NITROGEN UPTAKE AND BIOMASS AND ETHANOL YIELD OF BIOMASS CROPS AS FEEDSTOCK FOR BIOFUEL

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Robynn Elizabeth Anfinrud

In Partial Fulfillment
for the Degree of
MASTER OF SCIENCE

Major Department: Plant Sciences
Major Program: Plant Sciences

May 2012

Fargo, North Dakota

North Dakota State University
Graduate School

NITROGEN UPTAKE AND BIOMASS AND ETHANOL YIELD OF

BIOMASS CROPS AS FEEDSTOCK FOR BIOFUEL

By

Robynn Elizabeth Anfinrud

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

Plant Sciences

SUPERVISORY COMMITTEE:

Dr. Marisol Berti
Chair

Dr. Larry Cihacek

Dr. Edward Deckard

Dr. Burton Johnson

Approved by Department Chair:

7/9/2012
Date

Dr. Richard Horsley
Signature

ABSTRACT

Nitrogen fertilizers are extensively used to enhance the growth of biomass crops. This study was conducted to determine the effect of N rates on the biomass yield and quality, and N uptake of several crops. The experiment was conducted at Fargo and Prosper, ND, in 2010 and 2011. The crops studied were forage sweet sorghum [*Sorghum bicolor* L. Moench], sorghum x sudangrass [*Sorghum bicolor* var. *sudanense* (Piper) Stapf.], kenaf [*Hibiscus cannabinus* L.], and reed canarygrass [*Phalaris arundinacea* L.]. The different crops constituted the main plots and the nitrogen rates were regarded as subplots. The five N rates were 0, 75, 100, 150, and 200 kg N ha^{-1}.

Forage sweet sorghum and sorghum x sudangrass had the greatest dry matter biomass yield. Nitrogen fertilization increased biomass yield for each of the crops. The results indicate that forage sorghum and sorghum x sudangrass have the greatest potential as a feedstock.

ACKNOWLEDGMENTS

First and foremost, I would like to thank my advisor, Dr. Marisol Berti, from the bottom of my heart, for her continuous support and encouragement as I conducted my research. Her knowledge, expertise, patience, wisdom, strength, and faith gave me the courage to present my research at a number of meetings and to complete my thesis. I always felt welcome to come into her office, to ask questions or to talk about life. I could have never imagined having a more wonderful advisor; she is a blessing from the Lord.

I would also like to thank my committee members, Dr. Larry Cihacek, Dr. Edward Deckard, and Dr. Burton Johnson, for their time and for bringing in their expertise to guide me in my research. Their encouragement is invaluable. To the entire Plant Sciences Department, faculty and staff: thank you for the opportunity to complete my Master's degree.

I want to extend my deepest gratitude to the entire Forages and Biomass team. Many thanks to Bob Nudell for his expertise and encouragement as I learned about forage production. To my graduate student coworkers, Dulan Samarappuli, Osvaldo Teuber, and Alfredo Aponte: thank you for being my "big brothers," for your friendship and the laughter we shared.

Finally, I would like to thank my family and friends whose support has meant the most. Without them, I couldn't have finished this thesis. To mom, dad, and my sister, thanks for feeding me, loving me, and always encouraging me. To my brother, thanks for our weekly lunch dates, it's been fun being a Bison with you. And to my fiancé Lance Dillon, thank you for your constant love, support, and encouragement, for being there when I needed you, and giving me a reason to finish my thesis before August 3, 2012. I can't wait to spend the rest of my life with you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

LIST OF APPENDIX FIGURES

INTRODUCTION

In recent years, there has been interest in utilizing the biomass of agricultural crops for sustainable bioenergy production. Ethanol production has more than tripled since the year 2000 and is projected to displace 30 percent of petroleum production in the United States by 2030 with an annual supply of 1.18 billion dry metric tons of biomass. Though switchgrass (*Panicum virgatum* L.) and miscanthus (*Miscanthus* x *giganteus* hybrid) are widely quoted to be the most promising energy crops, many have reservations about their widespread utilization. Though these crops have been found to grow in areas unsuitable for food crop production, recent research suggests that these energy crops still require nitrogen fertilizer inputs to achieve greater yields.

The use of N fertilizers to enhance the growth and yield of crops is extensive. In the United States (US), almost 107 million hectares of crops, pasture, and range land were treated with commercial N fertilizers in 2007. In North Dakota, 7.4 million hectares were treated with fertilizers out of the 18 million hectares in the state. Extensive fertilizer use poses a risk for NO_3-N to leach into the water table and contaminate the ground water.

Research into using forage/fiber crops as potential feedstock for biofuel is gaining attention as they have multiple purposes and can obtain greater biomass yields than perennial grasses. Introducing forage biomass crops into an existing crop rotation adds diversification to the land, has a positive impact on soil health, and is a relatively easy process. Forage crops are ranked number one both in world production and in 18 states in the United States. Range and hay land accounts for 5.3 million hectares out of the 18 million hectares in North Dakota. The movement towards greater sustainability in agriculture has led to increased research and development in determining ways to use less agricultural chemicals, including synthetic fertilizers and to improve soil health.

Optimizing the use of fertilizers is important for maximum uptake by the plant with minimum residual NO_3-N loss from the root zone. Research is being conducted to study N fertility and the optimum application rate for maximum yield and growth of forage crops.

Forage sorghum (*Sorghum bicolor* L. Moench), sudangrass (*Sorghum sudanense* (Piper.) Stapf.), and sorghum x sudangrass hybrids are grown for their high quality silage, hay, and grazing properties. Reed canarygrass (*Phalaris arundinacea* L.) is grown for silage, hay, and grazing. Kenaf (*Hibiscus cannabinus* L.) is grown mainly as a fiber crop and for animal forage. The purpose of this study is to determine which annual forage/fiber crop produces the most biomass for bioenergy with the most economic rate of N fertilizer.

LITERATURE REVIEW

The emphasis on renewable energy sources has increased the value of research for dedicated energy crops that can be utilized in biofuel production. Long term energy security has been stated to come with increasing diversification of energy supplies (Gehlhar et al., 2010). To accomplish this, new technology is required to efficiently produce biofuels at levels that will have an economic impact and increase competitiveness with petroleum-based fuels. The Environmental Protection Agency (EPA) created and enforced the Renewable Fuel Standard (RFS) as part of the Energy Policy Act of 2005 (EPA, 2007). The goal of the RFS is to increase the volume of renewable fuel that is blended into transportation fuel, from 34 million m^3 of renewable fuel in 2008 to 136 million m^3 of renewable fuel by 2022. The RFS program was recently expanded to include biodiesel, establish new categories of renewable fuels, and to apply standards to ensure that the greenhouse gases emitted from renewable fuels are less than the petroleum it's replacing (EPA, 2007). Four separate standards include producing 60 million m^3 of cellulosic biofuel by 2022 and 3.8 million m^3 of biomass-based diesel by 2012, contributing to 79.5 million m^3 of advanced biofuel by 2022, and 136 million m^3 of total renewable fuel by 2022. This renewable fuel, including ethanol, is produced from cellulosic, hemicellulosic, or lignin components. Bioethanol is believed to be the most promising substitute for fossil energy because it is clean, renewable, and carbon neutral (Zhang et al., 2010). There are some important operations necessary to utilize biomass for energy production.

There are four operations that are required to process lignocellulosic material into ethanol: pretreatment, hydrolysis, fermentation, and purification (Mosier et al., 2005). Pretreatment technology prepares the biomass feedstock for further processing. It makes the cellulose in the

biomass more accessible to the enzymes required to convert the carbohydrates into fermentable sugars for ethanol production (Mosier et al., 2005). The lignin seal must be broken apart so the crystalline structure of the cellulose can be disrupted. Hydrolysis, or saccharification, is done in a series of steps which converts cell wall carbohydrates into monomeric sugars using various enzymes (Faaij, 2006; Mosier et al., 2005). Once sugars are released from the cell wall structure, they can be fermented into ethanol. When hydrolysis is conducted at the same time as fermentation, it is known as simultaneous saccharification and fermentation (SSF). This method is preferred over separate hydrolysis and fermentation as both operations can be done in the same processing tank, lowering the cost of processing. The ethanol is recovered from the fermentation process through distillation, which is the purification process, and the residual lignin, cellulose, hemicellulose, ash, enzymes, and other components are byproducts that can be burned as fuel or used for other applications.

An effective pretreatment avoids the need to reduce the size of the biomass material, preserves the hemicellulosic material as well as the organisms involved in fermentation, decreasing the energy demand in the production of ethanol (Mosier et al., 2005). Pretreatment methods can be either physical or chemical, mechanically reducing the size of the particles, or utilizing solvents, acids, or bases to promote the hydrolysis of the material. One of the most promising pretreatment methods includes ammonia fiber expansion (AFEX) (Li et al., 2010). It is a pretreatment process in which the biomass material is brought in contact with concentrated aqueous or anhydrous ammonia under moderately warm temperatures (80-150°C) and moderate pressure (1379-2758 kPa) for a short amount of time (Bals et al., 2010; 2011). After 5 to 30 minutes, the pressure is released quickly, resulting in the depolymerization of cellulose and the partial solubilization of hemicellulose. The cell wall structure is opened up to allow processing enzymes to enter. The goal

is to optimize fermentable sugar yields from lignocellulosic biomass, thus producing greater amounts of biofuel (Bals et al., 2011). The AFEX pretreatment process is less costly than other methods and due to its efficiency, could increase the amount of ethanol produced and decrease consumer costs (Li et al., 2010).

The technology for converting cellulosic biomass into ethanol on a commercial scale is complicated, as isolating the sugars from the cellulosic material can be a challenging task. A number of companies have been developing methods of converting biomass into ethanol using a variety of feedstocks, such as wheat (*Triticum aestivum* L.) straw, dry grains, and agricultural residues like corn (*Zea mays* L.) stover (Hettenhaus, 2006). Some of these same companies are working to make improvements to the processing technology, utilizing enzymes that break down the cellulosic material in a manner that is more cost effective and investigating other pretreatment options besides the AFEX process.

Biomass is also utilized in energy production through the gasification process. Gasification is the process by which biomass is converted to flue gas and syngas (Faaij, 2006). In this process, biomass, oxygen, and steam are reacted together to produce hydrogen and syngas that is environmentally clean (Digman et al., 2009). Syngas components are cleaned and processed so they are ready for conversion to methanol, hydrogen, or Fischer-Tropsch diesel (Faaij, 2006). So far, commercial production of liquid biofuels through this process is not taking place, though smaller-scale studies are being done with this technology. Currently, gasification of biomass has a low efficiency because it leaves behind tar and char components, requiring further research to identify its useful applications (Digman et al., 2009). Reducing the cost of converting biomass material into ethanol is an important consideration to make. Further biomass conversion cost reductions can be made by improving the quality of the feedstock itself, by increasing the

cellulosic components and reducing lignin to make the process of extracting ethanol more efficient (Lorenz et al., 2009).

The plant cell wall is the primary source of energy in forage feedstocks for bioenergy, yet the cell wall is a barrier to the carbohydrates it holds (Lorenz et al., 2009). Lignocellulosic plant material consists of the components cellulose, hemicellulose, and lignin (Lee et al., 2007; Hendriks and Zeeman, 2009). Cellulose makes up 30 to 50% of the total dry matter of the feedstock, hemicellulose being 20 to 40% of the dry matter, and lignin being 15 to 25% of the feedstock total dry matter.

Research has been done in recent years regarding forage quality components that are desirable for improving animal health and performance (Schroeder, 2004). Digestibility relates to how fast the material passes through the animal's digestive tract with faster passage indicating lower digestibility. Forage quality analysis uses techniques to measure characteristics such as crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid detergent lignin (ADL) (Lorenz et al., 2009). Crude protein is calculated from the nitrogen content of the forage which animals can utilize to some degree (Lorenz et al., 2009; Schroeder, 2004). The component NDF is an estimation of total cell wall components, which includes cellulose, hemicellulose, lignin, and silica. Acid detergent fiber indicates the cellulose and lignin components of the cell wall, so as ADF increases, forage digestibility decreases. The component ADL indicates the lignin component that prevents the digestibility of cellulose and hemicellulose in the forage. Silica is a major component of soils that is accumulated in the cell walls of grasses, increasing ash content and decreasing digestibility of the forage. These values provide useful information when screening the feedstock potential of forage and fiber crops for the conversion of biomass material into biofuel.

One factor that can influence biomass yield potential in plants is the photosynthesis pathway the plant contains, which is either the C3 or the C4 pathway. The CO_2 pressure at the site where Rubisco (Ribulose-1,5-bisphosphate carboxylase oxygenase) is located can be 5 to 10 times higher in C4 plants than in C3 plants, so the rate of photosynthesis and subsequent yield is higher in C4 plants than in C3 at higher temperatures (Lattanzi, 2010). Cool-season crops (C3) do most of their growth during the cooler portions of the growing season and are typically frost tolerant; whereas warm-season crops do most of their biomass growth during the warmer portions of the growing season may not tolerate frost temperatures as well. Perennial crops can overwinter and produce biomass for a number of years as energy is put into the root system during the growing season so the plant can survive the winter months, limiting the above-ground biomass yield of the crop. Annual crops do not overwinter, thus most of the plant's energy is put into above-ground biomass.

The goal of developing a good biomass feedstock is to produce a crop that has consistent composition of desirable quality components, high biomass yield, is economical by requiring lower inputs to provide maximum profit to growers, and can be produced close to the processing site to reduce transportation costs. In terms of quality, there are already methods available to measure these key quality components. Certain forages may have a quality composition that may give them an important place in future bioenergy production (Sanderson et al., 2006; Hettenhaus, 2006). The benefits of utilizing forages in biomass production are that they have the ability to produce high dry matter biomass yield and can survive a wide range of environmental conditions. Other benefits of forage crops are that perennial forages can offer excellent soil holding and decrease erosion; while annual forages can be integrated into traditional agricultural systems. There are also risks involved in utilizing forages for biomass production, which can include high storage dry matter

losses from 5 to 25%, variation in biomass yield from year to year, and high transportation costs. Transportation costs are estimated between $20 and $55 per dry metric ton, collected from a 24-km radius, and transported by short line rail to a processing plant up to 300 km distance (Hettenhaus, 2006; Li et al., 2011).

Sorghum

Sorghum has great potential as an important annual bioenergy crop. Worldwide, it is the fifth most important cereal crop and is a source of both feed and fuel in developing countries (Saballos, 2008; Ottman et al., 2001; Rooney et al., 2007). In 2010, the United States was ranked number one in world grain sorghum production, yielding 8,773,440 Mg total (FAOSTAT, 2012). The USA produces almost 7 million hectares annually of all the different sorghum cultivars including grain, forage, and sweet sorghum combined (Rooney et al., 2007).

Sorghum has the ability to grow in hot, dry environments because of its high water use efficiency and drought tolerance, yet it can also grow in areas of poor soil drainage nearing soil saturation (Saballos, 2008; Rooney et al., 2007). Sorghum has a deep root system that can reach an estimated 1.9 m depth (Robertson et al., 1993), which allows it to obtain water and nutrients from deeper layers of the soil profile and use those nutrients more efficiently (Jordan and Miller, 1980). The seeds are able to germinate at soil temperatures greater than 21°C, but can be planted when the soil temperature is at least 15°C (Saballos, 2008). Sorghum requires only 320 to 400 mm of water per season for optimal production, as compared with corn which requires 460 to 560 mm water per season (NDSU Ext. Serv., 1997). Sorghum is able to grow when planted in soils that are slightly saline (<3 dS m^{-1}), with vegetative yield decreasing at 3 to 7 dS m^{-1}, depending on the cultivar (Francois et al., 1984). Sorghum has a wide range for soil pH, from 6.0 to 7.5, with 6.5 considered optimal (Saballos, 2008; McClure, 2012). Sorghum has similar nutrient requirements as for corn

(McClure, 2012), and when the crop reaches maturity, it contains approximately 340 g kg^{-1} cellulose, 170 g kg^{-1} hemicellulose, and 160 g kg^{-1} lignin in its vegetative composition (Lee et al., 2007). Sorghum has high yield potential, with averages of 16 Mg ha^{-1} biomass yield in Ames, IA to 28 Mg ha^{-1} of dry-matter yield in Bushland, TX (Bennett and Anex, 2009; Rooney et al., 2007).

There is a great amount of genetic diversity in sorghum germplasm, which has created opportunities for continued genetic improvement in the last 50 years, focusing on high yield, improved quality traits, stress tolerance, including disease, drought, insect, and fertility stresses (Saballos, 2008; Rooney et al., 2007). Sorghum has diversity in traits that are important for energy production, such as stem sugar content, lignin and cellulose content, yield for grain or biomass, and drought tolerance. Several cultivars of sorghum have been developed with several of these traits targeted for bioenergy production. With all this in mind, the optimum type of sorghum for biofuel also depends on the process that is used to convert the cellulosic material into ethanol (Rooney et al., 2007).

Though there are many positive aspects of utilizing forage sorghum for bioenergy, there are some drawbacks as well. Sorghum does not grow at lower air temperatures as its base temperature is 10°C, the same as corn. Cool temperatures and poorly drained, wet soils in the first few weeks after planting can slow the process of seed germination and increase the incidence of fungal damping-off diseases of the seedlings (Saballos, 2008).

Sweet Sorghum

Sweet sorghum is a type of sorghum that accumulates high levels of sucrose in its stem and has been grown in areas where sugarcane (*Saccharum officinarum* L.) is unable to be produced (Rooney et al., 2007). It contains a high amount of soluble sugar in the plant sap of the stems, averaging around 7 to 8 Mg total sugar ha^{-1} (Saballos, 2008). Its chemical composition consists of

230 g kg^{-1} cellulose, 140 g kg^{-1} hemicelluloses, and 110 g kg^{-1} lignin of the dry matter (Lee et al., 2007). In a study by Massacci et al. (1996), sucrose and starch levels were found to be significantly higher in drought stressed plant stems as compared with the control. In the Upper Midwest, the dry matter yield of sweet sorghum can reach levels greater than 25 Mg ha^{-1} $year^{-1}$ as found in a study conducted at Iowa State University (Bennett and Anex, 2009). This has generated interest in the production of ethanol from the plant sap to supplement the ethanol production from sugarcane. Sweet sorghum has easily accessible fermentable sugars that can be used directly in ethanol production. Sweet sorghum bagasse, which is the remaining dry crop residue left after the juice is extracted, can also be utilized in bioenergy production (Rooney et al., 2007). Research conducted in China has determined the benefit of using sweet sorghum for the ethanol agro-industrial system (Guo et al., 2010; Zhang et al., 2010). In the study by Zhang et al., (2010) it was demonstrated that if all the common grain sorghum were replaced with sweet sorghum, the productive potentials of sweet sorghum ethanol would satisfy 63.2 to 84.9 % of the total demand for E10 ethanol in China, utilizing the grain and the juice from the sweet sorghum (Agassiz Seed, 2010).

Sorghum x Sudangrass

Hybrids of sorghum and sudangrass for forage production may be viable options for biomass production. Sudangrass (*Sorghum bicolor* var. *sudanense* L.) has thinner stems and tends to be leafier than forage or sweet sorghums, which have thicker stems and have limited regrowth capacity after being cut (Saballos, 2008). Sorghum x sudangrass hybrids have intermediate yield potential, though one goal of current research is to produce better cultivars with higher digestibility and greater daily average weight gain performance in cattle. Sorghum x sudangrass hybrids are adapted to drought, high temperatures, excess soil water, and low soil pH, and can yield anywhere from 7.8 to 11.8 Mg ha^{-1} in a season with a two- or three-cut system (Kilcer et al., 2005).

Sudangrass consists of 330 g kg^{-1} cellulose, 270 g kg^{-1} hemicellulose, and roughly 80 g kg^{-1} ADL, and 120 g kg^{-1} CP (Lee et al., 2007). A study by Beyaert and Roy (2005) found that the yield of forage sorghum x sudangrass was not significantly affected by lower N fertilizer inputs, though fertilizing the crop with up to 100 kg N ha^{-1} was found to achieve optimum biomass yield in sorghum x sudangrass hybrids. Sorghum-sudangrass quality has improved through the production of Brown Midrib (BMR) sorghum, characterized by its lower indigestible lignin content with greater digestibility of the fiber, and has the potential for higher ethanol yield as lignin impedes the conversion of lignocellulosic components into ethanol (Agassiz Seed, 2010; Dien et al., 2009; Producer's Choice Seed, 2007; Kilcer et al., 2005). Sorghum BMR varieties carry the *bmr* gene mutation, which changes how the cellular walls are built and turns the mid-rib of the leaves brown (Dien et al., 2009). Three of these mutations have been produced commercially, such as *bmr*-6, *bmr*-12, and *bmr*-18 and are currently incorporated into commercial sorghum hybrids.

Kenaf

Kenaf is a warm-season crop, C3 broadleaf annual that is utilized for a variety of applications (Danalatos and Archontoulis, 2010). Historically, it has been used as a fiber crop for cordage, and more recently, has been found useful in applications such as in the production of paper products, building materials, and livestock feed (Webber et al., 2002). Two distinctive fibers are harvested from the stalk: a long bast fiber from the bark which can be used to make burlap and the short, spongy core fiber is processed to make poultry bedding, for example (Geisler, 2011). Kenaf has high leaf protein levels, making it usable for livestock feed during the early stages of growth (Nielsen, 2004; Webber et al., 2002), and now may have potential as a biomass crop for bioenergy production. Kenaf can grow between 2- and 6-m in height in just 150 days in the southern United States (Geisler, 2011).

Kenaf grows optimally with adequate moisture, producing high yields (Danalatos and Archontoulis, 2010; Nielsen, 2004) and is adapted to a wide range of climate conditions and geographical locations. The appropriate time for kenaf to be sown is when the air temperature has reached 10°C, or is stabilized above 15°C, as that will support early and uniform seed germination and emergence (Danalatos and Archontoulis, 2010). Kenaf has the ability to tolerate drought conditions for limited periods of time, though the crop originates from tropical regions (Webber et al., 2002). According to Nielsen (2004), kenaf dry matter yield reached 2 Mg ha^{-1} with only 250 mm water use, whereas kenaf yield reached about 5 Mg ha^{-1} with 450 mm of water use during the growing season near Akron, Colorado. A study, conducted in Portugal, also evaluated the productivity of kenaf with different levels of irrigation (Fernando et al., 2004). This particular study also suggested that biomass productivity is greatly affected by irrigation levels. Higher yields between 4500 to 5500 kg ha^{-1} were achieved for plots irrigated with 301- and 401-mm of water, over the growing season.

There is conflicting information in regards to the relationship between harvest date and kenaf yield. Webber and Bledsoe (2002) stated that kenaf yield increased significantly from 60 days after planting (DAP) to 150 DAP, from 5.7 Mg ha^{-1} to 21.0 Mg ha^{-1} total biomass yield. Fernando et al. (2004) found that kenaf has the greatest yield (4000 kg ha^{-1}) at 90 days after sowing, but decreases to around 2500 kg ha^{-1} at 200 days after sowing. The author explained that the decrease in yield is most likely due to the desiccation of non-fiber components of the bark and loss of the leaf components of the plant as the temperature drops (Fernando et al., 2004), or because of lodging (Higgins and White, 1970), though the study by Fernando et al. (2004) was conducted in a warm temperate climate in Portugal where the average minimum temperature from July to January is 15.6°C, and a killing frost was not indicated. A study by Webber and Bledsoe

(2002), which was conducted in Lane, OK, also indicated a decrease in yield where a killing frost was not indicated to have occurred, which could possibly be explained by the translocation of nutrients back to the soil. A kenaf stem dry weight study conducted in Maryland found that maximum yields were obtained for the first harvest after a killing frost, but decreased thereafter (Higgins and White, 1970). In this particular study, higher average temperatures in 1966 than 1967 during the growing season contributed to greater average kenaf yield, from 13.7 Mg ha^{-1} to 13.0 Mg ha^{-1}, respectively.

Kenaf may require some N application to optimize yield (Webber et al., 2002), though if the soil is already rich in N, there could be little yield differences between 0 kg N ha^{-1} and 150 kg N ha^{-1} (Fernando et al., 2004). Soil type may also affect how much N fertilizer is required. Previous research suggests that NO_3-N levels in kenaf plant tissue decreases during the growing season, perhaps due to the dilution effect (Fernando et al., 2004), while biomass yield generally increases with increasing N fertilization (Webber et al., 2002).

Kenaf plant tissue quality components have been explored as well to determine the feasibility of their utilization as a biomass feedstock. Lignin makes up 79 g kg^{-1} of the kenaf dry matter, hemicelluloses makes up 184 g kg^{-1}, and cellulose makes up 532 g kg^{-1} of the dry matter cell wall components of the stem (Amaducci et al., 2000). When harvest was delayed in a study conducted in Northern Italy by Amaducci et al. (2000), kenaf cellulose content increased and hemicellulose decreased.

Reed Canarygrass

Reed canarygrass is another candidate for feedstock for bioenergy. It is considered a C3 crop that is best adapted to low-lying areas where there is short-term flooding or wetter soil conditions (Sheaffer et al., 1990; Sanderson et al., 2006). Reed canarygrass is a perennial crop

typically grown for forage production that can reproduce with either seed or through rhizomes (Saballos, 2008). Aboveground biomass consists of 240 g kg^{-1} cellulose, 360 g kg^{-1} hemicellulose, 20 g kg^{-1} ADL, and 100 g kg^{-1} CP in dry matter (Lee et al., 2007). The most important variable that regulates reed canarygrass establishment is seedling vigor (Casler and Undersander, 2006). Establishment rates are slow, so maximum yield is not achieved until the second or third year of production (Saballos, 2008; Anderson et al., 2008). Reed canarygrass is considered one of the most persistent perennial grasses adapted to the regions of North Dakota and Minnesota due to its exceptional winter hardiness and its ability to maintain yield and high forage quality under seasonal management of multiple cuttings (Sheaffer et al., 1990). Reed canarygrass establishes a profuse, shallow, but extensive root system.

Reed canarygrass is also used to uptake excess NO_3-N from the soil (Sheaffer et al., 1990), although more recent studies have shown that reed canarygrass does not increase NO_3-N accumulation in plant tissue and may have little effect on soil NO_3-N levels (Herr-Turoff and Zedler, 2005). This study showed that the total soil NO_3-N levels were significantly different between the high and the low N-treatment levels at various times during the study. The maximum difference between the high and low N-treatment levels occurred when the high N-treatment resulted in soil N levels of 17.3 mg NH_4-N kg^{-1}, and the low N-treatment level resulted in soil N levels of 6.7 mg NH_4-N kg^{-1}.

The profuse root system of reed canarygrass allows the crop to respond to nitrogen fertilizers with an increase in yield. Herr-Turoff and Zedler (2005) also found that with a high-N treatment (48 g N m^{-2} $year^{-1}$), the above-ground biomass of reed canarygrass was 90% greater than with the low-N treatment (12 g N m^{-2} $year^{-1}$), yielding 458 g $plot^{-1}$ versus 236 g $plot^{-1}$. For reed

canarygrass grown for biomass, there may be evidence of an internal recycling system for nitrogen, though further investigation is required (Sanderson et al., 2006; Partala et al., 2001).

Nitrogen is one of the most important macro-nutrients that plants need for optimal growth and yield (Mengel and Kirkby, 1982). In fact, nitrogen is considered the greatest growth limiting factor that can influence crop function and yield. Fertilizer recommendations for forage production may differ from the recommendations for the production of bioenergy fuels (Sanderson et al., 2006). The yield response from a fertilizer application, the price of the fertilizer, and the cost of the crop all help determine how profitable the crop will ultimately be (Johnson and Ali, 1979). The ultimate goal of fertilization for growers is to make money (Black, 1993). Lower fertilizer levels are more appropriate for biomass production as there is a low price received for the crop, making it more economical for the grower (Partala et al., 2001). Most crop yields increase with the addition of nitrogen fertilizer, but there is a point where the rate of the yield increase decreases at higher fertility levels (Johnson and Ali, 1979). The shape of the curve of the crop's response to nitrogen fertilizer shows how the price of N fertilizer and the price of the crop can determine the most profitable rate of application. Forage crops have yield response curves that level out less abruptly, making them more sensitive to the price of the fertilizer itself, though cultivars may respond differently to the same levels of fertilization as well (Mengel and Kirkby, 1982).

Both the NO_3^- and NH_4^+ forms of nitrogen can be taken up by plants, yet there may be a point when too much fertilizer has been applied to the soil and the plants are unable to take it all up, resulting in leaching into groundwater or denitrification into the atmosphere (Mengel and Kirkby, 1982). This can both hurt the environment and be a waste of money. When nitrogen fertilizer is limited, but applied in adequate amounts for crop uptake, the plants are better able to actively take up the nitrogen and use it more efficiently, reducing the cost of fertilizer. Nitrogen

use efficiency (NUE) refers to the proportion of nitrogen applied that the crops are able to take up (Mengel and Kirkby, 1982).

The most economic rate of fertilizer could mean one of two things: the rate that brings about maximum crop yield, or the rate that brings about the greatest economic return (Black, 1993). Both of these factors must be considered in crop production. The maximum net profit from fertilization input is reached when the value of the crop exceeds the total cost of fertilization. Thus, NUE is calculated along with agronomic and physiological efficiencies to determine the most optimum economic rate of fertilization. When determining the crop most suitable for the cheapest biomass feedstock production, economics must be considered, particularly for nitrogen fertilization.

CHAPTER 1. FORAGE SORGHUM AND KENAF BIOMASS YIELD RESPONSE TO NITROGEN FERTILIZATION

ABSTRACT

There is increasing interest in developing the technology to provide a constant supply of biomass for bioenergy production. Forage sweet sorghum (*Sorghum bicolor* L. Moench), sorghum x sudangrass (*Sorghum bicolor* var. *sudanense* (Piper.) Stapf.), and kenaf (*Hibiscus cannabinus* L.) were studied for their potential as feedstock for biofuel production as they can be integrated into existing cropping systems. They were evaluated for their response to different rates of nitrogen in their subsequent biomass output and quality components levels in four environments, Fargo and Prosper, ND, in 2010 and 2011 and at three growth stages (V8, R1, and harvest). The RCBD split-plot arrangement with three replicates was used, with the crops as the main plots, and N rates as the subplots. Results indicated that forage sweet sorghum and sorghum x sudangrass produced between 14 and 15 Mg ha^{-1} of dry matter biomass averaged across fertility rates, while kenaf yield was significantly lower at 7.4 Mg ha^{-1}. There was a significant response in biomass yield with increasing N fertility rates. Ash content for all three crops decreased significantly at the later growth stages, decreasing from 119 mg kg^{-1} at the earliest growth stage down to 79 mg kg^{-1} at harvest. The levels of neutral detergent fiber (NDF), acid detergent fiber (ADF), and hemicellulose increased at later growth stages for each crop. Kenaf had the greatest increase in NDF, from 238 mg kg^{-1} at V8 and increasing significantly to 583 mg kg^{-1} at harvest. Kenaf also had the greatest increase in ADF, from 156 mg kg^{-1} at V8 to 441 mg kg^{-1} at harvest. Forage sweet sorghum and sorghum x sudangrass both had higher NDF, ADF, and hemicellulose levels than kenaf at each growth stage. Sorghum may serve as a feedstock for bioenergy production.

INTRODUCTION

Bioenergy and biomass research has increased dramatically since the year 2000 and is predicted to continue to grow due to the higher cost of energy and the increasing demand of a limited supply of oil and natural gas. Currently, the main source of bioethanol comes from corn (*Zea mays* L.), yet there is concern about how effective this will be as a long-term energy source as it conflicts with the price of food and animal feed.

Annual forage crops, however, can be integrated into previous cropping systems and require minimal inputs to achieve maximum yield in a shorter period of time. The movement towards greater sustainability in agriculture has created more interest in utilizing annual crops as feedstock for biofuel production. Both educational and private industries are working together to reach the goal that the Renewable Fuel Standard (RFS) set forth: to produce 136 million m^3 of renewable fuel by 2022 (EPA, 2007). This fuel can come from cellulosic, hemicellulosic, or lignin components when processed accordingly. The goal of developing a biomass feedstock is to identify the crop with the greatest amount of dry matter yield, offer some level of drought tolerance, require minimal inputs and management costs, desirable quality components so the feedstock can be converted to bioenergy efficiently, and can be grown geographically close to processing site to reduce transportation costs (Hettenhaus, 2006). Annual crops such as sweet sorghum, sorghum x sudangrass hybrids, and kenaf may be able to serve as biomass feedstocks.

Sorghum has great potential as an important annual bioenergy crop. Sorghum is a C4 crop that has a deep root system, giving it the ability to obtain water and nutrients from deeper soil layers (Saballos, 2008; Rooney et al., 2007; Robertson et al., 1993). The crop requires only 320 to 400 mm of water per season for optimal yield production, as compared with corn which requires

460 to 560 mm of water per season (NDSU Ext. Serv., 1997). The root system of sorghum also gives it greater efficiency at utilizing nutrients from the soil (Saballos, 2008).

Nitrogen is one of the most important, but the most limiting growth factors that affect plant development, including sorghum (*Sorghum bicolor* L. Moench) (Mengel and Kirkby, 1982). Nitrogen is a constituent of a number of amino acids, amides, proteins, and nucleic acids (Taiz and Zeiger, 2006). There is a relationship between the concentration of nutrients in plant tissue and the yield of the crop. For example, when there is insufficient N fertilization, the concentration of nitrate will drop dramatically in the plant tissue and the crop will exhibit deficiency symptoms with a decrease in yield. When adequate amounts of soil nitrogen are available, the concentration of plant nitrate gives the crop the ability to achieve its maximum yield. Likewise, when there is too much N available for plant uptake, the nitrate content in the plant tissue reaches excessive or toxic levels, causing a decrease in plant yield. The use of nitrogen fertilizers is quite extensive in the United States, with the over-application of fertilizer wasting the producers' money, risking excessive levels in the plants and simultaneously posing a risk for NO_3-N to leach into the ground water system.

A study in Ames, IA, showed that the N rate of 140 kg N ha^{-1} caused forage sorghum biomass yield to reach 16 Mg ha^{-1}, whereas in Bushland, TX, the 120 kg N ha^{-1} rate caused biomass yield for the same crop to yield 24 Mg ha^{-1} (Rooney et al., 2007). Similar yield results were found for the sweet sorghum. The environmental conditions in Iowa differ from New Mexico, where Marsalis et al. (2010) showed that the fertilization rate of 218 kg N ha^{-1} resulted in forage sorghum biomass yield of 24.6 Mg ha^{-1} under irrigation. These varying yield responses to nitrogen fertilization show how increasing the N fertilization rate for forage sorghum can increase biomass yield to a greater degree in different environments. Sorghum x sudangrass hybrids

typically can yield between 7.8 to 11.8 Mg ha^{-1} in one season with a two- or three-cut system in New York (Kilcer et al., 2005). Beyaert and Roy (2005) found that the cumulative yield from three cuts of a sorghum x sudangrass hybrid in Delhi, Ontario increased from 3.5 Mg ha^{-1} to 5.5 Mg ha^{-1} from the 0 kg N ha^{-1} rate to 125 kg N ha^{-1} rate of fertilization. The authors suspected that the lower yields may be due to the timing of the fertilizer application.

Kenaf is a warm-season, C3 fiber crop that is utilized for a number of applications such as the production of paper products, cordage, building materials, and livestock feed (Danalatos and Archontoulis, 2010; Webber et al., 2002). There are two distinct types of fibers that can be harvested from the kenaf stalk: the long bast fiber and the spongy cord core fiber (Geisler, 2011). The growth pattern of kenaf gives it potential as a bioenergy crop, growing between 2- and 4-m in height in just 150 d. Kenaf also has the ability to survive drought conditions for limited periods of time (Webber et al., 2002). A study, in Colorado, showed how it can yield 2 Mg ha^{-1} with only 250mm water use during the growing season, and can reach 5 Mg ha^{-1} with 450 mm of water (Nielsen, 2004). Kenaf has the greatest yield (4 Mg ha^{-1}) around 90 days after sowing, then subsequently decreases with the loss of non-fiber components such as the leaves or lodging in a study conducted in Portugal (Fernando et al., 2004).

Webber et al. (2002) suggests that kenaf may require some N fertilization to optimize its yield, though there may not be significant yield differences between 0 kg N ha^{-1} and 150 kg N ha^{-1}. Danalatos and Archontoulis (2010) also found that there was no statistical significance between the four nitrogen fertility rates (0, 50, 100, and 150 kg N ha^{-1}). The authors suspected this may be due to the low nitrogen needs of the crop or the high fertility levels of the soil with high moisture levels.

Lignocellulosic biomass material dry matter consists of 300 to 500 g kg^{-1} cellulose, 200 to 400 g kg^{-1} hemicellulose, and 150 to 250 g kg^{-1} lignin (Lee et al., 2007; Hendriks and Zeeman, 2009). This material can be utilized in the production of bioenergy, specifically in the conversion of the plant material to ethanol (Mosier et al., 2005). Sorghum is a potential biomass feedstock for conversion to bioethanol due to its desirable quality components. At harvest, sorghum contains around 340 g kg^{-1} cellulose, 170 g kg^{-1} hemicellulose, and 160 g kg^{-1} lignin (Lee et al., 2007). Sudangrass quality components are slightly different with 330 g kg^{-1} cellulose, 270 g kg^{-1} hemicellulose, and roughly 80 g kg^{-1} acid detergent lignin (ADL) and 120 g kg^{-1} crude protein (CP). Kenaf dry matter consists of 530 g kg^{-1} cellulose, 180 g kg^{-1} hemicellulose, and 80 g kg^{-1} lignin (Amaducci et al., 2000). These three crops differ physiologically, but are worth exploring further for their potential for biomass production.

The specific objectives of this study are to (1) determine the annual biomass crop that produces the greatest biomass yield that can be utilized for bioenergy, to (2) determine the effect of different levels of nitrogen fertilizer on biomass yield in the different forage crops, and to (3) determine the biomass quality components of each crop at three stages of development for their response to nitrogen fertility. These objectives will be carried out in the following experimental approach.

MATERIALS AND METHODS

Field Establishment and Experimental Design

This research was conducted at the North Dakota State University (NDSU) research sites in Fargo, ND (-96°812'W, 46°897'N, 274 m elevation) and at the NDSU research site in Prosper, ND (-97°115'W, 47°002'N, 284 m elevation). The soil type in Fargo is Fargo-Ryan silty clay soil (fine, montmorillonitic, frigid, Vertic Haplaquoll, with a leached and degraded natric horizon); the Fargo series is fine, smectitic, frigid Typic Epiaquerts, while the Ryan series is fine, smectitic, frigid Typic Natraquerts (Soil Survey Staff, 2011). The soil type in Prosper is a Bearden silty clay loam (fine-silty, frigid Aeric Calciaquolls). Rainfall amounts were recorded automatically at both locations by the NDAWN system (NDAWN, 2012). Soil samples for analysis were taken at both locations the spring when the crop was planted. The soil analysis included pH, organic matter, N-NO_3, P, and K.

Previous crops in 2010 were corn at Prosper and soybean (*Glycine max* L. Merr.) at Fargo, and previous crops in 2011 were cereal crops at Fargo and corn at Prosper. The sweet sorghum (*Sorghum bicolor* L. Moench), sorghum x sudangrass (*Sorghum bicolor* var. *sudanense* Piper.), and the kenaf (*Hibiscus cannabinus* L.) were seeded at all locations with a cone plot planter. Planting dates at Fargo in 2010 and 2011 were 26 May, and planting dates at Prosper in 2010 and 2011 were 28 May and 7 June, respectively. The seeding rates were calculated based on the percentage of pure live seed. The seeding rate for the sweet sorghum and sorghum x sudangrass was 11 kg ha^{-1}, whereas the seeding rate for the kenaf was 5.5 kg ha^{-1}. The forage sweet sorghum (BMR Sweething) was obtained from Agassiz Seed (West Fargo, ND), sorghum x sudangrass (Forage King) was obtained from Producer's Choice Seed (Woodland, CA), and the kenaf was obtained from Tom Rymsza (VNS).

Soil analysis was done using the transnitration of salicylic acid method to determine the baseline N fertility level of each individual plot (Franzen and Cihacek, 1996; Vendrell and Zupancic, 1990). The initial soil analysis of each plot showed that none of the plots had 0 kg N ha^{-1} in the upper 0.62 m of soil, which suggests that a true baseline was not feasible. In each block, the plot with the least amount of nitrogen was selected as the control, with the treatments for the other four plots randomized. For the treatments, the amount of N fertilizer to add was calculated for each plot, so that the initial N levels of each plot would be fertilized up to the experimental rates of 75, 100, 150, and 200 kg N ha^{-1} (soil N + N fertilizer). If the initial soil N test indicated a higher level of N than the experimental rate in that particular plot, then no additional fertilizer was added. The source of N was urea [$CO(NH_2)_2$]. Urea was hand-broadcast in both locations in 2010 and 2011 on 11 June and 30 June, respectively. Each plot was fertilized individually. The experimental design in all sites was a randomized complete block design with three replicates and a split-plot arrangement where the crop was the main plot and the nitrogen rates were the sub-plots. Experimental units were 2m wide and 9m long with six rows separated at a 0.31-m row spacing. The seeding depth for all the crops was 20 mm.

Weed control for the plots in 2010 was hand-weeding as needed, and weed control in 2011 included pre-plant spraying of glyphosate (N-(phosphonomethyl)glycine) (1.4 kg a.i. ha^{-1}) followed by hand-weeding as needed.

Plant Sampling and Evaluations

Dependent variables evaluated were plant biomass and potential ethanol yield at harvest, and forage quality at three developmental stages. Immediately prior to harvest, biomass samples were collected from each plot. Biomass samples were taken from the 2^{nd} and 5^{th} row in each six-row plot, where plants were cut near the base of the stem, at the soil level. The two-center rows

from a 3.7 m^2 area were hand harvested in 2010 and the four-center rows were harvested in 2011 using a flail forage harvester.

The calculation of potential ethanol yield of forage sweet sorghum and sorghum x sudangrass from biomass dry matter yield data uses the following equation:

$$L\ ha^{-1}\ ethanol = (0.098 \times 1000 \times Mg\ ha^{-1}\ biomass\ yield)/0.78943$$

The conversion factor of 98.0 mg ethanol g^{-1} is for kenaf and non-BMR sorghums (0.098 in the equation), but the factor of 113 mg ethanol g^{-1} is used for BMR-sorghum genotypes (replacing 0.098 in equation with 0.113) (Dien et al., 2009).

Aboveground portions of whole forage sorghum and sorghum x sudangrass plants were collected at three developmental stages: vegetative or 8-leaf stage (V8), panicle initiation or flower bud (R1), and harvest (H) (Vanderlip, 1993). Aboveground portions of whole kenaf plants were collected at the same time as the sorghum, though kenaf developmental stages are based on days after planting (DAP) and all three kenaf samplings were during its vegetative growth (Webber and Bledsoe, 2002). The determination of plant tissue quality analysis was conducted to determine dry matter (DM) (AOAC Method 934.01), percentage of ash (AOAC Method 942.05), crude protein (CP) (AOAC Method 2001.11), and acid detergent lignin (ADL) (AOAC Method 973.18) (Horwitz and Latimer, 2010). Quality analysis was conducted to determine acid detergent fiber (ADF) (ANKOM A200 Method 5) and neutral detergent fiber (NDF) (ANKOM A200 Method 6). Quality analysis was also conducted to determine in-vitro dry matter disappearance (IVDMD) (Oh et al., 1966).

Statistical Analysis

Statistical analysis was conducted by using standard procedures for a randomized complete-block design with a split plot arrangement (Steel and Torrie, 1980). The biomass data

collected was analyzed by analysis of variance using the GLM procedure (SAS Institute, 2008), with each location-year combination considered an "environment" and a random effect, while crops and nitrogen fertility treatments were considered fixed effects in the analysis. Statistical analysis was also conducted by using standard procedures for a randomized complete-block design with a split-split plot arrangement (Steel and Torrie, 1980). All of the quality data collected was analyzed by analysis of variance using the GLM procedure (SAS Institute, 2008), with each location-year combination considered an "environment" and a random effect, while crops and nitrogen fertility treatments, and developmental stages were considered fixed effects in the analysis.

Analysis of variance was conducted within and across environments. Environments were considered homogenous when the mean square error variances for each trait differed by less than a factor of 10. If the environments were homogenous, then a combined analysis was conducted. A mean separation test was performed using the *F*-protected LSD at $P \leq 0.05$ level of significance for each evaluated trait. Regression analysis was done where there was a significant main effect. Linear and quadratic regression models were tested with the corresponding error. The regression models were all at $P \leq 0.05$ level of significance.

The plant biomass and ethanol data was analyzed according to a randomized complete block design with a split-plot arrangement, where the main plots were the crops (sweet sorghum, sorghum x sudangrass, and kenaf), and the subplots were the N fertility treatments (0, 75, 100, 150, 200 kg N ha^{-1}).

The plant quality sampling data was analyzed according to a randomized complete block design with a split-split plot arrangement, where the main plots were the crops (sweet sorghum,

sorghum x sudangrass, and kenaf), the N fertility treatments (0, 75, 100, 150, and 200 kg N ha^{-1}) were the subplots, and the phenological stages (V8, R1, and H) were the sub-sub plots.

RESULTS AND DISCUSSION

Rainfall, Temperature, GDD, and Soil Analysis

Total growing season rainfall varied between years and months, with greater rainfall occurring from April through August in 2011 than in 2010 in both Fargo and Prosper (Table 1.1).

Table 1.1. Monthly growing-season rainfall for four environments, Fargo and Prosper, ND, in 2010 and 2011, and the deviation from the 30-year average.

	Fargo†				Prosper†			
	2010		2011		2010		2011	
Month	Rainfall	Dev.	Rainfall	Dev.	Rainfall	Dev.	Rainfall	Dev.
	----------mm----------							
April	36.8	2.0	45.8	11.0	29.5	-6.6	45.0	8.6
May	68.1	1.8	109.7	43.4	69.9	2.0	80.0	12.2
June	86.1	-3.1	100.9	11.7	80.8	-10.7	131.6	40.1
July	105.1	32.0	103.6	30.5	103.4	21.1	150.1	67.8
Aug.	67.7	3.7	72.5	8.5	89.4	21.3	88.9	20.8
Sept.	151.4	96.0	4.0	-51.4	134.6	80.5	6.1	-48.0
Oct.	60.6	10.6	20.6	-29.4	36.1	-11.9	9.4	-38.6
Total	575.8		457.1		543.7		511.1	

† NDAWN, 2012.

The seasonal rainfall was greater in 2010 than 2011, though the rainfall in 2011 occurred in greater amounts in May, June, and July, when overland flooding delayed planting and saturated the soil. The amount of rainfall that fell during the month of July in Prosper, 2011, was the greatest amount of rainfall that occurred in the course of one month during the growing season. A thunderstorm in Prosper on July 19, 2011 delivered 50 mm of rain on the site according to the official NDAWN data (2012), yet visual observations soon after the storm hit suggested a greater amount fell, as the flooding in that area destroyed a number of research experiments. The widespread overland flooding was partially due to the saturated soil conditions that had been present for much of the spring and summer months. There was a reprieve from the moisture in time

for harvest in 2011 where both locations received below-average rainfall in September and October.

This above-average rainfall during in 2011 had resulted in a re-application of nitrogen fertilizer in Prosper, ND, as the nitrogen had leached out of the root zone. All of the crops exhibited yellowing symptoms and the smell of ammonia could be easily detected, suggesting that denitrification was taking place. This fertilizer re-application in Prosper was conducted on July 25, 2011.

Slightly above-average temperatures were observed in all four environments from June through August (Table 1.2).

Table 1.2. Growing-season average temperatures for four environments, Fargo and Prosper, ND, in 2010 and 2011, and the deviation from the 30-year average.

	Fargo†				Prosper†			
	2010		2011		2010		2011	
Month	Temp.	Dev.	Temp.	Dev.	Temp.	Dev.	Temp.	Dev.
	----------°C----------							
April	6.4	4.5	6.38	-0.3	5.8	4.4	5.8	-1.0
May	14.1	0.4	14.1	-1.2	13.5	0.4	13.5	-1.5
June	18.9	0.2	18.9	0.3	18.4	0.3	18.4	0.4
July	21.4	0.9	21.5	2.2	21.1	0.0	21.1	2.1
Aug.	20.6	1.6	20.6	1.2	20.1	1.0	20.1	1.0
Sept.	14.4	-0.8	14.4	1.0	14.4	-1.5	14.4	0.8
Oct.	7.4	2.8	7.4	3.9	7.5	1.8	7.5	3.3

† NDAWN, 2012.

Even though September temperatures were above average in 2011, an early frost occurred on September 15, 2011, which forced an early harvest the next day for the forage sweet sorghum, sorghum x sudangrass, and kenaf. Both of the Fargo and Prosper sites were hit by the frost, so harvest was conducted to avoid biomass lost when the leaves dried up. The planting dates in 2011 in Fargo and Prosper were May 26 and June 7, respectively, resulting in the growing season duration of 112 days and 100 days, respectively. Early-season moisture, mid-season flooding

rainfall in both locations and an early frost may have caused a reduction in plant growth in these environments.

The monthly accumulated growing degree days (AGDD) were below average in all four environments (Table 1.3).

Table 1.3. Growing-season growing degree-days (GDD) for four environments, Fargo and Prosper, ND, in 2010 and 2011, and the deviation from the 30-year average for forage sorghum, sorghum x sudangrass, and kenaf.

	Fargo†				Prosper†			
	2010		2011		2010		2011	
Month	GDD	Dev.	GDD	Dev.	GDD	Dev.	GDD	Dev.
	----------°C----------							
April	117	74	39	-4	115	68	33	-14
May	155	-13	131	-37	157	-20	124	-53
June	219	-47	210	-56	219	-34	210	-43
July	274	-81	287	-68	267	-76	287	-57
Aug.	268	-59	272	-55	266	-45	271	-40
Sept.	131	-36	188	21	134	-49	194	11
Oct.	112	56	118	62	112	42	118	48
Total	1276	-106	1245	-137	1270	-114	1237	-148

† NDAWN, 2012 where base temperature=10°C

Growing degree days (GDD), or heat units, were calculated based on the threshold temperatures for each crop. The base temperature for the three crops is 10°C (Archontoulis et al., 2011; Klein and Shapiro, 2011), while the maximum threshold temperature is 30°C. These values were used when calculating the monthly AGGD and total AGGD for each environment.

Each environment was different in terms of soil conditions, particularly initial soil N levels. The lowest soil NO_3-N level of the five plots in each block was chosen to be the check plot. The next lowest soil NO_3-N level of the five plots was chosen as the 75 kg N ha experimental rate, and so on. The initial soil NO_3-N results are indicated in Table 1.4.

In 2010, Fargo and Prosper locations had average soil NO_3-N levels that were higher than the experimental fertility rates of 0, 75, and 100. Fargo and Prosper had lower levels of nitrogen in

Table 1.4. Initial soil NO_3-N analysis for four environments and five N rates across three crops combined (forage sweet sorghum, sorghum x sudangrass, and kenaf) prior to planting for soil depths of 0 to 60 cm.

Environment	0	75	100	150	200
	----------kg NO_3-N ha^{-1}----------				
Fargo 2010	94.3	103.9	109.3	116.7	125.8
Fargo 2011	67.9	78.2	96.1	98.9	99.3
Prosper 2010	82.1	98.8	119.5	131.0	162.8
Prosper 2011	27.3	35.3	34.6	34.3	35.3

the soil in 2011 compared to the previous year, but still had average fertility levels higher than the experimental rate of 0 in Fargo and Prosper, as well as the 75 kg N ha^{-1} experimental rate in Fargo. For the analysis, the mean actual N levels for each crop and each treatment were calculated and used in the analysis. The average actual N rates for forage sweet sorghum were 70, 94, 108, 150, and 200 kg N ha^{-1}, the rates for sorghum x sudangrass were 68, 92, 111, 152, and 202 kg N ha^{-1}, and the rates for kenaf were 66, 89, 118, 155, and 203 kg N ha^{-1}.

The initial soil analysis for soil P, K, organic matter (OM), and pH was conducted for each environment before planting (Table 1.5).

Table 1.5. Initial soil analysis for four environments prior to planting for soil depths of 0 to 60 cm.

Environment	pH	OM	P	K
		----------mg kg^{-1}----------		
Fargo 2010	7.9	5.7	22.0	445.0
Fargo 2011	7.4	6.5	15.6	321.7
Prosper 2010	8.1	2.7	38.0	400.0
Prosper 2011	7.1	3.1	39.5	267.1

† OM: Organic matter

The organic matter levels were greater in Fargo than in Prosper locations in both 2010 and 2011. The pH was between 7 and 8 in all four environments, so N and K were most available for plant uptake (Mengel and Kirkby, 1982). The optimum soil pH for forage sorghum is between 6 and 7.5 (Saballos, 2008; McClure, 2012), sorghum x sudangrass optimal pH level is between 6 and 6.5 (Teutsch, 2009), and kenaf optimal pH level is between 6 and 6.8 (Rowell and Stout, 2006). There were lower pH levels in 2011 than in 2010. Phosphorus levels were between 15 and 25 mg

kg^{-1} in Fargo and between 35 and 40 mg kg^{-1} in both 2010 and 2011, so no additional P fertilization was required. Initial soil K levels were greater in 2010 than in 2011, with levels greater than the minimum K fertility requirements for all three crops, so no additional K fertilizer was required.

Biomass and Ethanol Yield

The crop and the N rate main effects were significant for biomass, relative biomass, and ethanol yield, though the interaction between the main effects was not significant (Table 1.6).

Table 1.6. Analysis of variance and mean squares for sorghum, sudangrass, and kenaf biomass, relative biomass, and ethanol yield for five N rates across four environments, Fargo and Prosper in 2010 and 2011.

Sources of variation	df	Biomass yield	Relative biomass yield	Ethanol yield
Env	3	1654.7	19295.3	31.86
Rep(env)	8	138.8	1270.0	2.81
Crop	2	1024.0**	268.4	27.16**
Env x crop	6	87.0	780.6***	2.02
Error (a)	16	33.4	303.1	0.68
N	4	106.2***	1294.8***	2.04***
Env x N	12	9.1	112.0	0.18
N x crop	8	7.0	66.7	0.16
Env x N x crop	24	9.5	100.7	0.19
Error (b)	96	5.4	52.7	0.11
CV, %		19.1	17.8	19.40

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively.

Forage sweet sorghum and sorghum x sudangrass hybrid biomass and ethanol yield did not differ significantly, but they both had significantly higher dry matter yield than kenaf (Table 1.7).

Sorghum and sorghum x sudangrass both had yield values between 14 and 15 Mg ha^{-1} across fertility rates. The literature stated that sorghum can reach greater biomass yields, between 16 and 28 Mg ha^{-1}, from Ames, IA to Bushland, TX, respectively (Bennett and Anex, 2009; Rooney et al., 2007). Sorghum reaches seed physiological maturity at 2350 GDD, where moisture

Table 1.7. Mean biomass and ethanol yield at harvest for three crops averaged across N rates and four environments in Fargo and Prosper, ND, in 2010 and 2011.

Crop †	Biomass yield	Relative biomass yield	Ethanol yield
	----------Mg ha^{-1}--------	----------%----------	----------m^3 ha^{-1}---------
Forage sweet sorghum	14.79	42.7	2.12
Sorghum x sudangrass	14.35	41.4	2.05
Kenaf	7.43	38.5	0.92
LSD (*P*=0.05) ‡	4.16	12.5	0.63

† Average actual N rates for forage sweet sorghum (70, 94, 108, 150, and 200); N rates for sorghum x sudangrass (68, 92, 111, 152, and 202); N rates for kenaf (66, 89, 118, 155, 203).
‡ To compare the means of different crops.

content of the seed is less than 400 g kg^{-1} (Klein and Shapiro, 2011), yet the sorghum crops were harvested at around 1257 GDD, when the sorghum was at panicle emergence or heading (R1), since it does not produce seed at this northern latitude. The rate of biomass accumulation decreased when the crop reached the reproductive stage, so it makes sense to harvest the biomass before the energy reserves are translocated to the seed and vegetative biomass accumulation rate declines.

Kenaf biomass yield averaged 7.4 Mg ha^{-1} across four environments and five nitrogen treatments, with an average of 522 mm of rainfall during the growing seasons. This data is consistent with the literature, where higher water availability resulted in greater kenaf yield. Kenaf did not have as great of biomass yield as the sorghum.

Plants typically have an increase in biomass yield as nitrogen fertility treatments increase. The analysis of variance did not show a significant interaction between treatment and crop because each crop responded similarly to higher nitrogen fertility rates. Instead of showing the combined response of the three crops to nitrogen rates, the three crops will be shown individually.

The regression model was calculated for each of the crops using relative yield with respect to the actual nitrogen rates present in each of the individual plots. The regression model (y=-0.7 + 0.6x - 0.002x^2, r^2=0.16) showed a polynomial increase in forage sweet sorghum biomass yield as

the rate of N fertility increased (Fig. 1.1). The greatest relative biomass yield according to the regression model was at 176 kg N ha^{-1}. The regression model ($y = 1.40 + 0.54x - 0.0015x^2$) showed a similar response to nitrogen fertilization for sorghum x sudangrass (Fig. 1.2).

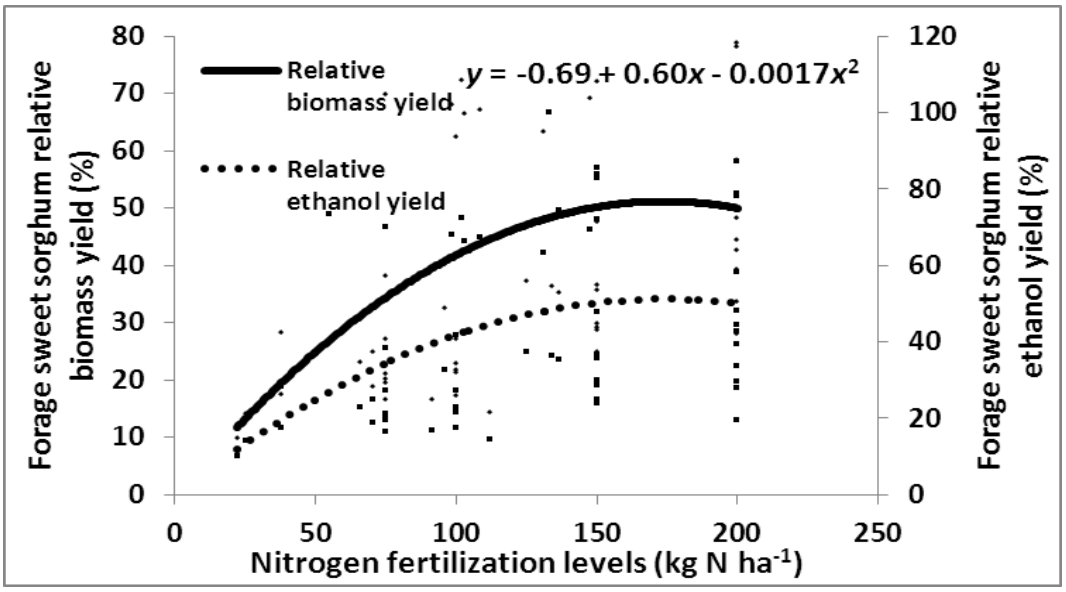

Fig. 1.1. Regression model for relative biomass and ethanol yield of forage sweet sorghum as affected by actual N rates averaged across four environments in Fargo and Prosper, ND, in 2010 and 2011.

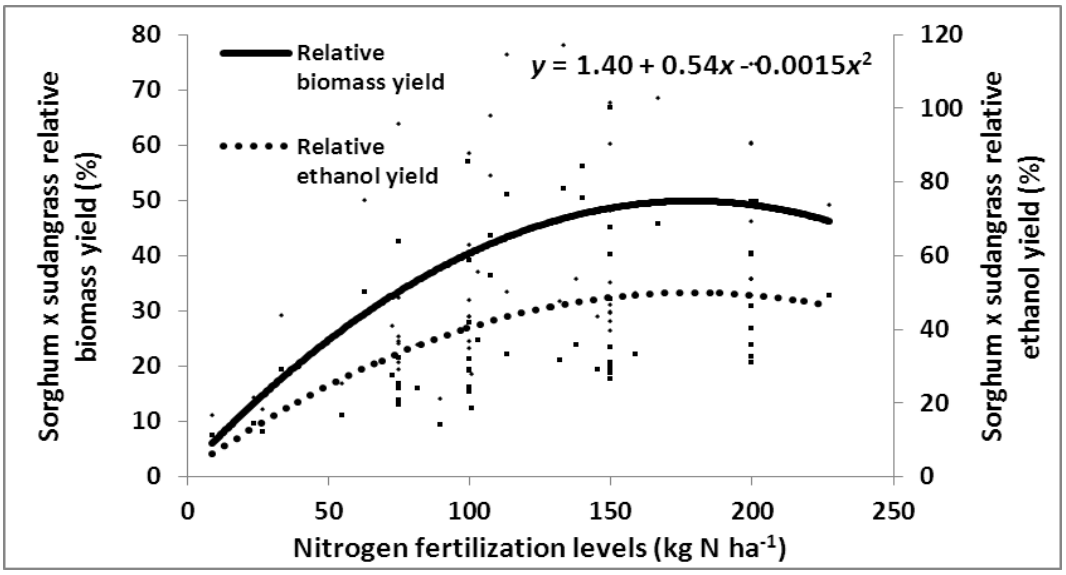

Fig. 1.2. Regression model for relative biomass and ethanol yield of sorghum x sudangrass as affected by actual N rates averaged across four environments in Fargo and Prosper, ND, in 2010 and 2011.

The greatest relative biomass yield for sorghum x sudangrass according to the regression model was found at the fertility rate of 180 kg N ha^{-1}. The regression model ($y = -3.83 + 0.59x - 0.0017x^2$) for relative biomass yield was also done for kenaf (Fig. 1.3).

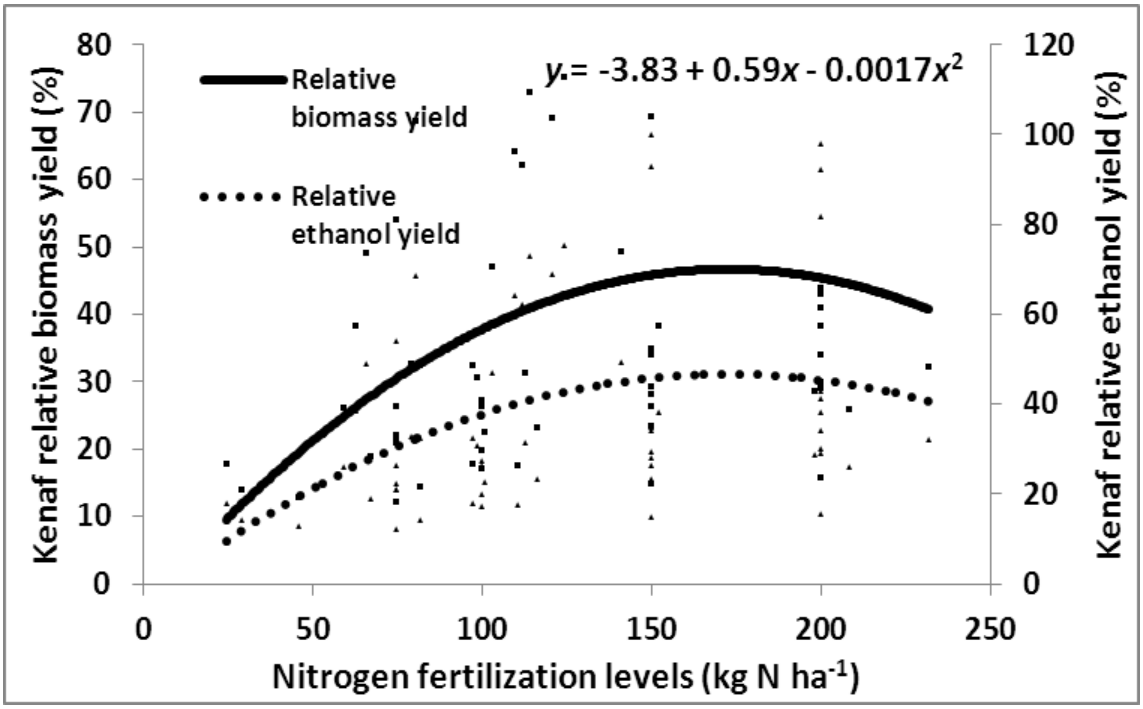

Fig. 1.3. Regression model for relative biomass and ethanol yield of kenaf as affected by actual N fertility levels averaged across four environments in Fargo and Prosper, ND, in 2010 and 2011.

This regression model showed that kenaf greatest relative biomass yield was found at the highest N rate of the three crops, at 174 kg N ha^{-1}. Because the initial available N levels were greater than some of the treatment levels (Table 1.4), the actual N levels after fertilization were used to calculate the regression curves for each crop. The average NO_3-N content in the soil was 87.6 kg NO_3-N ha^{-1} before planting. The plots with the check N fertility levels already contained between 27 and 94 kg N ha^{-1} in the plots before planting, and the 75 kg N ha^{-1} fertility rate already had between 35 and 104 kg N ha^{-1} before fertilization (Table 1.4). The environmental conditions may also have impacted biomass yield, as the late planting, early- to mid-season rainfall, and the

early-frost in 2011 provided less than optimal growth conditions, shortening the growing season duration.

Biomass yield responded to increasing N rates, so as N rates increased, dry matter biomass yield increased. Forage sweet sorghum and sorghum x sudangrass yield was significantly greater than kenaf biomass yield. But higher biomass is not the only factor that is important in determining a feedstock for bioenergy. Quality components play a role in how well the crop can be converted to ethanol.

Quality Analysis

The quality traits analyzed using the combined analysis included ash, CP, NDF, ADF, and hemicellulose, which were analyzed by the analysis of variance. The stage of sampling, the N fertility rates, and the crop main effects were significant for the combined analysis for plant tissue quality data (Table 1.8).

The stage by crop interaction and the stage by N rate interaction were significant. Environment by N rate, environment by crop, stage by environment, and stage by environment by crop were also significant, but since environment is considered a random effect, the discussion will focus on the significant main effects and the interaction between fixed effects. The interaction among the growth stages and the three crops was found to be significant for four of the quality components, as shown in Table 1.9.

In terms of the ash quality component, the means of the different crops at the same stages were not significantly different, but there was a decrease of ash content of the different growth stages within the same crop. Forage sweet sorghum and kenaf ash content decreased significantly between the V8 and H and the R1 and H growth stages.

Table 1.8. Analysis of variance and mean squares for forage sweet sorghum, sorghum x sudangrass, and kenaf plant-tissue quality analysis for five N rates and three developmental stages across four environments (env), Fargo and Prosper, ND, in 2010 and 2011.

Sources of variation	df	Ash	CP†	NDF	ADF	Hemi
Env	3	10840	85009	7170	9207	9188
Rep(env)	6	723	1353	3118	4141	573
Crop	2	1335**	120837***	1318729***	8047	1177318***
Env x crop	6	3526***	2343**	25081***	11952***	2545*
Error (a)	12	218	382	1486	965	584
N	4	592	10996**	970	161	607
Env x N	12	456*	1519***	1559	1049	456
N x crop	8	202	423	425	126	406
Env x N x crop	24	134	365	1062	450	374
Error (b)	72	220	348	1715	929	435
Stage	2	47866***	489952**	1009614***	912416***	4866*
Stage x env	6	1649***	41895***	16589***	12285***	964*
Stage x crop	4	693*	4683*	278508***	137733***	7114***
Stage x env x crop	12	583**	1336***	14967	7009***	1754***
Stage x N	8	337	2507***	1618	1071	343
Stage x env x N	24	189	449	820	657	264
Stage x N x crop	16	97	289	1540	873	349
Stage x env x N x crop	48	202	198	857	475	198
Error (c)	180	265	336	1379	842	422
CV, %		16	16	7	9	10

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively.
† Quality parameters: crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and hemicellulose (Hemi)

Sorghum x sudangrass ash content decreased significantly between all three stages, from 118 g kg^{-1} to 79.9 g kg^{-1}. Efficiency in the process of converting biomass to bioenergy is impacted by ash content, as ash is unable to be fermented in the ethanol production process (Anderson et al., 2010; Sanderson et al., 2006).

Ash content can come from the biomass material itself or from soil particles or dust on the plant, causing a negative economic impact on the conversion system (Bakker and Elbersen, 2005). Ash content of the crops may also be influenced by the amount of five specific inorganic constituents in the soil that can impact the biomass conversion systems. The five main components of ash are silicon, potassium, calcium, sulfur, and chlorine, and are important in many of the essential physiological processes that plants undergo (Bakker and Elbersen, 2005).

Table 1.9. Interaction among growth stages [vegetative 8-leaf (V8), reproductive (R1), and harvest (H)] and three crops in quality analysis components averaged across N rates and four environments, Fargo and Prosper, ND, in 2010 and 2011.

Crop/Stage	Ash†	CP	NDF	ADF	Hemi
	----------	----------	g kg^{-1}	----------	----------
Forage sweet sorghum					
V8	119.0	162.0	541.2	266.7	274.5
R1	108.8	89.7	634.4	361.1	273.3
H	78.8	45.1	591.9	353.5	238.3
Mean	102.2	98.9	589.1	327.1	262.0
Sorghum x sudangrass					
V8	118.8	167.7	518.0	251.2	266.8
R1	105.8	89.2	634.9	356.7	278.2
H	79.9	47.9	594.7	338.1	256.6
Mean	101.5	101.6	582.5	315.3	267.2
Kenaf					
V8	105.6	217.7	238.1	156.4	81.6
R1	106.6	152.4	459.0	349.5	109.5
H	76.2	76.5	583.2	441.4	141.9
Mean	96.1	148.9	426.8	315.8	111.0
LSD (*P*=0.05) ‡	16.4	17.3	58.3	39.9	19.2
LSD (*P*=0.05) §	12.7	52.6	54.5	40.1	17.2
LSD (*P*=0.05) ¶	18.0	52.3	63.3	45.6	19.7

† Quality parameters: crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and hemicelluloses (Hemi)
‡ To compare the means of different crops within a growth stage.
§ To compare the means of the different growth stages within a crop.
¶ To compare the means of different crops with different growth stages.
# Average actual N rates for forage sweet sorghum (70, 94, 108, 150, and 200); N rates for sorghum x sudangrass (68, 92, 111, 152, and 202); N rates for kenaf (66, 89, 118, 155, 203).

Magnesium is another component of ash that can impact biomass conversion systems (L. Cihacek, personal communication, 2012).

Ash content can affect thermochemical conversion processes at higher temperatures, melting the ash and damaging the mechanical equipment used (Biomass Energy, 2012). The biomass also has a lower energy value with greater levels of ash (Biomass Energy, 2012). A study was conducted on Bermuda grass (*Cynodon dactylon*), which found that the Pearson correlation coefficient for ash with theoretical ethanol yield is r= -0.40, significant at $P \leq 0.01$ (Anderson et al.,

2010). As ash content would decrease, theoretical ethanol yield would increase. Biomass yield was found to increase at later growth stages for the three crops, while ash content decreased, making harvest at the end of the growing season more desirable. A study by Sanderson et al. (2006) also found that ash content decreases as the crop matures. Extending the harvest date of the biomass crop to a later date in the fall increases the amount of translocation of some of the ash components: nutrients and other minerals, back to the root system, staying in the soil after the roots decompose (Bakker and Elbersen, 2005). The lower ash levels in plant tissue indicate that the mineral nutrients were left in the soil or were not taken up by the plant. The biomass material is then transported to the biorefinery in a more economical manner, because the refinery can use the material more efficiently and with less damage to the equipment, particularly in the process of gasification.

When subjecting biomass to the method of gasification, there can be damage to the equipment if there are high levels of ash (Bakker and Elbersen, 2005). There is an even greater negative impact on conversion systems that rely on biochemical reactions to convert the cellulosic material into ethanol.

The interaction among growth stages and the crops was significant for CP. Forage sweet sorghum and sorghum x sudangrass crops were not significantly different from one another at the same growth stages, but they both had significantly lower levels of CP than kenaf at all three stages. Kenaf had the highest level of CP at the earliest growth stage, 218 g kg^{-1}, and decreased significantly at R1 and H, with a harvest level of 76.5 g kg^{-1}. Forage sweet sorghum had decreasing levels of CP from V8 to H, decreasing from 162 g kg^{-1} to 45.1 g kg^{-1} at harvest. Crude protein is determined through measuring the total N content in the crop, and by multiplying that value by 6.25 for each of the crops. Nitrogen turnover in plants occurs when inorganic N is converted into organic N compounds, to produce high molecular weight proteins and nucleic acids (Mengel and

Kirkby, 1982). Crude protein levels then decrease in the older vegetation as hydrolyzing enzymes break down the higher molecular weight compounds into lower molecular weight components such as amino acids, amines, and amides. These nitrogen fractions can be transported through the xylem as NO_3, NH_4, and amino acids into the root system, decreasing the level of CP over time.

Crude protein is also affected by different levels of nitrogen fertilization. The main effect of N fertility on CP (Table 1.8) when averaged across the three crops was also found to be significant in the combined analysis.

A polynomial increase in CP in the plant tissue of forage sweet sorghum was observed at the earliest growth stage (V8) (y=109.5 + 0.5x - 0.0005x^2) (Figure 1.4).

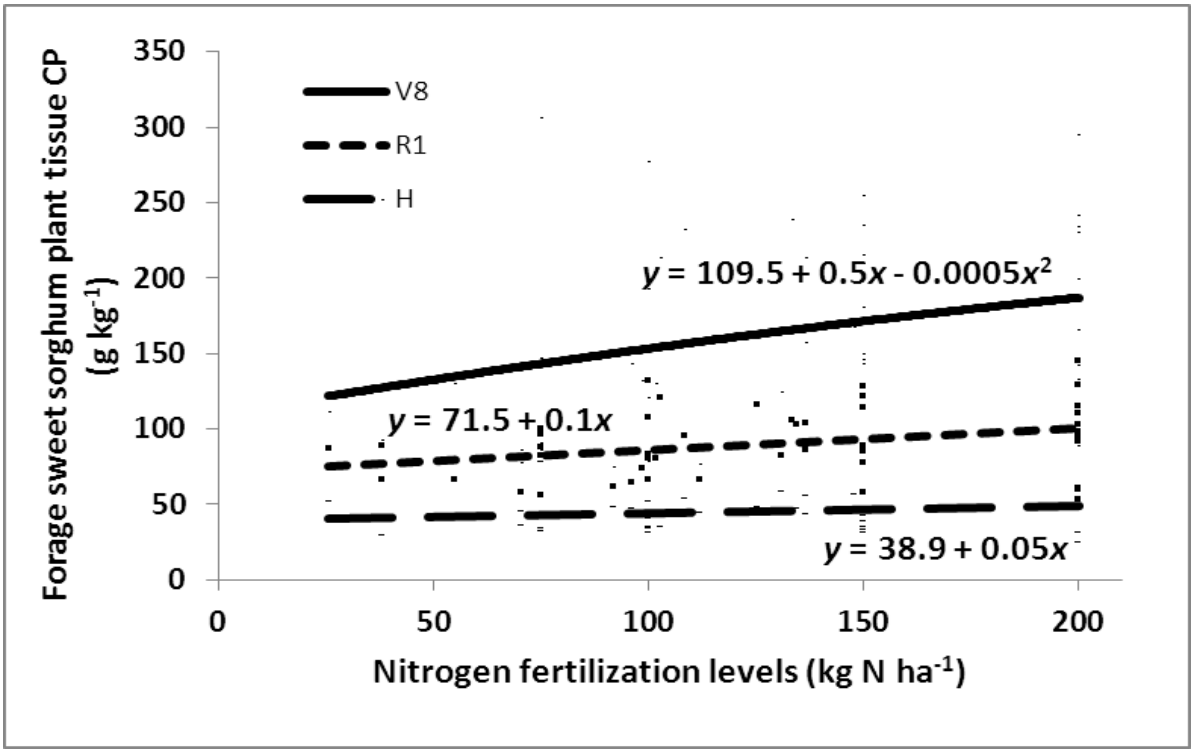

Fig. 1.4. Regression model for plant tissue crude protein (CP) of forage sweet sorghum at three growth stages: vegetative 8-leaf (V8), reproductive (R1), and harvest (H), as affected by N rates averaged across four environments, Fargo and Prosper, ND, in 2010 and 2011.

Crude protein levels increased from 135.8 g kg^{-1} at the 50 kg N ha^{-1} rate to 229.5 g kg^{-1} at the 200 kg N ha^{-1} fertility level. The amount of plant tissue CP increased at a greater rate when the level of fertility increased at the earliest growth stage. A linear increase was observed when the crops reached the reproductive stage (R1) (y=71.5 + 0.14x), but there was not a significant change in CP at harvest with increasing fertilization rates.

The CP regression model was also determined for sorghum x sudangrass (Fig. 1.5).

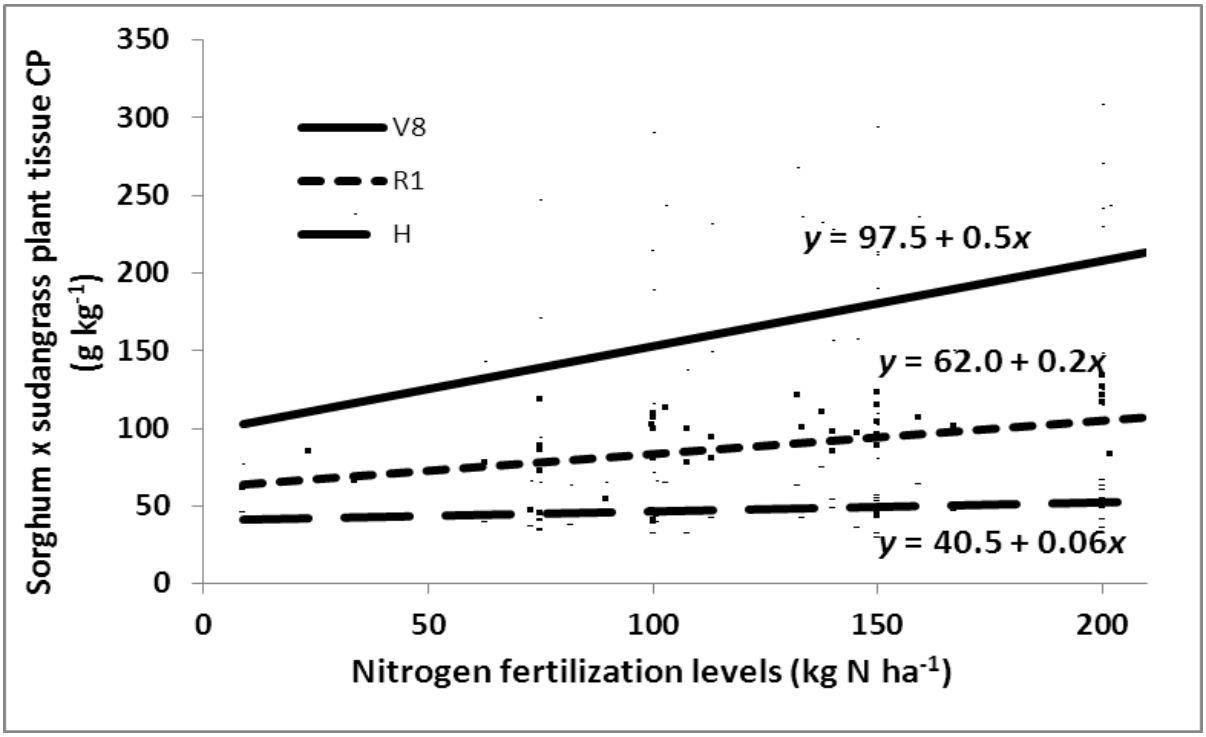

Fig. 1.5. Regression model for plant tissue crude protein (CP) of sorghum x sudangrass at three growth stages: vegetative 8-leaf (V8), reproductive (R1), and harvest (H), as affected by N rates averaged across four environments, Fargo and Prosper, ND, in 2010 and 2011.

A polynomial increase in CP in the plant tissue of sorghum x sudangrass was observed at the earliest growth stage (V8) (y=158.3 + 0.3x - 0.0008x^2). Crude protein levels increased from 135.8 g kg^{-1} at the 50 kg N ha^{-1} rate to 229.5 g kg^{-1} at the 200 kg N ha^{-1} fertility level. The amount of plant tissue CP increased at a greater rate when the level of fertility increased at the earliest growth stage. A linear increase was observed when the crops reached the reproductive stage (R1)

(y=71.5 + 0.14x), but there was not a significant change in CP at harvest with increasing fertilization rates.

The regression model for crude protein was also determined for kenaf (Fig 1.6).

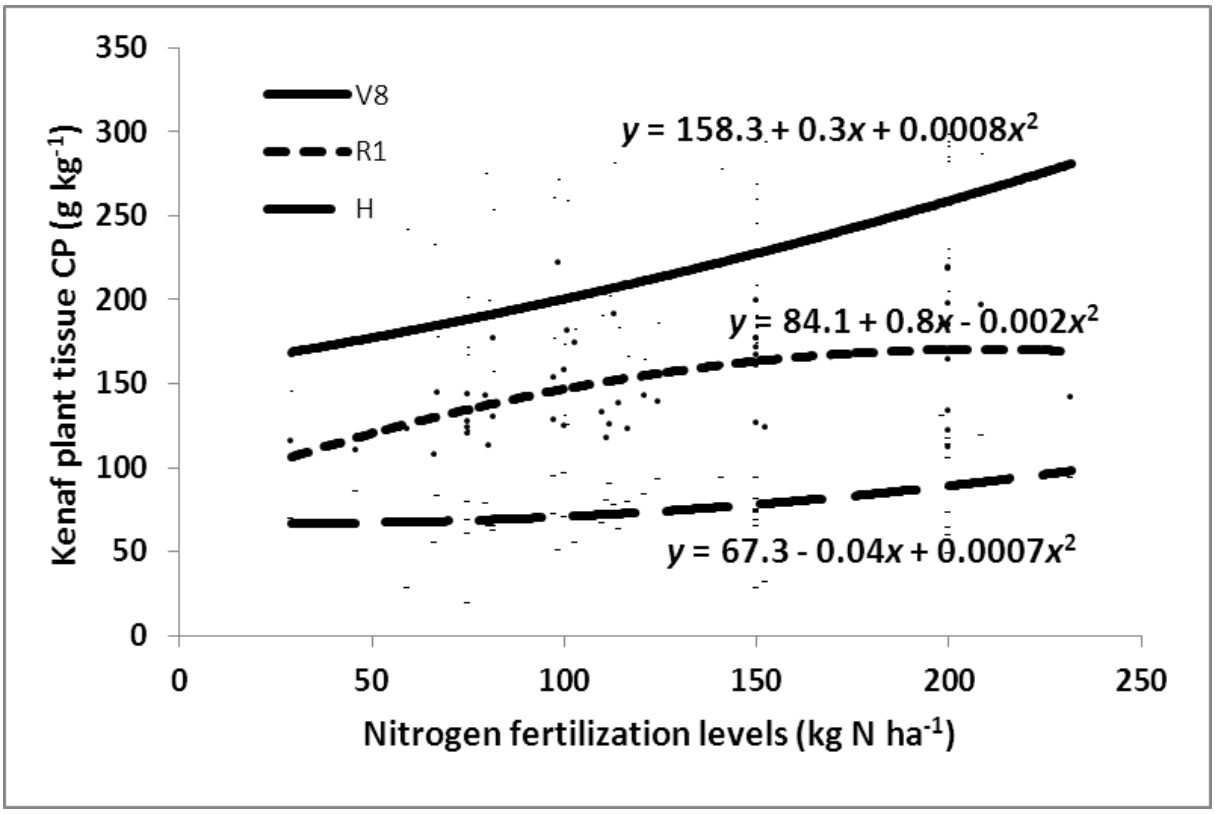

Fig. 1.6. Regression model for plant tissue crude protein (CP) of kenaf at three growth stages: vegetative 8-leaf (V8), reproductive (R1), and harvest (H), as affected by N rates averaged across four environments, Fargo and Prosper, ND, in 2010 and 2011.

At the reproductive stage, plant CP content increased from 96 g kg^{-1} at the control fertility rate to 110 g kg^{-1} at the 100 kg N ha^{-1} rate, reaching 124 g kg^{-1} of CP at the 200 kg N ha^{-1} rate.

The study by Anderson et al. (2010) stated that nitrogen content in the crop plant tissue did not have a significant impact on ethanol production in the process of simultaneous saccharification and fermentation (SSF), though other conversion systems may require different quality levels (Guretzky et al., 2011).

The quality component NDF increased significantly for forage sweet sorghum from the V8 to the R1 stage, but was not significant at the H stage (Table 1.9). For sorghum x sudangrass, NDF increased significantly between V8 and R1 and between V8 and H, but there was no significant difference in NDF levels between the later two stages. Kenaf had significantly lower levels of NDF at the earlier V8 and R1 stages when compared with the other two crops. They also increased significantly from V8 to R1 to H stages.

Acid detergent fiber (ADF) levels increased significantly from V8 to R1 and V8 to H stages for forage sweet sorghum as well as sorghum x sudangrass, but there was no significant difference between the latter two stages for either crop (Table 1.9). Kenaf also had significantly lower ADF levels at the V8 growth stage, but significantly higher ADF levels at the H stage when compared with the other two crops. Kenaf ADF levels significantly increased between each growth stage.

Both ADF and NDF quality components increased in these three crops at later growth stages. These two values are used to calculate hemicellulose, by subtracting ADF from NDF. Hemicellulose increased significantly from V8 to H growth stage and from R1 to H growth stage for forage sweet sorghum, but only increased significantly from R1 to H in sorghum x sudangrass (Table 1.9). Kenaf, on the other hand, had significantly lower hemicellulose levels than the other two crops at all three of its growth stages. Hemicellulose levels increased significantly at each growth stage for kenaf as well, staying at lower levels than the other two crops. Higher hemicellulose levels are cited to be more desirable for ethanol production in particular, as the sugars are fermented and converted to alcohol (Hettenhaus, 2006). The 5-C pentose sugars are linked to other 6-C sugars in hemicellulose and are broken down by cellulose enzymes through hydrolysis. Anderson et al. (2010) indicated that hemicellulose plays a role in reducing potential

ethanol yield as hemicellulose can block access to cellulosic fibers, which would explain the negative correlation observed in the study. Forage sweet sorghum and sorghum x sudangrass have significantly higher levels of hemicellulose than kenaf, giving them more potential for use as bioenergy feedstocks.

The NDF quality component provides the total cell wall levels, which includes cellulose, hemicellulose, and lignin. Hemicellulose binds to the surface of cellulose (Taiz and Zeiger, 2006). Cellulose is made of tightly packed microfibrils of linear chains of 1,4-β-glucose molecules that are acted on by enzymes in the conversion to ethanol. Lignin can prevent enzymes from acting upon the cellulosic material, but once that lignin seal is broken through the use of pretreatment, it is possible to disrupt the crystalline structure of cellulose. Both sorghum crops had the *bmr*-6 gene for lower lignin levels, which requires less pretreatment at the beginning of the conversion process. Cellulose itself is quite strong, but the process of hydrolysis can release the sugars from the cell wall.

CONCLUSIONS

First of all, forage sweet sorghum and sorghum x sudangrass had significantly higher biomass yield than kenaf. This is one of the main goals of developing a biomass feedstock. It is also important that this feedstock will not compete with food crops or acreage, and one that will not require intensive management practices to achieve maximum yield. These two sorghum crop have these characteristics, as long as they are planted in areas that are otherwise unsuitable for food production.

Another important aspect of biomass production is to produce a crop that requires only minimal chemical, fertility, or management inputs. Plant biomass yield showed a significant response to N fertilization treatments. As N fertility rates increased, dry matter biomass yield increased, when averaged across the three crops and four environments. The greatest sorghum yield was achieved at the highest fertility rate of 200 kg N ha^{-1}.

The third important aspect of biomass production is to produce a crop that has desirable quality characteristics that make conversion of the biomass to ethanol as efficient as possible. First of all, a later harvesting date would be ideal for reducing ash content for the sorghum crops. Kenaf had the lowest ash content, but because its biomass yield is significantly lower than the sorghum crops, the lower ash content doesn't make much of a difference when it can be lowered by using management techniques. The later harvest date may also decrease the amount of water left in the plant material, reducing transportation costs. When plant mineral nutrients have a chance to translocate back into the soil, there is a reduction in the subsequent transport of unusable minerals to the biomass processing plant that can impact the conversion process and take away valuable nutrients from the soil. Forage sweet sorghum and sorghum x sudangrass had significantly greater

levels of ADF, NDF, and hemicellulosic quality components than kenaf, suggesting that the higher cellulosic content can produce greater ethanol yield.

The recommendation for a potential biomass feedstock from this study is to produce either forage sweet sorghum or sorghum x sudangrass and fertilize it at the 180 kg N ha^{-1} rate. This rate will still produce adequate biomass yield (between 17.6 and 18.2 Mg ha^{-1}). A later harvest date is recommended to further reduce the amount of ash and mineral nutrients in the biomass material that can impact the process of conversion and the sustainability of the system. The level of cellulose and hemicellulose remain at levels adequate for processing, while the *bmr*-6 mutation gives the crop lower lignin content that can make the conversion of the biomass easier.

CHAPTER 2. NITROGEN UPTAKE AND NITROGEN USE EFFICIENCIES OF ANNUAL BIOMASS CROPS AND AN ECONOMIC ANALYSIS AS FEEDSTOCKS FOR BIOFUEL

ABSTRACT

Nitrogen can have the greatest effect in increasing crop production, but when nitrogen fertilizer is applied in excess to what crops can uptake, the excess may become unavailable for the next crop. This study was conducted to investigate the nitrogen uptake and nitrogen use efficiencies of three annual forage/fiber crops that have potential as biomass feedstock: forage sweet sorghum [*Sorghum bicolor* L. Moench], sorghum x sudangrass (*Sorghum bicolor* var. *sudanense* (Piper.) Stapf.), and kenaf (*Hibiscus cannabinus* L.). A field experiment was conducted in Fargo and Prosper, ND, in 2010 and 2011. The experimental design was a randomized complete block design with a split-plot arrangement and three replicates. The different crops were the main plots and the nitrogen rates were regarded as subplots. The independent variables in this study were the three crops and the five rates of fertilization (0, 75, 100, 150, and 200 kg N ha^{-1}). Aboveground portions of whole plants were collected at three developmental stages [vegetative (V8), flowering (VF), and harvest (H)] to determine NO_3-N uptake throughout the growing season.

Both forage sweet sorghum and sorghum x sudangrass had significantly higher biomass yield than kenaf. Plant biomass yield also showed a significant response to N rates, while nitrate content of the plant tissue did not respond significantly to fertility rates at harvest. The recommendation from this study is to produce either forage sweet sorghum or sorghum x sudangrass and fertilize it at the 150 kg N ha^{-1} rate, which will produce dry matter biomass yield around 16 Mg ha^{-1}. This rate will also be cheaper than fertilizing at the maximum rate, while reducing the amount of nitrogen that could leach out beyond the root zone if there is an excessive rainfall event.

INTRODUCTION

Bioenergy and biomass production has increased since the year 2000 and is predicted to continue to increase due to the greater cost of energy and the greater demand for a limited supply of oil and natural gas.

Various industries have been working to reach the goal of the Renewable Fuel Standard (RFS): to produce 136 million m^3 of renewable fuel by 2022 (EPA, 2007), which can be produced from cellulosic, hemicellulosic, or lignin components. A biomass feedstock must have high dry matter yield, is versatile to changing environmental conditions, is produced with characteristics that make converting the lignocellulosic material to bioenergy most efficient, requiring minimal inputs and management costs, and can be collected and delivered to a geographically close processing site to reduce transportation costs (Hettenhaus, 2006; Zegada-Lizarazu and Monti, 2012). Annual crops such as sweet sorghum, sorghum x sudangrass, and kenaf may serve as biofuel feedstocks. Annual forage crops can be integrated into established cropping systems that and may require less input costs to achieve maximum yield (Hettenhaus, 2006; Zegada-Lizarazu and Monti, 2011). The movement towards greater sustainability practices in agriculture has created more interest in utilizing annual crops as feedstock for biofuel production.

Sorghum has been found to be an important potential bioenergy crop. Sorghum is a C4 crop with a deep root system, providing the plant with the structure necessary to obtain water and nutrients from deeper layers of the soil (Saballos, 2008; Rooney et al., 2007; Robertson et al., 1993). The crop only requires 320- to 400-mm of water per season, as compared with corn which requires 460- to 560-mm of water per season (NDSU Ext. Serv., 1997). The root system of sorghum also increases its efficiency at obtaining nutrients from the soil (Saballos, 2008).

Sudangrass has thinner stems and tends to be leafier than forage or sweet sorghum, giving it the ability to re-grow after being cut (Saballos, 2008). Sorghum x sudangrass hybrids have intermediate growth yield potential, though there is currently work being done to produce better cultivars with quality characteristics that are more favorable for conversion to bioenergy. Sorghum x sudangrass is known to be more adapted to drought, high temperatures, excess soil water, and low soil pH, and can yield anywhere from 7.8 to 11.8 Mg ha^{-1} in a season using a two- or three-cut system (Kilcer et al., 2005).

Kenaf is a warm-season, C3 fiber crop that is utilized for paper and pulp production of paper, pulp, and cordage products (Danalatos and Archontoulis, 2010; Webber et al., 2002). Its growth pattern makes it a potential feedstock for bioenergy, as it can grow between 2- and 4-m in height in just 150 d. Kenaf also has the ability to survive drought conditions for limited periods of time (Webber et al., 2002). A study conducted in Colorado showed how it can yield 2 Mg ha^{-1} with only 250mm water use during the growing season, and can reach 5 Mg ha^{-1} with 450 mm of water (Nielsen, 2004). Growing season length is also a factor in determining the geographic areas in which certain crops can be grown. In regards to harvest time, kenaf has the greatest yield (4 Mg ha^{-1}) around 90 days after sowing, then subsequently decreases with the loss of non-fiber components such as the leaves, or through lodging, as shown in a study conducted in Portugal (Fernando et al., 2004).

The quality components of these three forage crops are also important in considering their potential for use as bioenergy crops. Lignocellulosic biomass material dry matter consists of 300 to 500 g kg^{-1} cellulose, 200 to 400 g kg^{-1} hemicellulose, and 150 to 250 g kg^{-1} lignin (Lee et al., 2007; Hendriks and Zeeman, 2009). This material is used in the conversion of lignocellulosic material to ethanol (Mosier et al., 2005). Sorghum is a potential biomass feedstock for conversion to

bioethanol due to its desirable quality components. At harvest, sorghum contains around 340 g kg^{-1} cellulose, 170 g kg^{-1} hemicellulose, and 160 g kg^{-1} lignin (Lee et al., 2007). Sudangrass quality components are slightly different with 330 g kg^{-1} cellulose, 270 g kg^{-1} hemicellulose, and roughly 80 g kg^{-1} acid detergent lignin (ADL) and 120 g kg^{-1} crude protein (CP). Kenaf dry matter consists of 530 g kg^{-1} cellulose, 180 g kg^{-1} hemicellulose, and 80 g kg^{-1} lignin (Amaducci et al., 2000).

One of the most important nutrients for crop growth and physiological development is nitrogen. Nitrogen is one of the most limiting growth factors for plants (Mengel and Kirkby, 1982). It is a constituent of amino acids, amides, proteins, and nucleic acids (Taiz and Zeiger, 2006). There is a relationship between the amount of N in plant tissue and crop yield. For example, the amount of nitrate will drop in the crop when there is insufficient nitrogen, so the plant will exhibit deficiency symptoms with a decrease in yield or leaf yellowing. On the contrary, when adequate amounts of nitrogen are available for plant uptake, the higher concentration of nitrate in the plant gives it the ability to achieve greater yield. When there is too much N available for plant uptake, the concentration of nitrate in the plant tissue may reach toxic or harmful levels, causing a reduction in plant yield.

Nitrogen fertilizers in the United States are used quite extensively, where over-application can be a waste of the producer's money and may simultaneously pose a risk for NO_3-N to leach into ground water (Hadas et al., 1999; Sainju et al., 2007). The primary goal of nitrogen management and nitrogen use efficiency is nitrogen uptake, so an adequate amount of fertilizer should be applied during the time when the plant is actively taking it up and utilizing it (Johnson et al., 2005).

The application of nitrogen fertilizers can significantly impact the yield of field and forage crops. A study in Ames, IA, showed that nitrogen fertilization increased biomass yield 3.5 Mg ha^{-1}

by adding 280 kg N ha^{-1} (Rooney et al., 2007). The sweet sorghum responded to additional N fertilizer in a similar way. Marsalis et al. (2010) showed that the N rate of 218 kg N ha^{-1} resulted in forage sorghum yield of 24.6 Mg ha^{-1} under irrigation. These varying yield responses to nitrogen rates show how different environments can make a significant impact on biomass yield at increasing N rates. Sorghum x sudangrass hybrids can yield between 7.8 to 11.8 Mg ha^{-1} in one season with a two- or three-cut system, as shown by a study conducted in New York (Kilcer et al., 2005). Beyaert and Roy (2005) found that the cumulative yield from a three cut system in Delhi, Ontario increased from 3.5 Mg ha^{-1} to 5.5 Mg ha^{-1} from the 0 kg N ha^{-1} rate to the 125 kg N ha^{-1} rate. Sorghum x sudangrass quality has improved with the development of Brown Midrib (BMR) sorghum, which has a lower indigestible lignin concentration, and has potential to produce higher ethanol yield, as lignin can impede the conversion of lignocellulosic components into ethanol (Agassiz Seed, 2010; Dien et al., 2009; Producer's Choice Seed, 2007; Kilcer et al., 2005).

Webber et al. (2002) suggested that kenaf may require some additional N fertilizer to optimize its yield, though there was not a significant yield difference between 0 and 150 kg N ha^{-1} treatments. Danalatos and Archontoulis (2010) did not find statistical significance between the four nitrogen fertility rates (0, 50, 100, and 150 kg N ha^{-1}) on kenaf yield. The authors suspected this may be due either to the low nitrogen needs of the crop or the high fertility levels of the soil with higher soil moisture levels.

Nitrogen fertility recommendations for forage production differ from the recommendations for bioenergy production (Sanderson et al., 2006). The yield response from a fertilizer application, the price of the fertilizer, and the cost of the crop all help determine how profitable the crop will ultimately be (Johnson and Ali, 1979). The ultimate goal of fertilization for growers is to make money (Black, 1993). Lower fertilizer levels are more appropriate for biomass production as there

is a low price received for the crop, making it more economical for the grower (Partala et al., 2001). Most crop yields increase with the addition of nitrogen fertilizer, but there is a point where the rate of the yield increase will reduce at higher fertility levels (Johnson and Ali, 1979). The shape of the curve of the crop's response to nitrogen fertilizer shows how the price of N fertilizer and the price of the crop can determine the most profitable rate of application. Forage crops have yield response curves that level out less abruptly, making them more sensitive to the price of the fertilizer itself, though cultivars may respond differently to the same levels of fertilization as well (Mengel and Kirkby, 1982).

Both the NO_3^- and NH_4^+ forms of nitrogen can be taken up by plants, yet there may be a point when too much fertilizer has been applied to the soil and the plants are unable to take it all up, resulting in leaching into groundwater or to the atmosphere (Mengel and Kirkby, 1982). This can both hurt the environment and be a waste of money. When nitrogen fertilizer is limited, but applied in adequate amounts for crop uptake, the plants are better able to actively take up the nitrogen and use it more efficiently, reducing the cost of fertilizer. Nitrogen use efficiency (NUE) refers to the proportion of nitrogen applied that the crops are able to take up (Mengel and Kirkby, 1982).

The most economic rate of fertilizer could mean one of two things: the rate that brings about maximum crop yield, or the rate that brings about the greatest economic return (Black, 1993). Both of these factors must be considered in crop production. The maximum net profit from fertilization input is reached when the value of the crop exceeds the total cost of fertilization. Thus, NUE is calculated along with agronomic and physiological efficiencies to determine the most optimum economic rate of fertilization. When determining the crop most suitable for the cheapest biomass feedstock production, economics must be considered.

The specific objectives of this study are to (1) determine the annual biomass crop that produces the greatest biomass yield and optimal quality components that make the crop a potential feedstock for bioenergy utilizing different levels of nitrogen fertilizer and to (2) calculate the most economical rate of fertilization at which the greatest yield will still yield the greatest return for the grower. These objectives will be carried out in the following experimental approach.

MATERIALS AND METHODS

Field Establishment and Experimental Design

This research was conducted at the North Dakota State University (NDSU) research sites in Fargo, ND (-96°812'W, 46°897'N, 274 m elevation) and at the NDSU research site in Prosper, ND (-97°115'W, 47°002'N, 284 m elevation). The soil type in Fargo is Fargo-Ryan silty clay soil (fine, montmorillonitic, frigid, Vertic Haplaquoll, with a leached and degraded natric horizon); the Fargo series is fine, smectitic, frigid Typic Epiaquerts, while the Ryan series is fine, smectitic, frigid Typic Natraquerts (Soil Survey Staff, 2011). The soil type in Prosper is a Bearden silty clay loam (fine-silty, frigid Aeric Calciaquolls). Rainfall amounts were recorded automatically at both locations by the NDAWN system (NDAWN, 2012). Soil samples for analysis were taken at both locations the spring when the crop was planted. The soil analysis included pH, organic matter, N-NO_3, P, and K.

Previous crop in 2010 was corn in Prosper and soybean (*Glycine max* L. Merr.) in Fargo, and previous crops in 2011 were cereal crops in Fargo and corn in Prosper. The sweet sorghum, sorghum x sudangrass, and the kenaf were seeded at all locations with a cone plot planter. Planting dates at Fargo in 2010 and 2011 were 26 May, and planting dates at Prosper in 2010 and 2011 were 28 May and 7 June, respectively. The seeding rates were calculated based on the percentage of pure live seed. The seeding rate for the sweet sorghum and sorghum x sudangrass was 11 kg ha^{-1}, whereas the seeding rate for the kenaf was 5.5 kg ha^{-1}. The forage sweet sorghum (BMR Sweething) was obtained from Agassiz Seed (West Fargo, ND), sorghum x sudangrass (Forage King) was obtained from Producer's Choice Seed (Woodland, CA), and the kenaf was obtained from Tom Rymsza (VNS).

Soil analysis was done using the transnitration of salicylic acid method to determine the baseline N fertility level of each individual plot (Franzen and Cihacek, 1996; Vendrell and Zupancic, 1990). The initial soil analysis of each plot showed that none of the plots had 0 kg N ha^{-1} in the upper 0.62 m of soil, which suggests that a true baseline was not feasible. In each block, the plot with the least amount of nitrogen was selected as the control, with the treatments for the other four plots randomized. For the treatments, the amount of N fertilizer to add was calculated for each plot, so that the initial N levels of each plot would be fertilized up to the experimental rates of 75, 100, 150, and 200 kg N ha^{-1} (soil N + N fertilizer). If the initial soil N test indicated a higher level of N than the experimental rate in that particular plot, then no additional fertilizer was added. The source of N was urea [$CO(NH_2)_2$]. Urea was hand-broadcast in both locations in 2010 and 2011 on 11 June and 30 June, respectively. Each plot was fertilized individually. The experimental design in all sites was a randomized complete block design with three replicates and a split-plot arrangement where the crop was the main plot and the nitrogen rates were the sub-plots. Experimental units were 2m wide and 9m long with six rows separated at a 0.31-m row spacing. The seeding depth for all the crops was 20 mm.

Weed control for the plots in 2010 was hand-weeding as needed, and weed control in 2011 included pre-plant spraying of glyphosate (N-(phosphonomethyl)glycine) (1.4 kg a.i. ha^{-1}) followed by hand-weeding as needed.

Plant and Soil Sampling and Evaluations

Dependent variables evaluated were plant biomass yield; plant NO_3-N content; plant N content and uptake; and soil NO_3-N at 0 to 0.15 and 0.15 to 0.60-m depths.

Immediately prior to harvest, biomass samples were collected from each plot. Biomass samples were taken from the 2nd and 5th row in each six-row plot, where plants were cut at the stem

base. The two center rows from a 3.7 m^2 area were hand harvested in 2010 and the four-center rows were harvested in 2011 using a flail forage harvester.

Aboveground portions of whole sorghum plants were collected at three developmental stages: vegetative or 8-leaf stage (V8), panicle initiation or flower bud (R1), and harvest (H) (Vanderlip, 1993). Aboveground portions of whole kenaf plants were collected at the same time as the sorghum, though kenaf developmental stages are based on days after planting (DAP) and all three kenaf samplings were during its vegetative growth (Webber and Bledsoe, 2002).

Plant tissue total N content was determined by using a Kjeldahl procedure with 0.1 g of dried, ground plant tissue. Nitrogen uptake in the biomass was determined by multiplying biomass N content by biomass yield.

The determination of plant tissue quality analysis was done to determine dry matter (DM) (AOAC Method 934.01) and crude protein (CP) (AOAC Method 2001.11) (Horwitz and Latimer, 2010).

Soil samples from 0- to 0.15- and 0.15- to 0.60-m were taken for the analysis of NO_3-N in each plot prior to fertilization as well as immediately after harvesting. The soil samples were analyzed for NO_3-N just as they were at the beginning of the season using the transnitration of salicylic acid method (Cataldo et al., 1975) by the Soil and Plant Analysis Laboratory, NDSU.

Data Analysis

Relative biomass yield was calculated for each crop to more accurately determine how actual N levels in each plot impact biomass yield. Relative biomass yield is determined by taking yield of the plot with the greatest yield, and setting that as 100% yield. The yield of each plot in all four environments is then recalculated into a percentage of the greatest yield of that crop. The

relative biomass yield (%) is calculated by taking the biomass value of each plot and dividing it by the greatest yield value for that crop and multiplying that value by 100.

$$\text{Relative yield (\%)} = (\text{plot yield}/\text{greatest plot yield}) \times 100$$

A number of equations were utilized to ultimately determine the most efficient and economical use of nitrogen fertilizer application with the biomass crops for potential conversion to bioenergy. Nitrogen use efficiency (NUE), or apparent nitrogen recovery, reflects the proportion of nitrogen that the different crops take up (Mengel and Kirkby, 1982). The calculation of NUE takes the nitrogen content of the plants treated with fertilizer (N_{fert}) and subtracting the nitrogen content of the plants not treated with fertilizer, or the check treatment (N_{check}), and dividing that value by the units of nitrogen fertilizer applied in kg N ha^{-1} ($N_{applied}$).

$$\text{NUE (\%)} = [(N_{fert} - N_{check})/N_{applied}] \times 100$$

Agronomic efficiency (AE) indicates the yield increment obtained per unit of N fertilizer applied in kg yield per kg N (Mengel and Kirkby, 1982). It is determined using the biomass yield of the fertilized crop (Y_{fert}) and subtracting the biomass yield of the unfertilized crop (Y_{check}), divided by the units of nitrogen applied as the fertilizer ($N_{applied}$).

$$\text{AE (kg kg}^{-1}\text{ N)} = [(Y_{fert} - Y_{check})/N_{applied}]$$

Physiological efficiency (PE) is the biomass produced per unit of nitrogen absorbed by the plant (Mengel and Kirkby, 1982). It's calculated by subtracting the biomass yield of the unfertilized crop (Y_{check}) from the biomass yield of the fertilized crop (Y_{fert}), and dividing that value by the difference between the nitrogen uptake of the unfertilized crop ($N_{uptake\ check}$) from the nitrogen uptake of the fertilized crop ($N_{uptake\ fert}$).

$$PE = [(Y_{fert} - Y_{check})/(N_{uptake\ fert} - N_{uptake\ check})]$$

Finally, the optimum economical rate of fertilization for each crop will be determined using the information from NUE, AE, and PE equations and the yield increase regression model (Johnson and Ali, 1979). The yield response equation plots the dry matter obtained at the different N fertility levels and determines which rate maximizes biomass yield for each crop. The cost per unit of nitrogen fertilizer will be factored in to determine the final cost of the fertilizer to produce a unit of dry matter for each crop. The crops will then be compared using this information to determine the cheapest source of biomass. The final cost of N fertilizer per unit dry matter per hectare ($ N_{fert}) is the result of the multiplication of the cost per unit of fertilizer ($\$_{per\ unit\ N}$) and the units of N to produce maximum biomass (N_{units}).

$$\$\ N_{fert} = \$_{per\ unit\ N} \times N_{units}$$

The price per unit N in kg in 2012 was $1.15 (Akron Services, 2012). This price is for urea (46-0-0).

The yield response equation uses the regression coefficients b_0, b_1, and b_2 and the nitrogen uptake of each crop per hectare (x) to determine biomass yield (Y).

$$Y = b_0 + b_1 x - b_2 x^2$$

The marginal value product ($Dy\ Dx^{-1}$) equation is used to determine the profit maximum, taking the cost of fertilizer, the applied nitrogen fertilizer rates, and the cost of the crop into consideration. The equation can be set to zero so the most economical rate of nitrogen fertilizer for the greatest yield can be determined. This is determined by subtracting the regression coefficient b_2 multiplied by x from the regression coefficient b_1.

$$Dy\ Dx^{-1} = b_1 - b_2 x$$

Statistical Analysis

Statistical analysis was conducted by using standard procedures for a randomized complete-block design with a split-plot arrangement (Steel and Torrie, 1980). All of the data collected were analyzed by analysis of variance using the GLM procedure (SAS Institute, 2008), with each location-year combination considered an "environment" and a random effect, while nitrogen rates, developmental stages, and crops were considered fixed effects in the analysis. Analysis of variance was conducted within and across environments. Environments were considered homogenous when the mean square error variances for each trait were less than a factor of 10. If the environments were homogenous, then a combined analysis was conducted. A mean separation test was performed using the F-protected LSD at $P \leq 0.05$ level of significance for each evaluated trait. Regression analysis was done where there was a significant main effect. Linear and quadratic regression models were tested with the corresponding error. The regression models were all at $P \leq 0.05$ level of significance.

Plant biomass, soil NO_3-N, CP, total plant N, N uptake, and the efficiencies were analyzed according to a randomized complete block design with a split-plot arrangement, where the main plots were the crops (sweet sorghum, sorghum x sudangrass, and kenaf), and the subplots were the N rates (0, 75, 100, 150, 200 kg N ha^{-1}).

The plant NO_3-N sampling data was analyzed according to a randomized complete block design with a split-split plot arrangement, where the main plots were the crops (sweet sorghum, sorghum x sudangrass, and kenaf), the N fertility treatments (0, 75, 100, 150, and 200 kg N ha^{-1}) were the subplots, and the phenological stages (V8, R1, and H) were the sub-sub plots.

RESULTS AND DISCUSSION

Rainfall, Temperature, and Soil Analysis

Total growing season rainfall varied between years and months, with greater rainfall occurring from April through August in 2011 than in 2010 in both Fargo and Prosper (Table 2.1).

Table 2.1. Monthly growing-season rainfall for four environments, Fargo and Prosper, ND, in 2010 and 2011, and the deviation from the 30-year average.

	Fargo†				Prosper†			
	2010		2011		2010		2011	
Month	Rainfall	Dev.	Rainfall	Dev.	Rainfall	Dev.	Rainfall	Dev.
	----------mm----------							
April	36.8	2.0	45.8	11.0	29.5	-6.6	45.0	8.6
May	68.1	1.8	109.7	43.4	69.9	2.0	80.0	12.2
June	86.1	-3.1	100.9	11.7	80.8	-10.7	131.6	40.1
July	105.1	32.0	103.6	30.5	103.4	21.1	150.1	67.8
Aug.	67.7	3.7	72.5	8.5	89.4	21.3	88.9	20.8
Sept.	151.4	96.0	4.0	-51.4	134.6	80.5	6.1	-48.0
Oct.	60.6	10.6	20.6	-29.4	36.1	-11.9	9.4	-38.6
Total	575.8		457.1		543.7		511.1	

† NDAWN, 2012.

The amount of rainfall that fell during the month of July in Prosper, 2011, was the greatest amount of rainfall that occurred in the course of one month during the growing season. A thunderstorm in Prosper on July 19, 2011 delivered 50mm rain on the site according to the official NDAWN data (2012), yet visual observations soon after the storm hit suggested a greater amount fell, as the flooding in that area destroyed a number of research experiments. The widespread overland flooding was partially due to the saturated soil conditions that had been present for much of the spring and summer months. There was a reprieve from the moisture in time for harvest in 2011 where both locations received below-average rainfall in September and October.

This above-average rainfall in 2011 had resulted in a re-application of nitrogen fertilizer in Prosper, ND, as the nitrogen had leached out of the root zone. All of the crops exhibited yellowing

symptoms and the smell of ammonia could be easily detected, suggesting that the process of denitrification was taking place. This fertilizer re-application was conducted on July 25, 2011.

Above-average temperatures were observed in all four environments from June through August (Table 2.2).

Table 2.2. Growing-season average temperatures for four environments, Fargo and Prosper, ND, in 2010 and 2011, and the deviation from the 30-year average.

	Fargo†				Prosper†			
	2010		2011		2010		2011	
Month	Temp.	Dev.	Temp.	Dev.	Temp.	Dev.	Temp.	Dev.
	----------°C----------							
April	6.4	4.5	6.38	-0.3	5.8	4.4	5.8	-1.0
May	14.1	0.4	14.1	-1.2	13.5	0.4	13.5	-1.5
June	18.9	0.2	18.9	0.3	18.4	0.3	18.4	0.4
July	21.4	0.9	21.5	2.2	21.1	0.0	21.1	2.1
Aug.	20.6	1.6	20.6	1.2	20.1	1.0	20.1	1.0
Sept.	14.4	-0.8	14.4	1.0	14.4	-1.5	14.4	0.8
Oct.	7.4	2.8	7.4	3.9	7.5	1.8	7.5	3.3

† NDAWN, 2012.

Even though September temperatures were above average in 2011, an early frost occurred on September 15, 2011, which forced an early harvest the next day for the forage sweet sorghum, sorghum x sudangrass, and kenaf. Both of the Fargo and Prosper sites were hit by the frost, so harvest was conducted to avoid biomass lost when the leaves dried up. The planting dates in 2011 in Fargo and Prosper were May 26 and June 7, respectively, resulting in the growing season duration of 112 days and 100 days, respectively. Early-season moisture, mid-season flooding rainfall in both locations and an early frost may have caused a reduction in plant growth in these environments.

Each environment was different in terms of soil conditions, particularly initial soil N levels. The initial soil NO_3-N results are indicated in Table 2.3.

Table 2.3. Initial soil NO_3-N analysis for four environments and five N rates across three crops combined (forage sweet sorghum, sorghum x sudangrass, and kenaf) prior to planting for soil depths of 0 to 60 cm.

Environment	0	75	100	150	200
	----------	----------	kg NO_3-N ha^{-1}	----------	----------
Fargo 2010	94.3	103.9	109.3	116.7	125.8
Fargo 2011	67.9	78.2	96.1	98.9	99.3
Prosper 2010	82.1	98.8	119.5	131.0	162.8
Prosper 2011	27.3	35.3	34.6	34.3	35.3

In 2010, Fargo and Prosper locations had average soil NO_3-N levels that were higher than the experimental fertility rates of 0, 75, and 100. Fargo and Prosper had lower levels of nitrogen in the soil in 2011 compared to the previous year, but still had average fertility levels higher than the experimental rate of 0 in Fargo and Prosper, as well as the 75 kg N ha^{-1} experimental rate in Fargo. For the analysis, the mean actual N levels for each crop and each treatment were calculated and used in the analysis. The average actual N rates for forage sweet sorghum were 70, 94, 108, 150, and 200 kg N ha^{-1}, the rates for sorghum x sudangrass were 68, 92, 111, 152, and 202 kg N ha^{-1}, and the rates for kenaf were 66, 89, 118, 155, and 203 kg N ha^{-1}.

The initial soil analysis for soil P, K, organic matter (OM), and pH was conducted for each environment before planting (Table 2.4).

The organic matter levels were greater in Fargo than in Prosper locations in both 2010 and 2011. The pH was between 7 and 8 in all four environments, so N and K were most available for plant uptake (Mengel and Kirkby, 1982).

The optimum soil pH for forage sorghum is between 6 and 7.5 (Saballos, 2008; McClure, 2012), sorghum x sudangrass optimal pH level is between 6 and 6.5 (Teutsch, 2009), and kenaf optimal pH level is between 6 and 6.8 (Rowell and Stout, 2006).

Table 2.4. Initial soil analysis for four environments prior to planting for soil depths of 0 to 60 cm.

Environment	pH	OM	P	K
		----------mg kg^{-1}----------		
Fargo 2010	7.9	5.7	22.0	445.0
Fargo 2011	7.4	6.5	15.6	321.7
Prosper 2010	8.1	2.7	38.0	400.0
Prosper 2011	7.1	3.1	39.5	267.1

† OM: Organic matter

There were lower pH levels in 2011 than in 2010. Phosphorus levels were between 15 and 25 mg kg^{-1} in Fargo and between 35 and 40 mg kg^{-1} in both 2010 and 2011, so no additional P fertilization was required. Initial soil K levels were greater in 2010 than in 2011, with levels greater than the minimum K fertility requirements for all three crops, so no additional K fertilizer was required.

Biomass Yield

The analysis of variance for biomass and relative biomass yield showed the main effects of crop and N rates were significant, though the interaction between them was not (Table 2.5).

Table 2.5. Analysis of variance and mean squares for sorghum, sudangrass, and kenaf biomass and relative yield for five N rates across four environ., Fargo and Prosper in 2010 and 2011.

Sources of variation	df	Biomass yield	Relative biomass yield
Env	3	1654.7	19295.3
Rep(env)	8	138.8	1270.0
Crop	2	1024.0**	268.4
Env x crop	6	87.0	780.6***
Error (a)	16	33.4	303.1
N	4	106.2***	1294.8***
Env x N	12	9.1	112.0
N x crop	8	7.0	66.7
Env x N x crop	24	9.5	100.7
Error (b)	96	5.4	52.7
CV, %		19.1	17.8

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively.

Forage sweet sorghum and sorghum x sudangrass hybrid biomass yield did not differ significantly from one another, but they both had significantly higher dry matter yield than kenaf (Table 2.6).

Table 2.6. Mean biomass and relative biomass yield at harvest for three crops averaged across N rates and four environments in Fargo and Prosper, ND, in 2010 and 2011.

Crop †	Biomass yield	Relative biomass yield
	----------Mg ha^{-1}----------	----------%----------
Forage sweet sorghum	14.79	42.7
Sorghum x sudangrass	14.35	41.4
Kenaf	7.43	38.5
LSD (*P*=0.05) ‡	4.16	12.5

† Average actual N rates for forage sweet sorghum (70, 94, 108, 150, and 200); N rates for sorghum x sudangrass (68, 92, 111, 152, and 202); N rates for kenaf (66, 89, 118, 155, 203).
‡ To compare the means of different crops.

Sorghum and sorghum x sudangrass both had of forage sweet sorghum in this study, when averaged across the N rates, was around 15 Mg ha^{-1}, which was half of what the literature states is possible (30 Mg ha^{-1}) (Rooney et al., 2007). Sorghum x sudangrass biomass yield was higher than the average stated in Kilcer et al. (2005) in NY, which is 8-12 Mg ha^{-1}, due to the plants ability to grow in more stressed conditions (excessive rainfall, high temperatures, then drought) and the use of an only one-cut system.

Plants typically have an increase in biomass yield as nitrogen fertility treatments increase. The analysis of variance did not show a significant interaction between treatment and crop because each crop responded similarly to higher nitrogen fertility rates. Instead of showing the combined response of the three crops to nitrogen rates, the three crops will be shown individually.

The regression model was calculated for each of the crops using relative yield with respect to the actual nitrogen rates present in each of the individual plots. The regression model (y=-0.7 +

$0.6x - 0.002x^2$, r^2=0.16) showed a polynomial increase in forage sweet sorghum biomass yield as the rate of N fertility increased (Fig. 2.1).

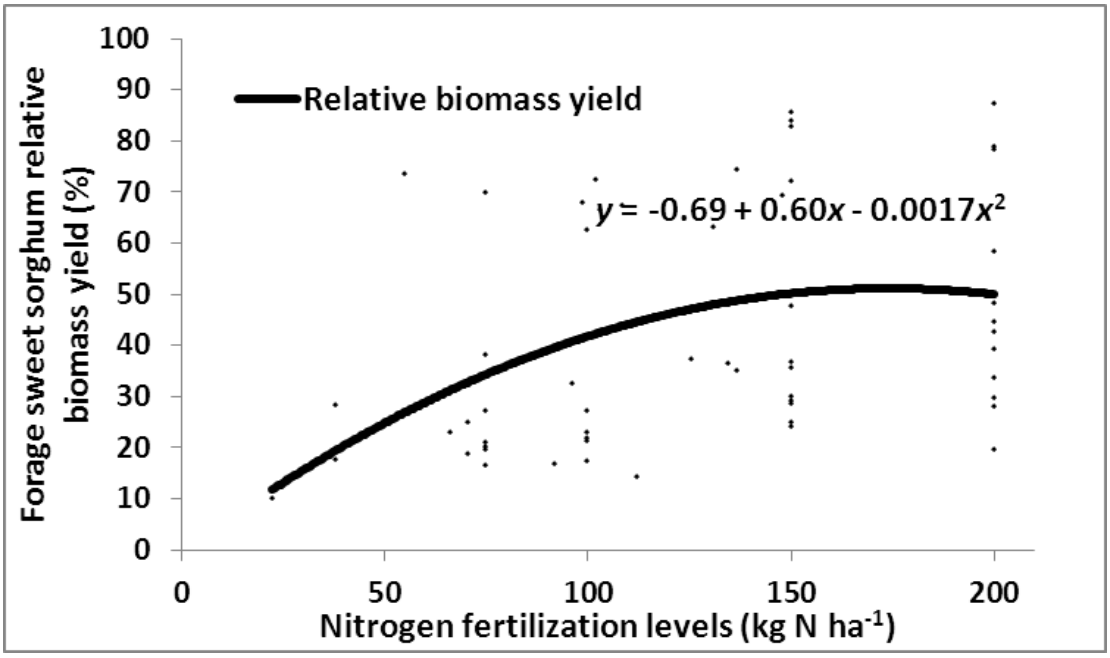

Fig. 2.1. Regression model for relative biomass yield of forage sweet sorghum as affected by actual N rates averaged across four environments in Fargo and Prosper, ND, in 2010 and 2011.

The greatest relative biomass yield according to the regression model was at 176 kg N ha^{-1}. The regression model ($y = 1.4 + 0.5x - 0.002x^2$) showed a similar response to nitrogen fertilization for sorghum x sudangrass (Fig. 2.2). The greatest relative biomass yield for sorghum x sudangrass according to the regression model was found at 180 kg N ha^{-1}.

The regression model ($y = -3.8 + 0.6x - 0.002x^2$) for relative biomass yield was also done for kenaf (Fig. 2.3). This regression model showed that kenaf greatest relative biomass yield was found at the lowest N rate of the three crops, at 174 kg N ha^{-1}. Because the initial available N levels were greater than some of the treatment levels (Table 2.3), the actual N levels after fertilization were used to calculate the regression curves for each crop.

The average NO_3-N content in the soil was 87.6 kg NO_3-N ha^{-1} before planting. The plots with the check N fertility levels already contained between 27 and 94 kg N ha^{-1} in the plots before planting, and the 75 kg N ha^{-1} fertility rate already had between 35 and 104 kg N ha^{-1} before fertilization (Table 2.3).

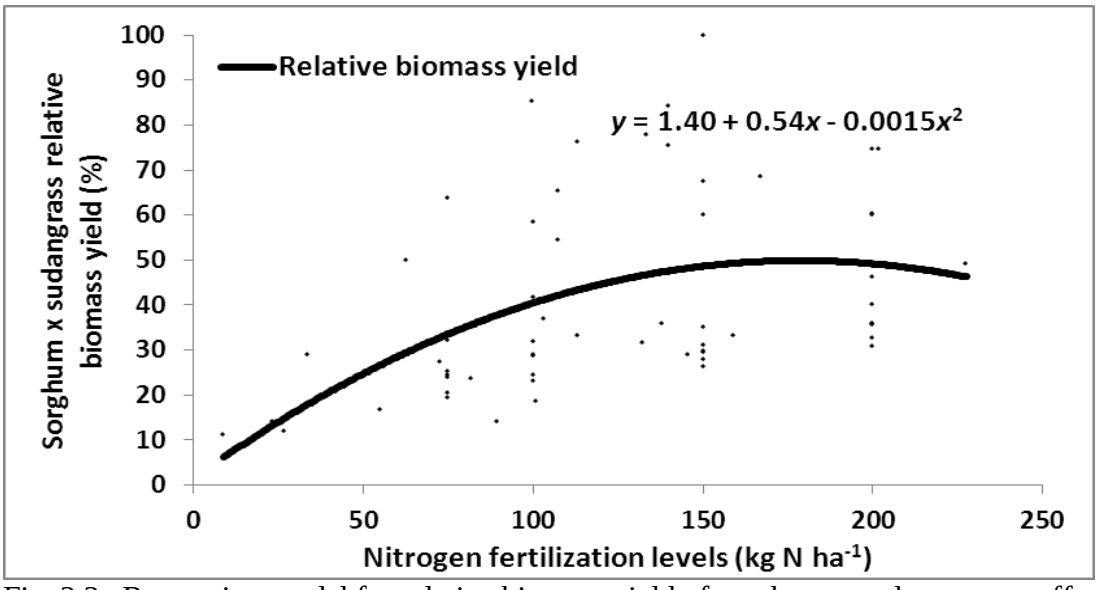

Fig. 2.2. Regression model for relative biomass yield of sorghum x sudangrass as affected by actual N rates averaged across four environments in Fargo and Prosper, ND, in 2010 and 2011.

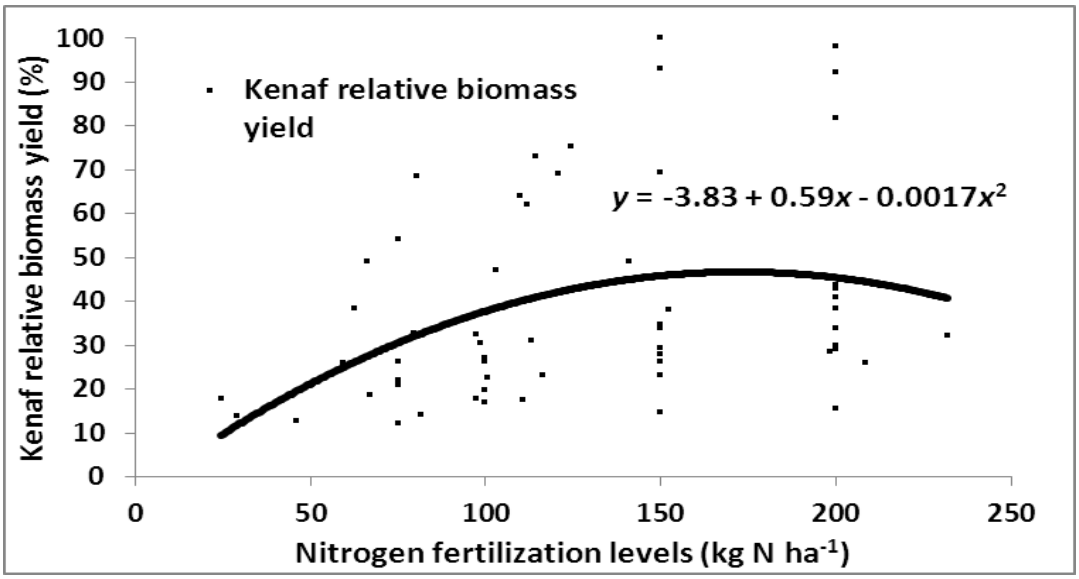

Fig. 2.3. Regression model for relative biomass yield of kenaf as affected by actual N fertility levels averaged across four environments in Fargo and Prosper, ND, in 2010 and 2011.

The environmental conditions may also have impacted biomass yield, as the late planting, early- to mid-season rainfall, and the early-frost in 2011 provided less than optimal growth conditions, shortening the growing season duration. Biomass yield responded to increasing N rates, so as N rates increased, dry matter biomass yield increased. Forage sweet sorghum and sorghum x sudangrass yield was significantly greater than kenaf biomass yield.

Plant NO_3-N Content

Plant tissue nitrate analysis was done to determine the amount of NO_3-N present in the tissue at the three growth stages. The N fertility rates were the only main effects that were significant for the combined analysis across environments for nitrate analysis (Table 2.7).

The stage by crop and the stage by N rate interactions were also found to be significant. Environment by crop, environment by N rate, environment by N rate by crop, stage by environment, stage by environment by crop, stage by environment by N rate, and stage by environment by N rate by crop are all interactions that were found to be significant, but will not be discussed as environment is considered a random effect. The discussion will focus on the main effects and the interaction between fixed effects.

In the interaction between crop and growth stage, kenaf was found to have the greatest content of NO_3-N in its tissue at the earliest growth stage (Table 2.8).

Kenaf had a significantly greater level of nitrate at V8 than forage sweet sorghum or sorghum x sudangrass at that time, where the two sorghum crops were not significantly different from one another. The majority of nitrogen present in plant leaves is found in proteins on the photosynthetic pathway. Kenaf is a broadleaf crop, which naturally takes up more NO_3-N than

Table 2.7. Analysis of variance and mean squares for forage sweet sorghum, sudangrass x sorghum, and kenaf plant-tissue nitrate analysis for five N rates and three developmental stages across four environments, Fargo and Prosper, ND, in 2010 and 2011.

Sources of variation	df	NO_3-N
Env	3	11772920.4
Rep(env)	8	1692733.9
Crop	2	992906.3
Env x crop	6	2712053.8**
Error(a)	16	418279.6
N	4	20200219.0**
Env x N	12	3770150.2***
N x crop	8	1054359.7
Env x N x crop	24	836840.6**
Error (b)	96	405979.6
Stage	2	42639096.2
Stage x env	6	9874466.6***
Stage x crop	4	5541925.8**
Stage x env x crop	12	616246.3**
Stage x N	8	5846909.9*
Stage x env x N	24	1788042.6***
Stage x N x crop	16	358187.2
Stage x env x N x crop	48	248520.5
Error(c)	240	265113.1
CV, %		41.8

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively.
† Average actual N rates for forage sweet sorghum (70, 94, 108, 150, and 200); N rates for sorghum x sudangrass (68, 92, 111, 152, and 202); N rates for kenaf (66, 89, 118, 155, 203).

Table 2.8. Interaction among growth stages [vegetative 8-leaf (V8), reproductive (R1), and harvest (H)] and three crops in NO_3-N levels averaged across N rates and four environments, Fargo and Prosper, ND, in 2010 and 2011.

Crop	V8	R1	H
	---------- mg kg^{-1} ----------		
Forage sweet sorghum	1548.4	956.2	1145.3
Sorghum x sudangrass	1606.2	854.1	1036.9
Kenaf	2225.4	1011.2	699.4
LSD (*P*=0.05) †	476		
LSD (*P*=0.05) ‡	823		
LSD (*P*=0.05) §	865		

† To compare the means of different crops within a growth stage.
‡ To compare the means of the different growth stages within a crop.
§ To compare the means of different crops with different growth stages.
† Average actual N rates for forage sweet sorghum (70, 94, 108, 150, and 200); N rates for sorghum x sudangrass (68, 92, 111, 152, and 202); N rates for kenaf (66, 89, 118, 155, 203).

grasses, like sorghum. Kenaf, as a C3 crop, CO_2 is fixed by the 1,5-Ribulose bisphosphate carboxylase (Rubisco) alone, which utilizes more N in the process of CO_2 fixation (Lindquist et al., 2007).

On the other hand, sorghum is a C4 crop which first fixes CO_2 by the phosphoenolpyruvate (PEP) carboxylase to form malate in the bundle sheath cells, then malate is transported to the mesophyll cells where the CO_2 is released into the cytoplasm and fixed by the Rubisco enzyme. There were no significant differences between the crops at the latter two stages, though the difference between the sorghum crops and kenaf approached significance. Kenaf NO_3-N content in its tissue was less than the sorghum crops at harvest, suggesting that NO_3-N had been assimilated and converted to other components.

Kenaf NO_3-N content decreased significantly from V8 to R1 growth stage and from R1 to H stage, as nitrate was assimilated and converted to amino acids (Taiz and Zeiger, 2006). First, NO_3^- is assimilated by the nitrate reductase into NO_2^-, then the nitrite reductase coverts NO_2^- into NH_4^+. Ammonium is then converted to amino acids to prevent ammonium toxicity within the cell. The nitrate content concentration thus decreases as it is converted during plant development. Forage sweet sorghum and sorghum x sudangrass nitrate content did not decrease significantly from one stage to another.

The interaction between growth stage and N rates also significantly affected plant tissue NO_3-N levels (Fig. 2.7). A regression analysis was done for each crop separately as the crops responded similarly at each stage to the increasing N rates.

The regression analysis showed a polynomial increase in plant tissue nitrate levels at the V8 growth stage with increasing rates of nitrogen fertilization for forage sweet sorghum (y=1854.6 – 21.6x + 0.1x^2) (Fig. 2.4).

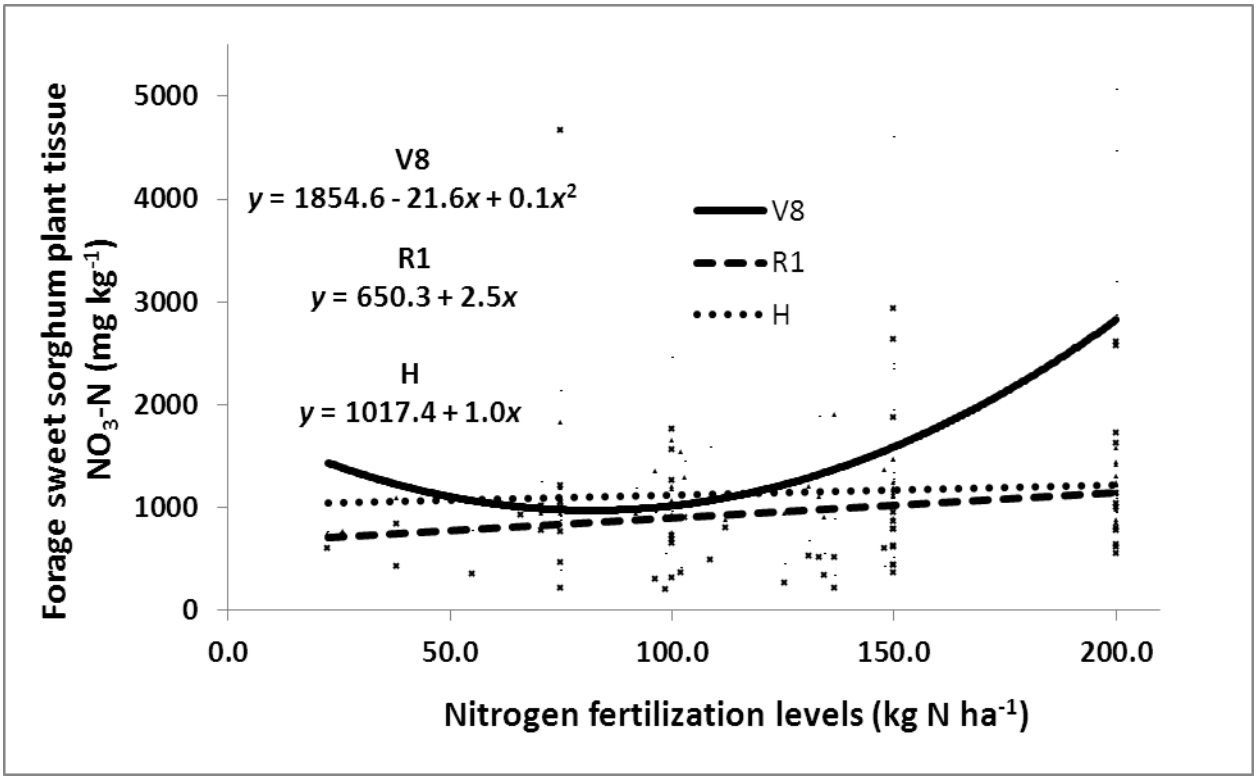

Fig. 2.4. Regression model for plant tissue NO_3-N of forage sweet sorghum at three growth stages [vegetative 8-leaf (V8), reproductive (R1), and harvest (H)] averaged across four environments, Fargo and Prosper in 2010 and 2011 as affected by actual N fertility levels.

When the plant tissue NO_3-N level of each plot were plotted against the actual N levels in the soil after fertilization, the regression line showed that at the V8 stage, forage sweet sorghum NO_3-N in the plant tissue decreased initially, but then increased at N rates greater than 100 kg N ha^{-1}. For every kg ha^{-1} of N that is applied, the rate of tissue nitrate accumulation increased. At the earlier growth stage, the plant tissue nitrate content increased from 1000 mg kg^{-1} to 2500 mg kg^{-1} from the 50 kg N ha^{-1} fertility rate to the 200 kg N ha^{-1}. At the later two stages, NO_3-N content did not change significantly at increasing N rates.

The regression analysis showed a polynomial increase in plant tissue nitrate levels at the V8 growth stage with increasing rates of nitrogen fertilization for sorghum x sudangrass (y= $1357.4 – 10.0x + 0.08x^2$) (Fig. 2.5).

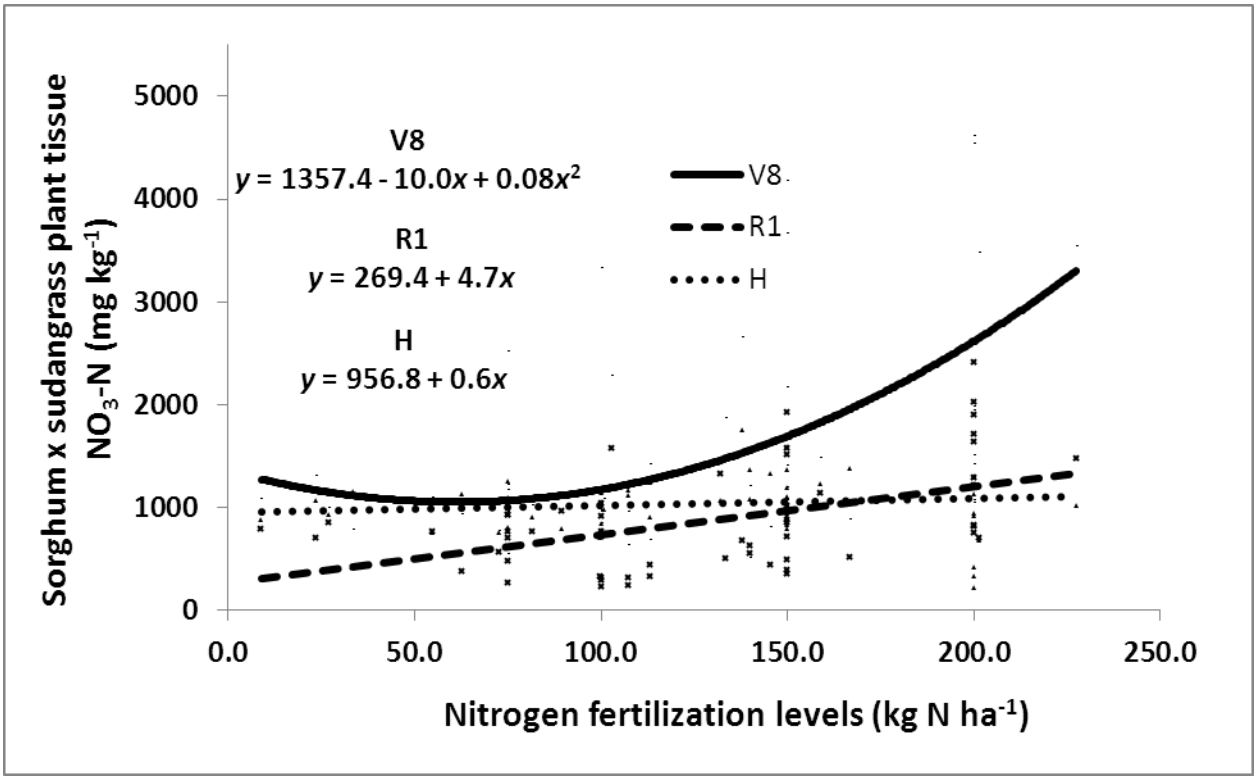

Fig. 2.5. Regression model for plant tissue NO_3-N of sorghum x sudangrass at three growth stages [vegetative 8-leaf (V8), reproductive (R1), and harvest (H)] averaged across four environments, Fargo and Prosper in 2010 and 2011 as affected by actual N fertility levels.

When the plant tissue NO_3-N level of each plot were plotted against the actual N levels in the soil after fertilization, the regression line showed that at the V8 stage, sorghum x sudangrass NO_3-N in the plant tissue increased at N rates greater than 62.5 kg N ha^{-1}. For every kg ha^{-1} of N that is applied, the rate of tissue nitrate accumulation increased. At the earlier growth stage, the plant tissue nitrate content increased from 1000 mg kg^{-1} to 3000 mg kg^{-1} from the 62 kg N ha^{-1} fertility rate to the 200 kg N ha^{-1}. At the later two stages, NO_3-N content increased linearly, but not significantly at increasing N rates.

The regression analysis showed a linear increase in plant tissue nitrate levels at the V8 growth stage with increasing rates of nitrogen fertilization for kenaf (y= -429.8 + 21.0x) (Fig. 2.6).

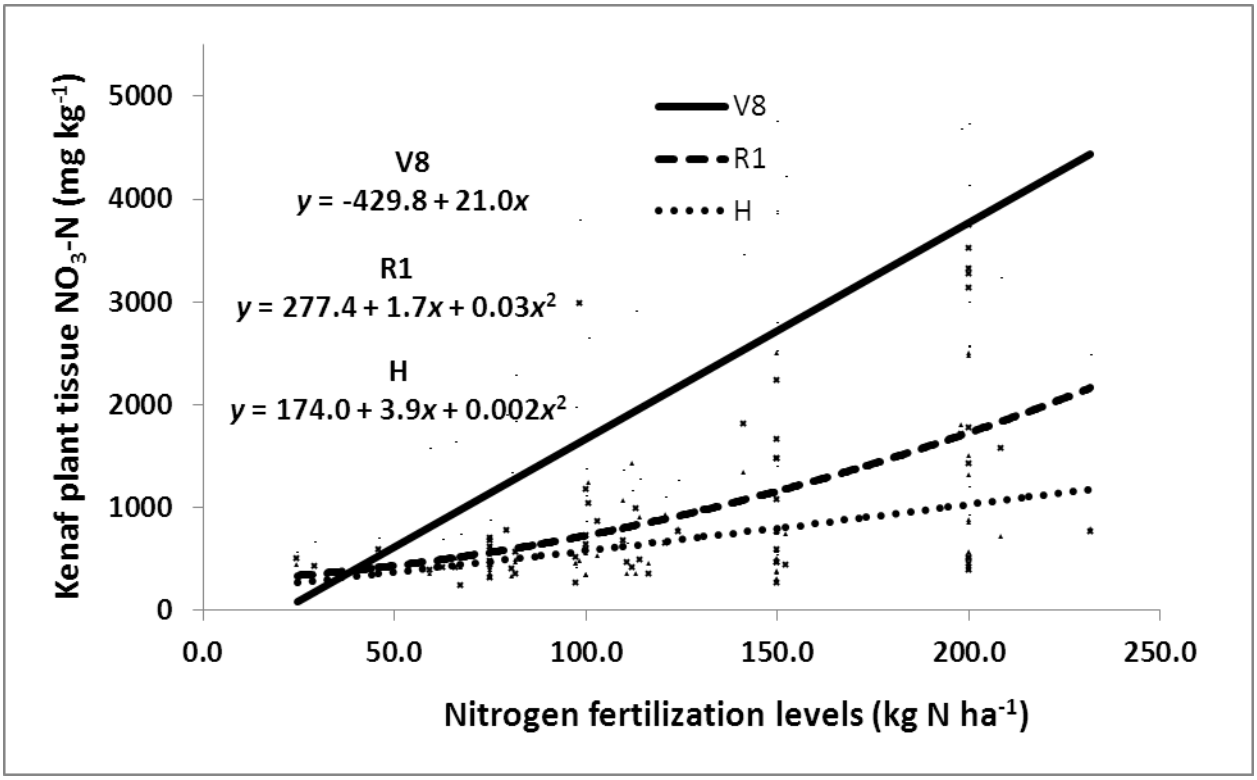

Fig. 2.6. Regression model for plant tissue NO_3-N of kenaf at three growth stages [vegetative 8-leaf (V8), reproductive (R1), and harvest (H)] averaged across four environments, Fargo and Prosper in 2010 and 2011 as affected by actual N fertility levels.

When the plant tissue NO_3-N level of each plot were plotted against the actual N levels in the soil after fertilization, the regression line showed that at the V8 stage, kenaf NO_3-N in the plant tissue increased at N rates increased. For every kg ha^{-1} of N that is applied, the rate of tissue nitrate accumulation in the kenaf increased. At the earlier growth stage, the plant tissue nitrate content increased greatly, but at the later two stages, there was a polynomial increase in NO_3-N content due to higher N rates.

The majority of nitrate uptake occurs during the initial vegetative growth of the crop. Soon after the nitrate is taken up into the plant, it is absorbed and rapidly converted to ammonium. Nitrate is converted to NO_2, catalyzed by the nitrate reductase enzyme (Taiz and Zeiger, 2006).

Because nitrite is highly reactive, and can be toxic, it is immediately transported from the cytosol into chloroplasts in leaves and the plastids in roots to be converted to ammonium, catalyzed

by nitrite reductase. To avoid ammonium toxicity if levels are too high, the plant can use one of two pathways to convert ammonium to amino acids glutamine and asparagine, which are then incorported into enzymes and proteins as needed throughout the plant.

The lower levels of nitrate at later growth stages indicate that the nitrate content decreased over time, as most of it is assimilated into proteins. The fertilization levels did not make any difference in the nitrate content of the crop tissue at harvest, which is desirable when considering how nitrate content can negatively impact biomass conversion to bioenergy. In a study conducted by Fernando et al. (2012), the author found that the higher the NO_3 in kenaf tissue, the greater the amount of ash present in the biomass. Better biomass quality has lower ash content and lower levels of nitrogen in above-ground biomass, which can make the conversion of biomass to various forms of bioenergy more efficient.

Tissue N Concentration and Nitrogen Uptake

A combined analysis was done of tissue N content, N uptake, and three efficiencies across three environments. The error variance for the fourth environment, Fargo in 2011, was very high and not homogenous with the other environments, so a combined analysis was conducted for only three environments, Fargo and Prosper in 2010, and Prosper in 2011.

For total N content, the crop and N rates main effects were found to be significant, as well as the interaction between crop and N rates (Table 2.9).

A regression analysis was conducted for the three crops to determine the total N content response to increasing nitrogen fertility rates. One of the crops that exhibited a significant response to N fertilization in its total N content at harvest, according to the regression analysis, was kenaf.

Table 2.9. Analysis of variance and mean squares for sorghum, sudangrass, and kenaf total N content, N uptake and efficiencies for five N rates across three environments, Fargo and Prosper, ND in 2010, and Prosper, ND in 2011. †

Sources of variation	df	Total N	N uptake	df	Agronomic efficiency	df	Physiological efficiency	NUE
Env	2	4.6	124223.7***	2	1746.4**	2	59569.0*	3591.1*
Rep(env)	5	21.2	13986.1	6	1128.9	5	25424.1	5165.0
Crop	2	276.2*	15914.4	2	4175.0	2	64362.8	1250.3
Env x crop	4	22.7	4799.7	4	2183.7	4	13591.1	1230.1
Error (a)	10	8.4	4430.8	12	2066.5	10	22027.9	2223.4
N	4	15.1*	11986.4**	3	216.3* ‡	3	19231.9	359.3
Env x N	8	4.2	1371.6	6	52.1	6	35861.4*	1234.4
N x crop	8	4.0*	1206.2	6	247.1	6	15071.0	407.3
Env x N x crop	16	1.2	1018.8	12	332.7	12	20779.0	462.6
Error (b)	60	7.7	1465.2	54	232.6	45	15469.6	927.6
CV, %		29.7	30.0		62.4		118.2	103.7

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively.
† Environment Fargo, 2011 was excluded from combined analysis
‡ Significant at $P \leq 0.065$

The crop showed a polynomial increase in the total N content (y=5.4 + 0.05x - 0.00007x^2), increasing from around 10 g N kg^{-1} to 15 g N kg^{-1} (Fig. 2.7). Forage sweet sorghum and sorghum x sudangrass exhibited similar responses to higher N rates in their total N content.

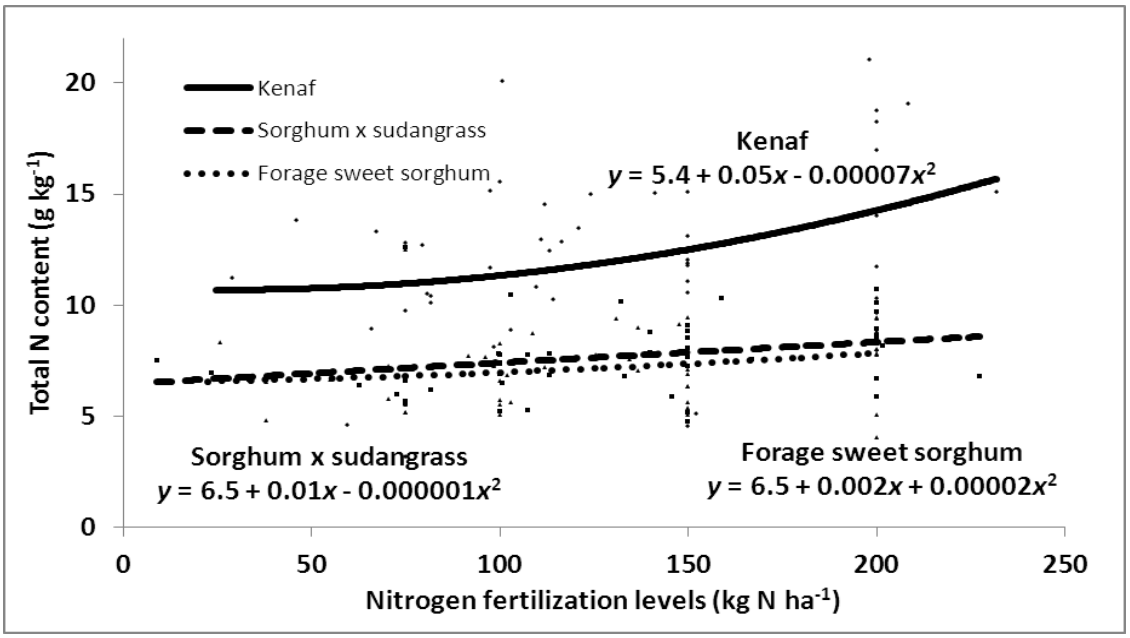

Fig. 2.7. Regression model for total N content of three crops averaged across four environments, Fargo and Prosper in 2010 and 2011 as affected by actual N fertility levels.

In the analysis of variance for N uptake, the only significant effect that was found was the main effect of N (Table 2.9). A regression analysis across environments was conducted to determine the response of each crop N uptake to N rates (Fig. 2.8).

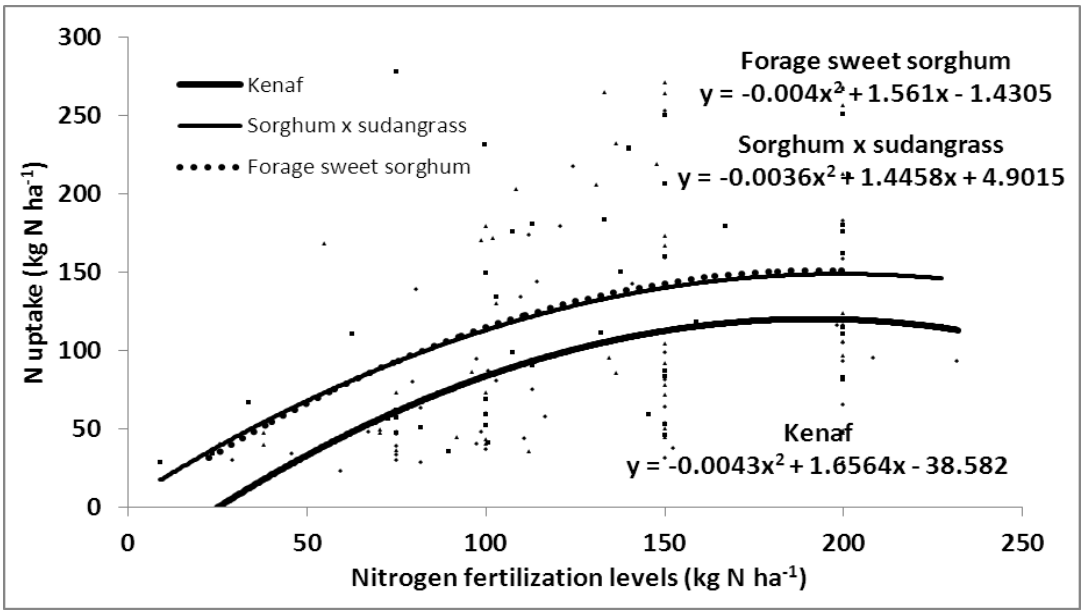

Fig. 2.8. Regression model for N uptake of three crops averaged across four environments, Fargo and Prosper in 2010 and 2011 as affected by actual N fertility levels.

The N uptake of each of the crops had a polynomial increase and responded similarly. Kenaf had lower N uptake than the sorghum crops, ranging from 25 kg N ha^{-1} to 100 kg N ha^{-1}, where the sorghum crops took up between 50 kg N ha^{-1} and 150 kg N ha^{-1}, depending on the nitrogen fertility level of the crop. Previous research suggests that total N uptake for kenaf can range from 50 to 65 kg N ha^{-1} (Muir, 2001). The average N uptake level reported for sorghum is 31 kg ha^{-1}, ranging from 13 to 54 kg ha^{-1}, depending on the soil conditions (Kaizzi et al., 2012).

Nitrogen is a mobile element in plants that is able to retranslocate during deficiencies (Taiz and Zeiger, 2006). Plants assimilate nitrogen through biochemical reactions involving oxidation and reduction processes to form covalent bonds with carbon, to ultimately create carbon and

organic compounds. Nitrogen is taken up by the roots and translocated through the xylem to the upper plant parts (Mengel and Kirkby, 1982) (Fig. 2.8).

Most of the ammonium is absorbed and assimilated in the root tissue and distributed in the form of amino acids. Nitrate, on the other hand, is able to be translocated to the leaves and shoots and assimilated there. Nitrogen is transported through the xylem in the forms of nitrate and amino acids most commonly. The intensity of nitrogen metabolism is dependent on the rate of protein synthesis which controls the import of nitrogen by the different plant parts.

These crops were fertilized with urea, a form of ammonia fertilizer. Yet plants are able to uptake both nitrate and the ammonia forms of nitrogen. Fertilizer urea goes through a number of reactions in the soil itself before it is taken up by the plants (Mengel and Kirkby, 1982). Urea is generally converted to NH_4-N by urease or split into NH_4^+ and CO_2. Ammonium can be taken up by the plants at a lower rate of absorption because of its strong bond to the negatively charged clay minerals in the soil, or it can be oxidized to form NO_3^-, which can be absorbed more quickly. This nitrate can be either taken up by the plants or leached out, depending on the soil and environmental conditions. Ammonia is rapidly converted to NO_3^- under normal soil conditions during the growing season (L. Cihacek, personal communication, 2012), which is why crops take up NO_3^-, even when NH_4^+ fertilizers are applied.

Ammonium is converted to amino acids through a process requiring two enzymes (Taiz and Zeiger, 2006). The primary pathway utilizes glutamine synthetase enzyme to combine ammonium with glutamate to form glutamine. When the level of glutamine increases, the enzyme glutamate synthase converts the glutamine to glutamate. The alternative pathway active in ammonium assimilation uses glutamate dehydrogenase to synthesize glutamine and glutamate. This reaction is catalyzed by aminotransferases to produce amino acids.

A number of factors can influence the loss of NH_3 from the soil. Soil pH can influence the loss of NH_3 from the soil, where higher pH soils have greater NH_3 loss due to volatilization (Mengel and Kirkby, 1982). Urea is rapidly converted to NH_3 in alkaline soils. Losses can also occur when urea is applied to the soil surface (not incorporated), and when there is little- to no-rain to incorporate it into the soil. If there is too much NO_3^- produced from the oxidation of NH_4^+, higher levels of rainfall can leach NO_3^- to deeper layers of soil where it is leached and lost.

Agronomic Efficiency, Physiological Efficiency, and Nitrogen Use Efficiency

The analysis of variance was done for agronomic efficiency for the three environments combined (Table 2.9). The only significance found was for the main effect of N fertility at $P \leq 0.065$. A regression analysis did not show any significance in the agronomic efficiency values to different N rates due to high variability. The crops averaged between 20 and 30 kg dry matter per kg N applied, but no conclusions can be made on the impact of these different N rates on agronomic efficiency in this particular study.

The high initial soil fertility levels at the 0 and 75 kg N ha^{-1} rates may have impacted the calculation of the efficiency, so the average actual N levels present after fertilization were used for each crop and each fertility level separately for the analysis (Table 2.10). Also, there may have been leaching of N out of the root zone of the crop, impacting the amount of N the crops were able to uptake.

However, a study conducted on sorghum in Uganda found that agronomic efficiency decreased with increased nitrogen fertility rates (Kaizzi et al., 2012). At the 50 kg N ha^{-1} fertility rate, sorghum biomass yield increased by 46.5 kg per unit N fertilizer, whereas at the 150 kg N ha^{-1} rate, sorghum biomass yield only increased 16.9 kg per unit N fertilizer. The low initial soil fertility levels may have impacted the efficiencies described in this study.

Table 2.10. Interaction among nitrogen fertilization rates and three crops for efficiencies averaged across three environments (env), Fargo and Prosper in 2010 and Prosper in 2011.

Crop/N rates	Agronomic efficiency†	Physiological efficiency	Nitrogen use efficiency
	kg bmass kg N^{-1} applied	kg bmass ha^{-1} per unit N kg ha^{-1} uptake	kg N ha^{-1} uptake per kg N ha^{-1} applied
Forage sweet sorghum			
94	38.6	115.9	52.4
108	31.7	215.7	27.8
150	40.3	195.6	33.6
200	27.3	81.3	36.4
Sorghum x sudangrass			
92	27.4	81.9	34.9
111	26.1	94.0	20.1
152	27.3	117.0	24.7
202	22.6	80.1	25.1
Kenaf			
89	6.1	58.4	16.2
118	9.8	80.9	24.4
155	18.1	49.9	24.7
203	18.2	92.7	31.8
LSD (*P*=0.05) ‡	28.7	NS	NS
LSD (*P*=0.05) §	NS	NS	NS
LSD (*P*=0.05) ¶	28.0	NS	NS

† To compare the means of different crops within an N rate.
‡ To compare the means of the different N rates within a crop.
§ To compare the means of different crops with different N rates.

Kenaf is a member of the mallow (Malvaceae) family, a relative of cotton (*Gossypium hirsutum* L.). There is a lack of substantial information about the agronomic efficiency of kenaf, but there is data on the subject available for cotton. In a study conducted in India, cotton was studied with two irrigation practices and four N rates with N applied in six rates during the growing season (Aujla et al., 2005). The agronomic efficiency also decreased with increasing N fertility rates. When N was applied at 100% of the recommended rate (six rates each with 12.5 kg N ha^{-1} totaling 75 kg N ha^{-1}), the agronomic efficiency was found to produce 28.6 kg seed cotton per unit N in kg ha^{-1}. When N was applied at 50% the recommended rate (six rates each with 6.25

kg N ha^{-1} totaling 37.5 kg N ha^{-1}), agronomic efficiency was calculated to produce 45 kg seed cotton per unit N in kg ha^{-1}.

The analysis of variance of physiological efficiency showed significance only for the interaction of environment by N rates (Table 2.9). The coefficient of variation for physiological efficiency was very high, at 118%. Environment is considered a random effect, as environmental conditions can change from year to year, location to location. Both Fargo and Prosper locations in 2010 had efficiency values between 40 and 180 kg biomass obtained per unit N uptake in kg N ha^{-1}, while Prosper in 2011 had efficiency values between 85 and 300 kg biomass produced per unit N taken up by the plant in kg N ha^{-1}. There was greater rainfall in Prosper in 2011, which leached some of the nitrogen out of the root zone as well as contributed to the denitrification process, decreasing the amount of N uptake in the crop, which would increase the physiological efficiency.

Physiological efficiencies for sorghum have been calculated in the study by Kaizzi et al. (2012). Sorghum physiological efficiency decreased at increasing fertility rates, but was not significant at $P \leq 0.05$ (Table 2.9). When N was applied at the 50 kg ha^{-1} rate, sorghum physiological efficiency was found to produce 50.8 kg biomass ha^{-1} per unit N in kg ha^{-1} taken up by the crop. At the 150 kg N ha^{-1} fertility rate, sorghum physiological efficiency was calculated to be 35.5 kg biomass ha^{-1} per unit N in kg ha^{-1} that was taken up by the crop.

Cotton physiological N use efficiencies were calculated for a study in California on cotton response to N fertility (Fritschi et al., 2004). The differences between N rates were generally not significant in individual years of the study, yet a repeated measures analysis found a significant trend ($P \leq 0.01$) for decreased physiological efficiency with increased N rates. For the cotton grown on Wasco, CA sandy loam, physiological efficiency decreased significantly from 8.3 kg lint yield per kg plant N uptake at the 56 kg N ha^{-1} rate to 5.9 kg lint yield per kg plant N at the 168 kg N ha^{-1}

rate. Varied physiological use efficiencies are not unexpected, as other researchers report (Fritschi et al., 2004).

The analysis of variance of nitrogen use efficiency showed no significance for any of the main effects or the interactions. The coefficient of variation was quite high as well, at 104%, which could explain the lack of significance, except for environment which is considered a random effect. Even though the interaction between environment and N fertility rates was not significant, there was an in increase in NUE in Prosper 2011 with increasing N fertility rates. This was not found for the locations in 2010.

Nitrogen use efficiency was calculated in the study on sorghum response to fertilizer and NUE by Kaizzi et al. (2012). Nitrogen use efficiency, as calculated previously, is designated by the term "recovery efficiency" in this particular study. Sorghum grain yield NUE decreased significantly at higher N fertility rates. When N was applied at the 50 kg N ha^{-1} rate, sorghum was found to take up 1.2 kg N ha^{-1} for every kg N applied ha^{-1}. At the 150 kg N ha^{-1} rate, sorghum was found to only take up 0.5 kg N ha^{-1} for every kg N applied ha^{-1}. This particular study only looked at sorghum grain yield. Maize NUE, on the other hand, is reported to be between 66 and 111 kg kg^{-1} (Danalatos and Archontoulis, 2010). Estimated kenaf NUE has been found to be 142 kg kg^{-1} in a study conducted on kenaf productivity in Greece. This value is greater than what is typically reported for the NUE of corn.

Nitrogen use efficiency is a useful tool to determine how well a crop is able to use the nitrogen available in the soil. Sorghum NUE is greatest at lower N fertility levels, which is important when considering that the sorghum for biomass production must be efficient at using N to minimize input costs.

Economic Analysis

An economic analysis was conducted to determine the most optimal rate of fertilization for each crop. The yield response equation was derived from the mean biomass yield at the actual N rate for each crop.

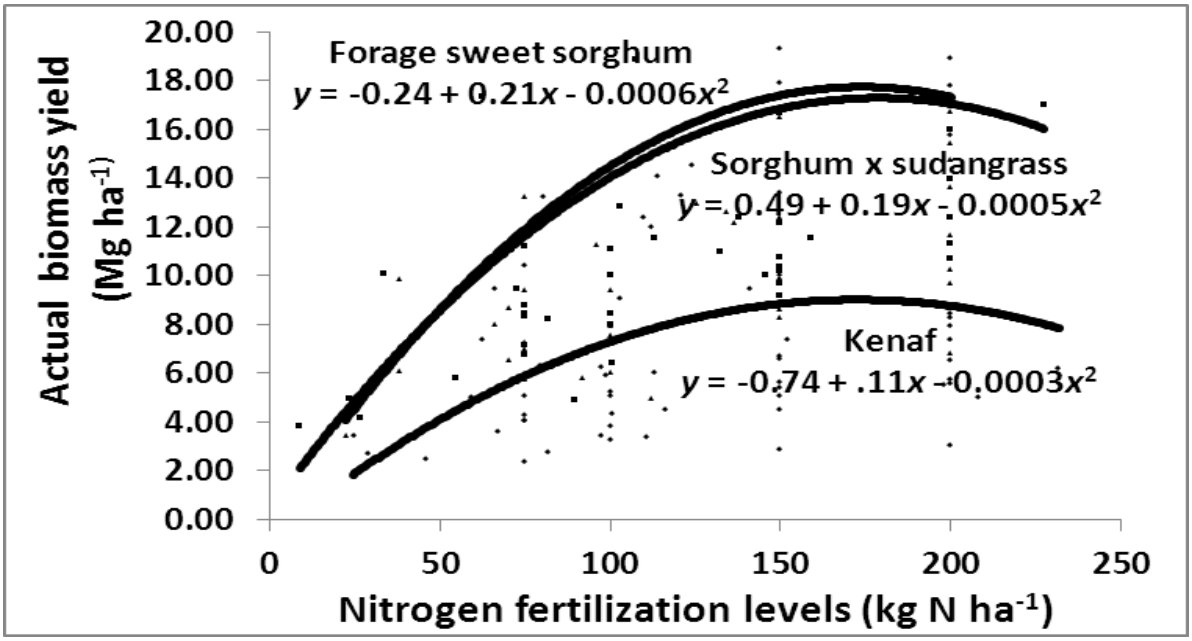

Fig. 2.9. Mean biomass yield of three crops with actual soil fertility levels in four environments in Fargo and Prosper, ND, in 2010 and 2011.

The yield response equation was used to determine the biomass yield at the economical fertility rate of fertilization. The derivative of the relative biomass yield equation, the marginal value product equation, was calculated and set to zero to determine the most economical rate of fertilization (Table 2.11).

The most economical rate of fertilization for forage sweet sorghum was calculated to be 176 kg N ha^{-1}. The most economical rate of N fertilization for sorghum x sudangrass was 180 kg N ha^{-1}, and the rate for kenaf was 174 kg N ha^{-1}. The 2012 rate for 46-0-0 urea fertilizer, the price per kg N is $1.15.

Table 2.11. Economic analysis of three crops using the yield response equation, deriving the economical fertility rate according to the marginal value product equation and the price to fertilize each crop at the economical fertility rate.

Crop	Yield response equation	Economical fertility rate	Price at economical fertility rate
		--------kg N ha^{-1}-----	----------$ Mg^{-1}----------
Forage sweet sorghum	$y= -0.2402 + 0.2069x – 0.0006x^2$	176	11.50
Sorghum x sudangrass	$y= 0.4852 + 0.1183x - 0.0005x^2$	180	11.37
Kenaf	$y= -0.7398 + 0.1132x - 0.0003x^2$	174	20.27

The cost to fertilize forage sweet sorghum was the same as fertilizing sorghum x sudangrass, but was less than the cost to fertilize kenaf, as the biomass yield obtained from kenaf at the most economical fertility rate was lower than yield obtained from the forage sweet sorghum or the sorghum x sudangrass. At the economical fertility rate, forage sweet sorghum can yield 17.6 Mg ha^{-1}, sorghum x sudangrass yields 18.2 Mg ha^{-1} at the economical fertility rate, while kenaf only yields about 9.9 Mg ha^{-1} at its most economical fertility rate. These values might not be accurate as there was nitrogen leaching out of the crop root zone and denitrification due to the excessive rainfall and high soil moisture in the 2011 growing season.

CONCLUSIONS

Forage sweet sorghum and sorghum x sudangrass had significantly higher biomass yield than kenaf. It is also important that this feedstock will not compete with food crops, and one that will not require intensive management practices to achieve maximum yield. Both of these sorghum crops have these characteristics. Another important aspect of biomass production is to produce a high yielding crop with the least amount of fertility inputs. Plant biomass yield showed a significant response to N rates. As N fertility rates increased, dry matter biomass yield increased, when averaged across the three crops and four environments.

Kenaf nitrate content had the lowest levels of the three crops at harvest, though its nitrate content was not significantly different from the other two crops at that stage. None of the fertilization rates impacted nitrate content of the crops at harvest, suggesting that much of the nitrate had been assimilated in each of the crops. This is desirable for any biomass feedstock as the mineral nutrient content must be at a minimum for more efficient conversion to ethanol.

The high variability of agronomic efficiency, physiological efficiency, and NUE suggests that more research is needed on the impact of N fertility levels on the efficiencies of each of the crops. The results of the economic analysis suggested that 180 kg N ha^{-1} can be applied to maximize biomass yield of forage sweet sorghum and sorghum x sudangrass biomass.

The recommendation for a potential biomass feedstock from this study is to produce forage sweet sorghum or sorghum x sudangrass at the rate provided by the economic analysis. This rate will produce adequate biomass yield (between 17.6 and 18.2 Mg ha^{-1}), and keep the cost per Mg biomass down. This rate will also lower the amount of nitrogen that may be leached out beyond the root zone or lost to denitrification if there is a high rainfall event.

CHAPTER 3. NITROGEN UPTAKE AND BIOMASS YIELD IN THE ESTABLISHMENT YEAR OF REED CANARYGRASS AS FEEDSTOCK FOR BIOFUEL

ABSTRACT

There is increasing interest in developing the technology to provide a constant supply of biomass for bioenergy production. This constant supply of biomass may come from a number of perennial crops. One of the crops that is being evaluated for its potential use as biomass feedstock is reed canarygrass (*Phalaris arundinacea* L.), commonly found in the Great Plains. This study looked at the response of this grass to different levels of nitrogen fertilization and its subsequent biomass output during the establishment year. This study was conducted in Fargo and Prosper, ND, in 2010 and 2011. The experimental design was a randomized complete block design with a split plot arrangement with three replicates. The independent variables in this study were the crops and five rates of fertilization (0, 75, 100, 150, and 200 kg N ha^{-1}). Aboveground samples were collected from each plot at three developmental stages [vegetative (V8), flowering (VF), and harvest (H)] to determine NO_3-N uptake throughout the growing season. The design used for analysis of developmental stages was an RCBD split-split design with stages as the sub-sub plots.

Results indicated that in the seeding year, reed canarygrass has a positive response to increasing N rates, producing up to 5 Mg ha^{-1} of dry matter per season with 200 kg N fertilizer ha^{-1}.

The level of crude protein in reed canarygrass increased at greater N rates, yet the amount of crude protein was lowest at harvest. Based on the yield data, it is suggested that 100 kg N ha^{-1} be applied to the crop to lower the cost of fertilizer per Mg biomass in the seeding year. A later harvest date is recommended to reduce the amount of ash and mineral nutrients that can impact the process of conversion.

INTRODUCTION

Bioenergy research has increased dramatically in the last decade with the emphasis on energy conservation. Long term energy security comes with a diversification of energy supplies, so new technology is being developed to produce biofuels at levels where they will be economical and competitive with petroleum (Gehlhar et al., 2010). The goal of the Renewable Fuel Standard (RFS) is to increase the volume of renewable fuels to 136 million m^3 of total renewable fuel by the year 2022 (EPA, 2007). Currently the greatest source of bioethanol comes from corn (*Zea mays* L.) grain, but there is concern with this feedstock due to the competition of food versus fuel. A biomass feedstock must have a number of characteristics such as consistent composition of quality components, high yield, low input costs, high profit, and production close to the feedstock processing site to reduce transportation costs (Hettenhaus, 2006).

Certain forage crops have the biomass yield, quality, and physiological components that make them suitable for bioenergy production. A benefit of utilizing forages for bioenergy includes their ability to produce high dry matter biomass yield with minimal inputs. Perennial forage grasses offer excellent soil holding capacities to decrease soil erosion and can be integrated into existing agricultural production systems (Sanderson et al., 2006; Hettenhaus, 2006; Wrobel, 2009). Perennial grasses have lower nutrient requirements because of nutrient retention and recycling (Wrobel, 2009). One perennial forage grass that is a candidate for bioenergy feedstock is reed canarygrass. It is a C3, cool-season crop that is typically grown for forage production and is well adapted to wetter environmental conditions (Sheaffer et al., 1990; Sanderson et al., 2006). It has a well developed intercellular air-space system that can supply oxygen to flooded roots (Wrobel et al., 2009). Reed canarygrass is well adapted to the regions of North Dakota and Minnesota due to its exceptional winter hardiness and its ability to maintain yield and high forage quality, even

through multiple cuttings (Sheaffer et al., 1990). It is also very responsive to nitrogen fertilization (Wrobel et al., 2009). Seedling vigor, however, is low and reed canarygrass is slow to establish, so maximum yield will not be achieved until the second or third year of production (Anderson et al., 2008; Casler and Undersander, 2006; Saballos, 2008;). Unlike annual crops, perennial grasses are able to store nutrients in their rhizomes, which can be advantageous at the beginning of the growing season (Wrobel et al., 2009). Early growth of reed canarygrass produces about twice as much biomass below-ground than above-ground, which can also increase competition with weeds (Wrobel et al., 2009).

Nitrogen is one of the limiting growth factors that influence plant development and growth (Mengel and Kirkby, 1982). Nitrogen is a constituent of a number of amino acids, amides, proteins, and nucleic acids that are important for crop physiological development and processes (Taiz and Zeiger, 2006). When there is an insufficient nitrogen level in the soil for plant uptake, the nitrate content will decrease in the plant tissue and the crop will exhibit deficiency symptoms, such as a decrease in yield or leaf yellowing. On the contrary, when there is too much N available for plant uptake, the nitrate content in the plant tissue may reach toxic levels, which can also decrease plant biomass yield.

The root system of the crop allows for a response in yield to nitrogen fertilizer applications. Herr-Turoff and Zedler (2005) found that with a high N-treatment of 48 g N m^{-2} $year^{-1}$, reed canarygrass yielded 458 g $plot^{-1}$ versus a low N-treatment of 12 g N m^{-2} $year^{-1}$, where it yielded 236 g $plot^{-1}$. The response to fertilizer also depends on other factors, including the amount of N already present in the soil before seeding or after overwintering, as well as environmental conditions such as rainfall, temperature during fertilizer application, and the timing of application.

With a delayed harvest system, nutrients such as N are able to translocate from the shoot to the rhizome, providing a growth advantage for the following season.

Even though nitrogen is considered one of the most important macro-nutrients for plant growth, there is a point when too much fertilizer is applied, so the crop is unable to take it all up (Mengel and Kirkby, 1982). This can result in the loss of nitrogen into the ground water system through leaching, loss of soil N to the atmosphere through volatilization, or may cause toxicity to the plants themselves. This can be a waste of the producers' money, so the fertilizer must be applied at strategic times and in limited rates, while still applying enough N to produce a high yielding crop. The yield response from different fertilizer applications, the price of the fertilizer, the cost of multiple applications, and the value of the crop all help determine how profitable the crop will be (Johnson and Ali, 1979), as this is the ultimate goal for the grower (Black, 1993). In biomass production, lower fertilizer yields are more appropriate because the crop does not command a high value (Partala et al., 2001).

Crop yield typically increases with the addition of nitrogen fertilizer, but the rate of the yield accumulation decreases at the higher fertility levels (Johnson and Ali, 1979). Both the equation of the crop yield response curve to higher fertility levels and the price of the fertilizer itself can help determine the most economical rate of application. Nitrogen use efficiency (NUE) refers to the proportion of applied nitrogen that the crops are able to uptake (Mengel and Kirkby, 1982). The maximum net profit from the input of N fertilizer is reached when the value of the crop exceeds the total cost of fertilization (Black, 1993). Nitrogen use efficiency, agronomic efficiency, and physiological efficiency are often calculated to help determine the most economic rate of fertilization.

Lignocellulosic biomass material typically consists of 300 to 500 g kg^{-1} cellulose, 200 to 400 g kg^{-1} hemicellulose, and 150 to 250 g kg^{-1} lignin (Lee et al., 2007; Hendriks and Zeeman, 2009). This can be utilized in the conversion of lignocellulosic material to ethanol (Mosier et al., 2005). The quality components of reed canarygrass include 240 g kg^{-1} cellulose, 360 g kg^{-1} hemicellulose, 20 g kg^{-1} ADL, and 100 g kg^{-1} crude protein (Lee et al., 2007; Wrobel et al., 2009). Because reed canarygrass is a C3 crop, it requires almost 50% more water to achieve comparable plant dry matter when compared with C4 grasses (Bakker and Elbersen, 2005; Wrobel et al., 2009). This higher water uptake can result in higher ash components, such as Si, and other dissolved inorganic components that are taken up into the plant biomass (Wrobel et al., 2009). These higher levels of ash (85 g kg^{-1}) can be detrimental in some thermochemical or biochemical conversion technologies (Bakker and Elbersen, 2005). Nitrogen fertilization also increases the uptake of N, P, K, S, and silica in the plant tissues, which are also components of ash. The ash can melt at high temperatures and damage the mechanical equipment by plugging it up (Biomass Energy, 2012). Delaying harvest until the spring can help minimize ash content as water-soluble elements are translocated from the aboveground biomass to the roots (Wrobel et al., 2009).

The specific objectives of this study are to (1) determine if reed canarygrass is a viable feedstock for bioenergy production during the seeding year by examining the nitrogen fertilization rates at which it has the greatest yield, to (2) determine the quality components of reed canarygrass at each of those fertility levels, and to (3) calculate the most economical rate of nitrogen fertilization at which the greatest yield will produce the greatest return for the grower.

MATERIALS AND METHODS

Field Establishment and Experimental Design

This research was conducted at the North Dakota State University (NDSU) research sites in Fargo, ND (-96°812'W, 46°897'N, 274 m elevation) and in Prosper, ND (-97°115'W, 47°002'N, 284 m elevation). The soil type in Fargo is Fargo-Ryan silty clay soil (fine, montmorillonitic, frigid, Vertic Haplaquoll, with a leached and degraded natric horizon); the Fargo series is fine, smectitic, frigid Typic Epiaquerts, while the Ryan series is fine, smectitic, frigid Typic Natraquerts (Soil Survey Staff, 2011). The soil type in Prosper is a Bearden silty clay loam (fine-slay, frigid Aeric Calciaquolls). Rainfall amounts were recorded automatically at both locations by the NDAWN system (NDAWN, 2012). Soil samples for analysis were taken at both locations the spring when the crop was planted. The soil analysis included pH, organic matter, N-NO_3, P, and K.

Previous crops in 2010 were corn in Prosper and soybean (*Glycine max* L. Merr.) in Fargo, and previous crops in 2011 were cereal crops in Fargo and corn in Prosper. The reed canarygrass (cv. Palaton) was seeded at all locations with a cone plot planter. Planting dates at Fargo in 2010 and 2011 were 26 May, and planting dates at Prosper in 2010 and 2011 were 28 May and 7 June, respectively. The seeding rate was calculated based on the percentage of pure live seed. The seeding rate for the reed canarygrass was 16.8 kg ha^{-1}. The reed canarygrass seed was obtained from Agassiz Seeds (West Fargo, ND).

Soil analysis was done using the transnitration of salicylic acid method to determine the baseline N fertility level of each individual plot (Franzen and Cihacek, 1996; Vendrell and Zupancic, 1990). The initial soil analysis of each plot showed that none of the plots had 0 kg N ha^{-1} in the upper 0.62 m of soil, which suggests that a true baseline was not feasible. In each block, the plot with the least amount of nitrogen was selected as the control, with the treatments for the other

four plots randomized. For the treatments, the amount of N fertilizer to add was calculated for each plot, so that the initial N levels of each plot would be fertilized up to the experimental rates of 75, 100, 150, and 200 kg N ha^{-1} (soil N + N fertilizer). If the initial soil N test indicated a higher level of N than the experimental rate in that particular plot, then no additional fertilizer was added. The source of N was urea [$CO(NH_2)_2$]. Urea was hand-broadcast in both locations in 2010 and 2011 on 11 June and 30 June, respectively. Each plot was fertilized individually. The experimental design in all sites was a randomized complete block design with three replicates. Experimental units were 2m wide and 9m long with six rows separated at a 0.31-m row spacing. The seeding depth for all the reed canarygrass was 20 mm.

Weed control for the plots in 2010 was hand-weeding as needed, and weed control in 2011 included pre-plant spraying of glyphosate (N-(phosphonomethyl)glycine) (1.4 kg a.i. ha^{-1}) to control existing vegetation, followed by hand-weeding as needed.

Plant Sampling and Evaluations

Dependent variables evaluated were plant biomass and predicted ethanol yield; plant NO_3-N content; plant total N content; plant N uptake; plant efficiencies; plant quality (CP, ash, NDF, ADF, ADL, and IVDMD); and soil NO_3-N content at 0 to 0.15 and 0.15 to 0.60-m depths.

Biomass samples were taken from the 2^{nd} and 5^{th} row in each six-row plot, where plants were cut at the stem base. Plant height was measured from the interior of each plot. Thereafter, the two center rows from a 3.7 m^2 area were hand harvested in 2010 and the four center rows were harvested in 2011 using a flail forage harvester.

The ethanol conversion factor for reed canarygrass is the standard for any cellulosic feedstock, which is 0.38 L kg^{-1} of dry matter (Schmer et al., 2008).

Aboveground portions of whole plants were collected at three developmental stages: vegetative or 8-leaf stage (V8), panicle initiation or flower bud (R1), and harvest (H) for plant NO_3-N determinations (Berti et al., 2008). The determination of plant tissue nitrate was done by the nitration of salicylic acid colorimetric method using 0.1 g of dried and ground plant tissue (Cataldo et al., 1975). Nitrogen uptake in the biomass was determined by multiplying biomass N content by biomass yield. The determination of plant tissue quality analysis was conducted to determine dry matter (DM) (AOAC Method 934.01), percentage of ash (AOAC Method 942.05), crude protein (CP) (AOAC Method 2001.11), and acid detergent lignin (ADL) (AOAC Method 973.18) (Horwitz and Latimer, 2010). Quality analysis was done to determine acid detergent fiber (ADF) (ANKOM A200 Method 5) and neutral detergent fiber (NDF) (ANKOM A200 Method 6). Quality analysis was also done to determine in-vitro dry matter digestibility (IVDMD) (Oh et al., 1966).

Soil samples from each plot from 0 to 0.15 and 0.15 to 0.60 m were taken immediately after planting for the analysis of NO_3-N, according to accepted procedures for soil analysis. Soil samples were collected at these depths from all plots immediately after harvesting. The soil samples were analyzed for NO_3-N just as they were at the beginning of the season using the transnitration of salicylic acid method (Cataldo et al., 1975) by the Soil and Plant Analysis Laboratory, NDSU.

Nitrogen use efficiencies, physiological efficiencies, and agronomic efficiencies were calculated for the economic analysis of reed canarygrass.

Data Analysis

Relative biomass yield was calculated for reed canarygrass to more accurately determine how actual N levels in each plot impact biomass yield. Relative biomass yield is determined by

taking yield of the plot with the greatest yield, and setting that as 100% yield. The relative biomass yield (%) is calculated by taking the biomass value of each plot and dividing it by the greatest yield obtained for reed canarygrass and multiplying that value by 100.

$$\text{Relative yield (\%)} = (\text{plot yield}/\text{greatest plot yield}) \times 100$$

A number of equations were utilized to determine the most efficient and economical use of nitrogen fertilizer application with reed canarygrass for potential conversion to bioenergy. Nitrogen use efficiency (NUE), or apparent nitrogen recovery, reflects the proportion of nitrogen that the different crops take up (Mengel and Kirkby, 1982). The calculation of NUE equals the nitrogen content of the plants treated with fertilizer (N_{fert}) and subtracting the nitrogen content of the plants not treated with fertilizer, or the check treatment (N_{check}), and dividing that value by the units of nitrogen fertilizer applied in kg N ha^{-1} ($N_{applied}$).

$$\text{NUE (\%)} = [(N_{fert} - N_{check})/N_{applied}] \times 100$$

Agronomic efficiency (AE) indicates the yield increment obtained per unit of N fertilizer applied in kg yield per kg N (Mengel and Kirkby, 1982). It's determined using the biomass yield of the fertilized crop (Y_{fert}) and subtracting the biomass yield of the unfertilized crop (Y_{check}), divided by the units of nitrogen applied as the fertilizer ($N_{applied}$).

$$\text{AE (kg kg}^{-1}\text{ N)} = [(Y_{fert} - Y_{check})/N_{applied}]$$

Physiological efficiency (PE) is the biomass produced per unit of nitrogen absorbed by the plant (Mengel and Kirkby, 1982). It's calculated by subtracting the biomass yield of the unfertilized crop (Y_{check}) from the biomass yield of the fertilized crop (Y_{fert}), and dividing that value by the difference between the nitrogen uptake of the unfertilized crop ($N_{uptake\ check}$) from the nitrogen uptake of the fertilized crop ($N_{uptake\ fert}$).

$$PE = [(Y_{fert} - Y_{check})/(N_{uptake\ fert} - N_{uptake\ check})]$$

Finally, the optimum economical rate of fertilization for reed canarygrass will be determined using the information from NUE, AE, and PE equations and the yield increase regression model (Johnson and Ali, 1979). The yield response equation plots the dry matter obtained at the different N fertility levels and determines which rate maximizes biomass yield for each crop. The cost per unit of nitrogen fertilizer will be factored in to determine the final cost of the fertilizer to produce a unit of dry matter for each crop. The crops will then be compared using this information to determine the cheapest source of biomass. The final cost of N fertilizer per unit dry matter per hectare ($\$ N_{fert}$) is the result of the multiplication of the cost per unit of fertilizer ($\$_{per\ unit\ N}$) and the units of N to produce maximum biomass (N_{units}).

$$\$ N_{fert} = \$_{per\ unit\ N} \text{ x } N_{units}$$

The price per unit N in kg in 2012 was $1.15 (Akron Services, 2012). This price is for 46-0-0 urea.

The yield response equation uses the regression coefficients b_0, b_1, and b_2 and the nitrogen uptake of each crop per hectare (x) to determine biomass yield (Y).

$$Y = b_0 + b_1x - b_2x^2$$

The marginal value product ($Dy\ Dx^{-1}$) equation is used to determine the profit maximum, taking the cost of fertilizer, the applied nitrogen fertilizer rates, and the cost of the crop into consideration. The equation can be set to zero so the most economical rate of nitrogen fertilizer for the greatest yield can be determined. This is determined by subtracting the regression coefficient b_2 multiplied by x from the regression coefficient b_1.

$$Dy\ Dx^{-1} = b_1 - b_2x$$

The calculation of potential ethanol yield from biomass dry matter yield data uses the following equation:

$$L\ ha^{-1}\ ethanol = (0.38L\ kg^{-1} \times 1000 \times Mg\ ha^{-1}\ biomass\ yield)$$

Statistical Analysis

Statistical analysis was conducted by using standard procedures for a randomized complete-block design (Steel and Torrie, 1980). All of the data collected were analyzed by analysis of variance using the GLM procedure (SAS Institute, 2008), with each location-year combination considered an "environment" and a random effect, while nitrogen fertility treatments and developmental stages were considered fixed effects in the analysis. Analysis of variance was conducted within and across environments. Environments were considered homogenous when the mean square error variances for each trait were less than a factor of 10. If the environments were homogenous, then a combined analysis was done. A mean separation test was performed using the *F*-protected LSD at $P \leq 0.05$ level of significance for each evaluated trait. Regression analysis was done where there was a significant main effect. Linear and quadratic regression models were tested with the corresponding error. The regression models were all at $P \leq 0.05$ level of significance.

The soil NO_3-N, plant biomass, total plant N, N uptake, and the efficiencies were analyzed according to a randomized complete block design, where the main factor was the N fertility treatments (0, 75, 100, 150, and 200 kg N ha^{-1}). The plant quality sampling data were analyzed according to a randomized complete block design with a split plot arrangement, where the main plots were the N fertility treatments (0, 75, 100, 150, and 200 kg N ha^{-1}) and the sub plots were the phenological stages (V8, R1, and H).

RESULTS AND DISCUSSION

Rainfall, Temperature, GDD, and Soil Analysis

Total growing season rainfall varied between years and months, with greater rainfall occurring from April through August in 2011 than in 2010 in both Fargo and Prosper. (Table 3.1)

Table 3.1. Monthly growing-season rainfall for four environments, Fargo and Prosper, ND, in 2010 and 2011, and the deviation from the 30-year average.

	Fargo†				Prosper†			
	2010		2011		2010		2011	
Month	Rainfall	Dev.	Rainfall	Dev.	Rainfall	Dev.	Rainfall	Dev.
	----------mm----------							
April	36.8	2.0	45.8	11.0	29.5	-6.6	45.0	8.6
May	68.1	1.8	109.7	43.4	69.9	2.0	80.0	12.2
June	86.1	-3.1	100.9	11.7	80.8	-10.7	131.6	40.1
July	105.1	32.0	103.6	30.5	103.4	21.1	150.1	67.8
Aug.	67.7	3.7	72.5	8.5	89.4	21.3	88.9	20.8
Sept.	151.4	96.0	4.0	-51.4	134.6	80.5	6.1	-48.0
Oct.	60.6	10.6	20.6	-29.4	36.1	-11.9	9.4	-38.6
Total	575.8		457.1		543.7		511.1	

† NDAWN, 2012.

The amount of rainfall that fell during the month of July in Prosper, 2011, was the greatest amount of precipitation that occurred in the course of one month during the growing season. A thunderstorm in Prosper on July 19, 2011 delivered 50mm rainfall on the site according to the official NDAWN data (2012), yet visual observations soon after the storm hit suggested a greater amount fell, as the flooding in that area destroyed a number of research experiments. The widespread overland flooding was partially due to the saturated soil conditions that had been present for much of the spring and summer months. There was a reprieve from the moisture in time for harvest in 2011 where both locations received below-average precipitation in September and October.

This above-average rainfall in 2011 resulted in a re-application of nitrogen fertilizer in Prosper, ND, as the nitrogen had leached out of the root zone. There was water standing in the plots and the smell of ammonia was in the air, suggesting that the process of denitrification was taking place. This fertilizer re-application was done on July 25, 2011. Soil tests were not taken for re-verification.

Above-average temperatures were observed in all four environments from June through August (Table 3.2).

Table 3.2. Growing-season average temperatures for four environments, Fargo and Prosper, ND, in 2010 and 2011, and the deviation from the 30-year average.

	Fargo†				Prosper†			
	2010		2011		2010		2011	
Month	Temp.	Dev.	Temp.	Dev.	Temp.	Dev.	Temp.	Dev.
	----------°C----------							
April	6.4	4.5	6.38	-0.3	5.8	4.4	5.8	-1.0
May	14.1	0.4	14.1	-1.2	13.5	0.4	13.5	-1.5
June	18.9	0.2	18.9	0.3	18.4	0.3	18.4	0.4
July	21.4	0.9	21.5	2.2	21.1	0.0	21.1	2.1
Aug.	20.6	1.6	20.6	1.2	20.1	1.0	20.1	1.0
Sept.	14.4	-0.8	14.4	1.0	14.4	-1.5	14.4	0.8
Oct.	7.4	2.8	7.4	3.9	7.5	1.8	7.5	3.3

† NDAWN, 2012.

The planting dates in 2011 in Fargo and Prosper were May 26 and June 7, respectively, and both sites were harvested on October 12, resulting in the growing season duration of 139 days and 127 days, respectively. Early season moisture, mid-season flooding rainfall in both locations that saturated the soil on multiple occasions, and lower than average AGDD caused a reduction in plant growth in these environments. The monthly accumulated growing degree days (AGDD) were below average in all four environments (Table 3.3).

Growing degree days (GDD), or heat units, were calculated based on the threshold temperatures for each crop. The base temperature for reed canarygrass is 5°C (Bosworth et al.,

2005). This value was used when calculating the monthly AGGD and total AGGD for each environment.

Table 3.3. Growing-season growing degree-days (GDD) for four environments, Fargo and Prosper, ND, in 2010 and 2011, and the deviation from the 30-year average for reed canarygrass.

	Fargo†				Prosper†			
	2010		2011		2010		2011	
Month	GDD	Dev.	GDD	Dev.	GDD	Dev.	GDD	Dev.
	----------°C----------							
April	347	144	178	-25	337	139	151	-47
May	348	-89	294	-143	349	-70	281	-138
June	384	-182	393	-173	381	-170	388	-163
July	454	-210	488	-177	440	-214	479	-175
August	462	-175	447	-190	453	-168	442	-179
September	291	-142	385	-48	292	-141	386	-47
October	309	74	331	96	301	59	325	83
TOTAL	2585	-587	2514	-658	2544	-569	2452	-661

† NDAWN, 2012 where base temperature=5°C

Each environment was different in terms of soil conditions, particularly initial soil N levels. The initial soil NO_3-N levels are indicated in Table 3.4.

Table 3.4. Initial soil NO_3-N analysis for four environments and five N rates for reed canarygrass prior to planting for soil depths of 0 to 60cm.

Environment	0	75	100	150	200
	----------kg NO_3-N ha^{-1}----------				
Fargo 2010	107.9	114.2	119.1	128.8	143.7
Fargo 2011	79.9	88.5	91.5	116.9	109.8
Prosper 2010	88.5	121.3	127.7	146.3	181.8
Prosper 2011	17.2	34.7	21.3	45.5	36.6

In 2010, Fargo and Prosper locations had average soil NO_3-N levels that were higher than the experimental fertility rates of 0, 75, and 100. Fargo and Prosper had lower levels of nitrogen in the soil in 2011 compared to the previous year, but still had average fertility levels greater than the experimental rate of 0 in Fargo and Prosper, as well as levels greater than the 75 kg N ha^{-1} experimental rate in Fargo.

The initial soil analysis for soil P, K, organic matter (OM), and pH was conducted for each environment before planting (Table 3.5).

Table 3.5. Initial soil analysis for four environments prior to planting for soil depth 0 to 60cm.

Environment	pH	OM†	P	K
		----------mg kg^{-1}----------		
Fargo 2010	7.9	5.7	22.0	445.0
Fargo 2011	7.4	6.5	15.6	321.7
Prosper 2010	8.1	2.7	38.0	400.0
Prosper 2011	7.1	3.1	39.5	267.1

† OM: Organic matter

The organic matter levels were greater in Fargo than in Prosper locations in both 2010 and 2011. The pH was between 7 and 8 in all four environments, so N and K were most available for plant uptake (Mengel and Kirkby, 1982). The optimum soil pH for reed canarygrass is between 5 and 8 (Sheaffer et al., 1990). There were lower pH levels in 2011 than in 2010. Phosphorus levels were between 15 and 25 mg kg^{-1} in Fargo and between 35 and 40 mg kg^{-1} in both 2010 and 2011, so no additional P fertilization was required. Initial soil K levels were greater in 2010 than in 2011, with levels greater than the minimum K fertility requirements for all three crops, so no additional K fertilizer was required.

Biomass and Ethanol Yield

The environment and the main effect of N fertility rate were significant for the combined analysis for biomass and ethanol yield data for reed canarygrass (Table 3.6).

Since environment is considered a random effect, the discussion will only focus on the significant main effect of N fertility on biomass and ethanol yield. There was an increase in biomass and ethanol yield as the N fertility treatments increased (Fig. 3.1).

Table 3.6. Analysis of variance and mean squares for reed canarygrass biomass, relative biomass, and ethanol yield for five N rates across four environments, Fargo and Prosper, ND, in 2010 and 2011.

Sources of variation	df	Biomass yield	Relative biomass yield	Ethanol yield
Env	3	134.97***	14677.1	2.080***
Rep(env)	8	2.93	319.0	0.045
N	4	6.10**	664.3	0.094**
Env x N	12	0.83	89.9	0.013
Error	32	1.05	114.0	0.016
CV, %		24.74	24.7	24.726

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively.

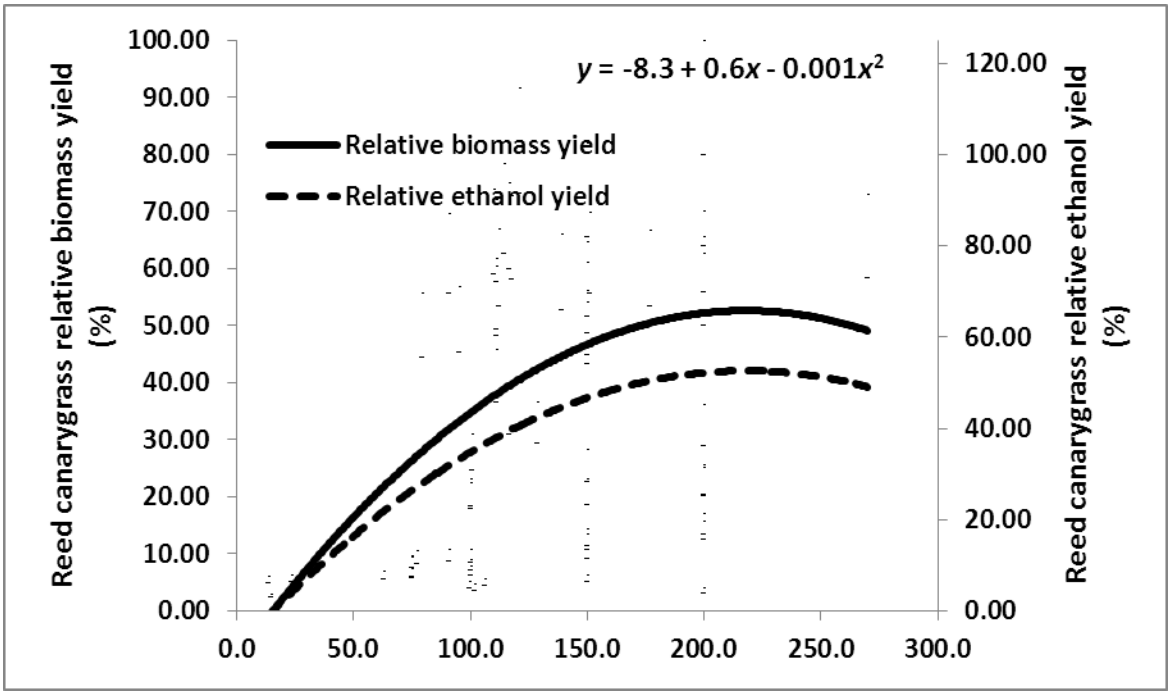

Fig. 3.1. Regression model for relative biomass and ethanol yield of reed canarygrass as affected by actual N rates averaged across four environments in Fargo and Prosper, ND, in 2010 and 2011.

Biomass yield of reed canarygrass increased as the fertility rate increased, reaching around 5 Mg ha^{-1} at the 200 kg N ha^{-1} fertility rate. Subsequent ethanol yield also increased with higher levels of fertility. The average initial soil N fertility level for three of the four environments was greater than 80 kg N ha^{-1} for the check plots and initially had greater than 89 kg N ha^{-1} at the 75 kg N ha^{-1} treatment level.

Environmental conditions may also have impacted biomass yield of reed canarygrass in the establishment year. The high soil moisture in 2011, that prevented the crop from being planted until the end of May, combined with the flooding conditions in Prosper, and high soil moisture in Fargo, probably caused much of the soil N to leach out of the soil. Prosper displayed greater symptoms of nitrogen deficiency (yellowing leaf tissue, while the air smelled like ammonium) when the flooding occurred, so the site was re-fertilized in 2011 with the same amount of N that was applied to each crop previously. The reed canarygrass in Fargo did not have the same symptoms as Prosper in 2011, so N was not reapplied at that location. In the establishment year, reed canarygrass is at risk for being damaged by stressful environmental conditions. Even though the crop is known for its ability to withstand higher moisture levels, excessive moisture still may drown the plants and the lack of oxygen in the root system can cause stunted growth and decreased biomass yield.

Quality Analysis

The environment, rep by environment, and stage by environment interactions, as well as the N fertility rate and the growth stage main effects were significant for the combined analysis for plant tissue quality data (Table 3.7).

Since environment is considered a random effect, the discussion will focus on the significant main effects. The quality traits analyzed using the combined analysis included CP, NDF, ADF, ADL, and IVDMD, which were analyzed by analysis of variance.

The analysis showed the significance of the effect of growth stage on leaf tissue quality components of reed canarygrass (Table 3.8).

Crude protein decreased significantly from V8 to R1 growth stages and from R1 to H growth stages. It is determined by measuring the total N content, then multiplying that value by

Table 3.7. Analysis of variance and mean squares for reed canarygrass plant-tissue quality analysis for five N rates and three developmental stages across four environments, Fargo and Prosper, ND, in 2010 and 2011.

Sources of variation	df	CP†	NDF	ADF	df	ADL	IVDMD
Env	3	279.36***	67.15***	13.88	1	1.88	4.38
Rep(env)	6	13.97***	3.61	9.61	4	0.89	4.22
N	4	24.28*	2.56	5.52	4	0.96	6.83
Env x N	12	6.99	2.86	6.83	4	0.67	3.15
Error (a)	72	3.29	3.42	4.03	16	0.95	4.80
Stage	2	2022.24**	330.19**	337.63**	1	63.37*	1736.13**
Stage x env	6	85.00***	24.02***	27.12***	1	0.32	0.15
Stage x N	8	6.14	2.83	2.71	4	1.21	11.30
Stage x env x N	24	5.51	2.82	5.69	4	0.89	2.32
Error (b)	180	2.18	1.90	5.95	20	0.99	3.74
CV, %		7.81	2.58	9.01		27.48	3.05

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively.
† Quality parameters: crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and in-vitro dry matter disappearance (IVDMD)

Table 3.8. Quality analysis of reed canarygrass at three growth stages [vegetative 8-leaf (V8), reproductive (R1), and harvest (H)] averaged across N rates (0, 75, 100, 150, and 200 kg N ha^{-1}) and four environments, Fargo and Prosper, ND, in 2010 and 2011.

Growth stages	CP	NDF	ADF	ADL	IVDMD
	----------	----------	g kg^{-1}	----------	----------
V8	260.2	508.1	240.2	25.9	721.9
R1	180.2	557.8	295.6	.	614.3
H	126.7	535.6	276.6	46.4	.
LSD (*P*=0.05)†	41.0	22.0	23.0	13.0	9.0

†Quality parameters: crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), in-vitro dry matter disappearance (IVDMD)

6.25. Crude protein levels were measured to be 260 g kg^{-1} at V8, but dropped to 127 g kg^{-1} at harvest. Reed canarygrass contained higher levels of nitrogen at earlier stages of development. Nitrogen turnover in reed canarygrass occurs when inorganic N is converted into organic compounds to produce high molecular weight proteins and nucleic acids (Mengel and Kirkby, 1982). Crude protein levels thus decrease in the older vegetation as hydrolyzing enzymes break down the higher molecular weight compounds into lower molecular weight components such as

amino acids, amines, and amides. These nitrogen fractions can be transported through the xylem as NO_3, NH_4, and amino acids through the xylem, decreasing the level of CP over time.

Nitrogen is one of the nutrients that is translocated down to the roots in the fall and stored in the plant rhizome for the following spring. A study was conducted on reed canarygrass N uptake grown for biomass (Partala et al., 2001). The uptake of N was studied using ^{15}N-labelled nitrogen as a fertilizer to track the movement of N from the soil to the above-ground and below-ground biomass of the crop. The recovery of the applied N in total above-ground and below-ground biomass reached its maximum level (71%) at midsummer during the seeding year, with most of the labeled N in the shoots (68%). There is rapid growth of the rhizomes of the young plants, as well as the lack of N stored in the undeveloped rhizomes that intensifies the uptake of nitrogen into the plant from the soil. The amount of nitrogen in the plant shoots decreased during the fall and winter months as the nutrient was translocated to the rhizomes for storage until the following spring, where the N was utilized for regrowth.

Different levels of N fertilization can also impact the plant tissue CP levels. The analysis of variance indicated that the main effect of N fertility was significant for CP (Fig. 3.2).

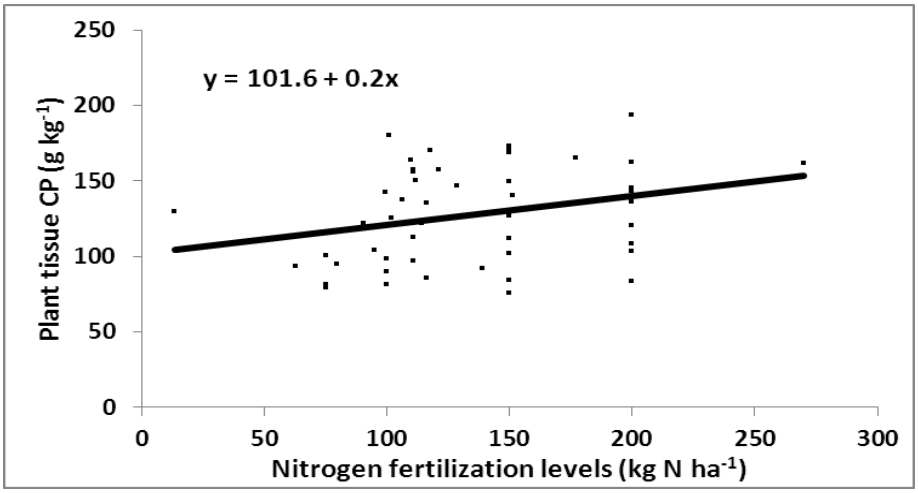

Fig. 3.2. Regression model for plant tissue crude protein of reed canarygrass as affected by N rates averaged across four environments, Fargo and Prosper, ND, in 2010 and 2011.

Crude protein in the plant tissue responded to increasing N fertility levels. The regression model (y= 101.6 + 0.2x), indicated a significant linear relationship between N and CP. Plant tissue CP generally increased from around 100 g kg^{-1} to 150 g kg^{-1} with soil from 50 kg N ha^{-1} to 200 kg N ha^{-1}, indicating that greater N rates can increase crude protein levels in the plant.

Neutral detergent fiber increased significantly from V8 to R1 and from V8 to H, but also decreased significantly from R1 to H growth stages. The highest level of NDF was at the R1 growth stage, where the plant tissue contained 558 g kg^{-1}. Acid detergent fiber, on the other hand, increased significantly from V8 to R1 and from V8 to H growth stages. The latter two growth stages measured significantly higher levels of NDF than at the V8 stage, which had 508 g kg^{-1}.

The components of NDF and ADF increased from V8 to both R1 and H growth stages. These two values are used to calculate hemicellulose, which is the subtraction of ADF from NDF. The 5-carbon sugars are linked to other 6-carbon sugars and broken down by cellulose enzymes through the process of hydrolysis. Hemicellulose is more readily hydrolyzed than cellulose because of its structure (Lee et al., 2007), though Anderson et al., (2010) made the statement that hemicellulose content actually plays a role in decreasing the potential ethanol yield as hemicellulose can block access to the cellulosic fibers, causing a negative correlation between them. Reed canarygrass hemicellulose levels decreased from 268 g kg^{-1} to 259 g kg^{-1} from V8 to H stages, though the difference between the three stages was not significant.

Acid detergent lignin was measured for reed canary grass at the V8 and H growth stages, but was not determined for the R1 growth stage. There was a significant difference between the two stages that were measured. The level of ADL increased significantly from the V8 to the H growth stages. Lignin is one of the cell wall constituents that can improve biofuel quality (Wrobel et al., 2009). Acid detergent lignin is the insoluble organic matter that remains after being treated

with an acidic solution (Lorenz et al., 2009; Schroeder, 2004). The lignin seal must be broken when pretreating the lignocellulosic material for conversion to ethanol, so that the cellulose can be accessed by enzymes to convert the carbohydrates into fermentable sugars for ethanol production (Mosier et al., 2005).

Lignin is one of the components of the cell wall that is the second most abundant organic substance in plants, after cellulose (Taiz and Zeiger, 2006). It is covalently bound to cellulose and other polysaccharides in the cell wall. Lignin strengthens plant stems, allowing plants to grow upward and to assist in water transport through the xylem. It reduces the digestibility of forage crops and can block access to cellulose unless pretreatment technologies are utilized when converting lignocellulosic biomass material into ethanol.

In-vitro dry matter disappearance levels decreased from the V8 to R1 growth stages. The quality component IVDMD measures digestibility of the forage by simulating the action of the rumen in-vitro. The greater the IVDMD, the more digestible the forage is. This characteristic is important when considering how easily the biomass can be converted to ethanol and other sources of bioenergy

Tissue Nitrate Content

Plant tissue nitrate analysis was done to determine the amount of NO_3-N present in the tissue at three growth stages. The analysis of variance was conducted for reed canarygrass plant tissue NO_3-N analysis (Table 3.9).

The interaction between environment by N rate, and environment by stage, and environment by stage by N rate were found to be significant, but since environment is a random effect, it will not be discussed.

The main effects of N fertility rate and growth stage were significant, as well as the interaction of growth stage and the N fertility rates, so the interaction between the two will be discussed (Fig. 3.3).

Table 3.9. Analysis of variance and mean squares for reed canarygrass plant-tissue nitrate analysis in the seeding year for five N rates and three developmental stages across four environments, Fargo and Prosper, ND, in 2010 and 2011.

Sources of variation	df	NO_3-N
Env	3	7051673.5***
Rep(env)	8	1807504.3
N	4	3573356.0*
Env x N	12	808525.0**
Error (a)	32	225265.3
Stage	2	27794562.1*
Stage x env	6	3123326.6**
Stage x N	8	1445087.8*
Stage x env x N	24	583443.0***
Error(b)	80	121149.4
CV, %		20.3

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively.

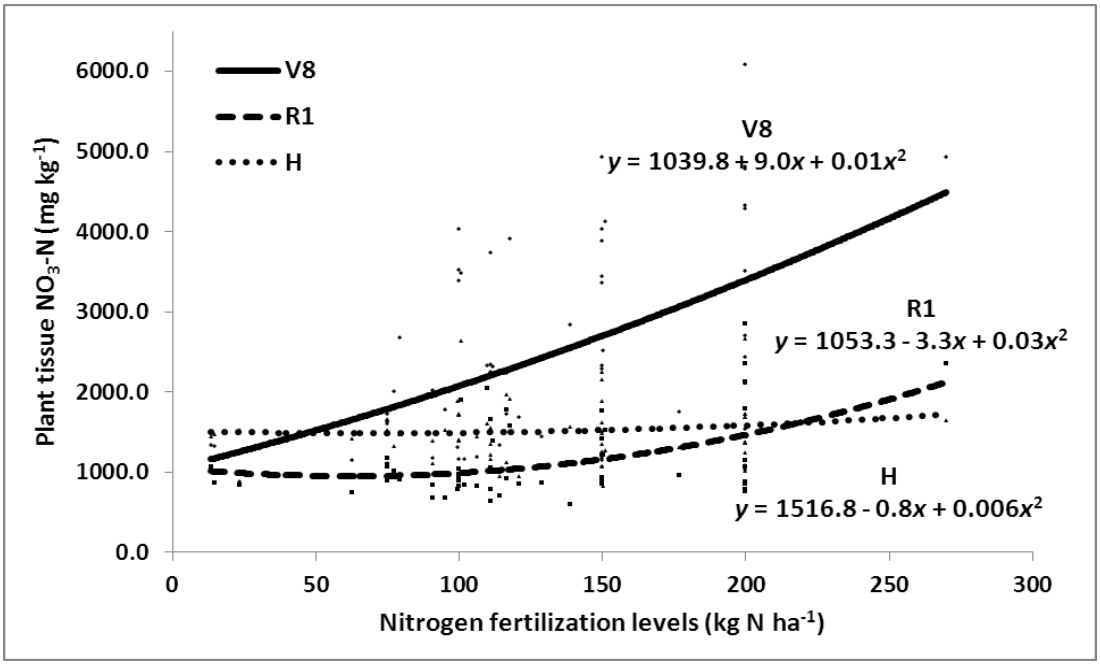

Fig. 3.3. Regression model for plant tissue NO_3-N of reed canarygrass at three growth stages [vegetative 8-leaf (V8), reproductive (R1), and harvest (H)] averaged across four environments, Fargo and Prosper in 2010 and 2011 as affected by actual N fertility levels.

The regression analysis ($y = 1039.8 + 9.0x + 0.01x^2$) showed a polynomial increase in plant tissue nitrate levels at the V8 growth stage for reed canarygrass with increasing levels of nitrogen fertilization. The latter two stages of development did not change significantly with increasing nitrogen fertility levels, indicating that the NO_3-N had been converted to another form and utilized by the plant.

The majority of nitrate uptake occurs during the early vegetative growth of the crop. Soon after the nitrate is taken up into the plant, it is absorbed and rapidly converted to NO_2, then to ammonium (Taiz and Zeiger, 2006). The conversion of nitrate to nitrite is catalyzed by the nitrate reductase enzyme, but because nitrite is so highly reactive and can be toxic to the plant, it is immediately transported from the cytosol into the chloroplasts in leaves, or the plastids in the roots, to be converted to ammonium, which is catalyzed by the enzyme nitrite reductase. Ammonium can be toxic to plants if at great enough levels, so the plant can use one of two pathways to convert the ammonium into amino acids, which are then incorporated into proteins. The lack of a significant impact of N fertility on NO_3-N levels in plant tissue at the later growth stages supports the idea that the nitrate has been assimilated and converted into usable proteins or amino acids. The fertilization levels didn't make a significant difference in nitrate content of the plant tissue at harvest, which is a desirable trait when considering how nitrate levels can impact the process of biomass conversion to bioenergy.

Total N Content and Nitrogen Uptake

An analysis of variance was conducted on tissue N content, N uptake, and three nitrogen efficiencies for reed canarygrass across three environments. The coefficient of variation for the fourth environment, Fargo in 2011, was very high and its error variance was not homogenous with

the other environments, so the combined analysis was conducted for Fargo and Prosper in 2010 and Prosper in 2011. For total N content, both environment and the N fertility main effects were significant, but because environment is considered a random effect, it will not be discussed (Table 3.10).

Table 3.10. Analysis of variance and mean squares for reed canarygrass N uptake and efficiencies for five N rates across three environments, Fargo and Prosper, ND, in 2010 and Prosper, ND in 2011. †

Sources of variation	df	Total N	N uptake	df	Agronomic efficiency	Physiological efficiency	NUE
Env	2	262.1***	90565.6***	2	76.5	3846.5**	1441.3
Rep(env)	5	19.3	5778.0	5	542.3	1080.6	2731.5
N	4	33.9‡	8328.1***	3	85.1	6.7	745.9
Env x N	8	11.7	599.9	6	47.2	777.9	318.7
Error	20	10.2	1842.9	15	80.2	382.9	905.0
CV, %		15.2	28.4		65.9	51.9	81.6

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively.
† Environment Fargo, 2011 was excluded from combined analysis
‡ Marginally significant at *P*=0.094

A regression analysis was conducted for reed canarygrass to determine the total N content response to nitrogen fertility rates (Fig. 3.4)

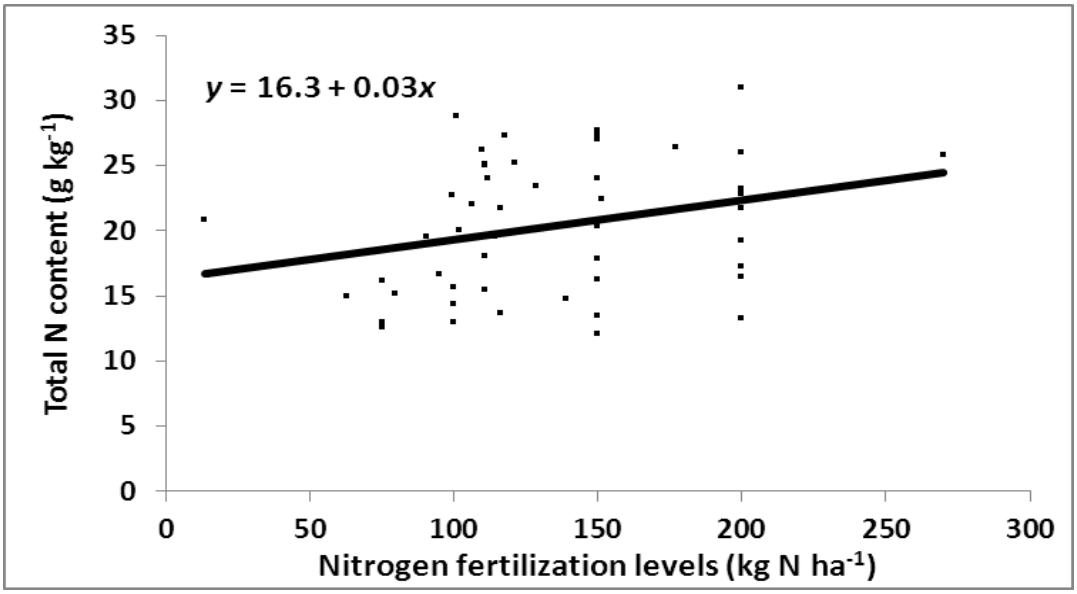

Fig. 3.4. Regression model for total N content of reed canarygrass averaged across four environments, Fargo and Prosper in 2010 and 2011 affected by actual N levels.

The regression analysis across environments did not show a significant response in total N content at harvest with increasing N fertility rates. One reason for a non-significant increase in total N content in the plant tissue is that much of the nitrogen in the plant tissue had already been converted to amino acids or proteins around harvest, so fertilization levels did not make as much of a difference later in the growing season.

In the analysis of variance for N uptake, the only significant effect was the main effect of N rates (Fig. 3.5).

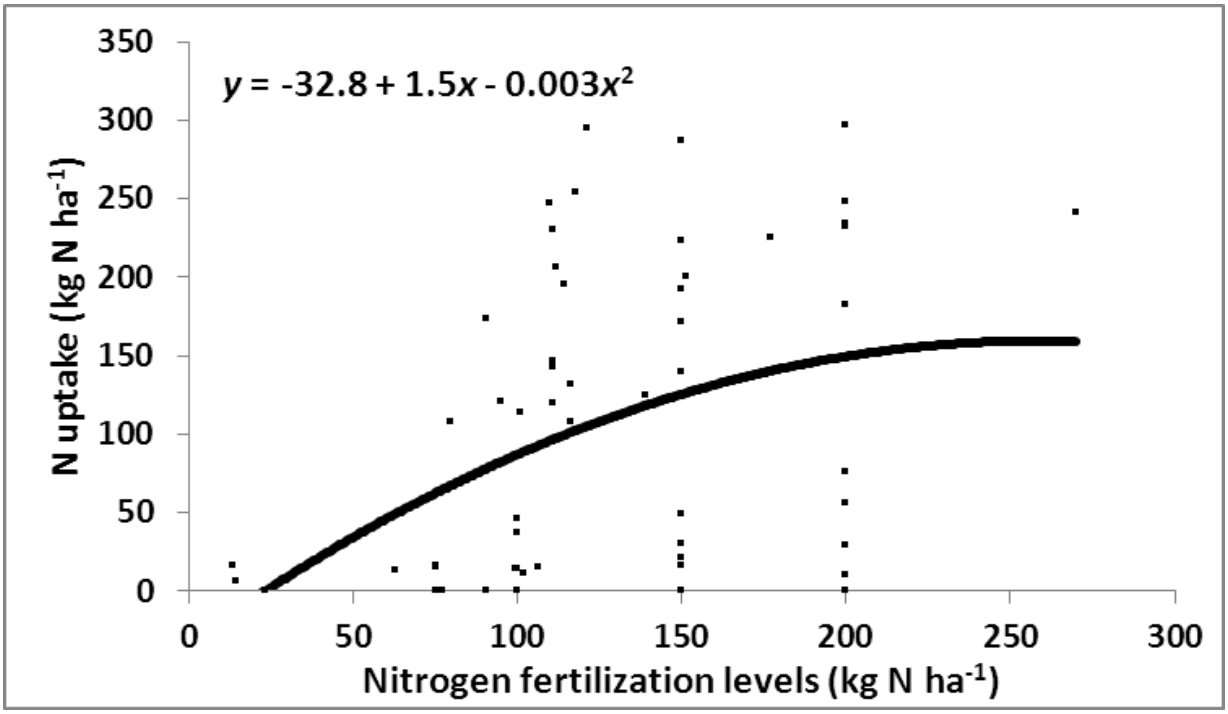

Fig. 3.5. Regression model for N uptake of reed canarygrass averaged across four environments, Fargo and Prosper in 2010 and 2011 as affected by actual N fertility levels.

According to the regression analysis, the N uptake of reed canarygrass did not increase significantly at increasing nitrogen rates at harvest. At 100 kg N ha^{-1}, reed canarygrass took up around 100 kg N ha^{-1}, which is almost three times greater than the N uptake at the same fertilization rate for the multi-year study on N uptake of reed canarygrass conducted by Partala et

al. (2001). The crop takes up greater amounts of N in the seeding year as it is getting established, and decreases in subsequent years.

Nitrogen is a mobile element in plants that is able to retranslocate during deficiency conditions (Taiz and Zeiger, 2006). Plants assimilate nitrogen through biochemical reactions involving oxidation and reduction processes to form covalent bonds with carbon, to ultimately create carbon and organic compounds. Nitrogen is taken up by the roots and translocated through the xylem to the upper plant parts (Mengel and Kirkby, 1982). Most of the ammonium is absorbed and assimilated in the root tissue and distributed in the form of amino acids. Nitrate, on the other hand, is able to be translocated to the leaves and shoots and assimilated there. Nitrogen is transported through the xylem in the forms of nitrate and amino acids most commonly. The intensity of nitrogen metabolism is dependent on the rate of protein synthesis controls the import of nitrogen by the different plant parts.

Reed canarygrass was fertilized with urea, a form of ammonium fertilizer. Yet the crop took up both nitrate and the ammonia forms of nitrogen. Fertilizer urea goes through a number of reactions in the soil itself before it is taken up by the plant (Mengel and Kirkby, 1982). Urea is generally converted to NH_4-N by urease or split into NH_4^+ and CO_2. Ammonium can be taken up by the plants at a lower rate of absorption because of its strong bond to the negatively charged clay minerals in the soil, or it can be oxidized to form NO_3^-, which can be absorbed by the plant more quickly. This nitrate can be either taken up by the plants or leached out, depending on the soil and environmental conditions. Ammonia can also rapidly convert to NO_3^- under normal soil conditions during the growing season (L. Cihacek, personal communication, 2012). This is the reason why crops still take up NO_3, even when NH_4 fertilizers are applied.

Ammonium is converted to amino acids through a process requiring two enzymes (Taiz and Zeiger, 2006). The primary pathway utilizes glutamine synthetase enzyme to combine ammonium with glutamate to form glutamine. When the level of glutamine increases, the enzyme glutamate synthase converts the glutamine to glutamate. The alternative pathway active in ammonium assimilation uses glutamate dehydrogenase to synthesize glutamine and glutamate. This reaction is catalyzed by aminotransferases to produce amino acids.

A number of factors can influence the loss of NH_3 from the soil. Soil pH can influence the loss of NH_3 from the soil, where higher pH soils have greater NH_3 loss due to volatilization (Mengel and Kirkby, 1982). Urea is rapidly converted to NH_3 in alkaline soils. Losses can also occur when urea is applied to the soil surface (not incorporated), and when there is little- to no-rain to incorporate it into the soil. If there is too much NO_3 produced from the oxidation of NH_4, higher levels of rainfall can leach NO_3 to deeper layers of soil where it is denitrified and lost.

Agronomic Efficiency, Physiological Efficiency, and Nitrogen Use Efficiency

The analysis of variance was conducted for agronomic efficiency, physiological efficiency, and nitrogen use efficiency for the three environments combined (Fargo and Prosper, 2010, and Prosper, 2011). None of the efficiencies were significant in the combined analysis. The coefficient of variation was high for each of the efficiencies (Table 3.11).

One reason for this may be that this study was conducted on a perennial grass in the establishment year. Much of the energy in the first year of production goes towards the production of rhizomes, and not for above-ground biomass, which is used to calculate agronomic efficiency. There is a lack of information on these efficiencies for reed canarygrass, though it was reported that reed canarygrass has a lower NUE than *Miscanthus x giganteus* (Lewandowski and Schmidt, 2006).

Table 3.11. Interaction among nitrogen rates for efficiencies of reed canarygrass averaged across three environments (env), Fargo and Prosper in 2010 and Prosper in 2011.

Actual N rates	Agronomic efficiency†	Physiological efficiency	Nitrogen use efficiency
	kg bmass kg N^{-1} applied	kg bmass ha^{-1} per unit N kg ha^{-1} uptake	kg N ha^{-1} uptake per kg N ha^{-1} applied
100	12.7	39.1	23.1
112	17.5	35.9	46.1
152	10.0	37.5	38.4
206	14.2	38.4	39.9
LSD (*P*=0.05) ‡	NS	NS	NS

† To compare the means of different N rates

Economic Analysis

Even though the efficiencies were not significant, an economic analysis was conducted to determine the most optimal rate of fertilization for reed canarygrass. The yield response equation was derived from the mean biomass yield at every actual N fertility level (Fig. 3.6).

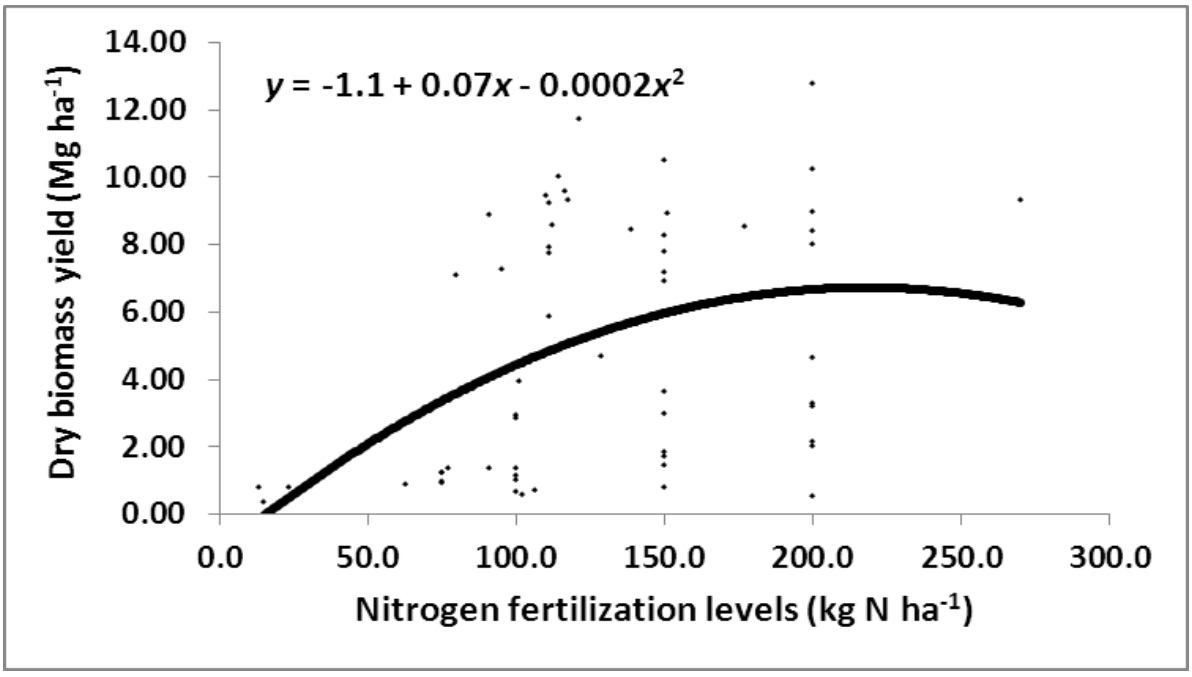

Fig. 3.6. Biomass yield of reed canarygrass with actual soil fertility levels in four environments at Fargo and Prosper, ND, in 2010 and 2011.

The derivative of the relative biomass yield equation, the marginal value product equation, was calculated and set to zero to determine the most economical rate of fertilization. The greatest biomass yield was obtained at 179 kg N ha^{-1}, which is the most economical fertility rate (Table 3.12). This rate may have been affected by nitrogen leaching out of the soil due to excessive rainfall and moisture in 2011, which could have impacted biomass yield.

Table 3.12. Economic analysis of reed canarygrass using the yield response equation, deriving the economical fertility rate according to the marginal value product equation and the price to fertilize the crop.

Yield response equation	Economical fertility rate	Price at 180 kg N ha^{-1} rate
	----------kg N ha^{-1}---------	----------$ Mg^{-1}----------
y= -1.0637 + 0.0715x - 0.0002x^2	179	38.62

With the base cost of fertilizer being $1.15 per kg N, the cost to fertilize reed canarygrass at the 179 kg N ha^{-1} rate was calculated to be $205.85 ha^{-1}, an estimated cost of $38.62 per Mg biomass produced. This may be the most economic rate for reed canarygrass in the seeding year, as biomass yield is not at its optimal level until the first or second production years.

CONCLUSIONS

Reed canarygrass obtained its greatest biomass yield at 179 kg N ha^{-1} in the seeding year. Its yield increased in response to greater N fertility rates, when averaged across the four environments.

Another important part of developing a feedstock for bioenergy production is to use a crop that has desirable quality characteristics to make the conversion of biomass to ethanol as efficient as possible. For reed canarygrass, a later harvesting date is recommended to give more time for plant mineral nutrients to translocate back into the soil or into the underground rhizomes, which reduces the ash content of the above-ground plant material. A later harvesting date will further reduce the transportation costs as well, as the grass will have more time to dry and decrease the amount of water weight that is a waste of energy to transport and that can reduce the efficiency of biomass conversion.

The level of crude protein in reed canarygrass increased at greater N fertility rates, yet the amount of CP was lowest at harvest. The crop also had higher levels of NDF, ADF, and ADL, indicating that higher cellulosic content can be produced for greater ethanol yield.

Reed canarygrass nitrate content was not affected significantly by increasing nitrogen fertility, suggesting that much of the nitrate had already been assimilated in the crop and translocated to the rhizomes for storage until the following spring, where the nitrogen was utilized for regrowth.

The high variability of agronomic efficiency, physiological efficiency, and NUE in this study suggests that more research is needed on the impact of N fertility regarding these efficiencies for reed canarygrass. It is suggested that the 179 kg N ha^{-1} rate be applied to achieve optimum

biomass in the seeding year. Greater amounts of N fertilization may be useful during the production years, but more research may be necessary before this conclusion can be made.

The recommendation for reed canarygrass as a potential biomass feedstock from this study is to fertilize it at the 179 kg N ha^{-1} rate during the seeding year. This rate will still produce adequate biomass yield for the seeding year (around 5.3 Mg ha^{-1}). A later harvest date is recommended to further reduce the amount of ash and mineral nutrients in the biomass material that can impact the conversion process. This lower rate will also decrease the amount of nitrogen that may be leached below the root zone if there is high rainfall during the seeding year, before the root biomass and rhizomes have had a chance to get fully established.

LITERATURE CITED

Agassiz Seed. 2010. Annual Forages. Agassiz Seed and Supply. Available at http://agassizseed.com/annual_forages.html (accessed 22 Nov. 2011).

Akron Services. 2012. Spring 2012 fertilizers and services. Akron Services, Inc. Available at http://akronservices.com/images/E0296801/2012_ASI_FERTILIZER_PRICES.pdf (accessed 15 May 2012).

Amaducci, S., M.T. Amaducci, R. Benati, and G. Venturi. 2000. Crop yield and quality parameters of four annual fibre crops (hemp, kenaf, maize, and sorghum) in the North of Italy. Ind. Crops Prod. 11:179-186.

Anderson, W.F., M.D. Casler, and B.S. Baldwin. 2008. Improvement of perennial forage species as feedstock for bioenergy. p. 309-345. *In* W. Vermerris (ed.) Genetic Improvement of Bioenergy Crops. Springer, New York.

Anderson, W.F., B.S. Dien, H.G. Jung, K.P. Vogel, and P.J. Weimer. 2010. Effects of forage quality and cell wall constituents of Bermuda grass on biochemical conversion to ethanol. Bioenerg. Res. 3:225-237.

ANKOM, 2011. Analytical procedures. ANKOM Technology. Available at http://www.ankom.com/analytical-procedures.aspx (accessed 8 May 2012).

Archontoulis, S.V., J. Vos, X. Yin, L. Bastiaans, N.G. Danalatos, and P.C. Struik. 2011. Temporal dynamics of light and nitrogen vertical distributions in canopies of sunflower, kenaf, and cynara. Field Crops Res. 122:186-198.

Aujla, M.S., H.S. Thind, and G.S. Buttar. 2005. Cotton yield and water use efficiency at various levels of water and N through drip irrigation under two methods of planting. Ag. Water Manag. 71:167-179.

Bakker, R.R., and H.W. Elbersen. 2005. Managing ash content and quality in herbaceous biomass: an analysis from plant to product. 14th European Biomass Conference, 17-21 Oct. 2005. WUR Bioenergy Papers and Project Reports. Paris, France. p. 210-213.

Bals, B., H. Murnen, M. Allen, and B. Dale. 2010. Ammonia fiber expansion (AFEX) treatment of eleven different forages: Improvements to fiber digestibility *in vitro*. Animal Feed Sci. and Technol. 155:147-155.

Bals, B., C. Wedding, V. Balan, E. Sendich, and B. Dale. 2011. Evaluating the impact of ammonia fiber expansion (AFEX) pretreatment conditions on the cost of ethanol production. Bioresource Technol. 102:1277-1283.

Bennett, A.S., and R.P. Anex. 2009. Production, transportation, and milling costs of sweet sorghum as a feedstock for centralized bioethanol production in the upper Midwest. Bioresource Technol. 100:1595-1607.

Berti, M.T., B.L. Johnson, R.W. Gesch, and F. Forcella. 2008. Cuphea nitrogen uptake and seed yield response to nitrogen fertilization. Agronomy J. 100:628-634.

Beyaert, R.P., and R.C. Roy. 2005. Influence of nitrogen fertilizer on multi-cut forage sorghum-sudangrass yield and nitrogen use. Agron J. 97:1493-1501.

Biomass Energy. 2012. Significant biomass properties. Part 2: Ash content. Available at: http://www.biomassenergy.gr/en/articles/technology/biomass/315-significant-biomass-properties-part-2-ash-content (accessed 10 May, 2012).

Black, C.A. 1993. Soil fertility evaluation and control. CRC Press, Boca Raton, FL. pp.746.

Bosworth, S., F. Jimenez-Serrano, and J. Aleong. 2005. Predicting quality of the first harvest of cool season grasses and grass/alfalfa mixtures grown for hay and silage. Univ. of Vermont Ext. Available at http://pss.uvm.edu/vtcrops/?Page=research/PredictingQualityFirstHarvest.html (accessed 2 May 2012). Burlington, VT.

Casler, M.D., and D.J. Undersander. 2006. Selection for establishment capacity in reed canarygrass. Crop Sci. 46:1277-1285.

Cataldo, B.A., M. Haroon, L.E. Schrader, and V.L. Youngs. 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun. Soil Sci. Plant Anal. 6:71-80.

Danalatos, N.G., and S.V. Archontoulis. 2010. Growth and biomass productivity of kenaf (*Hibiscus cannabinus* L.) under different agricultural inputs and management practices in central Greece. Ind. Crops Prod. 32:231-240.

Dien, B.S., G. Sarath, J.F. Pedersen, S.E. Sattler, H. Chen, D.L. Funnell-Harris, N.N. Nichols, and M.A. Cotta. 2009. Improved sugar conversion and ethanol yield for forage sorghum (*Sorghum bicolor* L. Moench) lines with reduced lignin contents. Bioenerg. Res. 2:153-164.

Digman, B., H.S. Joo, and D. Kim. 2009. Recent progress in gasification/pyrolysis technologies for biomass conversion to energy. Environ. Progress Sust. Energy. 28:47-51.

EPA. 2007. Regulation of fuels and fuel additives: renewable fuel standard. Environmental Protection Agency. Available at http://www.epa.gov/otaq/fuels/renewablefuels/index.htm (accessed 30 April 2012).

Faaij, A. 2006. Modern biomass conversion technologies. Mitigation and Adaptation Strategies for Global Change 11:343-375.

FAOSTAT. 2012. FAOSTAT database. Available at http://faostat.fao.org (accessed 4 March 2012)

Fernando, A., P. Duarte, J. Morais, A. Catroga, G. Serras, S. Pizza, V. Godovikova, and J.S. Oliveira. 2004. Characterization of kenaf potential in Portugal as an industrial and energy feedstock-the effect of irrigation, nitrogen fertilization and different harvest dates. p. 169-

172. *In* Van Swaaij, W.P.M. et al. (eds.), 2nd World Conference on Biomass for Energy, Industry and Climate Protection, Rome, Italy. 10-14 May 2004, Vol. I. ETA-Florence Renewable Energies. Florence, Italy.

Fernando, A.L., M. Ferreira, S. Boléo, E. Alexopoulou, and B. Mendes. 2012. Growth, productivity, and biomass quality of kenaf irrigated with wastewaters-the effect of nitrate ion. Presented at: 20th European Biomass Conference and Exhibition. Setting the course for a biobased economy. Milano, Italy. 18-22 June 2012.

Francois, L.E., T. Donovan, and E.V. Maas. 1984. Salinity effects on seed yield, growth, and germination of grain sorghum. Agron. J. 76:741-744.

Franzen, D.W., and L.J. Cihacek. 1996. North Dakota fertilizer recommendation tables and equations based on soil test levels and yield goals. Bull. SF-882. North Dakota State Univ. Ext. Serv., Fargo, ND.

Fritschi, F.B., B.A. Roberts, R.L. Travis, D.W. Rains, and R.B. Hutmacher. 2004. Seasonal nitrogen concentration, uptake, and partitioning pattern of irrigated Acala and Pima cotton as influenced by nitrogen fertility level. Crop Sci. 44:516-527.

Gehlhar, M., A. Winston, and A. Somwaru. 2010. Effects of increased biofuels on the U.S. economy in 2022. U.S. Dept. of Agriculture, Economic Research Service.

Geisler, M. 2011. Kenaf. Agricultural Marketing Resource Center. Available at http://www.agmrc.org/commodities__products/fiber/kenaf/ (accessed 17 April 2012)

Guo, Y., S. Hu, Y. Li, D. Chen, B. Zhu, and K.M. Smith. 2010. Optimization and analysis of a bioethanol agro-industrial system from sweet sorghum. Renewable Energy 35:2902-2909.

Guretzky, J.A., J.T. Biermacher, B.J. Cook, M.K. Kering, and J. Mosali. 2011. Switchgrass for forage and bioenergy: harvest and nitrogen rate effects on biomass yields and nutrient composition. Plant Soil. 339:69-81.

Hadas, A., A. Hadas, B. Sagiv, and N. Haruvy. 1999. Agricultural practices, soil fertility management modes, and resultant nitrogen leaching rates under semi-arid conditions. Agricultural Water Management. 42:81-95.

Hendriks, A.T.W.M., and G. Zeeman. 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresource Technol. 100:10-18.

Herr-Turoff, A., and J.B. Zedler. 2005. Does wet prairie vegetation retain more nitrogen with or without *Phalaris* invasion? Plant and Soil. 277:19-34.

Hettenhaus, J. 2006. Achieving sustainable production of agricultural biomass for biorefinery feedstock. For the Biotechnology Industry Organization Industrial and Environmental section. pp. 24. CEA Inc., Washington, D.C.

Higgins, J.J., and G.A. White. 1970. Effects of plant population and harvest date on stem yield and growth components of kenaf in Maryland. Agronomy J. 62(5): 667-668.

Horwitz, W., and G. Latimer, Jr., eds. 2010. Official methods of analysis of AOAC International. 18th ed., revision 3. AOAC Intl. Gaithersburg, MD.

Johnson, C., G. Albrecht, Q. Ketterings, J. Beckman, and K. Stockin. 2005. Nitrogen basics-the nitrogen cycle. Agronomy Fact Sheet Series-Fact Sheet 2. Cornell Univ. Coop. Ext. Ithaca, NY.

Johnson, R.G., and M.B. Ali. 1979. Economics of nitrogen use in crop production. ND Farm Research. 37(3):34-36.

Jordan, W.R., and F.R. Miller. 1980. Genetic variability in sorghum root systems: Implications for drought tolerance. p. 383-399. *In* P.C. Turner and P.J. Kramer (eds.) Adaptation of Plants to Water and High Temperature Stress. John Wiley & Sons, New York.

Kaizzi, K.C., J. Byalebeka, O. Semalulu, I. Alou, W. Zimwanguyizza, A. Nansamba, P. Musinguzi, P. Ebanyat, T. Hyuha, and C.S. Wortmann. 2012. Sorghum response to fertilizer and nitrogen use efficiency in Uganda. Agron. J. 104(1): 83-90.

Kilcer, T.F., Q.M. Ketterings, J.H. Cherney, P. Cerosaletti, and P. Barney. 2005. Optimum stand height for forage brown midrib sorghum x sudangrass in north-eastern USA. J. Agron. and Crop Sci. 191:35-40.

Klein, R.N., and C.A. Shapiro. 2011. Evaluating hail damage to grain sorghum. Bull. EC129. Univ. of Nebraska-Lincoln Ext. Serv. Lincoln, NE.

Lattanzi, F.A. 2010. C3/C4 grasslands and climate change. p. 3-13. *In* H. Schnyder, et al., (eds.) Proc. 23rd EGF General Meeting-Grassland in a Changing World. Grassland Science in Europe, vol. 15: Kiel, Germany. 29 Aug. to 2 Sept. 2010. European Grassland Federation, Duderstadt, Germany.

Lee, D., V.N. Owens, A. Boe, and P. Jeranyama. 2007. Composition of herbaceous biomass feedstocks. p. 16 Bull. SGINC1-07. North Central Sun Grant Center. South Dakota State University, Brookings, SD.

Lewandowski, I., and U. Schmidt. 2006. Nitrogen, energy, and land use efficiencies of Miscanthus, reed canary grass, and triticale as determined by the boundary line approach. Agric., Ecosystems, and Env. 112:335-346.

Li, B.-Z., V. Balan, Y.-J. Yuan, and B.E. Dale. 2010. Process optimization to convert forage and sweet sorghum bagasse to ethanol based on ammonia fiber expansion (AFEX) pretreatment. Bioresource Technol. 101:1285-1292.

Li, Y., J. Shi, and R. Reeder. 2011. Storing lignocellulosic biomass for bio-refining industry. Bull. AEX-651.1-11. The Ohio State Univ. Ext. Serv. Columbus, OH.

Lindquist, J.L., D.C. Barker, S.Z. Knezevic, A.R. Martin, D.T. Walters. 2007. Comparative nitrogen uptake and distribution in corn and velvetleaf (*Abutilon theophrasti*). Weed Sci. 55:102-110.

Lorenz, A.J., R.P. Anex, A. Isci, J.G. Coors, N. de Leon, and P.J. Weimer. 2009. Forage quality and composition measurements as predictors of ethanol yield from maize (*Zea mays* L.) stover. Biotechnol. Biofuels 2:5.

Marsalis, M.A., S.V. Angadi, and F.E. Contreras-Govea. 2010. Dry matter yield and nutritive value of corn, forage sorghum, and BMR forage sorghum at different plant populations and nitrogen rates. Field Crops Res. 116:52-57.

Massacci, A., A. Battistelli, and F. Loreto. 1996. Effect of drought stress on photosynthetic characteristics, growth, and sugar accumulation of field-grown sweet sorghum. Aust. J. Plant Physiol. 23:331-340.

McClure, B. 2012. Sorghum fertility management. Production Manual. Available at https://www.pioneer.com/home/site/ca/template.CONTENT/products/sorghum/production-manual/guid.AE3799A5-A380-492A-B5DF-1D49E8B1735B (accessed 5 April 2012)

Mengel, K. and E.A. Kirkby. 1982. Principles of Plant Nutrition. International Potash Institute, Bern, Switzerland. pp. 655.

Mosier, N., C. Wyman, B. Dale, R. Elander, Y.Y. Lee, M. Holtzapple, and M. Ladisch. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresource Technol. 96:673-686.

Muir, J.P. 2001. Dairy compost, variety, and stand age effects on kenaf forage yield, nitrogen and phosphorus concentrations, and uptake. Agron. J. 93:1169-1173.

NDSU Extension Service, North Dakota Corn Utilization Council. 1997. Irrigation management. Corn Production Guide. Bull. A-1130. North Dakota State Univ. Agriculture and University Extension. Fargo, ND. Available at http://www.ag.ndsu.edu/pubs/plantsci/rowcrops/a1130-8.htm#Irrigation (accessed 5 Jan. 2012).

Nielsen, D.C. 2004. Kenaf forage yield and quality under varying water availability. Agron J. 96:204-213.

North Dakota Agricultural Weather Network (NDAWN). 2012. NDAWN Center. North Dakota State Univ., Fargo, ND. Available at: http://ndawn.ndsu.nodak.edu (accessed 14 Apr. 2012).

Oh, H.K., B.R. Baumgardt, and J.M. Scholl. 1966. Evaluation of forages in the laboratory. Comparison of chemical analyses, solubility tests and in vitro fermentation. J. Dairy Sci. 49:850.

Ottman, M.J., B.A. Kimball, P.J. Pinter, G.W. Wall, R.L. Vanderlip, S.W. Leavitt, R.L. LaMorte, A.D. Matthias, and T.J. Brooks. 2001. Elevated CO_2 increases sorghum biomass under drought conditions. New Phytologist 150:261-273.

Partala, A., M. Timo, M. Esala, and E. Ketoja. 2001. Plant recovery of ^{15}N-labelled nitrogen applied to reed canary grass grown for biomass. Nutrient Cycling in Agroecosystems 61:273-281.

Producer's Choice Seed. 2007. HayKing Hybrid Sudangrass. Producer's Choice. Available at http://producerschoiceseed.com/docs/HayKingHybridSudangrass.pdf (accessed 22 Nov. 2011).

Robertson, M.J., S. Fukai, M.M. Ludlow, and G.L. Hammer. 1993. Water extraction by grain sorghum in a sub-humid environment. II. Extraction in relation to root growth. Field Crops Res. 33:99-112.

Rooney, W.L., J. Blumenthal, B. Bean, and J.E. Mullet. 2007. Designing sorghum as a dedicated bioenergy feedstock. Biofuels, Bioprod. Bioref. 1:147-157.

Rowell, R.M., and H.P. Stout. 2006. Jute and kenaf. p. 405-452. *In* M. Lewin (ed.) Handbook of Fiber Chemistry, 3rd ed. CRC Press, Taylor and Francis Group. Boca Raton, FL.

Saballos, A. 2008. Development and utilization of sorghum as a bioenergy crop, p. 211-248, *In* W. Vermerris et al., (eds.) Genetic Improvement of Bioenergy Crops. 6 ed. Springer Science+Business Media, LLC., New York.

Sainju, U.M., B.P. Singh, W.F. Whitehead, and S. Wang. 2007. Accumulation and crop uptake of soil mineral nitrogen as influenced by tillage, cover crops, and nitrogen fertilization.

Sanderson, M.A., N.P. Martin, and P. Adler. 2006. Biomass, energy, and industrial uses of forages. p. 635-647. *In* K. J. Moore et al., (eds.) Forages: The Science of Grassland Agriculture. 6th ed. Blackwell Inc., Ames, IA.

SAS Institute. 2008. The SAS system for windows. Release 9.2. SAS Inst., Cary, NC.

Schmer, M.R., K.P. Vogel, R.B. Mitchell, and R.K. Perrin. 2008. Net energy of cellulosic ethanol from switchgrass. PNAS. 105:464-469.

Schroeder, J.W. 2004. Interpreting composition and determining market value. Quality Forage. Bull. AS-1251. North Dakota State Univ. Ext. Serv. Fargo, ND.

Sheaffer, C.C., P.R. Peterson, and N.J. Ehlke. 1990. Reed canarygrass, *In* Univ. of Minnesota Agric. Exp. Stn., (ed.), St. Paul, MN.

Soil Survey Staff. 2011. Soil Survey. Available at http://soils.usda.gov/technical/classification/osd/index.html (accessed 4 Jan. 2011) USDA-NRCS, Lincoln, NE.

Steel, R.G.D., and J.H. Torrie. 1980. Principles and procedures of statistics: A biometrical approach. 2nd ed. McGraw-Hill, New York.

Taiz, L., and E. Zeiger. 2006. Assimilation of mineral nutrients. Plant Physiology, 4th ed. Sinauer Associates, Inc., Sunderland, MA.

Teutsch, C. 2009. Warm-season annual grasses for summer forage. Bull. 418-004. Virginia Tech and Virginia State Univ. Coop. Ext. Petersburg, VA.

Vanderlip, R.L. 1993. How a sorghum plant develops. Kansas State University Agricultural Experimental Station and Cooperative Extension Service. Manhattan, KS.

Vendrell, P.F., and J. Zupancic. 1990. Determination of soil nitrate by transnitration of salicylic acid. Commun. Soil Sci. Plant Analysis. 21:1705-1713.

Webber, C.L., H.L. Bhardwaj, and V.K. Bledsoe. 2002. Kenaf production: fiber, feed, and seed, p. 327-339, *In* J. Janick and A. Whipkey, (eds.) Trends in new crops and new uses. ASHS Press, Alexandria, VA.

Webber, C.L. and V.K. Bledsoe. 2002. Plant maturity and kenaf yield components. Ind. Crops Prod. 16:81-88.

Wrobel, C., B.E. Coulman, and D.L. Smith. 2009. The potential use of reed canarygrass (*Phalaris arundinacea* L.) as a biofuel crop. Acta Agriculturae Scandinavica Section B-Soil and Plant Sci. 59:1-18.

Zegada-Lizarazu, W., and A. Monti. 2011. Energy crops in rotation. A review. Biomass and Bioenergy. 35:12-25.

Zegada-Lizarazu, W., and A. Monti. 2012. Are we ready to cultivate sweet sorghum as a bioenergy feedstock? A review on field management practices. Biomass and Bioenergy. 40:1-12.

Zhang, C., G. Xie, S. Li, L. Ge, and T. He. 2010. The productive potentials of sweet sorghum ethanol in China. Applied Energy 87:2360-2368.

APPENDIX

Table A1. Analysis of variance and mean squares for sorghum, sudangrass, and kenaf N uptake and efficiencies for five N rates for one environment, Fargo, ND, in 2011. †

Sources of variation	df	Change in soil N	Biomass	df	CP	Tissue N	N uptake
Rep	2	604.7	8522051.4	1	0.00041	10.4	183.9
Crop	2	7.9	86587220.9*	2	0.0047*	119.6*	236.0
Rep x crop	4	141.6	8888911.6**	2	0.000057	1.5	582.5*
N	4	24287.4***	25867738.6**	4	0.000054	1.4	459.7 ‡
N x crop	8	205.8	3042731.7	8	0.000042	1.1	140.9
Rep x N x crop	24	184.0	1459062.1	12	0.000029	0.7	110.1
CV, %		17.5	16.1		10.9	10.9	21.3

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively.
† Environment Fargo, 2011 was excluded from the combined analysis
‡ Significant at 0.073

Table A2. Analysis of variance and mean squares for sorghum, sudangrass, and kenaf N uptake and efficiencies for five N rates for one environment, Fargo, ND, in 2011. †

Sources of variation	df	Physiological efficiency	NUE	df	Agronomic efficiency
Rep	1	39362.2	60.8	2	247.7
Crop	2	356441.8	39.9	2	378.8
Rep x crop	2	72223.8	43.8	4	141.6*
N	3	207588.9	95.7 ‡	3	347.9*
N x crop	6	126834.2	25.8	6	40.4
Rep x N x crop	9	405296.9	16.2	18	33.9
CV, %		219.6	147.2		66.1

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively.
† Environment Fargo, 2011 was excluded from the combined analysis
‡ Significant at 0.081

Table A3. Analysis of variance and mean squares for reed canarygrass N uptake and efficiencies for five N rates for one environment, Fargo, ND, in 2011.†

Sources of variation	df	Tissue N conc	N uptake	df	Agronomic efficiency	Physiological efficiency	NUE
Rep	1	112.2	61.3	1	21.8	1294.8	33.2
N	4	2.9	14.1	3	4.1	2005.1*	3.3
Rep x N	4	1.6	34.7	3	3.6	178.0	4.3
CV, %		7.2	36.4		91.7	19.8	82.5

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively.
† Environment Fargo, 2011 was excluded from combined analysis

Table A4. Analysis of variance and mean squares for sorghum, sudangrass, kenaf, and reed canarygrass height for five N rates across four environments, Fargo and Prosper, ND, in 2010 and 2011.

Sources of variation	df	Height
Env	3	41836
Rep(env)	8	2090
Crop	3	469520***
Env x crop	9	948
Error (a)	24	675
N	4	5829*
Env x N	12	1650
N x crop	12	609
Env x N x crop	36	486
Error (b)	127	216
CV, %		9

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively.

Table A5. Mean height for three crops at harvest averaged across N rates (0, 75, 100, 150, and 200 kg N ha^{-1}) and four environments, Fargo and Prosper, ND, in 2010 and 2011.

Crop	Height
	----------cm----------
Forage sweet sorghum	242.3
Sorghum x sudangrass	238.0
Kenaf	151.8
Reed canarygrass	54.6
LSD (*P*=0.05)†	12.7

† To compare the means of different crops.

Table A6. Analysis of variance for analyzing a single environment with two-factor treatment design conducted in a split-plot design.

Source of variation	df	Mean square		F-ratio
		Observed	Expected † ‡ §	
Replicate	2	M1	$\sigma^2 + n\sigma^2_{\gamma} + cn\sigma^2_{R}$	--
C	2	M2	$\sigma^2 + n\sigma^2_{\gamma} + r\sigma^2_{CN} + rn\Phi_C$	--
Error (a)	4	M3	$\sigma^2 + n\sigma^2_{\gamma}$	M3/M6
N	4	M4	$\sigma^2 + r\sigma^2_{CN} + rc\Phi_N$	M4/M5
C X N	8	M5	$\sigma^2 + r\Phi_{CN}$	M5/M6
Error (b)	24	M6	σ^2	
Total	44	M7		

† The letters N, C, and R, refer to N fertility levels, crop, and replicate, respectively.
‡ The letters n, c, and r, refer to the number of levels of factors N and C and the number of replications per environment, respectively.
§ $\Phi_C = \sum C_i^2/(c-1)$
$\Phi_N = \sum N_j^2/(n-1)$
$\Phi_{CN} = \sum\sum (CN)_{ij}^2/[(c-1)(n-1)]$

Table A7. Combined analysis of variance for analyzing the experiment with two-factor treatment design conducted in a split-plot design.

Source of variation	df	Mean square		F-ratio
		Observed	Expected † ‡ §	
Environment	3	M1		--
Rep (env)	8	M2		--
C	2	M3	$\sigma^2_{\varepsilon} + n\sigma^2_{\gamma} + rn\sigma^2_{CE} + re n\Phi_C$	M3/M4
C X E	6	M4	$\sigma^2_{\varepsilon} + n\sigma^2_{\gamma} + rn\sigma^2_{CE}$	M4/M5
Pooled error C	16	M5	$\sigma^2_{\varepsilon} + n\sigma^2_{\gamma}$	M5/M10
N	4	M6	$\sigma^2_{\varepsilon} + rc\sigma^2_{NE} + rec\Phi_N$	M6/M7
N X E	12	M7	$\sigma^2_{\varepsilon} + ra\sigma^2_{NE}$	M7/M10
C X N	8	M8	$\sigma^2_{\varepsilon} + r\sigma^2_{CNE} + re\Phi_{CN}$	M8/M9
C X N X E	24	M9	$\sigma^2_{\varepsilon} + r\sigma^2_{CNE}$	M9/M10
Pooled error N	96	M10	σ^2_{ε}	
Total	179			

† The letters E, N, C, and R, refer to environments, N fertility levels, crop, and replicates per environment, respectively.
‡ The letters e, n, c, and r, refer to the number of environments, the number of levels of factors N and C, and the number of replications per environment, respectively.
§ $\Phi_C = \sum C_i^2/(c-1)$
$\Phi_N = \sum N_j^2/(n-1)$
$\Phi_{CN} = \sum\sum (CN)_{ij}^2/[(c-1)(n-1)]$

Table A8. Analysis of variance for analyzing a single environment with single-factor treatment design conducted in a RCBD.

		Mean square		
Source of variation	df	Observed	Expected † ‡ §	F-ratio
Rep	2	M1		--
N	4	M2	$\sigma^2_\varepsilon + r\Phi_N$	M2/M3
Error	8	M3	σ^2_ε	
Total	14			

† The letters N and R, refer to N fertility levels and replicate, respectively.
‡ The letters n and r refer to the number of levels of factors N and the number of replications per environment, respectively.
§ $\Phi_N = \sum N_i^2/(n-1)$

Table A9. Combined analysis of variance for analyzing the experiment with single-factor treatment design conducted in a RCBD design.

		Mean square		
Source of variation	df	Observed	Expected † ‡ §	F-ratio
Environment	3	M1		--
Rep (env)	8	M2		--
N	4	M3	$\sigma^2_\varepsilon + r\sigma^2_{EN} + re\Phi_N$	M3/M4
Env X N	12	M4	$\sigma^2_\varepsilon + r\sigma^2_{EN}$	M4/M5
Error (a)	32	M5	σ^2_ε	
Total	59			

† The letters E, N, and R, refer to environments, N fertility levels, and replicates per environment, respectively.
‡ The letters e, n, and r, refer to the number of environments, the number of levels of factors N, and the number of replications per environment, respectively.
§ $\Phi_N = \sum N_i^2/(n-1)$

Table A10. Analysis of variance for analyzing a single environment with three-factor treatment design conducted in a split-split design.

Source of variation	df	Mean square		F-ratio
		Observed	Expected † ‡ §	
Replicate	2	M1		--
C	2	M2	$\sigma^2_\varepsilon + s\sigma^2_\gamma + ns\sigma^2_\delta + rsn\Phi_C$	M2/M3
Error (a)	4	M3	$\sigma^2_\varepsilon + s\sigma^2_\gamma + ns\sigma^2_\delta$	M3/M6
N	4	M4	$\sigma^2_\varepsilon + s\sigma^2_\gamma + rs\sigma^2_{CN} + rsc\Phi_N$	M4/M6
C X N	8	M5	$\sigma^2_\varepsilon + s\sigma^2_\gamma + rs\Phi_{CN}$	M5/M6
Error (b)	24	M6	$\sigma^2_\varepsilon + s\sigma^2_\gamma$	M6/M11
S	2	M7	$\sigma^2_\varepsilon + r\sigma^2_{CNS} + rn\sigma^2_{CS} + rc\sigma^2_{NS} + rcn\Phi_S$	M7/M8
C X S	4	M8	$\sigma^2_\varepsilon + r\sigma^2_{CNS} + rn\Phi_{CS}$	M8/M10
N X S	8	M9	$\sigma^2_\varepsilon + r\sigma^2_{CNS} + rc\Phi_{NS}$	M9/M10
C X N X S	16	M10	$\sigma^2_\varepsilon + r\Phi_{CNS}$	M10/M11
Error (c)	60	M11	σ^2_ε	
Total	134			

† The letters N, C, and R, refer to N fertility levels, crop, and replicate, respectively.

‡ The letters n, c, and r, refer to the number of levels of factors N and C and the number of replications per environment, respectively.

§ $\Phi_C = \sum C_i^2/(c-1)$

$\Phi_N = \sum N_j^2/(n-1)$

$\Phi_S = \sum S_k^2/(s-1)$

$\Phi_{CN} = \sum\sum (CN)_{ij}^2/[(c-1)(n-1)]$

$\Phi_{CS} = \sum\sum (CS)_{ik}^2/[(c-1)(s-1)]$

$\Phi_{NS} = \sum\sum (NS)_{jk}^2/[(n-1)(s-1)]$

$\Phi_{CNS} = \sum\sum\sum (CNS)_{ijk}^2/[(c-1)(n-1)(s-1)]$

Table A11. Combined analysis of variance for analyzing the experiment with three-factor treatment design conducted in a split-split design.

Source of variation	df	Mean square		F-ratio
		Observed	Expected † ‡ §	
Environment	3	M1		--
Rep (env)	8	M2		--
C	2	M3	$\sigma^2_\varepsilon + s\sigma^2_\gamma + ns\sigma^2_\delta + nrs\sigma^2_{CE} + nser\Phi_C$	M3/M4
Env X C	6	M4	$\sigma^2_\varepsilon + s\sigma^2_\gamma + ns\sigma^2_\delta + nrs\sigma^2_{CE}$	M4/M5
Error (a)	16	M5	$\sigma^2_\varepsilon + s\sigma^2_\gamma + ns\sigma^2_\delta$	M5/M10
N	4	M6	$\sigma^2_\varepsilon + s\sigma^2_\gamma + csr\sigma^2_{NE} + cser\Phi_N$	M6/M7
E X N	12	M7	$\sigma^2_\varepsilon + s\sigma^2_\gamma + csr\sigma^2_{NE}$	M7/M10
C X N	8	M8	$\sigma^2_\varepsilon + s\sigma^2_\gamma + rs\sigma^2_{CNE} + ser\Phi_{CN}$	M8/M9
E X C X N	24	M9	$\sigma^2_\varepsilon + s\sigma^2_\gamma + rs\sigma^2_{CNE}$	M9/M10
Error (b)	96	M10	$\sigma^2_\varepsilon + s\sigma^2_\gamma$	M10/M19
S	2	M11	$\sigma^2_\varepsilon + csr\sigma^2_{SE} + cser\Phi_S$	M11/M12
E X S	6	M12	$\sigma^2_\varepsilon + csr\sigma^2_{SE}$	M12/M19
C X S	4	M13	$\sigma^2_\varepsilon + nr\sigma^2_{CSE} + ner\Phi_{CS}$	M13/M14
E X C X S	12	M14	$\sigma^2_\varepsilon + nr\sigma^2_{CSE}$	M14/M19
N X S	8	M15	$\sigma^2_\varepsilon + cr\sigma^2_{NSE} + cer\Phi_{NS}$	M15/M16
E X N X S	24	M16	$\sigma^2_\varepsilon + cr\sigma^2_{NSE}$	M16/M19
C X N X S	16	M17	$\sigma^2_\varepsilon + r\sigma^2_{CNSE} + er\Phi_{CNS}$	M17/M18
E X C X N X S	48	M18	$\sigma^2_\varepsilon + r\sigma^2_{CNSE}$	M18/M19
Error (c)	240	M19	σ^2_ε	
Total	539			

† The letters E, N, S, C, and R, refer to environments, N fertility levels, stage, crop, and replicates per environment, respectively.

‡ The letters e, n, s, c, and r, refer to the number of environments, the number of levels of factors N, S, and C, and the number of replications per environment, respectively.

§ $\Phi_C = \sum C_i^2/(c-1)$

$\Phi_N = \sum N_j^2/(n-1)$

$\Phi_S = \sum S_k^2/(s-1)$

$\Phi_{CN} = \sum\sum (CN)_{ij}^2/[(c-1)(n-1)]$

$\Phi_{CS} = \sum\sum (CS)_{ik}^2/[(c-1)(s-1)]$

$\Phi_{NS} = \sum\sum (NS)_{jk}^2/[(n-1)(s-1)]$

$\Phi_{CNS} = \sum\sum\sum (CNS)_{ijk}^2/[(c-1)(n-1)(s-1)]$

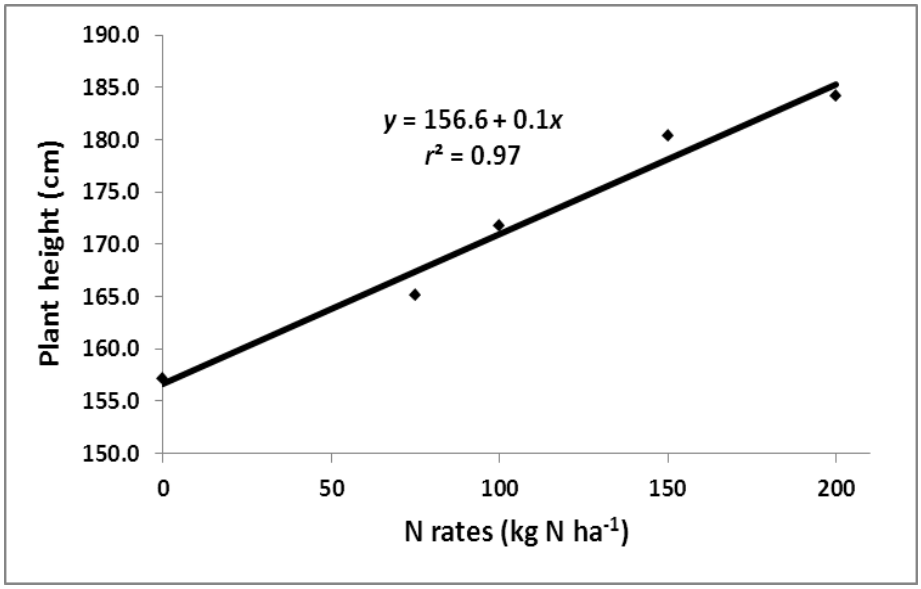

Figure A1. Regression model for plant height as affected by N rates averaged across four environments (Fargo and Prosper, ND, 2011 and 2012) and four crops (forage sweet sorghum, sorghum x sudangrass, kenaf, and reed canarygrass).

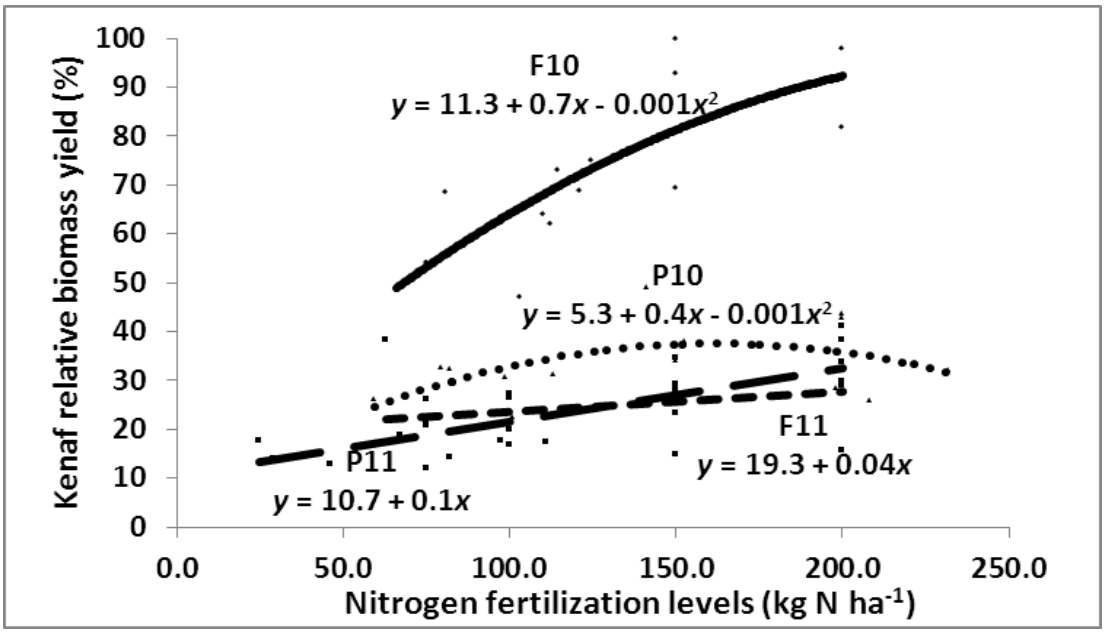

Figure A2. Regression model for relative biomass yield of kenaf as affected by N fertility levels for four environments, Fargo (F10-solid line, F11-short dash) and Prosper (P10-dotted line, P11-long dash), ND, in 2010 and 2011.

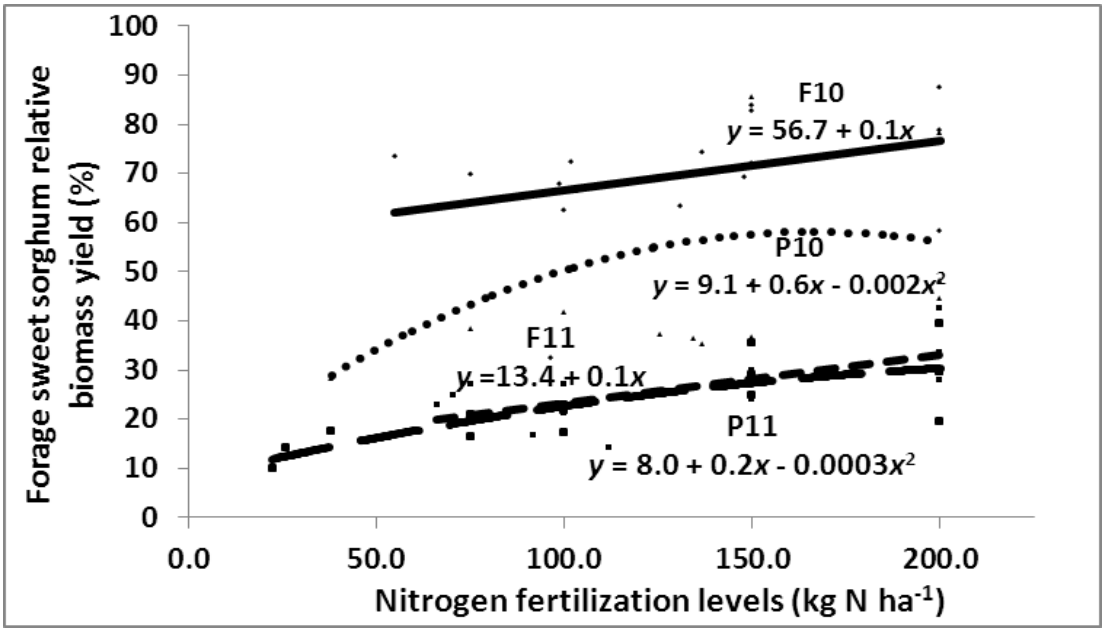

Figure A3. Regression model for relative biomass yield of forage sweet sorghum as affected by N fertility levels for four environments, Fargo (F10-solid line, F11-short dash) and Prosper (P10-dotted line, P11-long dash), ND, in 2010 and 2011.

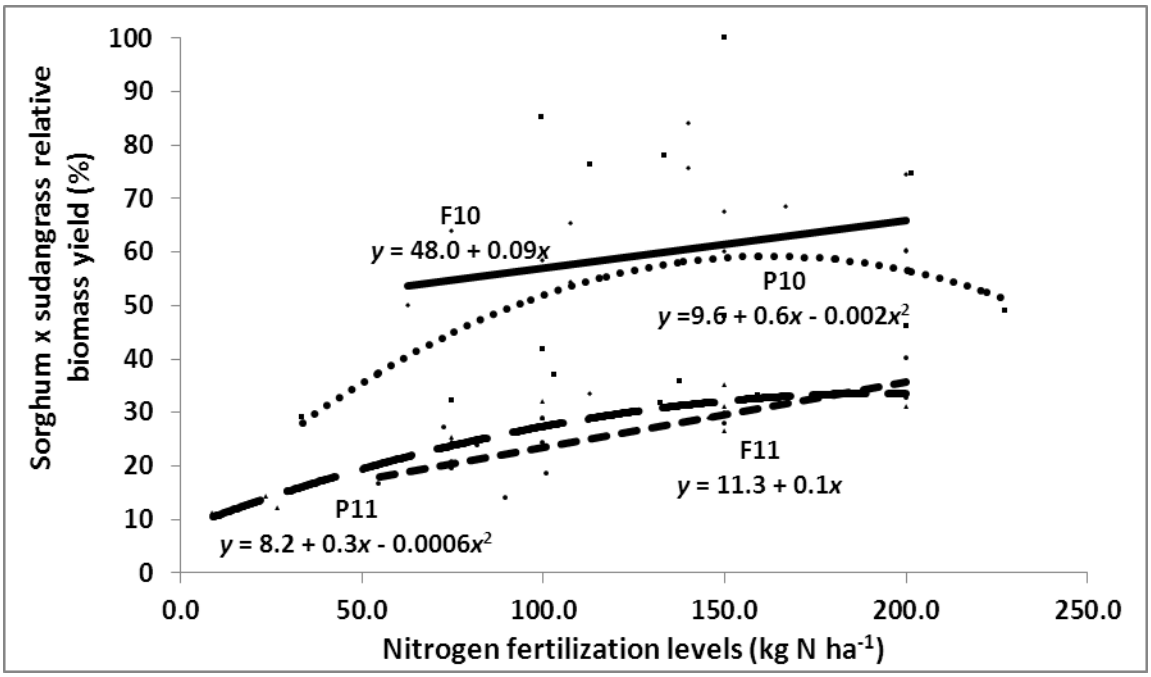

Figure A4. Regression model for relative biomass yield of sorghum x sudangrass as affected by N fertility levels for four environments, Fargo (F10-solid line, F11-short dash) and Prosper (P10-dotted line, P11-long dash), ND, in 2010 and 2011.