

Effects of Biosolids Application and Harvest Frequency on Switchgrass Yield, Feedstock
Quality, and Theoretical Ethanol Yield

Xiaojun Liu

Thesis submitted to the faculty of Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science
In
Crop and Soil Environmental Sciences

John H. Fike, Chair
John M. Galbraith, Co-Chair
Greg K. Evanylo
David J. Parrish
Brian D. Strahm

December 10, 2012
Blacksburg, VA

Keywords: switchgrass, biosolids, harvest frequency, yield, nitrogen, biofuel quality

Effects of Biosolids Application and Harvest Frequency on Switchgrass Yield, Feedstock Quality, and Theoretical Ethanol Yield

Xiaojun Liu

ABSTRACT

Switchgrass (*Panicum virgatum* L.) is a promising bioenergy crop for biofuel production. However, the effects of biosolids application on biomass yield, nitrogen (N) concentration, feedstock quality and theoretical ethanol yield (TEY) are rarely reported in the literature. The objectives of this research were: 1) to compare the effects of biosolids application on biomass yield, N concentration, feedstock quality and TEY, and 2) to compare the effects of harvest frequency on biomass yield, N concentration, feedstock quality and TEY. This experiment began in 2010 and tested four plant available N (PAN) rates of biosolids (0, 153, 306, 459 kg ha⁻¹), one urea rate (180 kg ha⁻¹), and two harvest frequencies (cut once in November or cut in July and November) on a Davidson soil at Orange, VA. Biosolids and urea applications increased biomass yield and TEY across years relative to control, but had no effects on measures of feedstock quality. Inconsistent biomass yield responses to harvest frequency were observed during three years. Cutting once per year consistently increased biomass lignin, cellulose, and hemicellulose concentrations, theoretical ethanol potential (TEP), and reduced N and ash concentrations compared to two cuts. Across years one cut increased TEY by 11% over the two cuts. The results demonstrate that biosolids can be applied as an N source to increase biomass yield and TEY. Two cuts increased biomass yield but reduced TEP, and had inconsistent effects on TEY.

ACKNOWLEDGEMENTS

I would like to thank my advisor Dr. John Fike and co-advisor Dr. John Galbraith, for their continuous guidance, support, and patience throughout my graduate studies. I sincerely appreciate all the time they have put in to help me craft and write research proposals, meeting abstracts, slides, and this thesis.

I would also like to thank my other committee members, Dr. Evanylo, Dr. Parrish, and Dr. Strahm for their thoughtful reviews and criticisms. Many other people helped make my thesis possible including Dr. Wonae Fike, Dr. Chao Shang, Dr. Xunzhong Zhang, and Steve Nagle for their vital assistance in the lab. Thanks also go to Dave Starner, Steve Gulick, Heather Taylor, Sami Owens, Chris Fields-Johnson, and Tianyu Lei for their help in the field.

I thank my mother Cuiqin Chang, father Zhangsheng Liu, and sister Xiaoli Liu, for lovingly supporting me all the time. Finally, I am greatly in debt to my family, especially my wife Xinhong Wu, who has been involved into the lab assistance during the first year in graduate school. Many thanks go to Xinhong as well for her consistent help in taking care of our beloved daughter Sophia while sacrificing her own time and career, and her continuous encouragement for me through the toughest of my graduate years.

TABLE OF CONTENTS

TITLE PAGE	i
ABSTRACT	ii
ACKNOWLEDGEMENTS	iii
TABLE OF CONTENTS	iv
LIST OF FIGURES	v
LIST OF TABLES	vi
LIST OF ABBREVIATIONS	vi
General Introduction	1
CHAPTER ONE. Literature Review	2
Characteristics and Potential of Switchgrass as a Biofuel Feedstock.....	2
Nitrogen Fertilization.....	4
Environmental Conditions	5
Harvest Frequency	6
Biosolids and Plant Growth	8
Biofuel and Conversion Systems.....	10
Reference	11
CHAPTER TWO. Effects of Biosolids Application and Harvest Frequency on Switchgrass Yield, Feedstock Quality and Theoretical Ethanol Yield	16
Abstract.....	16
Introduction.....	17
Materials and Methods.....	20
Results and Discussion	24
References.....	40
CHAPTER THREE. Conclusions.....	45

LIST OF FIGURES

Figure 1.1. Distribution of switchgrass in USA and Canada, reprinted from the USDA, NRCS. 2009 (http://luirig.altervista.org/schedenam/fnam.php?taxon=Panicum+virgatum).	3
Figure 2.1. Switchgrass yield in 2010, 2011, and 2012 as affected by the initial four biosolids rates of 0, 153, 306 and 459 kg N ha ⁻¹ and the urea rate of 180 kg N ha ⁻¹ . Data are averaged over two cutting frequencies in each year.	26
Figure 2.2. Switchgrass yield as affected by harvest frequencies. Data are averaged over all N treatments. 1X-Nov = one-cut system, November harvest; 2X-July and 2X-Nov = two-cut system, July and November harvests, respectively. Error bars for yields in the 2X system represent the standard errors for total season yields.	27
Figure 2.3. Switchgrass N concentrations in 2010, 2011, and 2012 as affected by the initial four biosolids rates of 0, 153, 306 and 459 kg N ha ⁻¹ and the urea rate of 180 kg N ha ⁻¹ . Data are averaged over two cutting frequencies in each year.	29
Figure 2.4. Switchgrass N concentrations as affected by harvest frequencies. Data are averaged over all N treatments. 1X-Nov = one-cut system, November harvest; 2X-July and 2X-Nov = two-cut system, July and November harvests, respectively. Error bars for yields in the 2X system represent the standard errors for total season yields.	30
Figure 2.5. Switchgrass lignin concentrations as affected by harvest frequencies. Data are averaged over all N treatments. 1X-Nov = one-cut system, November harvest; 2X-July and 2X-Nov = two-cut system, July and November harvests, respectively. Error bars for yields in the 2X system represent the standard errors for total season yields.	32
Figure 2.6. Switchgrass ash concentrations as affected by harvest frequencies. Data are averaged over all N treatments. 1X-Nov = one-cut system, November harvest; 2X-July and 2X-Nov = two-cut system, July and November harvests, respectively. Error bars for yields in the 2X system represent the standard errors for total season yields.	33
Figure 2.7. Switchgrass cellulose concentrations as affected by harvest frequencies. Data are averaged over all N treatments. 1X-Nov = one-cut system, November harvest; 2X-July and 2X-Nov = two-cut system, July and November harvests, respectively. Error bars for yields in the 2X system represent the standard errors for total season yields.	34
Figure 2.8. Switchgrass hemicellulose concentrations as affected by harvest frequencies. Data are averaged over all N treatments. 1X-Nov = one-cut system, November harvest; 2X-July and 2X-Nov = two-cut system, July and November harvests, respectively. Error bars for yields in the 2X system represent the standard errors for total season yields.	35

Figure 2.9. Switchgrass theoretical ethanol potential (TEP) as affected by harvest frequencies. Data are averaged over all N treatments. 1X-Nov = one-cut system, November harvest; 2X-July and 2X-Nov = two-cut system, July and November harvests, respectively. Error bars for yields in the 2X system represent the standard errors for total season yields. 36

Figure 2.10. Switchgrass theoretical ethanol potential yield (TEY) in 2010 (a), 2011 (b), and 2012 (c) as affected by the initial four biosolids rates of 0, 153, 306 and 459 kg N ha⁻¹ and urea rates of 180 kg N ha⁻¹. Data are averaged over two cutting frequencies in each year..... 37

Figure 2.11. Switchgrass theoretical ethanol yield (TEY) as affected by harvest frequencies. Data are averaged over all N treatments. 1X-Nov = one-cut system, November harvest; 2X-July and 2X-Nov = two-cut system, July and November harvests, respectively. Error bars for yields in the 2X system represent the standard errors for total season yields. 38

LIST OF TABLES

Table 2.1. Estimated plant available nitrogen (PAN) supplied by biosolids and urea. The estimated PAN was calculated based on Virginia Department of Conservation and Recreation equations for biosolids organic N mineralization and ammonia volatilization (VADCR, 2005). 21

Table 2.2. Calibration statistics for NIRS prediction of switchgrass feedstock quality for July harvests during 2010 and 2012at Orange, VA. 23

Table 2.3. Calibration statistics for NIRS prediction of switchgrass feedstock quality for November harvests during 2010 and 2012at Orange, VA. 23

Table 2.4. Monthly and 30-yr temperature and precipitation during 2010 and 2012at Orange, VA. 24

LIST OF ABBREVIATIONS

ADF	Acid Detergent Fiber	N	Nitrogen
ADL	Acid Detergent Lignin	NDF	Neutral Detergent Fiber
C	Carbon	PAN	Plant Available Nitrogen
EPA	U.S. Environmental Protection Agency	TEP	Theoretical Ethanol Potential
EISA	Energy Independence and Security Act	TEY	Theoretical Ethanol Yield
GHG	Greenhouse Gas	USDA	U.S. Department of Agriculture

General Introduction

Overall Hypothesis

Appropriate fertilization and harvest management practices are critical in boosting switchgrass biomass yield as well as improving feedstock quality, which in turn contribute to second-generation biofuel (non-food source) production. The experiments in this thesis test how biosolids application and harvest frequency affect biomass yield and certain feedstock qualities. The first hypothesis is that biosolids application will increase yield, N concentration, but have negative effects on feedstock quality and theoretical ethanol yield (TEY). In addition, a second hypothesis is that two cuts will increase yield, N concentration, and but have negative effects on feedstock quality and TEY.

Overall Objectives

- 1) To measure how different rates of biosolids affect yield, N concentration, feedstock quality, and TEY of switchgrass.
- 2) To determine if harvest frequencies affect yield, N concentration, feedstock quality, and TEY of switchgrass.

CHAPTER ONE. Literature Review

United States policy aims for production of 136 billion liters of biofuel per year by 2022 (EISA, 2007). According to the goal, 61 billion liters biofuel are required to be made from cellulosic feedstock and the production systems must meet greenhouse gas (GHG) reduction goals as determined by the US Environmental Protection Agency (EPA). Of these future cellulosic fuels, about half (30 billion liters) are expected to be made from switchgrass (*Panicum virgatum* L.) (USDA., 2010). To date, the EISA production goals for “cellulosic ethanol” have not been met and are limited by biomass-to-biofuel conversion technologies (biochemical and thermochemical processes).

Annual global ethanol production has reached 85.2 billion liters (GRFA, 2012). However, most of that biofuel has been produced from food crops such as corn. This has raised “food vs. fuel” concerns about the potential competition between land use for production of fuels, food, animal feed, and fiber (Somerville et al., 2010). To achieve US biofuel production goals, tremendous land and management resources will be needed. High productivity per land area will be important to minimize the landscape scale of the upcoming bioenergy industry. Further, producing biofuels on low productivity and marginal sites will be important to help attenuate land use competition between food and fuel.

Characteristics and Potential of Switchgrass as a Biofuel Feedstock

Switchgrass is a highly productive warm-season perennial grass native to North America (Figure 1.1). Originally researched as a forage crop, switchgrass has potential as a biomass crop for biofuels, fiber, electricity, and heat production (Parrish and Fike, 2005). Switchgrass’ characteristics of high productivity with limited inputs and tolerance to marginal environments make it an excellent choice for sustainable biomass production on marginal land (Parrish and Fike, 2005). Large amounts of biomass can be produced due to switchgrass’ perennial nature, extensive and deep root system. Compared to annual crops such as corn, switchgrass does not have to be replanted every year; it also requires fewer inputs and is productive on marginal lands,

resulting in more favorable net energy ratios (Ashworth, 2010). Switchgrass-based fuel systems also will reduce GHG emissions (Schmer et al., 2008), sequester CO₂ in the soil (Skinner and Adler, 2010), and improve soil properties (McLaughlin and Kszos, 2005). Because switchgrass does not directly compete with commodity food crops, its use as a feedstock for biofuel production will pose less of a threat to food security than using food crops to produce biofuel.

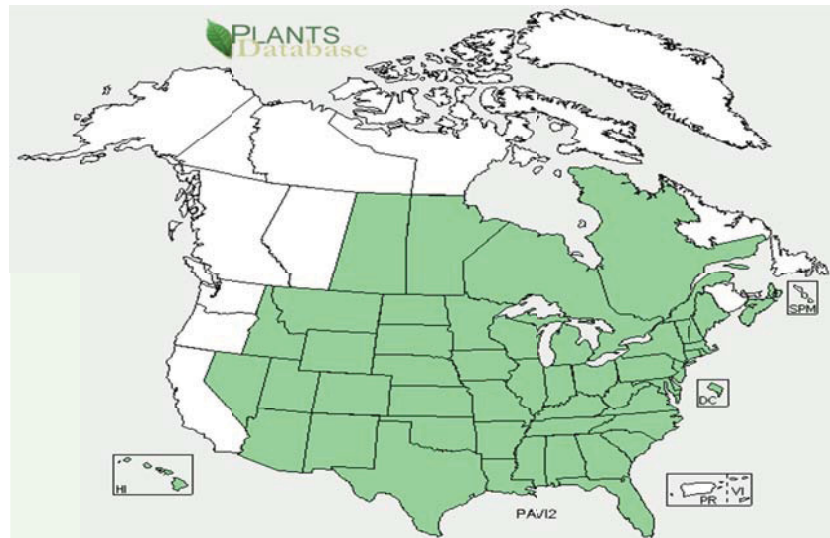


Figure 1.1. Distribution of switchgrass in USA and Canada, reprinted from the USDA, NRCS. 2009 (<http://luirig.altervista.org/schedenam/fnam.php?taxon=Panicum+virgatum>).

Growing switchgrass leads to several environmental benefits compared to growing grain crops, such as improve soil structure, water-holding capacity, nutrient availability, decrease soil erosion and CO₂ emissions (McLaughlin and Walsh, 1998). Low levels of N in switchgrass result in low fluxes of N₂O, which pose negative impacts on the environment and human health (Bransby et al., 2008). Switchgrass in a riparian buffer in Iowa has been reported to sequester C, improve soil quality, and reduce runoff, erosion, and associated N and P loss compared to bare soil surface (Simpson et al., 2008). Leaching losses of N were higher for corn cropping system than for switchgrass system (Anex et al., 2007). Switchgrass holds great promise for production on marginal land sites where water availability and soil fertility will limit yield of annual crops (Smith, 2009). However, there are a few limitations of growing switchgrass as a cellulosic

biofuel feedstock, including establishment difficulty, drought susceptibility (Parrish and Fike, 2005), low biomass-to-biofuel conversion efficiencies (Carroll and Somerville, 2009), logistical constraints in developing large-scale bioenergy systems (Cundiff et al., 2009).

Nitrogen Fertilization

Plants are surrounded by N in the atmosphere, but it is not directly available to the plants since they are inert N molecules. Nitrogen typically exists in soil in two forms – organic N and two inorganic N forms (NH_4^+ and NO_3^-). Inorganic N can be directly taken up by plants, while organic N requires mineralization for transformation to inorganic N forms. The major fertilizer nutrient required by switchgrass is N, which is affected by harvest timing and frequency (McLaughlin and Kszos, 2005; Vogel et al., 2002). Optimizing N fertilization rate is an essential aspect of developing switchgrass as a biofuel source because of the use of fossil energy in N fertilizer synthesis and the release of GHG during N use (Sinclair, 2009).

Literature reports of switchgrass yield responses to N vary substantially, owing to different soil textures, climate conditions, stand ages, harvest systems, and cultivars. Response of lowland cultivar appeared to plateau below 90 kg N ha^{-1} , while yields of upland ecotypes responded to N application up to 100 kg ha^{-1} and may then decrease above those rates (Gunderson et al., 2008). Vogel et al. (2002) tested switchgrass responses to N rate and reported a 28% increase in yield as N application increased from 60 to 120 kg ha^{-1} (Vogel et al., 2002). Nitrogen was used most efficiently by the switchgrass at levels between 56 and 112 kg N ha^{-1} (Lemus et al., 2008b). Switchgrass N fertilization (34 , 67 , 134 , and $269 \text{ kg ha}^{-1} \text{ yr}^{-1}$) produced 8.7 , 12.0 , 12.1 , and 12.3 Mg ha^{-1} , respectively, displaying a plateau in N response at 67 kg ha^{-1} in Oklahoma (Haque et al., 2009). Yield response to N was linear or quadratic, and optimum yield after 2 years was 13.5 Mg ha^{-1} at 140 kg N ha^{-1} for switchgrass, which maximized root biomass and favored allocation of nutrients to roots over shoots, but at 220 kg N ha^{-1} the plants shifted allocation of nutrients to shoots over roots (Conen and Smith, 1998). The N concentration of leaves was 13.5 g N kg^{-1} compared to 5.69 g N kg^{-1} for stems through the growing season (Shahandeh et al., 2011).

A wide range of biomass yields has been reported for switchgrass across diverse regions and varieties, but most of the N has come from chemical fertilizers. Lee and Boe (2005) reported maximum annual biomass yield ranged from 2 Mg ha⁻¹ to 9 Mg ha⁻¹ in South Dakota, depending on cultivar with no fertilizer applied. In contrast, single-season yields have reached 26.0 Mg ha⁻¹ in Texas (Sanderson et al., 1996), 34.6 Mg ha⁻¹ in Alabama (McLaughlin and Kszos, 2005), and 36.7 Mg ha⁻¹ in Oklahoma (Thomason et al., 2004) with chemical fertilizers. Switchgrass yield in Virginia ranged from 10 to 22 Mg ha⁻¹ across multiple locations and years (Lemus, 2004). In Iowa, the average switchgrass yield ranged from 6.4 to 11.8 Mg ha⁻¹ (Lemus et al., 2002) with urea applied. In Europe, lowland switchgrass (cv. 'Kanlow') yielded 17.1 Mg ha⁻¹ in Greece and 20 Mg ha⁻¹ in Italy (Alexopoulou et al., 2008). However, few studies have evaluated switchgrass performance, such as biomass yield and feedstock quality on marginal land amended with biosolids.

Nitrogen fertilization could affect switchgrass feedstock quality. Waramit et al. (2011) reported biomass N, cellulose, and lignin concentrations increased 25%, 3%, and 15% at 140 kg N ha⁻¹ in Iowa. However, high concentrations of N or ash reduce the effectiveness and chemical output of thermochemical conversion systems (Vogel et al., 2011). Another study in Iowa showed switchgrass cellulose, lignin, and N concentrations increased while hemicellulose and ash declined as N rates increased (Lemus et al., 2008a). Allison et al. (2012) illustrated that cellulose and lignin concentrations increased 8% and 5% at 100 kg N ha⁻¹ in England. Although these changes in feedstock quality are desirable, the actual significance of these results for overall feedstock quality was inconsistent among years. Understanding the interaction of N fertilization and different harvest frequencies on feedstock quality of switchgrass in Virginia is limited at present.

Environmental Conditions

Precipitation and temperature are important factors affecting switchgrass production. Several studies reported that temperature and precipitation had even more influence than did N

application and stand age (Gunderson et al., 2008; Wullschleger et al., 2010). In North Dakota, low rainfalls during growing season severely limited switchgrass yield to about 3 Mg ha⁻¹ compared with 12.5 Mg ha⁻¹ in a year with abundant precipitation (Berdahl et al., 2005). In areas with sufficient rainfall, sustainable yields of 15 Mg ha⁻¹ yr⁻¹ may be achievable (Parrish and Fike, 2005). Guretzky et al. (2011) reported that harvesting switchgrass twice per year resulted in greater biomass yield when late-summer precipitation was available to support regrowth in Oklahoma. Yield increased with increasing temperature and precipitation up to a point, and then decreased (Gunderson et al., 2008). However, clear links between precipitation and biomass yield have sometimes proved elusive (Fike et al., 2006a). A southern location of adaptation for switchgrass (cv. Alamo) in Texas was not well correlated with precipitation or soil types (Muir et al., 2001).

Harvest Frequency

Harvest management is an important, but not necessarily intuitive, factor affecting switchgrass yield. The upland cultivars produced more yield with two harvests than one, but two cuts per year may have less advantage for biomass yield of lowland cultivars in the southeastern USA (Fike et al., 2006b). The yields of one cut lowland and two cuts upland cultivars ranged from 12 to 19 Mg ha⁻¹ yr⁻¹ and 13.5 to 18.6 Mg ha⁻¹ yr⁻¹, respectively (McLaughlin and Kszos, 2005). The average yields of switchgrass (cv. 'Kanlow') were 12.3 (one cut) and 14.9 (two cuts) Mg ha⁻¹ yr⁻¹ in Virginia (Parrish and Fike, 2005). In Canada, the respective one cut yields for Cave-in-Rock, Pathfinder, and Sunburst were 12.2, 11.5, and 10.6 Mg ha⁻¹ (Madakadze et al., 1999). Biomass yields increased by 3.3, 3.8, and 8.8 Mg ha⁻¹ with 225 kg N ha⁻¹ when harvested after October, December and twice per year, respectively (Guretzky et al., 2011). However, multiple harvests of switchgrass may reduce total seasonal yields in some instances (Fike et al., 2006b; Sanderson et al., 1996). Furthermore, two cuts may place greater stress on the biofuels crop, particularly in dry years, and greater nutrient

concentrations in early-season biomass have negative consequences to biomass yield and feedstock quality in some conversion systems (Wright and Turhollow, 2010).

Harvest frequency greatly influences the feedstock quality. A single switchgrass harvest in fall produces a high-quality biofuel (Lemus et al., 2008b). Nitrogen concentrations in switchgrass were lower in late autumn compared to summer harvests, indicating that N_2O released during conversion processes would be lower from biomass harvested later in the season (Anex et al., 2007). Switchgrass yield decreased when harvest was delayed from fall to spring, while deferring harvest reduced ash, nutrient and water concentration, thus increasing the gross energy density of the biomass for conversion systems (Adler et al., 2006; Ashworth, 2010).

Harvest frequency has important interactions with biomass N concentration. Nitrogen concentration in the fall harvest was lower for the one cut (when it is taken at the end of the season) than the two cuts (Reynolds et al., 2000). Two harvests per year removed about $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in switchgrass biomass, even in plots with no N applied (Lemus et al., 2008b). Switchgrass loses very little biomass when harvested late in the fall, though the N removed from switchgrass is substantially lower when harvest is delayed until December (Carroll and Somerville, 2009).

Other factors may also affect biomass yield. The yield increased as date of first harvest was delayed throughout June, while the yield of biomass of regrowth consequently declined (Trocsanyi et al., 2009). Delayed harvest dates showed a trade-off with biomass yield, owing to leaf loss and stem lodging (Adler et al., 2006). Significant losses (35% to 45% of potential harvestable biomass) can occur during the harvest (Alexopoulou et al., 2008). Biomass not picked-up by the baler machine was up to 17%, while the uncut biomass due to the mower swinging averaged 29%; and it was also significantly affected by the field slope (Monti et al., 2009).

Biosolids and Plant Growth

Biosolids are nutrient-rich organic materials derived from domestic sewage in wastewater treatment facilities. When treated and processed, these organic materials can be applied to fields as alternative fertilizer to improve soil fertility and fuel plant growth (EPA, 1994). There are two types of biosolids with respect to pathogen concentrations: Class A biosolids are essentially free of pathogens prior to land application; Class B biosolids may have low levels of pathogens, which rapidly die off after applied to soils within a short period typically (EPA, 1994). Class A biosolids undergo heating, composting, digestion and pasteurization to reduce pathogens below detectable levels. Class B biosolids typically undergo digestion and sometimes lime stabilization. In addition, there are different rules for application of different classes of biosolids: Class A biosolids used in small quantities by general public have no buffer requirements, crop type, crop harvesting or site access restrictions, as long as such biosolids also meet restrictive pollutant limits. There are buffer requirements, public access, and crop harvesting restrictions for Class B biosolids, which must also meet pollutant standards.

There are potential benefits to apply biosolids as N and P fertilizers for bioenergy crops (Castillo et al., 2010). Total N and P in biosolids range from 35 to 68 g kg⁻¹ and 18 to 39 g kg⁻¹, respectively. About 80% of the N in the biosolids occurs in organic forms, whereas 78% of P is found in inorganic forms (García, 2009). Plant uptake of N from the soil occurs mainly in the forms of ammonium (NH₄⁺) and nitrate (NO₃⁻), and P is taken primary as orthophosphate (H₂PO₄⁻) and secondary orthophosphate (HPO₄⁻²). Because the initial forms of N are mainly organic in the biosolids, the main factors controlling availability of N to a crop are mineralization and immobilization.

The N mineralization process is controlled by the activities of soil microorganisms under aerobic and anaerobic conditions that use the N in their metabolism (Painter, 1970). Most soil microorganisms can increase N mineralization at optimum ranges of temperature (25 to 37°C) and moisture (50 to 75% of soil water holding capacity) (Jarvis et al., 1996). Other soil properties such as pH, salinity, and heavy metal concentration of the material may also affect

microbial activity and N mineralization. Once organic N has been mineralized, NH_4^+ can be taken up by plants, or nitrified to NO_3^- , immobilized by soil microorganisms, lost as a gas by NH_3 volatilization, held as an exchangeable ion by clays or other soils colloids, or fixed in the interlayers of certain clay minerals.

Estimation of N mineralization in biosolids is difficult due to quality differences in the initial material as well as the technique used to measure mineralization. The break-even point between net N mineralization and N immobilization can be found between C:N ratios of 20 to 40 (Whitmore, 1996). Several factors affect yearly N mineralization rates of biosolids, including initial concentration of organic N, season of application, length of treatment or stabilization method, and amount of lime (Cowley et al., 1999). When biosolids were applied in summer, organic N mineralization leveled off approximately 50 days after application compared with 150 to 250 days following spring application (Castillo et al., 2010). Accumulative amount of N mineralization of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ in biosolids, soil, and the difference in the organic N concentration of biosolids before and after field incubation were 49, 135, and 484 g kg^{-1} (He et al., 2000). In another study, application rate and C:N ratio as well as air temperature accounted for up to 90% of biosolids mineralization rate, but there was inadequate proof to conclude that soil organic N content and time contribute to N mineralization variability (Er et al., 2004).

Anaerobically-digested biosolids applied to winter wheat did not increase soil pH but increased levels of total C, total N, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, and electrical conductivity when application rates were greater than 180 kg N ha^{-1} (Schroder et al., 2008). Mean observed plant available N (PAN) released from biosolids applied in agronomic crop field during the growing season was 37% of the biosolids' total N (Gilmour et al., 2003). Biosolids PAN was related to the biosolids' C:N ratio, organic N, and total N (Gilmour and Skinner, 1999). Several laboratory techniques to determine N mineralization rates have been reported in the literature (Hanselman et al., 2004). However, there is uncertainty regarding the extrapolation of laboratory N mineralization data to field conditions due to dynamic and site-specific factors like drying and rewetting events and temperature changes (Gilmour et al., 2003; Hanselman et al., 2004). The

uncertainty of previous techniques has led to the measurement of N mineralization rates and PAN in actual production fields with in situ incubation devices (Hanselman et al., 2004). Information regarding the biosolids application to switchgrass is lacking. Most literature reported the biosolids used for reclamation purpose in abandoned mine sites or a few for main crops, but no one has ever investigated using biosolids as an alternative fertilizer source in biomass production system for biofuel production.

Biofuel and Conversion Systems

Biofuels include a wide range of fuels derived from biomass. They can be used as solid biomass (for combustion), liquid fuels (especially ethanol), and various biogases (Demirbas, 2009). With advanced technology being developed, cellulosic biomass is also used as feedstock for second-generation ethanol production. Ethanol is the most widely used biofuel for transportation worldwide so far, but it is made very heavily from food source (first-generation) fermentation of sugars from maize and sugar cane (Balat, 2008). Cellulosic ethanol is produced by either of two primary pathways: biochemical or thermochemical. Thermochemical conversion technologies are more flexible with respect to feedstock type because all C in the biomass can be converted to gaseous or oil forms (Bransby et al., 2008). As cellulosic ethanol provides health benefits from fine particulate matter reduction and reduces GHG emissions, a transition from gasoline to cellulosic ethanol has greater advantages than previously recognized (Hill et al., 2009).

Switchgrass biomass is called a cellulosic (or lignocellulosic) feedstock because the entire plant (which is largely lignin, cellulose and hemicellulose) is the feedstock. Switchgrass is generally composed of 3 to 6% ash, 30 to 34% cellulose, 24 to 27% hemicellulose, 16 to 19% Klason lignin (Bransby et al., 2008). Perennial warm-season grasses harvested after killing frost produced the greatest yields with high concentrations of lignocellulose and lower concentrations of N and ash (Mulkey et al., 2008). Lignin content was increased with increasing N fertilization, and cellulose increased with harvests made after maturity, while hemicellulose was affected by

harvest timing (Withers, 2010). Mean dry matter, fiber, and lignin concentrations increased with later harvest, hence the optimal harvest date was in late summer or early fall if aimed at bioenergy feedstock production (Casler and Boe, 2003). An advanced biochemical conversion system might be expected to yield 355 L ethanol Mg⁻¹ of dry switchgrass (Morrow et al., 2006). Expected yields for biochemical and thermochemical processes range from 250 to 333 L Mg⁻¹ up to 417 L Mg⁻¹ of biomass (Bransby et al., 2008; Varvel et al., 2008). In the mid-western US, hypothetical switchgrass ethanol yields were greater than 3500 L ha⁻¹ (Schmer et al., 2008).

Reference

- Adler P.R., Sanderson M.A., Boateng A.A., Weimer P.J., Jung H.-J.G. (2006) Biomass yield and biofuel quality of switchgrass harvested in fall or spring. *Agronomy Journal* 98:1518-1525.
- Alexopoulou E., Sharma N., Papatheohari Y., Christou M., Piscioneri I., Panoutsou C., Pignatelli V. (2008) Biomass yields for upland and lowland switchgrass varieties grown in the Mediterranean region. *Biomass and Bioenergy* 32:926-933.
- Allison G.G., Morris C., Lister S.J., Barraclough T., Yates N., Shield I., Donnison I.S. (2012) Effect of nitrogen fertiliser application on cell wall composition in switchgrass and reed canary grass. *Biomass and Bioenergy* 40:19-26.
- Anex R.P., Lynd L.R., Laser M.S., Heggenstaller A.H., Liebman M. (2007) Potential for enhanced nutrient cycling through coupling of agricultural and bioenergy systems. *Crop Science* 47:1327-1335.
- Ashworth A.J. (2010) Biomass production and nutrient removal of switchgrass as a bioenergy feedstock, *Crop, Soil, and Environmental Science*, University of Arkansas. pp. 82.
- Balat M. (2008) Global trends on the processing of bio-fuels. *International Journal of Green Energy* 5:212-238.
- Berdahl J.D., Frank A.B., Krupinsky J.M., Carr P.M., Hanson J.D., Johnson H.A. (2005) Biomass yield, phenology, and survival of diverse switchgrass cultivars and experimental strains in western North Dakota. *Agronomy Journal* 97:549-555.
- Bransby D.I., Samson R., Parrish D.J., Fike J.H. (2008) Harvest and conversion systems for producing energy from switchgrass: Logistic and economic considerations. *Forage and Grazinglands*.
- Carroll A., Somerville C. (2009) Cellulosic biofuels. *Annual Review of Plant Biology* 60:165-182.

- Casler M.D., Boe A.R. (2003) Cultivar-environment interactions in switchgrass. *Crop Science* 43:2226-2233.
- Castillo M.S., Sollenberger L.E., Vendramini J.M.B., Woodard K.R., O'Connor G.A., Newman Y.C., Silveira M.L., Sartain J.B. (2010) Municipal biosolids as an alternative nutrient source for bioenergy crops: II. Decomposition and organic nitrogen mineralization. *Agronomy Journal* 102:1314-1320.
- Conen F., Smith K.A. (1998) A re-examination of closed flux chamber methods for the measurement of trace gas emissions from soils to the atmosphere. *European Journal of Soil Science* 49:701-707.
- Cowley N., Thompson D., Henry C. (1999) Nitrogen mineralization study: Biosolids, manures, composts. Appendix D-Nitrogen Mineralization Study:1-7.
- Cundiff J.S., Fike J.H., Parrish D.J., Alwang J. (2009) Logistic constraints in developing dedicated large-scale bioenergy systems in the southeastern United States. *Journal of Environmental Engineering* 135:1086-1096.
- Demirbas A. (2009) Political, economic and environmental impacts of biofuels: A review. *Applied Energy* 86:108-117.
- EISA. (2007) Energy independence and security act of 2007. pp. 110-140.
- EPA. (1994) Land application of sewage sludge, in: EPA (Ed.). pp. 1-62.
- Er F., Ogut M., Mikayilov F.D., Mermut A.R. (2004) Important factors affecting biosolid nitrogen mineralization in soils. *Communications in Soil Science and Plant Analysis* 35:2327-2343.
- Fike J.H., Parrish D.J., Wolf D.D., Balasko J.A., Green J.T., Rasnake M., Reynolds J.H. (2006a) Long-term yield potential of switchgrass-for-biofuel systems. *Biomass and Bioenergy* 30:198-206.
- Fike J.H., Parrish D.J., Wolf D.D., Balasko J.A., Green J.T., Rasnake M., Reynolds J.H. (2006b) Switchgrass production for the upper southeastern USA: Influence of cultivar and cutting frequency on biomass yields. *Biomass and Bioenergy* 30:207-213.
- García M.S.C. (2009) Nutrient management of elephantgrass biomass production, University of Florida. pp. 109.
- Gilmour J.T., Cogger C.G., Jacobs L.W., Evanylo G.K., Sullivan D.M. (2003) Decomposition and plant-available nitrogen in biosolids: laboratory studies, field studies, and computer simulation. *Journal of Environmental Quality* 32:1498-1507.
- Gilmour J.T., Skinner V. (1999) Predicting plant available nitrogen in land-applied biosolids. *Journal of Environmental Quality* 28:1122-1126.
- GRFA. (2012) Global ethanol production to reach 85.2 billion litres in 2012.

- Gunderson C.A., Davis E., Jager Y., West T.O., Perlack R.D., Brandt C.C., Wullschlegel S.D., Baskaran L.M., Webb E., Downing M. (2008) Exploring potential US switchgrass production for lignocellulosic ethanol, in: ORNL. (Ed.).
- Guretzky J.A., Biermacher J.T., Cook B.J., Kering M.K., Mosali J. (2011) Switchgrass for forage and bioenergy: harvest and nitrogen rate effects on biomass yields and nutrient composition. *Plant and Soil* 339:69-81.
- Hanselman T.A., Graetz D.A., Obreza T.A. (2004) A comparison of in situ methods for measuring net nitrogen mineralization rates of organic soil amendments. *Journal of Environmental Quality* 33:1098-1105.
- Haque M., Epplin F.M., Taliaferro C.M. (2009) Nitrogen and harvest frequency effect on yield and cost for four perennial grasses. *Agronomy Journal* 101:1463-1469.
- He Z.L., Alva A.K., Yan P., Li Y.C., Calvert D.V., Stofella P.J., Banks D.J. (2000) Nitrogen mineralization and transformation from composts and biosolids during field incubation in a sandy soil. *Soil Science* 165:161-169.
- Hill J., Polasky S., Nelson E., Tilman D., Huo H., Ludwig L., Neumann J., Zheng H., Bonta a.D. (2009) Climate change and health costs of air emissions from biofuels and gasoline. *Proceedings of the National Academy of Sciences* 106:2077-2082.
- Jarvis S.C., Stockdale E.A., Shepherd M.A., Powlson D.S. (1996) Nitrogen mineralization in temperate agricultural soils: Processes and measurement. *Advances in Agronomy* 57:187-235.
- Lee D.K., Boe A. (2005) Biomass production of switchgrass in Central South Dakota. *Crop Science* 45:2583-2590.
- Lemus R., Brummer E.C., Burras C.L., Moore K.J., Barker M.F., Molstad N.E. (2008a) Effects of nitrogen fertilization on biomass yield and quality in large fields of established switchgrass in southern Iowa, USA. *Biomass and Bioenergy* 32:1187-1194.
- Lemus R., Brummer E.C., Moore K.J., Molstad N.E., Burras C.L., Barker M.F. (2002) Biomass yield and quality of 20 switchgrass populations in southern Iowa, USA. *Biomass and Bioenergy* 23:433-442.
- Lemus R., Parrish D.J., Abaye O. (2008b) Nitrogen-use dynamics in switchgrass grown for biomass. *Bioenergy Research* 1:153-162.
- Lemus R.W. (2004) Switchgrass as an energy crop: fertilization, cultivar, and cutting management, *Crop and Soil Environmental Sciences.*, Virginia Polytechnic Institute and State University, Blacksburg, VA. pp. 195.
- Madakadze I.C., Stewart K.A., Peterson P.R., Coulman B.E., Smith D.L. (1999) Cutting frequency and nitrogen fertilization effects on yield and nitrogen concentration of switchgrass in a short season area. *Crop Science* 39:552-557.

- McLaughlin S.B., Kszos L.A. (2005) Development of switchgrass (*Panicum virgatum*) as a bioenergy feedstock in the United States. *Biomass and Bioenergy* 28:515-535.
- McLaughlin S.B., Walsh M.E. (1998) Evaluating environmental consequences of producing herbaceous crops for bioenergy. *Biomass and Bioenergy* 14:317-324.
- Monti A., Fazio S., Venturi G. (2009) The discrepancy between plot and field yields: Harvest and storage losses of switchgrass. *Biomass and Bioenergy* 33:841-847.
- Morrow W.R., Griffin W.M., Matthews H.S. (2006) Modeling switchgrass derived cellulosic ethanol distribution in the United States. *Environmental Science & Technology* 40:2877-2886.
- Muir J.P., Sanderson M.A., Ocumpaugh W.R., Jones R.M., Reed R.L. (2001) Biomass production of 'Alamo' switchgrass in response to nitrogen, phosphorus, and row spacing. *Agronomy Journal* 93:896-901.
- Mulkey V.R., Owens V.N., Lee D.K. (2008) Management of warm-season grass mixtures for biomass production in South Dakota USA. *Bioresource Technology* 99:609-617
- Painter H.A. (1970) A review of literature on inorganic nitrogen metabolism in microorganisms. *Water Research* 4:393-450.
- Parrish D.J., Fike J.H. (2005) The biology and agronomy of switchgrass for biofuels. *Critical Reviews in Plant Sciences* 24:423-459.
- Reynolds J.H., Walker C.L., Kirchner M.J. (2000) Nitrogen removal in switchgrass biomass under two harvest systems. *Biomass and Bioenergy* 19:281-286.
- Sanderson M.A., Reed R.L., McLaughlin S.B., Wullschleger S.D., Conger B.V., Parrish D.J., Wolf D.D., Taliaferro C., Hopkins A.A., Ocumpaugh W.R., Hussey M.A., Read J.C., Tischler C.R. (1996) Switchgrass as a sustainable bioenergy crop. *Bioresource Technology* 56:83-93.
- Schmer M.R., Vogel K.P., Mitchell R.B., Perrin R.K. (2008) Net energy of cellulosic ethanol from switchgrass. *PNAS* 105:464-469.
- Schroder J.L., Zhang H., Zhou D., Basta N., Raun W.R., Payton M.E., Zazulak A. (2008) The effect of long-term annual application of biosolids on soil properties, phosphorus, and metals. *Soil Science Society of America Journal* 72:73-82.
- Shahandeh H., Chou C.Y., Hons F.M., Hussey M.A. (2011) Nutrient partitioning and carbon and nitrogen mineralization of switchgrass plant parts. *Communications in Soil Science and Plant Analysis* 42:599-615.
- Simpson T.W., Sharpley A.N., Howarth R.W., Paerl H.W., Mankin K.R. (2008) The new gold rush: fueling ethanol production while protecting water quality. *Journal of Environmental Quality* 37:318-324.
- Sinclair T.R. (2009) Taking measure of biofuel limits. *American Scientist* 97:400-407.

- Skinner R.H., Adler P.R. (2010) Carbon dioxide and water fluxes from switchgrass managed for bioenergy production. *Agriculture, Ecosystems and Environment* 138:257-264.
- Smith S.L. (2009) Bioenergy cropping systems on marginal land, *Crop & Soil Sciences*, Michigan State University. pp. 157.
- Somerville C., Youngs H., Taylor C., Davis S.C., Long S.P. (2010) Feedstocks for lignocellulosic biofuels. *PNAS* 329:790-792.
- Thomason W.E., Raun W.R., Johnson G.V., Taliaferro C.M., Freeman K.W., Wynn K.J., Mullen R.W. (2004) Switchgrass response to harvest frequency and time and rate of applied nitrogen. *Journal of Plant Nutrition* 27:1199-1226.
- Trocsanyi Z.K., Fieldsend A.F., Wolf D.D. (2009) Yield and canopy characteristics of switchgrass (*Panicum virgatum* L.) as influenced by cutting management. *Biomass and Bioenergy* 33:442-448.
- USDA. (2010) A USDA regional roadmap to meeting the biofuels goals of the renewable fuels standard by 2022, USDA Biofuels Strategic Production Report.
- Varvel G.E., Vogel K.P., Mitchell R.B., Follett R.F., Kimble J.M. (2008) Comparison of corn and switchgrass on marginal soils for bioenergy. *Biomass and Bioenergy* 32:18-21.
- Vogel K.P., Brejda J.J., Walters D.T., Buxton D.R. (2002) Switchgrass biomass production in the Midwest USA: harvest and nitrogen management. *Agronomy Journal* 94:413-420.
- Vogel K.P., Dien B.S., Jung H.G., Casler M.D., Masterson S.D., Mitchell R.B. (2011) Quantifying actual and theoretical ethanol yields for switchgrass strains using NIRS analyses. *Bioenergy Research* 4:96-110.
- Waramit N., Moore K.J., Heggenstaller A.H. (2011) Composition of native warm-season grasses for bioenergy production in response to nitrogen fertilization rate and harvest date. *Agronomy Journal* 103:655-662.
- Whitmore A.P. (1996) Modeling the release and loss of nitrogen after vegetable crops. *Netherlands Journal of Agricultural Science* 44:73-86.
- Withers K.K. (2010) Establishment, fertility and harvest management for optimizing switchgrass yield and quality as a cellulosic ethanol feedstock in the Great Lakes region, *Crop and Soil Science*, Michigan State University. pp. 110.
- Wright L., Turhollow A. (2010) Switchgrass selection as a "model" bioenergy crop : A history of the process. *Biomass and Bioenergy* 34:851-868.
- Wullschleger S.D., Davis E.B., Borsuk M.E., Gunderson C.A., Lynd L.R. (2010) Biomass production in switchgrass across the United States: database description and determinants of yield *Agronomy Journal* 102:1158-1168.

CHAPTER TWO. Effects of Biosolids Application and Harvest Frequency on Switchgrass Yield, Feedstock Quality and Theoretical Ethanol Yield

Abstract

Biofuel could be produced from high yield potential cellulosic crops, such as switchgrass (*Panicum virgatum* L.). Sustainable development of bioenergy industry will rely on high biomass yield and desirable feedstock quality. The objective of this study was to examine the effects of biosolids application and harvest frequency on switchgrass in a plot study between 2010 and 2012 in northern Virginia, USA. Class A biosolids (anaerobically digested, pasteurized, and dewatered) were applied once at 0 (control), 153, 306, and 459 kg N ha⁻¹ and urea was applied at 180 kg N ha⁻¹ in May 2010 for comparison. Switchgrass was cut once (November) or twice (July and November) per year. Biomass yield, concentrations of N, cellulose, hemicellulose, lignin, and ash were determined. Theoretical ethanol potential (TEP) and yield (TEY) were calculated based on concentrations of pentose and hexose estimated from concentrations of cellulose and hemicellulose. Biosolids at 153, 306, and 459 kg N ha⁻¹ increased yield by 26%, 37%, and 46%, N concentration by 17%, 34%, and 52%, and TEY by 27%, 37%, and 48% over the control, respectively. Urea increased yield, N concentration, and TEY by 41%, 27%, and 45% relative to the control. Both biosolids and urea had no effects on TEP. Two cuts produced greater biomass yield than one cut (11.9 vs. 9.8 Mg ha⁻¹) in all years except 2010, when yield was adversely affected by a late spring burn and summer drought. Cutting twice also obtained greater N concentration (8.6 vs. 4.1 g kg⁻¹) and yield (12.1 vs. 11.0 Mg ha⁻¹). One cut produced greater theoretical ethanol potential (TEP) (479 vs. 422 L Mg⁻¹) and TEY (4470 vs. 4020 L ha⁻¹). This experiment provides support that biosolids can be an alternative fertilizer and can increase biomass yield and TEY without having negative effect on feedstock quality. Also, these results suggest that single, end-of-season harvest in one-cut system produced the highest TEY and appeared to be affected more by feedstock quality rather than biomass yield.

Keywords: *Panicum virgatum*, bioenergy crop, N, biomass, biofuel quality, cellulose, hemicellulose

Introduction

Switchgrass is a perennial C₄ grass considered as a promising feedstock for second-generation biofuel production (Parrish and Fike, 2005). It has many desirable attributes for use as a bioenergy crop such as adaptive to marginal land with high biomass potential. Main costs associated with switchgrass production include N fertilization, harvesting (Vogel et al., 2002), and transportation (Cundiff et al., 2009). The N fertilization sources and application rates, number of harvest frequencies, and yields of each harvest affect the economics of switchgrass production system.

Research has been conducted on different sources of fertilizer requirements of switchgrass, mainly including chemical fertilizers. Generally, the major fertilizer requirement of switchgrass is N (Guretzky et al., 2011; Trocsanyi et al., 2009). Growing associated with mycorrhizae, switchgrass is a very efficient soil nutrients use such as N and P (Muir et al., 2001; Vogel et al., 2002). The N requirement of switchgrass largely depends on productivity of cultivars, yield potential of specific locations, and harvest management practices. Fike et al. (2006b) observed that switchgrass (lowland and upland cultivars) yields ranged from 10.4 to 19.1 Mg ha⁻¹ in response to 50 kg N ha⁻¹ in the upper southeastern USA. The optimum N fertilization rates of lowland switchgrass cultivar (Alamo) were 122 kg N ha⁻¹ in Alabama (McLaughlin and Kszos, 2005) and 168 kg N ha⁻¹ in Texas (Muir et al., 2001). Results from other studies suggest that switchgrass yields do increase in response to N rates at levels between 56 and 120 kg N ha⁻¹, before plateauing at higher rates (Gunderson et al., 2008; Haque et al., 2009; Lemus et al., 2008a). However, most switchgrass these N-response studies have been conducted using chemical fertilizers or animal manures. Although long-term yields were reported for switchgrass grown on a mine site reclaimed with biosolids (Evanylo et al., 2005), there are no published field

trials documenting switchgrass yield or quality responses to differing biosolids application rates in a biofuel production setting.

Switchgrass N concentration increases with increased N rates. Waramit et al. (2011) reported that N concentration increased by about 20 % and 30% with ammonium nitrate applied at 65 and 140 kg N ha⁻¹ in Iowa. In Nebraska, switchgrass N concentration increased by 75% with application of ammonium nitrate at 225 kg N ha⁻¹ (Guretzky et al., 2011). In a similar study in Virginia, switchgrass N concentration increased by about 30% with urea at 270 kg N ha⁻¹ (Lemus et al., 2008b). Yield and N uptake were affected by N applied and distributions in a high-rainfall growing season, where retention of N within the root zone accounted for residual N effects on yield and plant uptake which lasted to the end of the growing season (Kowalenko and Bittman, 2000). Although assuming N taken up by plants is mainly derived from newly applied N, the natural soil N pool can also contribute a significant portion of the N used for switchgrass growth (Overman, 1995).

Biosolids are high-moisture and nutrients-rich solids recovered from waste-water treatment facilities. The materials act as slow release organic fertilizers (mainly as sources of N and P) and can boost crop production and improve soil fertility (Christie et al., 2001; Gilmour et al., 2003). As an alternative N resource, biosolids are cost-effective materials which can displace synthetic fertilizer and be safely applied to improve soil properties and stimulate plant growth (Guo et al., 2012). Field application of biosolids can increase soil organic C (Brown and Leonard, 2004; Xu et al., 2006), N (Kelly et al., 2007), and improve soil physical and chemical properties (Hargreaves et al., 2008; Ojeda et al., 2003). When land applied, biosolids-derived nutrients contribute to increased plant productivity (Sigua et al., 2005). These characteristics may be particularly important in biofuel cropping systems because feedstock production is likely to be sited on marginal lands and the limited economic value of bioenergy crops themselves will require low (or low-cost) production inputs.

Harvest management plays an important role in switchgrass yield, N concentration, and feedstock quality. Several studies have shown about a 15% increase in switchgrass yield with

multiple harvests (Fike et al., 2006a; Haile-Mariam et al., 2008), but this is location, cultivar, or climate condition dependent. However, multiple harvests of switchgrass may reduce total seasonal yield, particularly in dry years, when environmental conditions place greater stress on the biofuel crop growth (Sanderson et al., 1996). Optimal harvest timing would be in late summer or early fall if aimed at maximizing biomass production (Ashworth, 2010; Lemus et al., 2008a). Feedstock quality parameters (e.g., cellulose, hemicellulose, lignin, and ash) are of interest for bioenergy systems because they affect conversion processes and determine the amount of biofuel that can be produced per unit biomass. When harvested in late fall biomass quality would be increased as switchgrass would have greater fiber and lignin concentration and reduced levels of ash and nutrient elements relative to earlier season harvests (Varvel et al., 2008; Waramit et al., 2011). However, management that seeks to maximize biomass yield (with earlier harvests) should be weighed against the degraded feedstock quality and increased costs associated with greater nutrient removal (Adler et al., 2006). Greater concentrations of cellulose and hemicellulose are preferable for biofuel production, but higher N and ash concentrations reduce the biomass conversion efficiency and biofuel production in thermochemical processes, and decrease feedstock quality for biofuel production.

Information regarding responses of biomass yield, N concentration, feedstock quality and TEY to biosolids application under different harvest frequencies is lacking. Based on existing published studies, we hypothesized that biosolids application and two cuts production systems would increase biomass yield and N concentration given normal climatic conditions, but may have varied effects on feedstock qualities and TEY. Four biosolids N rates, applied in a single application, and one urea rate for comparison, and two harvest frequencies were imposed in this study. The objectives of this study were to: 1) determine the effects of biosolids application on biomass yield, N concentration, feedstock quality, and TEY; 2) examine the effects of harvest frequencies on yield, N concentration, feedstock quality and TEY.

Materials and Methods

Site

The experiment was conducted in the Crop and Soil Environmental Sciences experiment station at Orange, VA (38° 13' N, 78° 07' W). The site elevation is 156 m above sea level, and the average annual rainfall is 780 mm. The soil was a Davidson clay (Fine, kaolinitic, CEC Activity class, thermic family of Rhodic Kandiudults), with pH 5.9, 23 to 40 g kg⁻¹ total C, 1.6 to 2.3 g kg⁻¹ total N, 7 to 22 mg kg⁻¹ P, and 32 to 111 mg kg⁻¹ K. Climate data were collected from an on-site weather station. Historical climate data were obtained from the National Oceanic and Atmospheric Administration's National Climatic Data Center using the weather station Orange, Virginia (<http://www.ncdc.noaa.gov/cdo-web/datasets/GHCNDMS/locations/ZIP:22960/detail>).

Experimental treatments

The experiment was begun on May 21, 2010, after burning a switchgrass (cv 'Cave-in-Rock') stand established in 1987, to which no fertilizer had been applied in the previous five years. A split plot with in a randomized complete block design was used with three replications; harvest frequencies of one cut and two cuts were used as main plots, and subplots consisted of application of biosolids and urea, and an unfertilized control. Plots (2.45 m × 4.9 m) were laid out with 0.62-m alleys. Class A biosolids (anaerobically digested, pasteurized, and dewatered) were obtained from Alexandria Sanitation Authority, Alexandria, VA.

Wet biosolids were weighed into buckets on a platform scale (Finemech, DE-300K50DL, Kern, CA), and distributed evenly across the plot-surfaces using hand rakes on May 27, 2010. Biosolids application rates were intended to deliver 90, 180, 270 kg plant available N (PAN) ha⁻¹. Urea was applied to compare biosolids with a chemical fertilizer source. Rates were 180 kg PAN ha⁻¹ and 30 kg PAN ha⁻¹ in 2010 and 2011 and no urea was applied in the third year. This was done with the intent of mimicking the available N mineralized from biosolids. Triple super phosphate was applied at 222 kg P ha⁻¹ in the first year to provide the same amounts of P as would be available in biosolids applied at 180 kg N ha⁻¹. Control plots received no biosolids or

chemical fertilizer. Biosolids application rates were calculated based on the most up-to-date analyses of biosolids N and dry matter (DM) concentrations (8.6 pH, 26.2% DM, 37.0% total C, 6.1% total N, 2.2% NH₄ – N, 4.0% P and 0.1% K) provided by the Alexandria Sanitation Authority, but a misapplication of the conversion equations (VADCR, 2005) resulted in application of higher biosolids PAN rates (153, 306, and 459 kg PAN ha⁻¹).

Table 2.1. Estimated plant available nitrogen (PAN) supplied by biosolids and urea. The estimated PAN was calculated based on Virginia Department of Conservation and Recreation equations for biosolids organic N mineralization and ammonia volatilization (VADCR, 2005).

Year	Estimated PAN, kg ha ⁻¹				
	Biosolids				Urea
2010	0	153	306	459	180
2011	0	27	54	81	30
2012	0	27	54	81	0

On the day of application, two biosolids samples were collected for analysis at a commercial soil testing laboratory (Eastern A&L Laboratory, Richmond, VA). Total Kjeldahl N (TKN) was determined by the method of Evanylo et al. (2005). Organic N calculated as: TKN – (NH₄ – N + NO₃ – N). NH₄ – N and NO₃ – N determined by shaking 2.5 g of biosolids with 25 mL of 1 M KCl for 1 h and analyzed with a Rapid Flow Analyzer (Quickchem 8000 Lachat, Loveland, CO, USA). Results from these analyses were used *a posteriori* to calculate total and PAN applied to each plot. TKN and PAN for each year were estimated using organic N mineralization rates (30%, 10%, and 10% for the first, second, and third years, respectively) and NH₄ – N availability coefficients (50% for the first year) as developed by VADCR (2005). Nitrogen availability from biosolids beyond the 3rd year was assumed to be minimal. Based on these analyses, the PAN application rates were 153, 306, and 459 kg N ha⁻¹ for the first year, and estimated N released in following years were shown in Table 2.1 (VADCR, 2005). Biosolids had 8.6 pH, 369.8 g kg⁻¹ total C, 61.4 g kg⁻¹ TKN, 39.7 g kg⁻¹ P, and 1.4 g kg⁻¹ K. The pH was tested with 1:1 water and solid ratio, and C was analyzed by combustion method, P and K were extracted with Mehlich I

(0.05 mol/L HCl in 0.0125 mol/L H₂SO₄) and analyzed with inductively coupled plasma atomic emission spectrometer.

Harvest treatments included one or two cuts per season. Plots in the one-cut system were harvested in November. Plots to be cut twice were harvested in both July and November. The study was conducted from 2010 through 2012.

Sample collection and biomass analysis

Switchgrass was cut to 15-cm height with a Carter flail-type forage harvester (Model FM08; Carter Manufacturing Co., Inc., Brookston, IN, USA). A 0.9-m-wide swath was cut the length of each plot. Harvest strips from each plot were weighed in the field with a hanging balance. Subsamples were randomly collected from the strip biomass, placed in cloth bags, weighed on a platform scale (Scout Pro SPE6001; Ohaus Corp., Pine Brook, NJ, USA), and dried in a forced-draft oven at 55 °C for least 72 hours or to a constant dry weight to determine dry matter content. Dried samples were ground to pass through a 2-mm screen with a Wiley mill (Thomas Wiley, Philadelphia, PA, USA). Subsamples were ground to pass through a 1-mm screen with an Udy cyclone mill (UDY Corporation, Fort Collins, CO, USA).

Samples were scanned with a near infrared reflectance spectrophotometer (NIRS) (Foss NIR System 6500M, Laurel, MD, USA). After scanning, two subsets of samples (Tables 3.2 and 3.3) were selected by WIN ISI software (Infrasoft International LLC, Port Matilda, PA, USA) for chemical analysis (Vogel et al., 2011). Biomass N concentration was determined by dry combustion method using a CNS elemental analyzer (Elementar, Vario MAX CNS Analyzer, Hanau, Germany). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined sequentially using the ANKOM 200 fiber analyzer (Ankom Technology Corporation, Fairport, NY, USA) according to the procedure described by (Mulkey et al., 2006). Cellulose concentration was calculated as ADF minus ADL and hemicellulose as the difference between NDF and ADF (Dien et al., 2006). Ash was measured as loss of weight

after combustion at 500°C for four hours in a muffle furnace (FA1730, Barnstead/Thermolyne, Dubuque, IA, USA).

Theoretical ethanol yield (TEY)

The theoretical ethanol potential (TEP; Goff et al. (2010)) of switchgrass was estimated as follows:

$$H = [\%Cellulose + (\%Hemicellulose \times 0.07)] \times 172.82$$

$$P = [\%Hemicellulose \times 0.93] \times 176.87$$

$$TEP (L Mg^{-1}) = [H + P] \times 4.17$$

where: H and P are hexose and pentose carbohydrates, respectively. The TEY ($L ha^{-1}$) was calculated by multiplying the TEP by the respective biomass yield ($Mg ha^{-1}$).

Table 2.2. Calibration statistics for NIRS prediction of switchgrass feedstock quality for July harvests during 2010 and 2012 at Orange, VA.

Components [†]	N [‡]	Mean	SD [§]	Est. Min	Est. Max	SEC [¶]	RSQ [#]	SECV ^{††}	1-VR ^{‡‡}
NDF	55	623	42.3	496	750	7.69	0.97	28.7	0.57
ADF	55	341	36.4	232	451	7.72	0.96	28.5	0.40
ADL	55	38.5	9.59	9.67	67.2	0.85	0.99	6.24	0.58
Ash	55	48.6	12.1	12.2	85.0	3.10	0.93	7.93	0.58

[†] NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin,

[‡] Number of samples, [§] Standard deviation, [¶] Standard error of calibration, [#] Coefficient of determination, ^{††} Standard error of cross validation.

Table 2.3. Calibration statistics for NIRS prediction of switchgrass feedstock quality for November harvests during 2010 and 2012 at Orange, VA.

Components [†]	N [‡]	Mean	SD [§]	Est. Min	Est. Max	SEC [¶]	RSQ [#]	SECV ^{††}	1-VR ^{‡‡}
NDF	96	706	46.3	567	845	11.6	0.94	24.2	0.73
ADF	98	402	45.3	266	538	10.4	0.95	16.5	0.87
ADL	95	51.2	13.2	11.6	90.9	3.17	0.94	4.83	0.87
Ash	96	29.7	9.65	0.82	58.7	1.92	0.96	3.57	0.86

[†] NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin,

[‡] Number of samples, [§] Standard deviation, [¶] Standard error of calibration, [#] Coefficient of determination, ^{††} Standard error of cross validation.

Data analysis and experimental design

This experiment was a randomized complete block design with a split plot treatment arrangement with three replications. Harvest frequencies were main plots, and N rates (biosolids and urea) were subplots. Nitrogen and harvest treatments were considered fixed effects, and replications and years were considered random effects. Data were analyzed using the PROC Mixed procedure of SAS software (version 9.2; SAS Institute, Cary, NC, USA). Least significant differences (LSD) were considered significant at $\alpha = 0.05$ level unless otherwise indicated. Relationships of yield and other feedstock quality parameters in response to N rates (biosolids and urea) were tested for simple linear or quadratic effects and their coefficients of determination (r^2) using regression analysis (PROC REG). Comparison of yield and other parameters affected by N rates across years was analyzed by averaging these data across years and then perform ANOVA test with SAS.

Results and Discussion

Climatic and soil conditions

Temperatures during the 2010 growing season were above historic averages for the research site, while precipitation during the growing season was about 200 mm below normal (Table 2.4).

Table 2.4. Monthly and 30-yr temperature and precipitation during 2010 and 2012 at Orange, VA.

	Year	Growing season (Months)								Mean
		M	A	M	J	J	A	S	O	
Temperature (°C)	2010	9.3	15.2	19.3	25.1	26.4	25.1	22.3	14.8	19.7
	2011	7.4	14.1	18.6	23.7	26.3	24.3	20.2	13.1	18.5
	2012	12.6	12.8	19.7	21.7	26.4	23.7	19.0	13.9	18.7
	30-yr mean	7	12	18	22	24	24	20	14	17.5
Precipitation (mm)	2010	101	41	78	62	37	66	99	89	574
	2011	172	95	119	87	67	193	163	156	1052
	2012	53	27	113	95	84	153	57	122	704
	30-yr mean	87	79	113	87	114	109	90	102	780

Temperatures during 2011 and 2012 were at or slightly below the 30-yr mean, while precipitation was above normal in 2011 and slightly less than the historic average in 2012. Before biosolids application, soils had pH 5.9, 23 to 40 g kg⁻¹ total C, 1.6 to 2.3 g kg⁻¹ total N, 7 to 22 mg kg⁻¹ P, and 32 to 111 mg kg⁻¹ K.

The response of yield, feedstock quality and TEY to biosolids application rates, harvest frequencies, and variable climatic conditions (Table 2.4) had interactions with year, so data for each year were analyzed separately. There were interactions among variables indicating the response to biosolids rates and harvest frequencies could not be evaluated independently of the others.

Biomass Yield

Figure 2.1 shows the best-fit quadratic responses (highest R² values to predict dependent variables) of yield to initial biosolids and urea N rates. Averaged across years, biosolids increased biomass yields by 26%, 37%, and 46% at application rates of 153, 306, and 459 kg N ha⁻¹, and urea applied at 180 kg N ha⁻¹ increased yield by 41% over the control. Compared with 2010, switchgrass yields were about 50 and 60% greater in 2011 and 2012 (Figure 2.2). Lower yields in 2010 were largely due to the late burning in May, which removed some new plant tissues and shortened the length of the plants' available growing season; low summer precipitation and higher temperature further limited switchgrass growth (Table 2.4). Fike et al. (2006a) reported that mean growing season temperature had negative relationships with biomass yields.

Plots receiving the highest biosolids N rate and plots receiving urea produced 19% and 13% more biomass than plots receiving biosolids at the 153 kg ha⁻¹ rate in 2011. No significant differences were detected among biosolids rates and urea in other years. The stronger responses to biosolids and urea in 2011 were probably due to greater precipitation (Table 2.4), as switchgrass yields may have positive relationship with rainfall early the growing season (Fike et al. (2006a) . Vogel et al. (2002) reported that across harvest frequencies (one- and two-cut

treatments) switchgrass yields were increased by 35% with applications of 180 kg N ha⁻¹ (as ammonium nitrate) in Nebraska. About a 33% yield increase was observed in Iowa at similar

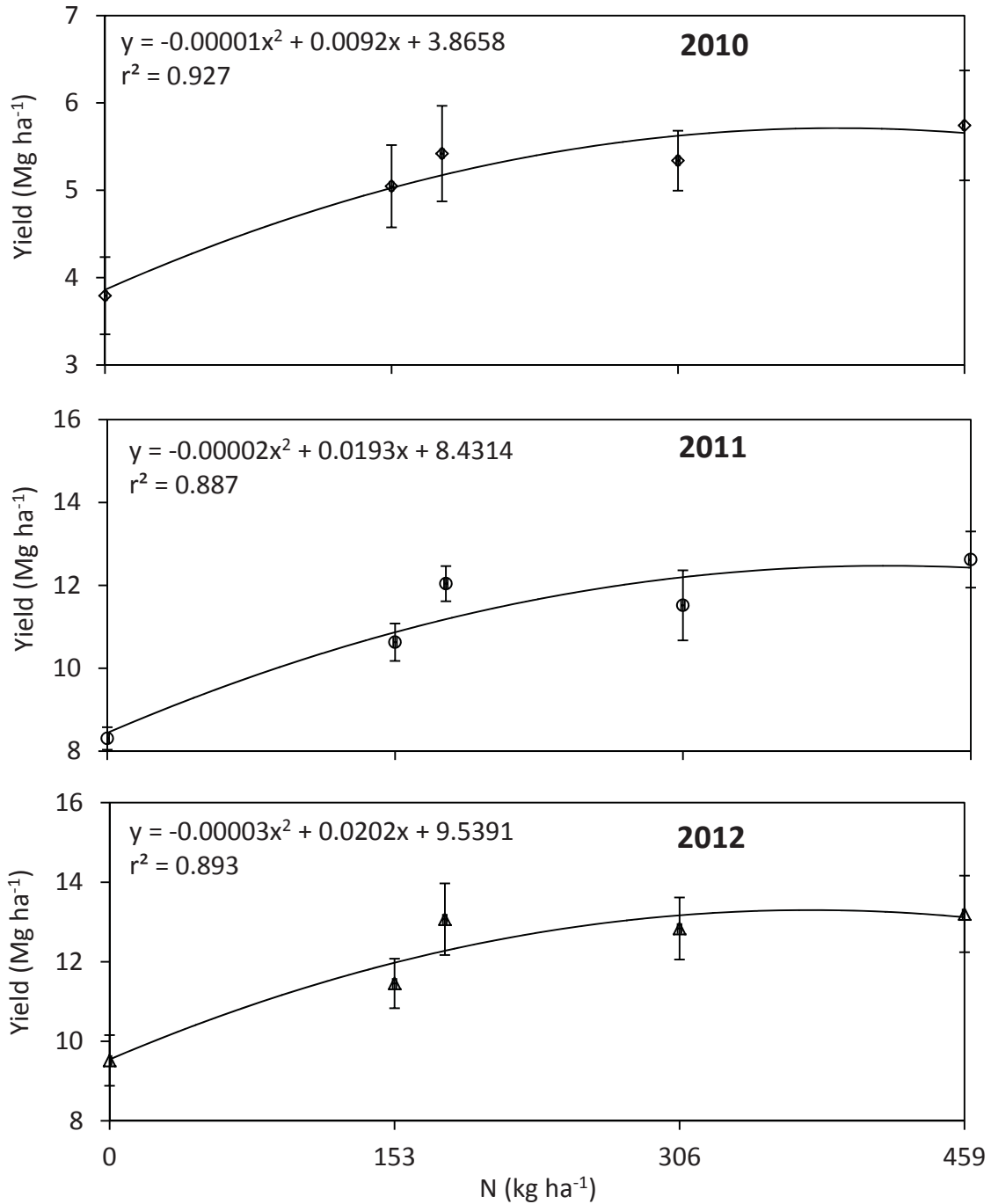


Figure 2.1. Switchgrass yield in 2010, 2011, and 2012 as affected by the initial four biosolids rates of 0, 153, 306 and 459 kg N ha⁻¹ and the urea rate of 180 kg N ha⁻¹. Data are averaged over two cutting frequencies in each year.

(200 kg N ha⁻¹) application rates (Lemus et al., 2008a). Across years the yield of switchgrass in response to urea at 180 kg ha⁻¹ did not differ from the yields of switchgrass plots receiving biosolids at rates of 306 and 459 kg N ha⁻¹, but yields with urea were 13% greater than yields in response to biosolids at 153 kg N ha⁻¹. These data suggest that N in urea was used more efficiently than the N in biosolids. However, these results also could reflect an overestimation of the PAN released from the organic N of biosolids, or an underestimation of the NH₄– N loss via volatilization predicted by the equations of VADCR (2005). Although not measured in this study, Robinson and Polglase (2000) reported that about 80% of the NH₄– N applied in biosolids was lost within three weeks of application in a simulation study under a low temperature regime (11 to 16 °C) in Australia.

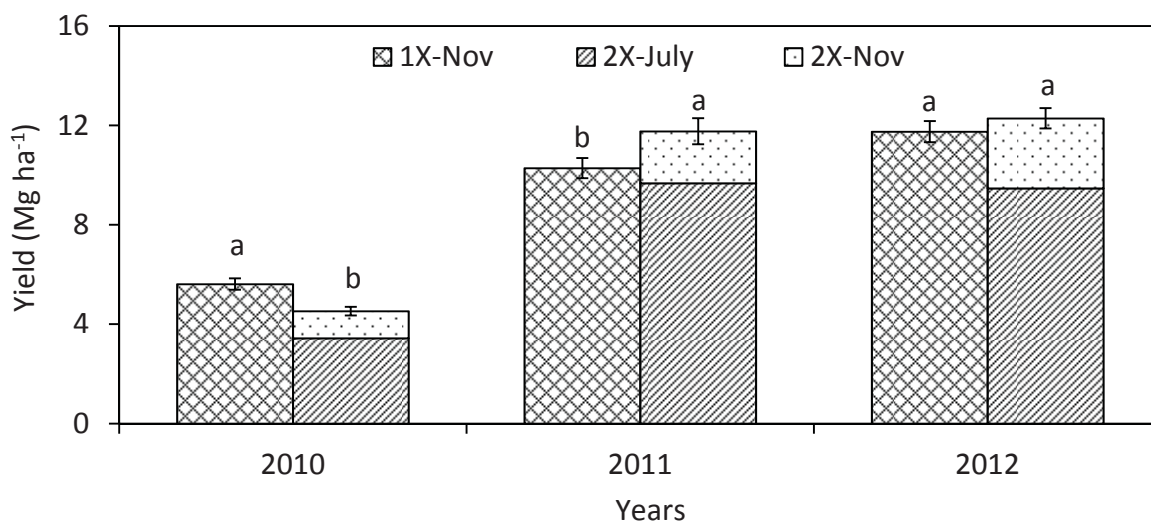


Figure 2.2. Switchgrass yield as affected by harvest frequencies. Data are averaged over all N treatments. 1X-Nov = one-cut system, November harvest; 2X-July and 2X-Nov = two-cut system, July and November harvests, respectively. Error bars for yields in the 2X system represent the standard errors for total season yields.

Ironically, the low apparent N use by switchgrass may reflect several factors that make this plant a promising bioenergy crop. First, the plant is inherently thrifty with N and mobilizes large amounts of N from belowground storage for shoot production (Parrish and Fike, 2005). The plant also may acquire large amounts of N from soil organic pools. Mineralization of soil organic

matter contributes significant amounts of N available to switchgrass and mineralization becomes most active as switchgrass grows quickly after soils are completely warmed. (Stout et al., 1991) reported that even at 180 kg N ha^{-1} , only about 25% of the N in harvested biomass was derived from the fertilizer applied, and the rest was from N available in soils. Atmospheric deposition of N can provide about $24 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in eastern USA (Coulston et al., 2004). The contribution of N from mycorrhizal and other microbial or rhizosphere processes might also exist. Arbuscular mycorrhizal fungi can increase switchgrass root length and surface area (Clark, 2002), and raise shoot N uptake (Schroeder-Moreno et al., 2012), and finally enhance biomass production (Ghimire et al., 2011). Rhizosphere microbes may also facilitate N supplies for switchgrass (Welbaum et al., 2004). For example, a combination of several rhizosphere bacteria were reported to increase switchgrass growth (Ker et al., 2012) in more nutrient-limited environments.

Switchgrass yields as affected by harvest frequency varied by year (Figure 2.2). Yield data for 2010 were atypical, with yields under two cut management 20% lower than with one cut. Lower yields with the two cuts in 2010 likely were caused by the combination of an artificially shortened growing season (plots were burned in May) and low summer precipitation, which limited switchgrass regrowth after the July harvest. Although not measured, the additional harvest under conditions of stress may have had more negative effects on switchgrass growth responses in the subsequent seasons. Averaged across 2011 and 2012, however, cutting twice per season increased yields by 9% over the single cut per season. This pattern is similar to but not as strong as the results of Fike et al. (2006b), who reported that cutting upland cultivars twice per season in the upper southeastern USA increased biomass yields >40% ($15.4 \text{ vs. } 10.8 \text{ Mg ha}^{-1}$). However, those authors used a split application of 100 kg N ha^{-1} , which may have supported greater switchgrass regrowth (50%) following the summer harvest than in this study (28%). Within the two-cut system, yields in November were consistently lower than yields in July in all years. This was similar to other reports (Fike et al., 2006a; Fike et al., 2006b; Lemus et al., 2008b; Vogel et al., 2002) in which most of the biomass accumulated was harvested in summer.

Biomass N concentration

Figure 2.3 shows the best-fit linear responses of biomass N concentration to initial biosolids

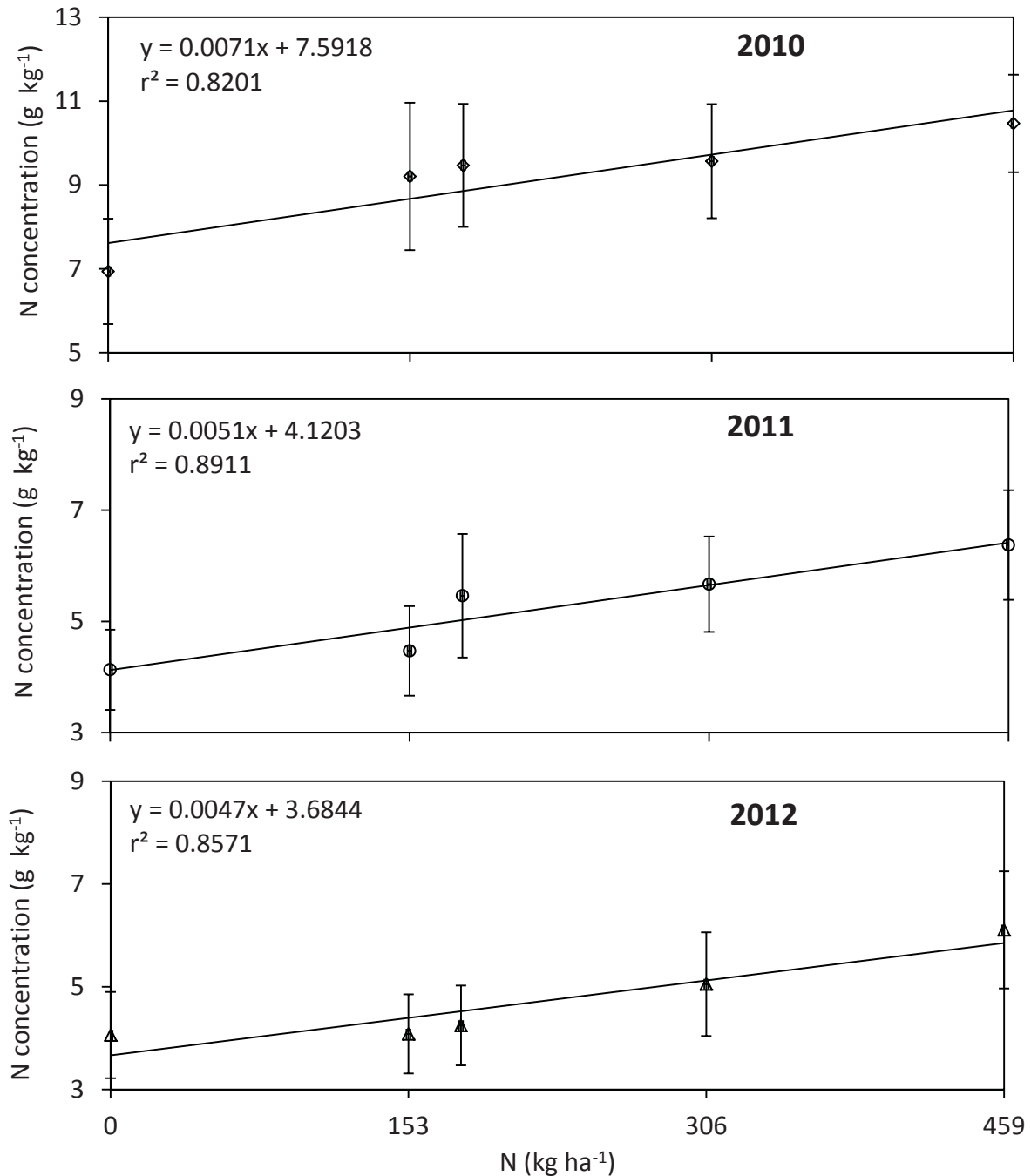


Figure 2.3. Switchgrass N concentrations in 2010, 2011, and 2012 as affected by the initial four biosolids rates of 0, 153, 306 and 459 kg N ha⁻¹ and the urea rate of 180 kg N ha⁻¹. Data are averaged over two cutting frequencies in each year.

and urea N rates (highest R^2 values to predict dependent variables). Biosolids N rates (from low to high) across years increased biomass N concentrations by 15%, 30% and 44% above the control (Figure 2.3). Differences in biomass N between urea and biosolids treatments increased in the third year, reflecting both the lack of urea application and the greater biomass production in the urea plots.

The literature generally suggests that switchgrass N concentrations increase in response to added N fertility. For example, applying ammonium nitrate at 150 kg N ha^{-1} increased N concentration by about 30% (3.0 g kg^{-1}) relative to the control in Canada when switchgrass was harvested at either four- or six-week intervals between July and November (Madakadze et al., 1999b). However, Lemus et al. (2008b) reported that applying urea at up to 270 kg N ha^{-1} did not affect biomass N concentrations when switchgrass was harvested twice in July and November in Virginia. In their study, the increased yields associated with added fertility may have countered any potential N concentration effects. As well, Lemus et al. (2008b) applied fertilizer only the first year of their study.

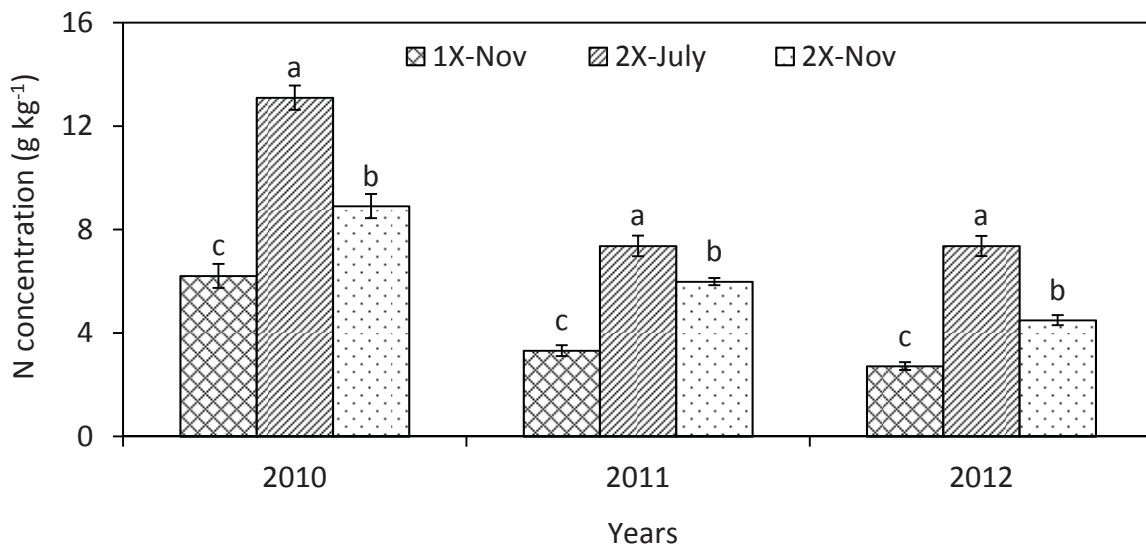


Figure 2.4. Switchgrass N concentrations as affected by harvest frequencies. Data are averaged over all N treatments. 1X-Nov = one-cut system, November harvest; 2X-July and 2X-Nov = two-cut system, July and November harvests, respectively. Error bars for yields in the 2X system represent the standard errors for total season yields.

The N concentrations in biomass from the one cut system were consistently lower and averaged 127% and 58% less than in the biomass harvested in July and November in the two-cut system (Figure 2.4) across the three years. Thus, cutting twice per season would remove more N from the soil than cutting once, as reported previously (e.g., Vogel et al., 2002; Fike et al., 2006ab; Lemus et al., 2008b). Similar results were reported in studies comparing N concentrations in biomass from one cut and two cuts using N fertilizer (Lemus et al., 2008b; Reynolds et al., 2000; Vogel et al., 2002). These results also imply feedstock from one cut would be more desirable for biofuel production via thermochemical process owing to less N₂O produced (Anex et al., 2007), but N concentrations may have no effects on biochemical conversion systems (Anderson et al., 2010).

Feedstock Quality and Ethanol Potential in Response to Fertility Treatments

Biosolids and urea applications did not affect biomass lignin, ash, cellulose, or hemicellulose concentrations. Lignin and ash concentrations in this study ranged from 23.1 to 67.9 g kg⁻¹ (mean = 43.0) and 20.5 to 58.8 g kg⁻¹ (mean = 41.1) across years and harvest frequencies. Similar results were reported in South Dakota; N fertilization did not affect switchgrass lignin and ash concentrations at rates up to 224 kg ha⁻¹ (Lee et al., 2007). The mean cellulose and hemicellulose concentrations were 326 g kg⁻¹ (range = 250 to 398 g kg⁻¹) and 291 g kg⁻¹ (range = 248 to 318 g kg⁻¹), similar to the data reported by Lemus et al. (2002) in which switchgrass was fertilized at 112 kg N ha⁻¹ and harvested in fall. Waramit et al. (2011) also reported no differences in cellulose concentrations in switchgrass fertilized with N at 65 and 140 kg ha⁻¹ in Iowa. Lemus et al. (2002) found no differences in hemicellulose concentrations for switchgrass in two years when N was applied at 140 kg ha⁻¹.

Across fertility treatments, the mean TEP of switchgrass was 449 L Mg⁻¹. This was lower than the TEP based on the concentrations of cellulose and hemicellulose data from other published papers. Calculated TEP ranged from 479 to 493 L Mg⁻¹ in studies from England testing Cave-In-Rock switchgrass (Allison et al., 2012) and in Iowa, USA, testing several switchgrass

cultivars (Lemus et al., 2002) . Values for TEP in this study were closer to the 436 L Mg⁻¹ predicted for biomass grown in Pennsylvania (Adler et al., 2006). The variation of TEP reflects differences in fiber and lignin concentrations which were affected by other factors such as harvest timing and environmental conditions.

Feedstock Quality and Ethanol Potential in Response to Harvest Treatments

Lignin and Ash

Lignin concentrations were consistently lower in July harvests within the two-cut system over years (Figure 2.5). One cut produced 49% and 31% greater lignin concentrations in 2011 and 2012 compared to the lignin concentrations of November harvests in two-cut system. However, the lignin concentration was 22% lower for one cut in 2010 than November harvest of two-cut system in 2010, likely due to dry climatic conditions. Allison et al. (2012) reported that

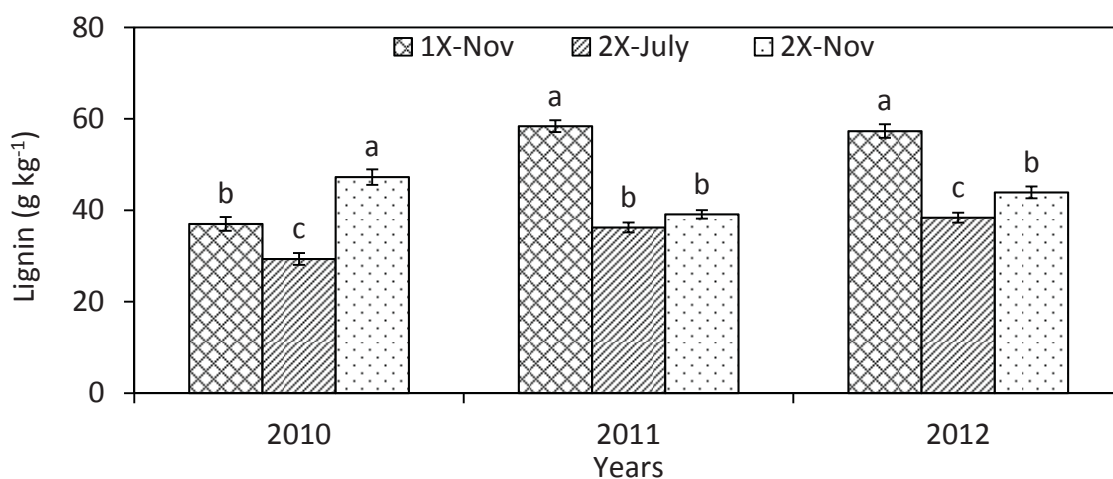


Figure 2.5. Switchgrass lignin concentrations as affected by harvest frequencies. Data are averaged over all N treatments. 1X-Nov = one-cut system, November harvest; 2X-July and 2X-Nov = two-cut system, July and November harvests, respectively. Error bars for yields in the 2X system represent the standard errors for total season yields.

all cell wall components increased in concentration in late fall harvests, likely owing to a combination of cell wall deposition and increased lignification during the growing season.

Increased lignin concentrations may have mixed impacts relative to the biofuel conversion system. Higher lignin concentrations are associated with greater energy density and can increase conversion efficiency for thermochemical conversion systems (McLaughlin and Kszos, 2005). However, lignin may reduce the availability of cellulose and hemicellulose in biochemical conversion processes (Allison et al., 2012; Waramit et al., 2011).

Ash concentrations as a function of harvest frequency were inconsistent among years (Figure 2.6). In 2010, the greatest ash concentration (49.0 g kg^{-1}) was observed in the July-harvested biomass, with the lowest concentration in the November harvest within the two-cut system. These lower ash concentrations may reflect limited nutrient uptake as a function of reduced mass flow given the quite low rainfall and higher-than-normal temperatures during the 2010 growing season. In 2011, ash concentrations were highest in the biomass from the November harvest and intermediate in biomass from the July harvest of two-cut system. Although we did not measure leaf-stem ratios, plant ash concentrations can be three times greater in leaves than in stems (Madakadze et al., 1999a). Thus, the greater ash in 2011 may reflect greater leaf production and

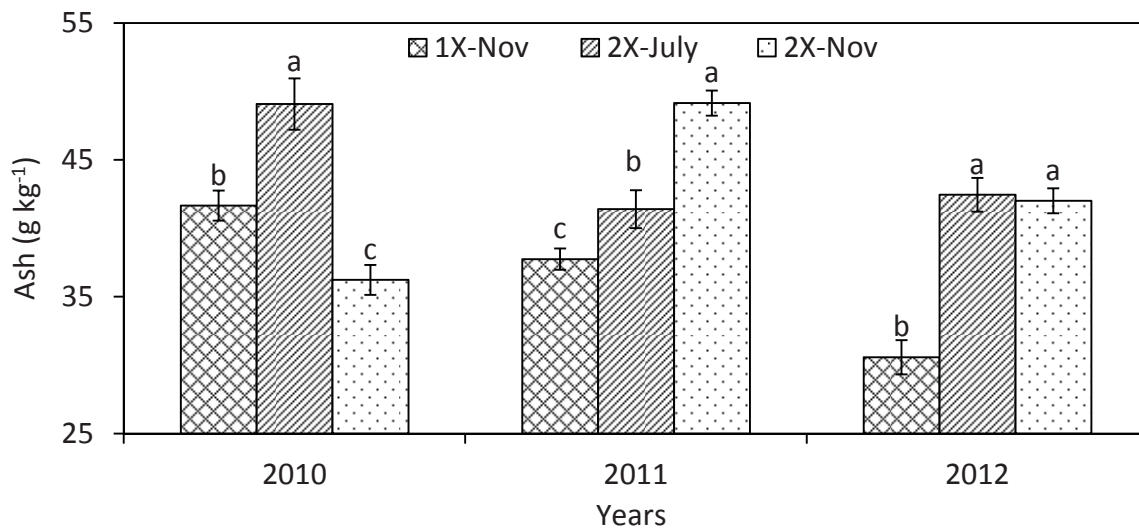


Figure 2.6. Switchgrass ash concentrations as affected by harvest frequencies. Data are averaged over all N treatments. 1X-Nov = one-cut system, November harvest; 2X-July and 2X-Nov = two-cut system, July and November harvests, respectively. Error bars for yields in the 2X system represent the standard errors for total season yields.

increased nutrient uptake due to higher summer rainfall. Lower ash in biomass from the one-cut system may be due both to greater nutrient translocation to storage organs and greater leaching losses which might occur with the more highly-senesced biomass (Parrish and Fike, 2005). Greater ash concentrations reduce oil yield and conversion efficiency, and increase corrosive chemicals and maintenance costs for thermochemical conversion systems (Sanderson et al., 1996). The lower ash concentration in biomass within the one cut was consistent with earlier studies (Mulkey et al., 2006; Waramit et al., 2011), except in 2010 when the ash concentration of November-harvested biomass in the two cuts had the lowest values.

Cellulose

The concentrations of cellulose increased with advanced maturity when harvested in November over three years, although this varied somewhat by year (Figure 2.7). Maximum cellulose concentrations occurred in the November harvest of the two cut system in 2010 but in

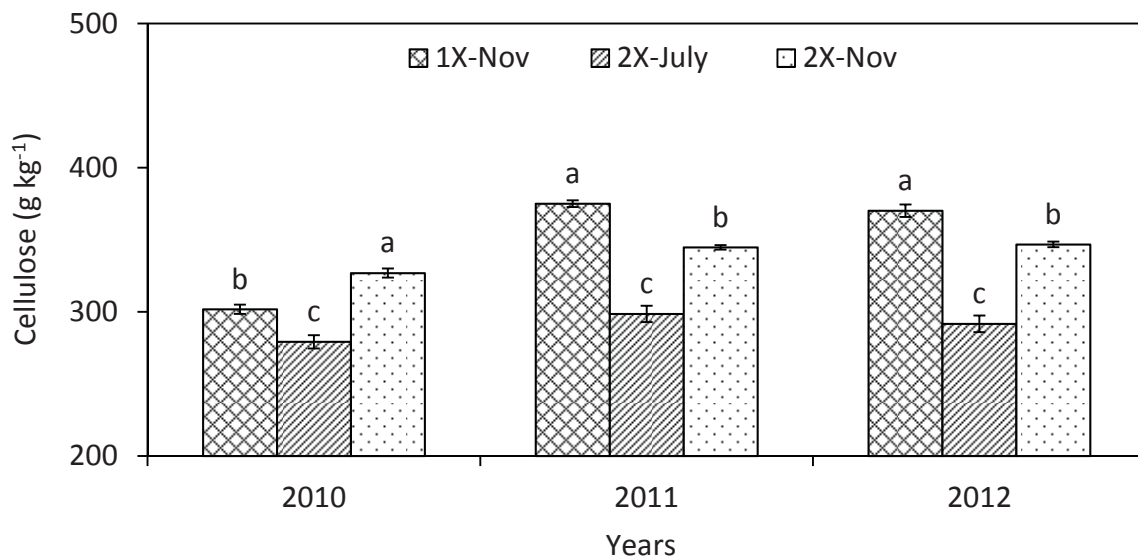


Figure 2.7. Switchgrass cellulose concentrations as affected by harvest frequencies. Data are averaged over all N treatments. 1X-Nov = one-cut system, November harvest; 2X-July and 2X-Nov = two-cut system, July and November harvests, respectively. Error bars for yields in the 2X system represent the standard errors for total season yields.

the one cut in 2011 and 2012. Across years, cellulose concentrations in biomass from the one cut system were 20% and 3% greater than concentrations in the biomass from the July and November harvests of the two cut system (349 vs. 290 and 339 g kg⁻¹).

Hemicellulose

Averaged across years, the hemicellulose concentration in once-cut biomass was 12% and 8% larger than in biomass from the July and November harvests of the two-cut system (309 vs. 276 and 286 g kg⁻¹; Figure 2.12). Differences in hemicellulose concentration were observed between July and November harvests of the two cuts only in 2012. In general, fiber concentrations increase with maturity because as grasses mature, more energy is partitioned to reproductive (stem) development which has greater concentrations of fibers than leaves (Waramit et al., 2011). However, our observations were that the second cut of switchgrass had little if any reproductive development and this would in part account for the limited difference in biomass hemicellulose concentrations observed between harvests in the two cuts.

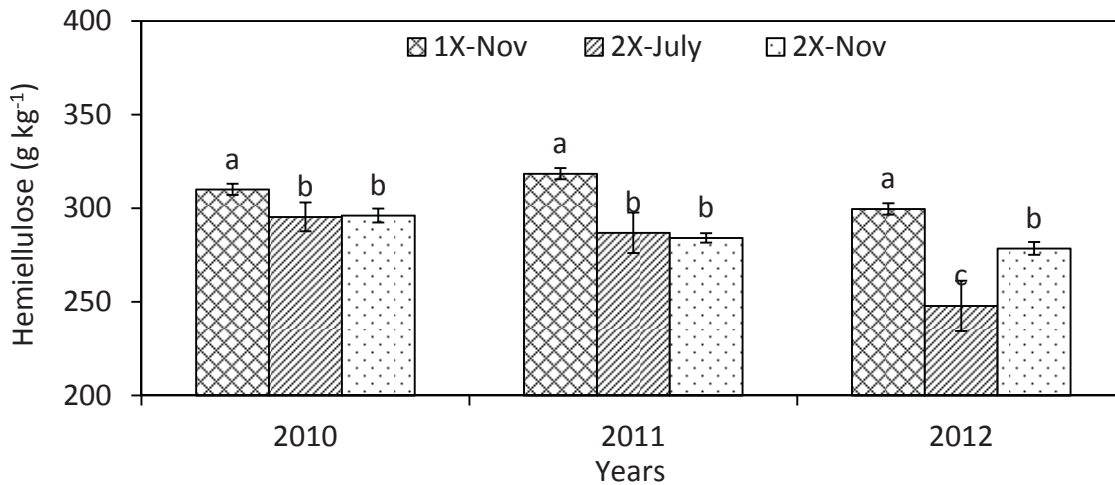


Figure 2.8. Switchgrass hemicellulose concentrations as affected by harvest frequencies. Data are averaged over all N treatments. 1X-Nov = one-cut system, November harvest; 2X-July and 2X-Nov = two-cut system, July and November harvests, respectively. Error bars for yields in the 2X system represent the standard errors for total season yields.

Theoretical Ethanol Potential

Across years the TEP of biomass harvested once in November was 16% and 5% greater than the TEP of biomass from the July and November harvests of the two cuts (479 vs. 412 and 455 L Mg⁻¹; Figure 2.9). Greater concentrations of cellulose and hemicellulose in the biomass harvested in November drove this difference in 2011 and 2012. No differences in TEP between November harvests were observed in 2010. The TEP ranged from 429 to 474 (mean = 449) L Mg⁻¹, which was higher than the 203 to 222 L Mg⁻¹ reported by Vogel et al. (2011) and 380 L Mg⁻¹ reported by Monono et al. (2013).

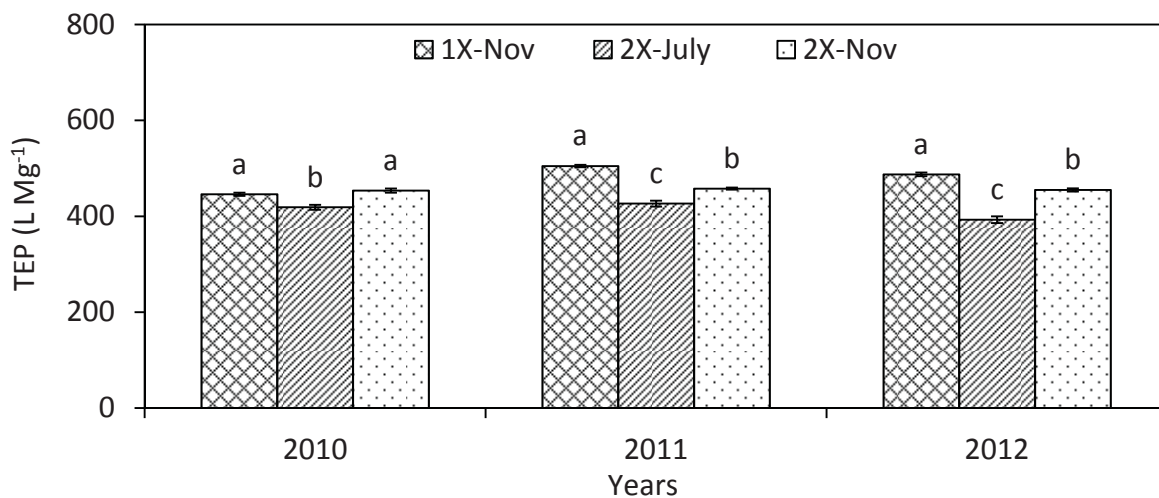


Figure 2.9. Switchgrass theoretical ethanol potential (TEP) as affected by harvest frequencies. Data are averaged over all N treatments. 1X-Nov = one-cut system, November harvest; 2X-July and 2X-Nov = two-cut system, July and November harvests, respectively. Error bars for yields in the 2X system represent the standard errors for total season yields.

Theoretical Ethanol Yield

Figure 2.10 shows the best-fit quadratic responses TEY to initial biosolids and urea N rates. Biosolids application increased TEY over the control by 38% (4440 vs. 3220 L ha⁻¹) across years (Figure 2.10). In 2010, TEY at the highest N rate was 48% greater than the control (2340 vs. 1710 L ha⁻¹), but no differences were found among biosolids treatments and urea. Across all

biosolids rates, TEY was increased by 630 L ha⁻¹ (37%) relative to control (1440 L ha⁻¹) in 2010. On average, biosolids increased TEY by 1580 L ha⁻¹ (41%) and 1430 L ha⁻¹ (34%) in 2011 and

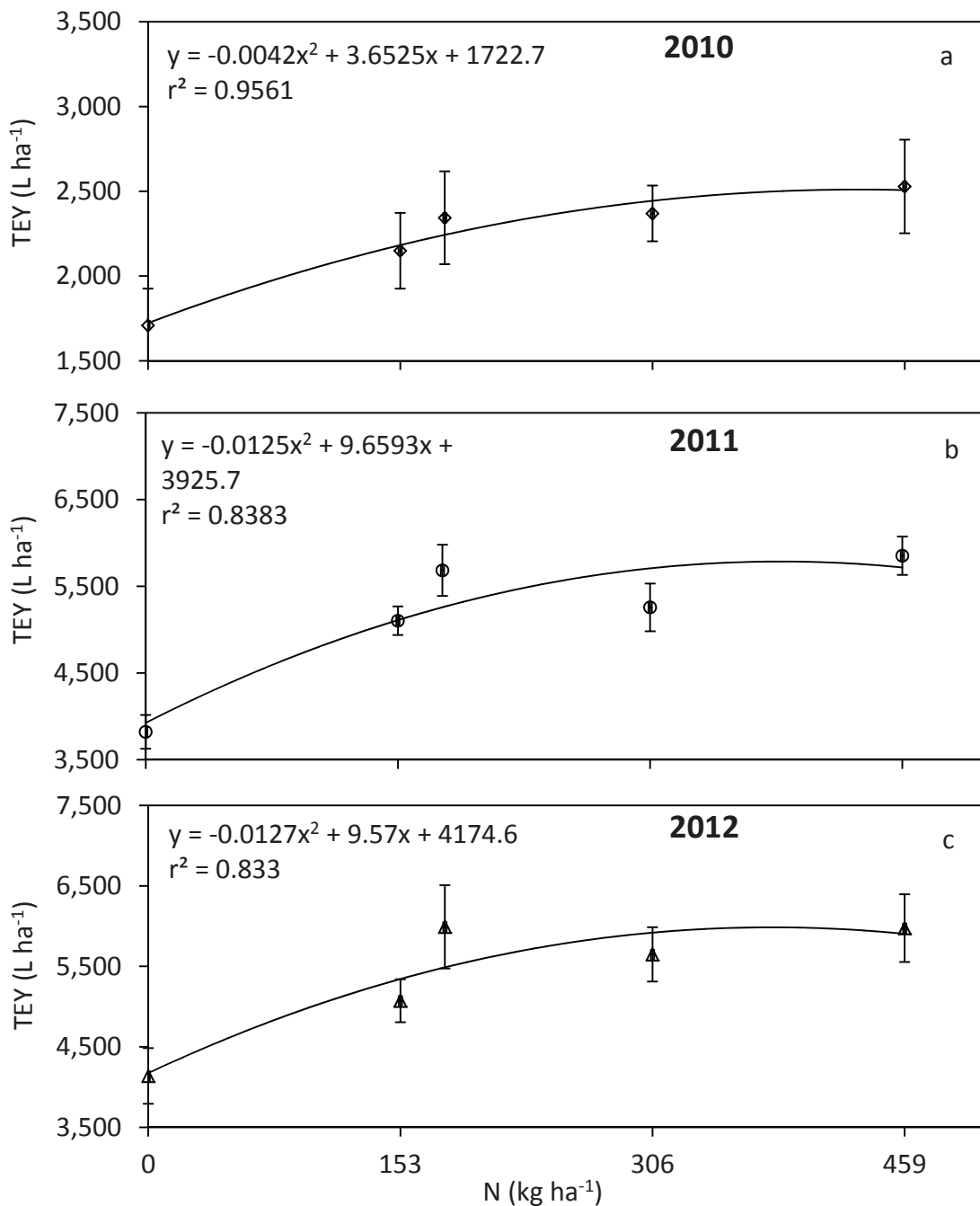


Figure 2.10. Switchgrass theoretical ethanol potential yield (TEY) in 2010 (a), 2011 (b), and 2012 (c) as affected by the initial four biosolids rates of 0, 153, 306 and 459 kg N ha⁻¹ and urea rates of 180 kg N ha⁻¹. Data are averaged over two cutting frequencies in each year.

2012 over the control, respectively. Across the later two years, Urea increased TEY by 1860 L ha⁻¹ (47%) relative to the control (3820 L ha⁻¹).

Averaged across years and N treatments, the TEY with a single harvest was about 11% greater (452 L ha⁻¹) than the TEY from two harvests (4020 L ha⁻¹; Figure 2.11). The TEY in 2010 was < 50% of the mean of 2011 and 2012 TEY, largely due to the lower biomass production in 2010 (5.1 vs. 12.1 Mg ha⁻¹). Cutting once increased TEY by 563 L ha⁻¹ (29%) and 712 L ha⁻¹ (14%) relative to the TEY of two cuts in 2010 and 2012, while no differences in TEY between harvest frequencies were found in 2011. Although harvesting once resulted in greater TEP (Figure 2.14) than the two-cut system in 2011, this was offset by lower biomass yield (Figure 2.2), and thus TEY was not different between the two harvest frequencies in 2011. However, feedstock quality plays the determinant role in TEY when biomass yields are not different between harvest frequencies.

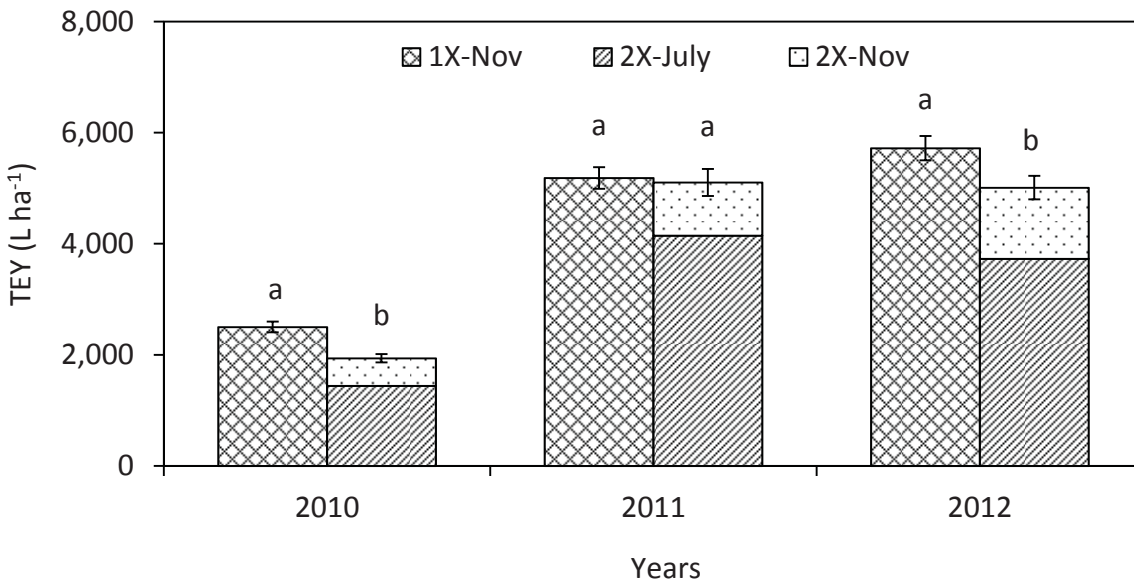


Figure 2.11. Switchgrass theoretical ethanol yield (TEY) as affected by harvest frequencies. Data are averaged over all N treatments. 1X-Nov = one-cut system, November harvest; 2X-July and 2X-Nov = two-cut system, July and November harvests, respectively. Error bars for yields in the 2X system represent the standard errors for total season yields.

Maximizing biomass cellulose, hemicellulose, and lignin and minimizing ash concentrations is thought the best way to maximize biofuel production through thermochemical conversion systems (Adler et al., 2006; Waramit et al., 2011). However, this may have detrimental effects on biochemical processes given that greater lignin concentration reduces the availability of cellulose and hemicellulose to saccharification and subsequent fermentation processes (Fu et al., 2011; Sarath et al., 2011). Adler et al. (2006) reported switchgrass ash and other mineral nutrients concentrations decreased, while fiber concentrations increased in spring compared to fall harvest. Biomass harvested in fall contained less ash and was of superior feedstock quality compared with switchgrass harvested in summer (Mulkey et al., 2006). The mean TEY was 4240 L ha⁻¹ in this study, which was higher than the 3740 and 4000 L ha⁻¹ reported by (Adler et al., 2006) and (Varvel et al., 2008) but lower than the switchgrass TEY of 6000 L ha⁻¹ (Vogel et al., 2011) and 4800 L ha⁻¹ (Monono et al., 2013) reported in other recent studies.

Summary

Overall, biosolids and urea application increased switchgrass biomass yield, N concentration, and theoretical ethanol yield (TEY) for three years. Biosolids applied at rates between 306 and 459 kg N ha⁻¹ would provide comparable biomass yield as urea applied at 180 kg N ha⁻¹. However, the two highest biosolids rates had greater N concentrations in biomass than urea after the third year, indicating that slow-releasing organic N from biosolids was still mineralizing. Biosolids had no detrimental effects on these measures of feedstock quality, suggesting TEY in response to biosolids was mainly a function of increased biomass yield. These data suggest that biosolids could be applied on an N-fertilizer basis at 150 kg N ha⁻¹ to achieve TEY comparable to higher biosolids rates or urea at 180 kg N ha⁻¹.

Cutting twice per year may increase biomass yield, but a single harvest in late fall could maximize concentrations of lignocellulose, and thus theoretical ethanol potential, while minimizing N and ash concentrations in switchgrass grown for biofuel production in Virginia. However, the benefits of single-harvest management for switchgrass feedstock quality must be

measured against the greater yields of lower-quality biomass obtained in a two cuts. To more fully evaluate harvest management implications will require a comparison of switchgrass production and quality in whole systems, including the logistics supply and biomass-to-biofuel conversion processes.

References

- Adler P.R., Sanderson M.A., Boateng A.A., Weimer P.J., Jung H.-J.G. (2006) Biomass yield and biofuel quality of switchgrass harvested in fall or spring. *Agronomy Journal* 98:1518-1525.
- Allison G.G., Morris C., Lister S.J., Barraclough T., Yates N., Shield I., Donnison I.S. (2012) Effect of nitrogen fertiliser application on cell wall composition in switchgrass and reed canary grass. *Biomass and Bioenergy* 40:19-26.
- Anderson W.F., Dien B.S., Jung H.-J.G., Vogel K.P., Weimer P.J. (2010) Effects of forage quality and cell wall constituents of Bermuda grass on biochemical conversion to ethanol. *Bioenergy Research* 3:225-237.
- Anex R.P., Lynd L.R., Laser M.S., Heggenstaller A.H., Liebman M. (2007) Potential for enhanced nutrient cycling through coupling of agricultural and bioenergy systems. *Crop Science* 47:1327-1335.
- Ashworth A.J. (2010) Biomass production and nutrient removal of switchgrass as a bioenergy feedstock, *Crop, Soil, and Environmental Science*, University of Arkansas. pp. 82.
- Brown S., Leonard P. (2004) Building carbon credits with biosolids recycling. *BioCycle* 45:25-29.
- Christie P., Easson D.L., Picton J.R., Love S.C.P. (2001) Agronomic value of alkaline stabilized sewage biosolids for spring barley. *Agronomy Journal* 93:144-151.
- Clark R.B. (2002) Differences among mycorrhizal fungi for mineral uptake per root length of switchgrass grown in acidic soil. *Journal of Plant Nutrition* 25:1753-1772.
- Coulston J.W., Riitters K.H., Smith G.C. (2004) A preliminary assessment of the Montreal process indicators of air pollution for the United States. *Environmental Monitoring and Assessment* 95:57-74.
- Cundiff J.S., Fike J.H., Parrish D.J., Alwang J. (2009) Logistic constraints in developing dedicated large-scale bioenergy systems in the southeastern United States. *Journal of Environmental Engineering* 135:1086-1096.
- Dien B.S., Jung H.-J.G., Vogel K.P., Casler M.D., Lamb J.F.S., Iten L., Mitchell R.B., Sarath G. (2006) Chemical composition and response to dilute-acid pretreatment and enzymatic

- saccharification of alfalfa, reed canarygrass, and switchgrass. *Biomass and Bioenergy* 30:880-891.
- Evanylo G.K., Abaye A.O., Dundas C., Zipper C.E., Lemus R., Sukkariyah B., Rockett J. (2005) Herbaceous vegetation productivity, persistence, and metals uptake on a biosolids-amended mine soil. *Journal of Environmental Quality* 34:1811-1819.
- Fike J.H., Parrish D.J., Wolf D.D., Balasko J.A., Green J.T., Rasnake M., Reynolds J.H. (2006a) Long-term yield potential of switchgrass-for-biofuel systems. *Biomass and Bioenergy* 30:198-206.
- Fike J.H., Parrish D.J., Wolf D.D., Balasko J.A., Green J.T., Rasnake M., Reynolds J.H. (2006b) Switchgrass production for the upper southeastern USA: Influence of cultivar and cutting frequency on biomass yields. *Biomass and Bioenergy* 30:207-213.
- Fu C., Mielenz J.R., Xiao X., Ge Y., Hamilton C.Y., Miguel Rodriguez J., Chen F., Foston M., Ragauskas A., Bouton J., Dixon R.A., Wang Z.-Y. (2011) Genetic manipulation of lignin reduces recalcitrance and improves ethanol production from switchgrass. *PNAS* 108:3803-3808.
- Ghimire S.R., Charlton N.D., Bell J.D., Krishnamurthy Y.L., Craven K.D. (2011) Biodiversity of fungal endophyte communities inhabiting switchgrass (*Panicum virgatum* L.) growing in the native tallgrass prairie of northern Oklahoma. *Fungal Diversity* 47:19-27.
- Gilmour J.T., Cogger C.G., Jacobs L.W., Evanylo G.K., Sullivan D.M. (2003) Decomposition and plant-available nitrogen in biosolids: laboratory studies, field studies, and computer simulation. *Journal of Environmental Quality* 32:1498-1507.
- Goff B.M., Moore K.J., Fales S.L., Heaton E.A. (2010) Double-cropping sorghum for biomass. *Agronomy Journal* 102:1586-1592.
- Gunderson C.A., Davis E., Jager Y., West T.O., Perlack R.D., Brandt C.C., Wullschleger S.D., Baskaran L.M., Webb E., Downing M. (2008) Exploring potential US switchgrass production for lignocellulosic ethanol, in: ORNL. (Ed.).
- Guo M., Song W., Kazda R. (2012) Fertilizer value of lime-stabilized biosolids as a soil amendment. *Agronomy Journal* 104:1679-1686.
- Guretzky J.A., Biermacher J.T., Cook B.J., Kering M.K., Mosali J. (2011) Switchgrass for forage and bioenergy: harvest and nitrogen rate effects on biomass yields and nutrient composition. *Plant and Soil* 339:69-81.
- Haile-Mariam S., Collins H.P., Higgins S.S. (2008) Greenhouse gas fluxes from an irrigated sweet corn (*Zea mays* L.)-potato (*Solanum tuberosum* L.) rotation. *Journal of Environmental Quality* 37:759-771.
- Haque M., Eppin F.M., Taliaferro C.M. (2009) Nitrogen and harvest frequency effect on yield and cost for four perennial grasses. *Agronomy Journal* 101:1463-1469.

- Hargreaves J.C., Adl M.S., Warman P.R. (2008) A review of the use of composted municipal solid waste in agriculture. *Agriculture, Ecosystems and Environment* 123:1-14.
- Kelly J.J., Favila E., Hundal L.S., Marlin J.C. (2007) Assessment of soil microbial communities in surface applied mixtures of Illinois River sediments and biosolids. *Applied Soil Ecology* 36:176-183.
- Ker K., Seguin P., Driscoll B.T., Fyles J.W., Smith D.L. (2012) Switchgrass establishment and seeding year production can be improved by inoculation with rhizosphere endophytes. *Biomass and Bioenergy* 47:295-301.
- Kowalenko C.G., Bittman S. (2000) Within-season grass yield and nitrogen uptake, and soil nitrogen as affected by nitrogen applied at various rates and distributions in a high rainfall environment. *Canadian Journal of Plant Science* 80:287-301.
- Lee D.K., Owens V.N., Doolittle J.J. (2007) Switchgrass and soil carbon sequestration response to ammonium nitrate, manure, and harvest frequency on conservation reserve program land. *Agronomy Journal* 99:462-468.
- Lemus R., Brummer E.C., Burras C.L., Moore K.J., Barker M.F., Molstad N.E. (2008a) Effects of nitrogen fertilization on biomass yield and quality in large fields of established switchgrass in southern Iowa, USA. *Biomass and Bioenergy* 32:1187-1194.
- Lemus R., Brummer E.C., Moore K.J., Molstad N.E., Burras C.L., Barker M.F. (2002) Biomass yield and quality of 20 switchgrass populations in southern Iowa, USA. *Biomass and Bioenergy* 23:433-442.
- Lemus R., Parrish D.J., Abaye O. (2008b) Nitrogen-use dynamics in switchgrass grown for biomass. *Bioenergy Research* 1:153-162.
- Madakadze I.C., Stewart K., Peterson P.R., Coulman B.E., Smith D.L. (1999a) Switchgrass biomass and chemical composition for biofuel in eastern Canada. *Agronomy Journal* 91:696-701.
- Madakadze I.C., Stewart K.A., Peterson P.R., Coulman B.E., Smith D.L. (1999b) Cutting frequency and nitrogen fertilization effects on yield and nitrogen concentration of switchgrass in a short season area. *Crop Science* 39:552-557.
- McLaughlin S.B., Kszos L.A. (2005) Development of switchgrass (*Panicum virgatum*) as a bioenergy feedstock in the United States. *Biomass and Bioenergy* 28:515-535.
- Monono E.M., Nyren P.E., Berti M.T., Pryor S.W. (2013) Variability in biomass yield, chemical composition, and ethanol potential of individual and mixed herbaceous biomass species grown in North Dakota. *Industrial Crops and Products* 41:331-339.
- Muir J.P., Sanderson M.A., Ocumpaugh W.R., Jones R.M., Reed R.L. (2001) Biomass production of 'Alamo' switchgrass in response to nitrogen, phosphorus, and row spacing. *Agronomy Journal* 93:896-901.

- Mulkey V.R., Owens V.N., Lee D.K. (2006) Management of switchgrass-dominated Conservation Reserve Program lands for biomass production in South Dakota. *Crop Science* 46:712-720.
- Ojeda G., Alcañiz J.M., Ortiz O. (2003) Runoff and losses by erosion in soils amended with sewage sludge. *Land Degradation & Development* 14:563-573.
- Overman A.R. (1995) Coupling among applied, soil, root, and top components for forage crop production. *Communications in Soil Science and Plant Analysis* 26:1179-1202. DOI: Doi 10.1080/00103629509369363.
- Parrish D.J., Fike J.H. (2005) The biology and agronomy of switchgrass for biofuels. *Critical Reviews in Plant Sciences* 24:423-459.
- Reynolds J.H., Walker C.L., Kirchner M.J. (2000) Nitrogen removal in switchgrass biomass under two harvest systems. *Biomass and Bioenergy* 19:281-286.
- Robinson M.B., Polglase P.J. (2000) Volatilization of Nitrogen from Dewatered Biosolids. *Journal of Environmental Quality* 29:1351-1355.
- Sanderson M.A., Reed R.L., McLaughlin S.B., Wullschleger S.D., Conger B.V., Parrish D.J., Wolf D.D., Taliaferro C., Hopkins A.A., Ocumpaugh W.R., Hussey M.A., Read J.C., Tischler C.R. (1996) Switchgrass as a sustainable bioenergy crop. *Bioresource Technology* 56:83-93.
- Sarath G., Dien B., Saathoff A.J., Vogel K.P., Mitchell R.B., Chen H. (2011) Ethanol yields and cell wall properties in divergently bred switchgrass genotypes. *Bioresource Technology* 102:9579-9585.
- Schroeder-Moreno M.S., Greaver T.L., Wang S., Hu S., Rufty T.W. (2012) Mycorrhizal-mediated nitrogen acquisition in switchgrass under elevated temperatures and N enrichment. *GCB Bioenergy* 4:266-276.
- Sigua G.C., Adjei M.B., Rechcigl J.E. (2005) Cumulative and residual effects of repeated sewage sludge applications: forage productivity and soil quality implications in South Florida, USA. *Environ Sci & Pollut Res* 12:80-88.
- Stout W.L., Staley T.E., Shaffer J.A., Jung G.A. (1991) Quantitative effects of soil depth and soil and fertilizer nitrogen on nitrogen uptake by tall fescue and switchgrass. *Communications in Soil Science and Plant Analysis* 22:1647-1660.
- Trocsanyi Z.K., Fieldsend A.F., Wolf D.D. (2009) Yield and canopy characteristics of switchgrass (*Panicum virgatum* L.) as influenced by cutting management. *Biomass and Bioenergy* 33:442-448.
- VADCR. (2005) Virginia nutrient management standards and criteria, Richmond, VA.
- Varvel G.E., Vogel K.P., Mitchell R.B., Follett R.F., Kimble J.M. (2008) Comparison of corn and switchgrass on marginal soils for bioenergy. *Biomass and Bioenergy* 32:18-21.

- Vogel K.P., Brejda J.J., Walters D.T., Buxton D.R. (2002) Switchgrass biomass production in the Midwest USA: harvest and nitrogen management. *Agronomy Journal* 94:413-420.
- Vogel K.P., Dien B.S., Jung H.G., Casler M.D., Masterson S.D., Mitchell R.B. (2011) Quantifying actual and theoretical ethanol yields for switchgrass strains using NIRS analyses. *Bioenergy Research* 4:96-110.
- Waramit N., Moore K.J., Heggenstaller A.H. (2011) Composition of native warm-season grasses for bioenergy production in response to nitrogen fertilization rate and harvest date. *Agronomy Journal* 103:655-662.
- Welbaum G.E., Sturz A.V., Dong Z., Nowak J. (2004) Managing soil microorganisms to improve productivity of agro-ecosystems. *Critical Reviews in Plant Sciences* 23:175-193.
- Xu L., Furtaw D.M., Madsen R.A., Garcia R.L., Anderson D.J., McDermitt D.K. (2006) On maintaining pressure equilibrium between a soil CO₂ flux chamber and the ambient air. *Journal of Geophysical Research* 111:1-14.

CHAPTER THREE. Conclusions

Biosolids proved an effective fertility source for increasing yields of switchgrass managed for bioenergy production. However, yield responses to biosolids were less than expected relative to commercial (urea) fertilizer, suggesting that losses were greater or that estimated plant available N (PAN) was less than predicted based on current models of N availability. Applying large amounts of biosolids on an infrequent basis also may have negative environmental consequences if much of this N is lost from the production system.

Cutting twice per year sometimes increased biomass yield, but this appeared dependent on sufficient rain during the growing season. A single harvest in late fall more often minimized ash and maximized lignocellulose concentrations – and thus boosted liters of theoretical ethanol per Mg biomass (theoretical ethanol potential, TEP). The theoretical ethanol yield (TEY) was largely affected by biomass yield rather than feedstock quality parameters. However, the yield gains sometimes seen with two harvests generally did not compensate for the lower feedstock quality, particularly in feedstock harvested in July, thus TEY was similar or lower in the two-harvest system over the three years of study. Although logistics issues were not considered, from a stand management perspective, data from this study provide support for once-per-year harvest management in order to achieve superior feedstock quality and greater theoretical ethanol yield in drier than normal growing seasons.