Biomass production, composition, and ethanol potential of switchgrass grown on reclaimed surface mines in West Virginia

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ABSTRACT

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Growing crops for biofuel production on agricultural land has caused a debate between whether we should grow corn grain on productive, agricultural lands to feed a growing human population or to fuel our vehicles. This has increased interest in growing cellulosic biofuel feedstocks on marginal lands. Switchgrass (*Panicum virgatum* L.), a warm-season perennial grass, has been shown to be a viable bioenergy crop because it produces high yields on marginal lands under low water and nutrient conditions. In previous studies, switchgrass yields on marginal croplands varied from 5.0 to 10.0 Mg ha^{-1} annually. West Virginia contains immense acreages of reclaimed surface mine lands and could offer enough area for the production of switchgrass as a feedstock for a biofuel industry.

The first study was established in 2008 to determine switchgrass yields of different cultivars on mine sites in West Virginia. Three varieties of switchgrass were tested on two mine sites, Hampshire and Hobet. The Hampshire site, which was reclaimed in the early 1990's using top soil and treated municipal sludge, consistently had the highest yield of the two sites with a sixth-year yield of 8.4 Mg Dry Matter (DM) ha^{-1} averaged across varieties. 'Cave-in-Rock' variety produced 13.0 Mg ha^{-1} of biomass which was more than the other two varieties, 'Shawnee' and 'Carthage', at 7.5 and 4.2 Mg ha^{-1}, respectively. The other site, Hobet, was prepared using crushed, unweathered sandstone in 2008. Yields of switchgrass were 1.0 Mg ha^{-1} for the sixth year of production, with Cave-in-rock producing the most biomass at 1.1 Mg ha^{-1}. The type and quality of soil and the variety of switchgrass selected for seeding should be considered when the goal is chiefly high yields of switchgrass for biofuel production.

The second study was conducted on Black Castle and Coal Mac mines in southern West Virginia. These two sites were reclaimed with a layer of topsoil over gray overburden. Sites were seeded with Cave-in-Rock on the newly reclaimed land in 2011. Fertilizer was applied at rates of 0, 33.6, and 67 kg N ha^{-1}. No fertilizer treatment yielded 0.32 Mg ha^{-1} while the low fertilizer treatments produced significantly higher yields averaging 1.4 Mg ha^{-1}. Doubling the fertilizer rate to 67 kg N ha^{-1} almost doubled the yield to 1.9 Mg ha^{-1}. Therefore, fertilizing with 67 kg N ha^{-1} may be best for switchgrass grown on reclaimed surface mines if high biomass production is the goal.

The objective of the third study was to determine if cultivars and samples from fertilizer treatments differed in composition and theoretical ethanol yield. Compositional analysis was done using near infrared reflectance spectroscopy. Carbohydrate contents were used to determine theoretical ethanol yield (L Mg^{-1}) and multiplied by DM yield to calculate the theoretical ethanol production (L ha^{-1}). It was determined that cultivars did not differ in theoretical ethanol yield

with averages ranging from 364 to 438 L Mg^{-1}. Theoretical ethanol production from Cave-in-Rock was significantly higher ranging from 6,092 to 7,348 L ha^{-1} due to its high biomass production. Fertilizer treatments did not greatly effect composition of switchgrass, but since it did improve yield this was reflected in greater ethanol production for fertilized treatments. Similar in other studies, biomass composition did not affect ethanol production as much as yield did. It should be pointed out, though, that the calculations used did not take into account constraints in conversion such as the recalcitrance of lignin. Based on the information presented here, high biomass should be the goal for switchgrass grown for biofuel production. With proper soil substrate and fertilizer regime, switchgrass grown on reclaimed surface mines may have high enough yield and quality to support ethanol production in the future.

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Chapter 1: Introduction

1.1: Background

Currently, most of the gasoline sold to power vehicles in the United States is about 10% ethanol (E10), which is the maximum allowable percentage of ethanol for non-flex fuel cars made before 2001 (U.S. EIA, 2012). In 2011, a historic high of 48.8 billion liters (L) of ethanol were consumed in the United States (U.S. EIA, 2012). Increased consumption of biofuels since 2001 has been driven by the desire to reduce and mitigate the environmental impacts of fossil fuels as energy sources and to increase energy security.

Many feel that the production of biofuels from food crops such as corn grain (*Zea mays L.*) is morally wrong since there are 870 million underfed and malnourished people in many parts the world (FAO, 2012). Nearly all the ethanol in the US is produced from corn grain, making it a 1st-generation biofuel (U.S. EIA, 2012). In 2010, 40% of the corn grain crop in the United States was used for ethanol and distillers grains (U.S. EIA, 2012). As human population and the demand for biofuel increase, the use of corn grain for ethanol and hence the potential shortage of food will be exacerbated. Many people believe our food crops and productive agricultural land should not be used for anything other than feeding the human population.

Life Cycle Assessments (LCAs) have shown that the production and use of biofuels emits less greenhouse gases (GHGs) than the production and use of petroleum-based gasoline (Blottnitz and Curran, 2007; Kim and Dale, 2005). However, Pimentel and Patzek (2005) argue that LCAs may not account for carbon emissions that occur when farmers convert forests and grasslands to produce food-based biofuels. They assert that the true amount of carbon emissions from the production and use of biofuels is larger. In fact, the Pimentel and Patzek study shows that ethanol produced from corn grain actually has a net negative energy return of 29%.

Pimentel and Patzek (2005) also accuse LCAs of not considering emissions of N_2O that come from nitrogen (N) fertilizer that are applied on biofuel crop fields and the decomposition of organic matter in the soil. Approximately 1.3% of N in synthetic fertilizer is emitted as N_2O (Cherubini and Jungmeier, 2010). This is cause for concern because N_2O has 298 times greater global warming potential than CO_2 (Cherubini and Jungmeier, 2010), making it a potent GHG that must be considered in all LCAs.

The use and production of biofuels may invite more negative environmental impacts than fossil fuels (Farrell et al. 2006). Additional negative environmental impacts that may result from biofuel production and use are eutrophication, acidification, and ozone depletion. However, these environmental impacts are thought to decrease with the use of cellulosic biofeedstocks rather than corn grain for the production of biofuel (Cherubini and Jungmeier; 2010; Tilman et al., 2006).

The desire to have 2nd-generation biofuels, produced from non-food ligno-cellulosic biofeedstock, as an alternative to 1st-generation biofuels, produced from starch- and sugar-based feedstocks, has increased research on different biofeedstocks, improving conversion processes, and finding commercial interest. Ligno-cellulosic feedstocks make-up the 2nd-generation biofuels and include byproducts (cereal, corn stover, straw, sugarcane bagasse, forest residues), wastes (organic components of municipal solids waste), and dedicated feedstocks (purposely-grown vegetative grasses, short rotation forests, and other energy crops) (Sims et al., 2010). The conversion technology for starch- and sugar-based biofeedstocks is well-researched and commercialized in converting the easily degradable starch and glucose molecules into ethanol and other by-products. Unfortunately, the conversion of ligno-cellulosic biofeedstocks is not as efficient as starch-based crops such as corn. This has prevented the transition into 2nd-generation liquid biofuels, but a commercial-scale industry is starting in coming years (Sims et al., 2010).

The Department of Energy's Bioenergy Feedstock Development Program researched and identified switchgrass (*Panicum virgatum* L.) as a viable alternative to corn grain for biofuel production (Wright and Turhollow, 2010). Switchgrass is a warm-season perennial bunchgrass that is native to North America. Being a C4 plant, it is able to grow during the water-limited summer months because it is water-use efficient; it does not need to keep its stomata open as much as a C3 plant in order to complete photosynthesis. Its ability to produce high yields on low-quality soils and with low energy inputs also makes it an ideal candidate as a bioenergy crop (Wright and Turhollow, 2010). A great advantage of switchgrass over corn is its perennial characteristics. Unlike corn, switchgrass does not need to be planted every year and its deep root structure helps protect the soil against erosion, losses of soil organic matter, and nutrient losses due to leaching.

Growing biofeedstocks on reclaimed mine land rather than agricultural lands is an opportunity to protect the nation's fertile agricultural soils while simultaneously restoring degraded lands into productive, economically viable uses. Extensive coal mining in the state of West Virginia produces large expanses of surface mined lands that were reclaimed to a designated post-mining land use. To meet standards by the Surface Mining Control and Reclamation Act of 1977 (SMCRA), these areas have historically been reclaimed with cool-season perennial grasses and legumes. If switchgrass was grown instead of the cool-season grasses, it would be a potential economic benefit to the area by providing a bioenergy feedstock. Unfortunately, full biomass production potential of switchgrass often is not achieved until the second or third growing season following establishment (Parrish and Fike, 2005). However, once mature switchgrass stands have established, they can produce consistent yields over 10 to 20 years with relatively low nutrient inputs (Kering et al., 2012).

In order to have the most efficient conversion process, differences in switchgrass composition should be taken into consideration by bio-refineries that will convert switchgrass into ethanol. Agricultural products including switchgrass generally vary in chemical composition and other properties based on a variety of factors such as plant type, genetics, growth environment, crop management, harvest practices, and post-harvest storage and processing (David and Ragauskas, 2010; Schmer et al., 2012; Jarchow et al., 2012; Vogel et al., 2011). Bio-refineries needing to predict theoretical and actual ethanol yields have relied on expensive and complex assays and analyses (Vogel et al., 2011). Near-infrared reflectance spectroscopy (NIRS) is a nondestructive technology that provides a fast, inexpensive, and accurate method to predict biomass quality and ethanol yields from a variety of feedstock types (Vogel et al., 2011). This technology uses spectral info and calibration equations to predict many constituents including water, crude protein, neutral detergent fiber, lignin, organic matter, nonstructural carbohydrates, and even potential ethanol production. This technology has already been used in agricultural areas to determine the composition of forages, grains, eggs, meat, fruits, coffee, and other agricultural products (McClure, 2007). Having a prediction of the quality of switchgrass before switchgrass bales come off the truck would could help bio-refineries determine the suitability of that particular switchgrass as a biofuel feedstock, whether it should be rejected or mixed with other materials, and the value and price of the material.

For this study, it was hypothesized that switchgrass grown on reclaimed mine land in West Virginia will achieve annual yields of more than 5.0 Mg dry matter (DM) ha^{-1}. Also, it was hypothesized that there will be compositional differences in switchgrass across cultivars and among reclaimed sites and these differences can be measured and evaluated by near-infrared reflectance spectroscopy.

1.1.1. Objectives

1. Determine yields, composition, and theoretical ethanol yields of three switchgrass cultivars on five and six year old stands established on two reclaimed mine sites with different reclamation techniques.
2. Determine if establishment methods for switchgrass affect yield, composition, and theoretical ethanol yields of Cave-in-Rock two and three years after planting.
3. Compare the yields of switchgrass from one- and two-harvest systems.

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Chapter 2: Yield of switchgrass grown on reclaimed surface mines in West Virginia under one-cut and two-cut harvest systems

2.1: Literature Review

2.1.1. Introduction

Switchgrass was first researched in the early 1900's as a native perennial in the Midwestern USA's tall-grass prairies (Parrish et al., 2012). This early agronomic work focused on factors related to forage value and yield with similar work continuing today (Anderson, 2000). It was only until the middle of the 20th century that switchgrass was grown as a monoculture and intensively researched as a forage crop (Parrish et al., 2012). Therefore, many of the commercially-available cultivars today have been chosen for their forage characteristics (Parrish et al., 2012). The first reports for switchgrass as a bioenergy crop were seen at the end of the 1980's, making it a relatively new research subject in the bioenergy field (Parrish et al., 2012).

Switchgrass was chosen as a 'model' bioenergy crop because of its ability to grow at commercial scales as a potentially profitable agricultural system (Wright and Turhollow, 2010). Initially, the main attraction to switchgrass as a bioenergy crop was its ability to be grown on many different soil types and climates throughout the USA (Parrish et al., 2012). It is a warm-season (C4) species, which means it grows well in summer temperatures in the Midwest and Great Plains. This grass is known as a bunchgrass, but it has short rhizomes which allow it to form a sod over time (Parrish et al., 2012). It is a long-shooted species and can grow up to 3 m in height (Zegada-Lizarazu et al., 2012). The ability to grow with low or little fertilizer inputs and less pesticide application means it is a more profitable system than annual crops that need to be seeded and fertilized annually (Wright and Turhollow, 2010). It can grow enough biomass to be harvested for biofuel production and it can be harvested using standard agricultural equipment for hay (Parrish et al., 2012).

As their name suggests, warm season grasses are able to grow well during periods of high temperatures due to their C4 photosynthetic pathway. When temperatures and potential evapotranspiration are high, plants respond by reducing the diameter of their stomata to minimize water loss. Even with their stomata openings reduced, photosynthesis continues, which lowers CO_2 concentrations and increases O_2 concentrations inside mesophyll cells. Unique to C4 plants, initial photosynthetic reactions occur in the mesophyll cells and the Calvin cycle occurs in the bundle sheath cells. This structure prevents O_2 from reacting with the enzyme Rubisco and out-competing CO_2 for its active site. Therefore, C4 plants greatly reduce photorespiration and have higher photosynthetic efficiency than their C3 counterparts. Being able to efficiently carry out photosynthesis with stomates reduced is the reason warm season grasses are more water-use efficient than cool season grasses.

Switchgrass is divided into two ecotypes: lowland and upland. Ecotype differences are based primarily on the environment in which they grow, their morphological characteristics, and the number of chromosomes they exhibit (Zegada-Lizarazu et al., 2012). Upland ecotypes are adapted to areas above 34°N latitude, while lowland varieties are generally found below 42°N latitude (Casler, 2012). Lowland types are more adapted to floodplains and the wetter soils that characterize these landscapes, while upland types are more adapted to dry to moderate soil moisture regimes (Koff and Tyler, 2011).

2.1.2. Potential yields on reclaimed mine lands

While the studies on switchgrass as a forage crop give evidence of the yields that can be achieved with this species under forage management, some of the data can also be used to evaluate switchgrass as a crop for bioenergy production. For example, Sanderson (2008) saw no difference between dry matter (DM) yield between Cave-in-Rock, ‘Shawnee’, and ‘Trailblazer’ switchgrass cultivars planted in Southeastern PA. This study showed annual switchgrass yield to be 6.0 Mg DM ha^{-1} averaged over the three cultivars during the first two years of growth for a 2-cut system (biomass harvested in mid-July, then again after senescence in October). Herbage from the 2-cut system contained lower amounts of crude protein than in the 3-cut system. More nitrogen (N) in switchgrass biomass is advantageous when used as a forage crop because of the value of proteins in feed. But high N concentrations are not advantageous when the biomass is used as a bioenergy feedstock because N is seen as an undesirable element in ethanol production and has to be replenished in soils if harvested and removed from site (Parrish and Fike, 2005). Therefore, it may not make sense to consider a 3-cut system when the biomass is used as a biofeedstock. This study also did not consider a 1-cut system (harvesting only once after senescence in October) which Parrish and Fike (2005) argue is the best harvesting system for bioenergy feedstocks, which contain lower amounts of nitrogen in biomass.

Research has shown that switchgrass grown in the Midwestern USA can produce high yields when managed as a biofeedstock. Switchgrass grown in Nebraska, North Dakota, and South Dakota had yields ranging from 5.2 to 11.2 Mg ha^{-1} averaged over the 3rd to 5th harvest years (Schmer et al., 2008). Fike et al. (2006) showed yields averaged over treatments and sites within the upper Southeastern USA were 14.2 Mg ha^{-1} for 10-year-old switchgrass stands managed for biomass production. Averaged over four cultivars and two management treatments, switchgrass planted in Morgantown, WV, yielded approximately 13.8 Mg ha^{-1}, while two sites in Blacksburg, VA, averaged 16.6 Mg ha^{-1} annual DM production (Fike et al., 2006). Three cultivars grown in Rock Springs, PA, and harvested in the fall had an average yield of 7.9 Mg ha^{-}

^{1} (Adler et al., 2006). Near Chariton, IA, 20 switchgrass cultivars yielded an average of 9.0 Mg ha^{-1} over a period of five years (Lemus et al., 2002).

Growing switchgrass on reclaimed surface mines in the Appalachian region could potentially be an alternative to growing biofeedstocks on agricultural lands. Surface mining for coal is estimated to affect approximately 4.9 million hectares of land across West Virginia, Kentucky, and a few counties in Tennessee (U.S. EPA, 2005). The goal for reclamation of these areas is to develop a suitable post-mining use of the land for landowners, to meet ground cover regulations and to minimize erosion as inexpensively and effectively as possible. A current, popular post-mining land use is hay land and pasture, which is relatively inexpensive to establish with agricultural grasses and legumes providing quick ground cover that is necessary to meet regulations.

Although there is much research on switchgrass production on productive USA agricultural land, very little research is available for switchgrass grown on reclaimed mine areas. Previous research that may be comparable is switchgrass grown on marginal lands. Marginal lands are those with low crop productivity due to inherent land or climatic limitations or because they are located in areas that are vulnerable to erosion (Gelfand et al., 2013). Switchgrass planted on marginal lands along newly-constructed highways was able to achieve good cover and soil stabilization after two years (Skousen and Venable, 2008). Schmer et al. (2008) produced annual average yields of 5.2 to 11.1 Mg DM ha^{-1} of switchgrass when managed as a biomass energy crop on marginal cropland. Even greater yields on marginal lands were shown by Kering et al. (2012), with switchgrass producing 16.0 Mg ha^{-1} during the fifth year of production.

2.1.3. Multiple harvest system

Switchgrass also has the potential to be harvested multiple times in one year as it regrows after cutting early in the growing season (Mitchell and Moser, 2000). Stem elongation occurs when intercalary meristems, located at the nodes below the apical meristem, cause the internodes to lengthen which raises up the apical meristem. If the stand is harvested after elongation, the apical meristem will be clipped off. New leaf development of that tiller will stop and regrowth will occur from other meristems where the rate of growth is much slower (Mitchell and Moser, 2000). Re-growth can come from axillary meristems near the soil surface, stem buds near the top of the remaining stubble, and new crown tillers (Anderson and Masters, 1983).

There have been successful multiple harvest systems for switchgrass fields. Averaged over eight sites in five states and four cultivars, it was shown that the 2-cut system outperformed the 1-cut system: 15.4 to 12.8 Mg ha^{-1} (Fike et al., 2006). Looking more closely at cultivars in this same study, Cave-in-Rock produced 10.8 and 15.4 Mg ha^{-1} with the 1- and 2-cut systems, respectively. In similar studies, Cave-in-Rock, an upland ecotype, and 'Alamo', a lowland ecotype, have been shown to have positive yield responses to the 2-cut harvest system (Vogel et al., 2002; Guretzky et al., 2011).

Conversely, there has been research that shows that the 2-cut harvest system does not outperform the 1-cut system and might reduce the viability of the stand. In one study, the 2-cut system (9.8 Mg ha^{-1}) produced less yield than the 1-cut system (11.1 Mg ha^{-1}) (Kering et al., 2013). For a Cave-in-Rock stand grown in South Dakota, after a first July harvest, not enough re-growth was available to harvest by machine later in October (Lee and Boe, 2005). Monti et al. (2008) saw greater yields in the 2-cut system the first two years, but saw reduced stand vigor for the next two years. They concluded that it might not make economic sense to make the second cut since it had such low biomass levels. It should also be noted that 2-cut systems may have to be fertilized more frequently than 1-cut systems as 2-cut systems remove more nutrients from soil (Kering et al., 2013). The economic advantage of a 2-cut system and fertilizer application would have to be determined on a site-by-site basis.

Timing of cut for a 2-cut system is critical. Enough time needs to be left between cuts to allow for re-growth to become significant enough to be harvested. Kering et al. (2013) harvested Alamo switchgrass for the first cut at vegetative stage in June and at boot stage in late July. Averaged over N treatments, cutting during the vegetative stage produced re-growth yields (2.2 Mg ha^{-1}) greater than the yield of regrowth after the first cut at boot stage (1.5 Mg ha^{-1}). Vogel et al. (2002) showed similar results with the greatest re-growth (approximately 3 Mg ha^{-1}) happening after first harvest in June and the least amount of re-growth (approximately 1 Mg ha^{-1}) happening after harvest in August. Drought may affect 2-cut system's yield more since soil moisture stress is often increased during this time (Kering et al., 2013, Sanderson et al., 1999).

2.1.4. Objectives

To determine the ability of switchgrass to be grown on reclaimed surface mines, we conducted field-scale experiments measuring the yield responses of three switchgrass cultivars grown on two reclaimed surface mines in West Virginia. One mine site represented reclamation conditions with no topsoil and no fertilizer application while the other mine site represented reclamation conditions with topsoil and sewage sludge amendments. Each mine site was seeded with three commercially-available upland switchgrass cultivars: Cave-in-Rock, 'Carthage', and Shawnee.

Our first objective was to determine if a less intensive reclamation process produced similar yields as a more intensive reclamation process. The second objective was to determine which of these three cultivars would produce the highest biomass on the reclaimed mine sites in WV. The third objective was to determine the effect of fertilizer on yield for the site with no topsoil. To evaluate the yield responses of the three switchgrass cultivars to timing and frequency of harvest on reclaimed surface mines, our last objective was to determine if 1- or 2-cut harvest systems would produce the highest biomass on the reclaimed mine sites in WV. This study was conducted from 2009-2013 on switchgrass plots established in 2008. We also monitored soil physical and chemical properties on these two sites.

2.2 Materials and Methods

2.2.1. Site Locations

This study was established in 2008 and was conducted on two reclaimed surface mine areas in West Virginia. The first site, Hampshire (39.4 N,-79.1 W) is located on a small contour mine in Mineral County, WV, which stopped operations in 1998. This site utilized smaller mining equipment and trucks. It is currently being managed by the Upper Potomac River Commission. At Hampshire, 30 cm of topsoil was placed over re-graded overburden. In 1998, 2003, and 2008, lime-treated sludge was applied at a rate of 225 Mg (dry) ha^{-1}. The sludge consisted of municipal sewage waste from the Westernport, MD municipal wastewater treatment facility and paper mill pulp from the nearby New Page paper mill. Because of the application of topsoil and sludge, the site had favorable soil properties which greatly enhanced revegetation potential. This site was selected for planting because it represented very favorable reclamation conditions available for establishing switchgrass. Precipitation and temperature amounts for Hampshire were averaged monthly using data from a weather station in Keyser, WV (39.4N, -79.0W) maintained by National Oceanic and Atmospheric Administration (NOAA) (Table 2.1).

Table 2.1. Average monthly precipitation and temperature at Keyser, WV, about 5 km southeast of the Hampshire site.

Month	2010	2011	2012	2013	Avg. (1981-2010)		2010	2011	2012	2013	Avg. (1981-2010)
			cm						°C		
Jan.	7.3	3.3	7.8	6.4	7.4		-2.1	-2.7	0.6	0.9	-1.2
Feb.	8.6	7.9	3.4	3.6	6.7		-2.6	1.3	3.2	-0.8	0.6
Mar.	10.5	14.2	14.1	8.3	9.2		7.1	5.0	10.6	2.5	5.1
Apr.	3.6	20.3	4.9	7.8	9.3		13.2	12.0	11.0	11.5	11.1
May	13.3	22.3	8.1	9.4	10.4		17.2	18.0	19.4	15.6	16.3
June	8.5	7.8	7.4	9.5	8.5		23.3	22.4	20.7	21.4	21.2
July	3.3	3.6	9.9	9.9	10.1		25.4	25.9	25.9	24.5	23.4
Aug.	4.1	9.6	7.3	9.3	8.5		24.4	23.7	22.9	21.6	22.5
Sept.	8.7	19.1	8.2	2.9	8.9		19.8	19.5	18.7	18.0	18.5
Oct.	7.2	12.7	20.4	8.2	6.9		13.3	12.0	12.2	12.7	11.8
Nov,	7.6	9.1	1.0	4.2	7.9		5.5	7.7	4.2	4.0	6.2
Dec.	5.9	7.3	9.4	8.6	7.3		-2.9	2.8	3.0	2.1	0.6
Total	88.5	137.2	102.1	88.2	101.3	Avg.	11.8	12.3	12.7	11.2	11.3

Table 2.2. Monthly precipitation and temperature at Madison, WV, about 11 km northeast of the Hobet site.

Month	2010	2011	2012	2013	Avg. (1981-2010)		2010	2011	2012	2013	Avg. (1981-2010)
			cm						°C		
Jan.	8.0	7.1	8.2	12.0	8.2		-0.6	-0.5	3.8	2.6	0.8
Feb.	7.6	6.3	6.5	4.0	8.4		-0.7	4.5	4.7	2.1	2.8
Mar.	11.9	13.5	14.0	8.2	9.9		7.7	7.9	13.6	4.2	7.2
Apr.	7.2	20.2	10.0	6.3	10.4		14.7	14.7	12.5	13.5	13.0
May	22.3	14.5	11.3	7.7	13.6		19.8	18.5	20.9	18.4	17.9
June	13.5	10.8	9.0	17.3	11.2		24.8	23.1	22.1	23.0	22.6
July	24.3	18.9	20.9	20.3	13.3		25.5	26.2	26.1	24.7	24.6
Aug.	11.2	7.9	2.8	9.7	10.8		25.3	23.9	23.6	23.0	24.0
Sept.	4.2	17.5	16.2	4.6	8.8		20.4	20.4	19.9	20.3	20.2
Oct.	5.9	12.9	15.7	5.4	7.5		14.3	13.1	13.3	14.2	13.6
Nov,	8.1	12.5	3.4	11.4	9.1		8.2	10.1	5.8	6.0	7.9
Dec.	11.9	10.4	14.4	20.3	9.3		-1.7	5.2	6.1	3.6	2.5
Total	136.0	152.3	132.3	127.1	120.7	Avg.	13.1	13.9	14.4	13.0	13.1

The second site, Hobet (38.1 N, -82.0 W), is located on a large mountaintop surface mine in Boone and Kanawha counties (near Madison, WV) operated by Hobet Mining Company. This mine utilizes a large dragline for overburden removal in order to reach the coal seam. The site area was reclaimed in 2008. The overburden was dumped with the dragline bucket and regraded to approximate original contour by large bulldozers after mining. The compacted overburden was covered with 1 m of crushed, unweathered rock material. Before planting, the heavily compacted soil was broken up with a large offset disk before planting. Hobet's reclamation strategy was selected to simulate reclamation conditions where no topsoil was applied. A 19-19-19 mixture of pelletized fertilizer was applied evenly by hand at a rate of 38 kg N ha^{-1} and was applied to one-half of each of Hobet's plot in 2009 and 2013 to determine the effects of N, P, and K on a reclamation strategy producing a lower quality site for forage production. Precipitation and temperature amounts for Hobet were averaged using data from a weather station in Madison, WV, (38.1025 N, -81.8464 W) maintained by the NOAA (Table 2.2).

2.2.2 Experimental Design

In 2008, nine plots were laid out on each site, each 0.4 ha in size. Seed of three cultivars of switchgrass was planted and replicated (three) in randomly assigned plots. Switchgrass cultivars Cave-in-Rock, Carthage, and Shawnee were broadcast-seeded at both sites. The seed was spread at a rate of 11.2 kg pure live seed (PLS) ha^{-1} using a hand broadcast seeder (Keene and Skousen, 2010). Aboveground biomass has been sampled annually since 2009 at the end of the growing season during post-anthesis stage of the switchgrass. Six sampling points were randomly chosen within each plot for aboveground biomass measurements by clipping at approximately 10 cm above ground level.

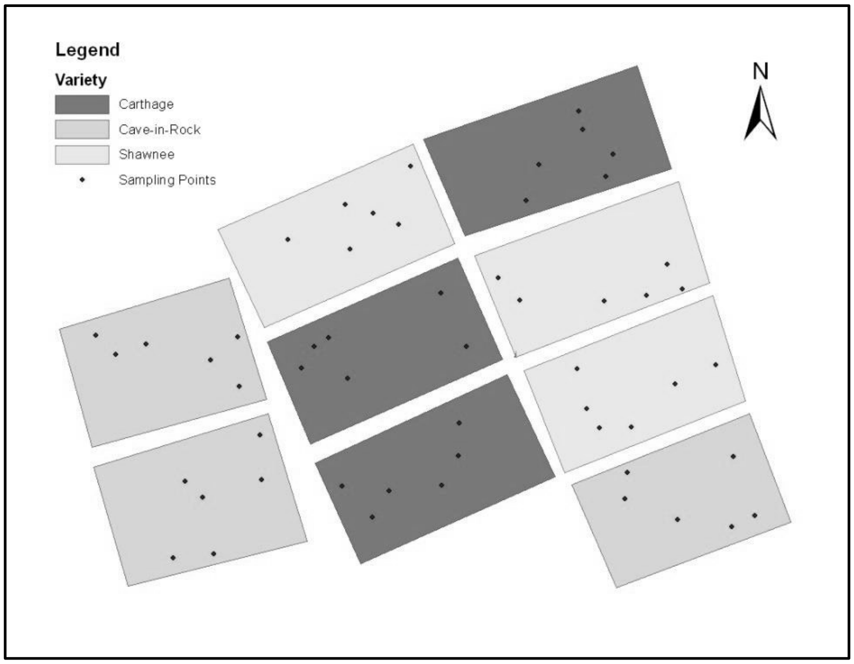

Figure 2.1. Schematic map of Hampshire showing the plots and sub-sampling points.

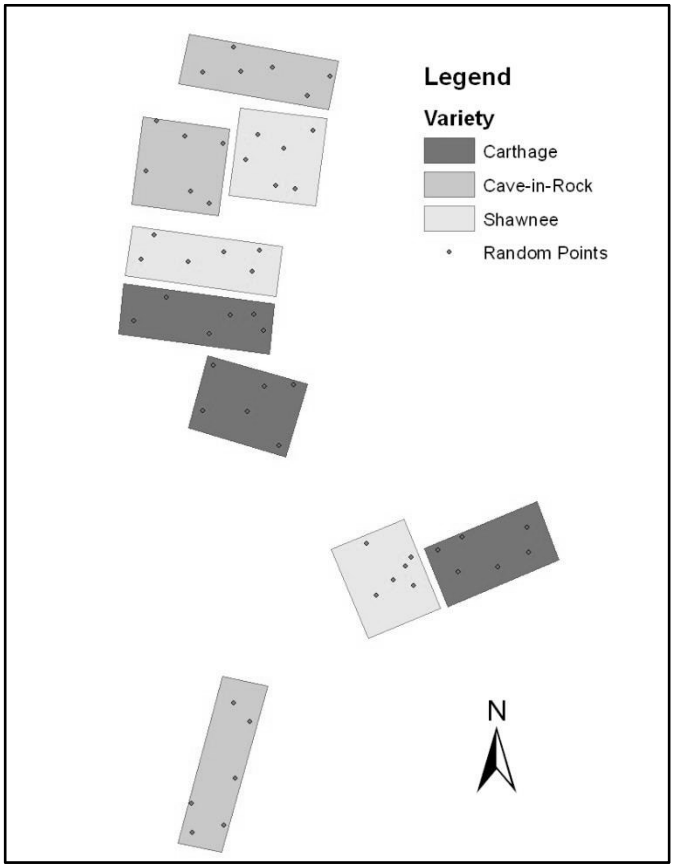

Figure 2.2. Schematic map of Hobet showing the plots and sub-sampling points.

2.2.3. Vegetation Measurement

For the 1-cut experiment, a 0.21-m^2 quadrat was placed on the ground at five paces south of each sampling point. All switchgrass inside the quadrat was clipped to a 10-cm stubble height (Mitchell and Schmer, 2012). Herbage from plants other than switchgrass that may have been located within the quadrat was discarded. Samples were oven dried at 60°C to a constant weight to determine dry weight.

For the 2-cut experiment, separate vegetative samples than the 1-cut harvest biomass clippings were taken in 2011, 2012 and 2013. A stake was driven into the ground at five paces north of each sampling point. This stake indicated the bottom left corner of the 0.21 m^2 quadrat with the quadrat facing north. The first harvest for the 2-cut system occurred in the middle of July at approximately boot stage. To try and prevent border effects, a 1-m area surrounding the quadrat for the first cut was clipped to a height of 10 cm. The second harvest occurred during the post-anthesis stage of switchgrass in October. This was done by placing the quadrat in the same position on the stakes to clip the same area where the first cut was done. Second-cut samples were also oven dried at 60°C to a constant weight to determine dry weight.

2.2.4. Soil Sampling

Soil samples were collected by taking a shallow shovel slice to approximately 15-cm depth at both sites annually since the inception of this study and analyzed for percent fines, pH, EC, Al, Fe, Mn, Mg, Ca, K, P, Ni, Cu, and Zn (Table 2.3 and Table 2.4). Marra et al. (2013) previously determined the particle size distribution for the sites (Table 2.3). For Hobet, three soil samples were composited per fertilized side giving two soil samples per plot. For Hampshire, two soil samples per plot were also obtained: three soil samples from the northern-most part of the plot were composited and three samples from the southern-most part of the part were composited. No fertilizer was applied at Hampshire. Soil samples were air dried and sieved to obtain a soil sample composed of 2 mm-sized material (fine fraction). Percent fines were determined by weighing whole soil sample and then weighing fine fraction. Subsamples of the fine fraction used for soil analysis were taken using a riffle splitter.

Table 2.3. Selected chemical and physical properties at Hampshire and Hobet averaged over 2008-2013.

Site	pH	EC	Percent Fines	Particle size distribution		
			%	% Sand	% Silt	% Clay
		(μS cm^{-1})				
Hampshire	7.3	648	78	42	47	11
Hobet	7.7	140	57	63	27	10

Table 2.4. Values of extractable soil nutrients using Mehlich 1 solution at Hobet and Hampshire averaged over 2008-2013.

Parameter	Hampshire	Hobet
	-------- cmol charge kg^{-1} --------	
Mg	0.84	0.73
K	0.16	0.10
Na	0.04	0.01
Ca	7.74	1.20
	------------- mg kg $^{-1}$ -------------	
Al	67.8	22.0
Fe	17.4	38.8
Mn	41.3	19.0
P	5.2	26.4
Ni	0.3	0.6
Cu	1.1	1.0
Zn	4.4	1.4

The fine fraction was used to determine pH, electrical conductivity (EC), and available nutrients. For pH, 5 g of soil were combined with 5 mL of distilled deionized (DDI) water. The mixture was placed on an orbital shaker table and mixed for 15 minutes, then allowed to equilibrate for at least 1 hour. A Mettler Toledo Seven Easy pH Meter was used to measure pH.

Electrical conductivity was determined by combining 5 g of soil with 10 mL DDI water. The mixture was placed on an orbital shaker table and mixed for 15 minutes, then allowed to equilibrate for at least 1 hour. A Seven Compact S230 Mettler Toledo Conductivity Meter was used to measure EC.

Soils were extracted with a Mehlich 1 solution to extract available elements from the soil (Wolf and Beegle, 1995). For extraction, 25 mL of Mehlich 1 solution were added to 5 g of soil, mixed on an orbital shaker for 5 minutes, and then allowed to equilibrate. Samples were filtered through Whatman No. 2 filter paper and a Perkin Elmer inductively coupled plasma emission spectrometer (ICP-ES) was used to analyze the filtrate for available nutrients: Al, Fe, Mn, Mg, Ca, K, P, Ni, Cu, and Zn.

2.2.5 Statistical Analyses

The experiment at each site had a completely randomized design with the three cultivars of switchgrass replicated three times in randomly assigned plots. For the 1-cut system comparing Hobet and Hampshire, the half-plots that were fertilized at Hobet were removed from the model because Hampshire had not been subjected to the same treatment. Hobet was then analyzed separately to see if fertilization affected yield. Hampshire was analyzed separately to see if the 1-cut system or the 2-cut system produced the highest yields. Data were analyzed by a repeated-measures ANOVA using PROC MIXED procedure of the Statistical Analysis System (SAS) (Statistical Analysis System, 2011). A randomized block design was used for the ANOVA with sites being blocks. Sites were fixed factors with replication year. Year and plot were random. Sub-sample weights were averaged to give one mean value per plot. No data were transformed because the assumptions of normal distributions for ANOVA were satisfied using the Shapiro-Wilk normality test (Shapiro and Wilk, 1965). For each analysis, statistical significance was based on a p value of ≤ 0.05. The least square (LS) mean yields are reported. When necessary, Tukey's Honestly Significant Difference multiple comparison test was used to determine significant differences among mean yields.

2.3 Results and Discussion

2.3.1. Unfertilized, 1-cut switchgrass yields

Due to the different reclamation techniques used prior to establishment, yields of switchgrass at the two mine sites were significantly different. Hampshire produced 8.4 Mg ha^{-1} compared to 1.0 Mg ha^{-1} for Hobet when averaged across cultivars and years ($p<0.01$) (Table 2.5). The topsoil and lime-treated sewage/paper sludge used to reclaim Hampshire provided a better soil medium for switchgrass than the unweathered overburden material used at Hobet, which accounted for the significantly different yields between the two sites. Hampshire would be expected to have more fine material and higher EC than Hobet because it was reclaimed with topsoil and amended with sewage and paper mill sludge. As previously described, Hobet was reclaimed with crushed overburden rock that had not been given much time to weather. The larger rock fragments translated into lower percent fines and lower water holding capacity. Switchgrass has been known for its soil moisture sensitivity and could explain the low yields (Fike et al., 2006). Hampshire also had a higher EC quantity which relates to more fertile soils.

Table 2.5. Statistical significance for yield by the main effects of site, cultivar, and year.

Effect		**P>F**	**Yield**
			Mg ha^{-1}
Site		<0.01	
	Hampshire		8.4
	Hobet		1.0
Cultivar		<0.01	
	Carthage		2.8^{b}
	Cave-in-Rock		7.0^{a}
	Shawnee		4.3^{b}
Year		<0.01	
	2010		4.2^{bc}
	2011		3.0^{c}
	2012		5.0^{ab}
	2013		6.5^{a}

Different letters denote significant difference at $p \leq 0.05$ level of probability according to Tukey's HSD. LS Means are reported. Interactions: cultivar x site ($p<0.01$); cultivar x year ($p>0.05$); site x year ($p<0.01$).

Yield differences were found for the three cultivars planted ($p<0.01$) and also a cultivar x site interaction ($p<0.01$, Figure 2.3). As shown in Figure 2.3, all cultivars grown on Hobet had similar yields and were significantly lower than Cave-in-Rock (CIR) and Shawnee grown on Hampshire. The high yields of these two cultivars at Hampshire caused the significant difference in yield among cultivars. The highest performing cultivar on Hampshire was CIR, which yielded 12.9 Mg ha^{-1} averaged over years. Carthage and Shawnee did not produce statistically different yields on this site with the average of their yields being 6.1 Mg ha^{-1}. At Hobet, no differences in average yield across years were found among cultivars (Figure 2.3). There was no cultivar x year interaction for yield averaged over sites (Figure 2.4). Every year CIR was consistently the highest yielding cultivar while Carthage was consistently the lowest (Figure 2.4). These figures show biomass grown at Hampshire drove the significant difference in yield between the main effect of cultivars (Figure 2.5).

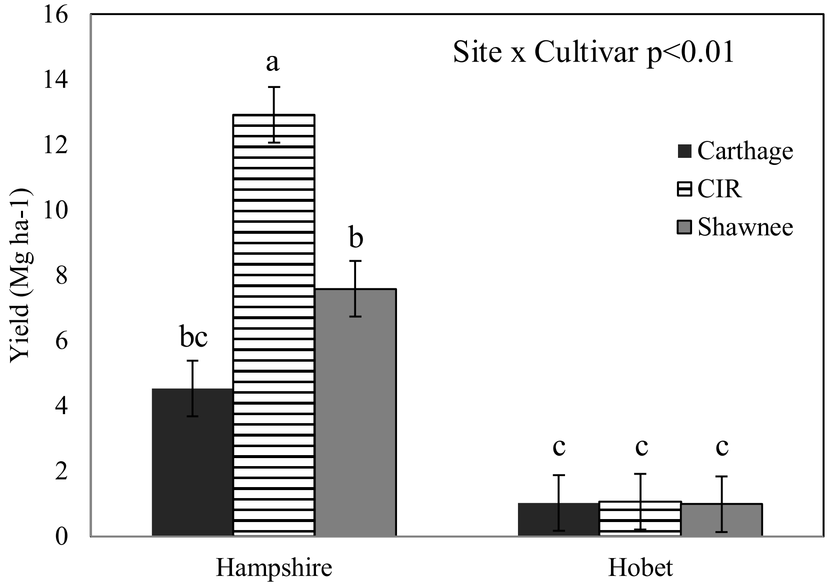

Figure 2.3. Yield of switchgrass cultivars at each site averaged over years (2009-2013). Vertical bars represent standard errors. Different letters on bars denote significant difference at p ≤0.05 level of probability according to Tukey's HSD. LS Means are reported.

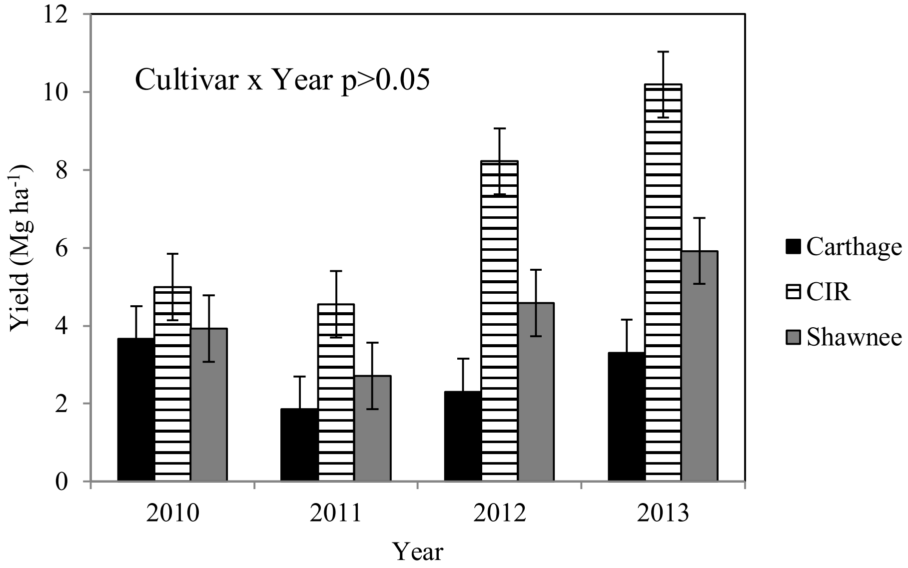

Figure 2.4. Yield of switchgrass cultivars for each year averaged over the two sites. There was no significant cultivar x year interaction. LS Means are reported.

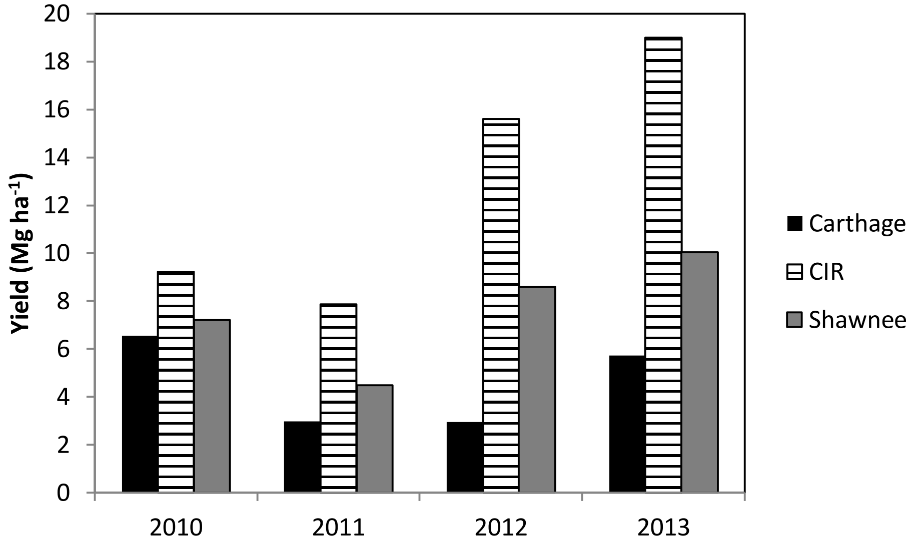

Figure 2.5. Yield of switchgrass cultivars for each year at Hampshire only. Raw means are reported. Not statistically analyzed.

Comparable yields for these cultivars grown in this region with better soils have been documented. For example, CIR grown in Morgantown, WV, and fertilized annually with 50 kg N ha^{-1} had comparable yields of 15 Mg ha^{-1} (Fike et al., 2006). CIR yielded 8.6 Mg ha^{-1} and Shawnee yielded 8.5 Mg ha^{-1} grown in Rock Springs, PA (Adler et al., 2006). All three cultivars were grown on experimental plots established on a research farm in Iowa, Carthage yielded 9.9 Mg ha^{-1}, CIR yielded 9.3 Mg ha^{-1}, and Shawnee yielded 8.8 Mg ha^{-1} (Lemus et al., 2002). These stands also received fertilizer in the form of ammonium nitrate at a rate of 112 kg N ha^{-1}.

There was a main effect of year on yield averaged across sites and cultivars ($p<0.01$) and also a site x year interaction ($p<0.01$). Sites were statistically different for all years except for Hampshire in 2011 and Hobet in 2013. Since these two sites were so different, sites are shown separately by year (Figures 2.6a and b). All years for Hobet had similar yields. These figures show yields at Hampshire caused the significant difference in yield between sites. Averaged across cultivars, yield decreased at Hampshire from 7.7 Mg ha^{-1} in 2010 to 5.1 Mg ha^{-1} in 2011, but increased to 11.5 Mg ha^{-1} in 2013. The decreased yield in 2011 is what accounted for the majority of the variation among years. It was expected that the year effect would be different as switchgrass stands continued to increase in biomass with time, and a trend of increasing yield with time can be seen, except for the year 2011. Yields for Hobet did not show a significant increase with time, although they are slowly trending upward.

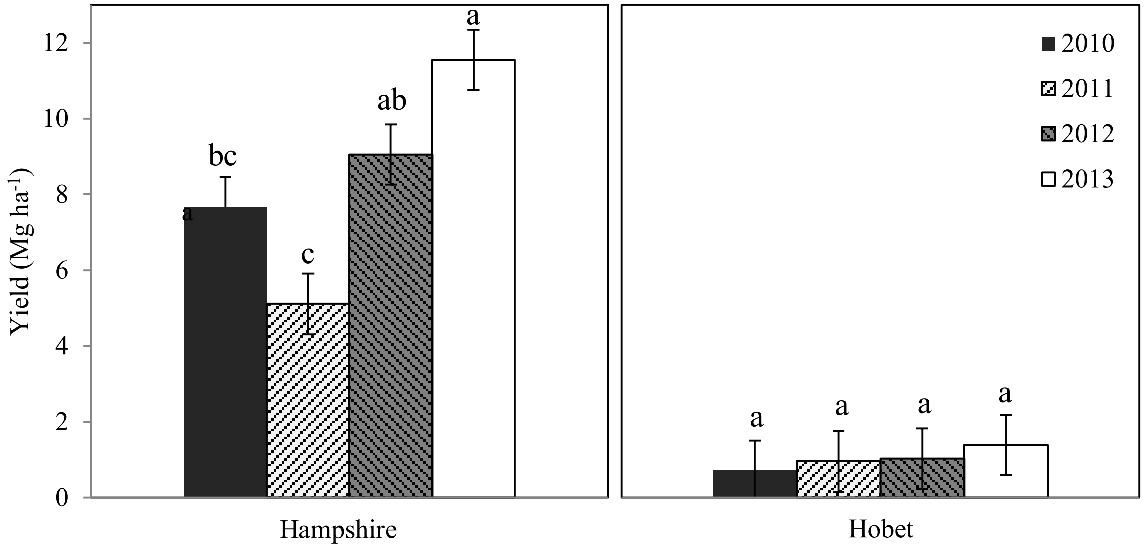

Figure 2.6. (a) Left. Yield of switchgrass at Hampshire averaged across cultivar. (b) Right. Yield of switchgrass at Hobet averaged across cultivar. Vertical bars represent standard errors. Different letters on bars denote significant differences at p ≤0.05 level of probability according to Tukey's HSD. LS Means are reported.

2.3.2. Comparison of fertilized and unfertilized split-plots of 1-cut on Hobet

To see if yields could be increased in the harsh soils at the Hobet site, fertilizer was added at a rate of 38 kg N ha^{-1} to half of each plot. Fertilizer was added the year after establishment (2009) and again in 2013 to the same half plots. This analysis includes all years from 2009 to 2013. Fertilizing increased switchgrass yields on Hobet ($p<0.01$, Figure 2.7). The fertilized sub-plots yielded 1.2 Mg ha^{-1} while the un-fertilized sub-plots yielded 0.85 Mg ha^{-1} averaged over years (Figure 2.7). There was a year effect on yields ($p=0.01$) but not a cultivar effect. There also was a significant cultivar x fertilizer x year interaction ($p<0.05$) (Figure 2.8). Even with fertilization, yields were still very low at Hobet. It does not seem that fertilizing could compensate for the poor water and nutrient holding capacity of the soils at Hobet toproduce good yields. Adding organic material, such as sewage sludge or paper mill sludge, may speed-up the transformation of soils into a suitable media for switchgrass growth.

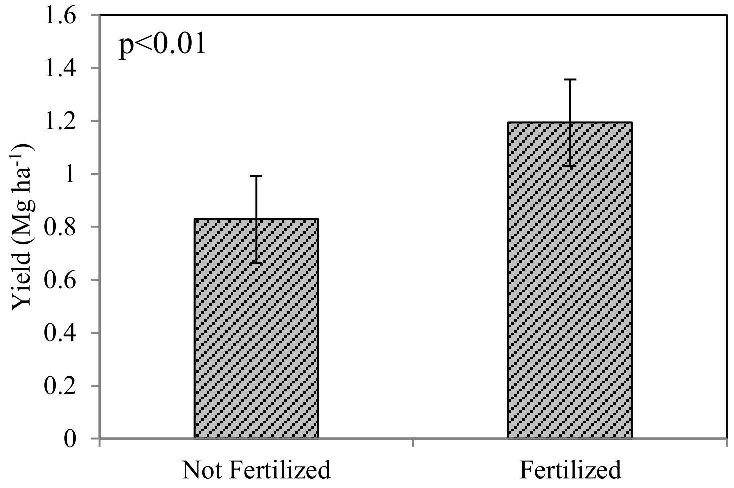

Figure 2.7. Comparison of fertilization and no fertilization treatments on the Hobet site. Vertical bars represent standard errors. Significant difference at $p \leq 0.05$ level of probability. LS Means are reported.

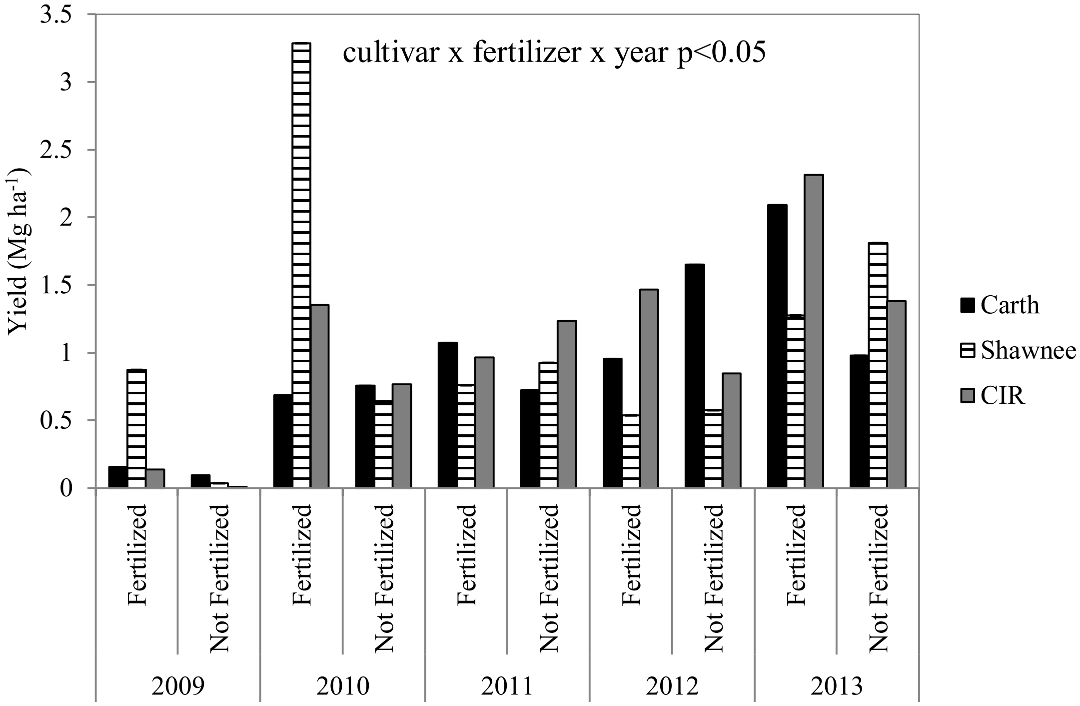

Figure 2.8. Interaction means yields for cultivar x fertilizer x year interaction on the Hobet site. LS Means are reported. Tukey's Honestly Significant Difference multiple comparison test was used to determine significant differences among mean yields.

2.3.3. Comparison of 1- and 2-cut system at Hampshire

The potential yield increase of a multiple harvest system was tested at Hampshire. A 2-cut harvest system was conducted in 2011, 2012, and 2013. The 1-cut yields taken in October from section 2.3.1. were compared to the total yield of the 2-cut harvest system. It was shown that a 2-cut system was not advantageous over the 1-cut system at Hampshire (Figure 2.9). The 2-cut harvest system had significantly lower yields than the 1-cut system ($p<0.05$, Figure 2.9). Averaged across years, the yield for the 1-cut harvest system was 8.5 Mg ha^{-1} and the yield for the 2-cut harvest system was 5.8 Mg ha^{-1}. None of the cultivars benefited from the 2-cut system as there was no significant difference between the yields ($p>0.05$, Figure 2.10). For each cultivar, the 1-cut system had higher yields than the 2-cut system.

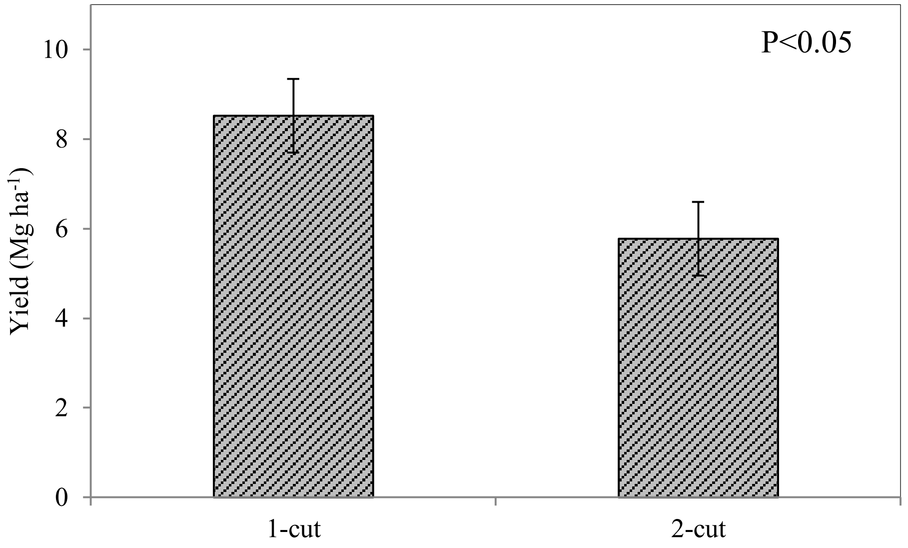

Figure 2.9. Comparison of 1- and 2-cut harvest system on the Hampshire site. Vertical bars represent standard errors. Different letters denote significant difference at p ≤0.05 level of probability. LS Means are reported.

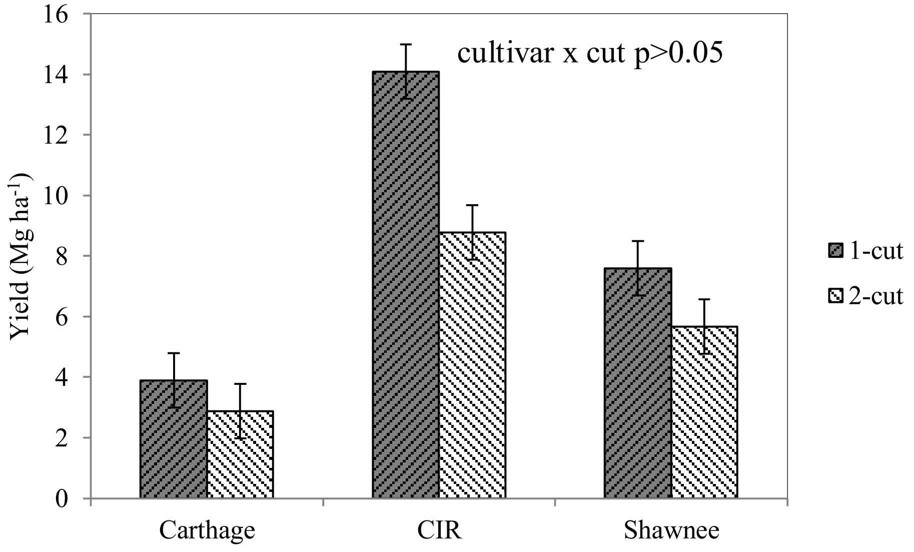

Figure 2.10. Yield of switchgrass cultivars for each harvest system averaged over years on the Hampshire site. There was no significant cultivar x harvest interaction. LS means are reported.

2.4 Conclusion

With the placement of a proper soil medium during reclamation, reclaimed surface mines can be suitable areas to grow switchgrass for biomass. As shown at Hampshire, reclaiming with topsoil and organic amendments created good soil conditions for switchgrass growth, while the unweathered overburden at Hobet produced poor growing conditions and poor switchgrass yields. It is clear that switchgrass would benefit from at least some topsoil or weathered overburden to provide for a thicker and higher yielding stand. CIR was the highest performing cultivar at Hampshire and would be an ideal cultivar to grow in West Virginia compared to Carthage and Shawnee. Fertilization was unable to compensate for the poor soil conditions (unweathered overburden) at Hobet, although yields were increased slightly. For these sites, harvesting switchgrass once per year produced more biomass than a 2-cut system. A 1-cut system reduces the time, effort and cost for harvesting compared to a 2-cut system, and would be preferable in terms of both yield and harvesting cost.

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Chapter 3: Yield of switchgrass grown with different establishment and nitrogen treatments on reclaimed surface mines in West Virginia

3.1: Literature Review

3.1.1. Nitrogen management

Optimizing the management of nitrogen (N) application on switchgrass stands has been a focus of research because N is often the most limiting nutrient in these systems (Muir et al., 2001; Thomason et al., 2004). Determining optimum N application rates will help switchgrass biomass producers achieve maximum profits by reducing inputs while obtaining high yields. Unfortunately, there is no consensus on the amount of fertilizer to apply to a field for switchgrass growth (Parrish and Fike, 2005). The amount of N input required by switchgrass is a function of the desired yield, N concentration of the biomass, potential productivity of the site, and management practices (Vogel et al., 2002). Fertilization in early spring will likely allow cool-season plants to obtain the N thereby creating greater competition from weeds and cool-season grasses.

It should be taken into consideration that not all N applied to a field will be available and used by the switchgrass. Understanding the whole N balance of a crop system will help in attaining adequate plant nutrient supply and reduce amount of fertilizer loss. Of the N added to a switchgrass system, general estimates are that <5% will be volatized, 1% will be lost in runoff water, 10% will be emitted as gas, up to 66% will be taken up and later harvested in the biomass, and 9% will be leached out of the soil (Bonin et al., 2012). The remaining N loss can be summed up to rounding from different sources and uncalculated N translocated from roots Nitrogen also has the ability to leach out of the soil from rain before it’s absorbed by plants. This is why the nitrogen-use efficiency should be taken into consideration instead of only the amount of nitrogen applied.

3.1.2. Switchgrass Nitrogen-use

Switchgrass is known to be efficient user of N. Switchgrass is an efficient user of N because at the end of the season, and also during times of drought, it is able to transport N from its shoots to belowground sections upon senescence (Parrish and Fike, 2005). Cutting the switchgrass too low, which effects carbohydrate storage, and also harvesting frequently will increase the amount of N needed by the plant (Parrish and Fike, 2005). Switchgrass biomass has been shown to respond better to fertilization when initial soil N is low (Owens et al., 2013). In Owen's study, only approximately 10% of the N was recovered by the switchgrass; leaving 90% of the N applied to the switchgrass fields unaccounted for (Owens et al., 2013). This is lower than the 30% N recovery shown by Stout and Jung (2005). More N recovery may have been shown had they determined how much N was sequestered by the plant.

3.1.3. Switchgrass yields with and without fertilizer input

Adequate switchgrass yields can be achieved with little or no fertilizer input. With only residual soil fertility, one study in Tifton, GA, saw 8.6 Mg ha^{-1} of biomass production averaged over the first four years of switchgrass growth (Knoll et al., 2011). Although Kering et al. (2013) showed no increase in biomass with N applied at a rate of 135 kg N ha^{-1}, they did show an 18% increase in Alamo production with a N rate of 135 kg N ha^{-1} and a potassium (K) rate of 68 kg K_2O ha^{-1} as compared to the control which did not have any added fertilizer. This indicates that switchgrass grown on marginal lands may benefit from the addition of K along with N. Switchgrass is able to grow without fertilization inputs, but yields respond positively to applied N (Mitchell et al., 2008).

The amount of fertilizer that is needed varies between systems with different cultivars, topography, and harvest timing. Vogel et al. (2002) reported maximum yields of Cave-in-Rock (CIR) at 120 kg N ha^{-1} in Nebraska even though they tested up to 300 kg N ha^{-1}. Another study in Nebraska that planted 'Sunburst', also an upland variety, showed maximum yields were reached with N rate up to 56 kg ha^{-1} even though 112 kg N ha^{-1} was tested (Owen et al., 2013). The critical N rate to reach maximum yield for three different harvests in Illinois all ranged from 50 to 165 kg N ha^{-1} (Anderson et al., 2013). All of these variable factors support the idea that N application is a site-specific management practice.

3.1.4. Management of switchgrass on mine soils

Many of these previously mentioned studies were conducted on sites that had residual N and might not apply to mine soils which typically have very low N amounts in their soils (Bendfeldt et al., 2001). Adding fertilizer to mine soils is important because low N concentration in mine soils is most frequently the growth limiting nutrient for plants (Bendfeldt et al., 2001). It was shown by Shrestha and Lal (2011) that a significant amount of total N concentration is lost due to mining activities. For soil depths of 0-15 cm, undisturbed sites had soil total N ranging from 1.10 to 2.96 g kg^{-1} while soils reclaimed after mining had soil total N concentrations ranging from 0.54 to 1.10 g kg^{-1}. The initial total Kjeldahl N concentration for the mine soils used in the Bendfeldt et al. (2001) experiment was 0.50 g kg^{-1} within the top 10 cm. This is much lower than the total N found in soils not disturbed by mining used for a switchgrass study in South Dakota, New York, and Oklahoma which averaged 1.93 g kg^{-1} within the top 15 cm (Owens et al., 2013). Owen's study showed that N-use efficiency for switchgrass was highest in the Virginia soils which had the lowest total N concentration of 0.835 g kg^{-1}. This may mean that switchgrass will respond well to fertilizer inputs on mine soils or soils with low N content.

Careful fertilizer management will likely reduce the economic investment and prevent pollution of the surrounding environment with nutrients not utilized by switchgrass. Applying the right amount of fertilizer should be the goal of any management plan to reduce environmental impact and profit loss. N that is leached from the soil can cause contamination of groundwater and eutrophication of water systems. To meet the standards of switchgrass cover, the best establishment method for switchgrass on mine soils should be found. Therefore, to reduce economic investment and prevent pollution, evaluating the optimum rate of N applied to grow switchgrass was the aim of this study.

3.1.5. Objectives

The objective of this study was to determine the best establishment methods and fertilizer rates for switchgrass growth on reclaimed surface mines in West Virginia. This experiment was conducted on two reclaimed surface mines in southern West Virginia that simulated common reclamation practices in the area. Cave-in-Rock was seeded the same year as the sites were backfilled and topsoiled. During establishment, different rates of mulch and fertilizer were applied during switchgrass seeding in 2011. The same fertilizer rates were applied in spring 2013. Biomass was collected annually from 2011 to 2013 on switchgrass plots established in 2011. We also monitored soil physical and chemical properties on these two sites.

3.2 Materials and Methods

3.2.1. Site Locations

Two sites in West Virginia, Coal Mac and Black Castle, were used in this study to see long-term effects of establishment techniques with different levels of mulch and fertilizer at planting (Figure 3.1). The first site, Coal Mac (37.7 N 82.0 W), is located on a large mountaintop surface mine in Mingo, Logan, and Boone counties operated by Coal-Mac, part of Arch Coal, Inc. This mining operation utilizes large earth moving equipment, such as draglines, shovels and loaders, to remove overburden and coal. The site of switchgrass planting was leveled and reclaimed in 2011 with 60 to 90 cm of topsoil and weathered sandstone mixture that was placed over gray sandstone overburden. Planting, fertilizing, and mulching were done at the end of May in 2011 as described below. The second site, Black Castle (38.1 N 81.7 W), is located in Boone County on a large mountaintop surface mine operated by Black Castle Mining Company and owned by Alpha Natural Resources. Reclamation was done in 2011 by leveling unweathered overburden and covering it with a 20 to 30 cm layer of topsoil mixed with crushed weathered rock. Planting, fertilizing, and mulching occurred in the middle of June in 2011.

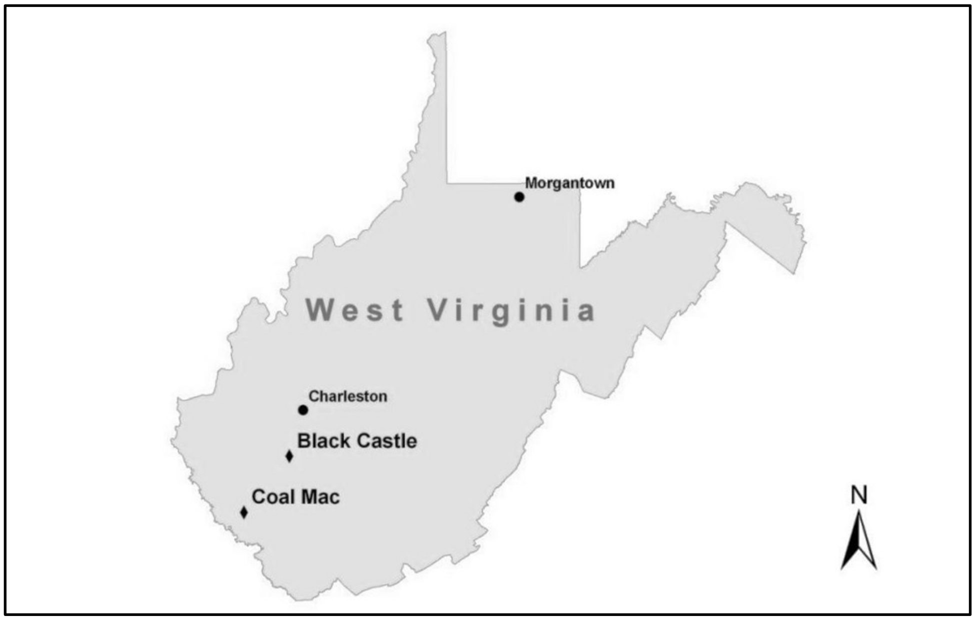

Figure 3.1. Schematic map showing location of Black Castle and Coal Mac sites in West Virginia.

3.2.2. Experimental Design

These sites were seeded with CIR switchgrass and four treatments were applied at planting to determine the effects of two levels of fertilizer and two levels of hydromulch. Diagrams of the treatments at the sites are shown in Figure 3.2 and 3.3. A randomized complete block design was used with five replications. Each block was 0.4 ha with four treatments randomly assigned to one of the quarters in each block. The four treatments were:

1. No fertilizer, light mulch: Control
2. Low fertilizer, light mulch: 33.6 kg N ha^{-1} and a light application of hydromulch
3. High fertilizer, light mulch: 67 kg N ha^{-1} and a light application of hydromulch
4. Low fertilizer, heavy mulch: 33.6 kg N ha^{-1} and a heavy application of hydromulch

Seed was applied at a rate of 11.2 kg PLS ha^{-1} to each plot using a Solo 421-S Portable Spreader. A 19-19-19 mixture of pelletized fertilizer was applied evenly by hand at the chosen rates to plots. After seeding and fertilizing, wood cellulosic mulch was applied at selected rates. Light mulch treatments received a coat of hydromulch approximately 1.7 Mg ha^{-1}, and the heavy mulch treatment received a coat of hydromulch approximately 3.0 Mg ha^{-1}. Three randomly selected sampling points were used to take biomass and soil samples from each treatment within blocks.

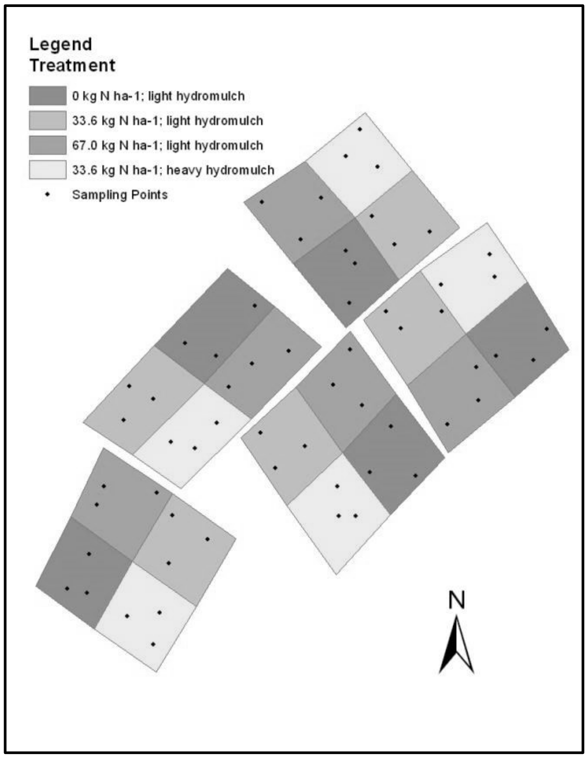

Figure 3.2: Schematic map of Coal Mac showing the plots and sub-sampling points.

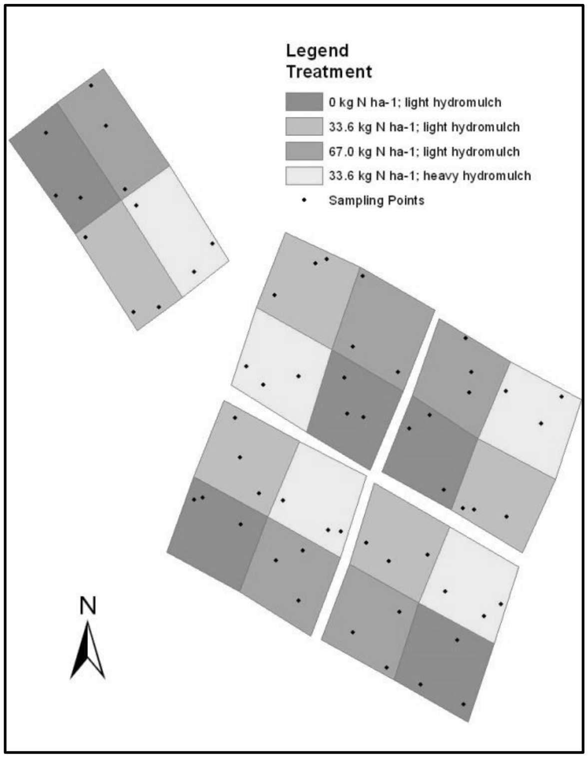

Figure 3.3: Schematic map of Black Castle showing the plots and sub-sampling points.

3.2.3. Vegetation measurement

Biomass samples were collected in 0.21-m^2 quadrats. These quadrats were placed five paces south of each sampling point. Samples were taken at the post-anthesis stage of switchgrass growth in October. All switchgrass within the quadrat was clipped to a stubble height of approximately 10 cm. No other plants were clipped for biomass samples. The samples were then oven dried at 60°C to a constant weight to determine dry weight.

3.2.4. Soil sampling

Soil samples were collected at both sites annually since the inception of this study. Soil samples were collected at each sampling point of the sites by taking a shallow shovel slice, approximately 15 cm in depth. For Black Castle and Coal Mac, the three sampling points within treatments were composited giving four soil samples per block (one sample per treatment). Soil samples were air dried and sieved to 2mm sized material (fine fraction). Percent fines were determined by weighing whole soil sample and then weighing fine fraction. Subsamples of fine fraction used for soil analysis were taken using a riffle splitter.

The fine fraction was used to determine pH, electrical conductivity (EC), and available nutrients. For pH, 5 g of soil was combined with 5 mL of distilled deionized (DDI) water. The mixture was placed on an orbital shaker table and mixed for 15 minutes, then allowed to equilibrate for at least 1 hour. A Mettler Toledo Seven Easy pH Meter was used to measure pH. Electrical conductivity was determined by combining 5 g of soil with 10 mL DDI water. The mixture was placed on an orbital shaker table and mixed for 15 minutes, then allowed to equilibrate for at least 1 hour. An Amber Science Inc. Digital Conductivity Meter was used to measure EC.

A Mehlich 1 solution was used to extract available elements from the soil (Wolf and Beegle, 1995). For extraction, 25 mL of Mehlich 1 solution was added to 5 g of soil, mixed on an orbital shaker for 5 minutes, and then allowed to equilibrate. The samples were then filtered through Whatman No. 2 filter paper. A Perkin Elmer inductively coupled plasma emission spectrometer as used to analyze the filtrate for available nutrients: Al, Fe, Mn, Mg, Ca, K, P, Ni, Cu, and Zn.

3.2.5. Statistical Analyses

The experimental design to test the treatment effects at Black Castle and Coal Mac was a randomized complete block design with five replications. Data were analyzed by a repeated-measures ANOVA using PROC MIXED procedures of Statistical Analysis System (SAS) (Statistical Analysis System, 2011). A randomized block design was used for the ANOVA with sites being blocks. Sites were fixed factors with replication year. Year and treatment blocks were random. Sub-sample weights were averaged to give one mean value per plot. Data were log transformed to satisfy the assumptions of normal distributions for ANOVA. The assumptions of normal distributions for ANOVA were satisfied using the Shapiro-Wilk normality test (Shapiro and Wilk, 1965). For each analysis, statistical significance was based on a p value of 0.05. The least square (LS) means are reported for yields. Tukey's Honestly Significant Different multiple comparison test was used to separate means for yields.

3.3 Results and Discussion

Soil tests showed slight differences between sites for pH, EC, and particle size (Table 3.1). Black Castle had much higher EC of 460 µS cm^{-1} when compared to Coal Mac that had an EC of 96 µS cm^{-1}. Black Castle and Coal Mac had similar soil pH (5.8 and 6.1, respectively).

Marra et al. (2013) previously determined the particle size distribution and determined the textural class for the sites. Black Castle is a loam and Coal Mac is a sandy loam. Black Castle had an average percent sand of 51, average percent silt of 35, and average percent clay of 13. Coal Mac had an average percent sand of 60, average percent silt of 28, an average percent clay of 12. Black Castle had mostly higher micro and macronutrients than Coal Mac (Table 3.2). Coal Mac had higher levels of K (20.8 cmol kg^{-1}) than Black Castle (0.11 cmol kg^{-1}). Each site had the same Na levels at 0.05 cmol kg^{-1}.

Table 3.1. Selected chemical and physical properties at Black Castle and Coal Mac averaged over 2011-2013.

	pH	EC	Percent Fines	Particle size distribution		
		µS cm^{-1}	%	% Sand	% Silt	% Clay
Black Castle	5.8	460	17	51	35	13
Coal Mac	6.1	96	42	60	28	12

Table 3.2. Values of extractable soil nutrients using Mehlich 1 solution at Black Castle and Coal Mac averaged over 2011-2013.

Parameter	Black Castle	Coal Mac
	-------- cmol charge kg^{-1} --------	
Mg	0.86	0.16
K	0.11	20.8
Na	0.05	0.05
Ca	1.32	0.24
	------------- mg kg^{-1} -------------	
Al	29.7	11.0
Fe	18.6	6.2
Mn	31.1	3.03
P	5.7	2.1
Ni	0.46	0.03
Cu	0.77	0.14
Zn	1.1	0.13

The two sites, Black Castle and Coal Mac, were comparable in the average amount of switchgrass they yielded over three years (Table 3.3). Black Castle produced 0.98 Mg ha^{-1} while Coal Mac produced 0.97 Mg ha^{-1} averaged over years and treatments ($p>0.05$). This is a positive result since the sites were reclaimed in a similar manner with a mixture of topsoil and brown weathered sandstone and soil properties were also shown to be similar (Table 3.1).

Table 3.3. Statistical significance for average yields for main effects of site, treatment, and year.

Effect		**P>F**	**Yield**
			Mg ha^{-1}
Site		0.94	
	Black Castle		0.98
	Coal Mac		0.97
Treatment		<0.01	
	No fertilizer; light mulch		0.32^{b}
	33.6 kg N ha^{-1}; light mulch		1.1^{a}
	33.6 kg N ha^{-1}; heavy mulch		1.1^{a}
	67 kg N ha^{-1}; light mulch		1.9^{a}
Year		<0.01	
	2011		0.28^{c}
	2012		0.95^{b}
	2013		2.7^{a}

Different letters denote significant difference at $p > 0.05$ level of probability according to Tukey's HSD. Untransformed, LS means are reported.

The treatments were statistically different in this experiment. As shown in Table 3.3, the treatment with no fertilizer and light mulch had the lowest yield with 0.32 Mg ha^{-1} averaged over years and sites. The other three treatments did not differ statistically. Averaged over years and sites, the plots fertilized with 33.6 kg N ha^{-1} averaged 1.1 Mg ha^{-1}, while the plots fertilized with 67 kg N ha^{-1} yielded 1.9 Mg ha^{-1}. It is clear that fertilizer added to the sites increased yield with the higher application rate producing higher yields. The fact that the yield for the 33.6 kg N ha^{-1}

was the same for both hydromulch rates gives evidence that the yield increase between the two fertilizer rates with the same hydromulch rates was due to the increase in fertilizer.

There was a main effect of year on yield averaged over sites and cultivars ($p<0.01$). Year 2013 had the highest biomass with 2.7 Mg ha^{-1} while 2011 produced the lowest yield with 0.28 Mg ha^{-1} (Table 3.3). This is to be expected as the year 2011 was the establishment year and switchgrass is slow to establish and generates only low amounts of biomass during the first two years after establishment. Higher yields were apparent by 2013, which was after the third growing season. There was no significant treatment x year interaction (Figure 3.4).

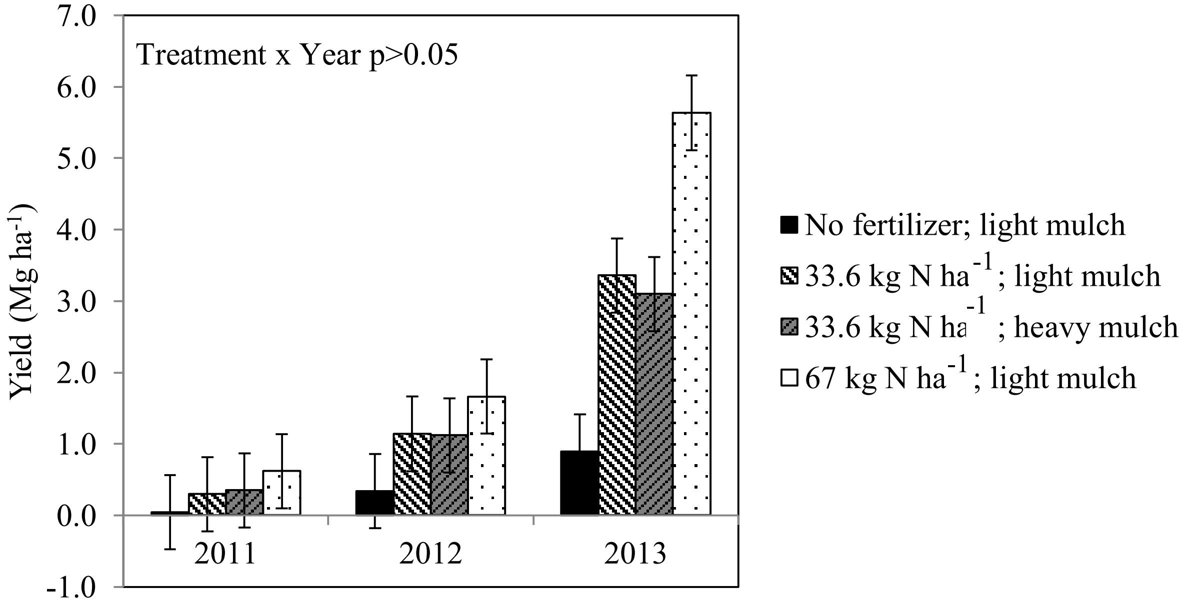

Figure 3.4. Yield of switchgrass treatments for each year averaged over the two sites. There was no significant treatment x year interaction. Untransformed, LS means are reported.

3.4 Conclusion

Determining the best establishment methods for switchgrass as a bioenergy feedstock grown on reclaimed mined lands is important information for mining operators in order to keep costs low and stand establishment high. The switchgrass cultivar Cave-in-Rock is able to grow on mine soils reclaimed similar to the sites used in this study. The control with no fertilizer and light mulch treatment yielded much lower biomass than the other treatments. This shows that adding at least 33.6 kg N ha^{-1} will be beneficial to establishing switchgrass and increasing yields. Switchgrass yield has also shown to respond to N applications by other studies. The treatments with fertilizer and mulch all had similar yields. Therefore, establishment with 33.6 N ha^{-1} and light mulch would be the best choice since it had high yields and is a lower cost and lower input system. The heavy mulch treatment did not seem to affect yield. The first three years of switchgrass growth are considered 'establishment years'. Future management of this study may show increased switchgrass yields and differences among the treatments with different fertilizer application rates.

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Chapter 4: Determining composition and ethanol potential of switchgrass grown on reclaimed surface mines in West Virginia using NIRS

4.1: Literature Review

4.1.1. Conversion technologies for cellulosic bioenergy materials

After biomass harvest of switchgrass, the technologies currently in place to convert switchgrass to heat energy or liquid fuels are either biochemical or thermochemical conversions (McKendry, 2002). Both technologies require more refinement to become economical for large-scale production. The four options available for the thermochemical conversion process for cellulosic crops to energy are combustion, pyrolysis, liquefaction and gasification (McKendry, 2002). Combustion provides direct thermal energy when switchgrass is burned with ample amounts of oxygen, but it is an inefficient way to generate energy from biomass (Digman et al., 2009). Pyrolysis is heating without oxygen at about 500°C and can produce liquid (bio-oil), gaseous, and solid (char) fractions. The liquefaction process does not require drying the biomass, which is a very costly part for combustion and pyrolysis (Brand et al., 2013). Under liquefaction, the biomass is thermally degraded in a solvent under temperatures of 250 to 400°C and pressures from about 4 to 5 MPa (Brand et al., 2013). To produce ethanol using gasification, switchgrass can be heated to produce syngas, which is composed of mainly carbon monoxide and hydrogen (Dwivedi et al., 2009). This gas can then either be fermented or catalytically converted into ethanol (Dwivedi et al., 2009). Production of ethanol through gasification is independent of the sugar present in the feedstock (Dwivedi et al., 2009).

Ethanol, through the biochemical platform, can be obtained using different processes, such as separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), and simultaneous saccharification and co-fermentation (SSCF) (Pessani et al., 2011). However, the products formed during the hydrolysis step in an SHF process, such as cellobiose and glucose, inhibit cellulase enzymes as well as the fermenting microorganisms (Alfani et al., 2000). In SSF, enzymatic hydrolysis and fermentation take place in the same vessel. The rationale for this approach is that since cellulase activity is inhibited by glucose, rapid fermentation into ethanol would increase the rate and efficiency of the overall process (Keshwani and Cheng, 2009). The SSF process has several other advantages, such as reduced operational costs, lower enzyme requirement, and increased productivity (Chen et al., 2007).

4.1.2. Switchgrass biofeedstock quality

The important determinants of biofuel conversion efficiency for the biochemical conversion process are the three major components of the switchgrass cell wall (hemicellulose, cellulose, and lignin). Cellulose is similar to starch in that it is a polymer of repeating glucose monomers. Glucose connected with β (1,4) bonds is what gives cellulose its strength and rigidity but also makes it much more difficult to break down than starch, which has glucose α (1,4) bonds. Hemicellulose is a hetero-polymer consisting of a main chain of xylan with branches of pentoses (arabinose), hexoses (glucose, galactose, mannose), and sugar acids (acetic) (Huber et al., 2006). Lignin is a complex polymer and differs in structure from plant to plant depending on the type of alcohols used to form it such as p-coumaryl, coniferyl, and sinapyl alcohols (Huber et al., 2006). The carbohydrate polymers, cellulose and hemicellulose, in ligno-cellulose are bound to lignin with mostly H bonds but also some covalent bonds (Lee, 1997). This complexity makes ligno-cellulosic material much more difficult to enzymatically degrade to fermentable sugars as compared to starch (Gray et al., 2006).

To ensure the most efficient conversion of plant biomass to biofuel, the carbohydrate fractions of ligno-cellulose (hemicellulose and cellulose) must be processed prior to hydrolysis (Keshwani and Cheng, 2009). This pre-treatment is not needed for sugar or starch crops but is necessary for cellulosic biofeedstocks because they have longer-chained polysaccharide molecules and there is a need to separate the recalcitrant lignin from the carbohydrate polymers (McKendry, 2002). Even after the biochemical pre-treatment, lignin cannot be processed by bacteria. Rather, the lignin can be used for producing bio-oil through pyrolysis, which can utilize lignin's high energy (David and Ragauskas, 2010). After pre-treatment, the hydrolysis process converts the ligno-cellulose into sugars. These sugar molecules are then converted into ethanol with the help of microorganisms. Fermentation of starch has been commercially dominated by the yeast *S. cerevisae* (Gray et al., 2006). The problem with this yeast and other commercially-used microorganisms is they do not metabolize C5 sugars, such as xylose and arabinose (Gray et al., 2006). Xylose from hemicellulose provides enough sugars to be included in ethanol conversion, but unfortunately right now the conversion of pentose sugars into ethanol is less efficient than the conversion of hexoses (Gray et al., 2006).

Switchgrass quality, in reference to biofuel production efficiency and yield, will be higher with greater carbohydrate fractions, fewer lignin constituents, and fewer inorganic metals (Vogel et al., 2011; Fahmi et al., 2007). Three sugars, glucose, mannose, and xylose, compose about 95 to 97% of the total sugars in ligno-cellulosic biomass (Dwivedi et al., 2009). The major fermentable sugars in biomass are glucose and xylose with arabinose, galactose, and mannose contributing significantly less (Gray et al., 2006). This is why a simple ethanol conversion calculation can be used to predict ethanol yield using the C6 sugars (mannose, galactose, glucose) and the C5 sugars (arabinose and xylose). A summary of the chemical composition of switchgrass and of corn stover are shown in Table 4.1.1.

Table 4.1.1. Compositional summary

				% Dry Matter									
Cultivars evaluated	**State**	**Time planted (Harvest date)**	**Ecotype**	**Ash**	**Lignin**	**Arabinan**	**Xylan**	**Mannan**	**Galactan**	**Glucan**	**Protein**	**Potential Ethanol Yield (L Mg^{-1})**	**Source Cited**
Alamo	TX	Planted 1985 (Fall 1992)	Lowland	5.7	17.4	2.6	22.2	0.3	0.9	34.5	3.5	394	DOE (2006)
Alamo	TX	Planted 1985 (Aug 1992)	Lowland	4.8	20.6	3.2	25.5	0.3	1	40.8	2.8	461	Wiselogel et al. (1995)
Cave-in-Rock	NE, IA, IN	Planted ? (Fall 1992)	Upland	5.9	17.9	3.1	22.2	0.3	1.1	32.5	5.7	385	DOE (2006)
Cave-in-Rock	NE	Planted ? (Fall 2003)	Upland	5.7	15.4	2.7	19.5	0.4	0.1	28.3	3.2	397	Dien et al. (2006)
Shawnee	OK	Planted 2005 (Dec 2006)	Upland	4.2	19.7	3.1	20	n/a	n/a	30.9	4.3	-	Kim et al. (2011)
Dacotah	SD	Planted 1999 (May 2008)	Upland	3.3	22.6	3.1	22.5	n/a	n/a	35.3	1.2	-	Kim et al. (2011)
			mean	4.9	18.9	3.0	22.0	0.3	0.8	33.7	3.5	400	
			min	3.3	15.4	2.6	19.5	0.3	0.1	28.3	1.2	334	
			max	5.9	22.6	3.2	25.5	0.4	1.1	40.8	5.7	488	
Hybrid Corn	MN, SD, IO, WI, MI, OH, IL, IN	Annually (2001-2003)		3.9	13.3	2.8	18.9	0.3	1.5	31.9	3.7	360	Templeton et al. (2009)

4.1.3. Chemical constituents of switchgrass

Switchgrass quality has been shown to have significant annual and spatial variation (Schmer et al., 2012). This can be due to precipitation (Jiang et al., 2012), management (Jarchow et al., 2012), and/or temperature (Adler et al., 2006). Differences in soil properties have been shown to change switchgrass ligno-cellulosic composition. Gillitzer et al. (2013) showed that grasslands in Minnesota with two different soil ratings had different lignin and ethanol production. Soils with high crop production index (CPI) ratings (CPI HIGH) grew plants with an average of 195 g kg^{-1} of lignin and soils with ratings of CPI LOW averaged lignin values of 192 g kg^{-1}. The CPI HIGH soils produced statistically higher yields (4.75 Mg ha^{-1}) than the CPI LOW soils (3.49 Mg ha^{-1}), which translated into the CPI HIGH soils having 1,873 L ha^{-1} ethanol production compared to 1,386 L ha^{-1} with CPI LOW soils. Within-field variation does not seem to have an effect on switchgrass quality because individual fields are subject to similar temperatures, precipitation, and harvest management (Schmer et al., 2012). This is useful because bio-refineries can run fewer analyses of switchgrass if shipments come to the refinery from the same fields.

Many studies have reported differences in switchgrass cultivars' ligno-cellulosic compositions (Adler et al., 2009; David and Ragauskas, 2010; Monono et al., 2013; Schmer et al., 2012), while others have shown similarities among cultivars (Liu et al., 2010; Yan et al., 2010). For example, Schmer et al. (2012) showed Cave-in-Rock (CIR) and Shawnee cultivars had higher hexose concentrations than Trailblazer cultivar, while Trailblazer had higher 5-yr mean glucose concentrations than the other two cultivars. Trailblazer had a higher theoretical ethanol yield (L Mg^{-1}) compared to Shawnee at one of three sites. Cultivar differences were also evident for theoretical ethanol production (L ha^{-1}) at two of the sites. Although different ligno-cellulosic compositions between cultivars may affect ethanol yield on a mass basis (L Mg^{-1}), greater aboveground biomass may likely be the determining factor in cumulative ethanol production values (L ha^{-1}) (Monono et al., 2013).

4.1.4. Wet Chemistry

Conducting wet chemistry analyses on individual biomass samples are expensive and time consuming processes. To create a comprehensive list of compositional analytes, it would cost approximately $300 per switchgrass sample without including equipment costs (Vogel et al. 2011). Although a biorefinery would not need an extensive list of forage traits and cell wall components to determine quality for ethanol production, analyzing large number of samples can become very costly. General forage quality information that might be important to a biorefinery are ash, structural and soluble carbohydrate content, lignin, and moisture. Generally, separate procedures are used to analyze each constituent or trait that is in question. For example, a procedure used by Monono et al. (2013) to extract main sugars starts by removing the non-structural components with 95% ethanol using a solvent extractor and heat. Dried biomass is hydrolyzed in 72% sulfuric acid for 1 hr which is then diluted to 4% sulfuric acid and autoclaved for another hour. Sugars glucose, xylose, arabinose, and galactose are obtained using an HPLC and a carbohydrate column. Sugar peaks are then detected by a refractive index detector. To determine Klason lignin, the sample must be ground through a 1-mm screen and mixed with solvents. The solution is then mixed with H_2SO_4 and stirred for 2 hrs. It is then diluted and boiled for 4 hrs. The lignin is then weighed, washed, and dried to a constant weight. Ash can be determined by weighing sample before and after being heated at 450 °C for 6 hrs (Vogel et al., 2011). Many procedures for compositional analyses can be found in National Renewable Energy Laboratory (NREL) analytical procedures (NREL, 2014). As is apparent from the foregoing discussion, analyzing individual sugars and forage traits requires a significant amount of time, equipment and money.

4.1.5. Near infrared reflectance spectroscopy

Recent research has demonstrated the feasibility of determining the chemical constituents of bio-feedstocks with an alternate method called Near Infrared Reflectance Spectroscopy (NIRS) rather than by traditional wet chemistry techniques. NIRS is a tool that can help distinguish the forage quality of bioenergy crops and help predict theoretical ethanol yields. NIRS analysis uses a spectrophotometer to help quantify chemical constituents of a sample by using the spectral characteristics of that sample (Vogel et al., 2011). This instrument can be simplified into three basic parts (Figure 4.1.1): a near-infrared (NIR) source (lamp), a wavelength isolator, and a detector (Tsuchikawa, 2007). The lamp transmits NIR light in wavelengths ranging from 2500 nm to 800 nm to the sample. The detector senses the wavelengths that are reflected-off or transmitted-through the sample, depending on the type of sample presentation used, and then a computer uses this information to make spectra.

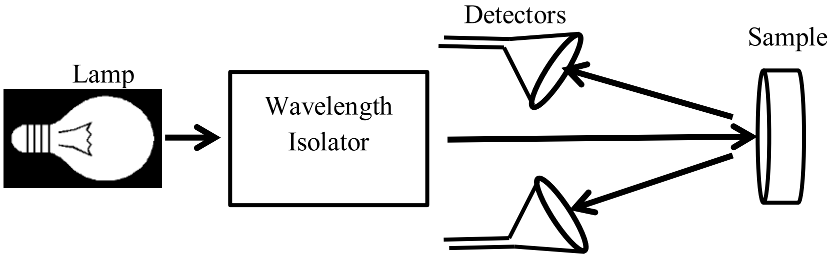

Figure 4.1.1. Simplified example of a near infrared spectrophotometer (McClure and Tsuchikawa, 2007).

Forage samples can have varying spectra because their molecular functional groups absorb NIR light differently, thereby giving absorption bands (Figure 4.1.2) (Murray, 1993). These highly overlapping bands make it difficult to differentiate specific functional groups from one another by looking at their absorption spectra (Murray, 1993). This is the main reason NIR was unexplored until the 1970's when computers were finally able to analyze these complex patterns for differentiation (Murray, 1993). NIR was initially found to be advantageous because water absorbs strongly in the NIR spectrum range (Murray, 1993), which provided an easy way to measure moisture in forages (Murray, 1993).

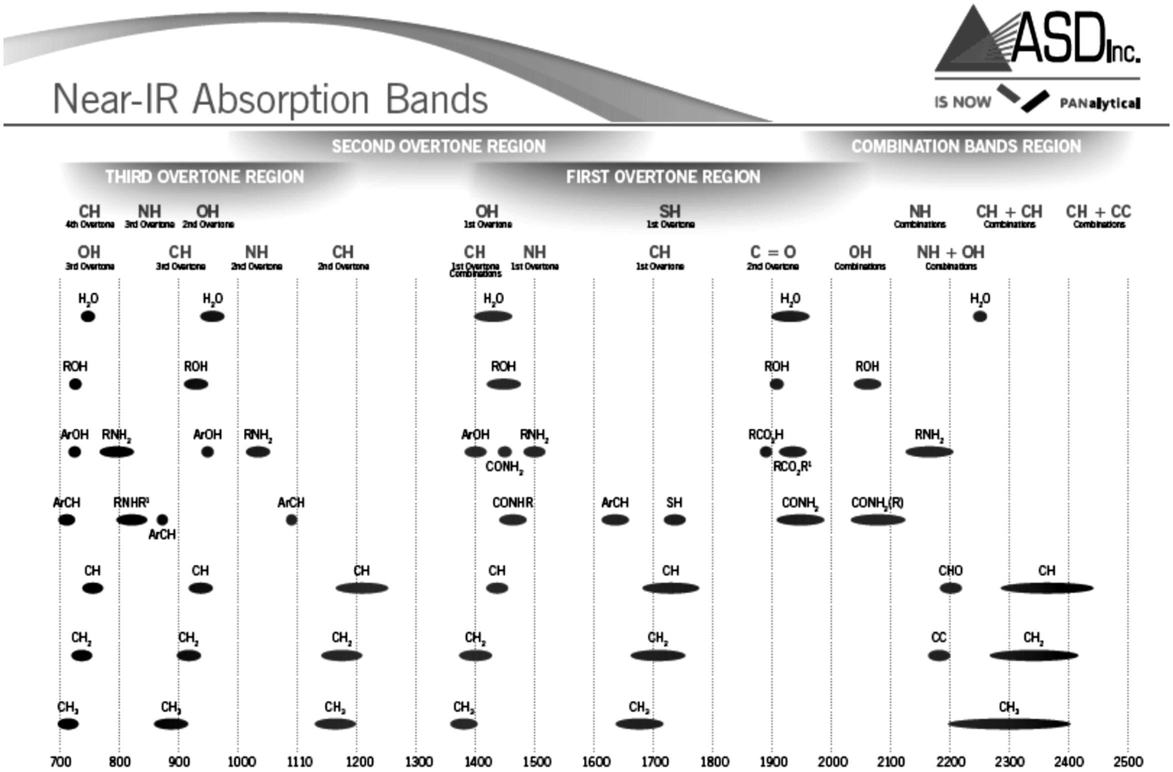

Figure 4.1.2. Absorption bands of functional groups in the near infrared spectra. Units are in nm wavelength. (ASD Inc., 2013).

Absorption bands of functional groups are created when NIR radiation vibrates at the same frequency as a molecular bond in the same sample (Shenk et al., 1992). Hydrogen-bearing functional groups, -CH, -OH, and –NH, dominate the NIR absorbance spectra (Murray, 1993) because these are the molecular vibrations that exist in the NIR region (Shenk et al., 1992). NIRS is able to detect hemicellulose, cellulose, and lignin because they contain hydrogen-bearing functional groups. The NIR spectrum (0.7 to 2.7μm) is divided into the overtone (0.7 to 1.8μm) and combination (1.8 to 2.7μm) bands (Figure 4.1.2) (Shenk et al., 1992). For each fundamental absorption band, a series of overtones is shown that get weaker by a factor of 10 to 100 (Murray, 1993; Shenk et al., 1992). For example, the first overtone of -CH occurs at nearly half the wavelength of the fundamental band while the second overtone occurs at nearly one third of the wavelength of the fundamental band (Murray, 1993).

Calibration is needed to correlate spectra with specific chemical constituents for development of prediction equations or models. Wet chemistry is used to determine the constituents present in the samples and the values are used for calibration. Utilizing laboratory and spectral data, prediction equations are developed and validated using mathematical and statistical procedures (Vogel et al., 2011). Forage samples for calibration are selected to represent the range of characteristics, such as maturity and crop management, in the population of interest (Vogel et al., 2011).

A major advantage of NIRS is the speed of the analysis, which allows testing of many samples in minutes as compared to chemical analysis that would take hours to days (Williams, 2007). A major disadvantage of NIRS is the need for calibration and also its lack of precision when measuring minerals and other constituents that are not hydrogen-bearing (Lupoi et al., 2014). NIRS has not been widely adopted for commercial use in bioenergy field because the NIRS spectrometer instrument is expensive and the calibration process is still being worked out for switchgrass models. However, once the device is purchased and properly set-up, it provides an inexpensive, non-destructive means to test many constituents from one sample (Williams, 2007). This makes the NIRS a less costly tool over the long-term, as it can perform the same functions as several different devices.

4.1.6. Objectives

Currently, there is no research regarding compositional analysis of switchgrass grown on reclaimed surface mines. There are two primary objectives of this study. The first objective was to determine if three switchgrass cultivars grown on surface mines reclaimed with a more intensive reclamation process had similar composition and theoretical ethanol yields. There were clearly different aboveground biomass levels among cultivars, but the higher yields may not have necessarily produced better forage quality for ethanol production. The second objective was to determine the effect of establishment techniques (fertilizer and mulch application) on the composition and theoretical ethanol yield of Cave-in-Rock grown on reclaimed surface mines.

4.2 Materials and Methods

4.2.1. Site Locations

This study investigates the compositional analysis of switchgrass samples collected from four reclaimed surface mine sites described in earlier chapters. To determine compositional differences of three switchgrass cultivars, forage samples were collected from Hampshire. Chapter 2 provides the locations and reclamation procedures for this site. To determine compositional differences of Cave-in-Rock grown with different fertilizer and mulch treatments, forage samples were collected from Black Castle and Coal Mac. Chapter 3 provides the locations and reclamation techniques used at these two sites.

4.2.2. Brief Description of Experimental Designs

For Hampshire mine site, nine plots were laid out in 2008, each 0.4 ha in size. A completely randomized design using three cultivars of switchgrass were replicated three times and randomly assigned to the nine plots. Switchgrass cultivars, Cave-in-Rock, Carthage, and Shawnee, were broadcast-seeded at a rate of 11.2 kg PLS. Six sampling points were randomly chosen within each plot for aboveground biomass measurements by clipping.

For Black Castle and Coal Mac mine sites, in 2009 these sites were seeded with Cave-in-Rock and four treatments were applied at planting to determine the effects of two levels of fertilizer and two levels of hydromulch. A randomized complete block design was used with five replications. Each block was 0.4 ha with four treatments randomly assigned to one of the quarters in each block. Three sampling points were randomly chosen within each treatment for aboveground biomass measurements by clipping. The four treatments were:

1. No fertilizer, light mulch: Control; light application of hydromulch
2. Low fertilizer, light mulch: 33.6 kg N ha^{-1} and a light application of hydromulch
3. High fertilizer, light mulch: 67 kg N ha^{-1} and a light application of hydromulch
4. Low fertilizer, heavy mulch: 33.6 kg N ha^{-1} and a heavy application of hydromulch

4.2.3. Biomass preparation and NIRS scanning

Switchgrass biomass samples were prepared before analysis with the spectrophotometer (Figure 2). Any biomass sample that appeared to be ≥ 3 grams was processed by the following procedure. The entire biomass sample clipped within the 0.21-m^2 quadrat was ground to pass a 2-mm screen of a shear (or cutting) mill (Wiley Laboratory Mill, Mod. 4, Thomas Scientific, Swedesboro, NJ 08085). Very large biomass samples were first downsized through a 5-mm screen of a Wiley Mill to a 5-mm particle size before being ground to a 2-mm particle size. A riffle splitter was used to take a sub-sample from the ground sample and this sub-sample was ground further in a cyclone mill through a 1-mm screen. These powder subsamples were packed into reflection mode sample cells, shown in Figure 4.2.1 (Tsuchikawa, 2007). Since a consistent packing density is very important for powder samples and the packing density significantly influences reflectance and spectral output, we were careful to pack each sample cell firmly and uniformly (Tsuchikawa, 2007).

The filled sample cell were placed on a SpectraStar 2400 RTW scanning monochromator (Unity Scientific, Brookfield, CT 06804) to collect spectra data. Spectral data were recorded as the reciprocal log of reflectance (log 1/R) at 1-nm increments over a range of 1250-2349 nm.

Figure 4.2.1. (Left) The NIR spectrophotometer that was used for this experiment. (Right) A reflection mode sample cell.

4.2.4. Calibration of Spectra

Spectra files were combined by site and year. The spectra were standardized to a master instrument (Foss model 6500) managed by the NIRS Forage & Feed Testing Consortium (NIRSC) and from which equations for compositional analysis were obtained. The spectra were then inserted into two calibration equations. The first was a grass hay equation (13ghu24.prd) which included cool- and warm-season grasses (with switchgrass) and the second was a switchgrass bioenergy equation based on samples from researchers at the USDA ARS (Vogel et al., 2011).

The calibrations that were used to predict bioenergy traits were created by Vogel et al. (2011) on a geographically-wide study of switchgrass cultivars in the Midwestern USA. These samples were grown in agricultural settings and represent diverse varieties, locations, and harvesting techniques and times. Many forage quality constituents can be found with this calibration but only 13 feedstock traits are of interest to predict ethanol yield (Table 4.2.1). The actual ethanol yield (ETOHL) via SSF was calculated using the ethanol that was reduced from hexoses. The total theoretical ethanol production was calculated from all biomass sugars (hexose and pentose). Since it is difficult to ascertain the exact conversion percentages, these equations assume 100% ethanol conversion even though this is not realistically possible (Vogel et al., 2011). Forage traits and cell wall components predicted with NIRS are shown in Table 4.2.1.

To decide if our switchgrass spectra 'fit' the two calibration equations and whether the constituents were considered accurate, the mean global H values, or global distance (GD) values, needed to be below 3. If these values were over 3, they were considered to be outliers and the prediction value for that constituent was not used. Another source of information that shows 'fit' is the nearest distance (ND) value. This relates how close the individual calibration spectra points are to the individual data in question. Prediction values for a constituent were deleted if they had an ND value greater than 1.2.

Table 4.2.1. Compositional traits predicted with NIRS.

Variable	Abbreviation	Reference or Equation
<u>Forage quality traits</u>		
In vitro dry matter digestibility	IVDMD	Vogel et al. (2011)
Neutral Detergent Fiber	aNDF	NIRSC (2013)
Acid detergent fiber	ADF	NIRSC (2013)
Nitrogen	N	CP = Nx6.25; NIRSC (2013)
48 hour In vitro dry matter digestibility	IVTDMD48	NIRSC (2013)
48 hour Neutral detergent fiber digestibility	NDFD48	NIRSC (2013)
Ash	ASH	NIRSC (2013)
Lignin	LIGNIN	NIRSC (2013)
<u>Cell wall constituents</u>		
Arabinose	ARA	Vogel et al. (2011)
Xylose	XYL	Vogel et al. (2011)
Mannose	MAN	Vogel et al. (2011)
Galactose	GAL	Vogel et al. (2011)
Glucose	GLC	Vogel et al. (2011)
Sucrose	SUC	Vogel et al. (2011)
Soluble glucose	GLCS	Vogel et al. (2011)
Fructose	FRU	Vogel et al. (2011)
Starch	STA	Vogel et al. (2011)

4.2.4. Estimation of Ethanol Yield

The individual sugar data obtained from compositional analysis was used to predict theoretical ethanol yield and production using the equations below. Two approaches were used to estimate theoretical ethanol yield and production from the switchgrass grown at Hampshire. The first equation from Vogel et al. (2011) is more comprehensive and includes main cell wall sugars and soluble carbohydrates. The second equation from the US Department of Energy (DOE, 2009) includes only the main cell wall hexose and pentose fermentable sugars. Not all constituents were able to be predicted for the Black Castle and Coal Mac data. Therefore, a simplified NREL equation was used taking into consideration only conversion from XYL and GLC.

Units for sugars in equations are in % of DM.

Equations used from Vogel et al. (2011) for Hampshire:

1. Theoretical ethanol yield (HEXEL) from all biomass hexoses:

$$\mathrm{HEXEL}\ (\mathrm{L\ Mg^{-1}}) = (((\mathrm{MAN+GAL+GLC+STA}) \times 0.57) + ((\mathrm{GLCS+FRU}) \times 0.51) + (\mathrm{SUC} \times 0.537))\ \ 1.267)\text{; assuming 100\% conversion}$$

2. Theoretical ethanol yield (PENTETL) from pentose sugars:

$$\mathrm{PENTETL}\ (\mathrm{L\ Mg^{-1}}) = (\mathrm{ARA} + \mathrm{XYL}) \times 0.579 \times 1.267$$

3. Total theoretical ethanol yield (ETOHTLT) from all biomass sugars:

$$\mathrm{ETOHTLT}\ (\mathrm{L\ Mg^{-1}}) = \mathrm{HEXEL} + \mathrm{PENTETL}$$

4. Total theoretical ethanol production (ETOHTLTH) from all biomass sugars:

$$\mathrm{ETOHTLTH}\ (\mathrm{L\ ha^{-1}}) = \mathrm{ETOHTLT} \times \text{biomass production yield of field or site } (\mathrm{Mg\ ha^{-1}})$$

Equations used from NREL for Hampshire:

5. Theoretical ethanol yield (NRELC6) from hexoses using NREL equation:

$$\mathrm{NRELC6} = (\mathrm{GLC+GAL+MAN})/100 \times 0.51 \times 3.79/2971 \times 1000000$$

6. Theoretical ethanol yield (NRELC5) from pentoses using NREL equation:

$$\mathrm{NRELC5} = (\mathrm{ARA+XYL})/100 \times 0.51 \times 3.79/2971 \times 1000000$$

7. Theoretical ethanol yield (NREL) using NREL equation:

$$\mathrm{NREL} = \mathrm{NRELC6} + \mathrm{NRELC5}$$

8. Theoretical ethanol production (EthProdNREL) using NREL equation:

$$\mathrm{EthProdNREL} = \mathrm{NREL} \times \text{biomass production yield of field or site } (\mathrm{Mg\ ha^{-1}})$$

Simplified Equations used from NREL for BC and CM

9. Theoretical ethanol yield (NREL_GLC) from hexoses using NREL equation:

NREL_GLC = (GLC)/100 × 0.51 × 3.79/2971 × 1000000

10. Theoretical ethanol yield (NREL_XYL) from pentoses using NREL equation:

NREL_XYL = (XYL)/100 × 0.51 × 3.79/2971 × 1000000

7. Theoretical ethanol yield (NREL) using NREL equation:

NREL = NREL_GLC + NREL_XYL

8. Theoretical ethanol production (EthProdNREL) using NREL equation:

EthProdNREL = NREL × biomass production yield of field or site (Mg ha^{-1})

4.2.5. Statistical Analyses

Data were analyzed by ANOVA using PROC MIXED procedures of Statistical Analysis System (SAS) (Statistical Analysis System, 2011). Data were not transformed because the assumptions of normal distributions for ANOVA were satisfied using the Shapiro-Wilk normality test (Shapiro and Wilk, 1965). Variables NRELPROD and STA for Black Castle and Coal Mac data were not normally distributed and were thus transformed before ANOVA. Ln_NRELPROD and Ln(STA/0.837 +0.2) were used for NRELPROD and STA. Transformation for STA was used because some rows had a zero value. Sites were fixed factors with replication year. Year and plot were random. For each analysis, statistical significance was based on a p value of <0.05. The least square (LS) mean yields are reported.

4.3. Results and Discussion

The NIRS compositional analysis statistics for switchgrass samples were presented on a whole biomass, % dry weight basis.

4.3.1. Differences in chemical composition of switchgrass cultivars

There were significant differences among each cultivars' forage quality traits (Table 4.3.1). CIR had the highest concentrations of aNDF, ADF, and LIGNIN while Carthage had the lowest (see Table 4.2.1 for forage trait abbreviations). Carthage had the highest concentrations in the remaining constituents. ASH was the only trait that was not significantly different among cultivars. The measured values for each constituent for Shawnee were consistently between the values measured for CIR and Carthage. Thus, the forage traits of Shawnee were similar to the other two cultivars for all forage quality traits. There were no cultivar x year interactions.

Our forage compositional values are comparable to those cited in other literature. Cortese and Bonos (2013) showed a significant year by cultivar effect for ash content across two years and 10 cultivars. Similar to our findings, CIR and Carthage did not differ statistically, but ash was reportedly much lower being 2.0%, averaged over the two cultivars and two years. Lemus et al. (2002) had similar results for NDF with CIR being statistically higher than Carthage, and Shawnee was similar to both cultivars. CIR had the highest NDF concentration with 76.6%, Shawnee was reported to be 75.1%, and Carthage was lowest at 74.8%. Our data for CIR seems a little higher for NDF and ADF than that reported by Lemus et al. (2002). CIR, Carthage, and Shawnee were not statistically different for ADF and averaged 43.8% (Lemus et al. (2002).

Table 4.3.1. Forage quality traits of switchgrass grown at Hampshire.

		Forage quality traits							
		IVDMD$^{¥}$	aNDF^{+}	ADF^{+}	N^{*+}	IVTDMD48^{+}	ASH^{+}	LIGNIN^{+}	NDFD48^{+}
		----------------% DM----------------							% NDF
Cultivar									
	CIR	33.6b	84.0a	51.5a	0.37b	49.4b	5.3	6.4a	30.4b
	Carthage	39.9a	76.9b	47.4b	0.83a	56.7a	6.3	5.1b	38.2a
	Shawnee	36.6ab	79.7ab	49.4ab	0.61ab	53.4ab	5.8	5.8ab	34.0ab
	Mean	36.7	80.2	49.5	0.61	53.2	5.8	5.8	34.2
	P value	0.05	*<0.01*	*0.05*	*0.02*	0.01	NS	*<0.01*	*0.04*
Year									
	2012	36.3	81.0	49.4	0.69	51.6	6.1	5.9	32.4
	2013	37.1	79.3	49.5	0.52	54.7	5.6	5.6	36.0
	Mean	36.7	80.2	49.5	0.61	53.2	5.8	5.8	34.2
	P value	NS	NS	NS	NS	NS	NS	NS	NS

Different letters denote significant difference at $p<0.05$ level of probability. LS means are reported.

$^{¥}$IVDMD = In vitro dry matter digestibility; NDF = neutral detergent fiber; ADF = acid detergent fiber; N = nitrogen; IVTDMD48 = 48 hour In vitro true dry matter digestibility; NDFD48 = 48 hour neutral detergent fiber digestibility as percentage of NDF; ASH = ash; LIGNIN = lignin.

$^{+}$ indicates constituent predicted with the 2013 NIRSC forage equation.

* N*6.25 = CP.

Cell wall constituents

Concentrations of MAN and GLC were significantly different across cultivars (Table 4.3.2). These compounds behaved similarly to previously measured forage traits, as Shawnee had the intermediate values making it similar to the other two cultivars. CIR recorded the highest values for both GLC and MAN. GLC averaged 30.0% and ranged from 31.7% for CIR and 28.5% for Carthage. MAN averaged 0.80% and ranged from 0.94% for CIR to 0.77% for Carthage. The GLC results seem to be within the range of previously reported data. Adler et al. (2006) showed statistically different GLC concentrations over years for CIR at 28.6% and 32.5%. Schmer et al. (2012) reported averages of 27.6% GLC for CIR, while Dien et al. (2006) found a post-frost harvest GLC concentration of 32.2%. Similar to our results, Shawnee was reported to have GLC concentrations of 28.1% (Schmer et al., 2012). Averaged over cultivars, MAN concentrations were statistically higher at 0.91% in 2012 than in 2013 with 0.78%. In other studies, MAN concentration for CIR was found to be closer to 0.50% with Adler et al. (2006) averaging 0.59% and Dien et al. (2006) reporting 0.50%.

The ARA, XYL, SUC, STA concentrations were all similar among cultivars. XYL ranged from 20.6 to 22.2 %, ARA ranged from 2.8 to 2.9 %, SUC from 2.7 to 3.0%, and STA from 0.18 to 0.43 %. XYL is a major fermentable sugar. Other studies reported XYL values for CIR and Shawnee within our range. For CIR, Adler et al. (2006) had an average XYL concentration of 22.2% and Dien et al. (2006) reported 22.3%. Schmer et al. (2012) showed Shawnee as having 20.6% GLC.

The remaining sugars, GAL, GLCS, and FRU, were different among cultivars. Carthage had the highest concentration for GAL with 0.93%, while CIR and Shawnee had similar values of 0.83%. GLCS was highest for both Carthage and Shawnee at 1.6%. Carthage showed the highest concentration of FRU with 1.1% while CIR and Shawnee had similar values. For GAL, 2012 had higher GAL concentration at 0.93% while 2013 was 0.80%. Years also differed for STA with 2012 having a lower concentration of 0.16% and 2013 with 0.42%. There were no cultivar x year interactions for any cell wall component.

Table 4.3.2. Compositional values of switchgrass grown on Hampshire.

		Cell wall constituents								
		ARA[¥]	XYL	MAN	GAL	GLC	SUC	GLCS	FRU	STA
		% DM								
Cultivar										
	CIR	2.8	22.2	0.94a	0.83b	31.7a	2.7	1.4b	0.58b	0.25
	Carthage	2.9	20.6	0.77b	0.93a	28.5b	3.1	1.6a	1.1a	0.18
	Shawnee	2.8	21.4	0.82ab	0.83b	29.7ab	3.0	1.6a	0.83b	0.43
	Mean	2.8	21.4	0.8	0.9	30.0	2.9	1.5	0.8	0.3
	P value	NS	NS	0.04	<0.01	<0.01	NS	<0.01	<0.01	NS
Year										
	2012	2.8	21.3	0.91	0.93	29.3	2.8	1.6	0.89	0.16
	2013	2.8	21.5	0.78	0.80	30.6	3.0	1.5	0.79	0.42
	Mean	2.8	21.4	0.8	0.9	30.0	2.9	1.5	0.8	0.3
	P value	NS	NS	0.02	<0.01	NS	NS	NS	NS	<0.01

Different letters denote significant difference at $p<0.05$ level of probability according to Tukey's HSD. LS means are reported.

[¥] ARA = arabinose; XYL = xylose; MAN = mannose; GAL = galactose; GLC = glucose; SUC = sucrose; GLCS = soluble glucose; FRU = fructose; STA = starch.

Ethanol prediction

Some ethanol yield characteristics showed differences among cultivars (Table 4.3.3). HEXEL (which included MAN, GAL, GLC, STA, GLCS, FRU, SUC) was highest for CIR at 274 L Mg^{-1} while Carthage and Shawnee were only slightly lower with an average value of 262 L Mg^{-1}. HEXEL was the only value that differed between years with 2013 having the higher value at 271 L Mg^{-1}. PENTETL (which included ARA and XYL) did not show differences among cultivars and ranged from 160 to 183 L Mg^{-1}.

ETOHTLT among cultivars was similar and ranged from 426 to 457 L Mg^{-1} with an average value of 437 L Mg^{-1}. Schmer et al. (2012) also did not show differences between CIR and Shawnee for ETOHTLT yields. Averaged over sites and years, they showed CIR yielded 420 L Mg^{-1} and Shawnee yielded 425 L Mg^{-1}. Ethanol production potential did show differences among cultivars. CIR had the highest EthProd with 7,348 L ha^{-1} while Carthage and Shawnee averaged 2,930 L ha^{-1}. Our values for CIR were much higher and Carthage and Shawnee were only slightly higher than the ethanol production values reported by Schmer et al. (2012). They did not find the two cultivars differed with CIR ranging from 3,286 to 2,310 L ha^{-1} and Shawnee ranging from 2,032 to 2,247 L ha^{-1} (Schmer et al., 2012).

Another more simplified ethanol equation was used to evaluate ethanol yield and production potential. NREL C6 (which included MAN, GAL, GLC) was highest for CIR at 217 L Mg^{-1} which was similar to Shawnee with 204 L Mg^{-1}. NREL C5 (which included ARA and XYL) did not show differences. Adler et al. (2006) used the same equation to estimate CIR ethanol yield and production. They had more constituents included into the hexose and only included XYL into the pentose equation.

Ethanol yield from the NREL equation was highest for CIR at 380 L Mg^{-1}, which was similar to Shawnee at 362 L Mg^{-1}, and Carthage had the lowest with 349 L Mg^{-1}. CIR also had the highest ethanol production value calculated from NREL with 6,092 L ha^{-1} while Carthage and Shawnee averaged 2,465 L ha^{-1}, which followed the same trend as that for HEXEL. Adler et al. (2006) showed CIR producing an average of 427 L Mg^{-1} averaged over two fall season harvests. Using the same NREL equation, two upland varieties averaged over a three-year period showed both Dacotah and Sunburst yielding approximately 350 L Mg^{-1} while Dacotah ethanol production was approximately 3,800 L ha^{-1} and Sunburst produced approximately 5,000 L ha^{-1} (Monono et al., 2013). A non-irrigated site from that same paper showed Sunburst yielding approximately 375 L Mg^{-1} and producing approximately 2,000 L ha^{-1} due to low yielding performance while Dacotah yielded 300 L Mg^{-1} and 1,800 L ha^{-1} (Monono et al., 2013). There were no cultivar x year interactions. CIR and Shawnee would be considered 'high yielding' by Schmer et al. (2008) since they had yielded similarly or greater than 4,000 L ha^{-1}.

The main factor that drove CIR having the highest ethanol production was its higher yield compared to Shawnee and Carthage. Our theoretical ethanol yields are much higher than values in the literature, which could be due to our sampling methods. Our estimates were based on clipped plots, where we carefully clipped and collected the biomass for determining dry weight. In conventional large-scale biomass harvesting, Adler et al. (2006) showed that 21% of biomass can be left behind. This loss of biomass during large-scale harvesting will lower our biomass yield and in turn our ethanol production values to those of other field studies.

Table 4.3.3. Switchgrass theoretical ethanol yield and ethanol production from Hampshire.

		Theoretical ethanol yield and production							
		HEXEL[¥]	PENTETL	ETOHTLT	EthProd	NRELC6	NRELC5	NREL	EthProdNREL
		L Mg^{-1}	L Mg^{-1}	L Mg^{-1}	L ha^{-1}	L Mg^{-1}	L Mg^{-1}	L Mg^{-1}	L ha^{-1}
Cultivar									
	CIR	274a	183	457	7348a	217a	162	380a	6092a
	Carthage	258b	173	430	1873b	196b	153	349b	1526b
	Shawnee	266ab	160	426	3988b	204ab	158	362ab	3405b
	Mean	266	172	438	4403	206	158	364	3674
	P value	<0.01	NS	NS	<0.01	<0.01	NS	0.02	<0.01
Year									
	2012	261	165	426	3556	202	157	359	3031
	2013	271	178	449	5250	210	158	368	4317
	Mean	266	172	438	4403.0	206	158	363	3674
	P value	<0.01	NS	NS	NS	NS	NS	NS	NS

Different letters denote significant difference at $p > 0.05$ level of probability. LS means are reported.

[¥] HEXEL = theoretical ethanol yield from all biomass hexoses; PENTETL = theoretical ethanol yield from pentose sugars; ETOHTLT = total theoretical ethanol yield from all biomass sugars; EthProd = total theoretical ethanol production from all biomass sugars; NRELC6 = theoretical ethanol yield from hexoses using NREL equation; NRELC5 = theoretical ethanol yield from pentose sugars using NREL equation; NREL = theoretical ethanol yield from NRELC6 and NREL C5; EthProdNREL = theoretical ethanol production from NREL C6 and NREL C5.

4.3.2. Differences in switchgrass chemical composition due to fertilizer applications at Black Castle and Coal Mac sites

For forage quality traits, CIR switchgrass grown at Black Castle differed for ADF, NDFD48, and LIGNIN concentrations compared to Coal Mac. The main effect of year differed significantly for all forage traits, but it varied as to which year had the highest and lowest values. NDF, ADF, and LIGNIN were statistically higher in 2013, while digestibility traits (IVDMD, IVTDMD48, and NDFD48) were statistically lower in 2013 compared to 2012. Although there were no Treatment x Year interactions, the NDF, ADF, and LIGNIN across treatments had higher values in 2013, IVDMD and IVTDMD48 treatments had lower values in 2013, and NDFD48 had similar values between years. Composition of switchgrass is known to vary among years because of environmental differences and harvesting times. Our forage trait differences are probably due to environmental causes, such as rainfall and temperature, but this pattern may relate to the age and maturity of the switchgrass. As switchgrass matures, lignin, hemicellulose, and cellulose all increase. An increase in cell wall and lignin will decrease the digestibility of the plant. A longer study would have to confirm that it was older switchgrass growth and not environmental differences between years that effected switchgrass composition.

Treatments differed statistically for all forage quality traits. No fertilizer with low mulch (NF:LM) had a significantly higher value for IVDMD at 42.8% as compared to the other treatments that averaged 37.5%. Guretzky et al. (2011) showed a different relationship with the lowland cultivar Alamo switchgrass with their treatment of 90 kg N ha^{-1} having the higher IVDMD value of 48.5% as compared to the no fertilized and 45 kg N ha^{-1} treatments having 47.9% and 47.3% IVDMD. The highest percentage of aNDF was for the low fertilizer with high mulch treatment (LF:HM) with 78.3% which was similar to the low fertilizer with low mulch treatment (LF:LM) and high fertilizer with low mulch (HF:LM). NF:LM had the lowest value of aNDF with 71.5%, which was similar to the HF:LM that had 75.0%. These values are lower than Alamo switchgrass reported to be 80.7% NDF averaged across three fertilizer treatments (Guretzky et al., 2011). For ADF, NF:LM had the lowest value of 37.9% and was different than the other three treatments which were all similar and averaged 41.3%. A similar pattern was shown by Guretzky et al. (2011) with their no fertilizer treatment having the lowest ADF value of 47.5% while 45 and 90 kg N ha^{-1} treatments averaged 48.1%. For IVTDMD48, NF:LM was the highest with 62.5% while the other three treatments having slightly lower values averaging 56.4%, and LF:HM having the lowest percentage of 55.2%. NF:LM and HF:LM had similar values for NDFD48 averaging 41.4% while treatments LF:LM, LF:HM, HF:LM were similar averaging 38.4%.

For N concentration, NF:LM was the highest at 0.57% while the other three had lower values averaging 0.31% with LF:HM having the lowest amount at 0.26%. Our N concentration for NF:LM was much higher than 0.34% reported by Sadeghpour et al. (2014) for CIR grown with no fertilizer. Our other treatments were similar to the 67 kg N ha^{-1} treatment in the Sadeghpour et al. (2014) study, which averaged an N concentration of 0.34%. Our results did not show that as N fertilizer rate increases so does N concentration in biomass, which was suggested by Lemus et al. (2008). Another study did not show an N-rate effect for N concentration of CIR over rates of 0, 65, and 140 kg N ha^{-1} (Waramit et al., 2011). The Waramit et al. (2011) study averaged 0.6% N which was similar to our NF:LM concentration.

ASH levels were highest for NF:LM at 8.1% which had similar percentage to HF:LM at 7.1% while LF:HM had the lowest percentage of ASH at 5.7%. These ASH levels for CIR are higher than the average ash concentration reported by Sadeghpour et al. (2014) and Wilson et al.

(2013) of 3.6% and 4.5%. Our ASH concentrations are similar to CIR grown by Waramit et al., (2011). With three N-rates of 0, 65, and 140 kg N ha^{-1}, they showed ASH concentration stayed constant across N-rates at around 0.7% in 2006 and declined slightly in 2007 to 0.65%. LIGNIN values were lowest for NF:LM which differed from all the other treatments. Treatments LF:LM, LF:HM, and HF:LM had similar LIGNIN values averaging 3.8% while NF:LM was significantly different with the lowest concentration of 2.9%. Waramit et al. (2011) showed LIGNIN increased from 0.4% to about 0.45% LIGNIN over N-rates from 0 to 140 kg N ha^{-1}.

Table 4.3.4. Forage quality trait values of switchgrass grown on Black Castle and Coal Mac

		Forage quality traits							
Effect		IVDMD$^{¥}$	aNDF^{+}	ADF^{+}	N*$^{+}$	IVTDMD48^{+}	ASH^{+}	LIGNIN^{+}	NDFD48^{+}
		---------------------------------------% DM--							% NDF
Site									
	Black Castle	39.6	74.1	39.1	0.34	58.6	7.3	3.3	41.8
	Coal Mac	38.0	76.3	41.8	0.40	57.3	6.5	3.8	37.4
	P value	*NS*	*NS*	*0.014*	*NS*	*NS*	*NS*	*0.002*	*0.0004*
Year									
	2012	42.3	71.2	37.5	0.56	60.7	7.8	2.9	42.9
	2013	35.4	79.1	43.3	0.18	55.2	6.0	4.2	36.3
	P value	*<.0001*	*<.0001*	*<.0001*	*<.0001*	*<.0001*	*<.0001*	*<.0001*	*<.0001*
Treat									
	NF:LM[1]	42.8^{a}	71.5^{b}	37.9^{b}	0.57^{a}	62.5^{a}	8.1^{a}	2.9^{b}	43.2^{a}
	LF:LM	37.8^{b}	75.9^{a}	41.0^{a}	0.31^{b}	57.0^{b}	6.8^{b}	3.7^{a}	38.8^{b}
	HF:LM	38.0^{b}	75.0^{ab}	40.7^{a}	0.35^{b}	57.1^{b}	7.1^{ab}	3.6^{a}	39.5^{ab}
	LF:HM	36.7^{b}	78.3^{a}	42.1^{a}	0.26^{b}	55.2^{b}	5.7^{c}	4.1^{a}	36.9^{b}
	P value	*0.006*	*0.002*	*0.002*	*0.001*	*0.002*	*<.0001*	*<.0001*	*0.004*
Site xTreat									
	Black Castle								
	NF:LM	45.5	68.3	35.6	0.6	64.0	8.8	2.4	45.4
	LF:LM	38.8	74.9	40.1	0.3	57.4	7.2	3.6	41.3
	HF:LM	38.3	73.9	39.6	0.3	57.8	7.2	3.4	41.9
	LF:HM	36.0	79.2	41.1	0.2	55.3	5.9	3.9	38.8
	Coal Mac								
	NF:LM	40.1	74.6	40.2	0.5	61.1	7.3	3.4	40.9
	LF:LM	36.7	76.9	42.0	0.4	56.7	6.3	3.9	36.3
	HF:LM	37.8	76.1	41.8	0.4	56.4	7.1	3.7	37.2
	LF:HM	37.5	77.4	43.0	0.3	55.0	5.5	4.3	35.1
	P value	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Treat xYear									
	2012								
	NF:LM	49.2	66.0	34.9	0.9	66.7	8.5	2.5	34.3
	LF:LM	41.6	71.7	37.9	0.5	60.1	7.7	3.1	34.6
	HF:LM	40.9	71.1	38.0	0.5	59.8	8.1	2.8	34.6
	LF:HM	37.3	76.0	39.2	0.3	56.0	6.8	3.3	34.8
	2013								
	NF:LM	36.4	76.9	40.9	0.2	58.3	7.7	3.4	34.8
	LF:LM	33.9	80.1	44.2	0.1	54.0	5.8	4.3	34.9
	HF:LM	35.1	79.0	43.4	0.2	54.4	6.1	4.3	34.8
	LF:HM	36.1	80.6	44.9	0.2	54.3	4.6	4.8	34.4
	P value	*0.0001*	*NS*	*NS*	*<.0001*	*NS*	*NS*	*NS*	*NS*
Mean		38.8	75.2	40.4	0.37	57.9	6.9	3.6	39.6

Different letters denote significant difference at p >0.05 level of probability according to Tukey's HSD. Untransformed, LS means are reported.

[1] NF:LM = No fertilizer, light mulch; LF:LM = Low fertilizer, light mulch; HF:LM = High fertilizer, light mulch; LF:HM = Low fertilizer, heavy mulch

[¥] IVDMD = In vitro dry matter digestibility; NDF = neutral detergent fiber; ADF = acid detergent fiber; N = nitrogen; IVTDMD48 = 48 hour In vitro true dry matter digestibility; NDFD48 = 48 hour neutral detergent fiber digestibility as percentage of NDF; ASH = ash; LIGNIN = lignin.

[+] indicates constituent predicted with the 2013 NIRSC forage equation

* N x 6.25=CP

Cell wall constituents

Ethanol compositional values also differed. The two sites were only different for SUC and STA. Year did not differ for MAN, SUC, GLCS, and STA. The only differences among treatments were for ARA and XYL, the two pentose sugars. For ARA, LF:LM and LF:HM were the only values that differed with all treatments ranging from 3.31 to 3.44%. For XYL, treatments LF:LM, LF:HM, HF:LM were not different and ranged from 21.8 to 22.0%. NF:LM had the lowest percentage of XYL with 20.4%. These values are similar to Adler et al. (2006) that reported XYL concentration in CIR to be 22.2% averaged over two fall harvest seasons. There were no Site by Treatment interactions while ARA was the only constituent with a Treatment by Year interaction.

Table 4.3.5. Compositional values of switchgrass grown on Black Castle and Coal Mac

		Cell wall constituents								
Effect		ARA[¥]	XYL	MAN	GAL	GLC	SUC	GLCS	FRU	STA
		% DM								
Site										
	Black Castle	3.4	21.4	0.9	Non-est	27.3	3.7	1.7	1.1	0.9
	Coal Mac	3.4	21.7	0.9	1.1	28.3	3.0	1.6	1.0	0.4
	P value	*NS*	*NS*	*NS*	-	*NS*	*0.010*	*NS*	*NS*	*0.001*
Year										
	2012	3.5	20.7	0.9	1.2	26.2	3.3	1.7	1.3	0.5
	2013	3.3	22.3	0.9	Non-est	29.4	3.3	1.5	0.7	0.9
	P value	*<.001*	*<.0001*	*NS*	-	*<.0001*	*NS*	*NS*	*<.0001*	*0.01*
Treat										
	NF:LM	3.36^{ab}	20.4^{b}	0.8	Non-est	26.2^{b}	3.1	1.6	1.2	0.5
	LF:LM	3.44^{a}	21.8^{ab}	0.9	1.1	28.3^{a}	3.3	1.5	1.0	0.7
	HF:LM	3.43^{ab}	22.0^{a}	0.9	1.1	28.0^{a}	3.3	1.6	1.0	0.6
	LF:HM	3.31^{b}	21.8^{a}	0.9	1.0	28.7^{a}	3.6	1.7	1.0	0.8
	P value	*0.038*	*0.013*	*NS*	*NS*	*<.01*	*NS*	*NS*	*NS*	*NS*
Site x Treat										
	Black Castle									
	NF:LM[1]	3.4	19.7	0.8	Non-est	25.1	3.3	1.5	1.2	0.7
	LF:LM	3.5	21.8	0.9	1.1	27.7	3.8	1.6	1.1	1.0
	HF:LM	3.5	21.9	0.9	1.1	27.6	3.8	1.7	1.1	1.0
	LF:HM	3.4	22.0	0.9	1.1	28.5	3.8	1.8	1.0	1.0
	Coal Mac									
	NF:LM	3.3	21.2	0.9	1.1	27.3	3.0	1.6	1.2	0.3
	LF:LM	3.4	21.8	0.8	1.1	28.8	2.8	1.4	0.8	0.5
	HF:LM	3.4	22.1	0.8	1.1	28.3	2.8	1.5	1.0	0.4
	LF:HM	3.3	21.7	0.9	1.0	28.9	3.4	1.7	1.0	0.7
	P value	*NS*	*NS*	*NS*	-	*NS*	*NS*	*NS*	*NS*	*NS*
Treat x Year										
	2012									
	NF:LM	3.42	19.2	0.8	1.2	24.4	3.4	1.8	1.6	0.3
	LF:LM	3.52	20.9	0.9	1.2	26.4	3.4	1.6	1.3	0.6
	HF:LM	3.60	21.2	0.9	1.2	26.4	3.2	1.6	1.3	0.5
	LF:HM	3.54	21.7	0.9	1.2	27.5	3.2	1.8	1.1	0.6
	2013									
	NF:LM	3.31	21.6	0.9	Non-est	28.0	2.9	1.4	0.8	0.7
	LF:LM	3.36	22.7	0.9	1.0	30.1	3.1	1.4	0.6	0.8
	HF:LM	3.26	22.8	0.8	1.0	29.5	3.4	1.6	0.7	0.8
	LF:HM	3.09	22.0	0.9	0.9	29.9	4.0	1.7	0.8	1.2
	P value	*0.003*	*NS*	*NS*	-	*NS*	*NS*	*NS*	*NS*	*NS*
Mean		3.4	21.5	0.9	1.1	27.8	3.3	1.6	1.0	0.6

Different letters denote significant difference at p >0.05 level of probability according to Tukey's HSD. Untransformed, LS means are reported.

[1] NF:LM = No fertilizer, light mulch; LF:LM = Low fertilizer, light mulch; HF:LM = High fertilizer, light mulch; LF:HM = Low fertilizer, heavy mulch

[¥] ARA = arabinose; XYL = xylose; MAN = mannose; GAL = galactose; GLC = glucose; SUC = sucrose; GLCS = soluble glucose; FRU = fructose; STA = starch.

Theoretical ethanol yield and production

Sites did not differ for any of the ethanol prediction values. Years were significantly different and 2013 was always greater than 2012 for all ethanol prediction values. This makes sense as 2013 had greater yields, XYL and GLC concentrations averaged over sites and treatments. Treatments differed for all ethanol prediction values and NF:LM had the lowest ethanol yield and production for NREL_GLC, NREL, and NRELPROD. For NREL_GLC, NF:LM had the lowest value of 170.6 L Mg^{-1} with the other three treatments ranging from 181.9 to 186.8 L Mg^{-1}. For ethanol predicted from XYL, LF:LM had similar yields to NF:LM which had the lowest yield of 133.0 L Mg^{-1}. Treatments LF:LM, LF:HM, and HF:LM were similar for NREL_XYL, averaging 142.4 L Mg^{-1}, and ranging from 141.9 to 143.2 L Mg^{-1}. NF:LM had the lowest yield for both NREL and NRELPROD at 302.5 L Mg^{-1} and 116.2 L ha^{-1}. Treatments had similar ethanol yield and production. For NREL, the three treatments LF:LM, LF:HM, HF:LM averaged 325.2 L Mg^{-1} with a range of 324.2-326.2 L Mg^{-1}. Adler et al. (2006) had a much higher ethanol yield of 427 L Mg^{-1} from CIR averaged over two fall season harvests. They used an equation that also only used XYL to estimate pentose ethanol yield, but they had more constituents included in the hexose calculation which may be the reason for higher ethanol production. For NRELPROD, they averaged 624.7 L Mg^{-1} with a range of 446.5 to 878.2 L ha^{-1}. NRELPROD had a Site by Treatment interaction that was driven by NF:LM at Black Castle having very low production compared to the other treatments.

A large percentage of constituents were not able to be predicted for each sample from Black Castle and Coal Mac. This caused us to only be able to account for ethanol yield from XYL and GLC sugars which were more complete data sets. Therefore, ethanol yields from Black Castle and Coal Mac were naturally lower compared to Hampshire and ethanol yields found in other studies since they included more sugars in their calculations. There are other options of predicting ethanol yield such as using a simple conversion rate like the one Schmer et al. (2008) used. They used a conversion of 0.38 L ethanol kg biomass $^{-1}$.

Table 4.3.6. Theoretical ethanol yields and production from Black Castle and CoalMac

		Theoretical ethanol yield and production			
Effect		NREL_GLC[¥]	NREL_XYL	NREL	NRELPROD
		L Mg^{-1}	L Mg^{-1}	L Mg^{-1}	L ha^{-1}
Site					
	Black Castle	177.3	138.9	314.7	446.5
	Coal Mac	184.3	141.2	324.2	423.9
	P value	*NS*	*NS*	*NS*	*NS*
Year					
	2012	170.4	135.0	304.6	254.3
	2013	191.2	145.1	334.4	744.2
	P value	*<.0001*	*<.0001*	*<.0001*	*<.0001*
Treat					
	NF:LM[1]	170.6^{b}	133.0^{b}	302.5^{b}	166.2^{b}
	LF:LM	183.9^{a}	141.9^{ab}	324.2^{a}	446.5^{a}
	HF:LM	181.9^{a}	143.2^{a}	325.1^{a}	549.5^{a}
	LF:HM	186.8^{a}	142.1^{a}	326.2^{a}	878.2^{a}
	P value	*0.002*	*0.013*	*0.006*	*0.0002*
Site x Treat					
	Black Castle				
	NF:LM	163.6	128.1	290.0	97.3
	LF:LM	180.3	141.9	321.2	573.5
	HF:LM	179.7	142.7	322.4	878.1
	LF:HM	185.6	143.0	325.5	810.8
	Coal Mac				
	NF:LM	177.7	137.8	315.0	283.9
	LF:LM	187.5	141.8	327.2	347.5
	HF:LM	184.1	143.8	327.8	372.4
	LF:HM	188.0	141.2	326.9	878.3
	P value	*NS*	*NS*	*NS*	*0.04*
Treat x Year					
	2012				
	NF:LM	159.0	125.1	284.2	80.8
	LF:LM	171.9	135.8	307.7	341.4
	HF:LM	179.0	141.1	316.8	456.6
	LF:HM	171.7	138.0	309.7	332.0
	2013				
	NF:LM	182.2	140.8	320.8	341.9
	LF:LM	195.9	148.0	340.7	583.9
	HF:LM	194.7	143.2	335.5	1689.2
	LF:HM	192.1	148.4	340.5	909.6
	P value	*NS*	*NS*	*NS*	*NS*
Mean		180.8	140.0	319.5	435.0

Different letters denote significant difference at $p > 0.05$ level of probability according to Tukey's HSD. Untransformed, LS means are reported.

[1] NF:LM = No fertilizer, light mulch; LF:LM = Low fertilizer, light mulch; HF:LM = High fertilizer, light mulch; LF:HM = Low fertilizer, heavy mulch

[¥] NREL_GLC = theoretical ethanol yield from glucose; NREL_XYL = theoretical ethanol yield from xylose; NREL = theoretical ethanol yield from GLC and XYL; NRELPROD = theoretical ethanol production from GLC and XYL.

4.4 Conclusion

Even though there were slight to no differences between cultivars for ethanol yields, CIR had significantly higher ethanol production due to its significantly larger biomass production at Hampshire compared to Hobet, Black Castle, and Coal Mac. Ideally, harvesting methods for this study would be used by machinery equipment to simulate field production scale. Fertilizing Black Castle and Coal Mac did show increased ethanol yield and production. It seems that the lower fertilization rate of 33.6 kg N ha^{-1} would produce the same results and be more cost effective for switchgrass grown on surface mines than fertilizing with 67.0 kg N ha^{-1}. It should be pointed out that these ethanol yields are assuming 100% conversion of sugars into ethanol and biomass production was estimated from carefully clipped plots. These theoretical ethanol yields do not account for recalcitrance from lignin and other large-scale production problems such as yeasts not being commercially available that can convert XYL to sugar. Not knowing realistic cellulosic ethanol conversion ratios are why many studies report maximum theoretical ethanol yields.

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APPENDIX 1

In this thesis, the ghu13 calibration equation created by the NIRSC was used to analyze aNDF, ADF, N, IVTDMD48, ASH, LIGNIN, and NDFD48 for both the 1-cut system at Hampshire and the treatments at Black Castle and Coal Mac for the 2012 and 2013 field seasons.

Another calibration equation ghu11 created by NIRSC was also used to predict the forage quality traits. Both equation's predictive qualities seemed similar and ultimately the ghu13 was chosen because it was said to include warm-season grasses and was an 'updated' version of the ghu11 equation. The averaged predicted values from both of these equations can be found in Appendix Table 1.1.

Both the ghu11 and ghu13 equations showed a high number of accurately predicted forage trait values as shown in Appendix Table 1.2. Per year, Black Castle and Coal Mac had the potential of 60 samples being predicted, while Hampshire had a potential total of 54 samples. Predicted values were determined to be accurate if they had a global H (GD) value below 3 and a nearest neighbor value (ND) below 1.2. If a predicted value had a GD value above 3 or a ND value above 1.2, this predicted value was determined inaccurate and was deleted.

The only concern surrounding the ghu13 equation stemmed from the graphical representation of our data set compared to the ghu13 calibration data set. Although the GD and ND values indicated that our switchgrass samples were similar to the calibration set, the graphics indicated otherwise. Looking at the graphical representation, our switchgrass samples looked much different from the calibration set which would indicate that the constituents could not be accurately predicted. This problem was initially thought to not affect the predictive quality of the equation; so ghu13 was used to predict the constituents.

It came to our attention recently that this discrepancy between the graphical representation and the GD and ND values were most likely caused by a computer glitch in the graphics software. Therefore, the ghu11 calibration equation would have been a better choice between the two calibration equations. We do not believe that this potential glitch gave imprecise predicted values of the samples. There may be potential for bias in accuracy, but relationships among treatments from both equations should be similar. Therefore, the predicted forage quality traits in this thesis are not necessarily 'wrong' but the ghu11 equation would be recommended to be used over ghu13 until this problem is assessed and fixed.

Appendix Table 1.1. Comparing forage quality traits of NIRSC calibration equations created in 2011 and 2013.

Comparing forage quality traits of calibration equations								
Equation	Site-year	aNDF⁺	ADF⁺	N*⁺	IVTDMD48⁺	ASH⁺	LIGNIN⁺	NDFD48⁺
		-------------------------------% DM					---------------------------	% NDF
ghu11								
	BC[1] 2012	71.4	46.8	0.3	56.0	8.9	2.1	41.2
	BC 2013	76.4	48.8	0.3	54.0	6.5	3.1	39.2
	CM 2012	70.7	45.2	0.7	58.8	8.1	2.1	41.4
	CM 2013	81.7	51.4	0.2	47.2	6.2	3.4	29.4
	HA 2012	81.6	51.5	0.8	48.2	6.7	3.8	30.4
	HA 2013	79.6	51.2	0.7	53.0	6.1	3.6	36.1
ghu13								
	BC 2012	71.8	44.4	0.3	58.9	8.1	2.8	42.0
	BC 2013	77.0	47.3	0.2	55.8	6.2	4.0	40.2
	CM 2012	71.1	43.5	0.6	60.8	7.2	3.0	42.7
	CM 2013	81.7	48.2	0.1	52.8	5.6	4.7	30.8
	HA 2012	81.1	49.5	0.7	51.2	6.0	6.0	32.0
	HA 2013	79.5	49.6	0.5	54.6	5.5	5.6	35.9

Values are averaged over treatments.

[1] BC- Black Castle; CM- Coal Mac; HA- Hampshire, 1-cut system

¥IVDMD = In vitro dry matter digestibility; NDF = neutral detergent fiber; ADF = acid detergent fiber; N = nitrogen; IVTDMD48 = 48 hour In vitro true dry matter digestibility; NDFD48 = 48 hour neutral detergent fiber digestibility as percentage of NDF; ASH = ash; LIGNIN = lignin.

⁺ indicates constituent predicted with NIRSC forage equation.

* N*6.25 = CP.

Appendix Table 1.2. Comparing the number of values accurately predicted by the NIRSC calibration equations created in 2011 and 2013.

Comparing number of predicated values								
Equation	Site-year	aNDF+	ADF+	N*+	IVTDMD48+	ASH+	LIGNIN+	NDFD48+
ghu11								
	BC[1] 2012	55	55	55	55	55	55	55
	BC 2013	53	53	53	37	51	48	51
	CM 2012	55	55	54	40	53	54	54
	CM 2013	59	59	59	59	59	59	59
	HA 2012	51	50	51	48	46	48	48
	HA 2013	49	49	49	49	49	49	49
ghu13								
	BC 2012	55	37	46	50	55	36	50
	BC 2013	53	39	50	50	51	44	50
	CM 2012	55	46	48	52	52	55	52
	CM 2013	59	36	59	31	55	52	47
	HA 2012	49	49	49	46	50	49	48
	HA 2013	49	49	49	49	49	49	49

Values are averaged over treatments.

[1] BC- Black Castle; CM- Coal Mac; HA- Hampshire, 1-cut system

¥IVDMD = In vitro dry matter digestibility; NDF = neutral detergent fiber; ADF = acid detergent fiber; N = nitrogen; IVTDMD48 = 48 hour In vitro true dry matter digestibility; NDFD48 = 48 hour neutral detergent fiber digestibility as percentage of NDF; ASH = ash; LIGNIN = lignin.

+ indicates constituent predicted with NIRSC forage equation.

* N*6.25 = CP.