

COVER CROPS IMPACT ON ENERGY AND FORAGE CROPS PRODUCTIVITY

IN NORTH DAKOTA

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

By

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In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Plant Science

April 2013

Fargo, North Dakota

North Dakota State University
Graduate School

Title

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University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Forage crops have gained interest as potential source of lignocellulosic feedstock to produce ethanol. More focus is needed on developing cropping systems to improve productivity. This study was conducted to identify the agronomic potential of six different cover crops on five different annual biomass crops. Results indicated that forage pea (*Pisum sativum* L. cv. Arvika) N uptake was 126 kg N ha⁻¹ and was able to fix approximately 60 kg of N ha⁻¹ in only 60 days in the fall. Results across locations indicated that forage sorghum (*Sorghum bicolor* L. Moench cv. FS-5), and sweet sorghum (*Sorghum bicolor* L. Moench cv. Theis) had the highest biomass yields among the forage crops with 17.8 Mg ha⁻¹ followed by sweet sorghum with 15.3 Mg ha⁻¹, respectively. Therefore forage sorghum and sweet sorghum can be considered as the most productive biomass sources, specially combined with a legume cover crop seeded the previous fall.

ACKNOWLEDGEMENTS

Firstly, my sincere appreciation goes to my advisor, Dr. Marisol Berti, from the bottom of my heart, for her continuous support, advice, and guidance provided throughout my research. Also, I would like to extend my heartfelt gratitude for her knowledge and expertise, and most of all the patience and encouragement she has given to me during the preparation of this thesis. I could have never hoped of having a more wonderful advisor than her.

My appreciation extends to all members of my graduate committee, Dr. Burton Johnson, Dr. Hans Kandel, and Dr. Kevin Sedivec for their assistance, advice, and time contribution rendered to this thesis.

I want to extend my deepest gratitude to the entire Forages and Biomass team; many thanks to Bob Nudell, our project technician for his help, expertise, and encouragement.

I would like to acknowledge my fellow graduate students; Osvaldo Teuber, Alfredo Aponte, and also our past graduate student Robynn Anfinrud; thank you for all the help, encouragement, and especially for your friendship and the laughter we shared. Special thanking goes to my dear friend Alfredo for all the help given to me in preparing this thesis.

Very special thanks to my parents back home for their love and encouragement which has helped me immensely in all aspects of my life.

My heartfelt gratitude and appreciation goes to my wonderful wife, Mihiri Mendis for all her loving and unwavering support and understanding that has made the fulfillment of this work possible.

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GENERAL INTRODUCTION

The increasing cost of energy and achieving energy independence has highlighted the need to develop alternative fuels from renewable sources. It has been estimated, that by the year 2025 world energy consumption will increase by 57%, forcing the expansion of alternative energy sources. There is a growing interest in using ethanol as a renewable transportation fuel. In the USA, it is proposed that biofuels should supply up to 30% of the US transportation fuel requirement by 2030 (Rooney et al., 2007). Currently, ethanol production is predominately from sugar and starch as this is a convenient and a technically proven option in most countries in the world. In 2009, the USA produced 38 billion L year⁻¹ (BLY) of ethanol, of which 95% came from maize (*Zea mays* L.) (Wu et al., 2010). However, use of maize as a source of fuel is limited, since it competes for food and feed, thus increasing global food prices.

As a result, lignocellulosic biomass is considered as a viable feedstock choice for ethanol production to meet the future demand. It has been estimated the USA would need approximately 1.2 billion Mg of dry biomass to achieve the goal by 2030 (Perlack et al., 2005). The need to generate a large and a sustainable supply of biomass profitably as feedstock will require identification and development of crops, as well as cropping systems, specifically for biofuel production. Several different crop species have been identified as dedicated bioenergy crops in different areas, based on factors such as climate, soil condition, cropping system, cost of production, and available conversion technology.

Using existing crop residues or forage crops as dedicated bioenergy crops, has been the first approach moving towards lignocellulosic feedstock production. Forage growers are already familiar with the crops agronomic management and already possess much of the machinery,

technology, and infrastructure to plant, harvest, store, and transport the biomass. For most of these crops, agronomic management as a bioenergy crop can be based on existing forage and grain production guidelines and recommendations until specific recommendations based on research data are available. Also, existing forage crops, especially annual crops, have the flexibility to be used as forage or as a bioenergy feedstock and also the land can be returned easily to other uses or into a rotation with other crops, giving growers a greater flexibility.

Unlike first generation biofuels, such as ethanol from maize starch, in which feedstock cost exceeds 50% of the total cost of production; the lignocellulosic-based ethanol industry will not be able to survive if the feedstock cost exceeds 40% of the production cost (Fales et al., 2007). The main reason behind this is that cellulose-to biofuel conversion technologies are not yet fully developed compared with that of maize-ethanol. Currently, the production and delivery cost of dedicated cellulosic feedstock exceeds this 40% margin (Kumara and Sokhansanj, 2007). Therefore, reducing feedstock production cost and supply costs are key issues for the development of this industry. Also, a cropping system designed for feedstock production should maintain or enhance soil fertility, productivity, and control soil erosion. Therefore, these issues need to be addressed, in developing a sustainable cropping system for dedicated energy-feedstock production.

Currently, crop fertilization is considered as a key component in any production system coupled with the high costs associated with fertilizers, efficient nutrient management could be critical in feedstock production. One option is the use of late-season cover crops to fix N, minimize nutrient leaching, and recycle nutrients from deeper soil layers back in to the root zone for the subsequent crop. The use of cover crops may reduce the needs for in-season application of fertilizer, thus significantly reducing the production costs of the feedstock. Further, cover

crops will provide other benefits to the cropping system such as soil quality improvement, erosion control, increase soil organic matter content, and weed suppression (Fisk et al., 2001).

This study, therefore, was carried out to examine the agronomic potential of six different cover crops on five different forage/energy crops, by analyzing the forage biomass yield and forage quality of both cover and biomass crops with the objective of identifying an efficient system to produce biomass as a source for biofuel.

The objectives of the study were to (1) Determine the biomass yield and forage quality of five annual forage species, grown after six different, leguminous and non-leguminous cover crop species (2) Determine the potential ethanol yield of the five annual forage crops to determine the most suitable source for the production of lignocellulosic ethanol.

LITERATURE REVIEW

Biomass as an Energy Source

Rapid increase in global population and widespread industrialization has resulted in rapid increase in energy consumption worldwide. Fossil fuels have been considered as the main source for energy production. It has been estimated, that by the year 2025, world energy consumption will increase by 57% compared with 2002 (Rooney et al., 2007). Increase in cost of energy due to high demand and limited oil and gas reserves, there is a need to develop alternative fuels from renewable sources. Bioenergy can be referred to as renewable energy derived from biological sources and co-products that can be used for heat, electricity, and fuel (Yuan et al., 2008). According to Abbasi and Abbasi (2010), biomass is the first ever fuel used by humans and until the mid-18th century the world's fuel economy depended on bioenergy derived from biomass. Biomass can be classified as *phytomass* (plant biomass) and *zoomass* (animal biomass), but animal biomass represent only a fraction in total biomass, thus the term 'biomass' generally represent plant derived biomass (Sanderson et al., 2006; Abbasi and Abbasi, 2010). Biomass resources can be categorized into waste material (agricultural residues, urban organic waste, and agricultural processing waste), forest products (long-rotation woody plantings, thinning material, and logging residues), and crops (traditional cereals, sugar-producing crops, dedicated-biomass or energy crops, and oilseed crops) (Demirbas, 2001; Yuan et al., 2008; Abbasi and Abbasi, 2010).

Biomass can be utilized directly by burning or indirectly by converting it into a biofuel. Different thermo-chemical processes could generate range of products which can be used as fuels. When biomass is heated in an oxygen-free environment, the resulting product is an organic liquid which can be refined or converted to produce liquid fuels. This process is known as pyrolysis (Digman et al., 2009). When biomass is heated under a low oxygen concentration, a

process known as gasification, a mixture of hydrogen and organic gases are produced (i.e. CO, CH₄, and CO₂). This product is called syngas. It can be used as a fuel or converted to liquid fuel forms by the Fisher-Tropsch process (Abbasi and Abbasi, 2010). Apart from the biomass produced, the main advantage is a cheap renewable energy sources that can provide a sustainable fuel supply with biomass-based energy that can provide a range of other potential benefits. Possible reduction of net CO₂ emissions has been associated with the use of biofuels (Farrell et al., 2006; Yuan et al., 2008). Biomass is considered neutral since the C in it comes from the atmosphere, not fossil sources. However, Searchinger et al. (2008) indicated that the conversion of existing grassland and forests into cropland for the production of biofuels actually may increase net greenhouse gas (GHG) emissions by releasing carbon sequestered in the soil. Furthermore, the biofuel production can improve rural employment and diversify rural economies (Hillring, 2002), lowering the dependency on foreign energy supplies as domestic biomass can substitute foreign imported oil.

Potential of Lignocellulosic Bioethanol in US

Ethanol and biodiesel can be used as a transportation fuel, and ethanol is a feedstock in many industrial processes (Yuan et al., 2008). In the USA, gasoline fuels which contain up to 10% ethanol by volume have been in use since 1980's (Cheng, 2001). Since then, the utilization of ethanol in the USA has rapidly increased. By 2009, the US ethanol consumption was over 38 BLY (RFA, 2009). Yuan et al. (2008) stated that ethanol can be considered the primary product when converting the present fossil-fuel-based energy to a biomass-based sustainable and environmentally friendly energy-production system. Starch and sugar-based ethanol represents the most convenient and technically advanced option for producing ethanol. Utilization of food crops such as sugarcane (*Saccharum officinale* L.), and maize as primary sources in ethanol production is

common in many parts of the world. According to Wu et al. (2010), 95% of the US ethanol was produced from maize and about 4% from sorghum grain (*Sorghum bicolor* L. Moench). In 2007, 15 to 20% of the US maize production was used to produce 22 BLY of ethanol. Even if the total maize production was destined for fuel production this would satisfy only 25% of the total transportation fuel needs in the USA (Rooney et al., 2007). Thus, a huge demand for suitable feedstock's to produce ethanol exists. Currently, the use of food crops, such as maize, and prime agriculture land for fuel production are facing increasing concern because they compete with food and feed, thereby increasing global food prices and having detrimental effects on food supply (Rooney et al., 2007; Abbasi and Abbasi, 2010). There is a growing interest in exploring more sustainable alternative sources than food crops to produce ethanol.

Currently, ethanol production from lignocellulosic biomass is a better choice than maize-ethanol. Lignocellulose is the term used to describe the three-dimensional polymeric composites produced by plant cells as structural material (Lee et al., 2007). Whereas, the starch-rich seed of maize represents a small fraction of the total biomass of the plant, lignocelluloses represent the most abundant renewable organic resource on the planet and fuels produced from it are considered as a second generation biofuels (Mabee and Saddler, 2010). The US Department of Energy (DOE) and US Department of Agriculture (USDA) estimated that the USA has the ability to generate approximately 1.2 billion Mg of dry lignocellulosic feedstock for biofuel production annually, which would provide one third of the annual US transportation fuel (Perlack et al., 2005).

Lignocellulose consists mainly of cellulose, hemicellulose, and lignin with the composition and proportions of these compounds varying among species (Abbasi and Abbasi, 2010; Lee et al., 2007). Cellulose is the largest component of plant biomass, followed by

hemicellulose and lignin. Cellulose consist of 30 to 50% of total dry matter and consists of glucose polymer linked by β -1,4 glycosidic bonds (Abbasi and Abbasi, 2010). Hydrolysis of cellulose results in individual glucose monomers. This process is also known as saccharification (Laureano-Perez et al., 2005). Hemicellulose is a short, highly branched polymer of five-carbon (C5) and six-carbon (C6) sugars. Hemicellulose content fluctuates between 20 and 40% of total dry matter. Hemicellulose is more readily hydrolyzed compared to cellulose because of its branched, amorphous nature. A major product of hemicellulose hydrolysis is the C5 sugar xylose (Lee et al., 2007). Lignin is a polyphenolic structural component of plants and is the largest non-carbohydrate fraction of lignocellulose. Lignin content can be as high as 25% of the dry matter depending on the feedstock (Abbasi and Abbasi, 2010). Unlike cellulose and hemicellulose, lignin cannot be utilized in fermentation processes (Lee et al., 2007). The main drawback in using lignocellulose compared to starch-based material for ethanol production is the complicated structure of the plant cell wall. In order to produce ethanol through fermentation, different components of the cell wall need to be separated before any chemical conversion can take place. The plant cell wall is designed to act as a barrier and resists any processes leading to its breakdown (Yuan et al., 2008; Abbasi and Abbasi, 2010). Therefore, lignocellulosic material requires two additional steps (pretreatment and enzymatic hydrolysis) prior to fermentation in order to produce ethanol (Yuan et al., 2008; Corredor et al., 2009). Corredor et al. (2009) further states that biofuel production using biomass will depend on five crucial factors; physical and chemical properties of the biomass, pretreatment methods, efficient microorganisms, process integration, and optimization of processing conditions. Pretreatment is critical because it releases cellulose from the lignocellulose matrix, hydrolyzes hemicellulose, modify chemically and/or removes lignin, and turns crystalline cellulose into an amorphous form (Laureano-Perez et al.,

2005). The microstructure and properties of cellulosic biomass have significant effects on bioconversion rate. Crystallinity, morphology, and surface area accessible for cellulose binding are considered the critical physical and structural factors which pretreatment and enzymatic hydrolysis depends on (Laureano-Perez et al., 2005). Hydrolysis, also known as saccharification, is the process where cellulose is broken down into single glucose molecules while hemicellulose is converted to a mixture of C5 and C6 sugars (Anex et al., 2007). During fermentation, these simple forms of sugars are converted to ethanol or other products through biological activities. When saccharification and fermentation are conducted in the same reactor it is known as simultaneous saccharification and fermentation (SSF). This dual reaction lowers the processing costs (Anex et al., 2007). When considering the importance of the lignin content, it can have both beneficial as well as detrimental effects on biofuel production depending on the type of biofuel and the generation process. Corredor et al. (2009) indicated that low-lignin biomass, thus less cross-linked lignin with other cell-wall compounds, is easier to hydrolyze and produce fermentable sugars. Lignin is an important by-product that can be burned or gasified to produce energy needed in ethanol conversion processes (Anex et al., 2007). Surplus energy produced by lignin can be easily converted into electricity to operate the facility or sold back to the grid.

The Biofuels Security Act of 2007 proposed a requirement of 37.9 Hm³ of renewable fuels by 2010, 113.6 Hm³ by 2020, and 227.1 Hm³ by 2030. Recently, a US congressional mandate requires that 79.5 Hm³ of renewable fuels has to come from feedstock other than maize (Ugarte et al., 2010). Such a mandate would necessitate the use of crop and forest residues as one potential source of biomass. But in order to produce large volumes of renewable fuel, there will be a need for large-scale cellulosic biomass feedstock production. Even though lignocellulosic biomass can be derived from almost all plant species known today, some plant species provide

greater yield and better quality of fuel than others (Abbasi and Abbasi, 2010). Sustainable energy from biomass programs should be based on those plant species. Another important consideration is the biomass production cost which must be sufficiently lower to make biofuels generation from lignocellulose profitable. Efficient harvesting, storage, transportation, and conversion processes must also be developed. Furthermore, a sustainable feedstock production system should maintain or enhance soil fertility, productivity, and control soil erosion. An ideal-bioenergy crop needs to be produced in areas not suited for food/feed grain production and thus, providing a way to minimize the food versus fuel utilization issues. Considering all these requirements, the choice of bioenergy crop will depend on different factors such as climate, soil condition, cropping system, cost of production, available processing technology, and etc. Currently, in the USA crops such as maize, switchgrass (*Panicum virgatum* L.), miscanthus (*Miscanthus x giganteus*), sugarcane, and the tree species poplar (*Populus trichocarpa* L.) have gathered the most attention (Rooney et al., 2007).

Annual Forages as Lignocellulosic Feedstock

In addition to perennial species, some of the most extensively studied crop species for lignocellulosic feedstock production include forages (Sanderson and Adler, 2008). Forage crops can be described as plants or parts of plants other than grain that are cultivated for grazing or harvested as hay or silage. Most studies of forages as energy crops have been conducted on perennial grass species. There has been a lack of interest in cultivating annual forages as lignocellulosic feedstocks. However, when considering the climate and short growing season common in places such as the northern Great Plains, annual forages have a distinct advantage over perennials. Most annual crops produce higher biomass yield per year because perennial crops channel their food reserves to underground structures to ensure their regrowth in the

subsequent year, rather than producing aerial biomass. Another advantage of using existing forage crops, as dedicated bioenergy crops, is that growers are already familiar with the agronomic management of those crops and possess much of the machinery, technology, and infrastructure to plant, harvest, store, and transport the crop. Agronomic management of these bioenergy crops can be based on existing forage and grain production guidelines and recommendations until specific recommendations based on research data are available (Rooney et al., 2007; Sanderson and Adler, 2008). Unlike perennial energy crops, annuals have the flexibility to be used as forage or as a bioenergy feedstock. The land can then be easily returned to other uses or back to food crops, if the grower desires. Some of these annual forages include sorghum, forage oat (*Avena sativa* L.), forage barley (*Hordeum vulgare* L.), and maize for silage.

Sorghum

Sorghum is an annual herbaceous grass evolved and domesticated in arid areas of northeastern Africa. Its cultivation spread to Asia and was introduced to the USA in the early 17th century (Rooney et al., 2007; Undersander et al., 2010). According to Undersander et al. (2010), sorghum was primarily grown as a source for producing sugar syrup, becoming popular with the demand for drought tolerant forage crops. Currently, Sub-Saharan Africa and India are considered to be the largest sorghum producers in the world where it provides food grain, feed grain, forage source, and as a combustion fuel (Rooney et al., 2007). In the USA, nearly 7.7 million ha of sorghum are grown annually (Rooney et al., 2007; Corredor et al., 2009) for various purposes. Sorghum grain is used to produce 4% of the total US ethanol production (Wortmann and Regassa, 2011). Currently, the southern Great Plains states such as Texas, Kansas, and Nebraska where conditions are not favorable for maize production due to low rainfall and higher temperatures are the main sorghum growing areas.

Shoemaker and Bransby (2011) describe sorghum as a universal crop due to its ability to grow in tropical, subtropical, temperate, and semi-arid areas of the world. There are many characteristics in sorghum which make it suitable for cultivation as a crop in both optimal crop production conditions as well as in marginal lands.

When considering the evolution and physiology of the plant, sorghum evolved in a semi-arid part of the world and possesses the C4 photosynthetic pathway, resulting in high and efficient productivity and water use efficiency. Compared with C3 pathway, crops with C4 pathway can produce 30% more dry matter per unit of water (Samson and Knopf, 1994), thus adapted to low rain fall areas. Furthermore, sorghum crop possess an extensive root system which can penetrate 1.5 to 2.5 m into the ground (Shoemaker and Bransby, 2011; Jordan and Miller, 1980). This results in higher water absorption efficiency during plant growth. Unlike most crops, sorghum has the ability to become dormant and remain for a substantial time period without wilting when in drought (Shoemaker and Bransby, 2011) and can resume normal growth after receiving adequate moisture. This response is due to the plants osmotic adjustment ability (Basnayake et al., 1995), efficiency in transpiration (Muchow et al., 1996), and presence of epicuticular wax (Buchanan et al., 2005; Undersander et al., 2010). In North Dakota, forage sorghum requires only 320 to 400 mm of water per season for optimal yield compared to maize which requires 460 to 560 mm of water per season (NDSU Ext. Serv., 1997), making sorghum a crop with higher water use efficiency.

Another advantage of sorghum as a bioenergy crop is its ability to be integrated into many production areas and cultivation systems (Rooney et al., 2007). Sorghum requires less nitrogen fertilizer than maize to obtain a high biomass yield (Lipinsky and Kresovich, 1980) and is more efficient in utilizing phosphorus and potassium than maize (Shoemaker and Bransby,

2011). Sorghum can tolerate a wide range of soil conditions, from heavy-clay to light-sand soils, with pH ranging from 5.0 to 8.5 (Smith and Frederiksen, 2000), and an optimal pH of 6.5 (McClure, 2012). Sorghum plants also have the ability to counterbalance different production situations. Habyarimana et al. (2004) reported that lower plant density results in higher leaf weight per plant, higher grain weight per panicle, and higher tillering ability, thus minimizing variations in grain yield.

Significant genetic improvements in sorghum have occurred in recent years (Rooney et al., 2007). Breeding programs have engaged in the development of high yielding grain cultivars, and forage cultivars with increased forage digestibility, improved tolerance to stresses such as diseases, and drought (Rooney et al., 2007). One such discovery is the brown midrib (BMR) character in sorghums. This character indicates lower lignin content in the plant. The BMR-sorghum cultivars have 25 to 50% less lignin (Dien et al., 2009), greatly improving forage digestibility and palatability (Bean and Marsalis, 2012). However, lower lignin content will increase the lodging potential of the plant (Bean and Marsalis, 2012). Unfortunately, many of the BMR sorghum types are associated with lower dry matter yield, plant height, and tillering ability compared with non-BMR types (Shoemaker and Bransby, 2011). As a result, there are new efforts to develop BMR-sorghum cultivars which are considerably shorter in height to minimize the lodging potential, while attaining a higher leaf to stem ratio to offset any yield loss.

Sorghum can be classified into four main groups based on the production characteristics, including grain sorghum, forage sorghum, high tonnage or biomass sorghum, and sweet sorghum (Shoemaker and Bransby, 2011).

Forage sorghum

Forage sorghum, which has similar plant characteristics to grain sorghum, tend to be taller, leafier, and late maturing compared with grain sorghum. Even though most forage sorghum cultivars accumulate sugar in the stalk, the main focus in forage sorghum breeding has been to obtain higher biomass yields for livestock production (Shoemaker and Bransby, 2011). Forage sorghum is used as single-cut hay and direct grazing, but primarily is used as silage (Undersander et al., 2010). Shoemaker and Bransby (2011) stated that in 2009, over 100,000 ha of forage sorghum were grown in the USA with an average yield of 13.7 Mg ha⁻¹. Forage sorghum is harvested at 1.2 m height for silage and soft dough stage if grain is produced. The quality components fluctuate between 520 to 650 g kg⁻¹ dry matter digestibility (DMD), 80 to 120 g kg⁻¹ crude protein (CP), 600 to 750 g kg⁻¹ neutral detergent fiber (NDF), and 340 to 400 g kg⁻¹ acid detergent fiber (ADF) (Undersander et al., 2010). Forage sorghum produces similar biomass yields per unit area compared with maize but sorghum contains less amount of grain in the biomass, thus has a lower digestibility and higher fiber content compared with maize silage (Undersander et al., 2010). Compared with maize, forage sorghum has several advantages to compensate its lower feed quality, such as, higher water use efficiency, ability to tolerate heat and drought stress, lower fertilizer needs, and lower seeding costs (Bean and Marsalis, 2012). Forage sorghum has been gaining importance as a potential feedstock for lignocellulosic ethanol production. Agronomic management of forage sorghum as a bioenergy crop can be based on existing forage and grain sorghum production guidelines and recommendations until specific recommendations for energy sorghum are available (Rooney et al., 2007).

According to previous research conducted in North Dakota, forage sorghum cultivars dry matter yield were over 30 Mg ha⁻¹ of above ground biomass with sufficient soil moisture, while

up to 15 Mg ha⁻¹ in dryland conditions (Berti et al., 2011). Maize will also yield 27 to 32 Mg ha⁻¹ with sufficient moisture (Bean and Marsalis, 2012). Unlike grain sorghum, forage sorghum does not go through a grain-filling stage due to the short growing season in North Dakota, resulting in more vegetative growth and less nutrient removal from the soil when compared with perennial energy crops. Forage sorghum biomass yield of 16 Mg ha⁻¹ and 28 Mg ha⁻¹ have been reported from Ames, IA and Bushland, TX, respectively (Rooney et al., 2007). According to Lee et al. (2007), forage sorghum biomass contains 340, 170, and 160 g kg⁻¹ of cellulose, hemicellulose, and lignin, respectively. This high cellulose and hemicellulose contents makes forage sorghum a good candidate for cellulosic feedstock.

Sweet sorghum

Sweet sorghum cultivars are characterized by the accumulation of high levels of soluble carbohydrates in the stalk that range from 150 to 230 g kg⁻¹ (Sarath et al., 2008). These carbohydrates are composed mainly of three types of sugars; sucrose (700 g kg⁻¹), glucose (200 g kg⁻¹), and fructose (100 g kg⁻¹) (Wortmann and Regassa, 2011). The content of the sugar types depends on cultivar and environmental conditions (Prasad et al., 2007).

Sweet sorghum was first introduced to the USA in the mid-19th century for the production of syrup; however production declined after the World War II due to low sugar prices (Wortmann and Regassa, 2011). In recent years, sweet sorghum has gained a new interest as a biofuel feedstock. One main reason for this interest is sweet sorghum's ability to provide different types of feedstock, which can be used to produce both first and second generation biofuels. Stalk sap can be fermented directly to produce ethanol and the remainder of the crop, bagasse, used as cellulosic feedstock to be fermented or for direct combustion (Saballos, 2008; Vermerris et al., 2011; Zegada-Lizarazu and Monti, 2012). Furthermore, Reddy et al. (2007)

stated that sweet sorghum is a beneficial biofuel crop for semiarid temperate climates since it requires less water and contains higher content of fermentable carbohydrates than maize stover.

Sweet sorghum biomass yields can vary significantly depending on cultivar, location (soil type, water availability, and temperature), and production practices (Vermerris et al., 2011). Putnam et al. (1991) studied 13 sweet sorghum cultivars in Minnesota. Sorghum yield components varied significantly among cultivars with total dry matter yield between 16.1 and 35.8 Mg ha⁻¹, and fermentable carbohydrates between 2.3 and 7.0 Mg ha⁻¹. Poornima et al. (2008) reported higher grain yield of 2483 kg ha⁻¹ and biomass yield of 37.2 Mg ha⁻¹ for early planting dates of sweet sorghum cultivars due to favorable environmental conditions during the early-growing season. The theoretical ethanol yield of sweet sorghum can be between 2100 to 5700 L ha⁻¹ in different US states with the highest amounts reported in the southern states (Zegada-Lizarazu and Monti, 2012).

Sweet sorghum is also a superior choice as a biofuel feedstock due to its drought tolerance and water use efficiency (Zegada-Lizarazu and Monti, 2012). Reddy et al. (2007) reported the water use efficiency of sweet sorghum was comparatively higher than maize. Geng et al. (1989) reported sweet sorghum can extract soil water to a 2.7 m depth in California and had a lower yield loss than maize under severe soil water deficit conditions.

Sweet sorghum is also known for its high N use efficiency. According to Geng et al. (1989) in a study conducted in California, sweet sorghum produced 23% more hexoses yield than maize utilizing only 36% of the N compared with maize. Sweet sorghum requires less than 50% of total N to produce similar ethanol yields compared with maize (Anderson et al., 1995). In Mississippi, stalk yield was 24% more with the application of 45 kg ha⁻¹ N compared with no N

application (Wortmann and Regassa, 2011). Nitrogen uptake by sweet sorghum was reported to be more gradual over a longer period of time than maize and grain sorghum, which had higher N uptake rates in a shorter time. This characteristic of sweet sorghum would allow for N take up later into the season, allowing more time for organic N to be mineralized in the soil and roots to obtain N from deep soil layers (Wortmann and Regassa, 2011). Bean et al (2008), reported sweet sorghum has the ability of removing 62% of total N.

Small grain cereal forages

Annual cereals are considered a main source of feed, especially in the Northern Prairies of USA, where 0.25 million ha were harvested in 1997 (Carr et al., 2004; Juskiw et al., 2000). Annual cereals include oat, barley, rye (*Secale cereale* L.), and wheat (*Triticum aestivum* L.). Silage, hay or green chop from cereals is mostly used as cattle feed in feedlots and dairies, and some for horse (*Equus ferus caballus*) and sheep (*Ovis aries*) feed (Juskiw et al., 2000). Nikkhah et al. (1995) found chemical composition and digestibility of cereal silage was similar to that of medium-quality alfalfa (*Medicago sativa* L.). The yield and feed quality of silage will depend on various factors such as the species, cultivar, agronomic practices and environmental conditions. As the cereal crop matures, increase in yield and decrease in quality can be observed (Acost et al., 1991), and the optimum stage to obtain maximum yield and quality is the soft-dough stage (Juskiw et al., 2000).

Forage barley

Barley is considered an ancient crop and currently is the fourth most important cereal in the world (Anderson et al., 2012). Barley has the C3 photosynthetic mechanism and is used as a human food, animal feed, and beer production. Forage barley has the highest yield and best forage quality among all annual cereal forages, used primarily as an energy and protein source

for beef cattle (Juskiw et al., 2000; Anderson et al., 2012). Compared with forage oat, forage barley contains higher crude protein and digestible dry matter at all stages of maturity (Harapiak et al., 2000). Barley cultivars are generally classified as two-row and six-row, malting and feed type, covered or hull-less, and floury or waxy-starch (Anderson et al., 2012). Barley is considered the most widely adapted cereal crop due its greater tolerance to drought, saline and alkali soil conditions, and frost (Deckard et al., 1994). In a four year study conducted in Lacombe, Canada, Juskiw et al. (2000) reported biomass yields between 10 to 16.5 Mg ha⁻¹. But in a study under dry land conditions in Dickinson, ND; Carr et al. (2004) obtained biomass yields of only 2.9 Mg ha⁻¹, confirming that biomass yields vary considerably among different environments and water availability.

Forage oat

Oat is another annual cereal crop considered to have desirable characteristics as forage because it is rich in crude protein and has the best balance of essential amino acids among all cereals (Deckard et al., 1994). The green plant contains high crude protein and soluble carbohydrates. In 1997, forage oat comprised nearly 80% of the area of cereals for hay (Juskiw et al., 2000). Production potential can be up to 7 Mg ha⁻¹ if adequate moisture is available and soil fertility high (Heyland and Werner, 2008). In dryland conditions, oat yield were considerably lower (3.8 Mg ha⁻¹) as reported by Carr et al. (2004). Oat is considered to be less-winter hardy than other main cereals such as barley or rye. Forage oat limits weed growth due to high biomass production.

The Need for Sustainable Cropping Systems

According to Sanderson et al. (2008), a main limitation for the increased use of forages for biomass is high cost relative to carbon fossil fuel sources such as coal and oil. However, the

added value of forages to the environment such as reduced soil erosion, carbon sequestration, and reduced production inputs can eventually make them a good source of energy. In order to achieve that situation input costs of forages needs to be substantially reduced.

Bioenergy crop production management has the objective of maximizing the biomass yield and lignocellulose content while minimizing the amounts of N and other minerals in the feedstock at harvest time. A sustainable feedstock production system must maintain or enhance soil fertility and productivity, control soil erosion, protect water quality, and protect or enhance local biodiversity. From the feedstock production point of view, one approach to address cost reduction and environment concerns is to develop a novel and innovative cropping systems.

Cropping systems including combinations of cool and warm-season annuals as dedicated bioenergy crops have been evaluated. Double-cropping cool-season cereals with warm-season annuals, such as maize and sorghum, were reported to be successful in the Midwestern USA, when the winter cereal crop was removed as forage early in the spring to allow early planting of maize for biomass (Sanderson and Adler, 2008). For systems which use annual grain crop residues as a biomass feedstock, use of living mulches would be beneficial as a soil cover after the removal of residue.

The inputs must be optimized in biomass cropping systems to reduce the cost of production of biofuels below what it would cost with fossil-fuel based fertilizers. When analyzing different cropping systems and management practices, all have a considerable requirement for N, P, and K fertilizer for optimum crop growth. Therefore, finding alternatives to substitute or minimize these fertilizer inputs is needed to reduce the cost of production. Nitrogen fertilizer is the most energy-intensive input, accounting for 41 to 64% of the energy inputs in annual crops, and 17 to 45% of inputs in perennial crops (Sanderson and Adler, 2008).

Additionally, N fertilizers may have negative environmental impacts such as leaching of nitrate to ground water and increased nitrous oxide emissions (Sanderson and Adler, 2008; Anex et al., 2007).

Due to their inherent symbiotic ability, leguminous cover crops can fix atmospheric N and reduce the use of N fertilizer and production costs. Nitrogen accumulation by leguminous cover crops can range from 60 to 200 kg N ha⁻¹ (Hargrove, 1986; Newman et al., 2007). The amount of N available from legumes depends on the species of legume grown, total biomass produced, and content of N in the plant tissue (Sullivan, 2003; Fageria, 2007).

Vetch species can be used as cover crops and green manure in organic farming and sustainable agriculture (Mihailovic et al., 2007). Biomass of annual legumes is considered to be easily degradable in comparison with other crops such as grasses and brassicas (Mihailovic et al., 2007), releasing higher amounts of nutrients for subsequent crops.

Non-leguminous cover crops such as brassica's are known for their potential to scavenge excess N and other nutrients from deep in the soil thus minimizing nitrate losses. Nutrients released from the biomass will be available for the crops the following season (Sundermeier, 2008). According to Sainju and Singh (1997), non-legumes can reduce nitrate leaching up to 94%. This could reduce ground water contamination and fertilizer inputs, which would improve the energy balance of bioenergy cropping systems.

Cover crops also provide a range of additional benefits to a cropping system. Accumulation of organic matter can increase the population of soil microbes and earthworms, which contribute to efficient nutrient cycling (Tiessen et al., 1994). Soil organic matter influences soil compactibility, friability, soil water holding capacity, regulating air and water infiltration, and soil permeability and erodibility (Carter, 2002). Deep rooted cover crops also

help alleviate soil compaction, which allows water infiltration (Williams and Weil, 2004; Newman et al., 2007; North Dakota State Univ. Ext. Serv., 2010). Cover crops have shown to be an effective means of suppressing weeds due to their rapid growth and leaf canopy closure (Fisk et al., 2001). Furthermore, members of the *Brassicaceae* family can form compounds toxic to plants, fungi, nematodes, and certain insects when incorporated into the soil (Haramoto and Gallandt, 2005). In addition, some leguminous and non-leguminous cover crops improve P availability and uptake (Sundermeier, 2008). This can be by mineralization of unavailable native phosphate, and unutilized-fertilizer derived phosphates (Cavigelli and Thien, 2003). This will enable the subsequent crop to utilize the readily available P for growth and development. Sharpley and Smith (1989) stated that cover crop residue can form H_2CO_3 during decomposition. Due to the acidification of the soil, P which is an immobile nutrient in neutral soils, will be more soluble and available for plants (Hargrove, 1986).

Studies have demonstrated the benefits of including cover crops in food crop rotations, but not in forage or energy crops. Therefore, there is a great importance to examine the effect of different cover crop species on the growth and production of different annual forage and energy crops.

CHAPTER 1. BIOMASS YIELD, NITROGEN UPTAKE, AND POTENTIAL ETHANOL YIELD OF ANNUAL BIOMASS CROPS FOLLOWING LATE-SEASON COVER CROPS

Abstract

In order to reduce the input costs and to improve the sustainability in lignocellulosic feedstock production, late-season cover crops can be included into existing crop rotations. The objectives of the study were to (1) Determine the biomass yield and quality of five annual biomass crops, grown after six different, leguminous and non-leguminous cover crop species (2) Determine the potential ethanol yield of the five annual crops to determine the most suitable source for the production of lignocellulose-derived ethanol. The experiment was conducted at two locations, Fargo and Prosper, ND, in 2010 and 2011. The experimental design was a randomized complete block with three replicates, in a split-plot arrangement where the cover crop treatment was the main plot and the forage crop was the sub-plot. Cover crop species were planted on 8 to 9 August in 2010 and 2011 following oat (*Avena sativa* L.). In the following spring, five biomass crops, maize (*Zea mays* L.), forage sorghum and sweet sorghum (*Sorghum bicolor* L.), and oat, and barley (*Hordeum vulgare* L.) were planted after the cover crops in each successive year, and their biomass yield and forage quality parameters were analyzed. Results across locations indicated that forage pea (*Pisum sativum* L. cv. Arvika) and forage radish (*Raphanus sativus* var. *niger* cv. Daikon), had the highest dry matter yield (3.3 Mg ha⁻¹). Further, forage pea N uptake was 126 kg N ha⁻¹ and forage turnip [hybrid forage brassica (forage turnip X forage rape)] N uptake was 65 kg N ha⁻¹. Accordingly, it can be estimated that forage pea fixed about 60 kg of N ha⁻¹ in only 60 days in the fall. Considering cover crop biomass yield and forage quality parameters, both legumes and non-legumes can be considered as good sources of forage, according to the needs of the livestock.

Results across locations indicated all forage crops had greater biomass, and ethanol yield, and N uptake when grown after a legume cover crop compared with a non-legume, in the previous year. However, forage sorghum had the highest biomass yields among the five forage crops, with 17.8 Mg ha⁻¹ followed by sweet sorghum with 15.3 Mg ha⁻¹, respectively. According to nitrogen uptake results, forage pea is the most suitable cover crop to provide additional N inputs for the subsequent crops. Forage sorghum and sweet sorghum can be considered as the most productive biomass sources, especially when combined with a legume cover crop.

Introduction

In order to meet the future demand for energy and to improve energy security in the USA, the Biofuels Security Act of 2007 proposed a requirement of 37.9 Hm³ of renewable fuels by 2010, 113.6 Hm³ by 2020, and 227.1 Hm³ by 2030. Also, a recent US congressional mandate requires that 79.5 Hm³ of renewable has to come from feedstock other than maize (Ugarte et al., 2010). Such a mandate would require the use of crop and forest residues as one potential source of biomass. However, in order to produce large volumes of renewable fuel for large-scale cellulosic biomass feedstock production is needed. To develop an economically viable feedstock production system, inputs must be optimized to reduce the cost of production of biofuels below the level of fossil-fuel based energy. Further, a sustainable feedstock production system should be able to maintain or enhance soil fertility, productivity and control soil erosion.

When analyzing different cropping systems and management practices, all have a considerably significant requirement for N, P, and K fertilizer for optimum crop growth. Therefore, finding alternatives to substitute or minimize these fertilizer inputs is needed, to reduce the cost of production and greenhouse gases emissions (GHG). Nitrogen fertilizer is the most energy intensive input and accounts for 41 to 64% of the energy inputs in annual crops and 17 to 45% of

inputs in perennials (Sanderson and Adler, 2008). According to Tonitto et al. (2005), humans are introducing 170 Tg of reactive N in to agrosystems annually, where synthetic fertilizers contribute 80 Tg of that amount. Further, synthesis of fertilizers also requires significant amounts of fossil fuel energy. However, only 45 to 55% of the reactive N entering agrosystems are recovered in crop biomass (Smil, 1999). The remaining N is lost through denitrification, leaching, and erosion. Denitrification is estimated to account for 26 to 60 Tg N, and leaching and erosion are attributed with 32 to 45 Tg N lost per year (Smil, 1999). Therefore it is important to reduce the N losses to reduce input costs and to mitigate negative environmental impacts associated with leaching of nitrate to ground and nitrous oxide emissions (Sanderson and Adler, 2008; Anex et al., 2007).

Cropping systems including combinations of cool and warm-season annuals as dedicated bioenergy crops have been evaluated. Double-cropping cool-season cereals with warm-season annuals, such as maize and sorghum, were reported to be successful in the Midwestern USA, when the winter cereal crop was removed as forage early in the spring to allow early planting of maize for biomass (Sanderson and Adler, 2008). For systems which use annual grain crop residues as a biomass feedstock, use of living mulches would be beneficial as a soil cover after the removal of residue.

The inputs must be optimized in biomass cropping systems to reduce the cost of production of biofuels below what it would cost with fossil-fuel based fertilizers. When analyzing different cropping systems and management practices, all have a considerable requirement for N, P, and K fertilizer for optimum crop growth. Therefore, finding alternatives to substitute or minimize these fertilizer inputs is needed to reduce the cost of production. Nitrogen fertilizer is the most energy-intensive input, accounting for 41 to 64% of the energy inputs in

annual crops, and 17 to 45% of inputs in perennial crops (Sanderson and Adler, 2008). Additionally, N fertilizers may have negative environmental impacts such as leaching of nitrate to ground water and increased nitrous oxide emissions (Sanderson and Adler, 2008; Anex et al., 2007).

Due to their inherent symbiotic ability, leguminous cover crops can fix atmospheric N and reduce the use of N fertilizer and production costs. Nitrogen accumulation by leguminous cover crops can range from 60 to 200 kg N ha⁻¹ (Hargrove, 1986; Newman et al., 2007). The amount of N available from legumes depends on the species of legume grown, total biomass produced, and content of N in the plant tissue (Sullivan, 2003; Fageria, 2007).

Vetch species can be used as cover crops and green manure in organic farming and sustainable agriculture (Mihailovic et al., 2007). Biomass of annual legumes is considered to be easily degradable in comparison with other crops such as grasses and brassicas (Mihailovic et al., 2007), releasing higher amounts of nutrients for subsequent crops.

Non-leguminous cover crops such as brassica's are known for their potential to scavenge excess N and other nutrients from deep in the soil thus minimizing nitrate losses. Nutrients released from the biomass will be available for the crops the following season (Sundermeier, 2008). According to Sainju and Singh (1997), non-legumes can reduce nitrate leaching up to 94%. This could reduce ground water contamination and fertilizer inputs, which would improve the energy balance of bioenergy cropping systems.

Cover crops also provide a range of additional benefits to a cropping system. Accumulation of organic matter can increase the population of soil microbes and earthworms, which contribute to efficient nutrient cycling (Tiessen et al., 1994). Soil organic matter influences soil compactibility, friability, soil water holding capacity, regulating air and water

infiltration, and soil permeability and erodibility (Carter, 2002). Deep-rooted cover crops also help alleviate soil compaction, which allows water infiltration (Williams and Weil, 2004; Newman et al., 2007; North Dakota State Univ. Ext. Serv., 2010). Cover crops have shown to be an effective means of suppressing weeds due to their rapid growth and leaf canopy closure (Fisk et al., 2001). Furthermore, members of the *Brassicaceae* family can form compounds toxic to plants, fungi, nematodes, and certain insects when incorporated into the soil (Haramoto and Gallandt, 2005). In addition, some leguminous and non-leguminous cover crops improve P availability and uptake (Sundermeier, 2008). This can be by mineralization of unavailable native phosphate, and unutilized-fertilizer derived phosphates (Cavigelli and Thien, 2003). This will enable the subsequent crop to utilize the readily available P for growth and development. Sharpley and Smith (1989) stated that cover crop residue can form H_2CO_3 during decomposition. Due to the acidification of the soil, P which is an immobile nutrient in neutral soils will be more soluble and available for plants (Hargrove, 1986).

Studies have demonstrated the benefits of including cover crops in food crop rotations (Holderbaum et al., 1990; Tonitto et al., 2005; Ort et al., 2013), but not in forage crops. Tonitto et al (2005), after conducting a meta-analysis of crop yields and N dynamics reported legume cover crops providing $\geq 110 \text{ kg N ha}^{-1}$ resulted in crop yields similar to that of conventional systems. Furthermore there was no differences between sorghum yields under conventional and legume N management systems. Ort et al (2013), reported cereal cover crops accumulated 18 to 29 kg N ha^{-1} and 174 to 339 kg C ha^{-1} , when seeded after silage corn. Therefore, there is a great importance to examine the effect of different cover crop species on the growth and production of different annual forage and energy crops.

This study, therefore, was carried out to examine the agronomic potential of six different cover crops on five different forage/energy crops, by analyzing the forage biomass yield and forage quality of both cover and biomass crops with the objective of identifying an efficient system to produce biomass as a source for biofuel.

The objectives of the study were to (1) Determine the biomass yield and forage quality of five annual forage species, grown after six different, leguminous and non-leguminous cover crop species (2) Determine the potential ethanol yield of the five annual forage crops to determine the most suitable source for the production of lignocellulosic ethanol.

Materials and Methods

Field establishment and experimental design

The experiment was conducted at two North Dakota State University research (NDSU) sites in Fargo, ND, (-96° 812' W, 46° 897' N, 274 m elevation) and Prosper, ND, (-97° 115 W, 47°002' N, elevation 284 m). The soil type in Fargo is a Fargo silty clay soil (fine, montmorillonitic, frigid, Vertic Haplaquoll, with a leached and degraded nitric horizon), while the soil in Prosper has a Kindred–Bearden silty clay loam (Perella: fine-silty, mixed, superactive Typic Endoaquoll; Bearden: fine-silty, mixed, superactive, frigid Aeric Calciaquoll) (Web Soil Survey, 2009). Daily temperature and rainfall amounts were recorded by the North Dakota Agriculture Weather Network (NDAWN) system at both sites (NDAWN, 2012). Soil samples were taken at both locations for analysis in the fall 2010 and 2011, and in spring 2001 and 2012 before crops were planted. The soil analysis for samples taken at 0- to 15-cm depth included pH, organic matter, P, and K (Franzen, 2010). The N-NO₃ analysis was done from the soil samples taken at 0- to 15-cm and 15- to 60-cm depth. Previous crop at both Prosper and Fargo in 2010 and 2011 were oat which was harvested for hay in late May in 2010 and early July in 2011.

The experimental design was a randomized complete block with three replicates, in a split-plot arrangement where the cover crop treatment was the main plot and the forage crop was the sub-plot. Experimental units were 9.1 m long and 1.5 m wide (Fig.1).

Cover crops treatments included; forage pea (*Pisum sativum* L. cv. Arvika), Austrian winter pea (*Pisum sativum* L. ssp. arvense (L.) Poir.), hairy vetch (*Vicia villosa* Roth.), forage turnip Pasja [hybrid forage brassica (*Brassica campestris* x *napus*)], purple top turnip (*Brassica rapa* var. *rapa*), forage radish (*Raphanus sativus* var. *niger* cv. Daikon), and a check with no cover crop.

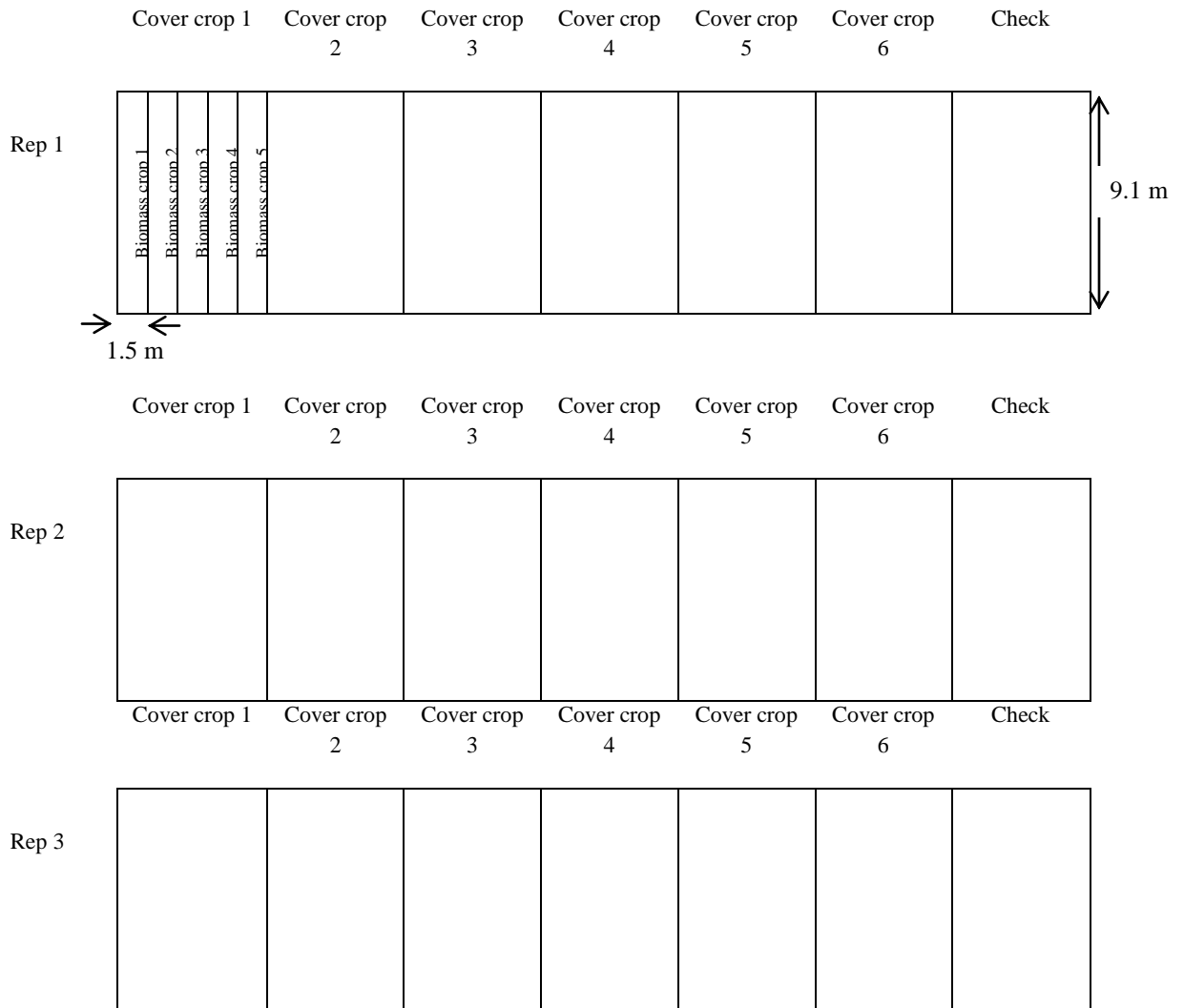


Fig. 1. Diagram of the experimental layout. The cover crop (forage pea, winter pea, hairy vetch, radish, turnip, and check) was the main plot and the biomass crop (sorghum, sweet sorghum, corn, barley, and oat) was the sub-plot. Biomass crops were planted the following spring on the same plot that had the cover crop. Experimental units were 9.1 m long and 1.5 m wide.

All cover crop seeds except forage pea were purchased from Agassiz Seed (West Fargo, ND), while forage pea seeds were obtained from Stock Seed Farms (Murdock, NE). Seeding rates were calculated based on the percentage of pure live seed (Table 1). All cover crops were seeded with an 8-cone plot planter with eight rows spaced 16 cm apart. The seeding depth for all the cover crops was 13 mm. Planting date for both Fargo and Prosper in 2010 was 9 August, and in 2011 the experiment was planted in 11 August at both locations.

Except for a pre-plant spraying of glyphosate (N-(phosphonomethyl) glycine) (1.4 kg a.i. ha⁻¹) in both locations and years, cover crops were grown without any post-emergence herbicides or fertilizers containing N, P, or K. Fertilization with sulfur at a rate of 20 kg of S ha⁻¹ was needed to correct symptoms of sulfur deficiency in Fargo, in 2010. The source of S was ZnSO₄ and each replicate was fertilized by hand-broadcasting. Soil tests indicated S levels of 3 to 14 mg kg⁻¹ before fertilizer application. Deficiency symptoms disappeared after the application.

In the spring of 2011 and 2012, five forage crops were planted directly (no-till) on top of residue of the cover crops planted on the previous fall. In the spring 2011, hairy vetch (the only cover crop that survived the previous winter) regrowth was terminated in both locations with glyphosate (N-(phosphonomethyl) glycine) (1.4 kg a.i. ha⁻¹), before seeding the spring forage crops, on 16 May 2011. In 2012, cover crop regrowth in the spring was not observed in any of the locations, thus glyphosate was not applied.

Forage crop treatments included; forage barley (cv. Hayes), forage oat (cv. Paul), maize (hybrid 56J86VT3), forage sorghum (cv. FS-5), and sweet sorghum (cv. Theis). Maize seeds were obtained from Peterson Farms Seeds (Harwood, ND), forage sorghum seeds from WinField Solutions (Grant, NE), sweet sorghum from Mississippi State University (Jackson, MS), and forage oat and forage barley seeds from NDSU seed stocks (Fargo, ND). Seeding rates were calculated based on the percentage of pure live seed (Table 1). In 2011, all five forage crops were planted using an 8-row-cone plot seeder on 26 May and 06 June in Prosper and Fargo, respectively. In 2012, forage barley, forage oat, and maize were planted on 01 May in both locations, while forage sorghum and sweet sorghum were planted on 15 May at both locations. Maize, forage sorghum, and sweet sorghum plot row spacing was 30 cm and had four rows per plot. Forage oat and barley row spacing was 15 cm and each plot had eight rows.

Table 1. Seed purity, germination percentage, and seeding rate of six different cover crops and five forage crops grown in Fargo and Prosper, ND, in 2010, 2011, and 2012.

Crop	Seed purity	Germination	Seeding rate
	(%)	(%)	(kg ha ⁻¹)
Forage pea	99	95	67
Austrian winter pea	99	90	67
Hairy vetch	99	85	41
Forage radish	99	90	14
Purple top turnip	99	94	4
Forage turnip	99	91	4
Forage oat	90	90	112
Forage barley	90	91	112
Maize	90	90	25
Sweet sorghum	90	95	7
Forage sorghum	90	98	23

All plots containing the forage crops were fertilized with N with a rate of 50 kg N ha⁻¹, broadcasted on top of the crop in both locations in in 01 July and 11 June, in 2011 and 2012, respectively. The source of N was urea [CO (NH₂)₂] and each plot was fertilized individually. Residual N volatilization in check plots was minimal since it rained soon after the application. Plots were hand weeded as needed both years, however in 2012 weed control included a post-plant spraying of bromoxynil + fluroxypyr [((4-amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy) acetic acid] (0.26 kg a.i. ha⁻¹) to control broad leaf weeds, followed by hand weeding as needed.

Plant sampling and analysis

Dependent variables evaluated for cover crops were biomass and forage quality at the end of the growing season. Shortly before the expected first hard frost, biomass samples were collected by hand clipping 1-m² from each cover crop, in each replicate on 14 October at both locations in 2010 and 2011. Radish and turnip enlarged hypocotyls, hereafter referred as roots, were not removed from the ground and only the portion of the root above ground was harvested

by separating it with a clipper. In the legume cover crops, all above ground biomass was harvested. Biomass samples were dried (70 °C for two days) to measure and record dry weight. Dried samples were then ground to 1-mm size particles with a mill.

For forage crops, plant height was measured for all the forage crops in all plots before harvesting. Both forage barley and forage oat were harvested at both locations when the grains were at the soft-dough stage on 10 August 2011 and 11 July 2012. The six-center rows of each plot were harvested with a flail forage harvester leaving a stubble height of 10 cm.

Maize, sweet sorghum, and forage sorghum were harvested on 17 September in both 2011 and 2012, at both locations. At the time of harvesting, maize seeds were in the process of forming the black-layer and forage sorghum and sweet sorghum grains were at the milk to soft-dough stage. Harvesting was done by hand-cutting all the plants in the two-center rows leaving a stubble height of approximately 10 cm. Whole plot biomass yields were calculated for each plot and a sample was also taken for moisture and quality analysis. Quality analysis was conducted only on samples of two replicates. Approximately, 10 to 12 days prior to the final harvesting, foliar samples were taken from all the plots to analyze foliar N-NO₃ content. In each plot, a 4th leaf from top of each of 10 plants in the two-center rows was taken. Harvested biomass and tissue samples were dried to measure and record dry weight and then ground to 1-mm size particles with a mill.

Total plant tissue N-NO₃ content for both cover crops and forage crops was determined by salicylic acid colorimetric method (Cataldo et al., 1975). The determination of plant tissue quality analysis was conducted to determine dry matter (DM) (AOAC Method 934.01). The total N content was measured with the Kjeldahl method, percentage of ash with AOAC Method 942.05), and crude protein (CP) with AOAC Method 2001.11. Quality analysis was conducted to

determine acid detergent fiber (ADF) (ANKOM, 2011. A200 Method 5), neutral detergent fiber (NDF) (ANKOM, 2011.A200 Method 6) and *in vitro* dry matter digestibility (IVDMD) (Tilley and Terry, 1963).

Nitrogen uptake of each cover crop was calculated multiplying the dry matter biomass yield by the total N content. The N uptake from N₂ fixation in the legume crops was estimated by subtracting the average N uptake of non-legume crops, although we are aware the N scavenging abilities of these crops can be different from each other.

The potential ethanol yield for all the forage crops was calculated with the following equation.

$$\text{L ha}^{-1} \text{ ethanol} = (0.098 \times 1000 \times \text{Mg ha}^{-1} \text{ biomass yield})/0.78943 \text{ (Dien et al., 2009)}$$

Growing degree days (GDD), or heat units, were calculated based on the threshold or base temperatures for each crop. The base temperature for the cool-season crops (oat and barley) was 0°C (Enz and Vasey, 2005) and for the warm-season crops (sorghum and corn) was 10°C (Geric et al., 2005). These values were used when calculating the monthly accumulated GGD (AGGD) and total AGGD for each environment.

Statistical analysis

Statistical analysis was conducted using standard procedures for a randomized complete-block design with a split-plot arrangement (Carmer et al., 1989). Biomass yield, potential ethanol yield, and forage quality data were analyzed using analysis of variance with the MIXED procedure of SAS (SAS Institute, 2012). Each location-year combination was considered an “environment” and a random effect, while both cover crops and forage crops were considered fixed effects in the analysis.

Analysis of variance was conducted within and across environments. If the error mean squares of 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.

Results and Discussion

Rainfall, temperature, GDD, and soil analysis

Total growing season rainfall varied between years and months, with greater total rainfall occurring from April to September in 2011 compared to 2012 at both Fargo and Prosper (Table 2). When comparing both growing seasons from April to October, the seasonal rainfall was greater in 2011 than 2012. . The 2012 growing season had well below-average rainfall. When considering the growing season for the cover crops (from August to October), both locations received above-average rainfall through the 2010 growing season, except Prosper for the month of October. This below-average rainfall was not expected to have a great impact on the plant growth since the crop was harvested 14 October. There was a marked difference in rainfall for the same time period in 2011. Although both locations received above average precipitation in May, June, and July, the months of August to October were very dry and received below-average rainfall; affecting the growth of the cover crops. This was especially observed in the Fargo location in 2011.

Slightly above-average temperatures were observed in all environments from June through August (Table 3). However, the monthly accumulated growing degree days (AGDD) were below average in all environments.

Table 2. Monthly rainfalls and deviation (Dev.) from the past 30 year average for Fargo and Prosper, ND, for the growing-seasons in 2010, 2011, and 2012 (NDAWN 2012).

Month	Fargo [†]						Prosper [†]					
	2010		2011		2012		2010		2011		2012	
	Rainfall	Dev.	Rainfall	Dev.	Rainfall	Dev.	Rainfall	Dev.	Rainfall	Dev.	Rainfall	Dev.
	-----mm-----											
April	36.8	2.3	45.8	11.3	28.9	-5.7	29.5	-7.4	45.0	8.1	30.0	-6.9
May	68.1	-3.3	109.7	38.3	43.4	-28.0	69.9	-7.6	80.0	2.5	46.2	-31.2
June	86.1	-13.0	100.9	1.8	56.9	-42.2	80.8	-19.6	131.6	31.2	67.3	-33.0
July	105.1	34.2	103.6	32.8	30.2	-40.7	103.4	15.5	150.1	62.2	16.3	-71.6
Aug.	67.7	2.7	72.5	7.5	20.9	-44.1	89.4	22.9	88.9	22.4	22.9	-43.7
Sept.	151.4	86.1	4.0	-61.3	1.3	-64.0	134.6	69.1	6.1	-59.4	14.7	-50.8
Oct.	60.6	6.0	20.6	-34.0	62.2	7.6	36.1	-25.7	9.4	-52.3	44.5	-17.3
Total	575.8		457.1		243.7		543.6		511.1		241.8	

[†]NDAWN, 2012.

Table 3. Growing-season average temperatures (Temp.) and growing degree days (GDD) for Fargo and Prosper, ND, in 2010, 2011 and 2012, for five different forage crops.

Month	2010			2011			2012		
	Temp.	GDD1 [†]	GDD2 [‡]	Temp.	GDD1	GDD2	Temp.	GDD1	GDD2
-----°C-----									
Fargo [†]									
April	10.9	51	326	6.1	10	182	9.0	34	269
May	14.5	140	451	12.9	112	399	16.0	189	496
June	19.1	274	574	19.2	275	575	21.0	331	631
July	22.3	382	692	23.7	424	734	24.9	463	773
Aug.	22.1	376	686	21.7	364	674	20.5	326	636
Sept.	13.6	109	409	15.4	172	463	15.3	172	458
Oct.	10.2	66	316	11.3	104	349	6.6	19	206
Total		1398	3452		1342	3375		1535	3470
Prosper [†]									
April	10.3	41	308	4.9	5	146	8.4	23	251
May	13.9	121	431	12.0	89	372	15.4	170	478
June	18.7	262	562	18.8	265	565	20.3	310	610
July	21.1	344	654	23.2	410	720	24.4	445	755
Aug.	21.0	342	652	21.0	342	652	20.1	313	623
Sept.	13.0	90	390	15.2	166	457	14.6	153	438
Oct.	9.3	51	289	10.8	64	336	5.8	11	179
Total		1251	3286		1366	3248		1426	3335

[†]GDD1 for maize, forage sorghum and sweet sorghum where base temperature =10 0C.

[‡]GDD2 for forage barley and forage oat where base temperature = 0 0C.

NDAWN, 2012.

In 2011, an early frost occurred on September 15, forcing an early harvest on September 16 for forage sorghum, sweet sorghum, and corn. Both the Fargo and Prosper sites were affected by the frost, so harvest was conducted immediately to avoid biomass loss. When taking account of the late planting dates in 2011 in Fargo and Prosper, (May 26 and June 7, respectively) a significant loss in growing days occurred for this year. However, cover crops were not affected by the frost and resumed growth until October.

The initial soil analysis for soil N, P, K, organic matter (OM), and pH was conducted for each environment before planting (Table 4). There was no variation in mineral content, soil pH, or organic matter content observed for each year or season.

Table 4. Initial soil analysis for six cover crops across a combined five forage crops prior to planting at the soil depth of 0 to 60 cm in Fargo and Prosper, ND, in 2010 to 2012.

Environment	pH	OM [†]	P	K	NO ₃ -N
		-----mg kg ⁻¹ -----			kg NO ₃ -N ha ⁻¹
Fargo					
Fall 2010	7.9	5.6	15.3	323.4	16.8
Spring 2011	7.0	6.2	17.7	317.8	14.7
Fall 2011	7.2	5.8	17.9	318.1	14.9
Spring 2012	7.1	6.4	18.1	315.7	14.8
Fall 2012	7.3	6.4	18.2	317.5	15.1
Prosper					
Fall 2010	7.0	3.9	19.9	293.0	12.2
Spring 2011	6.9	3.9	37.7	245.9	17.8
Fall 2011	7.1	3.9	33.4	241.3	18.0
Spring 2012	7.1	3.8	30.1	231.1	18.2
Fall 2012	7.0	3.9	31.2	230.1	18.2

[†]OM: Organic matter

The soil organic matter was greater in Fargo than in Prosper in both 2010 and 2011. The pH was between 7 and 8 in all six environments and considered adequate for all crops in this study (Mengel and Kirkby, 1982). Phosphorus levels were between 15 and 19 mg kg⁻¹ in Fargo and between 19 and 38 mg kg⁻¹ at Prosper, in both 2010 and 2011; thus, no additional P fertilization was required. No additional K fertilizer was required for any environment since initial soil K levels were greater than the minimum K fertility requirements. Average soil N levels range between 14 to 17 kg NO₃-N ha⁻¹ in both 2010 and 2011 in Fargo while, the range was 12 to 18 kg NO₃-N ha⁻¹ at Prosper for the time period. The cover crop main effects were different ($P \leq 0.05$) for biomass, plant nitrogen uptake, and for several forage quality parameters (Table 5).

Table 5. Analysis of variance and mean squares for six cover crop biomass yield, quality analysis, and nitrogen uptake across four environments, Fargo and Prosper in 2010 and 2011.

Sources of variation	df	Biomass yield	CP [†]	Ash	df	NDF	ADF	ADL	NDFD	IVDMD	Nitrogen uptake
Env	3	12.1	110.0	133.6	3	51.7	40.4	74.7	8.9	293.6	3818.4
Rep(env)	8	0.3	13.2	2.8	8	32.5	15.8	122.1	6.0	104.5	645.0
Cover crop	5	3.1*	425.8***	144.7***	5	422.0***	263.3***	208.4	328.3***	967.5**	3835.1*
Env x cover crop	15	0.9	17.3*	11.5**	15	34.0	22.6	131.1	7.9	145.0	1946.6
Error (a)	37	0.7	7.1	4.3	39	26.9	15.4	129.0	4.4	134.2	1418.9
CV, %		29.9	13.3	13.0		23.1	22.5	248.7	23.3	14.7	45.1

*, **, *** 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), neutral detergent fiber digestibility (NDFD), in vitro dry matter digestibility (IVDMD).

Cover crop biomass yield and nitrogen uptake

Biomass yields varied between 2.0 to 3.3 Mg ha⁻¹ for the six cover crops (Fig. 2). Results across four environments showed that forage pea, Forage radish, forage and purple top turnip had higher ($P \leq 0.05$) dry matter yield of 3.3 Mg ha⁻¹ than hairy vetch and Australian winter pea. In a study done in Aberystwyth, UK, Frasier et al, (2000) found forage pea would give a yield of 5.5 Mg ha⁻¹ after a 10 week growing period in the spring. It was predictable that hairy vetch and Austrian winter peas resulted in lower biomass than other cover crops since both of these have a prostrate growth compared to the upright growth of others. When taking account of the below-average rainfall and temperature during the fall growing season of 2010 and 2011, the results of both studies would have been similar. Leguminous cover crops had higher ($P \leq 0.05$) N uptake levels compared with non-leguminous cover crops (Fig. 3).

Forage pea had the highest N uptake level of 116 kg N ha⁻¹. There were differences ($P \leq 0.05$) among the leguminous cover crops on N uptake. Austrian winter pea and hairy vetch had similar N uptake levels of 86 kg N ha⁻¹ and lower ($P \leq 0.05$) than forage pea. There was no difference ($P > 0.05$) observed among the two turnips in N uptake; however, both turnips were lower ($P \leq 0.05$) than forage radish. Nitrogen uptake of forage radish was similar to that of Austrian winter pea and hairy vetch. Nitrogen uptake from N₂ fixation in the legume crops was estimated by subtracting the lowest N uptake of non-legume crops (Unkovich and Pate, 2000). Forage pea had 60 kg N ha⁻¹ from biological N-fixation during the 60-day growing season. This amount will be in the crop residue and would be released to the soil after mineralization. This N can be used to supplement N fertilizer used for the subsequent crops. Legume cover crops N credit is estimated to be approximately 72 kg N ha⁻¹ (Hargrove, 1986) Hairy vetch Nuptake is estimated in 88 kg N ha⁻¹. But only about 50 kg N ha⁻¹ are available through mineralization for

the subsequent crop (Sullivan and Andrews, 2012). Furthermore, non-legume cover crop N uptake range between 20 and 60 kg N ha⁻¹ and winter cover crops reduced nitrate leaching by 40-70% compared to bare fallow (Tonitto et al., 2006).

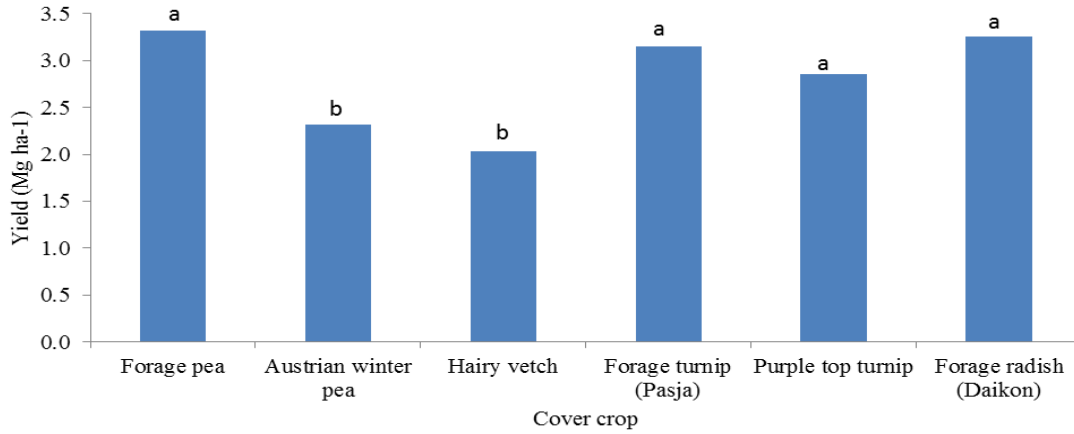


Fig. 2. Mean biomass yields of six different cover crops across four environments in Fargo and Prosper, ND, in 2010 and 2011. (Biomass yields with similar letters are not significantly different at $p = 0.05$)

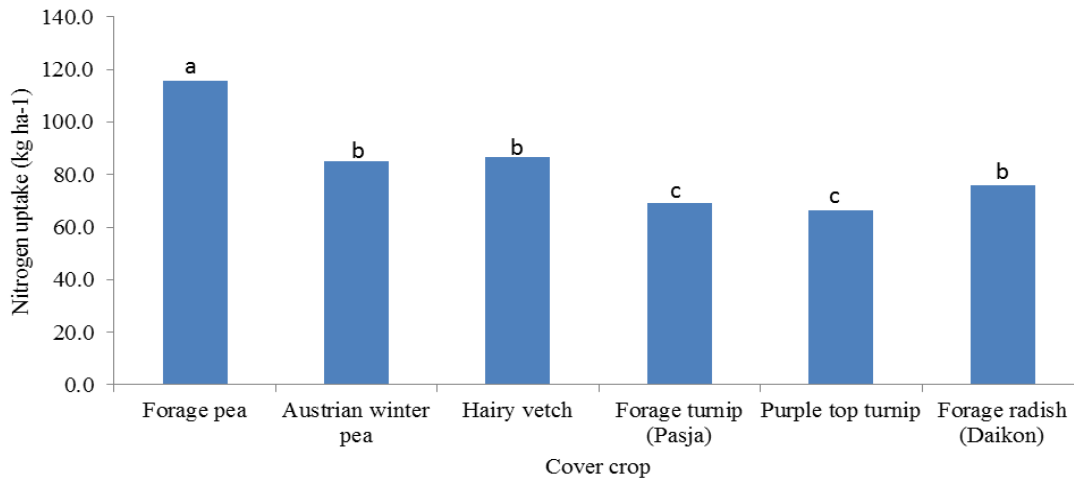


Fig. 3. Mean nitrogen uptake of six different cover crops across four environments in Fargo and Prosper, ND, in 2010 and 2011. (Nitrogen uptake levels with similar letters are not significantly different at $p = 0.05$)

Cover crop forage quality components

Forage quality parameters that included crude protein (CP), ash content, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), neutral detergent fiber digestibility (NDFD), and *in-vitro* dry matter digestibility (IVDMD) are presented in Table 6.

Table 6. Mean plant tissue nutritional quality parameters at harvest for six cover crop species averaged across four environments in Fargo and Prosper, ND, in 2010 and 2011.

Cover crop	CP [†]	NDF	ADF	Ash	NDFD	IVDMD
	-----g kg ⁻¹ -----					
Forage pea	239.6	319.8	252.8	106.6	779.6	657.4
Austrian winter pea	263.7	227.8	164.4	132.8	848.9	717.7
Hairy vetch	270.3	268.7	209.5	144.9	828.1	762.7
Forage turnip	136.1	173.4	134.9	188.0	900.9	856.6
Purple top turnip	161.3	166.8	129.2	183.5	906.4	860.7
Forage radish	147.5	191.9	158.9	196.0	910.1	874.8
LSD ($P=0.05$)	37.4	51.1	41.8	30.3	24.9	105.9

[†]Quality parameters: crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), ash content, neutral detergent fiber digestibility (NDFD), in vitro dry matter digestibility (IVDMD).

The CP content in legume cover crops was greater ($P \leq 0.05$) than non-legumes cover crop species. Hairy vetch had the highest CP content of 270.3 g kg⁻¹, although not different ($P >$

0.05) from the other legumes in the study. The CP content in non-legumes ranged from 136 and 147 g kg⁻¹, with none different ($P > 0.05$) among each other.

Forage pea had the highest NDF content at 319 g kg⁻¹, while purple top turnip the lowest content at 16.8 g kg⁻¹. The NDF content of forage pea was higher ($P \leq 0.05$) than the other legumes. Interestingly, NDF content of forage radish was greater ($P \leq 0.05$) than the other non-legumes, but similar to the content of Austrian winter pea.

The ADF content was highest in forage pea (252.8 g kg⁻¹), greater ($P \leq 0.05$) than hairy vetch (164.4 g kg⁻¹) and Austrian winter pea (209.5 g kg⁻¹). Non-leguminous cover crops did not differ ($P > 0.05$) and were similar ($P > 0.05$) to the ADF of hairy vetch.

The ash, NDFD, and IVDMD of non-leguminous cover crops were greater ($P \leq 0.05$) than those of legumes. Forage radish and forage pea had highest and lowest ash, NDFD, and IVDMD, respectively (Table 3.3). As expected, legumes and non-legumes crops have excellent forage quality indicated by the CP content and high digestibility by both NDFD and IVDMD. Forage brassicas are highly digestible and have high mineral nutrient content, which is reflected in the higher ash content.

Other researchers have reported similar quality components in these crops. According to a study conducted by Fraser et al. (2001), forage quality of forage pea for CP, ADF, and NDF values were 203, 304, and 382 g kg⁻¹, respectively. Buxton (1996) stated that NDF is an estimation of total cell wall components, which includes cellulose, hemicellulose, lignin, and silica. Therefore, NDF is negatively related to the intake potential of forages. High quality hay from legumes would normally have less than 390 g kg⁻¹ of NDF (Buxton, 1996). The ADF represents the cellulose and lignin components of the cell wall, so as ADF increases, forage digestibility decreases and hay quality decreases, ADF should be less than 290 g kg⁻¹ (Buxton, 1996). Brassica crops harvested

during the vegetative stage will result in CP, NDF, ADF, and IVDMD levels of 121, 395, 303, and 844 g kg⁻¹, respectively (Peiretti et al., 2005).

Forage crop biomass, ethanol yield, and plant tissue analysis

Combined analysis of variance across four environments for forage crop biomass, ethanol yield and several plant tissue composition parameters are presented in Table 7. The cover crop x forage crop interaction was significant ($P \leq 0.05$) for plant tissue nitrate content (Table 7 and Fig. 4.) and ash content (Fig. 5). In forage oat, average plant nitrate content was greater ($P \leq 0.05$) the year following forage pea than a non-legume brassica cover crops. Since forage pea had the highest N uptake among all cover crops, N availability after mineralization was expected to be higher after forage pea (Janzen and Kucey, 1988). However, having other leguminous cover crops such as Austrian winter pea or hairy vetch before forage oat was not different ($P > 0.05$) in tissue nitrate levels when compared with the brassica cover crops or the check. According to Smith (1960), tissue nitrate content in forage oat is highest during heading to milk stage and then declines gradually. Plant nitrate content in the other three crops did not differ within a same crop with different cover crop treatments.

Nitrate accumulation in annual forages depends on species, crop maturity, fertilization, and environmental conditions (Westcott et al., 1998). The critical factor influencing tissue nitrate content is nitrogen fertilization. In oat, the tissue nitrate content was reported highest at four-leaf stage, increasing gradually until a second peak during, heading- to early-milk stage, declining again thereafter (Smith, 1960). All the forage crops species in this study were fertilized with a 50 kg N ha⁻¹, but only oat following forage pea had an increase in tissue nitrate level. Therefore, it can be assumed that the additional nitrate in the oat crop came from the legume-fixed-N after mineralization of the pea residues.

Forage oat and barley nitrate content tend to increase under stress conditions such as drought (Cash et al., 2006). In 2012, crops at both experimental locations were under moisture stress due to lack of rainfall (Table 1) for most of the growing season. Also, nitrate accumulation increased immediately after a drought-ending rain as reported before (Cash et al., 2006 and Stoltenow and Lardy, 2008)

Nitrate accumulation above 1000 mg kg^{-1} can cause some deleterious effects on livestock (MacKown and Weik, 2004). In lower amounts, ingested N-NO_3 will enter the rumen, reduced to ammonia by rumen bacteria, and the ammonia excreted as urea (MacKown and Weik, 2004). However, higher amounts of N-NO_3 will by-pass this path and reduced to NO_2 . This NO_2 will enter the blood stream inhibiting the oxygen-transporting capacity of red blood cells (Westcott et al., 1998; MacKown and Weik, 2004), reducing animal performance.

Ash content in forage barley and oat was greater compared with corn, forage sorghum, and sweet sorghum. Ash content in forage crops was not different ($P > 0.05$) when grown after a legume or non-legume cover crop. Ash content in forage barley, from plots that had forage radish (146.6 g kg^{-1}) and purple top turnip (136.1 g kg^{-1}) cover crops in the previous year was significantly higher compared with the check (124.7 g kg^{-1}). However, ash content in forage and sweet sorghum that had forage radish previously, was the lowest.

Table 7. Analysis of variance and mean squares for five forage crops and six cover crops for biomass yield, tissue quality analysis, and nitrogen uptake across four environments (env), Fargo and Prosper, ND, from 2010 to 2012.

Sources of variation	df	Biomass yield	Ethanol yield	df	CP [†]	ADF	NDF	Ash	Nitrogen uptake	Plant tissue nitrate	Plant height
Env	3	3247.2	48451099.0	3	76.0	139.8	34.3	44.5	104045.0	235761.0	26070.0
Rep(env)	8	31.5	447596.0	6	2.8	8.3	16.3	0.5	2580.8	188510.0	1389.7
Cover crop	6	79.6**	1289006**	6	5.85***	2.84	6.12	2.41	8646.06**	24731	1867.5***
Env x cover crop	18	13.4	219204.0	18	0.8	8.5	21.1	1.9	1508.9	57870.0	210.0
Error (a)	48	23.7	381414.0	36	1.7	8.5	20.2	1.3	2611.9	29364.0	129.4
Forage crop	4	1588.7	24521723.0	4	377.5***	358.1*	157.90	530.5***	3131.1	801250	457340***
Env x forage crop	11	519.4***	8002762***	11	5.6***	93.6***	232.4***	15.3***	17807***	601705***	6367.53
Cover crop x forage crop	24	9.2	136107.0	24	0.6	5.9	14.1	2.05*	472.6	75078*	204.8
Env x cover crop x forage crop	66	14.3	218517.0	66	0.9	6.3	12.4	0.9	883.3	38862.0	518.2
Error (b)	205	12.6	190718.0	149	0.8	5.6	11.2	1.1	1188.2	39297.0	240.6
CV, %		29.9	29.7		14.5	7.6	6.1	12.5	33.1	21.6	27.9

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

[†]Quality parameters: crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF)

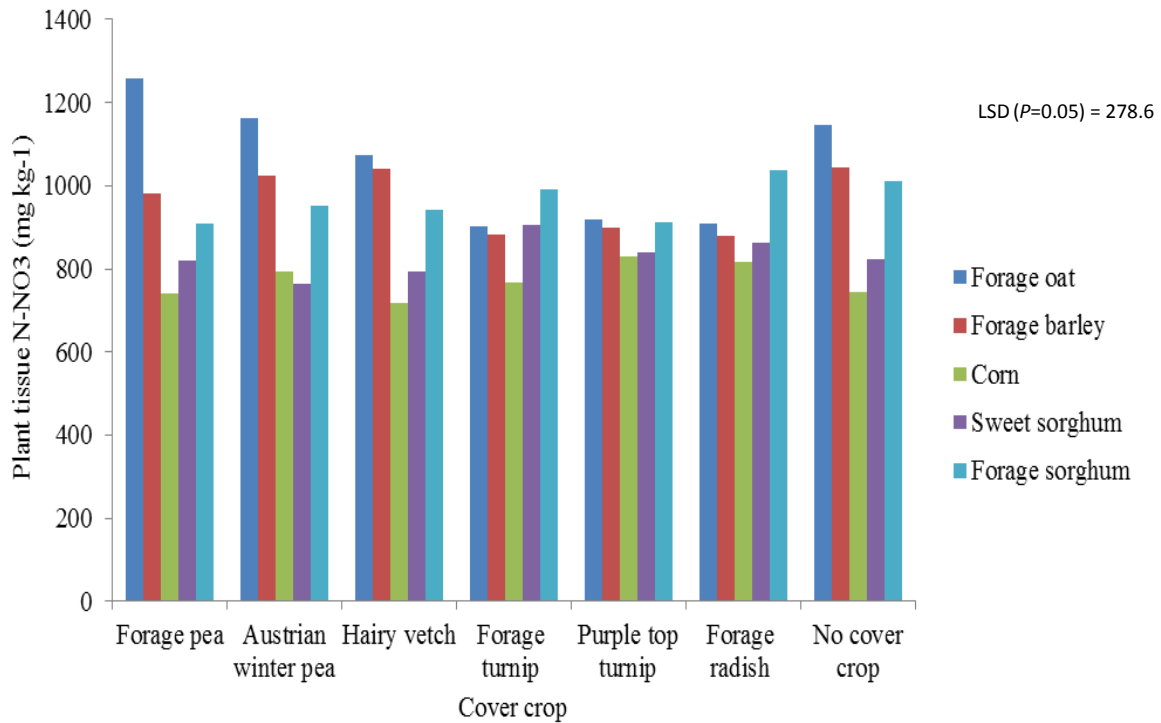


Fig. 4. Interaction among five forage crops and seven cover crops treatments in plant tissue nitrate averaged across four environments Fargo and Prosper, ND, from 2010 to 2012 (LSD value is to compare the means of the different cover crop treatments within forage oat; all other comparisons without a LSD value are NS).

Ash content is comprised of soil particles or dust on the plant, and also from several important plant nutrients such as silicon, potassium, calcium, sulfur, and chlorine (Bakker and Elbersen, 2005). According to Smith (1960) calcium, potassium, and tissue ash content in cereal crops decreases from early growth stages until early-dough stage, creating a higher ash content at soft-dough stage when the forage cereal crops are harvested. Compared with hand-harvested sorghum and corn, forage barley and forage oats harvested with a flail forage harvester could have more soil particles mixed in with the biomass. The harvest technique could contribute to the higher ash content in those two cereal crops. Forage sorghum and corn were also harvested after the hard frost, so most of the mineral nutrients probably had time to mobilize back to the soil before harvest.

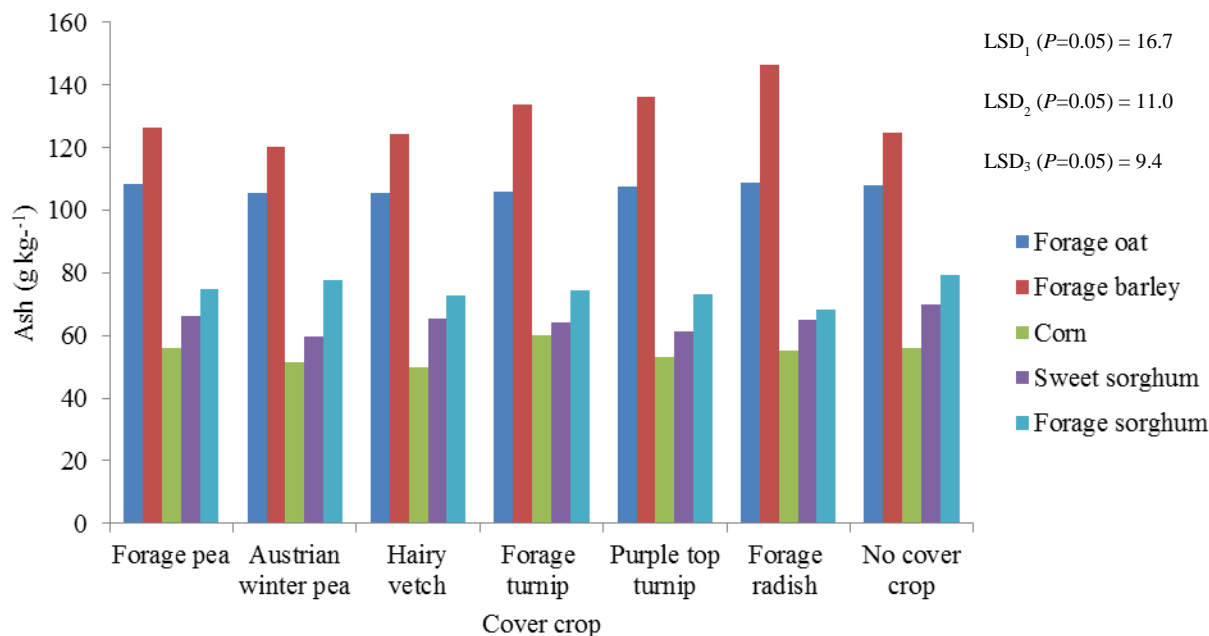


Fig. 5. Interaction among five forage crops and seven cover crops treatments in ash content averaged across four environments in Fargo and Prosper, ND, from 2010, to 2012 (LSD₁: To compare the means of different forage crops within a cover crop treatment; LSD₂: To compare the means of the different cover crop treatments within forage barley; LSD₃: To compare the means of the different cover crop treatments within corn, sweet sorghum and forage sorghum; All other comparisons without a LSD value are NS).

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 (Sanderson et al., 2006).

Furthermore, ash content can affect thermochemical conversion processes at higher temperatures. Melted ash can damage equipment and biomass with higher ash content may have lower energy value (Biomass Energy, 2012). Therefore, lower ash content in forage sorghum and sweet sorghum make these crops better candidates as biofuel feedstock than forage oat and barley. In addition to the above discussed interactions between forage crop and cover crop, forage crop main effects were significant at $P \leq 0.06$ for biomass yield (Fig. 6) and at $P \leq 0.05$ for CP, ADF, and plant height (Table 8).

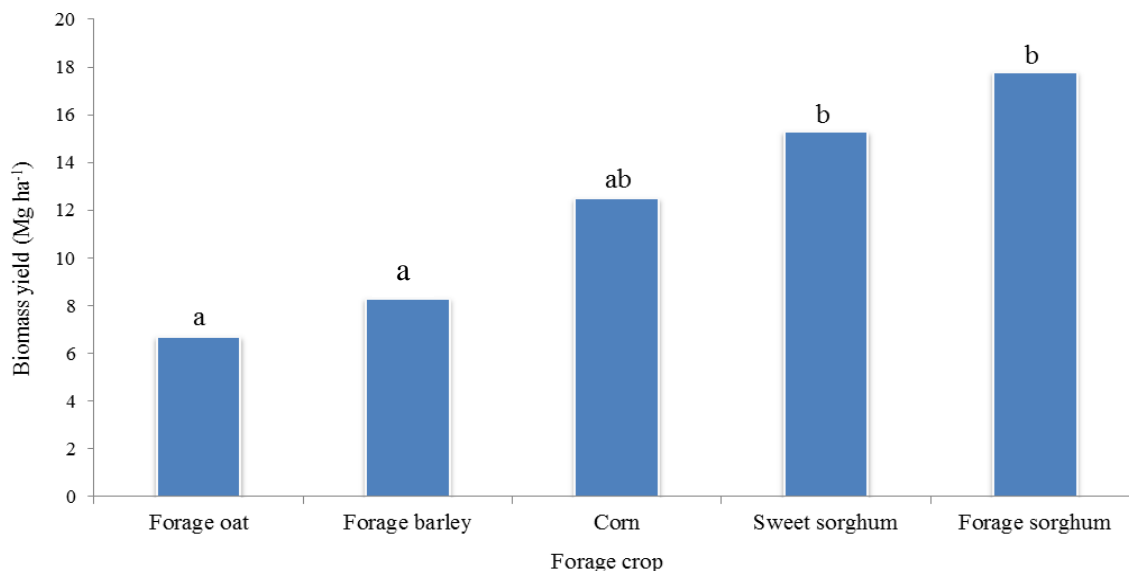


Fig. 6. Mean biomass yields of five different forage crops across four environments in Fargo and Prosper, ND, in 2011 and 2012. (Biomass yields with similar letters are not significantly different at $P=0.06$)

Table 8. Mean plant height, crude protein (CP), and acid detergent fiber (ADF) at harvest, for five forage crop species averaged across cover crops treatments and four environments in Fargo and Prosper, ND, from 2010 to 2012.

Forage crop	Plant height	CP	ADF
	m		g kg ⁻¹
Forage oat	0.9	89	324.5
Forage barley	0.7	88	314.1
Corn	2.1	48	269.7
Sweet sorghum	2.8	41	324.8
Forage sorghum	2.5	38	318.8
LSD ($P=0.05$)	0.3	09	39.2

Quality parameters: crude protein (CP), and acid detergent fiber (ADF) and ash content.

The mean plant biomass of forage sorghum and sweet sorghum were different ($P \leq 0.06$) than forage barley, and forage oat; but not different from corn ($P > 0.06$). Sorghum can reach biomass yields, between 16 and 28 Mg ha⁻¹, from Ames, IA to Bushland, TX, respectively (Rooney et al., 2007). Sorghum reaches physiological maturity at 2350 GDD (Klein and Shapiro,

2011), yet the sorghum crops were harvested prior to that stage (soft dough), since it does not normally produce seed at this northern latitude. Furthermore the lack of precipitation in 2012 growing season may also have affected plant growth, thus resulting in less biomass yields in corn and sorghum. However in this situation, sorghum has the ability to perform better than corn (NDSU Ext. Serv., 1997; Shoemaker and Bransby, 2011).

The mean plant height of forage sorghum and sweet sorghum were not difference ($P > 0.05$); however, both were taller ($P \leq 0.05$) than corn, forage barley, and forage oat. Mean plant height of forage sorghum and sweet sorghum were 2.5 and 2.8 m, respectively. Crude protein was greater ($P \leq 0.05$) in forage oat and forage barley. Lower ($P \leq 0.05$) CP content was observed in corn, sweet sorghum, and forage sorghum. Generally, it is considered that corn has a higher CP content compared with sorghum (Undersander et al., 2012), however, in our study the crops were harvested after the killing frost and similar in CP content ($P > 0.05$). Bean and Marsalis (2012) reported CP values for forage sorghum between 46 and 93 g kg⁻¹, which range is slightly greater than the range in protein observed in this study (38 and 89 g kg⁻¹). Sorghum CP content was 74 g kg⁻¹ in a study conducted in New Mexico, harvested at mid-dough stage (Bean and Marsalis, 2012). The CP content of corn was reported at 50 g kg⁻¹ by Lee et al. (2007). Carr et al. (2004) reported CP content of 90 and 61 g kg⁻¹ in forage barley and forage oat; respectively, in a study in Western North Dakota.

Crude protein content decreases in mature vegetation as hydrolyzing enzymes break down the higher molecular weight compounds to lower molecular weight components such as amino acids, amines, and amides (Mengel and Kirkby, 1982). These nitrogen containing compounds are transported through the xylem as NO₃, NH₄ and amino acids into the root system, decreasing the CP content in the aboveground biomass as the crop senesces

The mean ADF content was the lowest in corn and highest in sweet sorghum. However, there were no differences ($P > 0.05$) in ADF content between the other four crops. Bean and Marsalis (2012) reported values of ADF of around 320 g kg^{-1} in forage sorghum.

The component NDF is an estimation of total cell wall components; which are mainly cellulose, hemicellulose, lignin, and silica. Acid detergent fiber content indicates the cellulose and lignin components of the cell wall while the ADL content indicates the lignin component. Therefore, when ADF increases forage digestibility decreases. Also, higher lignin content in biomass decreases the digestibility of cellulose and hemicellulose. Furthermore, cover crop main effects were different ($P \leq 0.05$) for biomass yield, ethanol yield, CP, nitrogen uptake, and plant height (Table 9).

Forage crops planted on plots with forage pea and Austrian winter pea in the fall had a higher ($P \leq 0.05$) average plant height compared with forage crops grown on check plots. However, there was no difference ($P > 0.05$) between forage crops grown on plots with leguminous and non-leguminous cover crops.

Forage crops planted on leguminous cover crops had a higher ($P \leq 0.05$) biomass yields compared with non-leguminous cover crops and the check. Forage crops following Austrian winter pea cover crop had the highest ($P \leq 0.05$) average forage crop biomass yield of 14 Mg ha^{-1} . There was no difference ($P > 0.05$) within leguminous and non-leguminous cover crops for average forage crop biomass yields. Plant growth depends on the amount of N available from the soil.

Table 9. Mean of biomass yield, ethanol yield, nitrogen uptake, plant tissue nitrate content, crude protein content, and plant height of five forage crop species (averaged across), 10 days before the final harvest, averaged across four environments in Fargo and Prosper, ND, from 2010 to 2012.

Cover crop treatment	Biomass yield	Ethanol yield	Nitrogen uptake	CP	Plant height
	Mg ha ⁻¹	m ³ ha ⁻¹	kg ha ⁻¹	g kg ⁻¹	m
Forage pea	12.77	1.58	118.79	65.60	1.87
Austrian winter pea	13.97	1.73	116.06	61.78	1.87
Hairy vetch	12.99	1.61	107.85	62.71	1.81
Forage turnip	11.09	1.36	87.12	57.80	1.77
Purple top turnip	11.67	1.44	92.07	59.66	1.76
Forage radish	11.45	1.42	87.29	56.09	1.77
No cover crop	10.88	1.34	91.20	63.02	1.67
LSD ($P=0.05$)	1.46	0.19	16.70	4.00	0.06

(CP: crude protein)

It can be assumed that N from leguminous cover crop residue may have been mineralized and supplied the additional N for the subsequent forage crops. In a study conducted in Georgia, USA, on winter legumes and no-till sorghum production, Hargrove (1986), reported crimson clove (*Trifolium incarnatum* L.) added 120 kg N ha⁻¹ for the subsequent crop. Also, the study stated legumes were able to replace 72 kg N ha⁻¹, N fertilizer on average. Sweeny and Moyer (1994) reported a sorghum yield increase between 79 to 131% after a legume cover crop compared to continuous sorghum cultivation on a prairie soil of the eastern Great Plains.

Average potential cellulosic ethanol yields for forages after leguminous cover crop increased ($P \leq 0.05$) compared with forage crops grown after non-leguminous cover crops and check plots. Forage crops following Austrian winter pea had the highest potential ethanol yield of 1.7 m³ ha⁻¹, while the lowest ethanol yield of 1.3 m³ ha⁻¹ came from check plots. There was no difference ($P > 0.05$) in ethanol yields between the check and non-leguminous cover crops.

The CP content was highest ($P \leq 0.05$; 65.6 g kg⁻¹) after forage pea while the lowest ($P \leq 0.05$; 57.8 g kg⁻¹) after forage turnip when averaged across forage crops. Leguminous cover crop

treatments had a higher ($P \leq 0.05$) CP content compared with non-leguminous cover crops, although not different plant N uptake ($P > 0.05$). NO_3 or NH_4 are converted to organic- N forms in the plant and use for proteins and nucleic acids synthesis (Mengel and Kirkby, 1982). Therefore, greater content of CP will result from higher N levels in the soil. Most likely greater N availability for the crops will result from a longer growing season of these forage crops, thus providing adequate time for the mineralization of cover crop residue to occur.

Average N uptake of forage crops was greater ($P \leq 0.05$) when the forage crop followed a leguminous cover crop. The highest N uptake in forage crops was when crops followed forage pea ($118.8 \text{ kg N ha}^{-1}$) while crops following forage turnip had the lowest N uptake ($87.1 \text{ kg N ha}^{-1}$). A study conducted in Maryland, Holderbaum et al. (1990) tested the effect of several legume cover crops on corn N uptake and concluded peas and hairy vetch supplied over 30% or more, from the N taken up by the subsequent corn. Furthermore, Tonitto et al. (2006), in a meta-analysis of many previous publications, determined that 50% of the studies reported N uptake by a legume cover crop between 50 to 150 kg N ha^{-1} . According to that study crop yields showed no difference when grown under a conventional fertilizer recommendations or legume based N supply which provided $\geq 110 \text{ kg N ha}^{-1}$.

Conclusions

When considering the short growing season (approximately 60 days) of the cover crops, forage pea, forage radish, and turnips (forage and purple top) can be considered as good late-fall forage sources for western North Dakota and eastern Minnesota. These crops had biomass yields of 2.9 to 3.3 Mg ha⁻¹.

Legume cover crops, especially forage pea, were able to fix 60 kg N ha⁻¹ during the short growing season. Based on the forage quality parameters tested in this study, both legumes and non-legumes cover plant species studied in this trial were considered good quality forage resources.

Legume cover crops will provide high protein forage with lower fiber components. The non-leguminous brassica cover crops were considered high digestible and high mineral content forage species.

Forage sorghum and sweet sorghum had the highest biomass yields among the five forage crops evaluated and will be the most suitable feedstock for biomass. Plant nitrate contents of forage crops, except forage oat did not show a difference between cover crop treatments. Increases in ash content in forage barley and forage oat could be the result of excess soil during harvest.

All forage crops showed higher biomass, ethanol yield, and N uptake when grown after a legume cover crop compared with a non-legume from the previous year. The highest CP content was the forage crops that had a leguminous cover crop in the previous year. This can be attributed to the timely mineralization of legume crop residue which provided additional N input to the soil.

Therefore it can be recommended that biomass feedstock such as forage sorghum and sweet sorghum will be the best choice for the region to produce cellulosic ethanol. Further utilizing leguminous cover crops in the fall will provide sufficient amounts of N in the following year for the energy and forage crops, thus reducing the external N input.

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APPENDIX. INDIVIDUAL AND COMBINED ANALYSIS FOR THE EXPERIMENT WITH
TWO-FACTOR TREATMENT DESIGN CONDUCTED IN A SPLIT-PLOT DESIGN.

Source of Variation †	df	Mean square		F-ratio
		Observed	Expected ††	
Individual split-plot				
Replications	2	M1	$\sigma^2_\epsilon + b\sigma^2_r + ab\sigma^2_R$	
A	8	M2	$\sigma^2_\epsilon + b\sigma^2_r + br\Phi_A$	M ₂ /M ₃
Error (a)	18	M3	$\sigma^2_\epsilon + b\sigma^2_r$	M ₅ /M ₆
B	4	M4	$\sigma^2_\epsilon + ra\sigma^2_B + ar\Phi_B$	M ₄ /M ₆
A x B	32	M5	$\sigma^2_\epsilon + r\Phi_{AB}$	M ₅ /M ₆
Error (b)	72	M6	σ^2_ϵ	
Total	134			
Combined analysis				
Environment	3	M1		
Replications (Environment)	8	M2		
A	8	M3	$\sigma^2_\epsilon + b\sigma^2 + br\sigma^2_{AE} + ber\Phi_A$	M ₃ /M ₄
A X Environment	24	M4	$\sigma^2_\epsilon + b\sigma^2 + br\sigma^2_{AE}$	M ₄ /M ₅
Pooled error (a)	64	M5	$\sigma^2_\epsilon + b\sigma^2$	M ₅ /M ₁₀
B	4	M6	$\sigma^2_\epsilon + ar\sigma^2_{BE} + aer\Phi_B$	M ₆ /M ₇
B X Environment	12	M7	$\sigma^2_\epsilon + ar\sigma^2_{BE}$	M ₇ /M ₁₀
A x B	32	M8	$\sigma^2_\epsilon + r\sigma^2_{ABE} + er\Phi_{AB}$	M ₈ /M ₉
A X B X Environment	96	M9	$\sigma^2_\epsilon + r\sigma^2_{ABE}$	M ₉ /M ₁₀
Pooled error (b)	288	M10	σ^2_ϵ	
Total	539			

†The letters a, b, e, and r refers to the number of levels of factors A (cover crop) and B (forage crop), the number of environments (e), and the number of replicates per experiment (r), respectively.

$$\dagger\dagger \Phi_A = \sum A_i^2 / (a-1)$$

$$\Phi_B = \sum B_j^2 / (b-1)$$

$$\Phi_{AB} = \sum \sum (AB)_{ij}^2 / (a-1)(b-1)$$