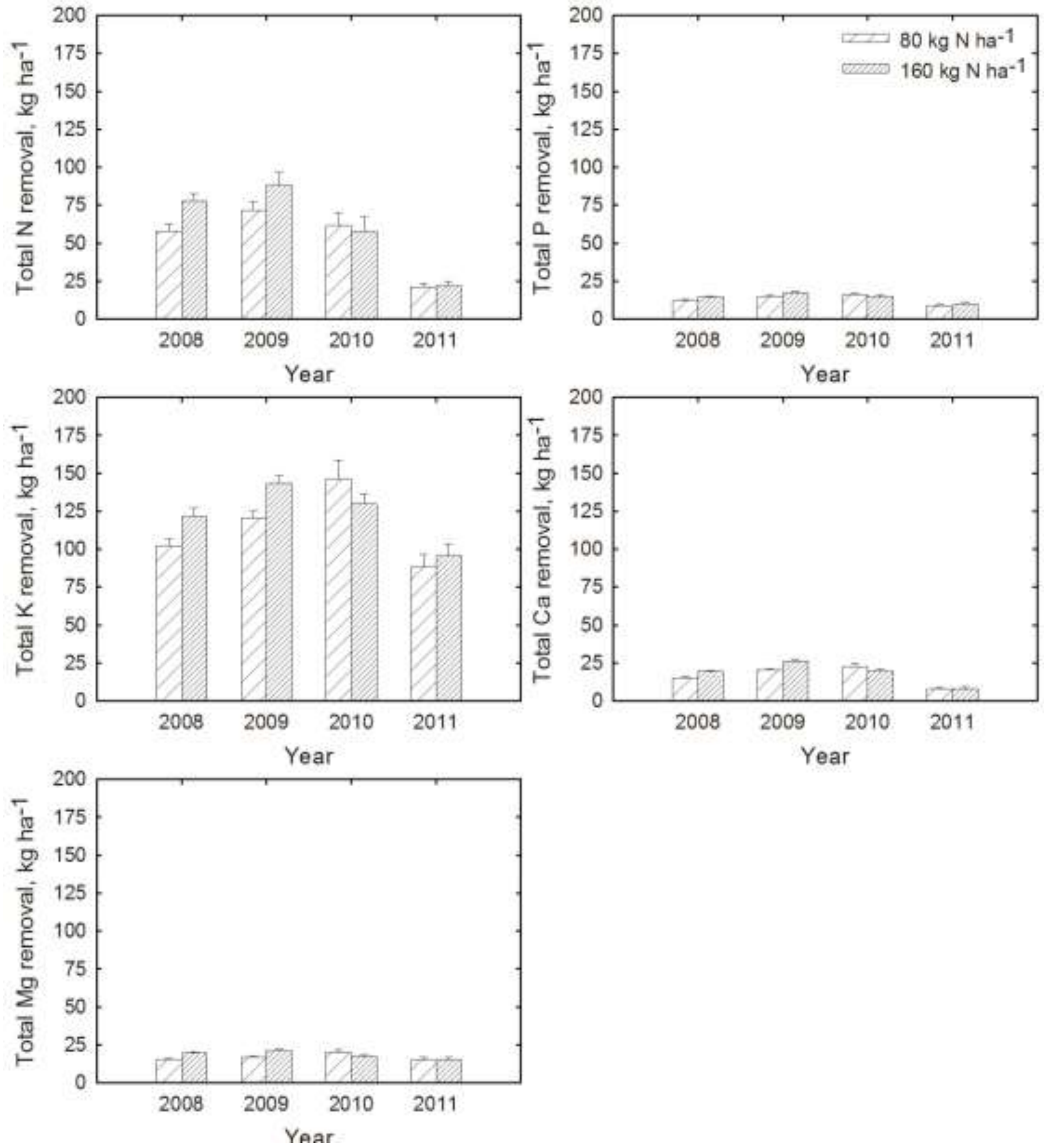

Figure 4.5 Nitrogen rate × year interaction effect on switchgrass total N, P, K, Ca, and Mg removal during four growing seasons (2008 to 2011) at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

The year × N rate interaction partly occurred because annual P removal increased with N rate in Years 1 and 2 but was not different between N rate in Years 3 and 4 (Figure 4.5). Other studies reported that P removal increased with N rate from 0 to 225 kg N ha^{-1} for a single (8 to 13 kg P ha^{-1}) and twice (16 to 28 kg P ha^{-1}) harvested switchgrass (Guretzky et al., 2011). Total P removal was positively correlated with DMY ($r = 0.63$; $P < 0.0001$) but there was no correlation ($P = 0.0865$) between P removal and P concentration, indicating that P removal was probably influenced by DMY rather than the tissue P concentration, even though P concentration was different among HF.

Total Potassium Removal

There was a year × HF ($P = 0.0018$) and a year × N rate ($P < 0.0008$) interaction effect on total annual K removal. Except in Year 3 where there was a linear decrease with increased HF, K removal generally increased from the 1- to the 2-cut HF then decreased as HF increased (Table 4.3). Differences across years within HF fluctuated but generally K removal was similar among the first 3 yr, peaked in Year 3, and was least in Year 4. Harvesting switchgrass twice annually was found to increase the K removal relative to a single midsummer harvest across 5 yr (Guretzky et al., 2011; Lemus et al., 2009). In the current study, harvesting twice increased the K removal by 20% relative to a single late fall harvest. Additional harvests decreased the K removal in two out of four years, reflecting inter-annual variability in DMY and K concentration in these years. Total K removal was positively correlated with DMY ($r = 0.69$; $P < 0.0001$) but there was no correlation ($P < 0.05$) between K removal and K concentration.

The year × N rate interaction occurred partly because annual K removal increased with N rate in Years 1 and 2 but there was no difference in Year 4 (Figure 4.5). In Year 3, the greater K removal (146.5 kg ha^{-1}) at the lower N rate is related to the greater DMY (Table 4.3). Other reports indicated that K removal had a limited response to N application whether harvested once in the late fall or twice annually (Guretzky et al., 2011).

Total Calcium Removal

There was a year × HF interaction ($P = 0.0417$) and a year × N rate ($P < 0.0008$) interaction effect on total annual Ca removal. During Years 1 and 4, there was no response to HF but in Year 2 there was a cubic response and in Year 3 there was a linear increase as HF increased (Table 4.3). The year × N rate interaction occurred partly because annual Ca removal increased with N rate in Years 1 and 2 but there were no differences in Years 3 and 4 (Figure 4.5). Calcium removal in a single late fall harvest was similar to two annual harvests. In addition, the multiple harvests systems removed similar quantities of Ca, despite differences in Ca concentration in all four years. Total Ca removal was positively correlated with Ca concentration ($r = 0.66$; $P < 0.0001$) but very weakly correlated with DMY ($r = 0.14$), indicating that Ca removal was probably influenced by tissue Ca concentration rather than DMY. Calcium removal increased linearly with Ca concentration in the biomass ($y = 6.78 + 48.45x$; $R^2 = 0.43$).

Total Magnesium Removal

There was a year × HF ($P < 0.0001$) and a year × N rate ($P < 0.0001$) interaction effect on the total annual Mg removal. During the first 3 yr there was a quadratic response to HF while in Year 4 there was a linear decrease (Table 4.3). Among years, Mg removal was similar within the 1-, 2-, and 3-cut HF for the first 3 yr but there was fluctuation among years at the 6-cut HF, increasing by 31% in Year 2 and decreased by 59% in Year 4 (Table 4.3). The year × N rate interaction occurred partly because annual

Mg removal increased with N rate in Years 1 and 2 but there were no differences in Year 4 (Figure 4.5). In Year 3, the greater Mg removal at the lower N rate was possibly due to the greater DMY. Total Mg removal was positively correlated with DMY ($r = 0.63$; $P < 0.0001$) but there was no correlation ($P < 0.05$) between Mg removal and concentration, indicating that Mg removal was probably influenced by DMY rather than the tissue Mg concentration.

Nutrient removal is function of yield and biomass nutrient concentration. In the current study, N removal was due to the effects of N application rate while P, K, Ca and Mg removal were due more to the effects of HF than N rate. Increased HF resulted in increased leaf:stem ratio and greater nutrient concentration in these tissues and is therefore responsible for the greater nutrient removal with increased HF. Nitrogen concentration was strongly correlated to P, K, Ca and Mg concentration ($r > 0.92$) and this led to the strong correlation between total N removal and P, K, Ca and Mg concentration ($r > 0.71$).

Plant lignocellulosics

ADF Concentration

There was a year × HF interaction effect ($P < 0.0001$) on weighted annual average ADF concentration. Generally, ADF decreased with increasing HF but the shape of the response curve was not the same each year. Also across years, ADF fluctuated within HF (Table 4.4). The ADF concentration results in the current study are comparable to previous findings, for example, Kering et al. (2013) reported ADF concentration of 465 g kg^{-1} from a single annual harvest and 388 g kg^{-1} when harvested twice annually.

Table 4.4 Harvest frequency × year interaction effect on switchgrass fiber properties grown during four growing seasons (2008 to 2011) at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Harvest frequency	2008	2009	2010	2011
	g kg^{-1}			
	Acid detergent fiber concentration			
1	451.8 c‡	494.9 b	525.6 a	497.3 b
2	436.1 a	437.4 a	436.8 a	431.1 a
3	424.6 a	430.6 a	414.9 b	416.2 b
6	381.8 c	407.8 a	398.1 ab	394.7 b
OPC†	L***, Q**	L***, Q***, C**	L***, Q***, C**	L***, Q***, C**
	Neutral detergent fiber concentration			
1	783.8 c	825.9 b	863.9 a	855.2 a
2	763.5 a	752.7 b	763.9 a	773.4 a
3	742.7 a	752.7 a	728.0 c	750.6 b
6	689.5 b	717.0 a	695.5 b	708.2 b
OPC	L***, Q*	L***, Q**, C***	L***, Q***	L***, Q**, C*
	Lignin concentration			
1	107.1 c	117.0 a	115.3 b	97.0 d
2	101.8 a	101.3 a	100.6 a	88.0 b
3	91.3 a	93.0 a	92.1 a	78.8 b
6	77.1 b	86.1 a	77.6 b	68.1 c
OPC	L***, Q**	L***, Q**	L***	L***
	Cellulose concentration			
1	344.7 d	377.9 c	410.3 a	400.3 b
2	334.2 a	336.2 a	336.2 a	343.1 a
3	333.3 a	337.5 a	322.7 b	337.4 a
6	304.8 b	321.7 a	320.5 a	326.6 a
OPC	L***, Q*, C*	L***, Q**, C**	L***, Q***, C**	L***, Q***, C**
	Hemicellulose concentration			
1	332.0 b	331.0 b	338.3 b	357.9 a
2	327.4 b	315.3 c	327.0 b	342.3 a
3	318.1 b	322.1 b	313.1 b	334.4 a
6	307.7 ab	309.3 ab	297.5 b	313.5 a
OPC	L***	L**, C*	L***	L***

† *, **, *** Orthogonal polynomial contrasts significant at the 0.05, 0.01, and 0.001 levels, respectively.

‡Means within a row followed by the same lowercase letter are not different (*P* > 0.05) using PDIFF option in PROC MIXED.

NDF Concentration

There was a year × HF interaction ($P < 0.0001$) effect on weighted annual average NDF concentration. Similar to ADF responses, NDF generally decreased but the shape of the response curves within years fluctuated (Table 4.4). These results are similar those reported in other studies. Sanderson et al. (1999) reported NDF values of 741 g $kg^{-1}$for a single annual harvest and 675 g kg^{-1} when 4 annual harvests were made for Alamo switchgrass. In the current study there was no response of NDF to N application rate, as reported in other studies (Allison et al., 2012; Guretzky et al., 2011) even at N rates greater than 160 kg ha^{-1}.

Lignin Concentration

There was a year × HF interaction ($P = 0.0128$) effect on weighted on annual average lignin concentration. Lignin concentration generally decreased with increasing HF but the shape of the response curve within year fluctuated and within HF fluctuated across years (Table 4.4). Mulkey et al. (2006) reported that lignin concentration was related to physiological maturity, being less at anthesis harvests than after a frost-killing harvest. Lignin deposition increased with physiological maturity and may also be as a result of lowered leaf:stem ratio in a single harvest relative to multiple harvests.

Cellulose Concentration

There was a year × HF interaction ($P < 0.0001$) effect on weighted annual average cellulose concentration. Generally, cellulose concentration decreased with increasing HF but differences within HF across year fluctuated (Table 4.4). For example, in the 1-cut HF cellulose concentration was different across years but in the 2-cut HF it was similar among years. Cellulose concentration increases with plant maturity (Mulkey et al., 2006) attributable to the decrease in the leaf:stem ratio as the growing season progressed. Increasing the number of annual harvests increases the leaf:stem ratio and decreases the cellulose concentration.

Hemicellulose Concentration

There was a year × HF interaction (*P* = 0.0388) effect on weighted annual average hemicellulose concentration. Generally, hemicellulose concentration decreased with increasing HF and the response was linear in all years except Year 2 (Table 4.4). Across years, hemicellulose concentration fluctuated but, except for the 6-cut HF, was consistently greater in Year 4 than all other years.

In this study, variation in switchgrass ADF, NDF, lignin, hemicellulose and cellulose concentrations were due to HF but not N application rate, which is supported by results reported by Allison et al. (2012). In other studies, switchgrass fiber properties responses to N rate are reportedly dependent on location, season, N application rate, and cultivar (Guretzky et al., 2011; Kering et al., 2013; Waramit et al., 2011).

Each HF differed in physiological maturity and this affected the fiber properties. All harvests except the final harvest of the 3- and 6-cut HF had transitioned from vegetative to flowering and this possibly had an effect on the fiber concentration in the harvested biomass. Since maximal concentrations of cellulose and hemicellulose are desirable for biofuel production, delaying harvests until senescence occurs will increase the cell wall constituents and thus improve feedstock quality factors. In this study, fiber concentration generally increased with physiological maturity resulting from the different HF and this supports previous findings (Sanderson et al., 1999; Waramit et al., 2011).

Physiological maturity at harvest has been reported to have an effect on the different concentrations of pyrolysis products with the activation energy of decomposition and estimated first order reaction kinetics linearly related to switchgrass physiological maturity (Boateng et al., 2006), therefore greater levels of fiber concentration rendered by senescent stages of maturity are highly desirable in feedstock. Also, greater yields are associated with fewer annual harvests. Yield was positively correlated with all fiber properties: ADF ($r = 0.78$; $P < 0.0001$), NDF ($r = 0.76$; $P < 0.0001$), lignin ($r = 0.67$; $P < 0.0001$), cellulose ($r = 0.74$; $P < 0.0001$), and hemicellulose, ($r = 0.52$; $P < 0.0001$) concentrations.

Ethanol Yield and Production from SSF

There was a year × HF ($P < 0.0001$) and a year × N rate interaction ($P = 0.0032$) (Table 4.5) effect on total ethanol yield (TEY) from SSF. During Year 1 there was no response to HF, however, in the last 3 yr, TEY response to HF was quadratic (Table 4.5). The year × HF interaction was partly due to differences in the responses to HF with year, possibly because of the interannual variability in growing conditions. From 2008 to 2009, TEY either decreased (1- and 6-cut HF) or was similar (2- and 3-cut HF), however, in the ensuing years TEY increased progressively at all HF.

Table 4.5 Harvest frequency × year interaction effect on switchgrass ethanol yield and production grown during four growing seasons (2008 to 2011) at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Harvest frequency	2008	2009	2010	2011
	Total ethanol yield from SSF (L Mg^{-1})			
1	203.1 b‡	178.2 d	184.4 c	209.3 a
2	205.8 c	203.3 c	214.1 b	235.4 a
3	198.4 c	197.1 c	210.7 b	234.3 a
6	204.8 b	192.1 c	204.9 b	227.2 a
OPC†	ns	L^{**}, Q^{***}, C^{**}	L^{***}, Q^{***}, C^{**}	L^{**}, Q^{***}
	Total ethanol production per ha from SSF (kL ha^{-1})			
1	1.8 c	1.8 a	2.7 b	3.2 a
2	2.0 b	2.2 ab	2.5 ab	2.5 a
3	1.4 b	1.6 ab	1.8 a	1.6 a
6	1.0 ab	1.3 a	1.3 a	0.8 a
OPC	L^{***}, Q^{*}, C^{*}	L^{***}, Q^{*}, C^{*}	L^{***}	L^{***}
	Total theoretical ethanol yield from all biomass sugars (L Mg^{-1})			
1	404.0 c	401.8 c	427.7 b	444.5 a
2	401.1 c	398.3 c	413.4 b	437.6 a
3	380.7 c	388.9 bc	398.6 b	423.7 a
6	370.5 c	368.8 bc	371.9 b	391.6 a
OPC	L^{***}	L^{***}, Q^{*}	L^{***}	L^{***}, Q^{**}
	Total theoretical ethanol production from all biomass sugars (kL ha^{-1})			
1	3.6 b	4.1 b	6.3 a	6.7 a
2	3.9 b	4.3 ab	4.5 a	4.6 a
3	2.6 b	3.0 ab	3.4 a	2.8 ab
6	1.9 b	2.5 a	2.4 a	1.4 b
OPC	L^{***}, Q^{**}, C^{*}	L^{***}	L^{***}	L^{***}

† *, **, *** Orthogonal polynomial contrasts significant at the 0.05, 0.01, and 0.001 levels, respectively.

‡Means within a row followed by the same lowercase letter are not different ($P > 0.05$) using PDIFF option in PROC MIXED.

Total ethanol yield was negatively correlated with ADF ($r = -0.47$; $P < 0.0001$), NDF ($r = -0.31$; $P < 0.0001$), lignin ($r = -0.56$; $P < 0.0001$) and cellulose ($r = -0.36$; P

< 0.0001) concentrations. Ethanol derived through SSF is influenced by soluble sugars including glucose, fructose and sucrose (Vogel et al., 2011).

There was a year × HF ($P < 0.0001$) and a year × N rate ($P = 0.0095$) interaction effect on total ethanol production (TEP) (Table 4.5). In the first 2 yr, TEP decreased linearly, however, in the last 2 yr, the response was quadratic. In the 1-cut HF, TEP was similar in Years 1 and 2 (3.6 kL ha^{-1}) and increased by 50% in Year 3 and further increased by 17% in Year 4. In the multiple harvest systems, TEP was relatively consistent in at least 3 of the 4 years (Table 4.5).

Ethanol production increased linearly with DMY ($y = 0.0985 + 0.1938x$; $R^2 = 0.95$), therefore, ≤ two annual harvests will maximize ethanol production. Ethanol production was positively correlated with all fiber properties: ADF ($r = 0.68$; $P < 0.0001$), NDF ($r = 0.71$; $P < 0.0001$), lignin ($r = 0.57$; $P < 0.0001$), cellulose ($r = 0.66$; $P < 0.0001$) and hemicellulose ($r = 0.57$; $P < 0.0001$) concentrations. The correlation between TEP and fiber concentration resulted from the relationship between DMY and ethanol production where greater yields are associated with fewer harvests and greater fiber concentrations.

Ethanol Yield and Production from all Biomass Sugars

There was a year × HF ($P = 0.0213$) interaction effect on total theoretical ethanol yield (TTEY) (Table 4.5). The interaction was partly due to the quadratic response to HF in Year 1 and linear decrease with HF in the subsequent years. Total theoretical ethanol yield was similar in Years 1 and 2 in the 1- and 2-cut HF as well as in the first 3 yr at the 3- and 6-cut HF, followed by increases in TTEY in subsequent years (Table 4.5). Ethanol yield was positively correlated with all fiber properties: ADF ($r = 0.62$; $P <$

0.0001), NDF ($r = 0.74$; $P < 0.0001$), lignin ($r = 0.38$; $P < 0.0001$), cellulose ($r = 0.66$; $P < 0.0001$) and hemicellulose ($r = 0.81$; $P < 0.0001$) concentrations.

There was a year × HF ($P < 0.0001$) and a year × N rate ($P = 0.0032$) interaction effect on total theoretical ethanol production (TTEP) (Table 4.5). During all years, TTEP decreased linearly with HF, however, the year × HF interaction occurred partly because TTEP was similar in the 1- (7.6 kl ha^{-1}) and 2-cut HF (8.2 kl ha^{-1}) in Years 1 and 2 and decreased with each additional harvest in multiple harvest systems. In Years 3 and 4, TTEP was greatest in the 1-cut HF and declined with each additional harvest (Table 4.5). Ethanol production increased linearly with DMY ($y = 0.3809 + 0.4505x$; $R^2 = 0.99$). Ethanol production was positively correlated with all fiber properties: ADF ($r = 0.79$; $P < 0.0001$), NDF ($r = 0.79$; $P < 0.0001$), lignin ($r = 0.65$; $P < 0.0001$), cellulose ($r = 0.77$; $P < 0.0001$) and hemicellulose ($r = 0.58$; $P < 0.0001$) concentrations.

Our data suggests that there is potential for increasing the ethanol output from lignocellulosic biomass. Only 50% of the potential ethanol is captured through the SSF process. Regardless of the HF, TTEY and TTEP from all sugars increased by a factor of two relative to the TEY and TEP from SSF. Maximum ethanol recovery is limited by current methods of conversion that cannot fully release the cellulose and hemicellulose from the lignin complex for chemical, microbial or enzymatic depolymerization (Jiang et al., 2013; Yang and Wyman, 2007). Current methods are not cost-effective to facilitate commercial biomass to ethanol conversion (Alvira et al., 2010; Yang and Wyman, 2007), therefore, novel pretreatment and conversion methods are required for improved and economical biofuel processing. Nitrogen concentration was negatively correlated with ethanol production from SSF ($r = -0.70$; $P < 0.0001$) and 100% sugar conversion ($r = -$

0.71; $P < 0.0001$). There was a weak negative relationship between N concentration and SSF ethanol yield. At 100% sugar conversion, as N concentration increased, ethanol yield decreased linearly ($y = 436.7 - 45.934x$; $R^2 = 0.86$).

Summary and Conclusions

Several conversion platforms for switchgrass to biofuels including biochemical and thermochemical exist, which use different pretreatment and conversion processes to produce bio-oil, syn-gas or ethanol. The quality parameters for feedstock can vary depending on the conversion platform. The increase in nutrient concentration with multiple harvests will have implications for nutrient management. Less frequent annual harvests produced greater yields with lower nutrient concentration and therefore may require less agronomic inputs resulting from the greater potential for nutrient translocation. A single post-frost kill harvest resulted in reduced tissue N, P, and K concentrations. Nitrogen removal differed as a result of N application rate effects while P, K, Ca and Mg removal differences were more due to HF than N application rate effects. Increased HF changed the plant physiology and phenology and increased the nutrient removal capacities from multiple harvest systems. In addition, less annual harvests led to increased cell wall constituents concentration thus improving feedstock quality factors. Generally, the feedstock quality increased with switchgrass maturity. Ethanol production increased linearly with dry matter yield. Fewer harvests will increase dry matter yield, fiber concentration, and ethanol production while reducing nutrient removal. These results indicate that less frequent harvest of Alamo switchgrass produced the greatest biomass yield but harvest management decisions likely will depend upon the potential for minimizing nutrient removal from the system, desired feedstock quality, and the economics of an additional harvest.

CHAPTER V

HARVEST TIMING AND N APPLICATION RATE EFFECTS ON SWITCHGRASS YIELD, REGROWTH POTENTIAL, NUTRIENT REMOVAL, CYCLING, AND PARTITIONING

Abstract

Environmental and economic benefits can be achieved from manipulating harvest time and nutrient management to exploit nutrient cycling in switchgrass (*Panicum virgatum* L.), a potential dual-use species with forage and feedstock applications. A 2-yr study was conducted to quantify the effects of harvest time (HT) and N application rates on changes in nutrients and partitioning, nutrient removal capacities and N-use and recovery. Treatments were all factorial combinations of four N application rates (0, 40, 80, and 120 kg ha^{-1}) and six monthly HT (May to October) in a RCBD experiment with a split-plot arrangement. Biomass yield increased with HT from May to October from 1.0 and 6.0 Mg ha^{-1} in Year 1 to 6.0 and 13.0 Mg ha^{-1} during Year 2. Stems contributed >70% to yield in both years. Regrowth yield decreased from 2.3 to 0.8 in May to 5.7 to 0.6 Mg ha^{-1} in August in Years 1 and 2, respectively. There was no N effect on yield in Year 1, however, in Year 2, yield increased by 22% with the addition of 40 kg N ha^{-1} but there were no further yield increase at the additional N rates. Nutrient concentration decreased from May to October in all treatments. Nutrient removal rates were largely a result of biomass yield. Nitrogen use efficiency and recovery were greatest at 40 kg

applied N ha^{-1} and declined with additional N inputs. Harvesting after a killing-frost produced greater biomass yield with relatively less nutrient concentration and nutrient removal rates compared with to pre-senescence harvests. Effective nutrient management in switchgrass depends on manipulating the harvest time with nutrient cycling to minimize nutrient removal and loss, improve feedstock quality, lower agronomic inputs, and enhance the sustainability of production systems.

Introduction

Switchgrass is a native perennial warm-season grass with high bioenergy potential and was selected for development as a bioenergy feedstock because of its perennial nature, high biomass production, adaptation to marginal sites with wide geographic distribution, and tolerance to water deficit and low soil nutrient concentrations (McLaughlin and Adams Kszos, 2005). It is a versatile feedstock that can be fermented to produce ethanol, direct combusted or co-fired to produce electricity (Larson et al., 2009), or thermochemically converted by gasification/pyrolysis to produce syngas (Choi et al., 2010). Among its earlier uses are livestock forage, erosion control, and habitat cover in wildlife management areas. Switchgrass is capable of producing high quality forage with similar nutritive value and animal performance to winter cereal grain pastures (Islam et al., 2011; Mosali et al., 2013) or summer bermudagrass pastures (Hill et al., 1993) and is used primarily for haying or summer grazing with high potential for regrowth after grazing (Beaty et al., 1978; Griffin and Jung, 1983).

The potential dual-use of switchgrass to provide early-season forage and late-fall feedstock to augment economic value has elicited questions on sustainable nutrient management. Intensively managed switchgrass production systems to produce acceptable quality and consistent forage and feedstock yields will require an understanding of nutrient cycling to minimize nutrient removal rates. Nutrient cycling in perennial grasses maintains the seasonal flux of mineral nutrients through translocation of minerals and carbohydrates from leaves and stems to the crown, roots, and rhizosphere (Holou et al., 2013) for overwintering and to be remobilized for spring regrowth. Reverse nutrient translocation augments greater N use efficiency (NUE) and has other benefits including a reduction in fertilizer requirements, enhanced feedstock quality and increased production energy system efficiencies by minimizing corrosion, slagging, fouling, and emissions upon combustion. Cyclical perturbations in this process through midseason harvests reduce the magnitude of translocated nutrients. Nitrogen concentration is usually greater in a midsummer harvest of a two-harvest system and less in the late-fall harvest of a single-harvest system (Lemus et al., 2008; Reynolds et al., 2000). This nutrient cycling therefore has an effect on the rate of annual nutrient removal. Among perennial grasses, switchgrass removes relatively low quantities of N (40 to 75 kg N ha^{-1}), P (5 to 12 kg P ha^{-1}), and K (44 to 110 kg K ha^{-1}) and is considered a high nutrient use efficiency crop (Kering et al., 2012). Tissue nutrient concentrations, however, affect both forage and feedstock quality. Nitrogen fertilizer application increases crude protein concentration (Cuomo and Anderson, 1996) and in vitro dry matter digestibility (IVDMD) (Perry and Baltensperger, 1979). High nutrient concentrations in feedstock, however, can be undesirable for energy conversion processes (Lewandowski and Kicherer, 1997).

Switchgrass responds positively to N application (Madakadze et al., 1999; Sanderson and Reed, 2000); therefore, understanding and quantifying the response of varying N application rates is important in developing an efficient nutrient management program for forage and feedstock production. Dry matter accumulation and changes in nutrient concentration affect the nutrient-use efficiency of all perennial grasses. The sustainability of bioenergy production systems will depend on the effects of fertilizer application rates on N use, remobilization, biomass nutrient concentration and removal capacity (Reynolds et al., 2000; Sanderson et al., 1999). The definition of NUE varied within the literature with two different perspectives: utilization efficiency is concerned with increasing yield with N while uptake efficiency relates to the internal nutrient requirements of the plants. Nutrient-use efficiency in the context of this research is defined as partial factor productivity (PFP) of the dry matter production per unit N applied and partial nutrient balance (PNB) as the N removal to N-use ratio. The latter indicates the relative balance between the amounts of N uptake and used by the crop versus the amount of fertilizer N "lost." Nitrogen use and recovery in grasses are affected by factors such as species, growth habit, N application rate, precipitation, and soil type (Zemenchik and Albrecht, 2002). Nutrient removal in perennial grasses can be relatively low if harvest timing is synchronized with nutrient and carbohydrate translocation dynamics (Boehmel et al., 2008). In switchgrass, NUE, N recovery and PFP decline with increasing N applications and depend on site-specific N management. For example, NUE measured by PFP decreased from 478 to 217 kg biomass kg N^{-1} at 90 and 180 kg N ha^{-1}, respectively, while at higher N rates N recovery decreased from 140 to 25% at similar N rates (Lemus et al., 2008).

Nitrogen is highly mobile in the soil and can be easily lost by leaching and/or run-off. Leached forms of N can enter waterways affecting surface and ground-water quality.

Modifications of fertilizer application practices to optimize NUE have potential for reducing run-off and leaching. In addition to environmental issues associated with N management, the monitoring of plant N status and appropriate N fertilizer management are essential to balance the factors of increasing cost of N fertilizer and crop demand (Jaynes et al., 2001). Therefore, from economic and environmental perspectives, switchgrass production systems should have high nutrient use efficiency with low nutrient removal rates and reduced capacity for nutrient loss to the environment. The objective of this study was to quantify the effects of harvest timing and N application rates on plant biomass, nutrient composition, nutrient partitioning, and nutrient removal capacities and N use and recovery.

Materials and Methods

Study Site

This study was conducted at the Brown Loam Branch Experiment Station, Raymond, MS (32^{o} 15’ N, 90^{o} 30’W). The soil at the site is classified as a Loring silt loam (fine-silty, mixed, thermic Typic Fragiudalfs) characterized by 2 to 5% slopes, eroded, moderately drained with a fragipan. Alamo switchgrass seeds were stratified at 5°C for 2 weeks and subsequently seeded into RLC4 plugs (Stuewe and Sons, Inc., Tangent, OR) containing Sungro Professional Growing Mix 1 (Seba Beach, Canada) on June 21, 2010. Trays were placed under direct sunlight and watered twice daily with full strength Hoagland solution (Hewitt, 1966). After 8 weeks of growth, seedlings were transplanted into prepared beds on August 16, 2010.

Weather

Annual growing-season (March-October) precipitation differed between the two years. In Year 1, total growing-season rainfall was 17% lower than the long-term (30-yr) average while in Year 2, rainfall was 12% greater than long- term average for the same period. Long-term average annual growing-season rainfall was 931 mm (Figure 5.1a). Mean monthly air temperature from January to April in Year 1 was 2.4°C lower than the long-term average while Year 2 was about 3.4°C higher than the long- term average for the same period (Figure 5.1b).

Treatments and Experimental Design

Treatment imposition and data collection were done during 2011 and 2012. Treatments were four N rates (0, 40, 80 and 120 kg N ha^{-1}) and six harvest times (HT; monthly harvests from late May to late October). The experimental design was a randomized complete block with four replications. Treatments were assigned in a split plot arrangement with N application rate as the whole plot and HT as the subplot. The first HT represented regrowth in spring until late May and subsequent HT followed at 1-mo intervals thereafter, with the final late October HT representing season-long growth. After each harvest, the plants were allowed to regrow and each plot with harvestable biomass was harvested after the first killing frost in both the years. The regrowth harvests were the May to August primary harvests.

Plot Management

Plots were 4.5 × 3.0 m with 1-m alleyways between subplots within a main plot, 2-m alleyways between plots within blocks, and 3-m alleyways between blocks.

Transplants were spaced 0.75 m within and across rows with a plant density of 24 (4 rows with 6 plants each) plants per plot yielding a harvestable area of 4.5 m^2 (inner 8 plants). Treatment N rates were imposed as a single application of urea 2 wk after spring regrowth began in both years.

Harvest management, tissue sampling and analysis

Tiller numbers, crown diameter and plant height were measured monthly on five plants per plot prior to each HT. Biomass yield was estimated by clipping the inner eight plants of each harvested plot at 10 cm stubble height. The total weight of the harvested material was recorded and a 1-kg subsample was collected and dried at 55-60 ºC in a forced-air oven until constant weight was achieved to determine dry matter concentration. For each plot harvested at each HT, vegetative and reproductive tillers per plant were counted and a subsample of these tillers was hand-separated into leaves and stems. Leaf area index was estimated from the leaf portion using a Li-3100 leaf area meter (Li-COR, Lincoln, NE) and leaf:stem ratio was computed from weight of the dried samples. Four plants were collected from each treatment: two complete plants were dried and ground whole while the other two were separated into leaf and stem components, oven-dried, ground and analyzed for nutritive value and chemical composition by NIRS using the Foss model 6500 spectrophotometer (Foss NIRSystems, Inc., Laurel, MD.) with procedures for grass hay and switchgrass developed by the NIRS Forage and Feed Testing Consortium.

Calculations and Statistical Analysis

Nutrient removal was calculated by multiplying biomass yield by elemental tissue concentration. Apparent N Recovery (ANR), PFP and PNB were calculated as follows:

$$\text{ANR} = [(\text{N removed at xN applied} - \text{N removed at 0N}) / \text{xN applied}] * 100 \tag{5.1}$$

$$\text{PFP} = \text{xN yield} / \text{xN applied} \tag{5.2}$$

$$\text{PNB} = \text{N removed at xN applied} / \text{xN applied} \tag{5.3}$$

where x represents the treatment N rate.

The data were analyzed by fitting mixed models using PROC MIXED in SAS (SAS Institute, 2008). Harvest time, N rate, year and their interactions were considered fixed effects while replication and replication × year were considered random effects. Year was considered as a repeated measure. Means were separated using the PDIFF option and responses were considered as different at the 0.05 probability level.

Results and Discussion

Yield

There was a year × HT interaction effect on total biomass ($P = 0.0042$), leaf ($P < 0.000$), and stem ($P < 0.0001$) component yield. In both years of the study, total biomass as well as the leaf and stem component of yield increased with increasing duration of HT. Yield accumulation was characterized by a cubic and quadratic response to HT in Years 1 and 2, respectively. In Year 1, following an early-season lag phase in growth, the most rapid growth occurred from June to July (2.2 to 3.3 Mg ha^{-1}) followed by a plateau and slight decline from September to October (6.1 to 5.9 Mg ha^{-1}) (Figure 5.2). In Year 2, yield increase was quadratic with rapid growth from July to August (10.4 to 13.1 Mg ha^{-1}), which subsequently plateaued (13.5 Mg ha^{-1}) (Figure 5.2). Rapid growth during this period resulted from more than average rainfall in Year 2. Early season growth in Year 2 was four times greater than Year 1 while midsummer to late fall biomass accumulation in Year 2 was double that of Year 1. Higher early-season temperatures in Year 2 possibly induced early-spring regrowth in March instead of late April as occurred in Year 1.

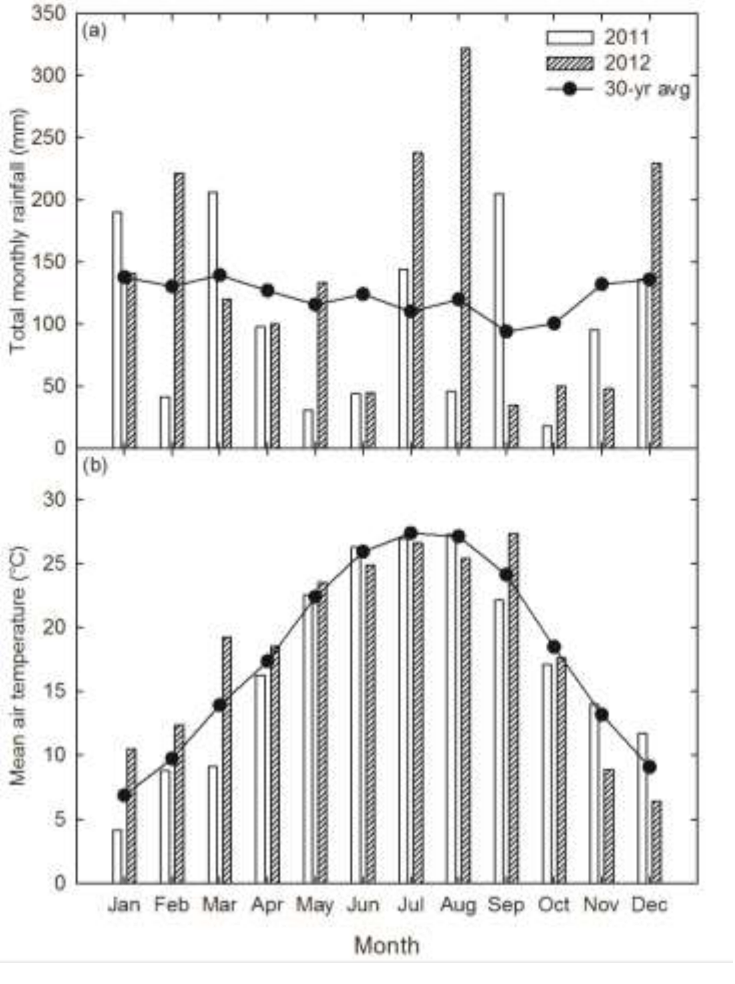

Figure 5.1 (a) Total monthly rainfall and (b) mean monthly air temperature at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA during the two years of this experiment (bars) and the average across 30 years, 1981 to 2011 (line).

Leaves contributed 55 and 37% of the total biomass in May of Years 1 and 2. Leaf weights increased across the growing season from 0.6 and 2.2 Mg ha^{-1} in May to 1.1 and 2.4 Mg ha^{-1} in October during Years 1 and 2, respectively (Figure 5.2). Concurrently, stems percent contribution to total yield increased with increasing HT across both years. Stem yield increased linearly from 0.4 to 4.7 Mg ha^{-1} in Year 1 while in Year 2 the increase was quadratic from 3.9 to 11.0 Mg ha^{-1} (Figure 5.2).

End-of-season regrowth harvests total aboveground biomass and the leaf and stem components decreased linearly from 2.3 to 0.8 in primary May harvests and 5.7 to 0.6 Mg ha^{-1} in primary August harvests of both years with greater yields recorded in Year 2 (Figure 5.3). The proportion of leaves in regrowth yield increased with later primary harvests as the season progressed reaching 50% in August of both years (Figure 5.3). There was no harvestable regrowth biomass from the September and October plots in both years since late season temperatures and rainfall limit the regrowth potential of switchgrass harvested during these later months in central Mississippi. Biomass production was relatively low compared with many other studies, attributed to the age of the switchgrass stands. With a relatively younger stand, biomass production is comparatively lower than some previous reports (Lemus et al., 2002; Muir et al., 2001), however, the trends in biomass accumulation are similar to other reports (Holou et al., 2011; Kering et al., 2012; Wilson et al., 2013).

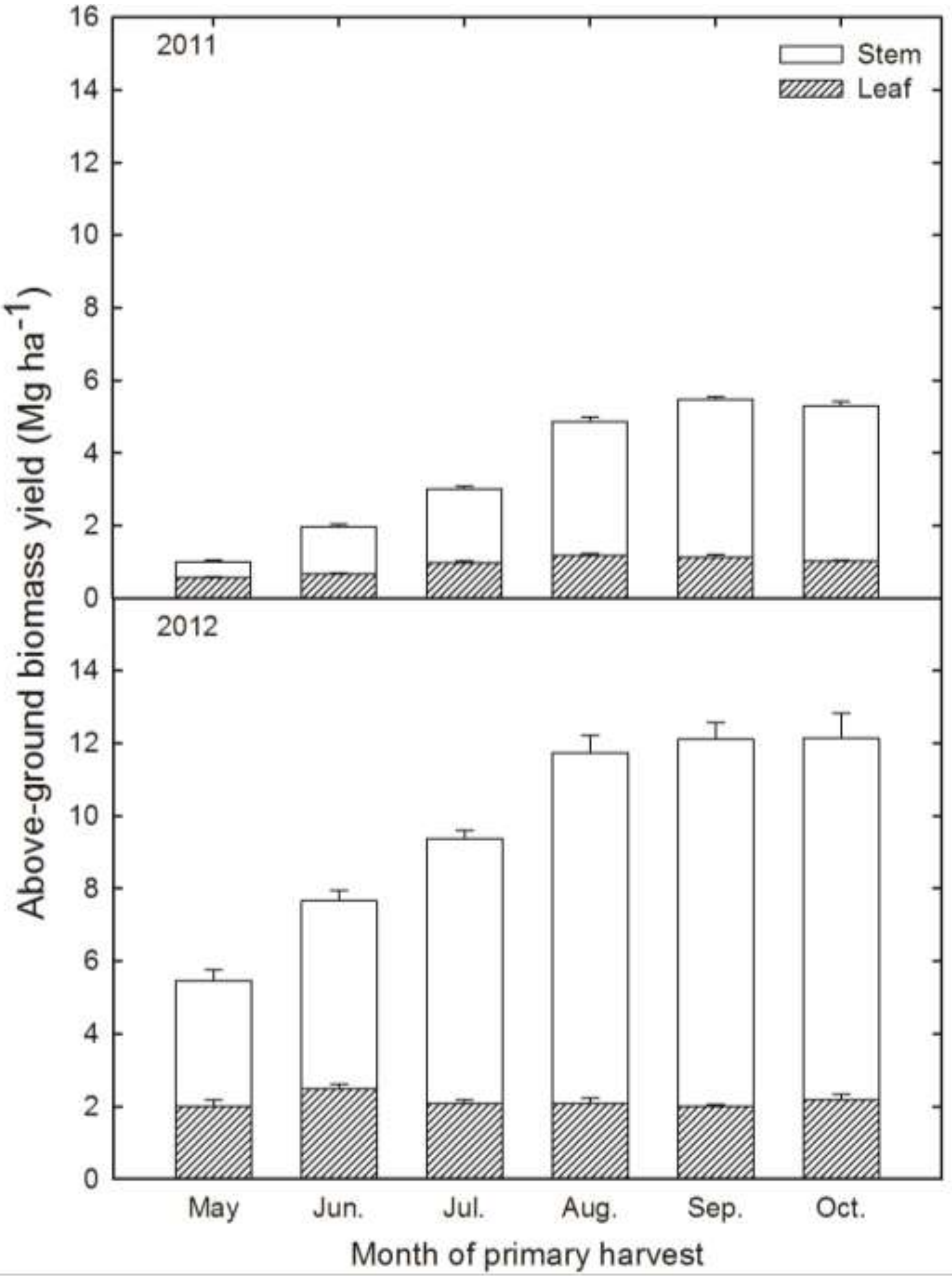

Figure 5.2 Harvest time × year interaction effect on switchgrass yield and biomass partitioning, averaged over N treatments, during the 2011 and 2012 growing seasons at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Error bars denote standard error.

There was also a year × N rate ($P = 0.008$) interaction effect on biomass yield. In Year 1, there was no response to N application, averaging 4.0 Mg ha^{-1}, but in Year 2, there was a quadratic response (Figure 5.4). There was no residual N benefit on the regrowth harvests ($P = 0.37$). Kering et al. (2012) reported that there was no effect of N application at rates high as 168 kg N ha^{-1} in switchgrass and other perennial bioenergy species. In another study with multiple native grasses and N application rates as high as 220 kg N ha^{-1}, optimum yield after 2 yr occurred at 140 kg N ha^{-1} (Heggenstaller et al., 2009). Alamo produced 22.5 Mg ha^{-1} at 168 kg N ha^{-1}, which was found to be necessary for sustainable biomass production in Texas (Muir et al., 2001). Results from this study indicate that N fertilizer application at this central Mississippi location would be between 40 to 80 kg N ha^{-1} in establishment year with precise recommendations based on soil nutrient status, forage/feedstock enterprises and yield goal. In post establishment years, N residual effects (Lemus et al., 2008) and microbial activity (Ghimire et al., 2009; Kim et al., 2012; Kleczewski et al., 2012) may reduce the N input requirements. However, a dual forage/feedstock production scenario, which translates to more than one annual harvest, will require higher N inputs to sustain biomass yield. Biomass production can be achieved with minimal N inputs but stands require adequate harvest and nutrient management to maintain the soil N status (Lemus et al., 2008). Research on temporally splitting fertilizer application specific to a forage/feedstock production system is required to increase the nutrient use and minimize loss that may occur with a single spring application.

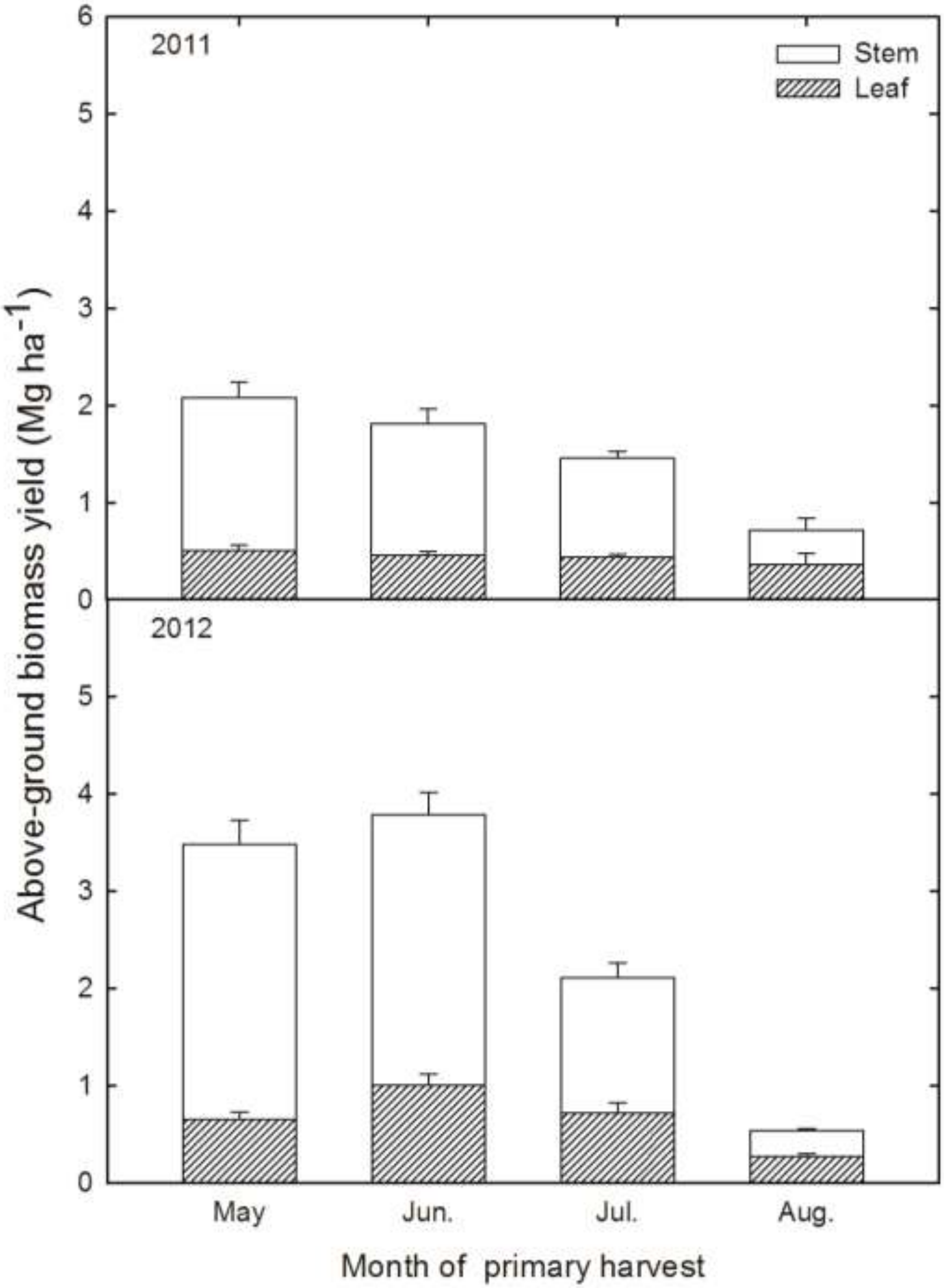

Figure 5.3 Harvest time × year interaction effect on switchgrass regrowth yield and biomass partitioning during the 2011 and 2012 growing seasons at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Error bars denote standard error.

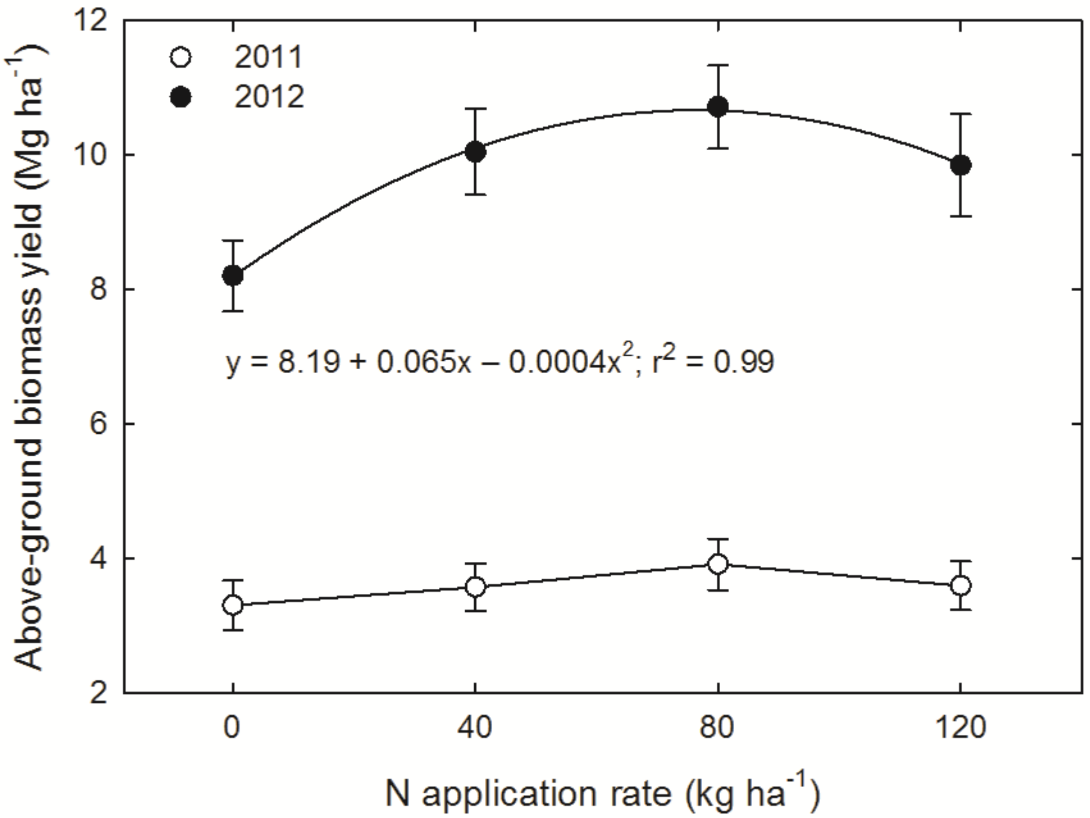

Figure 5.4 Year × N rate interaction effect on switchgrass yield during the 2011 and 2012 growing seasons at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Error bars denote standard error.

Nutrient concentration

There was a year × HT ($P < 0.05$) interaction effect on concentration of all nutrients but not by N rate or its interaction with HT. All elements decreased with increasing length of HT from May to October in both years but fluctuation in the pattern of response and the magnitude of decrease in different years was partially responsible for the interaction (Table 5.1).

In Year 1, N concentration decreased from 10.4 and in Year 2 from 8.0 g kg^{-1} at the earliest HT to an average of 4.2 g kg^{-1} at the last HT (Table 5.1). Early season N concentration in Year 1 was greater than the same period in Year 2, however, midsummer to late fall concentrations were not different across years. Also, there was a quadratic increase in regrowth N concentration with shorter regrowth periods for total biomass and its components in both years (Table 5.2). Similar decline in nutrient concentration with increasing maturity was reported by Dohleman et al. (2012), Heaton et al.(2009), Holou et al. (2011), and Kering et al. (2012) and is related to the translocation of nutrients from leaves and stems to the crown, roots, and rhizosphere at the end of the growing season (Holou et al., 2013). Partitioning total biomass into leaves and stems revealed that the N concentration was greater in leaves than stems; therefore post frost leaf abscission can be a viable breeding objective to minimize feedstock N concentration (Wilson et al., 2013) and increase soil N cycling. Nutrient translocation is associated with plant senescence and dormancy and the physiological changes associated with plant maturity (Holou et al., 2013; Wilson et al., 2013), indicating the reverse flux of nutrients. These studies have reported that nutrient concentration in the belowground biomass was inverse to the nutrient concentration in the aboveground biomass with late season increases in belowground biomass nutrient concentration.

Table 5.1 Harvest time × year interaction effect on switchgrass whole plant, leaf and stem N, P, K, Ca, and Mg concentration during the 2011 and 2012 growing seasons at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

HT	Nutrient concentration							
	Whole plant		Leaf		Stem		Leaf vs Stem	
	2011	2012	2011	2012	2011	2012	2011	2012
	$\mathrm{g\ kg^{-1}}$						(P value)	
	Nitrogen concentration							
May	10.4 a†	8.0 b	15.1 a	13.6 b	5.4 a	2.9 b	< 0.0001	< 0.0001
Jun.	7.2 a	5.6 b	11.5 a	11.7 a	2.0 a	2.1 a	< 0.0001	< 0.0001
Jul.	7.4 a	5.2 b	15.3 a	12.2 b	2.9 a	1.9 b	< 0.0001	< 0.0001
Aug.	6.1 a	6.3 a	12.9 a	13.6 a	1.9 a	1.5 b	< 0.0001	< 0.0001
Sep.	6.4 a	5.8 a	13.1 a	13.5 a	1.3 a	1.5 a	< 0.0001	< 0.0001
Oct.	4.2 a	4.3 a	10.1 b	11.6 a	1.0 a	1.2 a	< 0.0001	< 0.0001
OPC‡	L^{***}, Q^{*}, C^{***}	L^{***}, C^{***}	L^{***}, Q^{***}, C^{***}	C^{***}	L^{***}, Q^{***}, C^{***}	L^{***}, Q^{*}		
	Phosphorus concentration							
May	2.2 a	1.9 b	2.2 a	2.0 b	2.3 a	1.9 b	0.2234	< 0.0001
Jun.	1.7 a	1.7 a	1.7 a	1.8 a	1.9 a	1.7 b	< 0.0001	0.0226
Jul.	1.7 a	1.5 b	1.8 a	1.6 b	1.9 a	1.6 b	0.0132	0.526
Aug.	1.6 a	1.5 b	1.8 a	1.5 b	1.8 a	1.5 b	0.7511.	0.6049
Sep.	1.4 a	1.5 a	1.8 a	1.4 b	1.6 a	1.4 b	< 0.0001	0.0924
Oct.	1.3 b	1.4 a	1.1 a	1.2 a	1.5 a	1.3 b	< 0.0001	0.0634
OPC	L^{***}, Q^{***}, C^{*}	L^{***}, Q^{***}, C^{*}	L^{***}, Q^{***}, C^{***}	L^{***}, Q^{**}, C^{*}	L^{***}, C^{***}	L^{***}, Q^{*}		
	Potassium concentration							
May	22.3 a	17.3 b	18.5 a	18.6 a	21.6 a	17.8 b	< 0.0001	0.0893
Jun.	16.1 a	14.6 b	15.0 b	16.2 a	17.7 a	14.9 b	< 0.0001	0.0046
Jul.	15.0 a	11.4 b	14.2 a	11.2 b	17.0 a	13.3 b	< 0.0001	< 0.0001
Aug.	15.2 a	10.7 b	14.7 a	10.2 b	16.3 a	11.2 b	0.0006	0.0371
Sep.	12.6 a	9.9 b	14.3 a	9.8 b	14.0 a	9.8 b	0.5875	0.9485
Oct.	11.3 a	9.2 b	9.8 a	7.6 b	13.3 a	8.5 b	< 0.0001	0.047
OPC	L^{***}, Q^{***}, C^{***}	L^{***}, Q^{***}	L^{***}, C^{***}	L^{***}, Q^{***}	L^{***}, Q^{**}, C^{*}	L^{***}, Q^{*}		
	Calcium concentration							
May	2.7 a	2.0 b	3.6 b	4.1 a	1.0 a	0.8 a	< 0.0001	< 0.0001
Jun.	2.0 a	1.6 b	4.1 b	4.5 a	0.4 a	0.6 a	< 0.0001	< 0.0001
Jul.	1.8 a	2.0 a	3.8 b	4.3 a	0.4 a	0.7 a	< 0.0001	< 0.0001
Aug.	1.6 b	2.2 a	3.9 b	4.4 a	0.2 a	0.5 a	< 0.0001	< 0.0001
Sep.	1.8 a	2.0a	3.7 b	4.4 a	0.2 b	0.6 a	< 0.0001	< 0.0001
Oct.	1.7 b	2.1 a	3.8 b	4.6 a	0.2 b	0.9 a	< 0.0001	< 0.0001
OPC	L^{***}, Q^{***}	L^{***}, Q^{***}, C^{***}	C^{***}	L^{*}, C^{*}	NS	NS		
	Magnesium concentration							
May	2.7 a	2.3 b	2.9 a	3.0 a	2.4 a	2.3 a	< 0.0001	< 0.0001
Jun.	2.1 b	2.3 a	3.0 a	3.3 a	1.6 b	2.2 a	< 0.0001	< 0.0001
Jul.	2.2 a	2.2 a	3.0 a	3.1 a	1.8 b	2.1 a	< 0.0001	< 0.0001
Aug.	2.1 b	2.4 a	3.2 a	3.0 a	1.6 b	2.0 a	< 0.0001	< 0.0001
Sep.	2.2 b	2.8 a	3.1 a	3.0 a	1.6 b	2.3 a	< 0.0001	< 0.0001
Oct.	1.8 b	2.7 a	2.9 a	3.1 a	1.5 b	2.5 a	< 0.0001	< 0.0001
OPC	L^{***}, Q^{*}, C^{***}	L^{***}, Q^{*}, C^{*}	Q^{*}	NS	L^{***}, Q^{**}, C^{***}	Q^{***}		

†Means within a row followed by the same lowercase letter are not different between 2011 and 2012 for whole plant, leaf or stem ($P > 0.05$) using PDIFF option in PROC MIXED.

‡ *, **, *** Orthogonal polynomial contrasts significant at the 0.05, 0.01, and 0.001 levels, respectively.

Table 5.2 Harvest time × year interaction effect on switchgrass regrowth whole plant, leaf and stem N, P, K, Ca, and Mg concentration during the 2011 and 2012 growing seasons at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Primary harvest HT	Nutrient concentration							
	Whole plant		Leaf		Stem		Leaf vs Stem	
	2011	2012	2011	2012	2011	2012	2011	2012
	g kg^{-1}						(*P* value)	
	Nitrogen concentration							
May	4.1 a†	3.6 a	10.8 a	10.5 a	1.3 a	2.1 a	< 0.0001	< 0.0001
Jun.	4.1 a	3.4 a	10.2 a	10.5 a	1.3 b	4.5 a	< 0.0001	< 0.0001
Jul.	5.1 a	6.2 a	12.6 b	14.4 a	1.4 b	3.6 a	< 0.0001	< 0.0001
Aug.	14.4 a	13.4 a	18.7 a	18.2 a	7.3 b	2.7 a	< 0.0001	< 0.0001
OPC‡	$L^{***}, Q^{***}, C^{***}$	L^{***}, Q^{***}	L^{***}, Q^{***}	L^{***}, Q^{***}	L^{***}, Q^{***}, C^{**}	Q^{***}		
	Phosphorus concentration							
May	1.1 b	1.3 a	1.2 b	1.3 a	1.2 b	1.5 a	0.4119	< 0.0001
Jun.	1.2 b	1.4 a	1.1 b	1.3 a	1.2 b	1.5 a	0.0246	< 0.0001
Jul.	1.1 b	1.3 a	1.2 b	1.4 a	1.2 b	1.4 a	0.0408	0.2187
Aug.	1.0 b	1.4 a	1.0 b	1.3 a	1.2 b	1.4 a	< 0.0001	< 0.0001
OPC	L^{***}, Q^{***},	L^{***}, C^{**}	L^{***}, Q^{***}, C^{**}	L^{*}, C^{**}				
	Potassium concentration							
May	7.2 b	10.4 a	6.4 b	8.0 a	8.1 b	11.9 a	< 0.0001	< 0.0001
Jun.	7.8 b	11.6 a	6.8 b	9.0 a	8.6 b	11.8 a	< 0.0001	< 0.0001
Jul.	7.2 b	10.4 a	6.6 b	8.5 a	8.7 b	11.6 a	< 0.0001	< 0.0001
Aug.	3.8 b	8.7 a	2.6 b	6.8 a	6.3 b	11.5 a	< 0.0001	< 0.0001
OPC	L^{***}, Q^{***},	L^{***}, Q^{***}	L^{***}, Q^{***}, C^{**}	L^{***}, Q^{***}	L^{***}, Q^{***}	L^{**}		
	Calcium concentration							
May	2.5 a	2.6 a	4.2 a	4.3 a	1.9 a	2.0 a	< 0.0001	< 0.0001
Jun.	2.6 a	2.8 a	4.5 a	4.3 a	1.8 b	2.3 a	< 0.0001	< 0.0001
Jul.	2.8 b	3.1 a	4.8 a	4.7 a	1.9 b	2.3 a	< 0.0001	< 0.0001
Aug.	3.5 a	3.6 a	4.5 b	4.9 a	1.8 b	2.3 a	< 0.0001	< 0.0001
OPC	L^{***}, Q^{*}	L^{***}	L^{***}, Q^{**}	L^{***}				
	Magnesium concentration							
May	1.7 a	1.4 b	2.3 a	2.0 b	1.0 b	1.3 a	< 0.0001	< 0.0001
Jun.	1.6 a	1.6 a	2.5 a	2.3 b	1.1 b	1.7 a	< 0.0001	< 0.0001
Jul.	1.7 b	1.9 a	2.5 a	2.6 a	1.1 b	1.5 a	< 0.0001	< 0.0001
Aug.	2.8 a	2.6 a	3.7 a	3.5 b	1.6 a	1.4 b	< 0.0001	< 0.0001
OPC	L^{***}, Q^{***}, C^{*}	L^{***}, Q^{**}	L^{***}, Q^{**}	L^{***}, Q^{**}	L^{***}, Q^{***}	Q^{***}		

†Means within a row followed by the same lowercase letter are not different between 2011 and 2012 for whole plant, leaf or stem (*P* > 0.05) using PDIFF option in PROC MIXED.

‡ *, **, *** Orthogonal polynomial contrasts significant at the 0.05, 0.01, and 0.001 levels, respectively.

In both years, whole plant, leaf, and stem P concentration all decreased with increasing maturity due to HT (Table 5.2). Interaction with year was mainly because of the different magnitude of the decrease each year. There was no effect of HT on regrowth stem P concentration but it was greater in Year 2 than Year 1 (Table 5.2).

Similar to P, whole plant, leaf, and stem K concentration decreased with increased HT (Table 5.1). The interaction with year was partially because of the magnitude of the decrease each year. Also, K concentration was greater in Year 1 than in Year 2. In both years, K concentration in the regrowth harvest increased with decreasing regrowth interval after the initial harvest and generally was greater in Year 2 than Year 1 (Table 5.2). Calcium and Mg concentrations decreased with increasing maturity due to HT. The fluctuation in the pattern of responses between the two years contributed to the interaction effect. In both years, Ca and Mg concentrations in the regrowth harvest increased from May to August primary harvests and fluctuated between the years (Table 5.2). The amount of P and K decline was greater in leaves than stems while with Ca and Mg decline the response was opposite. Kering et al. (2012) report >50% decline in N, P and K concentration while Ca and Mg declined at 19 and 36% from May to October.

Post-frost harvest N concentration in both years was nine times greater in leaves than stems. Since high N causes fouling and slagging of processing equipment (Qin et al., 2006), cultivars with lowered leaf:stem ratio may be developed for improved feedstock. Harvesting switchgrass after frost-induced senescence produced biomass with the least nutrient concentration. In a dual-use scenario where switchgrass is grazed or hayed from May to July and the regrowth harvested after a frost kill for feedstock, biomass is produced with similar or less nutrient concentration in the regrowth harvest compared to the primary harvest. If the first harvest is delayed beyond July, however, the N concentration in the regrowth harvest will increase from 6 to 14 g kg^{-1} and may be unsuitable for feedstock processing. Harvesting switchgrass after a killing frost will produce feedstock that is low in nutrient concentration and be ideal for biofuel processing. Although harvesting can be delayed until spring of the following year prior to regrowth, there is no additional reduction in nutrient concentration (Wilson et al., 2013) and will incur a 11 to 44% yield penalty from leaf shedding (Adler et al., 2006; Wilson et al., 2013).

Nutrient Removal

For all removal rates of all nutrients measured, there were year × HT and year × N rate interaction effects ($P < 0.05$) but there were no HT × N rate interactions ($P > 0.05$). For the regrowth harvests, there was a year × HT interaction effect ($P < 0.05$) on yield and nutrient concentration.

Nitrogen removal increased from 12 and 49 kg ha^{-1} in May to 24 and 59 kg ha^{-1} in October during Years 1 and 2, respectively (Table 5.3). The response was cubic with the period of most rapid N removal occurring from June to July in Year 1 and from July to August in Year 2 (Table 5.3). Nitrogen removal in Year 2 was greater than Year 1 across all months with early season removal in Year 2 being two to three fold greater than Year 1. Nitrogen removal in regrowth harvests increased from 9 and 18 kg ha^{-1} in May primary harvest to 12 and 8 kg ha^{-1} in August primary harvest during Years 1 and 2, respectively (Table 5.4). Low N removal in August in Year 2 is related to the low biomass yield in that year. Nitrogen removal was greater in Year 2 than Year 1 for all months except August when both years were similar resulting from low yields in that month.

Table 5.3 Harvest time × year interaction effect on switchgrass whole plant N, P, K, Ca, and Mg removal during the 2011 and 2012 growing seasons at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

HT	Nutrient removal	
	2011	2012
	kg ha^{-1}	
	Nitrogen removal	
May	11.7 b[†]	48.9 a
Jun.	15.6 b	47.5 a
Jul.	24.7 b	54.0 a
Aug.	32.9 b	82.6 a
Sep.	39.2 b	77.6 a
Oct.	24.6 b	58.5 a
OPC[‡]	L***, Q***, C**	L***, Q***, C***
	Phosphorus removal	
May	2.4 b	11.5 a
Jun.	3.7 b	14.7 a
Jul.	5.5 b	16.0 a
Aug.	8.9 b	19.6 a
Sep.	8.8 b	20.2 a
Oct.	7.4 b	19.1 a
OPC	L***, Q***, C**	L***, Q***
	Potassium removal	
May	24.9 b	106.4 a
Jun.	35.3 b	124.1 a
Jul.	49.9 b	119.2 a
Aug.	82.5 b	139.2 a
Sep.	76.5 b	133.1 a
Oct.	66.8 b	123.1 a
OPC	L***, Q***, C**	L**, Q**
	Calcium removal	
May	3.1 b	12.3 a
Jun.	4.4 b	13.9 a
Jul.	6.0 b	20.8 a
Aug.	9.0 b	29.3 a
Sep.	10.7 b	27.2 a
Oct.	9.9 b	28.9 a
OPC	L***	L***, Q**
	Magnesium removal	
May	3.1	14.1 a
Jun.	4.6	19.8 a
Jul.	7.1	23.4 a
Aug.	11.4	31.5 a
Sep.	13.4	37.7 a
Oct.	10.7	36.5 a
OPC	L***, Q*, C**	L***, Q*, C**

[†] Means within a row followed by the same lowercase letter are not different between 2011 and 2012 for whole plant, leaf or stem ($P > 0.05$) using PDIFF option in PROC MIXED.

[‡] *, **, *** Orthogonal polynomial contrasts significant at the 0.05, 0.01, and 0.001 levels, respectively.

Nitrogen removal was also influenced by a year × N rate (P = 0.0479) interaction effect. During Year 1, N removal increased linearly with N rate from 20 to 26 kg N ha^{-1} while in Year 2 the response was quadratic (Table 5.4). Phosphorus removal increased from May to October with the greatest P removal occurring from July to August in both years (Table 5.3). Year 2 recorded a two- fold increase in P removal relative to Year 1 (Table 5.3). The quantity of P removed from regrowth harvests decreased linearly with HT in both years (Table 5.5). Phosphorous removal increased quadratically with N application rate in both years (Table 5.4).

Table 5.4 Year × N rate interaction effect on switchgrass whole plant nutrient removal during the 2011 and 2012 growing seasons at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

N rate	Nitrogen removal		Phosphorous removal		Potassium removal		Calcium removal		Magnesium removal	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
	——kg ha^{-1}——									
0	20.4 b[†]	49.9 a	5.4 b	14.2 a	49.5 b	105.8 a	6.3 b	17.3 a	7.1 b	22.0 a
40	24.3 b	65.3 a	6.1 b	17.4 a	56.0 b	128.3 a	7.4 b	23.2 a	8.4 b	28.2 a
80	28.7 b	69.2 a	6.8 b	18.7 a	62.0 b	137.2 a	7.8 b	25.0 a	9.1 b	30.3 a
120	25.7 b	61.3 a	6.2 b	17.0 a	56.4 b	124.8 a	7.2 b	22.4 a	9.0 b	27.5 a
OPC‡	L*	L***, Q***	Q***	L***, Q***	Q**	L***, Q***	Q**	L**, Q***	Q**	L***, Q***

†Means within a row followed by the same lowercase letter are not different between 2011 and 2012 for whole plant, leaf or stem (P > 0.05) using PDIFF option in PROC MIXED.

‡ *, **, *** Orthogonal polynomial contrasts significant at the 0.05, 0.01, and 0.001 levels, respectively.

Potassium removal in the primary harvest increased with HT from 25 and 106 kg ha^{-1} to 67 and 123 kg ha^{-1} in Years 1 and 2 (Table 5.3). In regrowth harvests, K removal decreased linearly from May primary harvest to October primary harvest during both years (Table 5.5). Potassium removal response to N rate was quadratic among all biomass components (Table 5.4). Primary harvest Ca removal increased linearly with HT in Year 1 and quadratically in Year 2 (Table 5.3) and decreased in the regrowth harvests as regrowth harvest interval decreased from May to August primary HT (Table 5.5). Calcium removal also increased quadratically with N rate (Table 5.4). Similar to the other elements, Mg removal also increased with plant maturity (Table 5.3). Magnesium removal decreased linearly with decreased harvest regrowth duration in both years; however, the magnitude of removal was greater in Year 2 than Year 1 (Table 5.5). Magnesium removal increased quadratically with N application rate in both years (Table 5.4).

Table 5.5 Harvest time × year interaction effect on switchgrass regrowth whole plant, N, P, K, Ca, and Mg removal during the 2011 and 2012 growing seasons at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Primary harvest HT	Nitrogen removal		Phosphorous removal		Potassium removal		Calcium removal		Magnesium removal	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
	kg ha^{-1}									
May	9.3 b†	17.5 a	2.6 b	7.1 a	17.0 b	55.7 a	5.7 b	14.2 a	3.9 a	7.3 b
Jun.	8.0 b	14.5 a	2.3 b	5.7 a	16.0 b	48.9 a	5.3 b	11.7 a	3.2 a	6.6 b
Jul.	8.4 b	13.5 a	1.8 a	3.1 a	11.9 a	24.9 a	4.6 a	7.3 a	2.8 a	4.4 b
Aug.	11.6 a	8.1 a	0.8 a	0.8 a	2.5 a	5.1 a	2.7 a	2.2 a	2.3 a	1.6 b
OPC‡	Q^{*}	L^{***}	L^{***}, Q^{***}	L^{***}	L^{***}, Q^{***}	L^{***}	**, Q^{*}	L^{***}	L^{**}	L^{***}

†Means within a row followed by the same lowercase letter are not different between 2011 and 2012 for whole plant, leaf or stem ($P > 0.05$) using PDIFF option in PROC MIXED.

‡ *, **, *** Orthogonal polynomial contrasts significant at the 0.05, 0.01, and 0.001 levels, respectively.

Nutrient removal increases in dual-use switchgrass stands resulting from the increases in nutrient concentration in the first or second harvest, which depends on the timing of the harvest. For example in a two-harvest system, haying in July removed 24 and 54 kg N ha^{-1} in Years 1 and 2, respectively while the regrowth feedstock harvest removed 8 and 14 kg N ha^{-1} in Years 1 and 2, totaling 32 and 68 kg N ha^{-1} in Years 1 and 2, respectively. Midsummer harvests remove greater quantities of nutrients than late fall harvests (Guretzky et al., 2011; Lemus et al., 2009; Reynolds et al., 2000). Harvest timing is critical to minimize nutrient removal, however, weather conditions can influence the nutrient uptake capacity (Holou et al., 2011). The annual changes in biomass yield resulting from interannual weather variability and stand aging will influence the extent of nutrient removal. Nutrient removal increased linearly with biomass yield (Figure 5.5) supporting previous findings that nutrient removal is related to biomass yields in switchgrass (Wilson et al., 2013) and other perennial grasses (Dohleman et al., 2012; Vogel et al., 2002). There was a weak negative correlation between nutrient removal and N ($r = -0.12$; $P = 0.09$), P ($r = -0.35$; $P < 0.0001$), K ($r = -0.50$; $P < 0.0001$), Ca ($r = -0.36$; $P < 0.0001$) and Mg ($r = -0.48$; $P < 0.0001$) concentrations. Therefore, removal rates are more influenced by biomass yield than the nutrient concentration. Dual-use systems will therefore require additional fertilizer application, either increased fertilizer rates or in-season split applications, to sustain soil nutrient availability and achieve annual yield goals.

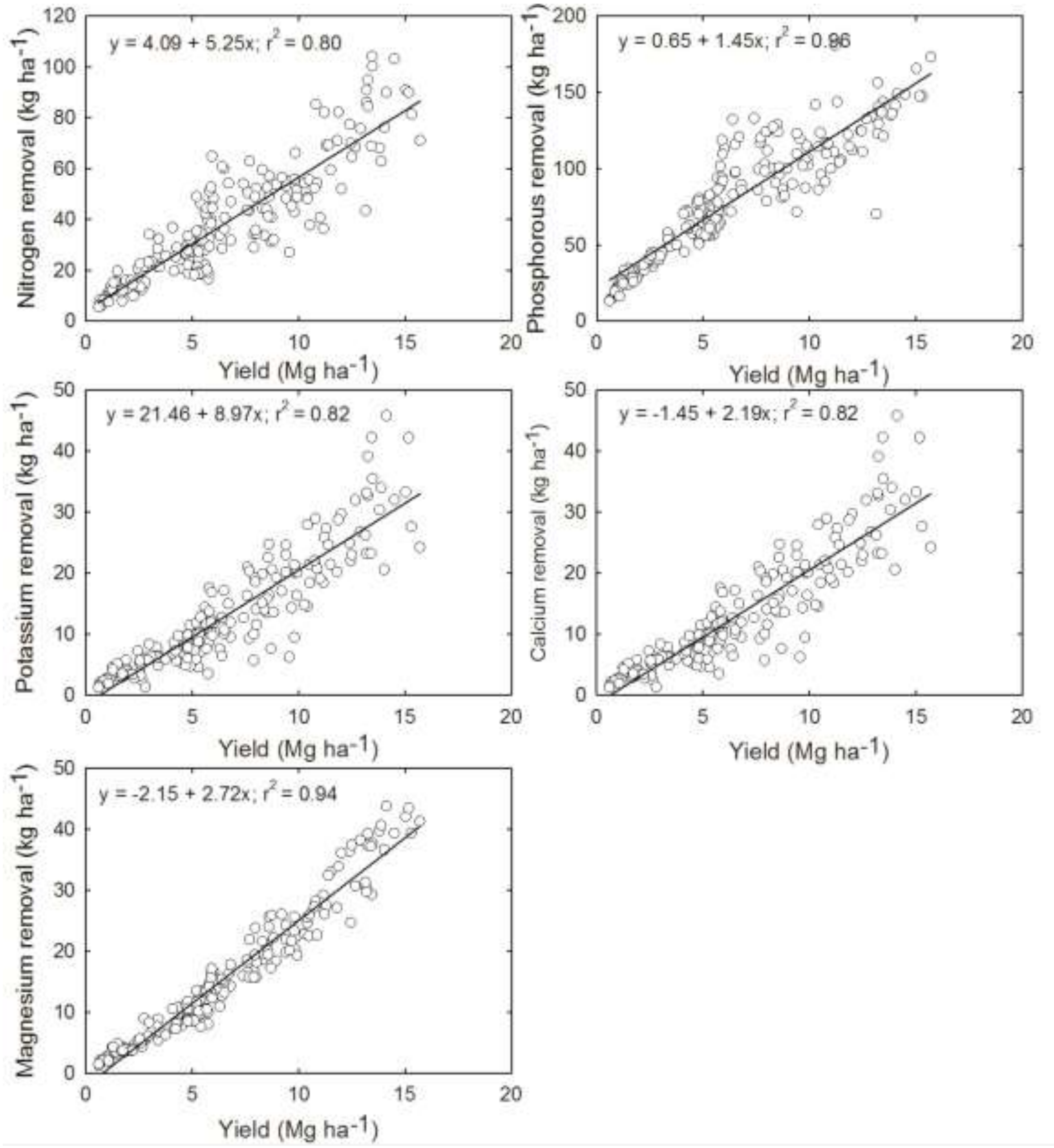

Figure 5.5 Relationships between N, P, K, Ca and Mg removal and biomass yield; data points from four N rates, six harvest times and two years.

N-use indices

There was year × HT and year × N rate interaction ($P < 0.05$) effect on PFP, ANR, PNB. Monthly yields and N removal were used in calculating the N-use indices.

Partial factor productivity, measures the ratio of biomass yield per unit of applied N fertilizer, increased linearly with increasing length of growth period dictated by HT in both years (Table 5.6). A two-fold increase in PFP in Year 2 was related to the greater biomass yields in that year. In the regrowth harvests, PFP declined with decreasing length of the regrowth interval by 43 and 53% in Years 1 and 2, respectively (Table 5.6). Averaged across HT, PFP for initial harvests increased linearly with increasing N application rate in both years. For initial harvests, ANR response to HT in Year 1 was cubic while in Year 2, the response was quadratic (Table 5.6). In the regrowth harvests, ANR response was quadratic in both years. Partial N balance, another N recovery efficiency metric, is the ratio of N removal to N-use, increased with HT in both years. Averaged across HT, PNB increased linearly with N rate in the primary harvests.

Nitrogen use and recovery generally followed the law of diminishing returns with increasing N application rate, supporting the findings of Lemus et al. (2008), who reported that approximately 80% N recovery occurred at 90 kg ha^{-1} N over a 3-yr period. Lower N recovery rates of 31 and 23% for 90 and 180 kg N ha^{-1}, respectively, was found in another study (Stout et al., 1991). Nitrogen-use and recovery in the first and second year after planting seems to be relatively low compared with well-established switchgrass stands (Lemus et al., 2008; Stout et al., 1991). Nitrogen-use indicators are biomass dependent, therefore N use and recovery will increase as biomass yield peak 3 to 4 yr post –establishment. Our data implies that applications of N above 40 kg ha^{-1} results in less N recovery rates and therefore creates a potential for N leaching.

Table 5.6 Nitrogen application rate effect on partial factor productivity (PFP), apparent N recovery (ANR) and partial nitrogen balance (PNB) of switchgrass, averaged over six harvests, at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Effects	Nitrogen-use metrics					
	PFP		ANR		PNB	
	2011	2012	2011	2012	2011	2012
	—kg biomass kg N applied—		———— % ————			
	Year x HT interaction					
May	17.9 b	101.9 a	7.0 b	30.6 a	0.19 b	0.79 a
Jun.	35.2 b	132.7 a	5.1 b	36.0 a	0.23 b	0.75 a
Jul.	53.0 b	159.5 a	16.4 b	34.0 a	0.41 b	0.80 a
Aug.	83.6 b	211.6 a	20.8 b	59.4 a	0.52 b	1.39 a
Sep.	93.5 b	216.8 a	17.6 b	50.8 a	0.62 b	1.31 a
Oct.	91.2 b	216.0 a	9.1 b	36.2 a	0.40 b	0.99 a
OPC‡	L**, Q**, C**	L**, Q**, C***	C***	C***	C***	C***
	Year x N rate interaction					
40	99.5 b	279.1 a	16.9 b	69.9 a	0.61 b	1.63 a
80	54.4 b	148.9 a	11.7 b	29.7 a	0.36 b	0.87 a
120	33.3 b	91.2 a	6.7 b	19.9 a	0.21 b	0.51 a
OPC	L***	L***	L***	L***	L***	L***
	Regrowth nitrogen-use metrics					
	Year x HT interaction					
May	40.7 b	90.8 a	17.2 a	19.1 a	0.15 b	0.28 a
Jun.	29.5 b	59.2 a	5.5 a	4.1 a	0.10 b	0.21 a
Jul.	26.5 b	39.4 a	2.4 a	6.5 a	0.13 b	0.20 a
Aug.	18.2 a	9.7 b	3.4 a	5.4 a	0.26 a	0.12 b
OPC	L***	L***, Q***	Q***	Q***	Q***	L***, Q**
	Year x N rate interaction					
40	45.4 b	67.4 a	16.9 a	11.9 b	0.31 a	0.32 a
80	20.4 b	58.0 a	5.3 b	9.6 a	0.10 b	0.18 a
120	14.5 b	23.9 a	3.1 a	3.4 a	0.07 a	0.10 a
OPC	L***	L***	L**	L***	L**	L***

†Means within a row followed by the same lowercase letter are not different between 2011 and 2012 for whole plant, leaf or stem ($P > 0.05$) using PDIFF option in PROC MIXED.

‡ *, **, *** Orthogonal polynomial contrasts significant at the 0.05, 0.01, and 0.001 levels, respectively.

Summary and Conclusions

Biomass yield responded to N application rate and could have been influenced by weather conditions. There was no effect of N in Year 1, however, in Year 2, yield increased by 22% with the addition of 40 kg N ha^{-1} but there were no further increases at additional N rates. There was no residual N effect on regrowth yield, nutrient concentration, and removal rates. Yield and nutrient removal varied with maturity determined by HT and length of the regrowth interval. Stems contributed more than 70% of total biomass and had less N concentration than leaf. Nutrient concentration decreased with plant maturity as nutrients are translocated to below-ground sinks. Harvesting after a killing frost produced greater biomass with less nutrient concentration and therefore, less nutrient removal rates relative to pre-senescence harvests. Nitrogen-use efficiency and recovery decreased with N application rate. Switchgrass has potential for dual-purpose use, with the first harvest optimally targeted for forage use occurring before July and the final harvest after a killing frost for bioenergy processing. Effective nutrient management in switchgrass depends on manipulating the HT with nutrient cycling to minimize nutrient removal and loss, optimize feedstock quality, lower agronomic inputs, and enhance the sustainability of production systems.

CHAPTER VI

SWITCHGRASS BIOMASS AND ITS COMPONENTS FORAGE NUTRITIVE VALUE, FEEDSTOCK COMPOSITION AND ETHANOL YIELD

Abstract

Biomass chemical composition dynamics can influence the potential dual purpose use of switchgrass. This 2-yr research conducted at the Brown Loam Branch Experiment Station, Raymond, MS on a Loring silt loam soil (fine-silty, mixed, thermic Typic Fragiudalfs) during the 2011 and 2012 growing seasons quantified the effects of harvest time (HT) and N application rates on forage nutritive value, feedstock chemical composition, and ethanol yield of switchgrass. Treatments were all factorial combinations of four N application rates (0, 40, 80 and 120 kg ha^{-1}) and six monthly harvest times (May to October) in a RCBD with treatments allocated in a split-plot arrangement. In the primary harvest, ADF, NDF, lignin, cellulose and hemicellulose concentrations in the total aboveground biomass, stem and leaves increased from the May to October HT in both years while in the regrowth harvest, fiber concentration decreased from May to August HT with stems components accumulating greater fiber concentrations than leaf. Crude protein and in vitro dry matter digestibility decreased with plant maturity and were greatest in the leaf component. Similar to fiber concentration, the concentrations of major sugars increased with maturity and were greatest in the most mature stand in the regrowth harvest. Total ethanol yield was

greatest in the least mature stands, however, total ethanol production increased with maturity and is strongly influenced by the biomass yield. Nitrogen rate did not influence the forage nutritive value or feedstock composition in either the first or regrowth harvests. These results indicate that a two-harvest system (early season and end-of-season) has potential for both forage and feedstock use, however, dual use will depend on the economics of forage vs. feedstock use.

Introduction

Season-long animal production requires the use of different forages that differ in their growth habits and period of maximum production to supply forage throughout the season as well as maintain pasture carrying capacity and annual seasonal gains (Krueger and Curtis, 1979). Warm-season grasses provide forage production during summer and can complement cool-season grasses to improve season-long forage availability (Anderson et al., 1988). Studies have demonstrated the utility of switchgrass either as monoculture forage (Krueger and Curtis, 1979; Mosali et al., 2013), in a sequential tall fescue (*Festuca arundinacea* Schreb.)-switchgrass pasture system (Smart et al., 1995), and a binary mixture with legumes (Hudson et al., 2010). Smart et al. (1995) reported average daily gain (ADG) of 0.52 kg d^{-1} grazing ‘Blackwell’ switchgrass from mid-June to late July or early August. Mosali et al. (2013) reported ADG ranging from 0.83 to 1.05 kg d^{-1} for beef steers grazing Alamo switchgrass beginning towards the end of April and ending 28 to 81 d later, depending on the varying stocking rates in their study. Switchgrass forage nutritive value declines with plant physiological maturity (Kering et al., 2013; Mosali et al., 2013; Waramit et al., 2012), therefore switchgrass can be utilized to maintain summer productivity, however, its forage use will be restricted to vegetative stage (Burns et al., 1997).

Switchgrass is a high biomass producer especially in the high temperature summer months and is relatively more water efficient as well as N, P, and K efficient than cool-season grasses, by virtue of its C4 physiology (Barney et al., 2009; Brown, 1978; Lemus et al., 2008a; Sanderson and Reed, 2000). These characteristics confer drought tolerance, high nutrient use efficiencies, and the ability to proliferate and persist on marginal and eroded lands, making switchgrass an ideal candidate for biofuel production (Sanderson et al., 1996). When managed as a bioenergy crop, switchgrass produced ethanol yields comparable to corn grain with greenhouse gas emissions averaging 94% lower than gasoline (Schmer et al., 2008; Varvel et al., 2008). Switchgrass chemical composition changes with plant maturity, influencing its suitability as feedstock (Kering et al., 2013; Waramit et al., 2011).

The adoption of switchgrass as a forage and feedstock species is limited by the unavailability and inaccessibility of biorefineries in close proximity of production fields (Yacobucci and Schnepf, 2007) preventing to date the widespread adoption of switchgrass (Hipple and Duffy, 2002). To minimize risks associated with feedstock production inherent in a fledgling lignocellulosic ethanol industry, producers can exploit the forage and feedstock qualities of switchgrass for early-season forage use and post-frost feedstock harvest. This will require an understanding of changes in forage and feedstock chemical composition, however, in order to optimize both forage use and conversion efficiencies. The objective of this study was to quantify the effect of harvest time and N application rate on total biomass, forage nutritive value, and feedstock composition of Alamo switchgrass.

Materials and Methods

Stand establishment, experimental design and treatments

The stand establishment, experimental design and treatments, and field procedures are described in Chapter 3. Plots were harvested according to harvest treatment starting in May for harvest time 1 (HT), followed by June for HT 2, etc., until the final harvest in October. Each plot was allowed to regrow after its respective harvest until after a killing frost in November and was harvested if regrowth occurred. For the remainder of this Chapter, the first harvest refers to the May, June, July, August, September or October harvest, for each HT, while regrowth harvest refers to the November harvest.

Data collection and analysis

At each monthly harvest, a 1-kg subsample was collected and oven-dried at 55-60 ºC until constant weight was achieved to determine DM concentrations. A second subsample taken from the harvested material was dried similarly and ground to pass a 2-mm stainless steel screen using a Wiley Mill (Model 4; Thomas Scientific, Swedesboro, NJ) and stored for subsequent laboratory analysis. Leaf and stem samples were separated to determine leaf:stem ratio and were ground in a similar manner.

Nutritive value and chemical characteristics were determined using NIR analysis. Whole plant, leaf, and stem tissue samples were scanned using FOSS ISIScan software version 4.4 (Vision, 2007) and prediction equations for grass hay and switchgrass developed by the NIRS Forage and Feed Testing Consortium (Hillsboro, WI). Calibration statistics for trait characterization are presented in Table 6.1 and were adapted from Vogel et al. (2011). Hemicellulose and cellulose concentrations were calculated as the difference between NDF and ADF and the difference between ADF and ADL, respectively.

Table 6.1 Calibration statistics for near infrared reflectance spectroscopy (NIRS) prediction of switchgrass biomass quality traits.

Abbreviation	Mean g kg^{-1}	N	SEC† g kg^{-1}	r^2	SECV‡ g kg^{-1}
ADF§	382.76	108	9.71	0.95	12.79
ADL	55.56	109	4.65	0.84	6.2
Ash	77.45	107	5.54	0.84	8.6
ETOH	91.91	102	3.5	0.94	4.96
Gal.	9.55	107	0.38	0.93	0.61
Glu.	265.9	107	5.7	0.95	7.82
Hex.	367.14	103	7.36	0.92	10.33
IVDMD	494.76	109	12.45	0.98	22.68
NDF	713.59	109	11.98	0.93	16.42
Pent.	196.22	104	6.47	0.95	10.4
Xyl.	191	110	6	0.93	8.41

†SEC standard error of calibration
‡SECV standard error of cross validation, and SEP standard error of prediction
§ADF = acid detergent fiber; ADL = acid detergent lignin; ETOH = ethanol g-1 dry forage; Gal. = galactose; Glu. = glucose; Hex. = hexose; IVDMD = in vitro dry matter digestibility; NDF = neutral detergent fiber; Lig = lignin; Cell = cellulose; Hemi = hemicellulose; NDF = neutral detergent fiber; Pent. = pentose; and Xyl. = xylose

Ethanol yields were calculated as per the methods of Vogel et al. (2011) using the following equations:

$$\text{Total ethanol yield} = (\text{ETOH} \times 1.267) + ((\text{Ara.} + \text{Xyl.}) \times 0.579 \times 1.267 \qquad (6.1)$$

$$\text{Total theoretical ethanol yield} = [(((\text{Man.} + \text{Gal.} + \text{Glc.} + \text{Sta.}) \times 0.57) + (\text{Glcs.} + \text{Fru.}) \times 0.51) + (\text{Suc.} \times 0.537) \times 1.267)] + [(\text{Ara.} + \text{Xyl.}) \times 0.579 \times 1.267)] \quad (6.2)$$

where ETOH: ethanol/g dry forage, Ara.: arabinose, Fru.: fructose, Gal.: galactose, Glc.: glucose, Glcs.: soluble glucose, Man.: mannose, Sta.: starch, Suc.: sucrose, and Xyl.: xylose (g kg^{-1}).

Ethanol production calculations are presented in Chapter 3.

The data were analyzed by fitting mixed models with repeated measures using PROC MIXED in SAS (SAS Institute, 2008). Harvest time, N application rate, year, and their interactions were considered fixed effects while replication and replication × year were considered random effects. Year was considered as a repeated measure and responses were considered as different at 0.05 probability level. Means were separated using the PDIFF option. Correlations among forage nutritive value and feedstock chemical composition parameters and ethanol yield and production were done separately for whole plant, leaf, and stem using PROC CORR of SAS. Regression analyses were performed on the forage nutritive value and feedstock chemical composition parameters that were correlated with ethanol yield and production in the whole plant only.

Results and Discussion

Forage Nutritive Value

Crude Protein and IVDMD concentration

There was year × harvest time interaction effect on primary and end-of-season harvest CP and IVDMD concentration. During both years, whole plant, leaf, and stem CP and IVDMD concentration decreased with increasing maturity from May to October primary harvest and decreased with the length of the regrowth period in the end-of-season harvest (Table 6.2). Both CP and IVDMD concentrations was greater in leaf than stem component(Table 6.2). Also, CP and IVDMD showed a strong negative correlation with ADF and NDF concentrations and IVDMD was negatively correlated with cellulose and hemicellulose concentrations (Table 6.5). In the leaf component, CP was negatively correlated with NDF while in the stem component, IVDMD was negatively correlated with ADF, NDF, and cellulose concentrations (Table 6.6 andTable 6.7).

Table 6.2 Harvest time × year interaction effect on switchgrass whole plant biomass, leaf and stem crude protein and in vitro dry matter digestibility concentrations during the 2011 and 2012 growing seasons at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

	Forage nutritive value							
Month	Whole-plant biomass		Leaf		Stem		Leaf vs. Stem§	
	2011	2012	2011	2012	2011	2012	2011	2012
	g kg-1						(*P* value)	
	Crude protein concentration							
May	64.8 a†	50.2 b	94.3 a	85.0 b	33.5 a	18.1 b	< 0.0001	< 0.0001
Jun.	45.0 a	34.8 b	72.0 a	73.2 a	12.3 a	13.0 a	< 0.0001	< 0.0001
Jul.	46.0 a	32.7 b	95.5 a	76.0 b	18.0 a	11.6 b	< 0.0001	< 0.0001
Aug.	38.0 a	38.9 a	80.8 a	85.1 a	11.9 a	9.2 b	< 0.0001	< 0.0001
Sep.	40.1 a	36.0 a	81.9 a	84.2 a	7.8 a	9.3 a	< 0.0001	< 0.0001
Oct.	26.1 a	27.1 a	63.4 b	72.7 a	6.5 a	7.7 a	< 0.0001	< 0.0001
OPC‡	L^{***}, Q^{*}, C^{***}	L^{***}, C^{***}	L^{***}, Q^{***}, C^{***}	C^{***}	L^{***}, Q^{***}, C^{***}	L^{***}, Q^{*}		
	In vitro dry matter digestibility concentration							
May	576.2 a	487.4 b	651.3 a	590.9 b	523.7 a	425.6 b	< 0.0001	< 0.0001
Jun.	491.8 a	425.3 b	563.9 a	524.4 b	401.8 a	367.3 b	< 0.0001	< 0.0001
Jul.	461.8 a	410.5 b	564.3 a	495.2 b	422.4 a	383.7 b	< 0.0001	< 0.0001
Aug.	460.0 a	398.3 b	560.2 a	487.4 b	394.9 a	385.1 a	< 0.0001	< 0.0001
Sep.	452.8 a	380.6 b	572.8 a	471.1 b	411.8 a	375.6 b	< 0.0001	< 0.0001
Oct.	393.7 a	342.4 b	467.4 a	432.6 b	347.4 a	304.4 b	< 0.0001	< 0.0001
OPC	L^{***}, Q^{***}, C^{***}	L^{***}, Q^{*}, C^{***}	L^{***}, C^{***}	L^{***}, Q^{**}, C^{***}	L^{***}, Q^{***}, C^{***}	L^{***}, Q^{**}, C^{***}		
	Regrowth							
	Crude protein concentration							
May	24.8 a	24.1 a	56.1 b	68.2 a	8.6 a	12.0 a	< 0.0001	< 0.0001
Jun.	25.6 a	24.3 a	53.4 b	67.4 a	7.4 b	11.2 a	< 0.0001	< 0.0001
Jul.	30.3 b	40.4 a	64.9 b	84.9 a	9.0 b	18.7 a	< 0.0001	< 0.0001
Aug.	71.3 a	73.7 a	86.4b	97.1 a	36.7 a	35.2 a	< 0.0001	< 0.0001
OPC	L^{***}, Q^{***}, C^{**}	L^{***}, Q^{***}	L^{***}, Q^{***}	L^{***}, Q^{***}, C^{*}	L^{***}, Q^{***}, C^{***}	L^{***}, Q^{***}		
	In vitro dry matter digestibility concentration							
May	319.6 a	307.2 a	415.6 a	404.6 b	264.9 a	272.4 a	< 0.0001	< 0.0001
Jun.	324.2 a	316.0 a	419.1 a	412.1 a	270.6 b	291.5 a	< 0.0001	< 0.0001
Jul.	332.8 a	341.1 a	428.4 a	429.1 a	267.6 b	292.9 a	< 0.0001	< 0.0001
Aug.	386.6 a	392.1 a	437.0 a	434.7 a	306.9 b	346.6 a	< 0.0001	< 0.0001
OPC	L^{***}, Q^{***},	L^{***}, Q^{***}	L^{***}	L^{***}	L^{***}, Q^{**}, C^{*}	L^{***}, Q^{**}, C^{**}		

†Means within a row followed by the same lowercase letter are not different between 2011 and 2012 for whole, leaf, or stem (*P* > 0.05) using PDIFF option in SAS.

‡ *, **, *** Orthogonal polynomial contrasts significant at the 0.05, 0.01, and 0.001 levels, respectively.

§Comparison between leaves and stems in 2011 and 2012.

The rate of decline in CP concentration with increasing maturity (60% in Year 1 and 46% in Years 2) was similar to those previously reported (Mosali et al., 2013; Mullahey et al., 1992; Waramit et al., 2012). Several studies have shown that switchgrass forage nutritive value decreases with plant maturation (Burns et al., 1997; Sanderson et al., 1999; Twidwell et al., 1988). Crude protein and IVDMD concentration exhibited an inverse linear relationship to fiber concentration (Figure 6.1and Figure 6.2). Initiation of grazing early in the season will allow for the ultilzation of forage with better nutritive value than later in the season. Reid et al.(1988) reported that grazing beef steers on 'Kanlow' switchgrass with 672 g kg^{-1} NDF and 90 to 130 g kg^{-1} CP produced ADG of 0.92 kg d^{-1}. Mosali et al., (2013) reported that beef steers on moderately stocked (4.9 head ha^{-1}) Alamo switchgrass had ADG of 1.04 kg d^{-1} during a 43-d grazing period. Although the first harvest in both years of our study occurred in May while still in the vegetative stage of development, the nutritive value was lesser than the Mosali et al. (2013) study with grazing initiated in late April. Therefore, optimum management will require grazing or harvesting earlier during the season but the timing will be detemined on whether the biomass acummulated can adequaltely support grazing or clipping.

Fiber and Ash Concentrations

There was a year × harvest time interaction effect on primary and regrowth harvest ADF, NDF, lignin, cellulose and hemicellulose concentration ($P < 0.0001$). In both years, ADF, NDF, lignin, cellulose and hemicellulose concentration of primary harvests increased with increasing maturity (0) and similarly increased with longer regrowth interval for the end-of-season harvest from May to August primary harvests in both years (0).

Table 6.3 Harvest time × year interaction effect on switchgrass whole plant, leaf, and stem ADF, NDF, lignin, hemicellulose, cellulose and ash concentrations during the 2011 and 2012 growing seasons at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

HT	Fiber concentration							
	Whole plant		Leaf		Stem		Leaf vs. Stem§	
	2011	2012	2011	2012	2011	2012	2011	2012
	g kg^{-1}						(*P* value)	
	ADF concentration							
May	369.3 b†	421.1 a	309.9 b	348.1 a	384.3 b	439.6 a	< 0.0001	< 0.0001
Jun.	403.7 b	453.5 a	353.1 b	389.1 a	459.9 b	474.8 a	< 0.0001	< 0.0001
Jul.	415.7 b	458.5 a	342.4 b	387.2 a	437.7 b	465.3 a	< 0.0001	< 0.0001
Aug.	419.9 b	457.6 a	348.6 b	389.6 a	461.2 a	460.6 a	< 0.0001	< 0.0001
Sep.	412.8 b	473.5 a	335.0 b	402.7 a	441.4 b	463.5 a	< 0.0001	< 0.0001
Oct.	458.0 b	500.9 a	404.4 b	433.6 a	484.5 b	512.7 a	< 0.0001	< 0.0001
OPC‡	L^{***}, C^{***}	L^{***}, C^{***}	L^{***}, Q^{**}, C^{***}	L^{***}, C^{***}	L^{***}, Q^{***}, C^{***}	L^{***}, Q^{***}, C^{***}		
	NDF concentration							
May	683.2 b	760.7 a	583.2 b	650.8 a	750.6 b	838.6 a	< 0.0001	< 0.0001
Jun.	738.7 b	804.6 a	650.4 b	692.5 a	856.0 b	874.3 a	< 0.0001	< 0.0001
Jul.	757.4 b	793.5 a	645.1 b	692.2 a	819.9 b	845.7 a	< 0.0001	< 0.0001
Aug.	753.3 b	782.1 a	650.3 b	677.6 a	837.6 a	826.6 a	< 0.0001	< 0.0001
Sep.	735.4 b	798.5 a	638.1 b	686.7 a	804.5 a	813.0 a	< 0.0001	< 0.0001
Oct.	804.2 b	828.3 a	724.6 a	714.2 a	859.4 a	871.0 a	< 0.0001	< 0.0001
OPC	L^{***}, C^{***}	L^{***}, C^{***}	L^{***}, C^{***}	L^{***}, C^{***}	L^{***}, Q^{***}, C^{***}	Q^{**}, C^{***}		
	Lignin concentration							
May	67.1 a	40.7 b	64.0 a	23.4 b	74.6 a	43.8 b	< 0.0001	< 0.0001
Jun.	74.4 a	45.5 b	62.7 a	30.2 b	92.9 a	53.6 b	< 0.0001	< 0.0001
Jul.	79.2 a	49.8 b	59.1 a	28.1 b	92.8 a	54.6 b	< 0.0001	< 0.0001
Aug.	80.9 a	50.1 b	60.3 a	30.9 b	97.5 a	56.3 b	< 0.0001	< 0.0001
Sep.	81.3 a	49.0 b	59.9 a	32.9 b	96.3 a	56.0 b	< 0.0001	< 0.0001
Oct.	90.5 a	56.1 b	69.9 a	35.9 b	103.9 a	63.3 b	< 0.0001	< 0.0001
OPC	L^{***}, C^{***}	L^{***}, C^{**}	L^{*}, Q^{***}, C^{**}	L^{***}, C^{*}	L^{***}, Q^{***}, C^{***}	L^{***}, C^{***}		
	Hemicellulose concentration							
May	313.9 b	339.5 a	273.3 b	302.8 a	366.4 b	399.0 a	< 0.0001	< 0.0001
Jun.	335.1 b	351.1 a	297.3 a	303.4 a	396.1 a	399.6 a	< 0.0001	< 0.0001
Jul.	341.7 a	335.0 a	302.7 a	305.0 a	382.2 a	380.5 a	< 0.0001	< 0.0001
Aug.	333.4 a	324.6 b	301.7 a	288.0 b	376.5 a	366.0 b	< 0.0001	< 0.0001
Sep.	322.6 a	325.0 a	303.1 a	284.0 b	363.1 a	349.4 a	< 0.0001	< 0.0001
Oct.	346.2 a	327.3 b	320.2 a	280.5 b	374.9 a	358.3 b	< 0.0001	< 0.0001
OPC	L^{***}, C^{***}	L^{***}, C^{***}	L^{***}, C^{***}	L^{***}, C^{**}	L^{*}, Q^{*}, C^{***}	L^{***}, C^{***}		
	Cellulose concentration							
May	302.2 b	380.5 a	245.9 b	324.6 a	309.7 b	395.9 a	< 0.0001	< 0.0001
Jun.	329.3 b	408.0 a	290.4 b	358.9 a	367.0 b	421.2 a	< 0.0001	< 0.0001
Jul.	336.4 b	408.7 a	283.3 b	359.1 a	344.9 b	410.6 a	< 0.0001	< 0.0001
Aug.	339.0 b	407.5 a	288.3 b	358.6 a	363.7 b	404.3 a	< 0.0001	< 0.0001
Sep.	331.5 b	424.4 a	275.1 b	369.8 a	345.0 b	407.5 a	< 0.0001	< 0.0001
Oct.	367.5 b	444.9 a	334.5 b	397.7 a	380.6 b	449.4 a	< 0.0001	< 0.0001
OPC	L^{***}, C^{***}	L^{***}, C^{***}	L^{***}, C^{***}	L^{***}, C^{***}	L^{***}, Q^{**}, C^{***}	L^{***}, Q^{***}, C^{***}		
	Ash concentration							
May	87.2 a	72.3 b	89.3 a	85.7 a	80.5 a	67.0 b	< 0.0001	< 0.0001
Jun.	72.1 a	66.6 b	99.4 a	90.9 b	62.3 a	55.7 b	< 0.0001	< 0.0001
Jul.	68.2 a	64.7 a	93.1 a	88.4 b	62.8 a	52.1 b	< 0.0001	< 0.0001
Aug.	65.4 a	69.2 a	102.0 a	93.7 b	54.9 a	44.4 b	< 0.0001	< 0.0001
Sep.	64.5 a	68.6 a	94.6 a	95.8 a	49.4 a	45.1 b	< 0.0001	< 0.0001
Oct.	66.3 b	61.3 a	105.8 a	100.5 b	50.5 a	43.8 b	< 0.0001	< 0.0001
OPC	L^{***}, Q^{***}, C^{*}	Q^{***}	L^{***}, C^{***}	L^{***}	L^{***}, Q^{***}	L^{***}, Q^{***}		

†Means within a row followed by the same lowercase letter are not different between 2011 and 2012 for whole, leaf, or stem (*P* > 0.05) using PDIFF option in SAS.

‡ *, **, *** Orthogonal polynomial contrasts significant at the 0.05, 0.01, and 0.001 levels, respectively.

§Comparison between leaves and stems in 2011 and 2012.

Table 6.4 Harvest time × year interaction effect on regrowth switchgrass whole plant biomass, leaf and stem ADF, NDF, lignin, hemicellulose, cellulose and ash concentrations during the 2011 and 2012 growing seasons at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Primary HT	Fiber concentration							
	Whole plant		Leaf		Stem		Leaf vs. Stem§	
	2011	2012	2011	2012	2011	2012	2011	2012
	——————g kg^{-1}——————						(*P* value)	
	ADF concentration							
May	494.6 b†	527.2 a	448.2 b	463.1 a	519.9 a	526.1 a	< 0.0001	< 0.0001
Jun.	496.9 b	529.1 a	446.3 b	468.2 a	519.1 a	521.8 a	< 0.0001	< 0.0001
Jul.	490.4 b	501.7 a	441.9 b	450.8 a	517.9 a	520.6 a	< 0.0001	< 0.0001
Aug.	437.7 b	458.1 a	433.9 b	443.1 a	474.1 b	515.5 a	< 0.0001	< 0.0001
OPC‡	L^{***}, Q^{***}	L^{***}, Q^{***}	L^{***}, Q^{***},	L^{***}, Q^{*}, C^{*}	L^{***}, Q^{***}, C^{*}	Q^{*}		
	NDF concentration							
May	858.8 b	883.5 a	760.8 b	777.8 a	914.3 a	894.7 b	< 0.0001	< 0.0001
Jun.	854.6 b	880.0 a	753.0 b	766.7 a	911.9 a	891.1b	< 0.0001	< 0.0001
Jul.	840.5 a	847.2 a	743.2 b	753.4 a	890.9 a	885.4 b	< 0.0001	< 0.0001
Aug.	783.2 a	791.2 a	738.9 b	746.6 a	843.3 b	875.4 a	< 0.0001	< 0.0001
OPC	L^{***}, Q^{***}	L^{***}, Q^{***}	L^{*}	L^{*}	L^{***}, Q^{***}, C^{*}	Q^{*}		
	Lignin concentration							
May	56.9 b	69.6 a	24.1 b	38.1 a	70.1 a	68.7 a	< 0.0001	< 0.0001
Jun.	58.7 b	69.5 a	23.9 b	38.3 a	69.7 a	64.1 b	< 0.0001	< 0.0001
Jul.	56.8 a	60.1 a	24.3 b	35.5 a	69.0 a	57.7 b	< 0.0001	< 0.0001
Aug.	28.5 b	44.1 a	17.7 b	29.8 a	51.3 a	50.2 a	< 0.0001	< 0.0001
OPC	L^{***}, Q^{***}, C^{**}	L^{***}, Q^{***}	L^{***}, Q^{***}, C^{**}	L^{***}, Q^{***}	L^{***}, Q^{***}			
	Hemicellulose concentration							
May	364.1 a	356.3 b	312.6 a	314.8 a	394.3 a	368.6 b	< 0.0001	< 0.0001
Jun.	357.7 a	350.9 a	306.7 a	298.5 b	392.8 a	369.3 b	< 0.0001	< 0.0001
Jul.	350.1 a	345.4 a	301.3 a	302.6 a	373.9 a	364.8 b	< 0.0001	< 0.0001
Aug.	345.5 a	333.1 a	305.1 a	303.4 a	369.3 a	359.9 a	< 0.0001	< 0.0001
OPC	L^{***}	L^{***}	Q^{***}		L^{***}, Q^{**}			
	Cellulose concentration							
May	437.7 b	457.6 a	424.1 a	425.0 a	449.9 b	457.4 a	< 0.0001	< 0.0001
Jun.	438.2 b	459.6 a	422.4 b	429.9 a	449.5 b	457.7 a	< 0.0001	< 0.0001
Jul.	433.6 b	441.6 a	417.6 a	415.3 a	449.0 a	452.9 a	< 0.0001	< 0.0001
Aug.	409.2 a	414.0 a	416.2 a	413.3 b	422.7 a	427.3 a	< 0.0001	< 0.0001
OPC	L^{***}, Q^{***}	L^{***}, Q^{***}	L^{*}, Q^{***}	L^{*}, C^{***}	L^{***}, Q^{***}	Q^{*}		
	Ash concentration							
May	72.4 a	72.2 a	56.1 b	68.2 a	36.7 b	59.0 a	< 0.0001	< 0.0001
Jun.	74.4 a	77.6 a	53.4 b	67.4 a	35.9 b	62.4 a	< 0.0001	< 0.0001
Jul.	78.7 b	96.0 a	64.9 b	84.9 a	44.8 b	63.5 a	< 0.0001	< 0.0001
Aug.	80.3 b	89.8 a	86.4 b	97.1 a	61.8 a	64.3 a	< 0.0001	< 0.0001
OPC	L^{**}	L^{***}, Q^{*},	L^{***}, Q^{***}	L^{***}	L^{***}, Q^{*}			

†Means within a row followed by the same lowercase letter are not different between 2011 and 2012 for whole, leaf, or stem ($P > 0.05$) using PDIFF option in SAS.

‡ *, **, *** Orthogonal polynomial contrasts significant at the 0.05, 0.01, and 0.001 levels, respectively.

§Comparison between leaves and stems in 2011 and 2012.

Similar response patterns were observed in the leaf and stem components in both years but was greater in stem than leaf (0 and 0). Fiber accumulation increases with physiological maturity (Griffin and Jung, 1983) and is related to the increase in both parenchyma and sclerenchyma cell walls, increased lignin concentrations, increased ratios of xylose to arabinose and *p*-coumaric acid to ferulic acid with plant maturation (Grabber et al., 1991; Sarath et al., 2007). Ash concentration decreased with maturity in the primary harvests and increased with length of regrowth interval until the end-of-season harvest. In addition, the ash concentration was 68 and 86% greater in leaf than stem in Years 1 and 2, respectively. Interactions with year resulted from differences in the magnitude of the response and not a change in the direction of the responses. Whole plant, leaf, and stem fiber concentration of first harvests samples increased rapidly from the early season to midsummer and the rate of change declined from midsummer to late fall. Similar patterns in changes in fiber concentrations in switchgrass and other native warm-season grasses have been previously reported (Mosali et al., 2013; Waramit et al., 2012). Greater ADF, NDF and cellulose concentrations in Year 2 than Year 1 may be associated with the greater early season growth temperatures in Year 2 (3.4°C higher in Year 2 vs. 2.4°C lower than the long-term average in Year 1) similar to Xue et al., (2011), which can lead to reduced leaf:stem ratio, increased lignification, and accelerated physiological maturity (Buxton and Casler, 1993). The range in ADF and NDF concentrations are comparable to previous findings (Mosali et al., 2013).

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Table 6.5 Pearson’s correlation coefficients for mean switchgrass total plant forage nutritive value, feedstock chemical composition, and ethanol yield and production values (n = 319) during 2011 and 2012 growing seasons at Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Total biomass	Yield	ADF	NDF	Lig.	Cell.	Hemi.	CP	IVDMD	Ash	ETOH	Glc.	Hex.	Pent.	Xyl.	TEY	TEP	TTEY
ADF	ns‡																
NDF	ns	0.96															
Lig.	ns	ns	ns														
Cell.	ns	0.94	0.9	ns													
Hemi.	ns	0.47	0.7	-0.42	ns												
CP	ns	-0.62	-0.64	ns	ns	-0.44											
IVDMD	ns	-0.95	-0.94	ns	-0.9	-0.56	0.57										
Ash	ns	ns	ns	ns	ns	ns	0.47	ns									
ETOH	ns	-0.95	-0.93	ns	-0.9	-0.49	0.49	0.92	ns								
Glc.	ns	0.79	0.86	ns	0.71	0.69	-0.71	-0.72	ns	-0.72							
Hex.	ns	ns	ns	ns	ns	ns	ns	ns	-0.69	ns	ns						
Pent.	ns	ns	0.41	ns	ns	0.51	-0.61	-0.45	-0.42	ns	0.44	ns					
Xyl.	ns	0.7	0.79	ns	0.65	0.7	-0.8	-0.73	-0.42	-0.61	0.83	ns	0.76				
TEY	ns	-0.86	-0.81	ns	-0.81	ns	ns	0.78	ns	0.95	-0.59	ns	ns	ns			
TEP	0.99	ns	ns	ns	ns	ns	ns	ns	-0.45	ns	ns	0.39	ns	ns	ns		
TTEY	ns	ns	ns	ns	ns	0.5	-0.66	ns	-0.62	ns	0.55	0.64	0.63	0.63	ns	ns	
TTEP	1	ns	ns	ns	ns	ns	ns	ns	-0.44	ns	ns	ns	ns	ns	ns	0.99	ns

†ADF, acid detergent fiber; NDF, neutral detergent fiber; Lig., lignin; Cell., cellulose; Hemi., hemicellulose; CP, crude protein; IVDMD, in vitro dry matter digestibility; ETOH, ethanol concentration, Glc., glucose; Hex., hexose; Pent., pentose; Xyl., xylose; TEY, total ethanol yield from SSF; TEP, total ethanol production; TTEY, total theoretical ethanol yield from all biomass sugars; TTEP, total theoretical ethanol production

‡ ns, not significant; where correlation coefficients are given, they all were significant at the 0.001 probability level.

Table 6.6 Pearson's correlation coefficients for mean switchgrass leaf forage nutritive value, feedstock chemical composition, and ethanol yield and production values (n = 319) during 2011 and 2012 growing seasons at Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Leaf	Yield	ADF†	NDF	Lig.	Cell.	Hemi.	CP	IVDMD	Ash	ETOH	Glc.	Hex.	Pent.	Xyl.	TEY	TEP	TTEY
ADF	ns‡																
NDF	ns	0.94															
Lig.	ns	-0.47	-0.48														
Cell.	ns	0.96	0.92	-0.68													
Hemi.	ns	ns	0.48	ns	ns												
CP	ns	-0.46	-0.51	ns	-0.4	ns											
IVDMD	ns	ns	ns	ns	ns	ns	ns										
Ash	ns	ns	ns	ns	ns	-0.45	ns	ns									
ETOH	ns	-0.87	-0.8	ns	-0.81	ns	ns	ns	-0.4								
Glc.	ns	0.68	0.77	ns	0.65	0.48	-0.59	ns	ns	ns							
Hex.	ns	ns	ns	ns	ns	ns	ns	ns	-0.56	ns	ns						
Pent.	ns	0.41	0.55	ns	0.4	0.53	-0.81	ns	ns	ns	0.58	ns					
Xyl.	ns	0.57	0.73	-0.042	0.6	0.62	-0.7	ns	ns	ns	0.75	ns	0.86				
TEY	ns	-0.87	-0.82	0.51	-0.87	ns	ns	ns	ns	0.79	-0.61	ns	ns	ns			
TEP	0.96	ns	ns	ns	ns	ns	ns	ns	ns	ns	-0.41	ns	ns	ns	0.49		
TTEY	ns	ns	ns	ns	ns	0.55	-0.54	ns	ns	ns	0.63	0.57	0.67	0.7	ns	ns	
TTEP	0.94	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.98	ns

†ADF, acid detergent fiber; NDF, neutral detergent fiber; Lig., lignin; Cell., cellulose; Hemi., hemicellulose; CP, crude protein; IVDMD, in vitro dry matter digestibility; ETOH, ethanol concentration, Glc., glucose; Hex., hexose; Pent., pentose; Xyl., xylose; TEY, total ethanol yield from SSF; TEP, total ethanol production; TTEY, total theoretical ethanol yield from all biomass sugars; TTEP, total theoretical ethanol production

‡ ns, not significant; where correlation coefficients are given, they all were significant at the 0.001 probability level.

Table 6.7 Pearson's correlation coefficients for mean switchgrass stem forage nutritive value, feedstock chemical composition, and ethanol yield and production values (n = 319) during 2011 and 2012 growing seasons at Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Stem	Yield	ADF	NDF	Lig.	Cell.	Hemi.	CP	IVDMD	Ash	ETOH	Glc.	Hex.	Pent.	Xyl.	TEY	TEP	TTEY
ADF	ns‡																
NDF	ns	0.9															
Lig.	ns	ns	ns														
Cell.	ns	0.92	0.82	ns													
Hemi.	ns	ns	0.48	ns	ns												
CP	-0.43	ns	ns	ns	ns	ns											
IVDMD	ns	-0.67	-0.58	ns	-0.74	ns	ns										
Ash	ns	ns	-0.41	ns	ns	ns	0.49	ns									
ETOH	ns	-0.86	-0.8	ns	-0.83	ns	ns	0.74	ns								
Glc.	ns	0.59	0.72	ns	0.62	0.46	ns	-0.46	ns	-0.69							
Hex.	0.69	-0.52	-0.51	ns	-0.49	ns	-0.48	0.61	ns	0.66	-0.4						
Pent.	ns	ns	0.4	ns	ns	ns	ns	ns	-0.51	ns	0.46	ns					
Xyl.	ns	0.48	0.67	ns	0.47	0.56	-0.54	ns	ns	-0.59	0.85	ns	0.67				
TEY	ns	-0.8	-0.71	ns	-0.78	ns	-0.42	0.66	ns	0.97	-0.59	0.64	ns	-0.42			
TEP	0.99	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.75	ns	ns	ns		
TTEY	ns	ns	ns	ns	ns	ns	-0.46	ns	ns	ns	ns	0.45	0.57	0.42	0.45	ns	
TTEP	1	ns	ns	ns	ns	ns	-0.44	ns	ns	ns	ns	0.71	ns	ns	ns	0.99	ns

†ADF, acid detergent fiber; NDF, neutral detergent fiber; Lig., lignin; Cell., cellulose; Hemi., hemicellulose; CP, crude protein; IVDMD, in vitro dry matter digestibility; ETOH, ethanol concentration, Glc., glucose; Hex., hexose; Pent., pentose; Xyl., xylose; TEY, total ethanol yield from SSF; TEP, total ethanol production; TTEY, total theoretical ethanol yield from all biomass sugars; TTEP, total theoretical ethanol production

‡ ns, not significant; where correlation coefficients are given, they all were significant at the 0.001 probability level.

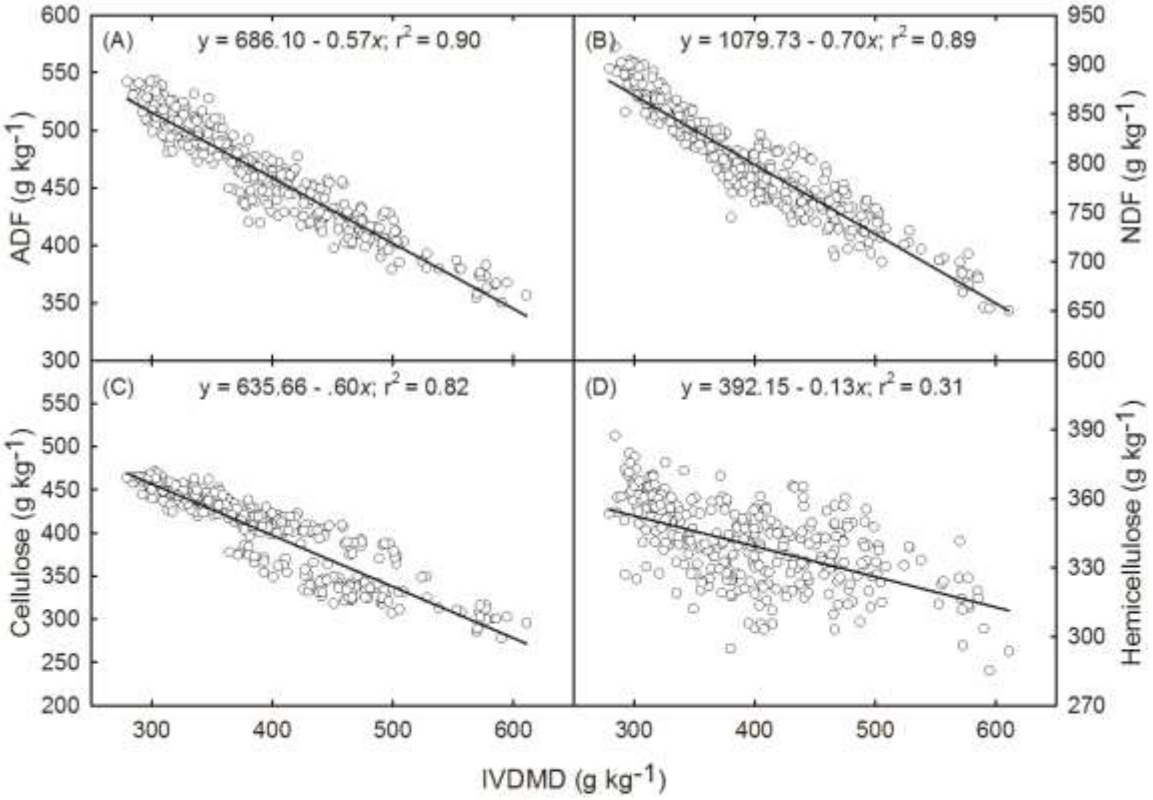

Figure 6.1 Relationships between switchgrass in vitro dry matter digestibility (IVDMD) concentration and fiber concentrations.

Relationships between switchgrass in vitro dry matter digestibility (IVDMD) concentration and (A) acid detergent fiber (ADF), (B) neutral detergent fiber (NDF), (C) cellulose and (D) hemicellulose concentrations measured monthly from May to October during 2011 and 2012 growing seasons at Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

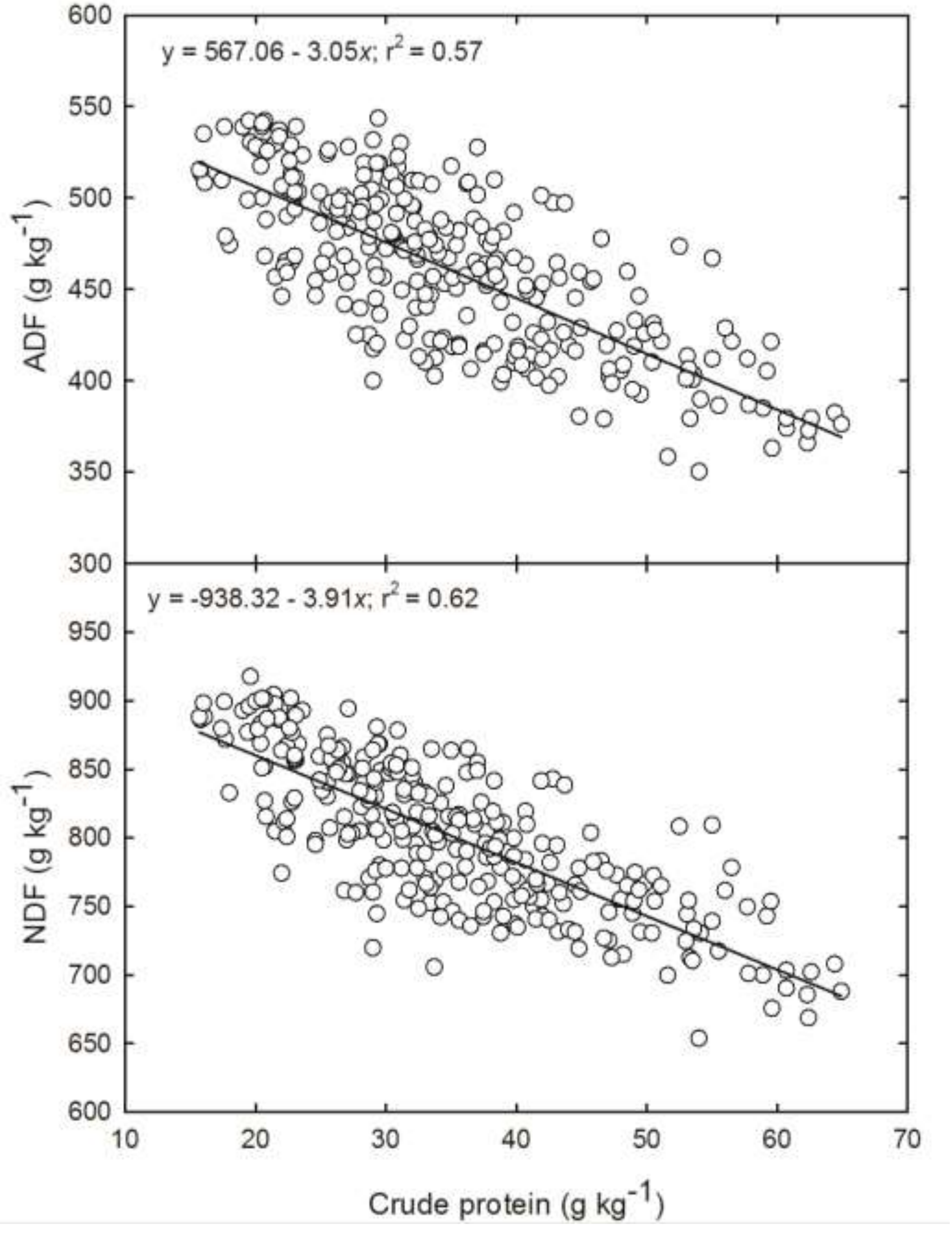

Figure 6.2 Relationships between switchgrass crude protein concentration and fiber concentrations.

Relationships between switchgrass crude protein concentration and (A) acid detergent fiber (ADF) and (B) neutral detergent fiber (NDF) concentrations measured monthly from May to October during 2011 and 2012 growing seasons at Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Feedstock Chemical Composition

Ethanol and Sugar Concentrations

There was a year × harvest time interaction effect on whole plant, leaf, and stem ethanol and sugar concentrations ($P < 0.0001$). Ethanol concentration decreased with increasing maturity of May to October harvests by 32 and 44% during Years 1 and 2, respectively (Table 6.8). The interaction was due in part to the magnitude of the response in different years. In the end-of season harvests, ETOH concentration decreased with increasing length of regrowth period based on May to August primary harvests by 30 and 48% in Years 1 and 2, respectively (Table 6.8). In the primary harvests, ETOH concentration of leaf was 15% greater than stem, while in the regrowth harvest it was 70% greater (Table 6.8 andTable 6.9). The decrease in ethanol concentration may be related to the increase in fiber concentration. Dien et al., (2006) found that glucose conversion efficiency declined with switchgrass maturity. In addition, as lignin increased, the total sugars released from saccharification decreased linearly (Chen and Dixon, 2007; Dien et al., 2006); thereby increasing the recalcitrance to saccharification and lowering the ethanol concentration. Ethanol concentration was negatively correlated with ADF, NDF, cellulose, and hemicellulose concentrations and positively correlated with CP and IVDMD) in the total aboveground biomass (Table 6.5).

Table 6.8 Harvest time × year interaction effect on switchgrass primary harvest whole plant, leaf and stem ethanol, glucose, hexose, pentose and xylose concentrations during the 2011 and 2012 growing seasons at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Month	Whole plant		Leaf		Stem		Leaf vs. Stem§	
	2011	2012	2011	2012	2011	2012	2011	2012
	g kg-1						(*P* value)	
ETOH concentration								
May	106.4 a†	93.8 b	118.5 a	102.4 b	105.5 a	82.5 b	< 0.0001	< 0.0001
Jun.	98.6 a	82.8 b	102.8 a	91.2 b	81.3 a	70.8 b	< 0.0001	< 0.0001
Jul.	91.0 a	84.2 b	100.7 a	92.5 b	87.2 a	79.4 b	< 0.0001	< 0.0001
Aug.	93.8 a	80.1 b	99.3 a	88.4 b	84.6 a	87.7 a	< 0.0001	< 0.0001
Sep.	95.9 a	75.9 b	102.6 a	85.5 b	97.0 a	88.6 b	< 0.0001	< 0.0001
Oct.	80.8 a	65.2 b	87.9 a	78.9 b	76.1 a	66.9 b	< 0.0001	< 0.0001
OPC‡	L^{***}, C^{***}	L^{***}, C^{***}	L^{***}, C^{***}	L^{***}, C^{*}	L^{***}, Q^{*}, C^{***}	Q^{***}, C^{***}		
Glucose concentration								
May	267.4 b	287.6 a	227.2 b	240.1 a	293.4 b	316.2 a	< 0.0001	< 0.0001
Jun.	274.7 b	296.5 a	234.1 b	245.1 a	311.2 b	319.7 a	< 0.0001	< 0.0001
Jul.	277.8 b	286.5 a	231.1 a	234.9 a	301.0 b	307.6 a	< 0.0001	< 0.0001
Aug.	281.7 a	277.7 a	231.0 a	219.3 b	306.2 a	296.2 b	< 0.0001	< 0.0001
Sep.	289.8 a	281.4 b	228.3 a	222.2 a	294.8 a	294.7 a	< 0.0001	< 0.0001
Oct.	284.8 b	299.3 a	224.7 a	231.2 b	307.1b	314.4 a	< 0.0001	< 0.0001
OPC	L^{***}, Q^{***}, C^{**}	Q^{***}	L^{***}, Q^{*}, C^{***}	L^{***}, Q^{***}, C^{***}	C^{***}	L^{***}, Q^{***}, C^{***}		
Hexose concentration								
May	405.5 b	429.7 a	369.7 a	359.7 b	451.4 b	473.2 a	< 0.0001	< 0.0001
Jun.	414.3 b	438.6 a	339.4 a	343.3 a	465.1 a	472.6 a	< 0.0001	< 0.0001
Jul.	409.3 b	435.7 a	331.3 b	341.5 a	465.9 b	484.4 a	< 0.0001	< 0.0001
Aug.	431.3 a	419.2 a	326.6 a	329.6 a	479.7 b	506.3 a	< 0.0001	< 0.0001
Sep.	428.9 a	418.6 a	334.5 a	327.8 a	501.5 a	504.1 a	< 0.0001	< 0.0001
Oct.	424.4 b	385.9 a	330.8 a	323.6 a	472.7 a	476.3 a	< 0.0001	< 0.0001
OPC	L^{***}, Q^{***}, C^{*}	L^{***}, Q^{***}, C^{*}	L^{***}, Q^{***}, C^{**}	L^{***}	L^{***}, Q^{***}, C^{***}	L^{***}, Q^{***}, C^{***}		
Pentose concentration								
May	178.2 b	191.7 a	164.2 a	167.7 a	189.6 a	192.5 a	< 0.0001	< 0.0001
Jun.	201.8 a	199.9 a	180.8 a	183.6 a	201.1 a	198.4 a	< 0.0001	< 0.0001
Jul.	200.1 a	206.6 a	177.9 b	186.2 a	197.1 a	198.8 a	< 0.0001	< 0.0001
Aug.	201.2 a	203.0 a	177.9 a	177.0 a	199.7 a	196.5 a	< 0.0001	< 0.0001
Sep.	198.1 a	202.7 a	178.6 a	178.0 a	201.2 a	198.9 a	< 0.0001	< 0.0001
Oct.	205.9 a	200.4 a	199.6 a	192.7 a	205.5 a	205.3 a	< 0.0001	< 0.0001
OPC	L^{***}, Q^{***}	L^{*}	L^{***}, C^{***}	L^{***}, C^{***}	L^{***}, C^{***}	L^{***}, C^{**}		
Xylose concentration								
May	180.2 a	199.3 a	151.0 b	164.7 a	200.5 b	220.4 a	< 0.0001	< 0.0001
Jun.	196.7 a	211.8 a	165.9 b	175.3 a	224.0 b	228.9 a	< 0.0001	< 0.0001
Jul.	199.6 a	209.8 a	159.0 b	170.3 a	214.9 b	220.8 a	< 0.0001	< 0.0001
Aug.	200.6 a	204.0 a	164.5 a	160.0 a	218.6 a	212.4 b	< 0.0001	< 0.0001
Sep.	191.9 a	205.5 a	162.4 a	161.5 a	209.3 a	210.4 a	< 0.0001	< 0.0001
Oct.	205.8 b	240.9 a	176.5 a	173.8 a	221.5 a	224.0 a	< 0.0001	< 0.0001
OPC	L^{***}, Q^{***}, C^{**}	L^{***}, Q^{***}, C^{***}	L^{***}, C^{***}	Q^{*}, C^{***}	L^{***}, Q^{***}, C^{***}	L^{***}, Q^{***}, C^{***}		

†Means within a row followed by the same lowercase letter are not different between 2011 and 2012 for whole, leaf, or stem ($P > 0.05$) using PDIFF option in SAS.

‡ *, **, *** Orthogonal polynomial contrasts significant at the 0.05, 0.01, and 0.001 levels, respectively.

§Comparison between leaves and stems in 2011 and 2012.

Table 6.9 Harvest time × year interaction effect on switchgrass end-of-season whole plant biomass, leaf and stem ethanol, glucose, hexose, pentose, and xylose concentrations during the 2011 and 2012 growing seasons at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

HT	Whole plant		Leaf		Stem		Leaf vs. Stem§	
	2011	2012	2011	2012	2011	2012	2011	2012
	g kg-1						(*P* value)	
				ETOH concentration				
May	65.2 a[†]	50.3 b	88.9 a	71.8 b	51.4 a	42.8 b	< 0.0001	< 0.0001
Jun.	64.6 a	50.5 b	87.8 a	73.9 b	53.3 a	45.6 b	< 0.0001	< 0.0001
Jul.	69.1 a	59.6 b	89.8 a	77.2 b	52.1 a	48.7 a	< 0.0001	< 0.0001
Aug.	84.8 a	74.6 b	93.1 a	81.8 b	77.3 a	65.6 b	< 0.0001	< 0.0001
OPC[‡]	L^{***}, Q^{***}	L^{***}, Q^{***}	L^{***}, Q^{*}	L^{***}	L^{***}, Q^{***}, C^{***}	L^{***}, Q^{***}		
				Glucose concentration				
May	305.7 a	308.8 a	264.3 a	263.1 a	321.3 a	320.6 a	< 0.0001	< 0.0001
Jun.	304.1 a	307.2 a	262.6 a	260.9 a	322.3 a	320.8a	< 0.0001	< 0.0001
Jul.	299.3 a	295.7 a	256.9 a	250.8 b	322.5 a	314.9 b	< 0.0001	< 0.0001
Aug.	270.7 a	276.9 a	248.8 a	250.8 a	312.9 a	305.1 b	< 0.0001	< 0.0001
OPC	L^{***}, Q^{***}	L^{***}, Q^{**}	L^{***}	L^{**}	L^{**}, Q^{**}	L^{***}, Q^{**}		
				Hexose concentration				
May	403.1 b	415.7 a	342.2 a	327.8 b	439.6 b	438.9 a	< 0.0001	< 0.0001
Jun.	403.2 b	416.6 a	347.7 a	343.0 a	436.2 b	443.7 a	< 0.0001	< 0.0001
Jul.	398.0 a	393.9 a	344.2 a	337.7 a	432.6 a	426.6 b	< 0.0001	< 0.0001
Aug.	387.5 a	382.3 a	413.9 a	374.7 b	428.2 a	418.0 b	< 0.0001	< 0.0001
OPC	L^{***}, Q^{***}, C^{**}	L^{***}, Q^{*}, C^{**}	L^{***}, Q^{***}, C^{***}	L^{***}, Q^{**}, C^{***}	Q^{*}	L^{***}, Q^{**}, C^{**}		
				Pentose concentration				
May	219.8 a	198.4 b	212.7 a	183.7 b	220.2 a	196.1 b	< 0.0001	< 0.0001
Jun.	215.9 a	193.9 b	208.1 a	183.8 b	219.1 a	193.5 b	< 0.0001	< 0.0001
Jul.	216.2 a	192.8 b	201.1 a	172.6 b	219.0 a	192.9 b	< 0.0001	< 0.0001
Aug.	204.3 a	179.7b	193.9 a	167.6 b	218.3 a	191.4 b	< 0.0001	< 0.0001
OPC	L^{***}, Q^{*}, C^{*}	L^{***}, Q^{*}	L^{***}	L^{***}				
				Xylose concentration				
May	220.7 a	217.2 a	198.8 a	183.2 b	234.9 a	225.0 b	< 0.0001	< 0.0001
Jun.	218.0 a	213.2 a	185.9 a	174.3 b	234.3 a	223.6 b	< 0.0001	< 0.0001
Jul.	214.6 a	201.3 b	180.9 a	164.7 b	234.2 a	216.5 b	< 0.0001	< 0.0001
Aug.	203.4 a	181.3 b	190.5 a	166.8 b	220.3 a	202.0 b	< 0.0001	< 0.0001
OPC	L^{***}, Q^{*}	L^{***}, Q^{***}	Q^{***}, C^{*}	L^{***}, C^{**}	L^{***}, Q^{***}, C^{*}	L^{***}, Q^{***}		

[†] Means within a row followed by the same lowercase letter are not different between 2011 and 2012 for whole, leaf, or stem ($P > 0.05$) using PDIFF option in SAS.

[‡] *, **, *** Orthogonal polynomial contrasts significant at the 0.05, 0.01, and 0.001 levels, respectively.

§Comparison between leaves and stems in 2011 and 2012.

In both leaf and stem components, ethanol concentration was negatively correlated with ADF, NDF, and cellulose concentrations (Table 6.6 andTable 6.7). Glucan concentration was reported to be negatively correlated with convertibility (percentage of measured glucose converted to ethanol) in corn stover (Lorenz et al., 2009), which supports our findings. Anderson et al. (2010) suggested that the enzymes involved in ruminal digestion are similar to the enzymes used in converting cellulosic biomass to ethanol, suggesting that forage digestibility is related to ethanol yield derived from the SSF process.

Ethanol production from cellulosic biomass is achieved through a two-conversion process involving cellulose hydrolysis to produce fermentable reducing sugars and fermentation of these sugars to ethanol (Badger, 2002). The efficiency of hydrolysis is reduced when lignin and hemicellulose are present reducing the access and activity of cellulase enzymes (Sun and Cheng, 2002). Current methods of conversion limit the achievable maximum ethanol recovery (Jiang et al., 2013; Yang and Wyman, 2007). Because fiber concentration in switchgrass biomass increased with increasing maturity, it is expected that ethanol concentration will decrease as harvests are done later in the growing season, evident from the inverse linear relationship between fiber and ethanol concentration (Figure 6.3). Ethanol concentration reported here was determined assuming the direct fermentation of hexoses with *S. cerevisiae,* which do not ferment pentose and xylose (Vogel et al., 2011). A more recent novel yeast strain, *K. marxianus*, is capable of recovering 15% more ethanol than *S. cerevisiae* (Suryawati et al., 2008). Molecular techniques to improve cell wall properties for improved saccharification has been recently reviewed (Nookaraju et al., 2013) and show promise to improve ethanol recovery rates. In addition, recurrent breeding methods for increased IVDMD have increased ethanol yields by 39% in plants with low lignin (Sarath et al., 2011).

In both years, glucose, hexose, pentose and xylose concentrations increased with increasing plant maturity from May to October in primary harvests and decreased with decreasing length of the regrowth interval in the end-of-season harvest (Table 6.8 and Table 6.9). Similar responses were observed for leaf and stem (Table 6.8).

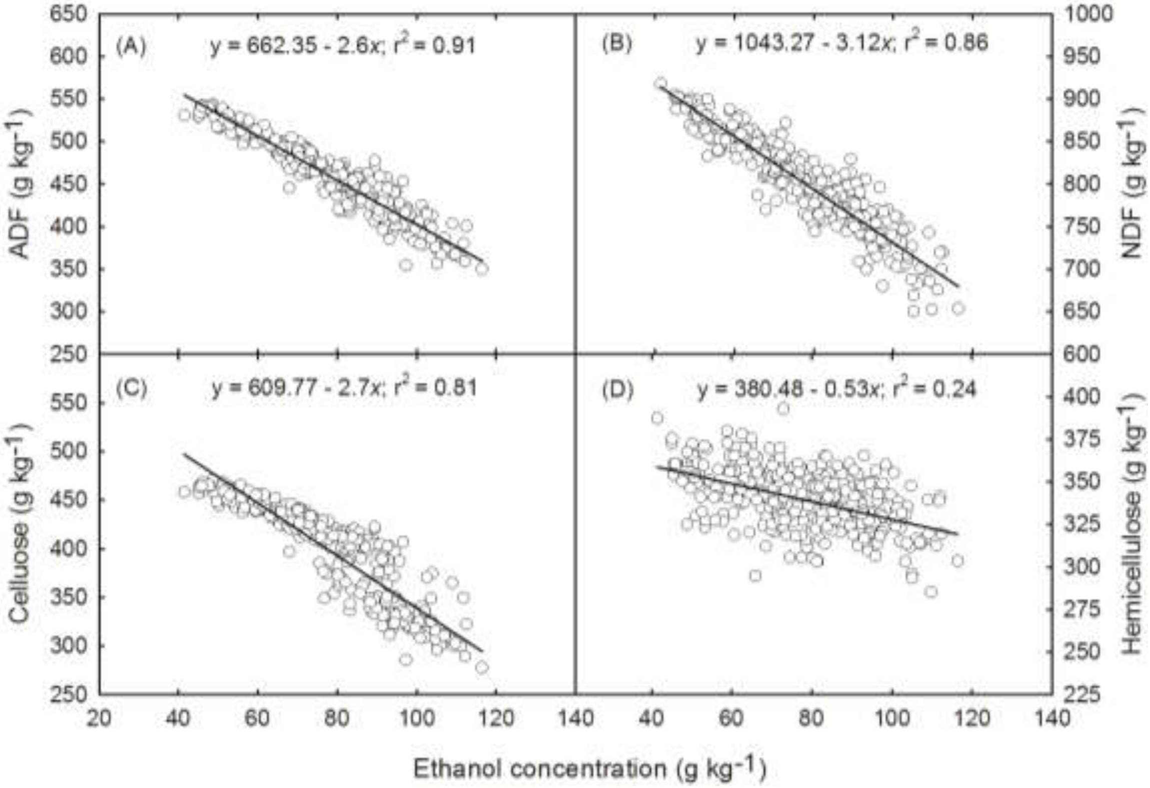

Figure 6.3 Relationships between switchgrass ethanol concentration and fiber concentrations.

Relationships between switchgrass ethanol concentration and (A) acid detergent fiber (ADF), (B) neutral detergent fiber (NDF), (C) cellulose and (D) hemicellulose concentrations measured monthly from May to October during 2011 and 2012 growing seasons at Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Pentose and xylose concentrations increased with increasing maturity in both the leaf and stem components in the primary harvest, however, stems were 10% greater in pentose and 31% greater in xylose compared with leaves (Table 6.8). Except for leaf hexose concentration, leaf and stem sugar concentrations in the regrowth harvest decreased with decreasing length of the regrowth period in both years (Table 6.9). Similar to primary harvest, end-of-season harvest sugar concentration was greater in stem than leaf. Whole-plant, leaf, and stem glucose and xylose concentrations were positively correlated with ADF, NDF, cellulose, and hemicellulose and negatively correlated with CP and IVDMD (Table 6.5). Hexose concentration was negatively correlated with ADF, NDF and cellulose concentrations but positively correlated with ethanol concentration in the stem component (Table 6.7). The increase in the stem percentage and increased fiber accumulation with increasing plant maturity (reported in Chapter 3) is mainly responsible for the increase in glucose, hexose, pentose and xylose concentrations. The ranges of glucose (219 – 323 g kg^{-1}), hexose (324 – 506 g kg^{-1}), pentose (164 – 220 g kg^{-1}) and xylose (151 – 341 g kg^{-1}) concentrations in the primary and end-of-season harvests found in the current study are comparable to those reported by Schmer et al. (2012).

Switchgrass utilizes N efficiently relative to C3 grasses (Staley et al., 1991) but N use and recovery decline at N rate greater than 90 kg N ha^{-1} (Lemus et al., 2008a; Staley et al., 1991). In our study, N use and recovery were greater at the 40 kg N ha^{-1} application rate and declined with additional N inputs (Chapter 3). Although a yield benefit was realized at that application rate, there was no effect on whole plant forage nutritive value and sugar compositions, which supports the findings of Guretzky et al. (2011) for Alamo in Oklahoma using N rates of 0, 45, 90, 135, 180, 225 kg N ha^{-1} and

Lemus et al. (2008b) for upland Cave-in-Rock in Virginia using 0, 56, 112, 224 kg N ha^{-1}. Waramit et al. (2011; 2012) reported an N effect on NDF, CP, cellulose, lignin but no effect on hemicellulose for Cave-in-Rock in Iowa using application rates of 0, 65, 140 kg N ha^{-1}. Kering et al. (2013) found a linear increase in CP with N rates from 0 to 270 kg N ha^{-1}, and marginal or negative effects on IVDMD, ADF and NDF concentrations for Alamo switchgrass in Oklahoma. Forage nutritive value responses to fertilizer N application are subjected to spatial variation which may be dependent on soil N mineralization rates and availability and mycorrhizal activity.

Ethanol Yield and Production

There was a year × HT interaction effect on ethanol yield and production in the primary and end-of-season harvests ($P < 0.05$). In both years, whole plant, leaf, and stem total ethanol yield SSF decreased with increasing maturity (Table 6.10 andTable 6.11). In the first harvest, stem total ethanol yield (TEY) was greater by 2 to 3% than stem while in the regrowth harvest, stem TEY was 10 to 12% greater than leaves (Table 6.10).

In both years, whole plant, leaf, and stem total ethanol production (TEP) from SSF increased with increasing plant maturity with May to October primary harvests and with increasing length of the regrowth interval in the end-of-season harvests (Table 6.10 and Table 6.11). In the primary harvests, TEP was 2.7 and 3.7 times greater in the stem component in Year 1 and 2 relative to the leaf component, while in the end-of-season harvest; it was 3.5 and 3.3 times greater in stems than leaves in Year 1 and 2 (Table 6.11).

Primary harvest total theoretical ethanol yield (TTEY) from all biomass sugars increased with increasing maturity in the primary harvest during Year 1, while in Year 2, TTEY decreased perhaps due to the relative reduction in hexose sugars in Year 2 (Table 6.10). In both years, whole plant, leaf, and stem TTEY decreased with decreasing length of the regrowth interval in the end-of-season harvests. In the first harvests, stem TTEY was 24 % greater than leaves, while in the regrowth harvest it was 20% greater (Table 6.11). The ranges of TTEY (343 – 449 L Mg^{-1}) in our study are similar to those reported by Schmer et al. (2012).

Total theoretical ethanol production from all biomass sugars (TTEP) increased from May to October harvests across total biomass and its components in the first harvest while in the regrowth harvest, TTEP increased with delayed regrowth period. TTEP in stem component in both the first and regrowth harvest was 2 to 3 fold greater than the leaf component (Table 6.10). In the first harvest, TTEY and TTEP in total biomass was 88 and 100% greater than TEY and TEP while in the regrowth harvest TTEY and TTEP were 108 and 133% greater than TEY and TEP (Table 6.11).

There was a N rate effect on primary harvest TEY and TTEY among total biomass and its components ($P < 0.05$) and a year × N rate interaction effect on TEP and TTEP in the total above-ground biomass ($P < 0.05$). There was an N application rate effect on leaf and stem component TEP and TTEP. In the whole plant, leaf, and stem, TEY and TTEY decreased with increasing N rate (Table 6.12).

Table 6.10 Harvest time × year interaction effect on switchgrass primary harvest whole plant biomass, leaf, and stem ethanol yield and production during the 2011 and 2012 growing seasons at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

HT	Whole plant		Leaf		Stem		Leaf vs. Stem§	
	2011	2012	2011	2012	2011	2012	2011	2012
	Total ethanol yield from SSF (L Mg^{-1})						(*P* value)	
May	226.9 a[†]	217.9 b	235.1 a	216.3 b	231.7 a	204.1 b	< 0.0001	0.0002
Jun.	229.2 a	208.3 b	223.7 a	210.4 b	206.9 a	192.3 b	< 0.0001	< 0.0001
Jul.	218.7 a	213.5 a	219.6 a	213.5 a	212.3 a	203.4 b	< 0.0001	0.0021
Aug.	222.8 a	206.3 b	217.8 a	203.5 b	210.4 a	212.7 b	0.0233	0.0049
Sep.	223.9 a	200.9 b	222.3 a	200.3 b	226.9 a	215.1 b	< 0.0001	< 0.0001
Oct.	208.8 a	188.1 b	214.5 a	199.6 b	202.7 a	190.9 b	< 0.0001	0.0104
OPC[‡]	L^{***}, C^{**}	L^{***}, Q^{*}, C^{*}	L^{***}, Q^{*}, C^{*}	L^{***}	L^{***}, C^{***}	Q^{**}, C^{***}		
	Total ethanol production per ha from SSF (L ha^{-1})							
May	251.8 b	1306.6 a	144.7b	477.1 a	115.0 b	773.6 a	< 0.0001	< 0.0001
Jun.	502.7 b	1764.6 a	163.7 b	583.4 a	300.9 b	1098.6 a	< 0.0001	< 0.0001
Jul.	728.0 b	2219.9 a	236.7 b	495.8 a	478.4 b	1645.9 a	< 0.0001	< 0.0001
Aug.	1205.5 b	2689.0 a	286.8 b	471.1 a	859.6 b	2284.7 a	< 0.0001	< 0.0001
Sep.	1361.5 b	2695.2 a	277.8 b	444.2 a	1095.4 b	2408.5 a	< 0.0001	< 0.0001
Oct.	1229.2 b	2542.2 a	242.3 b	488.6 a	962.3 b	2109.4 a	< 0.0001	< 0.0001
OPC	L^{***}, Q^{**}, C^{**}	L^{***}, Q^{***}, C^{*}	L^{***}, Q^{**}	L^{*}, C^{***}	L^{***}, Q^{*}, C^{**}	L^{***}, Q^{***}, C^{***}		
	Total theoretical ethanol yield from all biomass sugars (L Mg^{-1})							
May	400.5 b	417.6 a	362.2 a	366.2 a	437.9 a	443.4 a	< 0.0001	< 0.0001
Jun.	417.5 a	423.5 a	364.7 a	367.2 a	446.4 a	436.4 b	< 0.0001	< 0.0001
Jul.	415.9 a	420.3 a	358.4 a	360.9 a	443.6 a	441.2 a	< 0.0001	< 0.0001
Aug.	425.4 a	407.2 b	358.8 a	342.8 b	449.7 a	439.3 b	< 0.0001	< 0.0001
Sep.	417.8 a	400.5 b	361.4 a	342.8 b	452.7 a	439.2 b	< 0.0001	< 0.0001
Oct.	424.2 a	400.5 b	366.3 a	352.3 b	449.3 a	437.5 b	< 0.0001	< 0.0001
OPC	L^{***}, Q^{**}	L^{***}, C^{**}		L^{***}, Q^{**}, C^{***}	L^{**}			
	Total theoretical ethanol production from all biomass sugars (L ha^{-1})							
May	443.9 b	2536.2 a	223.2 b	814.5 b	217.9 b	1700.7 a	< 0.0001	< 0.0001
Jun.	916.7 b	3596.1 a	267.6 b	1021.1 b	651.3 b	2497.6 a	< 0.0001	< 0.0001
Jul.	1384.9 b	4373.5 a	386.1 b	840.1 b	999.6 b	3566.1 a	< 0.0001	< 0.0001
Aug.	2302.5 b	5305.8 a	472.3 b	789.3 b	1840.5 b	4713.8 a	< 0.0001	< 0.0001
Sep.	2540.4 b	5382.6 a	450.9 b	759.5 b	2187.3 b	4922.3 a	< 0.0001	< 0.0001
Oct.	2497.5 b	5384.2 a	414.4 b	861.0 b	2135.9 b	4824.3 a	< 0.0001	< 0.0001
OPC	L^{***}, Q^{*}, C^{*}	L^{***}, Q^{***}	L^{***}, Q^{**}	L^{**}, C^{***}	L^{***}, C^{*}	L^{***}, Q^{***}, C^{**}		

†Means within a row followed by the same lowercase letter are not different between 2011 and 2012 for whole, leaf, or stem ($P > 0.05$) using PDIFF option in SAS.

‡ *, **, *** Orthogonal polynomial contrasts significant at the 0.05, 0.01, and 0.001 levels, respectively.

§Comparison between leaves and stems in 2011 and 2012.

Table 6.11 Harvest time × year interaction effect on regrowth switchgrass end-of-season whole plant, leaf, and stem ethanol yield and production during the 2011 and 2012 growing seasons at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

HT	Whole plant		Leaf		Stem		Leaf vs. Stem§	
	2011	2012	2011	2012	2011	2012	2011	2012
	Total ethanol yield from SSF (L Mg^{-1})						(*P* value)	
May	196.3 a†	166.3 b	163.0 a	139.3 b	179.0 a	155.6 b	< 0.0001	< 0.0001
Jun.	193.5 a	164.2 b	161.9 a	142.0 b	180.7 a	157.8 b	< 0.0001	< 0.0001
Jul.	199.4 a	175.2 b	165.4 a	147.8 b	179.2 a	161.9 b	< 0.0001	< 0.0001
Aug.	213.0 a	187.5 b	169.2 a	153.9 b	211.2 a	182.1 b	< 0.0001	< 0.0001
OPC‡	L^{***}, Q^{***}	L^{***}, Q^{***}	L^{***}	L^{***}	L^{***}, Q^{***}, C^{***}	L^{***}, Q^{***}		
	Total ethanol production per ha from SSF (L ha^{-1})							
May	449.9	961.2	89.8	100.2	310.5	486.1	< 0.0001	< 0.0001
Jun.	385.9	691.1	82.1	158.2	270.8	487	< 0.0001	< 0.0001
Jul.	322.4	405.7	80.8	119.1	202.2	248.8	< 0.0001	< 0.0001
Aug.	167.8	111.2	66.5	46.6	84.1	52.8	< 0.0001	< 0.0001
OPC		L^{***}		L^{***}	L^{***}	L^{***}, Q^{**}, C^{*}		
	Total theoretical ethanol yield from all biomass sugars (L Mg^{-1})							
May	425.6 a	416.9 b	377.1 a	363.5 b	449.0 a	439.2 b	< 0.0001	< 0.0001
Jun.	421.9 a	412.4 b	372.2 a	359.7 b	447.6 a	429.2 b	< 0.0001	< 0.0001
Jul.	417.0 a	401.3 b	365.8 a	348.7 b	447.4 a	422.5 b	< 0.0001	< 0.0001
Aug.	399.2 a	379.7 b	356.1 a	345.1 b	441.7 a	411.9 b	< 0.0001	< 0.0001
OPC	L^{***}, Q^{*}	Q^{**}	C^{**}	Q^{**}		L^{***}, C^{*}		
	Total theoretical ethanol production from all biomass sugars (L ha^{-1})							
May	984.4	2397	208.8	257.8	777.7	1346.4	< 0.0001	< 0.0001
Jun.	849.9	1734.4	188.9	402	674.4	1329.1	< 0.0001	< 0.0001
Jul.	675.4	939.2	178.8	281.1	506.1	653.3	< 0.0001	< 0.0001
Aug.	316.9	224.1	149.4	107.1	174.9	120.2	< 0.0001	< 0.0001
OPC		L^{***}		L^{***}	L^{***}	L^{***}, Q^{**}, C^{*}		

†Means within a row followed by the same lowercase letter are not different between 2011 and 2012 for whole, leaf, or stem ($P > 0.05$) using PDIFF option in SAS.

‡ *, **, *** Orthogonal polynomial contrasts significant at the 0.05, 0.01, and 0.001 levels, respectively.

§Comparison between leaves and stems in 2011 and 2012.

Table 6.12 Year × N rate interaction effect on primary harvests whole plant ethanol production and N main effect on whole plant ethanol yield and leaf and stem ethanol yield and production during the 2011 and 2012 growing seasons at the Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

N rate	TEY	TEP		TTEY	TTEP	
		2011	2012		2011	2012
	L Mg^{-1}	L ha^{-1}		L Mg^{-1}	L ha^{-1}	
			Whole plant			
0	216.8	808.6 b[†]	1903.1 a	418.6	1556.4 b	3796.2 a
40	214.3	876.2 b	2273.3 a	414.2	1671.2 b	4545.2 a
80	210.7	953.1 b	2372.0 a	411.8	1825.9 b	4840.0 a
120	208.9	881.2 b	2236.7 a	412.8	1670.4 b	4471.3 a
OPC[‡]	L^{*}	Q^{**}	L^{***}, Q^{***}	L^{**}, Q^{*}	L^{*}, Q^{***}	L^{***}, Q^{***}
			Leaf			
	TEY	TEP		TTEY	TTEP	
0	219.1	329.5		362.2	551.9	
40	217.0	364.3		360.9	620.9	
80	214.6	395.2		359.3	670.7	
120	213.6	345.1		356.5	583.4	
OPC	L^{**}	Q^{**}		L^{*}	L^{*}, Q^{***}	
			Stem			
0	212.6	1015.6		448.3	2163.6	
40	209.2	1179.2		441.7	2551.5	
80	207.2	1289.5		441.6	2756.6	
120	207.4	1203.6		440.9	2558.7	
OPC	Q^{*}	L^{***}, Q^{****}		Q^{**}	L^{***}, Q^{***}	

[†] Means within a row followed by the same lowercase letter are not different between 2011 and 2012 for whole, leaf, or stem ($P > 0.05$) using PDIFF option in SAS.

[‡] *, **, *** Orthogonal polynomial contrasts significant at the 0.05, 0.01, and 0.001 levels, respectively.

The TEP and TTEP reflected the trends in biomass yield response to N rate for both years in the total above-ground biomass (Chapter 4). Total ethanol yield was positively correlated with ethanol concentration but negatively correlated with ADF, NDF and cellulose concentrations in total aboveground biomass and leaf and stem components (Table 6.5, Table 6.6Table 6.7). The minimum ethanol yield of 152 L Mg^{-1} occurred at 42 g kg^{-1} ethanol concentration, while the maximum value of 242 L Mg^{-1} occurred at 116 g kg^{-1} ethanol concentration. Ethanol yield increased by 1.2 L Mg^{-1} per unit increase in ethanol concentration (Figure 6.4).

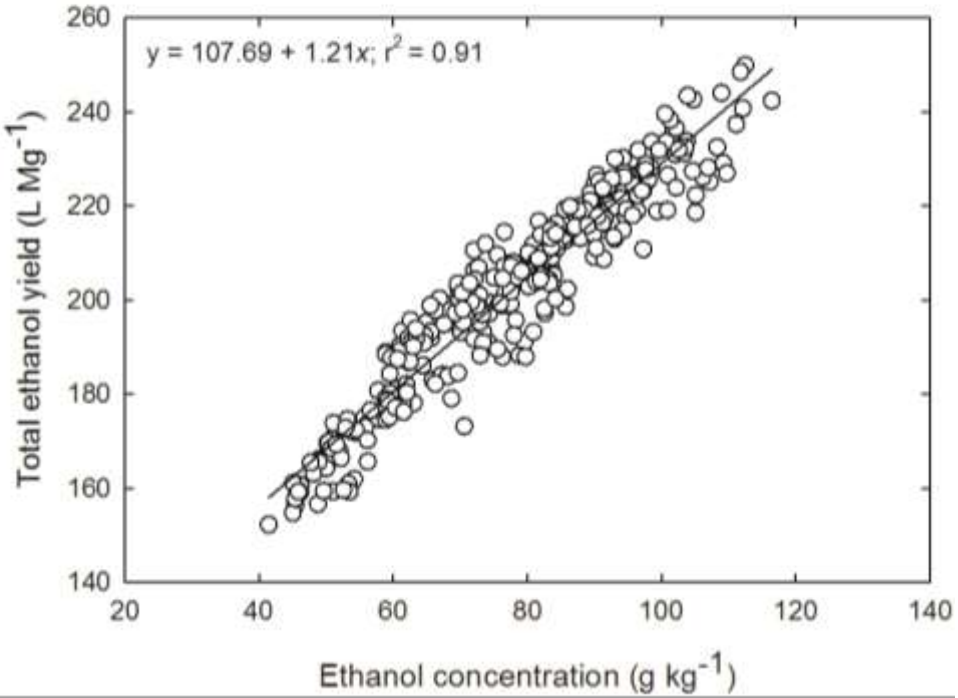

Figure 6.4 Relationship between switchgrass ethanol concentration and total ethanol yield.

Relationship between switchgrass ethanol concentration and total ethanol yield from simultaneous scarification and fermentation estimation of ethanol from May to October primary harvests during 2011 and 2012 growing seasons at Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Ethanol concentration declined linearly by 2.6, 3.12 and 2.7 g kg^{-1} per unit increase in ADF, NDF and cellulose concentrations, respectively (Figure 6.5). Furthermore, as fiber concentration increased, total ethanol yield decreased by 1.9, 2.2 and 2 L Mg^{-1} per unit increase in ADF, NDF and cellulose concentrations, respectively (Figure 6.5).

Anderson et al. (2010) reported that there was no correlation between glucose concentration and ethanol yields in bermudagrass [*Cynodon dactylon* (L.) Pers.] while Lorenz et al. (2009) found no relationship between glucan concentration and ethanol yield in corn stover. Glucose is the fermentation substrate for yeasts, therefore high levels of glucose should increase ethanol concentration, which is contrary to our findings and those of Anderson et al. (2010) and Lorenz et al. (2009). Simultaneous scarification and fermentation assay measures only the conversion of hexoses to ethanol, however, Anderson et al. (2010) posits that in the SSF assay, not all of cellulose is converted to glucose. These authors suggest that the glucose to ethanol conversion efficiency is less than 100% because of the presence of soluble sugars and fermentation inhibitors, therefore the glucose converted to ethanol may not be directly proportional to the cellulose content.

Total theoretical ethanol yield, which assumes 100% conversion of all biomass sugars, was positively correlated with hemicellulose as well as glucose, hexose, pentose and xylose concentrations (Table 6.5,Table 6.6 and Table 6.7). For each unit increase in glucose, hexose, pentose, and xylose concentrations, TTEY increased by 0.6, 0.9, 0.5, and 0.6 L Mg^{-1} respectively (Figure 6.6). Similar positive correlations between TTEY and glucose, hexose, pentose and xylose concentrations occurred in the leaf and stem components (Table 6.6 and Table 6.7).

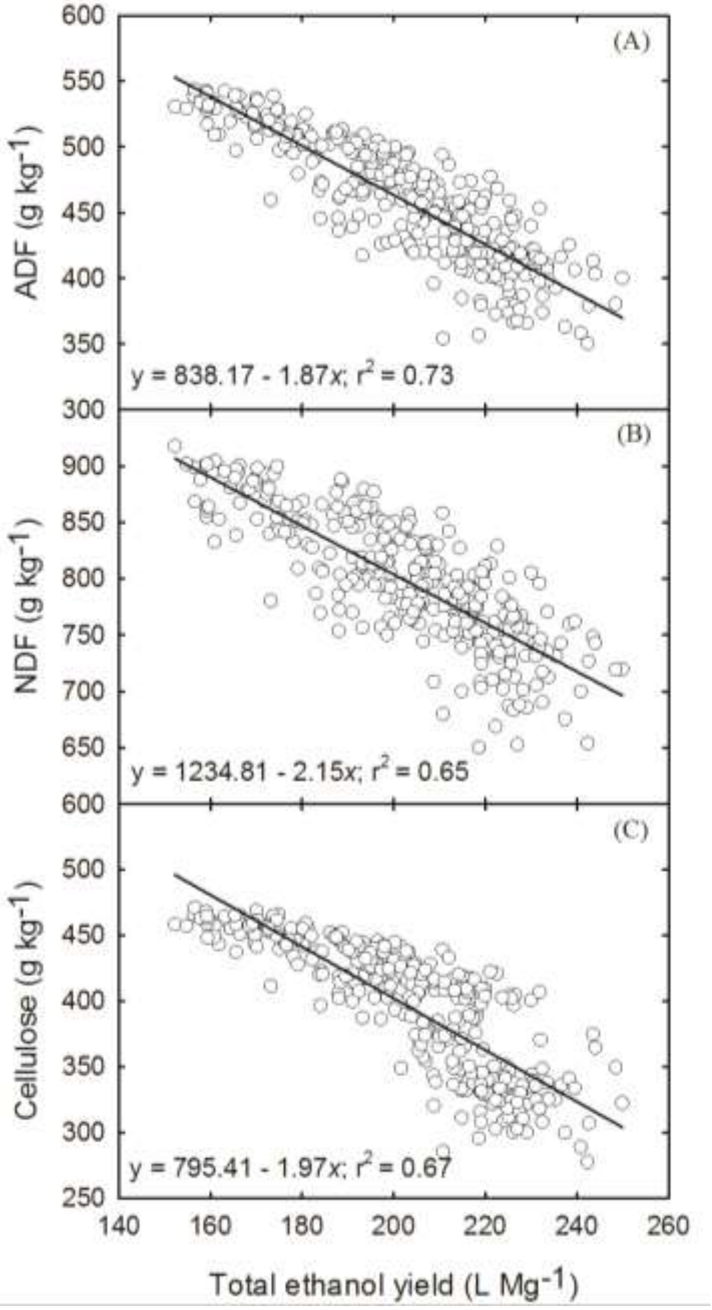

Figure 6.5 Relationships between switchgrass total ethanol yield and fiber concentrations.

Relationships between switchgrass total ethanol yield, from simultaneous scarification and fermentation estimation of ethanol, and (A) acid detergent fiber (ADF), (B) neutral detergent fiber (NDF) and (C) cellulose concentrations measured monthly from May to October during 2011 and 2012 growing seasons at Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Both TEP and TTEP were strongly positively correlated with yield in the total biomass and its components (Table 6.5,Table 6.6Table 6.7). There was no correlation with fiber or sugar concentrations, except in the stem component where TTEP was positively correlated with hexose concentration.

Both estimates of ethanol yield, TEY and TTEY, linearly increase with biomass yield (Figure 6.7). Switchgrass produced an estimated TEY and TTEY of 4 and 8.4 kL ha^{-1} at maximum biomass production (15.7 Mg ha^{-1}; Figure 6.7). Every incremental Mg ha^{-1} increase in biomass production, 0.2 and 0.41 kL ha^{-1} ethanol is produced under TEY and TTEY conversion scenarios respectively (Figure 6.7).

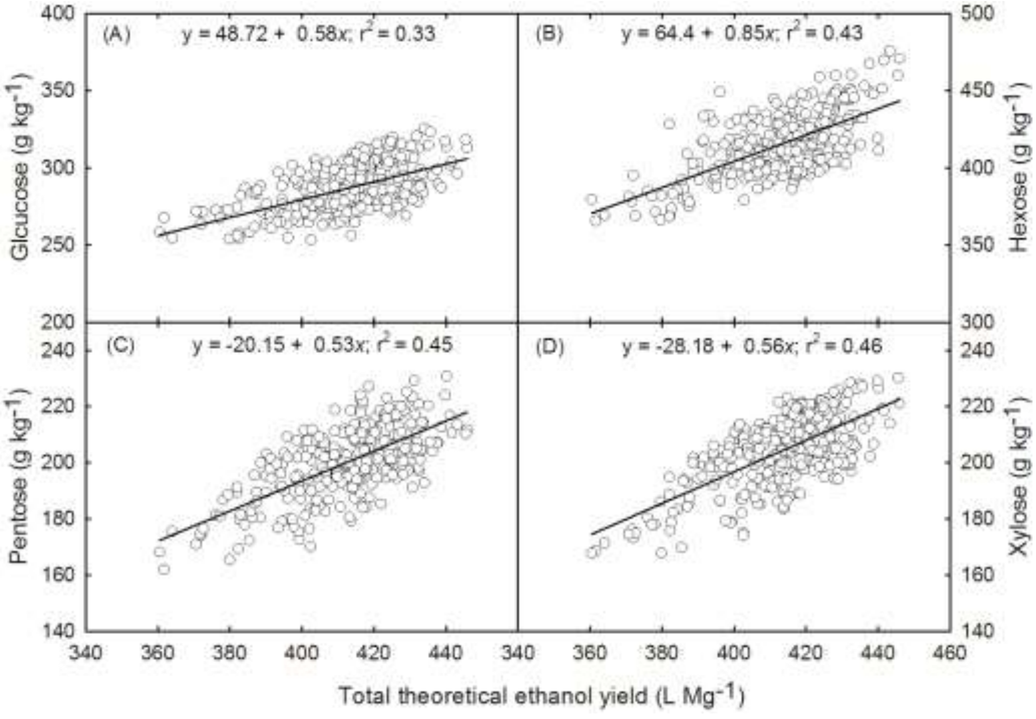

Figure 6.6 Relationships between switchgrass total theoretical ethanol production and sugar concentrations.

Relationships between switchgrass total theoretical ethanol production which assumes 100% conversion of biomass sugars and (A) glucose, (B) hexose, (C) pentose and (D) xylose concentrations from May to October primary harvests during 2011 and 2012 growing seasons at Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

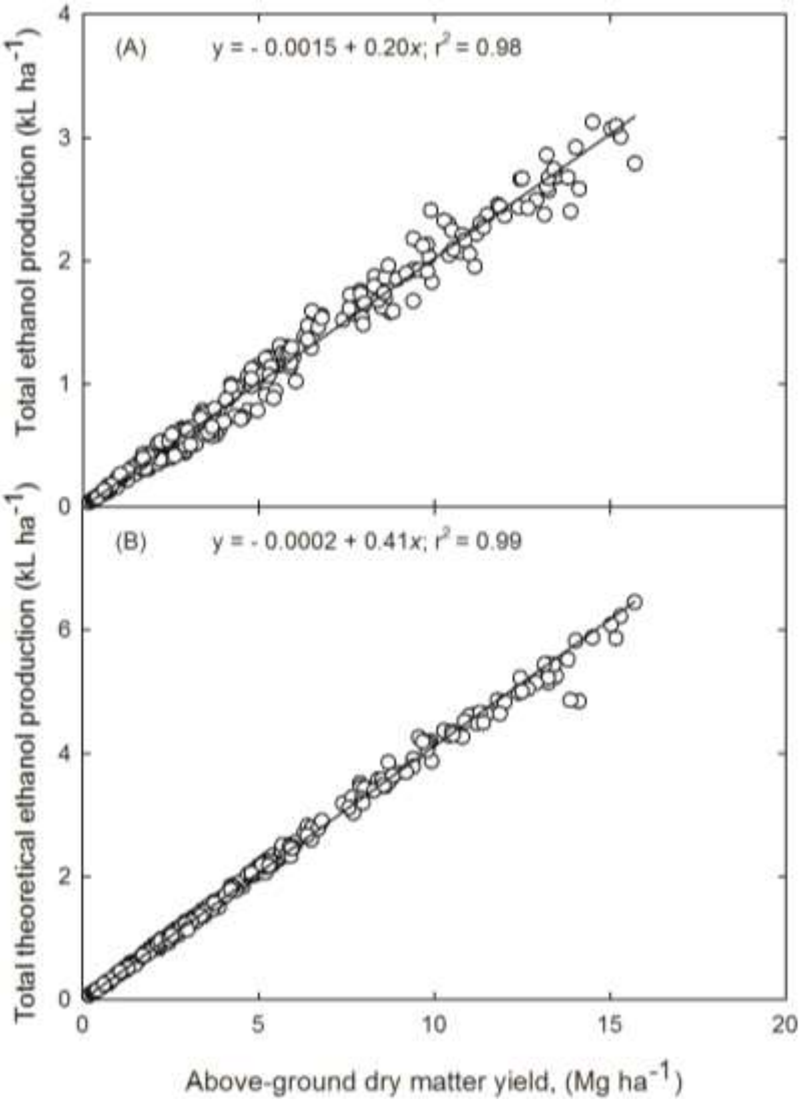

Figure 6.7 Relationships between switchgrass biomass production and ethanol production.

Relationships between switchgrass biomass production and (A) total ethanol production from simultaneous scarification and fermentation estimation of ethanol and (B) total theoretical ethanol production that assumes 100% conversion of biomass sugars from May to October primary harvests during 2011 and 2012 growing seasons at Brown Loam Branch Experiment Station, Raymond, Mississippi, USA.

Summary and Conclusions

Whole plant, leaf, and stem fiber concentrations increase with plant maturity with stem components accumulating greater fiber concentrations as switchgrass transitions from vegetative to reproductive and dormant stages. Crude protein and IVDMD decreased with increasing plant maturity and were greatest in the leaf component. Concentrations of major sugars increased with plant maturity. Greater sugar concentrations are associated with greater fiber concentrations in the mature stands. Total ethanol yield was greatest in the least mature stands, however, TEP increased with increasing length of the growth period and is strongly influenced by the biomass yield. Similar trends in TTEY and TTEP were observed, although these were twice as much as the ethanol yield estimated from SSF methods, indicating that there is potential for increasing ethanol recovery and efficiency with improved and novel pretreatment and fermentation methods. Nitrogen rate did not influence the forage nutritive value or feedstock composition in either the primary or end-of-season harvests. There was an N rate effect on ethanol production in the primary harvest with no residual effect in the end-of-season harvest. Our results suggest that switchgrass can provide early season forage in spring and early summer with regrowth harvested for feedstock in the late fall, however, a dual-use integrated system will depend on the economics of forage vs. feedstock use.

CHAPTER VII

GENERAL SUMMARY AND CONCLUSIONS

Switchgrass has been earmarked as a potential biofuel feedstock, however, agronomic and economic feasibility will be determined by its ability to persist as well as maintain high yields and feedstock quality under different management systems. This dissertation research aimed to determine the effect of cultivar selection, N application management and harvest frequency and timing on switchgrass yields, biofuel quality, and nutrient removal capacities. Three field experiments were conducted at the Brown Loam Branch Experiment Station during the 2008 to 2012 growing seasons to address these objectives. The first study evaluated the effects of four N application rates (0, 80, 160 and 240 kg ha^{-1}) and four genotypes (two commercial cultivars, 'Alamo' and 'Cave-in-Rock,' and two advanced breeding lines, NF/GA001 and NF/GA992) on biomass production, composition, nutrient removal capacity, and N use and recovery. The second study quantified the yield, nutrient removal, ethanol and composition responses of Alamo switchgrass to two N rates (80 and 160 kg N ha^{-1}) and four harvest frequencies (HF; 1, 2, 3 or 6 harvests annually). The third study quantified the effects of harvest time (monthly intervals from May through October) and N application rates (0, 40, 80 and 120 kg ha^{-1}) on the changes in nutrients and partitioning, nutrient removal capacities, N use and recovery, biomass yield, ethanol yield and composition.

In the first study, the advanced breeding lines NF/GA001 (9.3 Mg ha^{-1}), NF/GA992 (8.4 Mg ha^{-1}) had greater yields than Alamo (7.9 Mg ha^{-1}) and Cave-in-Rock (4.6 Mg ha^{-1}) in 2008. In 2009, differences among entries were the same but yield were greater: (NF/GA001, 13.8 Mg ha^{-1}; NF/GA992, 15.0 Mg ha^{-1}; Alamo, 12.6 Mg ha^{-1}; and Cave-in-Rock, 6.3 Mg ha^{-1}). In the second study, Alamo yield was 8.8 Mg ha^{-1} in 2008, 10.2 Mg ha^{-1} in 2009, 14.7 Mg ha^{-1} in 2010, and 14.7 Mg ha^{-1} in 2011. In the third study, Alamo yield averaged across N rate and harvest time treatments were 5.3 Mg ha^{-1} in 2011 and 12.1 Mg ha^{-1} in 2012. These results indicate high biomass production can be obtained at this central Mississippi location using Alamo or its breeding derivatives, NF/GA001 and NF/GA992.

Harvest frequency (HF) also has an effect on biomass production. In the 1-cut HF, yield increased from 8.8 Mg ha^{-1} in 2008 to 14.7 Mg ha^{-1} in 2010 and plateaued thereafter. Yield averaged 10 Mg ha^{-1} in the 2-cut HF across the 4 years of the study. In the 3- and 6-cut HF, yield increased from 2008 to 2009 but declined thereafter. Relative to the 1-cut HF, total yields decreased by 29, 36 and 50% as HF increased to 2, 3, and 6-cut HF, respectively, when averaged across all years and N rate. Therefore, a single annual harvest may produce the greatest biomass while multiple annual harvests will have implications for plant persistence.

The timing of harvesting will also influence the biomass production. In 2011, following an early season lag phase in growth, rapid growth occurred from June to July (accumulation of 2 to 3 Mg ha^{-1}) followed by a plateau and slight decline from September to October (5.5 to 5.3 Mg ha^{-1}). In 2012, rapid growth occurred from July to August (9.4 to 11.7 Mg ha^{-1}), which subsequently plateaued (12.1 Mg ha^{-1}). Leaves contributed 55 and 37% of the total biomass in May of 2011 and 2012 and declined across the growing season to 19 and 18% in October of 2011 and 2012. On the contrary, stems percent contribution to total yield increased with increasing maturity across both years from 45 (2011) and 63% (2012) in May to 81 and 82% in October 2011 and 2012, respectively. These results have implications for dual-use switchgrass production systems.

Biomass yield responded to the effects of N application rate. In the first study, averaged across genotype, yield increased by 29% (2008) and 69% (2009) at 80 kg N ha^{-1} compared to the control, but there was no further increase in yield with the additional N rates. In the second study, Alamo yield was unresponsive to N rate in 2008 (7.5 Mg ha^{-1}) and 2011 (9.0 Mg ha^{-1}) but increased in 2009 (8.0 to 9.5 Mg ha^{-1}) and decreased by 14% in 2010 (11 to 9.5 Mg ha^{-1}) as N rate increased from 80 to 160 kg N ha^{-1}. In the third study, there was no yield response to N rate Year 1 averaging 3.6 Mg ha^{-1}, however, in Year 2, yield increased quadratically from 8.2 to 9.9 Mg ha^{-1}. Yield increased by 22% with the addition of 40 kg N ha^{-1} but there were no further yield increases at additional N rates in Year 2.

In the first study, tissue N concentration did not vary with genotype or N rate but tissue P, K, Ca, and Mg concentrations were affected by a year × genotype interaction. In the second study, N application rate had an effect on the N concentration but not P, K, Ca, and Mg concentrations. Nitrogen fertilizer application will be required to sustain biomass production without increasing P, K, Ca and Mg concentrations, an attribute that may be highly desired if switchgrass is thermochemically converted or co-combusted. Nitrogen concentration increased with HF in all years and is related to the physiological maturity of the harvested material. Less frequent harvests had greater yields with lesser nutrient concentration, indicating there is greater potential for nutrient translocation with fewer harvests annually. Tissue nutrient concentration decline with plant maturity is related to the translocation of nutrients from leaves and stems to the crown, roots, and rhizosphere at the end of the growing and is associated with plant senescence and dormancy and the physiological changes related to plant maturity. In the third study, the rate of decline in nutrient concentration with increased stand maturity differed between the two years. In 2011, all elements declined by 33 to 60% while in 2012 the decline was 5 to 45% in primary harvests from May to October. Nitrogen concentration decreased by 15 to 33% in leaves and 59 to 81% in the stems. The amount of P and K decline was greater in leaves than stems while Ca and Mg decline responses were opposite. Post-frost harvests N concentration in both years was about nine times greater in leaves than stems. Harvesting switchgrass after frost-induced senescence produced biomass with the least nutrient concentration.

Nutrient removal is a function of yield and nutrient concentration. In our studies it was obvious that yield played the major role in nutrient removal. Removal rates of all elements increased linearly with biomass yield in all three experiments. In the first study, Alamo, NF/GA001, and NF/GA992 N removal was 112% greater than that of Cave-in-Rock. In the second study, N removal differed with N application rate while P, K, Ca, and Mg removal responded more to HF than N rate effects. Increased HF resulted in greater leaf:stem ratio and nutrient concentration leading to greater nutrient removal.

Nitrogen use generally followed the law of diminishing returns with increasing N application rate. Nitrogen use efficiency declined at N application rates above 80 kg ha^{-1} N for Alamo, NF/GA001 and NF/GA992. Nitrogen use efficiency among genotypes varied in response to N application rate with linear responses observed for Cave-in-Rock and NF/GA011 and quadratic responses for Alamo and NF/GA992. Averaged across genotypes, NUE declined by 39% from 80 to 160 kg N ha^{-1} and further declined by 23% from 160 to 240 kg N ha^{-1}. Alamo, NF/GA001 and NF/GA992 are more efficient in utilizing N and converting to biomass production than upland Cave-in-rock at this central Mississippi location. Apparent nitrogen recovery responses were similar to NUE, decreasing with increasing N rate. At 80 kg N ha^{-1}, Cave-in-Rock recovered 77.2 percentage points less N than the average recovery of the other three genotypes, which recovered approximately 99% of the applied N. Partial factor productivity (PFP), which measures the ratio of biomass yield per unit of applied N fertilizer, increased linearly from May to October primary harvests in both years. Averaged across harvest time, PFP decreased linearly with increasing N rates in both years. Partial factor productivity decreased between 39 and 47% with each 40 kg increase in N fertilizer applied in both years. In both years of the study, N recovery decreased linearly with increasing N rates. Nitrogen use indicators are biomass dependent, therefore N use and recovery will increase as biomass increases. Our data implies that applications of N above 40 kg ha^{-1} results in lower recovery rates and potential for N leaching.

Feedstock quality parameters acid detergent fiber, lignin, and hemicellulose differed among genotypes but there was no effect of N application rates. Cell wall properties may change in different years in response to precipitation and temperature with genotypes responding dissimilarly. Fiber concentration differed among harvest frequency and generally increased with physiological maturity resulting from the different harvesting frequencies. Since maximal concentrations of cellulose, hemicellulose and lignin are desirable for biofuel production, delaying harvests to senescence will increase the cell wall constituents and thus improve feedstock quality factors. Fiber concentration among biomass and its leaf and stem components increased rapidly from the early season to midsummer and the rate of change declined from midsummer to late fall.

Total ethanol yield (TEY) as determined by near infrared spectroscopy (NIRS) with equations developed from the simultaneous saccharification and fermentation (SSF) method in the first study was greatest for Alamo (165.8 L Mg^{-1}) and averaged 162 L Mg^{-1} for Cave-in-Rock, NF/GA001 and NF/GA992 . All lowland genotypes ethanol production was similar but differed from Cave-in-Rock in 2008 (1.7 vs. 0.85 kL ha^{1}) and 2009 (2.6 vs. 1.1 kL ha^{-1}) and this relationship was correlated with biomass yield. Across N application rates, TEY averaged 162 L Mg^{-1}. In the second study, total ethanol production (TEP) in the 1-cut HF system was similar in 2008 and 2009 (1.8 kL ha^{-1}) and increased by 50% in 2010 and further increased by 17% in 2011. In the multiple harvest systems, TEP was relatively consistent in at least 3 of the 4 yr. In the third study, ethanol concentration decreased from May to October primary harvests by 32 and 44% during 2011 and 2012, respectively. Primary harvest total ethanol production was 2.7 and 3.7 times greater in stem component than leaf in both years. If 100% of biomass sugars are converted to ethanol, the recovery will increase by a factor of 2 to 2.5 relative to total ethanol production from SSF. Since ethanol production is linearly related to yield, we can estimate theoretical ethanol production from SSF ethanol production. Therefore, there is tremendous potential in increasing the ethanol output from lignocellulosic biomass with the development of novel pretreatment and conversion technologies.

Adaptable and high yielding cultivars and economically and environmentally feasible N and harvest management will be necessary to optimize and maintain feedstock yield and stand persistence in switchgrass production systems. Generally, nutrient removal was a function of biomass yield that was, in turn, dependent on cultivar-inherent biomass production capacities and their response to N rather than tissue nutrient concentration. Less frequent annual harvests produced greater yields with lower nutrient concentration and therefore may require less agronomic inputs resulting from the greater potential for nutrient translocation. Fewer harvests per season will also increase fiber concentration and ethanol production while lowering nutrient removal.

Nitrogen use efficiency and ANR decreased with increased N application. Nitrogen application rates above 80 kg N ha^{-1} reduced NUE and ANR and are likely to result in increased N leaching and volatilization. Feedstock grown from Alamo and its derivatives, NF/GA001 and NF/GA992 had the greatest biomass production, NUE, ANR and ethanol yield at 80 kg N ha^{-1} under the experimental conditions in these field studies. The adoption of recommended management practices likely will depend upon the potential for minimizing nutrient removal from the system, desired feedstock quality, and the economics of forage vs. feedstock use particularly considering the cost incurred by additional harvests. Findings from this research will allow us to (1) develop a database on the effects of N application and harvest management on switchgrass biomass yield, chemical composition characteristics, and nutrient removal capacities (2) functional algorithms developed can be used to amend currently used predictive biomass models, (3) effectively manage inputs in a biomass production system, and (4) feasibly develop alternative fuels. With Mississippi investing in the bioenergy sector in the near future to meet the Renewable Fuel Standard mandate, this dissertation will provide critical information that can be used to make management decisions to optimize feedstock yield, quality and nutrient removal from production systems.

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