NITROGEN FERTILIZATION, YIELD COMPONENTS, AND SPECIES SELECTION OF PERENNIAL GRASS BIOENERGY CROPPING SYSTEMS ACROSS EASTERN NORTH AMERICA

BY

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DISSERTATION

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ABSTRACT

Perennial grasses are being studied as a fuel source and potential replacement for fossil fuels. Perennial grasses are a versatile group of potential bioenergy crops that have the ability to produce large yields on relatively low-quality land or with lower inputs than traditional annual row crops such as maize. Grasses can be directly combusted to produce heat and/or electricity or can be processed to produce cellulosic ethanol similarly to corn grain ethanol that is combined with gasoline for use as a liquid transportation fuel. *Miscanthus* x *giganteus* (Miscanthus), *Panicum virgatum* (switchgrass), and recreated, multi-species tallgrass prairies are possible perennial systems for high-yield production in crop-growing regions of North America. However, past research has reported mixed findings regarding species selection and responses of perennial energy crops to nitrogen fertilization. This dissertation examines several of these aspects in three studies:

1) An in-depth look at the effects of N fertility rates on biomass yield and individual yield components of Miscanthus across several seasons. Results demonstrated that applying 60 or 120 kg N $ha^{-1}$ provides a 2x yield increase compared to unfertilized Miscanthus in a long-term study at Urbana, IL, USA. Total tillers per $m^2$ were strongly correlated with increasing biomass yield, with tiller height, diameter, and phytomer number also correlating well with yield.
2) The productivity of side-by-side plots of Miscanthus and switchgrass was evaluated over two years in 11 locations in eastern North America. Results showed Miscanthus to be a greater producer of biomass than switchgrass across all sites, and showed variability among sites on the effect of applied nitrogen fertilizer. Most

yield components were correlated with yield changes, but in some cases were affected differently by added fertility.  Overall, proper selection of species or switchgrass cultivar played a role in biomass production at a given site.

3) A long-term field-scale comparison of three perennial grass systems: Miscanthus, switchgrass, and a high-diversity recreated tallgrass prairie.  Monoculture stands of Miscanthus and switchgrass produced more biomass than the prairie system while the stands were young, but yields of monoculture crops declined with stand age. The monoculture stands produced more biomass than the prairie stand during the drought year of 2012, indicating that diverse systems are not necessarily more resistant to drought.  Application of nitrogen fertilizer to declining Miscanthus stands starting in year six generated a nearly 2x yield increase over unfertilized plots, which still produced more biomass than the prairie.  In addition, average annual energy production was greater in switchgrass and Miscanthus stands than in a corn-corn-soybean rotation system, due to the inconsistency of corn yields and the low energy potential of soybean.  Finally, as a sidebar, harvest results using a plot-scale combine and a commercial harvest system were strongly correlated, which indicates that the plot-scale combine is a good estimator of overall yields.

These results show that applied nitrogen fertilizer increases yields of bioenergy crops in certain situations, and measuring certain yield components may provide a good estimate of total biomass yield.  Perennial grass systems are at least comparable to annual row-crop systems, and in many cases may exceed them in overall energy production.

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# Chapter I

## General Introduction

The world requires large amounts of energy to function; energy is required to do everything from cooking to driving to flying a jet.  In fact, according to the U.S. Department of Energy (2008), in 2006 the world consumed 498 billion Gigajoules of energy.  Currently, fossil fuels operate most of the power plants, transportation systems, and factories that make life "normal" for everyone living in the developed world.  While other energy sources such as nuclear, wind, and hydroelectric power play a significant role, the world relies upon the three primary fossil fuels, oil, coal, and natural gas, to meet its current energy demands.

Although humans rely heavily upon fossil fuels, political and environmental issues leave future energy sources in question.  Much of the world's oil supply is located in regions that, at best, have strained relations with many western countries.  These geopolitical problems have contributed to volatile energy prices, especially in the mid- to late-2000s.  Moreover, oil is a dwindling resource; one projection estimates that it could be depleted within 50 years (Keller, 2000).  Coal, while relatively abundant, is often surface mined, which removes all the soil above a deposit, resulting in environmental damage.  Natural gas has become a popular and less expensive energy source in recent years, but increased extraction relies on hydraulic fracturing or "fracking" techniques, which are controversial and linked to causing earthquakes in some regions, particularly in Oklahoma (Weingarten et al, 2015).

When burned, all three primary fossil fuels release pollutants and greenhouse gases such as carbon dioxide ($CO_2$) into the atmosphere (EIA, 2016).  Carbon dioxide is released naturally by life on earth through respiration, or through the combustion of carbon-based fossil fuels that were formed over millions of years from decomposing life forms and buried deep underground by natural geologic processes. However, since this carbon has been out of the aboveground ecosystem for millions of years, combustion releases the previously stored carbon dioxide, increasing atmospheric levels. The US NOAA Earth System Research Laboratory has reported a steady year-over-year increase in atmospheric carbon dioxide at their observing station at Mauna Loa, Hawaii since the late 1950s (ESRL, 2016).

The overall effects of increased atmospheric carbon dioxide are a hotly debated topic. In 1988, the World Meteorological Organization and the United Nations formed the Intergovernmental Panel on Climate Change to report on peer-reviewed studies involving climate change (IPCC, 2013). These reports provide recommendations on policy positions to many western governments. For example, in late 2015, nearly 200 nations adopted the Paris Agreement, which pledges to reduce worldwide carbon emissions in an effort to reduce climate change (NPR, 2015). But some also feel that the predicted effects of increasing atmospheric carbon dioxide are overstated. Either way, the debate has contributed to an increased interest in alternative and renewable energy sources.

**Biofuels**

Biofuels are energy sources produced from living plants. Bioenergy crops have two primary advantages compared to fossil fuels. First, biofuels are renewable because plants can be grown and harvested on set intervals. As long as proper land-use techniques are followed, biofuel crops can be grown indefinitely and unlike fossil fuels, are not finite resources. Secondly, bioenergy crops recycle greenhouse gases from the atmosphere. When either fossil fuels or biofuels are burned, $CO_2$ is the main greenhouse gas released. The release of carbon dioxide from burning fossil fuels, which originated from deep beneath the earth, is not re-absorbed; it is released into the atmosphere. However, since the plants grown to produce biofuels utilize atmospheric $CO_2$ for growth, there is not a net atmospheric carbon increase.

A variety of crops can be used to produce biofuels, ranging from annual row crops to woody tree species to herbaceous perennials. At present in the United States, the most common biofuel crops are maize or corn (*Zea mays* L.) from which ethanol is produced and soybeans (*Glycine max* (L.) Merr.), which produces biodiesel. Corn ethanol production has steadily increased in the U.S., with production of approximately 140 million liters per day in late 2013 (EIA, 2013). While maize and soybeans will likely remain important components of U.S. biofuel production, there is significant and increasing interest in the production of other biofuel sources, particularly those that can maximize production with limited inputs.

An example of increased interest in biofuels production is evidenced in the 2007 US Renewable Fuel Standard-2 (RFS-2) which states that corn grain ethanol production should not exceed 56.8 billion liters (15 billion gallons) per year by the year 2022 (EPA, 2010). This standard emphasizes that an additional 79.5 billion liters (21 billion gallons) should be produced from other biofuel sources, including 60.6 billion liters (16 billion gallons) from cellulosic biofuels (EPA, 2010). While annual cropping systems could fill this need, they require large energy inputs for their production (Hulsbergen et al., 2001). Since plant cell walls are made of cellulose, fuels can be produced directly from total above-ground plant biomass, not just the grain. Many plants could be used to produce bioenergy, but those that maximize annual biomass production with minimal inputs or land-use change are optimal candidates, with perennial grasses being among the most promising and studied across much of North America.

**Perennial Grasses**

Perennial grasses will likely be a primary source of cellulosic biofuel production because these plants have many benefits compared to annual row crops. Most obviously, being perennial eliminates yearly establishment costs after the initial planting and allows growers to realize multiple years of production from a single establishment. Perennial grasses are relatively easy to maintain, with many requiring limited water and fertilizer inputs, which also reduce costs and environmental impacts. Since there are many different species of perennial grasses adapted across many environments, the grasses can potentially be grown on a wide range of land types. Moreover, these grasses are relatively easy to harvest, since modern haying equipment can usually be used (Lewandowski et al., 2003). Finally, the grasses can be used to produce energy in more than one way; by producing ethanol by breaking down carbon-containing molecules in the plant cell wall, or by direct combustion for heat and electricity production, either alone or co-fired with fossil fuels such as coal.

Beyond the agronomic advantages, growing perennial grasses can benefit the environment through reduced equipment, water, and fertilizer inputs, as well as through decreased net greenhouse gas emissions (Hill et al., 2006). Perennial grasses can also provide increasingly scarce wildlife habitat, especially when native tallgrass prairie species are used (Werling et al., 2013). In addition, atmospheric carbon can be captured and stored below ground in plant root or rhizome biomass, further enhancing environmental benefits. While there are many crops that could be potentially grown for biofuel production, the three perennial systems of interest to this work are Giant Miscanthus, switchgrass, and mixed tallgrass prairie grasses.

**Miscanthus**

The warm-season grass *Miscanthus* x *giganteus* Greef et Deu ex. Hodkinson et Renvoize (Hodkinson and Renvoize, 2001), hereafter Miscanthus, has been studied extensively in Europe and is now the focus of much research in the U.S. Miscanthus, a perennial $C_4$ grass native to eastern Asia (Jones and Walsh, 2001), shows great potential as a biofuel crop. Originally brought to Denmark from Japan in 1935, it eventually was grown throughout Europe and eventually made its way to North America as a landscape plant (Scally et al., 2001). It is believed to be a naturally occurring hybrid between *Miscanthus sinensis* and *M. sacchariflorus* (Linde-Laursen, 1993). While the hybrid Miscanthus is a sterile triploid that does not produce fertile seed that can invade non-crop sites, vegetative propagation, usually by rhizome division, is more costly and less efficient than seed establishment.

In central Illinois, Miscanthus typically grows to 4 meters in height each year, requires minimal nutrient inputs, and to date, has been resistant to most disease and insect problems (Anderson et al., 2011). At harvest, the stalks produce a relatively dry biomass (Jones and Walsh, 2001), which allows immediate use or storage for later use.

Although Miscanthus is a fairly new biomass feedstock crop in the U.S., and European scientists have studied it for several years, published research findings, however, are still relatively limited on both continents. In a summary of European research, Lewandowski et al. (2000) reported yields as high as 25 Mg $ha^{-1}$ without irrigation and more than 30 Mg $ha^{-1}$ on irrigated sites. These authors also confirm the crop's low fertilizer requirements and report its high establishment costs due to vegetative propagation (Lewandowski et al., 2000). However, Heaton et al. (2004) indicate that its future as a biofuel crop is positive when compared to other crops, specifically switchgrass (*Panicum virgatum*). A summary of reported Miscanthus yields is shown in Table 1.1.

The difficulty and cost of establishing Miscanthus may be one factor that limits its expanded use. Other biomass crops, such as switchgrass, produce viable seeds and can be propagated by conventional planters. Conversely, Miscanthus rhizomes must be dug, separated, and replanted, requiring much more labor. Mechanical harvesting and planting equipment that increases speed and efficiency is being refined. However, even these improvements will likely not match the economy, speed, and efficiency of seed-propagated crops.

Greenhouse and *in vitro* propagation of Miscanthus are also options, albeit on a limited scale. Lewandowski (1998) showed that rhizome-propagated plants tended to fare better than micropropagated plants initially, but these differences decreased with age. One propagation method gaining popularity within the young industry is the production of rhizome-derived plugs (Anderson et al., 2011). Plugs are established in protected areas (greenhouses or hoop houses) in late winter. By planting time, small plants have already begun to grow. This method gives the plants a head start on the growing season, and eliminates issues with rhizomes failing to survive and sprout. However, young plug plants may be more prone to damage and drought injury than direct rhizome planting because they are actively growing at planting time.

Given these propagation limitations, a key question for Miscanthus researchers is whether the increased establishment costs are justified by significantly increased plant yields in comparison to alternative perennial crop species. Since it is still a fairly new crop, large-scale research trials have yet to establish the full extent of the optimal growth environment for Miscanthus production, particularly in comparison to switchgrass, which is native to the U.S. and has a well-known, extensive range. It is also unclear which components of Miscanthus growth have the largest impact on overall yield potential.

**Switchgrass**

Switchgrass (*Panicum virgatum* L.) is a $C_4$ warm-season perennial grass native to much of the continental U.S. In addition to being a widely used forage crop, it has also become one of the first extensively studied bioenergy crops in the U.S. The U.S. Department of Energy (DOE) first identified switchgrass as a "model" energy crop as early as 1991 (Wright and Turhollow, 2010). In 1997, the DOE's Bioenergy Feedstock Development Program (BFDP) suggested that switchgrass, as a bioenergy crop, should be produced on a large scale because of its wide native range, high potential yields, good nutrient-use efficiency, benefits to wildlife, and positive effects on the soil (McLaughlin and Walsh, 1997). Switchgrass research has slowly increased over the ensuing years, and the grass gained even greater attention as a bioenergy crop after former President George W. Bush mentioned it during his 2006 State of the Union address (Bush, 2006).

Since the BFDP expressed interest in switchgrass, several studies have examined many aspects of switchgrass biomass production for bioenergy. For example, Vogel et al. (2002) found that at the optimal nitrogen fertilization rate of 120 kg N $ha^{-1}$, the cultivar 'Cave-in-Rock' could yield up to 12.6 Mg $ha^{-1}$ in Iowa. Thomason et al. (2004) showed even greater yield potential (16.9 Mg $ha^{-1}$) with the cultivar Kanlow in Oklahoma without fertilization. In a multi-location U.S. study, Fike et al. (2006) reported that several cultivars could be grown effectively for a long time period with average yields of approximately 14 Mg $ha^{-1}$ in the upper southeastern U.S. After the 10-year duration of the BFDP program, McLaughlin and Kszos (2005) reviewed switchgrass research literature and found that by choosing the proper varieties, properly timing harvests, and improving breeding methods, all while reducing nitrogen fertilizer inputs, could significantly increase yields. In short, switchgrass is a promising bioenergy crop.

Aside from cultivar selection and fertilizer use, significant impacts on productivity are linked to switchgrass yield components including tiller density, tiller height, tiller diameter, tiller mass, and phytomer number. Determining which component(s) have the greatest effect on overall yield could guide breeding efforts to further boost biomass yields of switchgrass. Casler et al. (2004) stated that rapidly expanding stems were more important to higher yields in southern switchgrass cultivars (lowland ecotypes) than in northern cultivars (upland ecotypes). Similarly, other studies have found a link between high yields and the density and size of reproductive tillers (Boe and Casler, 2005; Boe, 2007; Boe and Beck, 2008). However, these yield component effects have not been extensively studied in other biomass crops such as Miscanthus.

**High-Diversity Recreated Tallgrass Prairie**

Although much bioenergy research has been conducted using Miscanthus and switchgrass monocultures, other candidate crops could also become productive biomass feedstocks in the U.S. Of great interest here is the perennial high diversity recreated tallgrass prairie. High-diversity native tallgrass prairie plantings comprised of a number of perennial grass, forb, and sedge species are another potential option for producing biomass on a large scale. There is evidence that these diverse systems may be better adapted to environmental stresses (Tillman and Downing, 1994), and may have a positive impact on the soil (Tillman et al., 1996), as well as improving other biological systems (Werling et al., 2013). High-diversity native tallgrass prairie plantings, however, weren't studied as a biomass feedstock until interest in bioenergy as a whole increased in the mid-2000s.

Results published by Tillman et al. (2006) became the catalyst for many discussions on the possibilities of using diverse plantings instead of monocultures. In that article, the authors stated that high-diversity plantings produced more than twice the biomass yields of a comparable monoculture over a 10-year timespan. The authors also found that these plants have a greater ability to perform well on low-quality lands, while also sequestering a large amount of carbon in plant root systems (Tillman et al., 2006). The opportunity to grow crops on marginal land not used to produce cash crops is important in the "food vs. fuel" debate in which the advantages and disadvantages of replacing food-producing crops with energy crops are discussed. The benefits of producing more biomass per acre are obvious, creating much interest in using diverse plantings of perennial natives for biomass.

**Summary**

The balance between global energy supply and demand is uncertain. Fossil fuels, currently the world's primary energy sources, are becoming more expensive. Oil supplies are often dependent upon politics, and even in a good geopolitical environment, are becoming more limited. Burning fossil fuels contribute to air pollution and greenhouse gas buildup. Alternative energy sources will be a necessary component to alleviate these problems.

Biofuels are an excellent way to mitigate current energy concerns. Because growing biofuel crops absorb carbon dioxide, there is theoretically no net atmospheric carbon increase as the biomass is burned. These crops can also be grown in a variety of locations, so countries with enough farmland could conceivably become much less dependent on other countries for their energy needs. Biofuel crops can be used to produce ethanol or biodiesel, allowing the current transportation system to function with only minimal changes. Biofuels can be burned outright, directly replacing coal and fuel oil in such applications. Perennial grasses are good options for large-scale biomass production across much of the U.S. because the crops are low-input and high-output.

While a number of potential bioenergy crops have been investigated, there are still important gaps in our knowledge. A more detailed look may determine the optimal planting range across the continent for Miscanthus, as well as identify which growth parameters play the most important role in productivity and how Miscanthus compares directly with other perennial grass species such as switchgrass or native prairies. Perhaps with this knowledge the U.S. can develop a robust bioenergy economy that eventually replaces a portion of fossil fuels, while supporting the rural communities in which these crops would be grown.

**Objectives**

This dissertation will examine three key areas of bioenergy research and production.

1) Determine how different yield components of *Miscanthus* x *giganteus* impact overall productivity, and how these components change in response to nitrogen fertilization.
2) Compare the biomass productivity from replicated *Miscanthus* x *giganteus* and switchgrass plots from across the United States and southern Canada. Specifically, determine how

location, fertility, individual yield components, and species/cultivar selection compare across environments using the same harvest methods.

3) Compare the overall biomass yields, total energy output, and harvest methods from large-scale plots of *Miscanthus* x *giganteus*, switchgrass, recreated high-diversity tallgrass prairie, and a corn/soybean rotation.

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## Tables and Figures

**Table 1.1. Examples of reported yields of *Miscanthus* x *giganteus* in European and North American research to date. Results are highly variable across studies.**

| Yield (Mg ha$^{-1}$) | Fertilization Notes | Location | Authors |
|---|---|---|---|
| 5-10 | Fertilization had no effect | Denmark | Jorgensen, 1997 |
| 29.6 | No fertilization | Illinois | Heaton et al., 2008 |
| 17.9 | No fertilization | Illinois | Dohleman et al., 2009 |
| 23.4 | No fertilization | Illinois | Arundale et al., 2014a |
| 28.9 | Fertilized with 202 kg N ha$^{-1}$ | Illinois | Arundale et al., 2014b |
| 17.7 | Fertilization had no effect | United Kingdom | Christian et al., 2008 |
| 13.4 | Fertilization had no effect | Ireland | Clifton-Brown et al., 2007 |
| 8-30 | Variable based on site | Germany | Lewandowski and Kicherer, 1997 |

## Chapter II

## Nitrogen Fertilization Effects on Biomass Production and Yield Components of *Miscanthus* x *giganteus*


### Abstract

Perennial grasses such as *Miscanthus* x *giganteus* can potentially be used to produce cellulosic ethanol on a large scale, particularly in the U.S. Midwest. Miscanthus biomass productivity can be substantial, even when grown with limited management inputs on marginal lands. However, the literature varies regarding the fertilizer requirements of Miscanthus, and there have been no published results that discern which yield components provide the greatest contribution to its high yields. This study examines the effects of added nitrogen on yield and individual yield components. Results show that applying nitrogen at rates of 60 or 120 kg N $ha^{-1}$ provides a 2x yield increase compared to unfertilized Miscanthus in a long-term study at Urbana, IL, USA. Additionally, the total number of tillers per $m^2$ was strongly correlated with increasing biomass yield, with additional yield components, particularly reproductive stems, also correlating strongly. These results indicate that Miscanthus yields can increase when nitrogen is applied and that measuring tiller density and mass may provide a good estimate of total biomass yield.


### Introduction

When growing crops for cellulosic bioenergy, efficient production of high-yielding biomass feedstocks is a primary goal. In the U.S. Midwest, *Miscanthus* x *giganteus* Greef et Deu ex. Hodkinson et Renvoize (hereafter Miscanthus), a sterile, warm-season, perennial grass, shows potential as a bioenergy crop due to its high biomass production (Heaton et al., 2008).

Miscanthus is a rhizomatous grass native to East Asia that was first cultivated as an energy crop in Europe in the early 1980s (Lewandowski et al., 2000). It is believed to be a cross between the fertile species *M. sinensis* and *M. sacchariflorus* (Hodkinson et al., 2002). As it is sterile, Miscanthus must be propagated vegetatively using rhizome cuttings, rhizome-derived plugs, or *in vitro* micro propagation (Lewandowski, 1998; Anderson et al., 2011). Rhizome propagation has produced more robust plants than *in vitro* propagation (Lewandowski, 1998).

Miscanthus has high yield potential. In Europe, Miscanthus has produced 25 to 30 Mg $ha^{-1}$ (Lewandowski et al., 2000). In the U.S., University of Illinois bioenergy studies began in 2002 using Miscanthus rhizomes originally harvested from a Chicago Botanic Garden (Glencoe, Illinois, U.S.A.) landscape planting in 1988 (Heaton et al., 2008). Miscanthus biomass production has ranged between 15 and 30 Mg $ha^{-1}$ in several Illinois field studies (Heaton et al., 2004; Heaton et al., 2008; Maughan et al., 2012).

Nitrogen applications to Miscanthus have had variable productivity results. Two long-termed Miscanthus fertility studies in Europe found no productivity response to N fertilization over many years (Himken et al., 1997; Christian et al., 2008), while a third reported a N response as the plot aged beyond ten years (Clifton-Brown et al., 2007). The Illinois Miscanthus studies were initially designed to compare Miscanthus yields with those of switchgrass (*Panicum virgatum* L.) with no added fertility (Heaton et al., 2008). As stands aged to approximately 8-10 years, Miscanthus and switchgrass productivity both declined (Arundale et al., 2014a). However, when nitrogen was applied to the aged stand in unfertilized plots, Miscanthus productivity rose as nitrogen rates increased (Arundale et al., 2014b).

A North America native, switchgrass (*Panicum virgatum* L.) has received much attention as a potential bioenergy crop in the U.S. (Sanderson et al., 2006). Like Miscanthus, switchgrass is a rhizomatous, $C_4$ perennial grass that is a promising biomass crop. Unlike Miscanthus, however, switchgrass is fertile and can be readily established by seed (Heaton et al., 2009). Switchgrass produced approximately 10 Mg of biomass $ha^{-1}$ $year^{-1}$ in a direct comparison with Miscanthus in Illinois (Arundale et al., 2014a; Arundale et al., 2014b). Because switchgrass is seed established, is a native in much of the continental U.S., is used in conservation plantings, and is commonly used as a hay crop, it is grown on many acres across the country.

Grass phenotypic traits such as tiller density, tiller length, the number of phytomers (vegetative units of growth that include a internode, node, and leaf nodes) per tiller, the reproductive:vegetative tiller ratio, and tiller weight all play a role in determining productivity in cellulosic bioenergy grass crops. To date, these yield components have been evaluated in the bioenergy grasses, switchgrass and prairie cordgrass (*Spartina pectinata* Link).

A study of three switchgrass cultivars showed strong correlation between increasing yield and both tiller density and phytomer mass, and a weak correlation with the number of phytomers per tiller (Boe and Beck, 2008). Similar studies also found that the number of reproductive tillers per $m^2$ and the number of phytomers per tiller were also good selection criteria for increased biomass production (Boe, 2007). Das et al. (2004) reported a positive correlation between yield and tiller density. Much of the overall variation in switchgrass yield, however, results from genetic variability among cultivars (Boe and Beck, 2008). In prairie cordgrass, another warm-season rhizomatous perennial grass, Guo et al. (2015) found that tiller mass, tiller density, heading date, plant height, and phytomer number were all positively correlated with yield in some manner, but also found that much of the phenotypic variation was from the genetic diversity of the germplasm.

Because Miscanthus is sterile and clonally propagated, it is possible that individual yield components could be a greater indicator of overall yield than in more genetically diverse switchgrass and prairie cordgrass populations.  Additionally, switchgrass yield-component research found that plants with greater numbers of larger, reproductive stems tended towards higher yields (Boe, 2007). There is no similar published information on Miscanthus yield components, nitrogen fertilizer effects on yield components, and the yield component and N fertility roles on biomass productivity.  Therefore, the objective of this study was to examine the effects of fertilization on Miscanthus biomass yield and individual yield components.

**Materials and Methods**

The study site was located near Urbana, Illinois, U.S.A., at the University of Illinois Energy Farm (40.0624 N, -88.1915 W) in Dana silt loam soil (fine-silty, mixed, superactive, mesic Oxyaquic Argiudolls).  In July 2008, potted 'Illinois' Miscanthus were planted on one-meter spacing in twelve, 10 m x 10 m plots (100 plants per plot) with three nitrogen fertility treatments applied annually early spring at or near the time of emergence at 0, 60, and 120 kg N $ha^{-1}$ using urea as the N source (Maughan et al., 2012).  Due to winterkill during the 2008-09 winter the site was partially replanted in Spring 2009 to fill plots to 100 plants each. The study was planted as a randomized complete block design with four replications with the three N application levels in each replication (Maughan et al., 2012).

This study reports on 2011-2014 growing-season findings.  Plots were harvested at two different times, depending on objective.  In 2011 and again in 2014, each plot was sampled for peak standing biomass in summer (July or August), while after each growing season the plots were harvested during the typical bioenergy crop harvest timing of over winter post-senescence (typically December through February). Biomass was cut by hand in 1-$m^2$ quadrats with three replications per plot in peak standing biomass harvests, and five replications per plot in senesced biomass harvests. Quadrats were selected throughout the plots in an attempt to produce samples representative to the plot as a whole and were not selected from border rows. Stems were cut at a height of 10 cm and each quadrat was bundled individually. The detailed component measurements were total plant fresh weight, subsample wet and oven-dry weights, number of vegetative and reproductive tillers per plant, tiller diameter, tiller height, and tiller phytomer number. Five vegetative and five reproductive tillers were randomly selected from each replication for detailed measurements. Tiller diameter was measured at the midpoint of the lowest complete phytomer. Tiller height was measured to the top node of vegetative stems and to the base of the flower in reproductive stems. Dry biomass weight was determined by drying a subsample to 60 °C for up to 72 hours until dry weight was constant.

Normality of the residuals and homogeneity of variances were tested by plotting the residuals against their predicted values in SAS software version 9.4 (SAS Institute, Cary, NC, USA). Data was analyzed using Proc Mixed in SAS. Tukey's studentized range test was used to compare phenotypic traits at $\alpha$=0.05. In senesced harvests, N-rate (N), year (Y), and the interaction of N-rate and year (YN) were considered fixed effects and block (B) as random in the following model:

$$y = \mu + Y + B + N + YN + e$$

In peak harvests, due to discontinuous harvesting, year (Y) was considered random. Pearson correlations were calculated for yield components using SAS.

**Results**

Added nitrogen fertilizer was highly significant ($P<0.0001$) in increasing yields between fertilized and unfertilized plots, but there was no significant difference between the two N rates (60 and 120 kg N $ha^{-1}$) when averaged across both 2011 and 2014. Nitrogen application increased peak biomass yields by more than two-fold (Fig. 2.1). Interactions between years are likely due to the declining yield of the unfertilized plots over time (Fig. 2.1).

Miscanthus harvested post-senescence showed similar trends to peak biomass. Nitrogen-fertilized plots produced more total biomass than unfertilized plots ($P<0.01$), but the 60 and 120 kg N $ha^{-1}$ rates did not differ. Year effects were also seen between the 2013 season and other years, possibly due to a substantial yield increase across all fertility levels after the drought year of 2012 (Figs. 2.2 and 2.3). A notable drop in yield was observed during 2012 in the unfertilized plots, which was not observed in the fertilized plots. All plots rebounded in yield for 2013, followed by a drop in 2014 across all fertility treatments (Fig. 2.3). An N-rate by year interaction was also seen, likely due to the overall decline in yield in unfertilized plots during the study while fertilized plots increased. Total average biomass yields for the duration of the study were nearly double for fertilized plots compared to the unfertilized plots.

Individual yield-component effects on total biomass yield from peak-harvested plots in 2011 and 2014 differed between fertilized and unfertilized plots. In unfertilized plots, tiller diameter, height, and weight were all correlated with total biomass yield (Table 2.1), and tiller number was not correlated with yield.  In the fertilized plots, however, biomass yield and tiller number were correlated and less so between yield and the other components examined.

Senesced biomass yields were lower than peak biomass yields (Fig. 2.4).  Separated by added nitrogen, the average yield decline across both 2011 and 2014 was 31%, 34%, and 42% for the 0, 60, and 120 kg $ha^{-1}$ nitrogen rates, respectively.  This decrease was likely due to in-plant nutrients being cycled to belowground root and rhizome systems during senescence, as well as to leaf drop.

Yield component correlations from senesced biomass were similar, with one notable exception.  Unlike in peak biomass, the correlation between yield and tiller number was highly significant for senesced biomass across all fertility levels (Table 2.1).  Reproductive tiller density and tiller mass also correlated with yield across all nitrogen treatments (Fig. 2.5).  Tiller height and phytomer number were correlated with biomass yield at varying degrees across fertility levels, especially with reproductive tiller number (Table 2.1).  Nitrogen fertility also affected most other yield components.  For example, tiller mass (Fig. 2.6) and tiller number (Fig. 2.7) increased significantly with added nitrogen compared to the unfertilized Miscanthus. Interestingly, this response differed in response to drought.  Even though biomass yield increased in fertilized plots in 2012 (Fig. 2.3), tiller mass decreased (Fig. 2.6).  This change was made up for by an increase in tiller number in 2012 (Fig. 2.7).

**Discussion**

Nitrogen fertilization impacted Miscanthus biomass productivity in this study, similar to yield improvements shown in aging stands in other research (Arundale et al., 2014a).  Total harvested biomass was greater across all years for both peak and senesced crops when fertilized, but there were no differences between yields of plots fertilized with 60 and 120 kg N $ha^{-1}$. This indicates these Miscanthus plots do not require 120 kg N $ha^{-1}$ to reach full yield potential, but the precise amount required for maximum yield is yet to be determined. In addition, the optimum N fertilization level is likely to vary based on environment and soil type.

During the 2012 growing season, much of the central U.S. experienced an "extreme" drought as classified by the US National Drought Mitigation Center. The study site received 59% of its normal precipitation between January and July 2012 and 43% during the primary growing period of May - July (Fig. 2.2). As most annual biomass production occurs by the end of August, the effects of a severe drought on Miscanthus growth should be noticeable. Emerson et al. (2014) noted a decline in Miscanthus yield in Nebraska due to the 2012 drought of 14% over the average growing season of 2010. Interestingly, in this study dry biomass yields from the senesced harvest only declined in the unfertilized plots, from an average of 17 Mg $ha^{-1}$ down to 12 Mg $ha^{-1}$. More interesting, however, are the 2012 yield increases in the fertilized plots. This indicates Miscanthus has the ability to adapt to drought conditions when supplied with adequate nitrogen fertility. The exact mechanism is unknown, but increased root growth in previous years under optimum fertility would help plants retrieve more water from dry soils or at greater depth.

In addition to reduced yields in the unfertilized plots during the 2012 drought, overall productivity trends downward, while the yield trends in fertilized plots was upward (Fig. 2.3). This is also likely the cause of the year by nitrogen rate interactions in the peak biomass harvests, since overall yields declined more in the unfertilized than fertilized plots in 2014. This trend appears similar to other Illinois Miscanthus studies showing a decrease in yield with stand age and a subsequent increase with added fertility (Heaton et al., 2008; Arundale et al., 2014a), as well as one study from Europe showing the same effect (Clifton-Brown et al., 2007). Even though yields decreased year-over-year for all fertility levels in 2014, the greater drop in unfertilized plots and the overall negative yield trend could indicate a potential for overall stand decline if not properly fertilized. Soil type, mineralized nitrogen, and climatic conditions may have altered this effect in other soils, but the possibility of permanently affecting a stand's health should be considered.

Although the total dry biomass for Miscanthus is greater when harvested during the growing season with leaves present, part of the benefit of using perennial grasses for energy production is the potential sustainability. Perennial grass systems have the ability to move nutrients belowground into roots and rhizomes during the winter (Jorgensen, 1997; Dubeux et al., 2007). The remaining aboveground biomass stalks consist primarily of cellulose. There is also evidence that Miscanthus plots decline with repeated harvests of live biomass during the growing season (Parrish, 2013). Although leaves and stems can be used in biomass conversion to ethanol (da Costa et al., 2014), harvesting senesced biomass during winter months should create a more sustainable cropping system while still producing high yields.

Most yield components played a role in productivity differences in this study. In fresh biomass harvested at peak harvest, the greatest correlation with yield in unfertilized Miscanthus was tiller weight ($R^2$=0.76). Tiller diameter and height were also correlated with yield; this is expected because thicker and taller tillers are likely to be heavier. Tiller number was positively correlated with yield ($R^2$=0.40) but not significantly (P=0.054) in unfertilized peak-harvested biomass. However, fertilized Miscanthus yield was strongly correlated with tiller number ($R^2$=0.91 for 60 kg $ha^{-1}$ added nitrogen) as well as with other components. This could indicate Miscanthus is able to produce a greater number of average tillers given sufficient nitrogen, and produces fewer, larger tillers during the growing season when nitrogen is lacking.

In senesced biomass grasses, several component effects shift in importance. All fertility levels showed significant correlations between yield and tiller number and tiller weight, indicating that greater numbers of tillers and larger tillers both play a significant role in increased yield. For example, across all years, the mean tiller number in unfertilized Miscanthus was 44.6, tiller mass was 27.8 grams, and weight per $m^2$ was 1.3 kg. In fertilized Miscanthus, however, the mean tiller number across both fertility levels was 59.9, the tiller mass was 42.8 grams, and the weight was 2.5 kg $m^2$. Therefore, a yield increase of 92% is correlated with a tiller number increase of 34% and a tiller mass increase of 54%. Adding 34% more tillers to the unfertilized plots at the same average weight would potentially increase the average weight per $m^2$ to 1.7 kg, while adding 54% more mass to the existing tiller number would increase the total weight to 1.9 kg per $m^2$.

In this study, both tiller number and weight were important factors in Miscanthus productivity, while tiller number was the most important yield component in switchgrass (Boe, 2007; Boe and Beck, 2008; Das et al., 2004). The tendency of switchgrass to produce many small stems, compared to fewer large stems in Miscanthus could amplify the importance of tiller weight in Miscanthus and minimize it in switchgrass. Even though an understanding of Miscanthus yield components may not be necessary because its sterility means it can’t be improved by traditional breeding methods, a better understanding of how nitrogen impacts overall growth components can help producers and researchers estimate yield potential during the season and maximize its genetic potential to produce large amounts of biomass and further develop the bioenergy economy.

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## Tables and Figures

**Table 2.1. Significance (α=0.05) and R values for yield component by average biomass production in peak (2011 & 2014) and senesced (2011-2014) biomass.**

| Harvest | Fertility (kg N $ha^{-1}$) | Component | P value | R value |
|---|---|---|---|---|
| **Peak** | 0 | Tiller Mass | <0.0001 | 0.76 |
| **Peak** | 0 | Tiller Diameter | 0.0033 | 0.57 |
| **Peak** | 0 | Tiller Height | 0.0004 | 0.66 |
| **Peak** | 60 | Total Tiller No. | <0.0001 | 0.91 |
| **Peak** | 60 | Phytomer No. | 0.0026 | 0.59 |
| **Peak** | 120 | Total Tiller No. | <0.0001 | 0.76 |
| **Peak** | 120 | Tiller Mass | 0.0081 | 0.53 |
| **Peak** | 120 | Tiller Diameter | 0.0034 | 0.57 |
| **Senesced** | 0 | Total Tiller No. | <0.0001 | 0.83 |
| **Senesced** | 0 | Rep. Tiller No. | <0.0001 | 0.68 |
| **Senesced** | 0 | Tiller Mass | <0.0001 | 0.87 |
| **Senesced** | 0 | Rep. Tiller Diameter | 0.0003 | 0.41 |
| **Senesced** | 0 | Rep. Tiller Height | <0.0001 | 0.63 |
| **Senesced** | 0 | Veg. Tiller Diameter | 0.0033 | 0.33 |
| **Senesced** | 0 | Veg. Tiller Height | <0.0001 | 0.58 |
| **Senesced** | 60 | Total Tiller No. | <0.0001 | 0.70 |
| **Senesced** | 60 | Veg. Tiller No. | 0.0441 | -0.23 |
| **Senesced** | 60 | Rep. Tiller No. | <0.0001 | 0.81 |
| **Senesced** | 60 | Tiller Mass | 0.0001 | 0.43 |
| **Senesced** | 60 | Rep. Tiller Height | 0.0252 | 0.25 |
| **Senesced** | 120 | Total Tiller No. | <0.0001 | 0.78 |
| **Senesced** | 120 | Rep. Tiller No. | <0.0001 | 0.84 |
| **Senesced** | 120 | Tiller Mass | <0.0001 | 0.54 |
| **Senesced** | 120 | Rep. Tiller Height | 0.0259 | 0.26 |
| **Senesced** | 120 | Rep. Tiller Phytomer No. | 0.0077 | 0.30 |

**Figure 2.1. Average peak biomass yields of *Miscanthus* x *giganteus* at added nitrogen rates of 0, 60, and 120 kg N $ha^{-1}$ in the 2011 and 2014 growing seasons at Urbana, Illinois.**

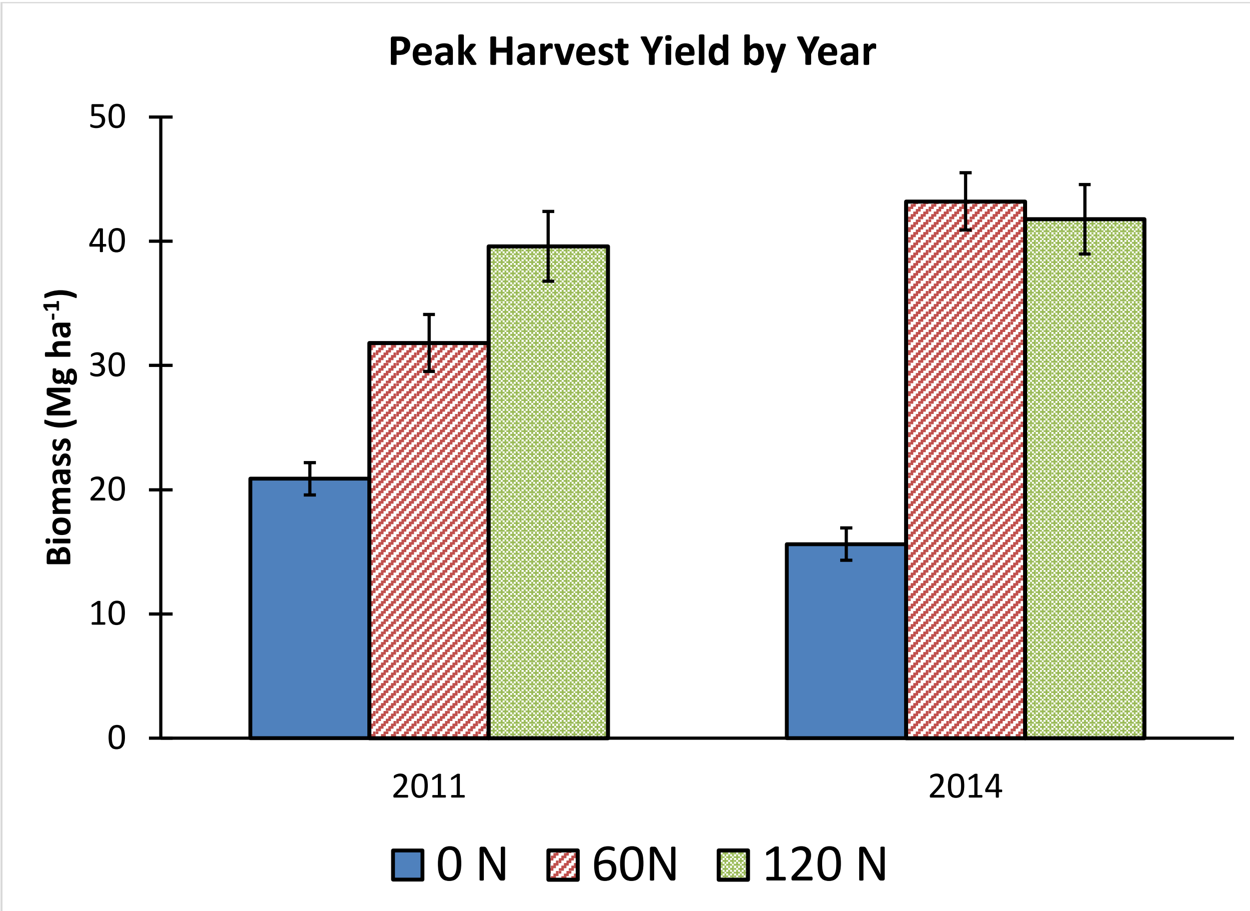


**Figure 2.2. Monthly actual vs. average rainfall rates and 20-year average rainfall for 2011-2014 in Urbana, Illinois.**

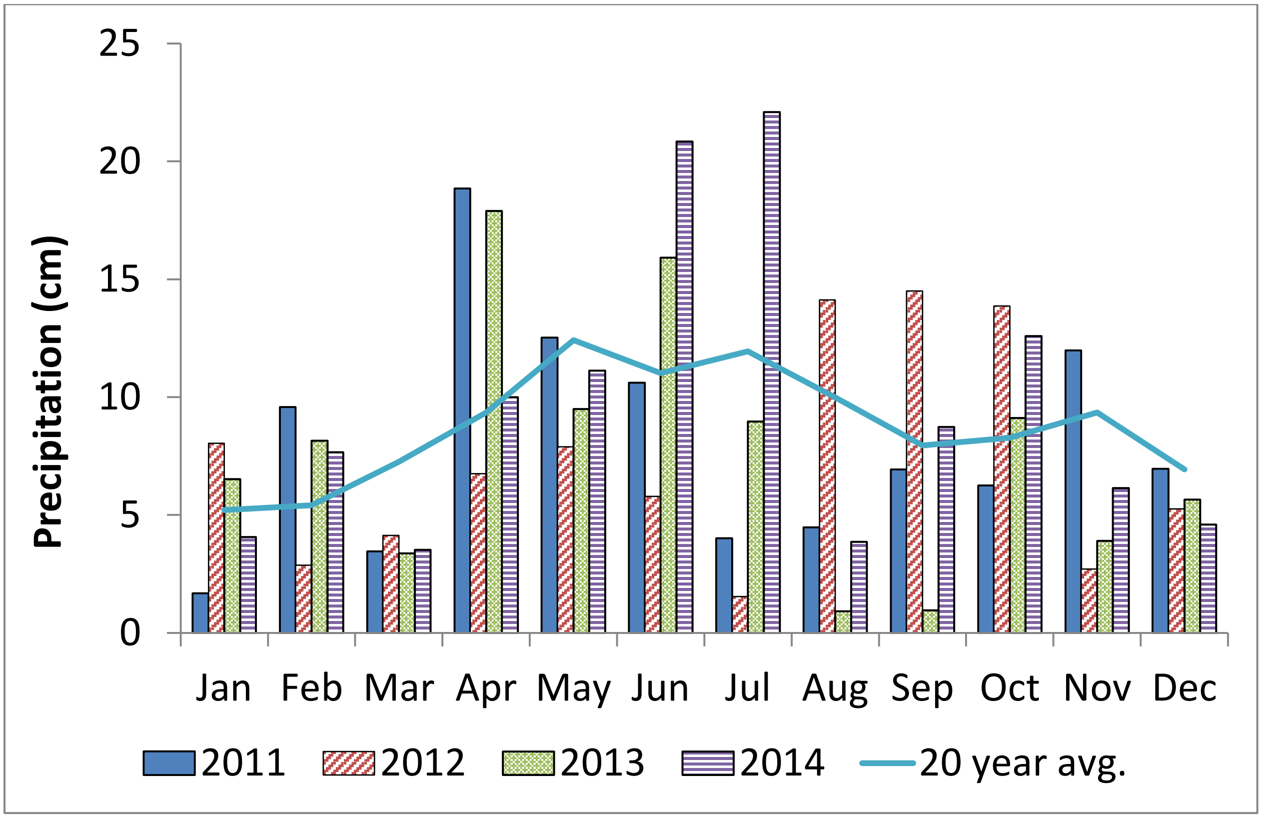


**Figure 2.3. Effect of nitrogen rate on biomass yield of *Miscanthus* x *giganteus* harvested after complete senescence in Urbana, Illinois. Three nitrogen rates (0, 60, and 120 kg ha$^{-1}$) were compared across four crop years (2011-2014). Linear trendlines for each nitrogen rate are also displayed.**

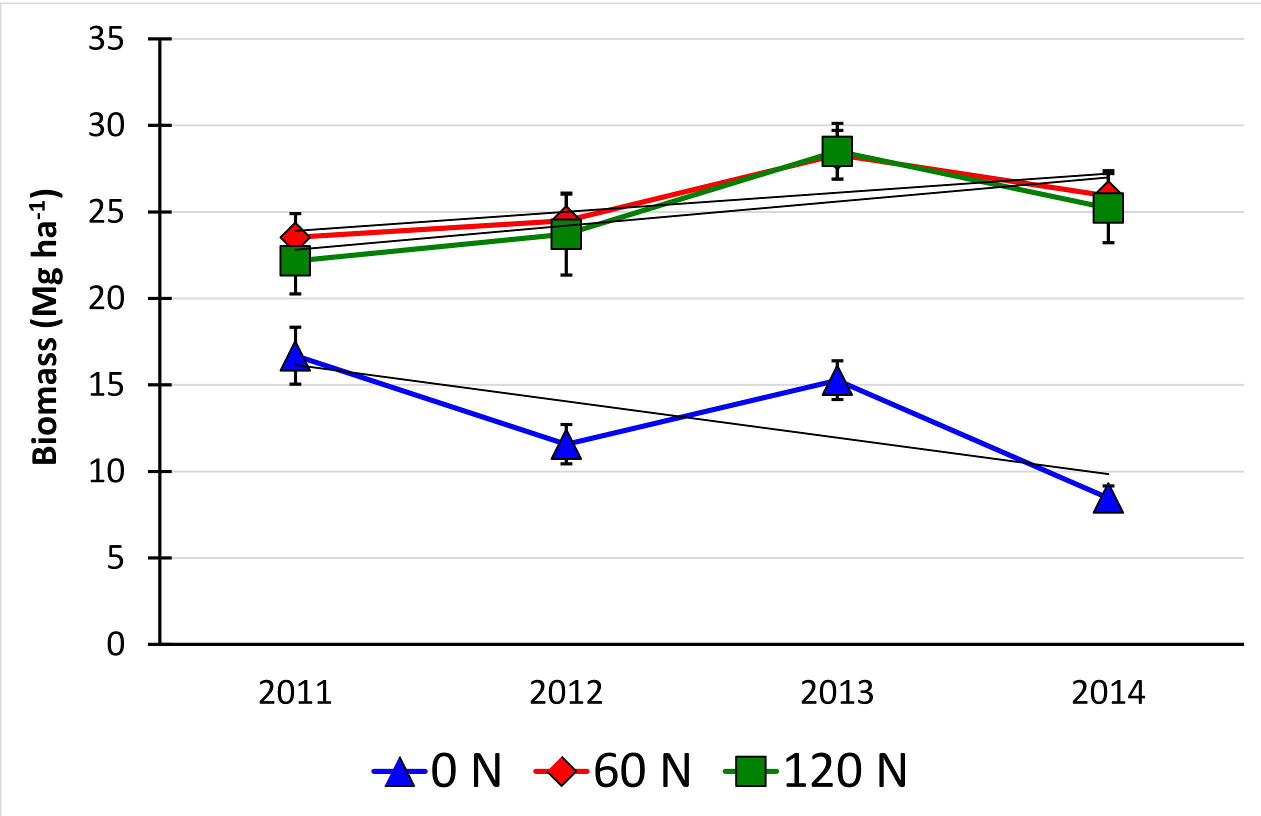


**Figure 2.4. Yields of average peak and senesced *Miscanthus* x *giganteus* biomass yields for three fertility levels in 2011 and 2014 growing seasons at Urbana, Illinois.**

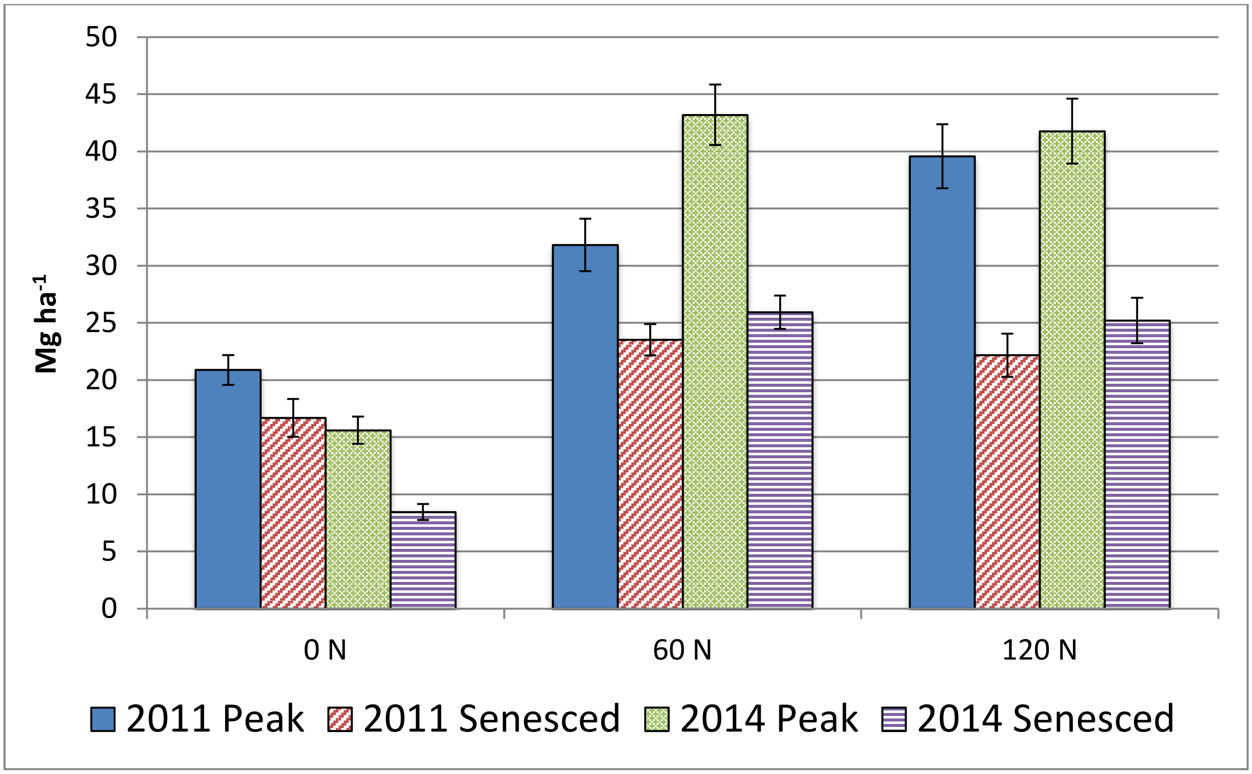
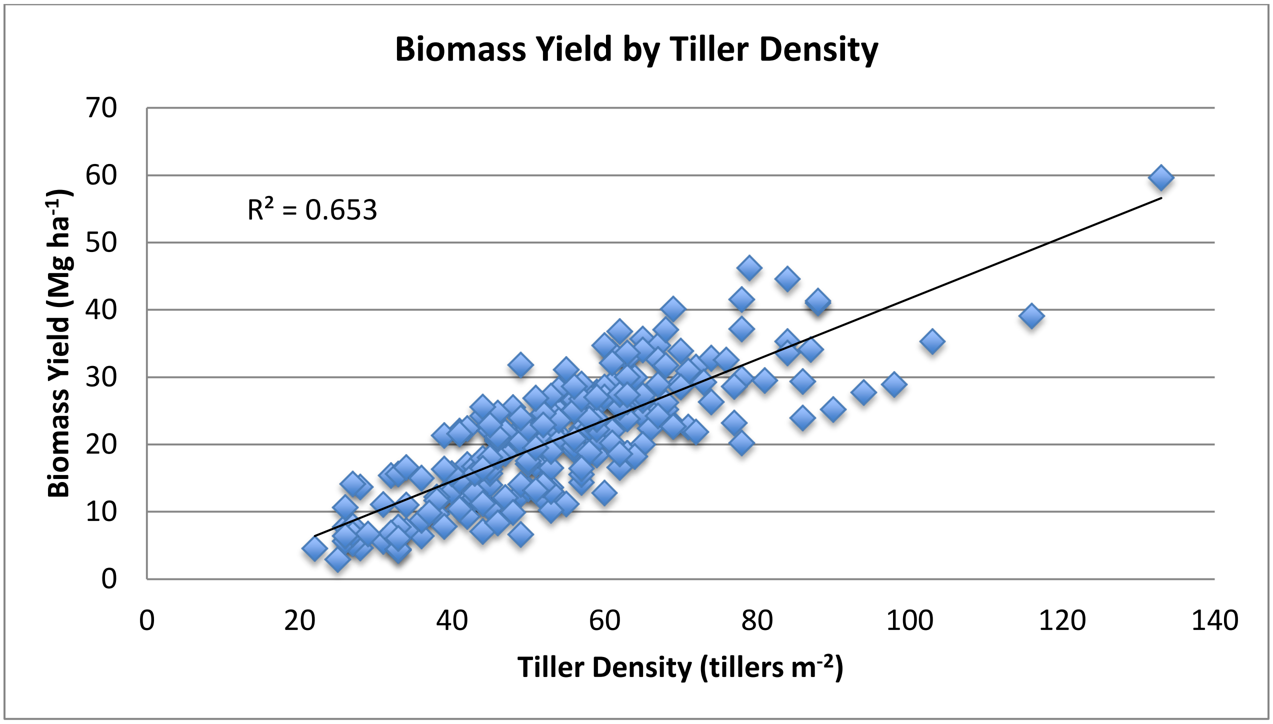


**Figure 2.5. Examples of senesced biomass yield component correlations of *Miscanthus* x *giganteus* with average biomass yield across all nitrogen rates (0, 60, and 120 kg N ha$^{-1}$) in Urbana, Illinois, 2011-2014.**



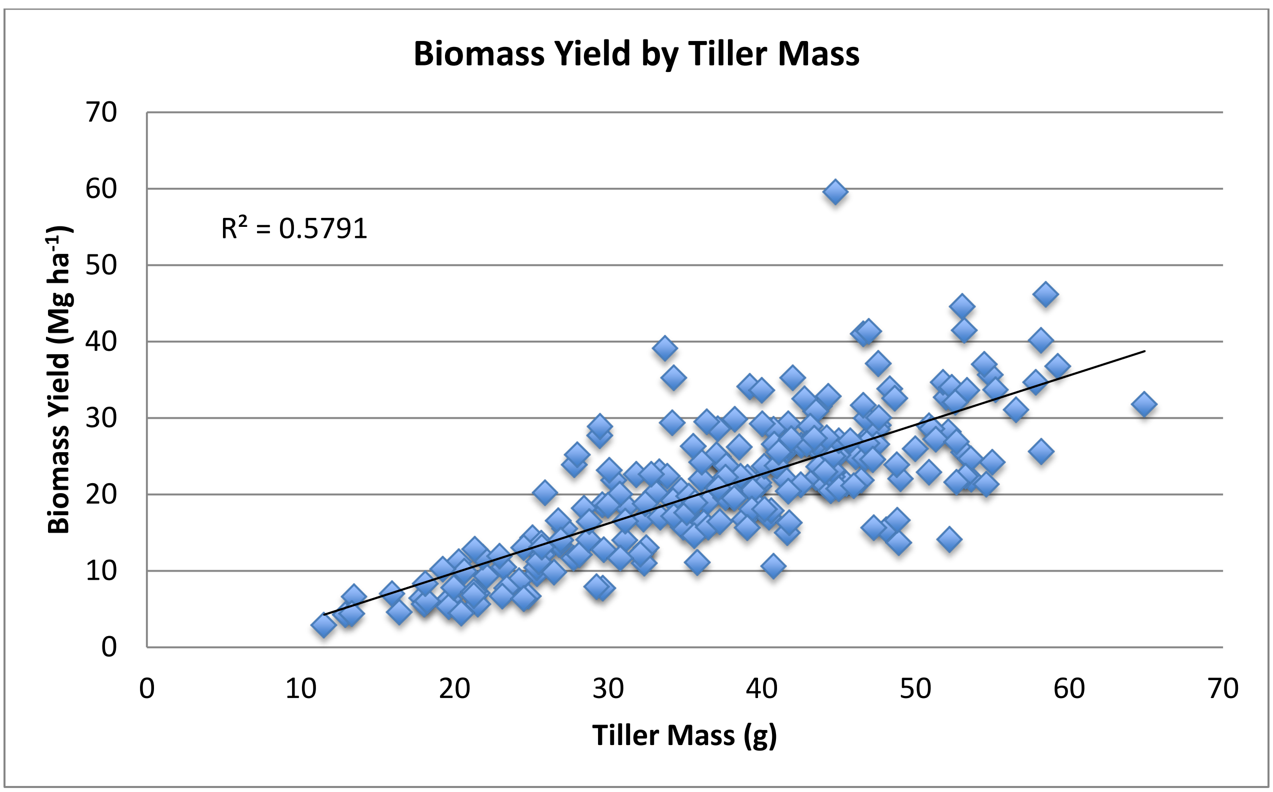



**Figure 2.6. Response of average *Miscanthus* x *giganteus* tiller mass to added nitrogen at rates of 0, 60, and 120 kg N ha$^{-1}$ by year (2011-2014) in Urbana, Illinois. The notable drop in tiller mass corresponds to the extreme drought year of 2012.**

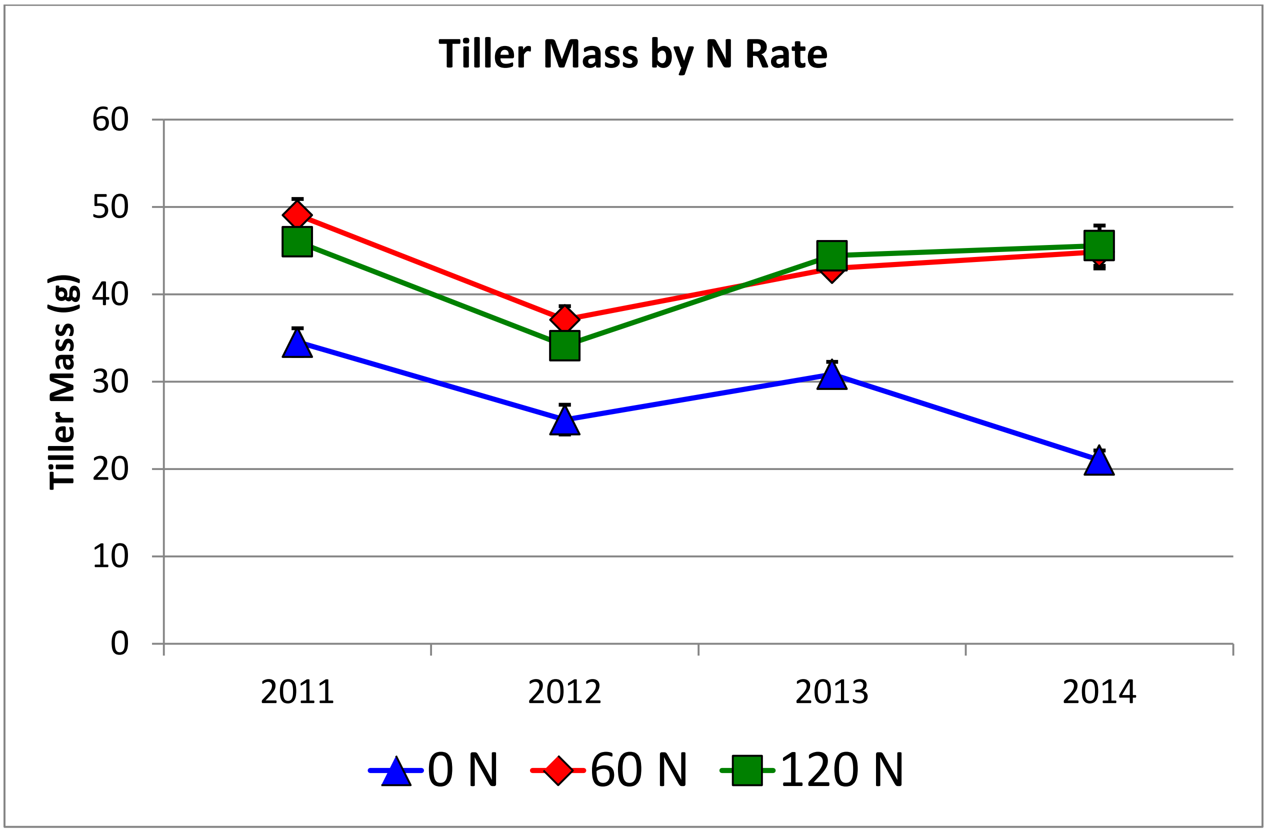
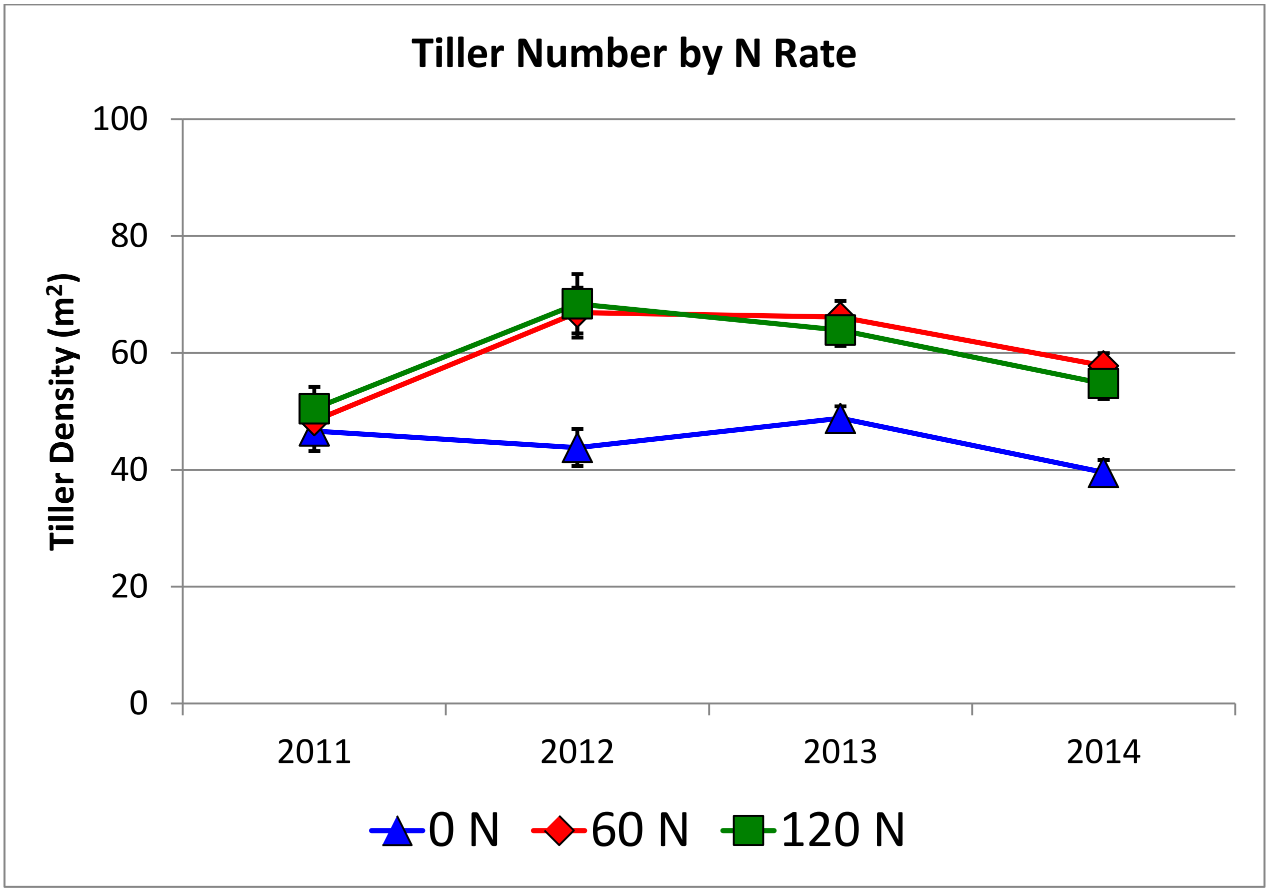


**Figure 2.7. Response of average *Miscanthus* x *giganteus* tiller number to added nitrogen at rates of 0, 60, and 120 kg N ha$^{-1}$ by year (2011-2014) in Urbana, Illinois. The notable rise in tiller number corresponds to the extreme drought year of 2012, when tiller mass had a significant decline.**



## Chapter III

## Perennial Grass Yield Variability Across Eastern North America

### Abstract

In North America, perennial bioenergy crops are being studied as potential replacements for fossil fuels. Given the diversity among perennial grasses, there are many species adapted to most agronomic regions that are potentially high-yielding sources of bioenergy feedstocks for combustion and production of biofuels . *Miscanthus* x *giganteus* (Miscanthus) and *Panicum virgatum* (switchgrass) are two candidate species for widespread bioenergy production. However, previous research on species and cultivar selection, fertility, and effects of yield components on total yield research results have shown mixed results. In this study, the productivity of side-by-side plots of Miscanthus and switchgrass were evaluated over two years in 11 locations in eastern North America. Miscanthus produced more biomass than switchgrass across all sites, while response to N fertilizer applications were variable among sites. Most individual yield component measurements were correlated with yield, and in some cases were affected by N applications. Overall, proper selection of species or switchgrass cultivar plays a large role in biomass production at a given site, while the effect of added N is highly location-dependent.

### Introduction

Although humans rely heavily on fossil fuels, political and environmental issues leave future energy sources in question. Much of the world's oil supply is located in countries that sometimes have strained relations with the U.S. Moreover, geopolitical problems have contributed to volatile energy prices, especially in the mid- to late-2000s. In reality, oil is a dwindling resource; one estimate projects that it could be depleted within 50 years (Keller, 2000). Coal, while relatively abundant, is commonly surface mined, which removes all the soil above a deposit, which can result in environmental damage. Natural gas has become a popular and inexpensive energy source in recent years, but increased extraction relies on hydraulic fracturing or "fracking" techniques, which are controversial and linked to earthquakes in some regions, particularly in Oklahoma (Weingarten et al, 2015).

When burned, fossil fuels release pollutants and greenhouse gases including carbon dioxide, methane, and nitrous oxide into the atmosphere. Carbon dioxide ($CO_2$) is the primary greenhouse gas and is released naturally by respiring living organisms and via combustion of carbon-based fossil fuels that were formed millions of years ago from decomposing life forms and buried deep underground by natural geologic processes. Because this carbon has not been in the aboveground ecosystem for millions of years, combustion releases the previously stored carbon dioxide, increasing atmospheric levels. The U.S. National Oceanic and Atmospheric Administration's Earth System Research Laboratory has reported steady, year-over-year atmospheric carbon dioxide increases at the Mauna Loa, Hawaii observing station since the late 1950s (ESRL, 2016). In 1988, the United Nations formed the Intergovernmental Panel on Climate Change (IPCC) that reported on peer-reviewed studies involving climate change and provided recommendations on policy positions to western governments in an effort to reduce carbon emissions.

Concerns about the continued use of fossil fuels have encouraged consideration of renewable alternatives. While wind and hydroelectric power are currently in use, these renewable energy sources work best in regions where it is continuously windy or where there are rivers on which to build dams. Biofuels produced from biomass could potentially be an energy source on croplands of the U.S. and around the world.

Biofuels are energy sources produced from living plants. Perennial bioenergy crops have several potential advantages compared to fossil fuels. First, perennial biofuels are renewable because plants can be grown and harvested on set intervals. As long as proper land-use techniques are followed, these crops can be grown indefinitely. Second, plants recycle greenhouse gases from the atmosphere. When either fossil fuels or biofuels are burned, $CO_2$ is the primary greenhouse gas released. The carbon released as a result of burning fossil fuels is not re-absorbed into the earth, resulting in a buildup of atmospheric $CO_2$. However, since bioenergy crops utilize $CO_2$ from the atmosphere for growth, there is not a net atmospheric carbon increase. Third, the ability to reduce the amount of fuel being purchased from other countries would help improve U.S. energy security. The development of this new bioenergy market could also help increase investments in rural areas where most crops are grown.

In the U.S., ethanol, produced from corn (*Zea mays* L.) grain, is the most common biofuel with a production of 140 million liters per day in late 2013 (EIA, 2013). Using food crops as energy sources, however, may contribute to global hunger and increase food prices (Babcock, 2011). One potential solution is to grow non-food crops such as perennial grasses for biomass production on marginal, less-productive lands. In fact, the U.S. 2007 Renewable Fuels Standard (RFS2) limited the amount of ethanol production from corn grain to 56.8 billion liters (15 billion gallons) per year by 2022, and encouraged the production of cellulosic ethanol sources (EPA, 2010).

Perennial grasses have a number of production benefits compared to corn and other conventional, annual row crops. Most obviously, being perennial eliminates yearly establishment costs after the initial planting and allows growers to realize multiple years of production from a single planting. There are many species and varieties of perennial grasses that are adapted to different regions, reducing input requirements such as water or fertilizer. These grasses can be used to produce ethanol by breaking down carbon-containing molecules in the plant cell wall, as well as produce heat and electricity through direct combustion, either alone or co-fired with fossil fuels such as coal. In addition, some perennial grasses can also create natural environments for wildlife (Werling et al., 2013).

While there are many perennial grass species that could be grown for biofuel production, the two that have been studied with the greatest interest in the fertile Midwestern U.S. are the warm-season grasses *Miscanthus* x *giganteus* Greef et Deu ex. Hodkinson et Renvoize (Hodkinson and Renvoize, 2001) (hereafter Miscanthus) and *Panicum virgatum* L. (hereafter switchgrass).

Miscanthus has been studied extensively in Europe since the 1980s and is now the focus of much research in the U.S. It is a perennial $C_4$ grass native to eastern Asia (Jones and Walsh, 2001) that shows great potential as a biofuel crop. Originally brought to Denmark from Japan in 1935, it eventually was planted throughout Europe and ultimately made its way to North America as a landscape plant (Scally et al., 2001). It is believed to be a naturally occurring hybrid of *Miscanthus sinensis* and *M. sacchariflorus* (Linde-Laursen, 1993). Since the hybrid Miscanthus is a sterile triploid and does not produce fertile seed, asexual propagation, usually by rhizome divisions, is necessary and the process is difficult and expensive.

In central Illinois, Miscanthus typically grows to 4 meters each year, requires minimal nutrient inputs, and to date, has been resistant to most disease and insect problems that could decrease yield (Anderson et al., 2011). In addition, the stalks dry down well, yielding relatively dry biomass (Jones and Walsh, 2001), a desirable trait for immediate use or storage for later use.

Miscanthus productivity has been studied by both European and U.S. researchers. Lewandowski et al. (2000) report annual yields in Europe as high as 25 Mg dry biomass $ha^{-1}$ on non-irrigated land, and more than 30 Mg $ha^{-1}$ on irrigated land. These authors also confirmed the crop's low fertilizer requirements and reported its high establishment costs due to vegetative propagation (Lewandowski et al., 2000). Despite the high establishment costs, Heaton et al. (2004) indicated that its future as a biofuel crop is positive when compared to other crops, specifically switchgrass. Miscanthus yields in the U.S. are comparable to those from Europe and generally range from 15 to 30 Mg dry biomass $ha^{-1}$ $year^{-1}$ (Heaton et al., 2004; Heaton et al., 2008; Maughan et al., 2012).

Switchgrass is a warm-season perennial grass native to much of the continental U.S. Although it has been used as a forage crop for many years, it also became one of the first extensively studied bioenergy crops in the U.S. The U.S. Department of Energy (DOE) first identified switchgrass as a "model" energy crop as early as 1991 (Wright and Turhollow, 2010). In 1997, the DOE's Bioenergy Feedstock Development Program (BFDP) identified several reasons for producing switchgrass as a bioenergy crop on a large scale. It's wide native range, high potential yields, good nutrient-use efficiency, benefits to wildlife, and positive effects on the soil were among the reasons it was singled out for further study (McLaughlin and Walsh, 1997). Switchgrass research has increased over the ensuing years, and the grass gained even greater attention as a bioenergy crop after former President George W. Bush mentioned its bioenergy potential and use in the 2006 State of the Union address (Bush, 2006).

Switchgrass is commonly divided into upland and lowland types. Lowland switchgrasses are native to more southern U.S, regions, and commonly exhibit a taller growth habit, more robust growth, larger vegetative components, and lower fertilizer requirements than upland cultivars (Porter, 1966). Upland switchgrasses were traditionally more desirable as forage crops due to finer texture (Porter, 1966). In the early days of bioenergy research, it was generally unknown how well lowland cultivars would survive in more northern climates (personal observation). As research progressed, it became clear that many lowland cultivars could survive and thrive further north than anticipated.

Numerous studies have examined various aspects of switchgrass biomass production for bioenergy. For example, Vogel et al. (2002) found that at the optimal nitrogen fertilization rate of 120 kg N $ha^{-1}$, the upland cultivar 'Cave-in-Rock' produced as much as 12.6 Mg dry biomass $ha^{-1}$ $year^{-1}$ in Iowa, U.S.A. Thomason et al. (2004) reported unfertilized lowland Kanlow switchgrass produced 16.9 Mg dry biomass $ha^{-1}$ $year^{-1}$ in Oklahoma, U.S.A. In a multi-location U.S. study, Fike et al. (2006) wrote that several switchgrass cultivars could be grown over a long time period and with average annual yields of approximately 14 Mg dry biomass $ha^{-1}$. After the 10-year Biomass Feedstock Development Program, McLaughlin and Kszos (2005) concluded that the selection of the proper varieties, proper timing of harvests, improved breeding methods, and reductions in nitrogen fertilization could significantly increase yields. In short, switchgrass has been shown to be a very promising bioenergy crop.

The first side-by-side evaluations of Miscanthus and switchgrass in the U.S. were established in 2002 (Heaton et al., 2008). Miscanthus and ‘Cave-in-Rock’ switchgrass were planted at northern, central, and southern locations in Illinois, U.S.A. Results indicated Miscanthus yields were significantly higher than those of switchgrass, suggesting that the high yields of Miscanthus could be sufficient to offset a large amount of U.S. energy use if planted on sufficient acreage (Heaton et al., 2008). Four additional Illinois sites were added in 2004 and the effects of nitrogen fertilization were evaluated at all 7 sites (Arundale et al., 2014b). Results indicated a long-term yield decline in aging stands of both crops (Arundale et al., 2014a), but one that could be partially arrested by nitrogen fertilizer applications (Arundale et al., 2014a).

In 2009 and 2010, additional testing sites were established across sites in eastern North America to determine how these crops compared over a larger geographic area with differing soil types and climatic conditions (Arundale, 2012). Results from the young plots reported the same trends, with Miscanthus producing more biomass annually than switchgrass, even when locally adapted switchgrass cultivars were planted (Arundale, 2012). However, these plots were only sampled in their early years; not long enough to determine whether the yield decline seen at the IL sites would occur across a broader area (Arundale, 2012).

The morphology of these grasses, in particular the individual yield components, could play a role in helping to estimate total yield. Switchgrass yield component effects have been studied in a number of regions (Casler, 2004; Boe and Casler, 2005; Boe, 2007; Boe and Beck, 2008), but never compared directly to Miscanthus. Tiller density, height, type (reproductive or vegetative), and phytomer (vegetative stem unit from node to node) number are all aspects that could potentially be correlated with yield. For example, Boe (2007) found that the number of reproductive tillers per $m^2$ was important in selecting varieties for greater biomass production.  While switchgrass is fertile and breeding efforts can target improvement of specific yield components for increased yields, the sterility of Miscanthus hinders breeding efforts. However, determination of changing component effects across regions and fertilization treatments, especially when compared to another species in the same environment, may prove useful.

This study aimed to examine the effects of fertilization on biomass yield and individual yield components in side-by-side replicated plots of Miscanthus and switchgrass across 11 locations in eastern North America.  It compared Miscanthus and switchgrass productivity at several locations, evaluated the effects of nitrogen fertilization on biomass yield, and determine if individual yield components were correlated with biomass yields.

**Methods**

All plots were established and managed as in Heaton, et al. (2008), Arundale (2012), and Arundale et al. (2013).  Briefly, individual 10m x 10m plots were planted in a completely randomized design at each location.  The Miscanthus was established using rhizomes or small plugs propagated from rhizomes.  The switchgrass was seeded into rotary-tilled seedbeds. Depending on location, weeds were controlled mechanically or chemically for one or two growing seasons; crop growth was sufficient to suppress weed growth thereafter.  Following crop sampling, the remaining biomass was removed annually following plant senescence.

In Table 3.1, the study sites' locations and latitudes, establishment years, switchgrass cultivars, and average annual temperatures and precipitation are listed.  In 2013 and 2014, half of each plot either remained unfertilized or received 60 kg N $ha^{-1}$ using granular urea (46-0-0).

All plots were manually harvested for analysis by cutting five representative 1-$m^2$ quadrats following senescence. Samples were not taken from borders. Each quadrat was individually bundled, weighed, and taken off-site for additional measurements. For each bundle, the total dry weight and reproductive and vegetative tiller counts were determined. As reproductive tillers tend to make up the majority of total tillers in mature Miscanthus, ten representative reproductive tillers were randomly selected from each bundle to make phytomer counts and measure tiller heights. If ten reproductive tillers were not available, vegetative tillers were selected to bring the total measured tiller number to ten.

Results were analyzed by using Proc Mixed in SAS Version 9.3 (SAS Institute, Cary, NC, USA). Location (L), crop (C), and added nitrogen fertility (F) were considered fixed effects. Year (Y) and block (B) were considered random. By considering year and its interactions as random effects, differences in weather from year to year are taken into account. Each factor, their interactions, and whole plot (d) and subplot (e) errors were analyzed with the following model:

$$y = \mu + L + C + F + B + (LC) + (LF) + (CF) + (LCF) + d + Y + (YL)$$
$$+(YC) + (YF) + (YLC) + (YCF) + (YLF) + (YLCF) + e$$

In order to analyze specific differences by location, a similar model with location effects removed was used:

$$y = \mu + C + F + B + (CF) + d + Y + (YC) + (YF) + (YCF) + e$$

**Results**

Across all locations and in both years, dry yield effects by crop were significant (P=0.0303) (Table 3.2). When each site was analyzed separately, crop effect was highly significant at all locations (P<0.0001) (Table 3.3). Total average Miscanthus yields were greater than switchgrass yields at all locations in this study (Fig. 3.1). Averaged over both fertility levels, the highest Miscanthus yield was at Brownstown (22.3 Mg $ha^{-1}$) and the lowest yields were at New Jersey (13.81 Mg $ha^{-1}$). The greatest switchgrass average yield in this study was at Kentucky where the cultivar 'Alamo' produced 11.98 Mg $ha^{-1}$ biomass. Direct comparison between switchgrass yields at all sites was difficult because of cultivar differences, but when comparing the seven sites with 'Cave-in-Rock' switchgrass, yields were lower than any of the other cultivars. The greatest Cave-in-Rock switchgrass yield was at Michigan (6.26 Mg $ha^{-1}$) and the lowest at Urbana (3.87 Mg $ha^{-1}$). The lowest-producing non-'Cave-in-Rock' switchgrass was 'Timber' at New Jersey (8.87 Mg $ha^{-1}$). Only Alamo switchgrass in Kentucky fertilized with 60 kg N $ha^{-1}$, was more productive (13.21 Mg $ha^{-1}$) than the lowest-yielding, unfertilized Miscanthus (11.93 Mg $ha^{-1}$) at New Jersey (Fig. 3.2), but not greater than the average from both fertility levels.

There were no site effects (P=0.0593) across species or years, likely due to the wide range of site environments and because Miscanthus yielded more biomass than switchgrass across all locations. But there were site by crop interection effects (P=0.0005) (Table 3.2), indicating a yield difference between Miscanthus and switchgrass in different environments or between different switchgrass varieties (Fig. 3.1). For example, switchgrass yields at Brownstown and Urbana were only 28% and 20% of Miscanthus yields, respectively. At Kentucky and New Jersey, switchgrass yields were 73% and 64% of Miscanthus yields, respectively. It is likely that switchgrass cultivar played a large role in this effect, since 'Cave-in-Rock' stands exhibited a much greater yield gap with Miscanthus than the other cultivars.

There were also key differences in fertility effect based on location. Across all locations and both species, main effects of fertility (P=0.0004) and interaction effects of site by fertility (P=0.0148) were significant. But when separated by site, the significance of applied nitrogen varied (Table 3.3). This would be expected in sites that had different environments, soil types, or native fertility levels where one location would mute a response from applied fertilizer. Individually, New Jersey, Orr, Illinois, and Kentucky sites showed increased Miscanthus yields with 60 kg N $ha^{-1}$, but Michigan yields declined with applied N (Fig. 3.3). Other sites generally showed numerical, but not significant yield increases. There were switchgrass yield increases in Ontario, Kentucky, and Mississippi with applied N (Fig. 3.3). Kentucky was the only site in which Miscanthus and switchgrass yields both increased with nitrogen fertilization. There were no fertilizer yield effects at sites where Cave-in-Rock switchgrass was grown.

Significant crop effects on yield components would be expected since the growth habits of the two crops were very different. Therefore, when separated by species, individual yield components of Miscanthus and switchgrass showed differing relationships to biomass yield. Even within switchgrass, a direct comparison between sites was difficult due to different cultivars planted. For example, switchgrass reproductive tiller number was not significant across all locations (Table 3.4). But when separated out by site, reproductive tiller number was the highest-correlated component with biomass yield (Table 3. 5).

Miscanthus, however, showed an interesting trend. Biomass yield was highly correlated with tiller number at several sites, while simultaneously not well correlated with tiller height (Table 3.6). The opposite was true at other sites; Miscanthus biomass yield was correlated with tiller height, but not tiller number. For example, Miscanthus biomass at Kentucky correlated with total tillers ($R^2$=0.51), but not with tiller height ($R^2$=0.02) (Fig. 3.4). At Michigan, Miscanthus biomass was correlated with tiller height ($R^2$=0.71), but not tiller number ($R^2$=-0.00) (Fig. 3.5). Interestingly, Kentucky Miscanthus biomass increased significantly when N was applied, but Michigan biomass decreased as N was applied (Fig. 3.3), indicating a potential role of added fertility on component correlations.

**Discussion**

Miscanthus was more productive than switchgrass at most locations previously studied (Heaton et al., 2008; Arundale et al., 2014b). This study confirmed those findings, with total average switchgrass yields being lower than Miscanthus at all locations, even when locally selected and adapted switchgrass varieties were planted. Some sites produced greater fertilized-switchgrass yields than other sites' unfertilized Miscanthus yields (Table 3.3), but the average of fertilized and unfertilized Miscanthus was always greater (Table 3.2).

The possibility of yield decline with stand age was another factor in determining proper agronomic practices in perennial grass bioenergy systems (Arundale et al., 2014a). Plot stand-age differences made it difficult to determine precisely, but a few general observations can be made. First, yields of the five older IL Miscanthus stands were stable or decreased slightly compared to yields reported by Arundale et al. (2014a), even though harvest methods differed between the studies. Specifically, the 0.19-$m^2$ subsample quadrats in the earlier study showed greater variability than our 1-$m^2$ harvested quadrats. Switchgrass yields appeared to be much lower in our study with yields of approximately 5 Mg $ha^{-1}$, compared to approximately 10 Mg $ha^{-1}$ in previous work (Arundale et al., 2014a). Secondly, this study was the first time the six newer sites across North America had been sampled in multiple years when the plantings were mature. Arundale et al. (2012) reported on productivity following the 2011-growing season (third growing season) for five of the six sites (except Ontario), which could generally be considered a “mature” stand. In our study, yields were less than in Arundale et al. (2012).

Stand age alone, however, does not appear to matter to the extent of species and location selection. The greatest average Miscanthus productivity across both years was from the 2004 planting at Brownstown, IL (22.3 Mg $ha^{-1}$), and the lowest average Miscanthus yield was from New Jersey (13.8 Mg $ha^{-1}$) planted in 2009. Additionally, the New Jersey Miscanthus planting was very similar to Miscanthus at Urbana (14.5 Mg $ha^{-1}$), which was one of the oldest sites, planted in 2002. The same trend was seen in switchgrass, with the biomass production at the newer Illinois and Michigan (‘Cave-in-Rock’) sites planted in 2009 produced similarly to the older Illinois sites planted in 2002 and 2004. The other young switchgrass sites produced more biomass, including New Jersey where Miscanthus yields were lowest, indicating that cultivar plays a much larger role in switchgrass productivity than stand age.

Even though the relative importance between Miscanthus and switchgrass yield components could be similar, individual yield component effects were inherently difficult to compare directly between species due to the greater number of small stems produced by switchgrass, requiring separate evaluations of each. Most switchgrass yield components, especially reproductive tiller numbers, correlated with total biomass productivity across both fertility levels when separated by location. These results compare favorably to other research on switchgrass yield components, particularly to results reported by Boe (2007).

The variability in correlations between biomass yield and tiller number and tiller height in Miscanthus was more difficult to analyze due to the lack of Miscanthus yield component research to date. However, there may be a pattern here. As previously noted, Miscanthus biomass in Kentucky was correlated with tiller number, but not height, while the opposite was true in Michigan (Table 3.6). The Kentucky site also showed a significant increase in yield when N was applied, with the opposite true in Michigan. It is possible that when nitrogen is limiting, as was the case in Kentucky, Miscanthus was not able to produce its full compliment of tillers. When nitrogen is not limiting, as was the case in Michigan, tiller numbers remained constant with added fertility, but height increased. The average unfertilized Kentucky tiller numbers (55.1 tillers per $m^2$) were lower than fertilized (62.8 tillers per $m^2$), while average tiller height was identical between fertilizer treatments (2.82 m). At Michigan, tiller numbers decreased (61.1 to 57.2 tillers per $m^2$), while height increased (14.75 to 14.87 m) with added nitrogen. Further research would help determine if this is a repeatable effect.

Even with consistently greater Miscanthus yields across all locations, it was clear that local environments played a role in crop selection and fertilizer response. 'Cave-in-Rock' switchgrass was a poor choice for substantial biomass production when compared to Miscanthus. But in Kentucky and New Jersey, for example, where locally adapted varieties of switchgrass were planted, the biomass yield differences between the two species were smaller. In recent years, a seed smut disease (*Tilletia maclaganii*) found in much of the U.S. (Farr et al., 1995) caused substantial yield decreases in switchgrass (Thomsen et al., 2008). Sites were not specifically monitored for the disease, but it has been identified on 'Cave-in-Rock' switchgrass in Illinois.

Overall switchgrass yields in this study were lower than the potential yields reported in previous studies, particularly those that grew lowland switchgrass cultivars, which are able to produce greater biomass. With proper switchgrass cultivar selection, it may be possible to create a better bioenergy cropping systems in some locations by planting switchgrass, particularly when accounting for the added difficulty of establishing Miscanthus rhizomes and the potential “backup” forage market for switchgrass. But the consistently higher yields of Miscanthus across many locations, even with its lower projected yields than in earlier studies, make it an attractive choice if maximizing biomass for bioenergy is the goal.

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## Tables and Figures

**Table 3.1. Description of planting sites for Miscanthus and switchgrass side-by-side study sites. Partially adapted from Arundale (2012). Weather data is from the National Oceanic and Atmospheric Administration National Centers for Environmental Information (2016) and Environment Canada (2016).**

| Site Name, Nearest Town (Establishment Year) | Latitude, Longitude | Switchgrass Cultivar | Ave. Annual Temp °C | Ave. Annual Rainfall (cm) | Soil Type |
|---|---|---|---|---|---|
| **Dixon Springs, Simpson, IL (2002)** | 37.4535° N, 88.7229° W | Cave-in-Rock | 14.6 | 123.8 | Grantsburg silt loam |
| **Urbana, Urbana, IL (2002)** | 40.0425° N, 88.2378° W | Cave-in-Rock | 10.8 | 104.3 | Flanagan silt loam |
| **Brownstown, Brownstown, IL (2004)** | 38.9509° N, 88.9601° W | Cave-in-Rock | 12.2 | 110.6 | Cisne silt loam |
| **Havana, Havana, IL (2004)** | 40.2952° N, 89.9438° W | Cave-in-Rock | 11.0 | 101.2 | Sparta loamy sand (87%) + Plainfield sand (13%) |
| **Orr, Perry, IL (2004)** | 39.8062° N, 90.8195° W | Cave-in-Rock | 11.7 | 102.7 | Winfield silt loam (97%) |
| **U. of I. Energy Farm, Urbana, IL (2009)** | 40.0652° N, 88.1907° W | Cave-in-Rock | 10.8 | 104.3 | Thorp silt loam (80%) + Wyanet silt loam (20%) |
| **U. of Kentucky, Lexington, KY (2009)** | 38.1277° N, 84.4971° W | Alamo | 12.9 | 116.6 | Bluegrass-Maury silt loams |
| **Michigan State U., Hickory Corners, MI (2009)** | 42.3947° N, 85.3766° W | Cave-in-Rock | 9.4 | 101.2 | Kalamazoo loam |
| **Mississippi State U., Starkville, MS (2009)** | 33.4245° N, 88.7952° W | Ceres EG1101 (improved Alamo) | 16.8 | 140.8 | Marietta fine sandy loam |
| **Rutgers U., New Brunswick, NJ (2009)** | 40.4636° N, 74.4269° W | Timber | 10.2 | 126.7 | Sassafras-Urban land complex |
| **U. of Guelph, Guelph, ON, Canada (2010)** | 43.6414° N, 80.4108° W | Carthage | 6.3 | 93.9 | London silt loam |

**Table 3.2. Significant effects of individual yield components on total biomass yield across both Miscanthus and switchgrass at all locations and both years (2013 and 2014). Significance levels indicated are P <0.0001 (***), P ≤0.005 (**), P ≤0.05 (*), or not significant (ns).**

| Effect | Dry Wt. | Total Tillers | Veg. Tillers | Rep. Tillers | Phytomers | Tiller Height |
|---|---|---|---|---|---|---|
| Site | ns | * | ** | ** | *** | *** |
| Crop | * | * | *** | *** | *** | *** |
| Site x Crop | ** | ** | ** | *** | ** | *** |
| N-rate | ** | ns | ns | ns | ** | ns |
| Site x N-rate | * | * | ns | *** | * | ns |
| Crop x N-rate | ns | *** | * | ns | ns | ns |
| Site x Crop x N-rate | * | * | * | ns | ns | ns |

**Table 3.3. Significant effects of yield components on total biomass yield across both Miscanthus and switchgrass in 2013 & 2014, separated by sites. Significance levels indicated are P <0.0001 (***), P ≤0.005 (**), P ≤0.05 (*), or not significant (ns).**

| Effect | BT | DS | HA | IL | KY | MI | MS | NJ | ON | OR | UR |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Crop | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| N-rate | ns | ns | ns | * | *** | ns | ns | * | ** | * | * |
| Crop x N-rate | ns | ns | ns | * | ns | ns | ns | ns | ns | * | ns |
| Year | * | *** | *** | * | ns | *** | ns | ns | * | * | * |
| Crop x Year | ns | *** | ns | * | ns | * | * | ns | * | ns | ns |

BT = Brownstown, IL; DS = Dixon Springs, IL; HA = Havana, IL; IL = Illinois, KY = Kentucky, MI = Michigan, MS = Mississippi, NJ = New Jersey, ON = Ontrario, OR = Orr Center, IL; UR = Urbana, IL

**Table 3.4. Significant effects on yield and individual yield components of switchgrass across all locations for both years (2013 and 2014). Significance levels indicated are P <0.0001 (***), P ≤0.005 (**), P ≤0.05 (*), or not significant (ns).**

| Effect | Dry Wt. | Total Tillers | Reproductive Tillers | Vegetative Tillers | Phytomers | Tiller Height |
|---|---|---|---|---|---|---|
| Site | *** | ** | ns | *** | ** | ns |
| N-rate | ** | *** | *** | *** | *** | *** |
| Site x N-rate | *** | * | ** | ns | ns | * |
| Year | ns | ns | ns | *** | *** | *** |
| Year x Site | *** | *** | *** | *** | ** | *** |

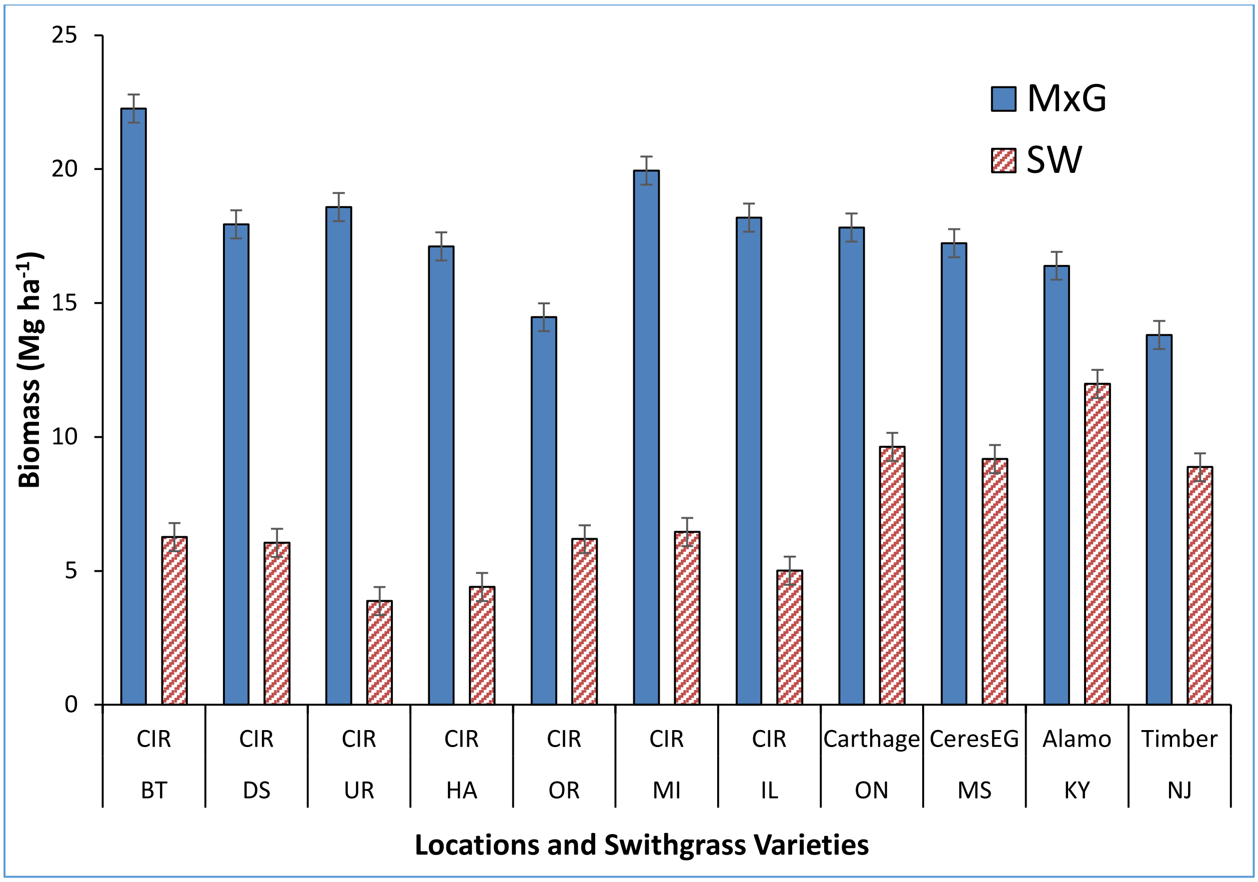
**Table 3.5. Correlation coefficients (R value) of individual yield components by biomass yield in switchgrass across the 11 locations of this study.**

| Site | Total Tillers | Reproductive Tillers | Vegetative Tillers | Phytomers | Tiller Height |
|---|---|---|---|---|---|
| Brownstown, IL | 0.59 | 0.75 | 0.00 | 0.25 | 0.40 |
| Dixon Springs, IL | 0.38 | 0.58 | 0.05 | 0.30 | 0.32 |
| Havana, IL | 0.93 | 0.95 | 0.31 | 0.93 | 0.86 |
| Illinois | 0.39 | 0.64 | 0.01 | 0.38 | 0.53 |
| Kentucky | 0.41 | 0.63 | -0.33 | 0.24 | 0.48 |
| Michigan | 0.51 | 0.76 | -0.05 | 0.58 | 0.43 |
| Mississippi | 0.69 | 0.77 | 0.19 | 0.30 | 0.36 |
| New Jersey | 0.82 | 0.92 | -0.15 | 0.53 | 0.63 |
| Ontario | 0.84 | 0.89 | -0.22 | 0.48 | 0.64 |
| Orr Center, IL | 0.85 | 0.92 | 0.55 | -0.67 | -0.21 |
| Urbana, IL | 0.89 | 0.94 | 0.47 | 0.52 | 0.79 |

**Table 3.6. Correlation coefficients (R value) of total tillers and tiller height of Miscanthus at each of 11 locations in this study. Results show a pattern of one component being highly correlated while the other is not.**

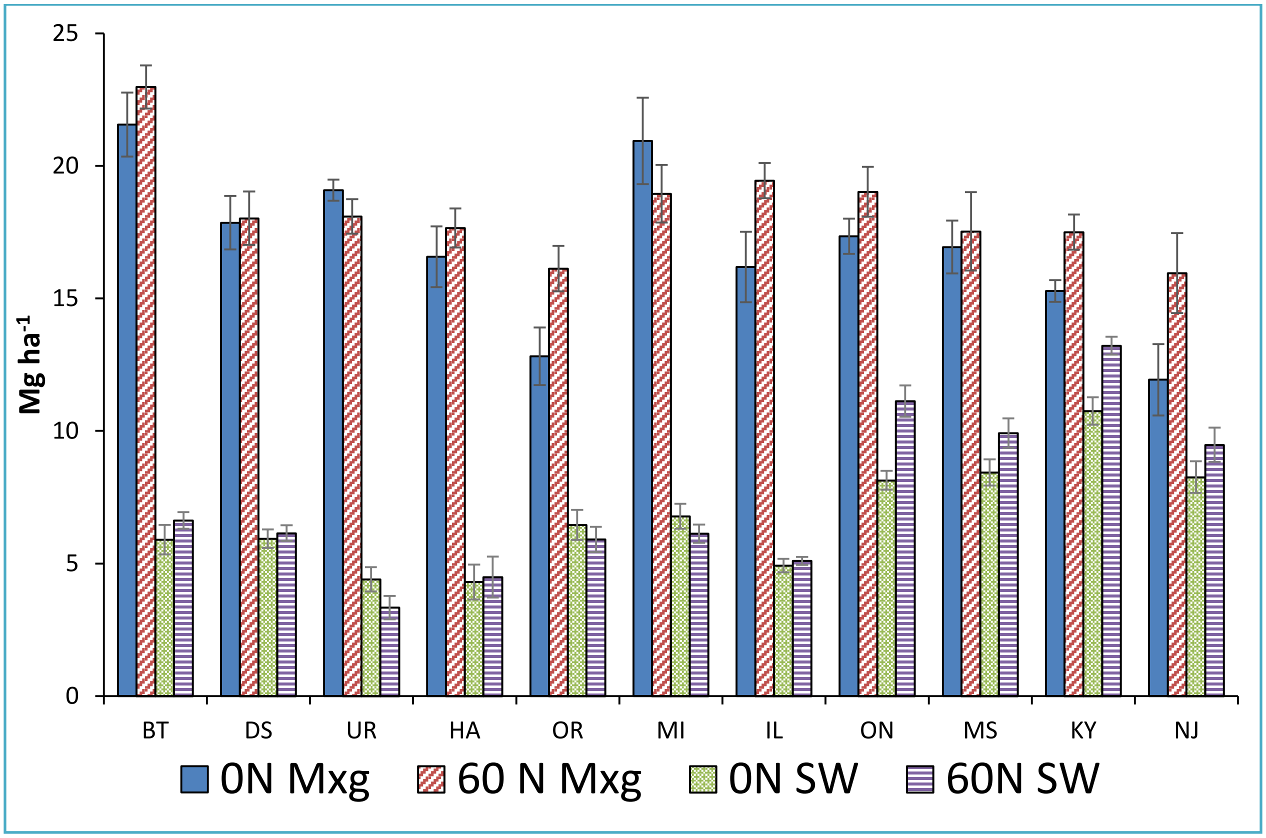
| Site | Total Tillers | Tiller Height |
|---|---|---|
| Brownstown, IL | 0.82 | 0.11 |
| Dixon Springs, IL | -0.58 | 0.90 |
| Havana, IL | 0.39 | 0.76 |
| Illinois | 0.36 | 0.29 |
| Kentucky | 0.72 | 0.14 |
| Michigan | -0.05 | 0.84 |
| Mississippi | 0.52 | 0.85 |
| New Jersey | 0.78 | 0.38 |
| Ontario | -0.19 | 0.92 |
| Orr Center, IL | 0.76 | 0.58 |
| Urbana, IL | 0.72 | 0.30 |

**Figure 3.1. Total biomass yields from 2013 and 2014 of Miscanthus (Mxg) and switchgrass (SW) across 11 locations, with fertilization treatments averaged together. Switchgrass cultivar (CIR stands for 'Cave-in-Rock') and site locations are listed underneath for comparison. The first five locations (BT, DS, UR, HA, and OR) are Illinois sites first planted in 2002 or 2004. The latter six locations (MI, IL, ON, MS, KY, and NJ) are spread across eastern North America and planted in 2009 or 2010.**



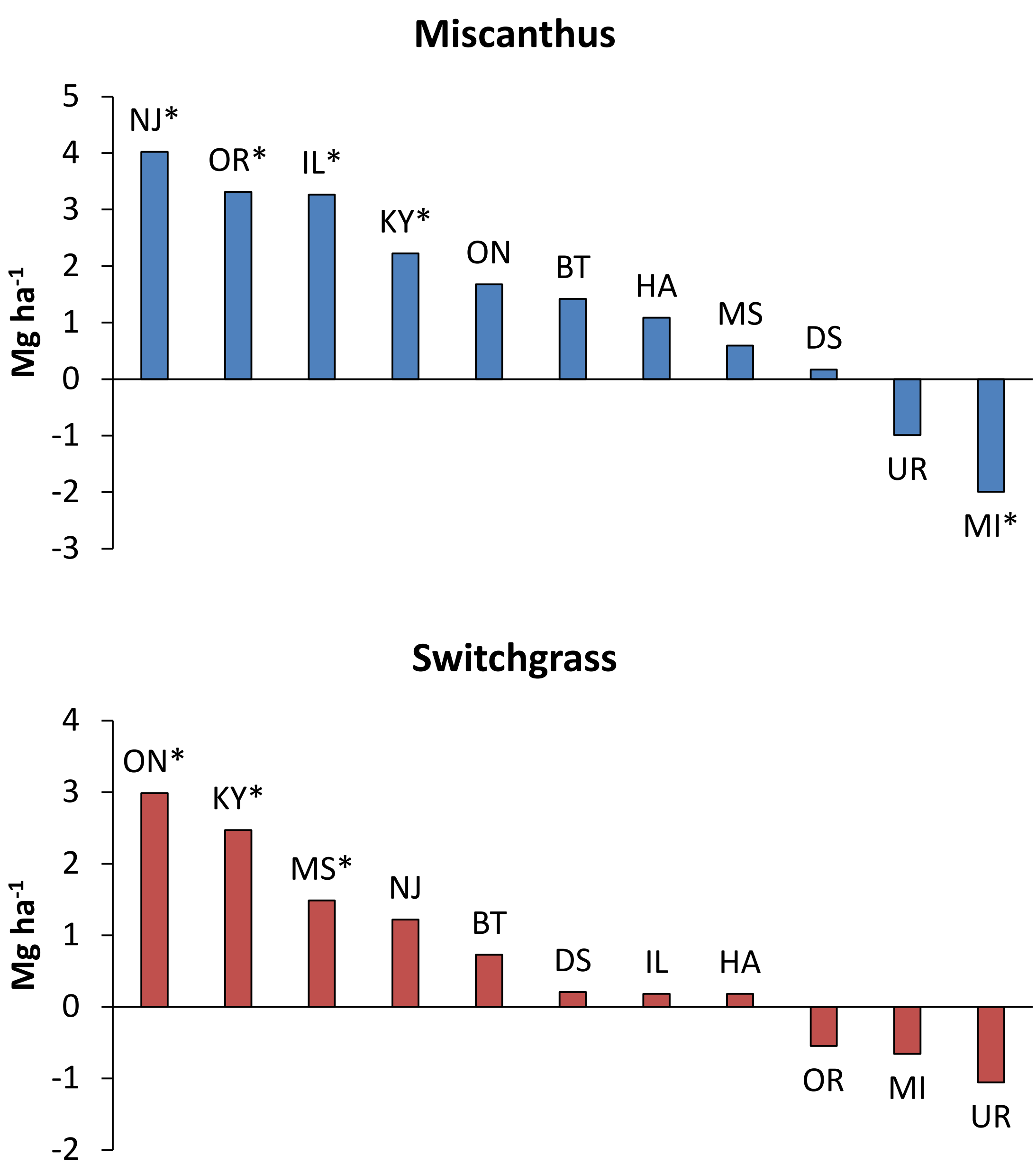

BT = Brownstown, IL; DS = Dixon Springs, IL; HA = Havana, IL; IL = Illinois, KY = Kentucky, MI = Michigan, MS = Mississippi, NJ = New Jersey, ON = Ontrario, OR = Orr Center, IL; UR = Urbana, IL

**Figure 3.2. Total biomass yields from 2013 and 2014 of Miscanthus (Mxg) and switchgrass (SW). Switchgrass cultivar and site locations are listed underneath for comparison. The first five locations (BT, DS, UR, HA, and OR) are Illinois sites first planted in 2002 or 2004. The latter six locations (MI, IL, ON, MS, KY, and NJ) are spread across eastern North America and planted in 2009 or 2010.**




BT = Brownstown, IL; DS = Dixon Springs, IL; HA = Havana, IL; IL = Illinois, KY = Kentucky, MI = Michigan, MS = Mississippi, NJ = New Jersey, ON = Ontrario, OR = Orr Center, IL; UR = Urbana, IL

**Figure 3.3. Change in biomass yield with application of 0 and 60 kg ha$^{-1}$ of added nitrogen fertilizer for Miscanthus and switchgrass across both years of the study (2013-2014). The 0 Mg ha$^{-1}$ line represents no change in yield with added N fertilizer. Numbers above and below zero indicate an increase or decrease in yield, respectively, with added N fertilizer. The Illinois locations (BT, DS, UR, HA, and OR) were first planted in 2002 or 2004. The other six locations (MI, IL, ON, MS, KY, and NJ) are spread across eastern North America and planted in 2009 or 2010. A star by the location name indicates a significant difference in yield due to added N.**




BT = Brownstown, IL; DS = Dixon Springs, IL; HA = Havana, IL; IL = Illinois, KY = Kentucky, MI = Michigan, MS = Mississippi, NJ = New Jersey, ON = Ontrario, OR = Orr Center, IL; UR = Urbana, IL

**Figure 3.4. Correlations ($R^2$) of Miscanthus tiller number and tiller height with biomass yield at Kentucky across both years (2013 and 2014).**

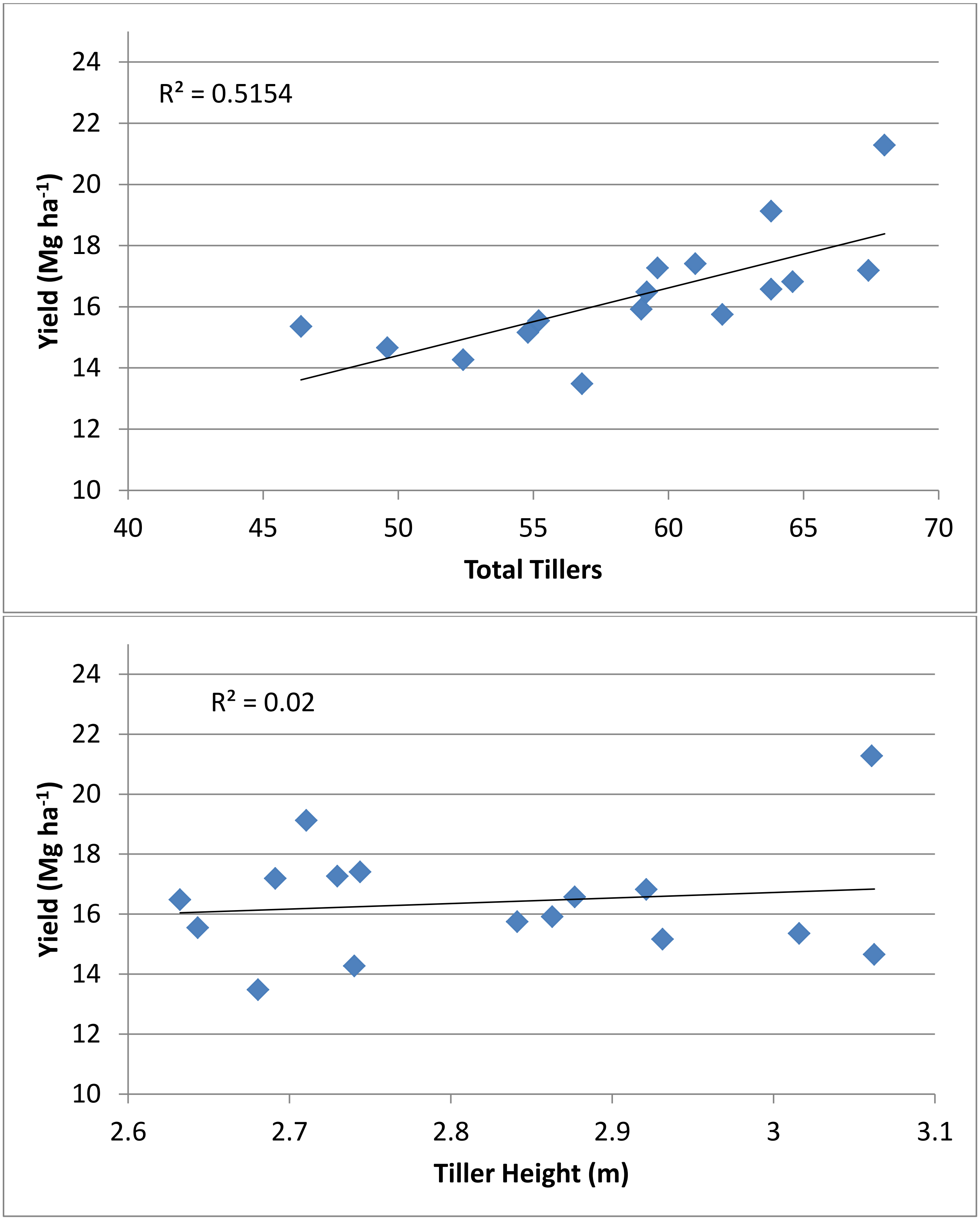
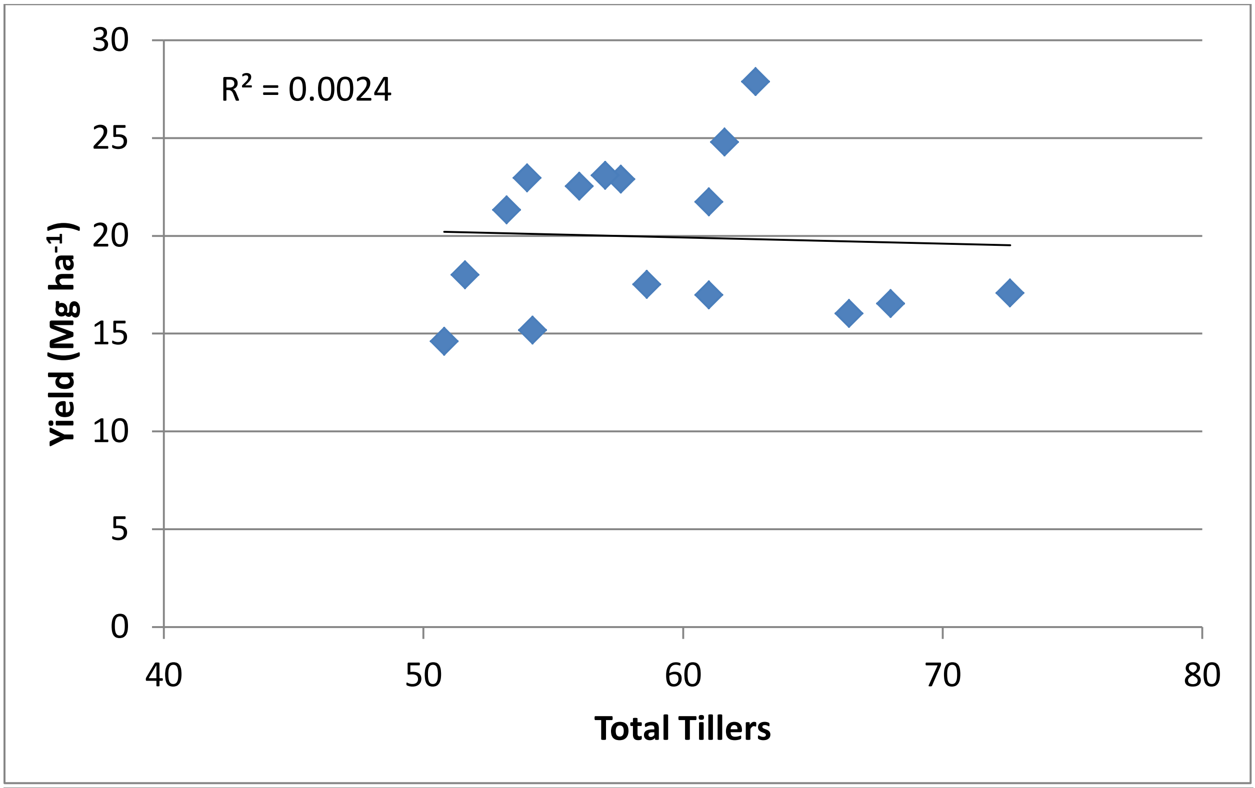
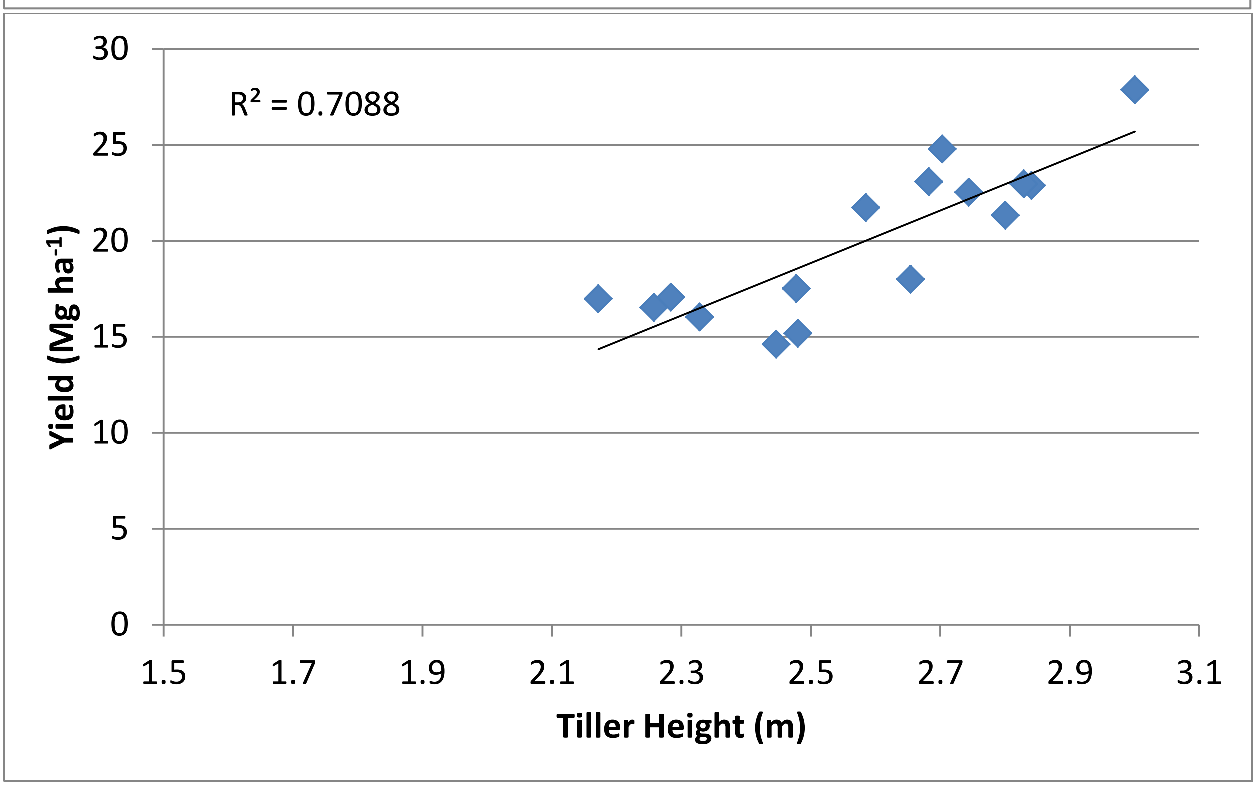


**Figure 3.5. Correlations of Miscanthus tiller number and tiller height with biomass yield at Michigan across both years (2013 and 2014).**



## Chapter IV

## Large-Scale Comparison of Four Potential Energy Cropping Systems


### Abstract

Bioenergy cropping systems are of interest because of fossil fuel price volatility, U.S. energy security, rural economic development, and environmental damage reductions. Creating biofuels from perennial grass systems is a potential alternative to using corn grain based ethanol in the U.S. In this study, the long-term biomass and potential energy productivity of *Miscanthus* x *giganteus* (Miscanthus) and *Panicum virgatum* (switchgrass) monostands, and a high-diversity tallgrass prairie system were compared using field-scale and plot-scale harvesting equipment. Total potential energy output of all three perennial systems was compared to a corn/corn/soybean rotation-cropping system. The Miscanthus and switchgrass monocultures were more productive than the high-diversity tallgrass prairie system when stand ages were young, but productivity declined to a level comparable to the prairie polyculture system by year six without N fertilization. The prairie system did not perform as well as the monoculture systems in the drought year of 2012, only producing 2.66 Mg $ha^{-1}$, while switchgrass produced 7.20 Mg $ha^{-1}$ and Miscanthus produced 8.64 Mg $ha^{-1}$. Applications of nitrogen to the Miscanthus plots starting in year six resulted in a nearly 2x yield increase, again producing greater yields than the prairie system. Harvesting grass plots using commercial-scale equipment was highly correlated to harvests using a small plot harvester. Total energy production from the Miscanthus and switchgrass systems was higher and more consistent than the corn/corn/soy and prairie systems averaged over the life of the study due to inconsistent corn yields and the soybean's lower energy potential.

## Introduction

As world energy demand increases and fossil fuel reserves decrease, alternative energy sources will be required. It is likely that the alternative energy sources will vary depending on location. For example, hydroelectric power must be generated from water sources and solar power from areas with abundant sunshine. Similarly, potential sources in the agronomic regions of the world include biofuels produced from bioenergy crops.

Biofuels are energy sources obtained from living plants. Most bioenergy crops are renewable because plants can be grown and harvested on set intervals. Given proper land use and land management, biofuel crops can be grown indefinitely, and thus, are not the finite resource that fossil fuels are. Biofuels also recycle atmospheric carbon dioxide ($CO_2$), a greenhouse gas released when fossil fuels and biofuels are burned to produce energy. The $CO_2$ released when fossil fuels are burned originated as carbon that has been stored for millions of years within the earth. The $CO_2$ released when bioenergy crops are converted to energy, however, was taken up from the atmosphere into the crops and used in photosynthesis. Therefore, there is not a net atmospheric carbon increase.

Currently, the most common biofuels used in the U.S. are ethanol produced from corn (*Zea mays* L.) and biodiesel produced from soybean (*Glycine max* (L.) Merr.). Corn ethanol production has steadily increased in the U.S., with production of nearly 140 million liters per day in late 2013 (EIA, 2013). Corn ethanol is produced by conversion of the sugars in corn grain into alcohol using yeast fermentation. Since the primary sugar form in corn grain is starch, it must be broken down to simple sugars for the yeast to digest (Moser and Ileleji, 2006). In the U.S., the primary uses of ethanol are increasing octane levels and as an oxygenator additive to gasoline, to reduce air pollution (DOE, 2016). Most light vehicles can burn ethanol as a 10% blend with gasoline, while other vehicles are designed to burn up to 85% ethanol (DOE, 2016).

Biodiesel can be produced from several different types of organic oils that are converted into fuel through a refining process called transesterification (Pedersen, 2007). While biodiesel can be produced from several vegetable oils (e.g., canola, palm, or sunflower) (Hay, 2016), soybean oil is the most commonly used for U.S. biodiesel production (Pedersen, 2007); more than 4.45 billion liters were produced in the U.S. in 2015 (EIA, 2016).

Using corn and soybean for bioenergy, however, is not without controversy. The use of food crops to produce energy can increase commodity prices, which in turn can affect food prices (Babcock, 2011). While there is no clear consensus on how to resolve the “food vs. fuel” debate, one potential solution is to produce non-food perennial crops on marginal agronomic lands. Whether used to produce cellulosic ethanol or to produce heat and electricity via direct combustion of dried plant material, these potential energy sources have received increasing attention in the past several years.

The U.S. government supports this effort. The U.S. Renewable Fuel Standard-2 (RFS-2) states that corn ethanol production should not exceed 56.8 billion liters per year by the year 2022. This standard emphasizes that an additional 79.5 billion liters should be produced from other biofuel sources, including 60.6 billion liters from cellulosic biofuels (EPA, 2010). Cellulosic ethanol is produced by breaking the complex carbon-containing molecules in plant cell walls into sugars that are then fermented into ethanol (Badger, 2002). Maximizing the total biomass produced in a given area of land with the fewest inputs makes cellulosic ethanol most profitable. In the fertile regions of the central U.S., perennial grasses are of interest for creating these cellulosic biofuels.

Growing perennial grasses can benefit the environment through reduced equipment, water, and fertilizer inputs and decreased net greenhouse gas emissions by producing much greater net energy output than input (Hill et al., 2006). In addition, perennial grasses can provide diverse ecosystems for wildlife, especially with grasses native to the tallgrass prairies of the Midwest and Great Plains (Werling et al., 2013). Finally, atmospheric carbon can be captured and stored belowground in plant root or rhizome biomass, further enhancing their environmental benefits (Anderson-Teixeira et al., 2012). While there are many perennial grass species that can be grown for biofuel production, the three perennial systems that have been studied the most in the U.S., particularly in the fertile Midwest include Miscanthus, switchgrass, and mixed tallgrass prairie.

The warm-season grass *Miscanthus* x *giganteus* Greef et Deu ex. Hodkinson et Renvoize (Hodkinson and Renvoize, 2001) (hereafter Miscanthus) has been studied extensively in Europe and is now the focus of much research in the U.S. Miscanthus is a perennial $C_4$ grass native to eastern Asia (Scally et al., 2001) that shows great potential as a biofuel crop. Originally brought to Denmark from Japan in 1935, it spread throughout Europe and made its way to North America as a landscape plant (Scally et al., 2001). It is believed to be a naturally occurring hybrid of *M. sinensis* and *M. sacchariflorus* (Linde-Laursen, 1993). The hybrid *M.* x *giganteus* is a sterile triploid that does not produce fertile seed, and therefore, propagation is inherently more difficult than for seed-established perennials because it is done vegetatively, usually by rhizome division.

Miscanthus has the capability to produce high yields. In Europe, Lewandowski et al. (2000) reported yields of 25 to 30 Mg $ha^{-1}$ in a summary of research on that continent. In the U.S., the first studies on Miscanthus began in 2002 in Illinois (Heaton et al., 2008). This research confirms a high Miscanthus yield potential, with yields ranging from 15 to 30 Mg $ha^{-1}$ in small plots (Heaton et al., 2004; Heaton et al., 2008; Maughan et al., 2012), and that its future as a biofuel crop is positive (Heaton et al., 2008).

Switchgrass is a warm-season perennial grass native to much of the continental U.S. Although it has been used as a forage crop for many years, it also became one of the first extensively studied bioenergy crops in the U.S. The U.S. Department of Energy first identified switchgrass as a “model” energy crop as early as 1991 (Wright and Turhollow, 2010). In 1997, the U.S. Department of Energy’s Bioenergy Feedstock Development Program (BFDP) reported that switchgrass should be produced as a bioenergy crop on a large scale and was singled out for further study because of it’s large native range, high potential yields, good nutrient-use efficiency, benefits to wildlife, and positive effects on the soil (McLaughlin & Walsh, 1997). Switchgrass research increased over the ensuing years, and the grass received great attention as a bioenergy crop after former President George W. Bush mentioned it during his 2006 State of the Union address (Bush, 2006).

Since the BFDP expressed interest in switchgrass, several studies have examined many aspects of switchgrass biomass production for bioenergy. For example, Vogel et al. (2002) found that at the optimal nitrogen fertilization rate of 120 kg N $ha^{-1}$, ‘Cave-in-Rock’ switchgrass can yield as much as 12.6 Mg $ha^{-1}$ in Iowa. Thomason et al. (2004) found greater yield potential (16.9 Mg $ha^{-1}$) with the cultivar Kanlow in Oklahoma without fertilization. In a multi-location study across the country, Fike et al. (2006) reported that several switchgrass cultivars could be used effectively over a long time period and with average yields of approximately 14 Mg $ha^{-1}$ across multiple years and management practices. After the 10-year duration of the BFDP program, McLaughlin and Kszos (2005) reviewed the switchgrass literature and found that by choosing proper varieties, correctly timing harvests, and improving breeding methods, yields could be further increased, even with reduced N inputs. In short, switchgrass is a very promising bioenergy crop.

Mixed native tallgrass prairie plantings are potential options for producing biomass on a large scale. Comprised of perennial grasses, forbs, and sedges, these plantings are more diverse than monocultures. There is evidence that diverse systems may be better able to adapt to environmental stresses than those with fewer species (Tillman and Downing, 1994) and may have a positive impact on the soil (Tillman et al., 1996) and other biological systems (Werling et al., 2013). Mixed native tallgrass prairie plantings, however, were not studied in detail until interest in bioenergy increased in the mid-2000s when Tillman et al. (2006) reported the potential use of diverse plantings (polystands) for bioenergy production. The authors stated that high-diversity plantings produced more than twice the biomass yields of a comparable monoculture over a 10-year timespan. The authors also found that these plants have a great ability to perform well on low-quality lands, while also sequestering a large amount of carbon in plant root systems (Tillman et al., 2006). The ability to grow a crop on limited-quality land not being used for cash crops is an important issue in the “food vs. fuel” debate, which argues the pros and cons of replacing food-producing crops with energy crops. Moreover, sequestering large amounts of carbon, especially amounts that surpass the amounts used to produce a crop, can potentially reduce the challenges of climate change from atmospheric carbon dioxide. Plus, the benefits of producing more biomass per acre are obvious.

To date, there have been no studies that compare perennial grass bioenergy-cropping systems to a corn/soy system in a systematic or direct method. As the grass and corn grain systems can be converted into ethanol, but the soy system can only be converted to biodiesel, a direct comparison requires conversion to a common energy unit such as megajoules. Grass crop ethanol yields can be calculated at 417.3 liters of ethanol per megagram of biomass (DOE, 2006; Heaton et al., 2008). Corn ethanol yield was estimated at 2.8 gallons per bushel (EIA, 2015), or 417.3 liters of ethanol per megagram of corn grain. Soy grain can be estimated to produce 1.5 gallons of biodiesel per bushel (Sadaka, 2016). One liter of ethanol is equivalent to 21.1 megajoules of energy, while one liter of biodiesel is equivalent to 32.6 megajoules (ISU, 2008).

Determination of the relationship between harvesting perennial bioenergy crops on a large (commercial agronomic) scale and small-plot research scale is necessary, as many perennial grass bioenergy studies were conducted on a limited scale. Previous research comparing the systems is limited; but one study found harvesting switchgrass using commercial equipment to be highly variable in the amount of biomass left in the field (Monti et al., 2009). That study found nearly 50% of available biomass was not making it into the baler compared to hand-harvested biomass estimates. But a comparison between three cropping systems and a plot harvester vs. commercial harvester has not yet been studied in detail.

Comparing perennial energy crop systems with an annual corn/soy cropping system will help determine the total potential energy output from each, as well as the potential contributions each can make to the U.S. bioenergy portfolio. Thus, the objective of this study was to determine the total energy production of perennial Miscanthus, switchgrass, and reproduced native prairie systems and an annual corn/corn/soybean rotation system.

**Methods**

This study was established in 2008 at the University of Illinois Energy Farm near Urbana, Illinois, USA (40.0624 N, -88.1915 W) in Dana silt loam soil (fine-silty, mixed, superactive, mesic Oxyaquic Argiudolls). Plantings of Miscanthus, 'Cave-in-Rock' switchgrass, a recreated native prairie consisting of 26 species (Table 4.1), and a corn/corn/soybean annual row crop rotation were established in 0.7-hectare plots with four replications.

In early June 2008, Miscanthus rhizomes were planted on a 1-meter spacing; replanting was necessary in May 2009 using rhizomes and small potted plants due to poor establishment caused by poor quality rhizomes and winterkill. Additional rhizomes were again planted in Spring 2010 in the 3.6-hectare plot to ensure commercial density. Plots were irrigated once in June 2009 to encourage establishment. In 2009-2011, Bicep herbicide (26.1% S-metolachlor and 33.0% atrazine) was applied at 4.9 liters per hectare to control weeds as the crops established. Additionally, 2,4-D herbicide was applied at varying rates in the 2009 planting year as needed. No herbicides were applied after 2011. Beginning in 2014, half of each Miscanthus plot received 56 kg N $ha^{-1}$ using granular urea and the other half remained unfertilized.

'Cave-in-Rock' switchgrass was seeded in May 2008 with a seed drill at a rate of 13.5 kg PLS per hectare, with one block partially replanted in July. Also in 2008, the switchgrass was mowed and 2,4-D herbicide was applied to encourage stand-fill and control weeds. In 2009, the plots were spot-treated with 2,4-D as needed. Urea was applied annually at a rate of 56 kg N $ha^{-1}$.

The prairie plots were planted with a seed drill in May 2008 and mowed the first season to encourage plot fill and discourage weed growth. Species and seeding rates are shown in Table 4.1. The plots received no fertilizer or chemical herbicides during the study and were hand weeded as necessary to maintain the integrity of the native planting.

The corn/corn/soybean annual row crop rotation was planted and managed with conventional row crop equipment common to the central U.S. Corn was planted annually with Dekalb brand hybrids (DKC62-63, DKC64-69, DK63-33, and DKC62-77 in 2011, 2012, 2014, and 2015, respectively) at approximately 84,000 seeds per hectare. A rate of 168-201 pounds of nitrogen per hectare was applied each year with lime, potassium, and phosphorous fertilizer applied in 2012. Lime was applied at a rate of 4.5 to 6.7 Mg $ha^{-1}$ to bring the soil pH up to 6.0. Phosphorous was applied as monoammonium phosphate at a rate of 168 kg $ha^{-1}$ and potassium was applied as potash at a rate of 224 kg $ha^{-1}$. Soybeans were planted every third year with Asgrow varieties (AG3431, AG3555 in 2010 and 2013, respectively) at a rate of approximately 346,000 seeds per hectare. Chemical weed controls were applied as needed to both annual crops, including glyphosate, S-Metolachlor, atrazine, and mesotrione.

Harvests were conducted annually post-senescence for all crops. The corn and soybeans were harvested in late fall with a combine as whole plots. Only grain was harvested; stover was left on the field and not considered in this study. The perennial systems were harvested in late fall through winter with commercially available haying equipment including a mower-conditioner (New Holland H8080 Discbine, New Holland, PA) and square baler (New

Holland BB9080, New Holland, PA). All bales from the entire plot of each were weighed and crop percent moisture were recorded. Additionally, strips from each plot were harvested with a plot harvester (Wintersteiger Cibus S, Ames, IA) prior to whole-field harvest. Biomass harvest weights were recorded for four sample strips per plot, with subsamples taken from each to determine percent moisture and dry weight. Strip lengths were measured to determine the total area harvested for each.

Results were analyzed using LS Means in SAS software version 9.4 (SAS Institute, Cary, NC, USA) to determine significant differences between yields of the three perennial grass crops, and to determine the difference in total ethanol production between the grass crops and corn. Differences in yields between grass crops were analyzed using the following basic model:

$$y = \mu + Y + B + N + YN + e$$

N-rate (N), year (Y), and the interaction of N-rate and year (YN) were considered fixed effects and block (B) as random. Average yields from commercial-scale and plot-scale harvesting methods were correlated using Proc Corr in SAS to determine the comparability between the two.

**Results**

Biomass yields in the replicated 0.7-ha plots across the three perennial cropping systems increased substantially following the establishment year, but varied in later years (Fig. 4.1). In 2010, Miscanthus and switchgrass yields did not differ when harvested commercially, but both were nearly double the yield of the prairie mixture. In 2011, the yields of both switchgrass and prairie declined year-over-year, but yields of Miscanthus increased to the highest level in the study, averaging nearly 12 Mg ha$^{-1}$. In the severe drought year of 2012, rainfall during the primary growing months of May-July was 43% of normal (Fig. 4.2). Yields responded accordingly, with all three perennial systems declining (Fig. 4.1). However, the diverse prairie system showed the lowest yields at less than half that of switchgrass or Miscanthus. Yields rebounded in 2013 for the prairie mixture, but the increases for the perennial grasses were not significant. However in 2014, all three cropping systems showed a significant drop in overall biomass yield. By 2015, all three perennial systems were nearly comparable in overall yield.

The same trends were seen in these plots when the smaller plot harvester was used (Fig. 4.3). Miscanthus yields were higher in 2010 when using the plot harvester, but afterwards the yields were comparable to those of the commercial-scale harvesting system (Figs. 4.1 and 4.3). There was a strong correlation ($R^2$=0.88) between the two harvest systems, indicating that the plot harvester results could reasonably be used to estimate commercial yields (Fig. 4.4).

Beginning in 2014, the replicated Miscanthus plots were split and nitrogen was applied to one-half of each plot with yield increases occurring when N was applied (Fig. 4.5). Yields in the fertilized portions of the plots were nearly double the unfertilized portions with biomass yields of 4.97 Mg $ha^{-1}$ and 8.78 Mg $ha^{-1}$ and 5.81 to 11.34 Mg $ha^{-1}$ for the unfertilized and fertilized plot halves in 2014 and 2015, respectively.

Corn was planted for two consecutive years, followed by a year of soybean in the corn/corn/soybean rotation treatments. The corn plantings in 2011, 2012, 2014, and 2015 were compared to the mature perennial grass stands in this study, as were the soybean plantings of 2010 and 2013. Since energy conversion processes differ among corn ethanol, cellulosic ethanol, and biodiesel, total energy production was converted to megajoules per hectare based on estimated conversion efficiencies. Results show a high variability year to year in the annual corn/corn/soy rotation when compared to the perennial systems, particularly to Miscanthus and switchgrass (Fig. 4.6). Miscanthus energy output peaked in 2011, producing an average of nearly 100,000 megajoules of energy per acre. As yields of the perennial systems declined, so did the potential energy output, producing approximately 40,000 to 50,000 megajoules per hectare by 2015. From 2010 through 2013, for example, Miscanthus produced more average annual energy than the corn/corn/soy rotation. Only in 2014 was corn higher than the perennial systems, producing nearly 120,000 megajoules per hectare (Fig. 4.6).

Averaged across all years, both Miscanthus and switchgrass produced more energy per hectare than the corn/corn/soy rotation (Fig. 4.7). Corn grain has the potential to produce more in a given year, as seen in 2014, but the limited soy energy production and the major decline of corn yields during the drought year of 2012 (Fig. 4.6) lowered the corn/corn/soy rotation average.

**Discussion**

In the early years of this study, the young Miscanthus and switchgrass monostands produced more total biomass than the diverse prairie system. Moreover, the study's fourth growing season, the drought year of 2012, the plantings could be considered to be mature. The drought affected the corn, prairie, Miscanthus, and switchgrass productivity and yields of all treatments declined. In the commercially-harvested system, Miscanthus yields declined 24% year-over-year (YOY) from 2011 to 2012 from 11.3 to 8.6 Mg $ha^{-1}$, prairie yield declined 30% YOY from 3.79 to 2.66 Mg $ha^{-1}$, corn yield declined from 8.8 to 5.2 Mg $ha^{-1}$, and switchgrass yield only declined 4% YOY from 7.51 to 7.20 Mg $ha^{-1}$. This indicates that diverse systems may not tolerate drought stress better than less-diverse systems, as was indicated by Tillman and Downing (1994).

As the stands aged, differences emerged. Yields for Miscanthus and switchgrass began to decline, while yields of the prairie system did not. The declining yields of the Miscanthus and switchgrass were similar to the findings by Arundale et al. (2014) that showed yield decreases with stand age in side-by-side Miscanthus and switchgrass plots throughout Illinois, and one study in Europe showing declining yields which could be corrected with added nitrogen (Clifton-Brown et al., 2007). The study by Arundale et al. (2014) found a yield decrease in both fertilized and unfertilized plots, so the trend is comparable even though the switchgrass in this study was fertilized annually and the Miscanthus was not. Although the duration of this present study was shorter than the 10-year study performed by Tillman et al. (2006), it appears that the unfertilized prairie plots could perform at least as well as the fertilized switchgrass and unfertilized Miscanthus in this study.

However, in 2014 and 2015, the Miscanthus stands were split and half of each plot was fertilized with 56 kg N $ha^{-1}$, as previous research (Arundale 2014) and direct observations indicated yield decline in Miscanthus in Illinois as stands aged. Although 2014 and 2015 unfertilized Miscanthus yields were comparable to prairie yields those years, the fertilized Miscanthus yields were much higher (Fig. 4.5) than either prairie or switchgrass plots in this study. Determination of the economic threshold for added nitrogen fertility in bioenergy crops has not been definitively determined for all regions and crop prices, but it appears that it may be important for maintaining elevated yields over the long-term at this study site.

Even though the switchgrass monostand in this study was fertilized annually at a rate of 56 kg $ha^{-1}$, yields became comparable to the unfertilized prairie system over time. These differences likely depend on switchgrass cultivar. The upland ecotype, Illinois-native 'Cave-in-Rock' was selected for this study, but is less productive than other types such as the lowland Kanlow and Alamo (Fike et al., 2006). In recent years, the seed smut disease *Tilletia maclaganii* caused premature flowering and substantial yield decreases in switchgrass (Thomsen et al., 2008), and is endemic across much of the U.S. (Farr et al., 1995). This disease was identified on 'Cave-in-Rock' switchgrass at this site, but disease impacts were not specifically monitored. Improved breeding lines specifically for biomass production may also differentiate a switchgrass system from a high-diversity prairie system over time. Finally, the yield effects of nitrogen applications to the prairie plots are unknown.

In corn, grain yields followed a somewhat different trend to that of the perennial grasses in this study. When young and vigorous, the perennial grass monocultures competed well with corn grain as a potential ethanol producer. In the severe drought year of 2012, the perennial grasses fared much better in comparison to corn. However, corn yields in 2014 were much higher than other years, and 2015 yields were similar to 2011. Over the same time period, the perennial stands began to decline in productivity unlike the annual corn, further highlighting the difference. Unlike the Miscanthus and prairie plots, the corn treatments were fertilized based on common agricultural practices in central Illinois. Even with added nitrogen, switchgrass productivity was less than corn in later years. Soy produces substantially less grain than corn, and much less soy oil than corn produces ethanol, and therefore less overall energy.

Soy energy production did not compare to either the corn or grass systems in either year it was grown.

These results compare favorably to the work of Schmer et al. (2008), in which intensively-managed switchgrass produced approximately 2,800 l ethanol ha$^{-1}$, low-input prairie approximately 1,400 l ethanol ha$^{-1}$, and corn grain 3,751 l ethanol ha$^{-1}$. Our yields of 2,690, 1,690, and 3,000 l ethanol ha$^{-1}$ for switchgrass, prairie, and corn, respectively, are similar. However, in Schmer et al. (2008), the estimate for corn grain was an average of Nebraska, South Dakota, and North Dakota corn production, not a direct comparison to on-site corn plots.

**Conclusions**

Younger stands (2-5 years old) of Miscanthus and switchgrass, whether fertilized or unfertilized, produce greater biomass yields than a low-input high-diversity prairie planting. The prairie planting also performed more poorly than Miscanthus or switchgrass under drought stress, showing that greater diversity did not provide for greater stress tolerance. However, as stands aged, the differences in biomass yield were less evident, with prairie yields approaching the productivity of the perennial monocultures by year six. Yields of fertilized stands of 'Cave-in-Rock' switchgrass equaled unfertilized prairie stands and unfertilized Miscanthus. If these trends continue long-term, it is possible that low-input high-diversity plantings can provide at least equal biomass production to the more intensively managed switchgrass stands, depending on switchgrass cultivar. Higher-yielding or improved switchgrass varieties selected specifically for biomass production may still out-produce prairie systems, but planting a mixed prairie with a different ratio of species that flower later in the summer may boost prairie yields further.

When N was applied in 2014 and 2015, the fertilized Miscanthus produced much greater yields than prairie plots. Adding N to aging Miscanthus stands may be necessary for sustaining high yields in some areas, and productivity of a Miscanthus monoculture could remain higher than a switchgrass monostand or prairie polyculture over time.

When converted to estimated total energy output, perennial grass systems compared favorably to our corn/corn/soybean grain system, especially during drought stress. It is possible that by including corn stover as part of the study the total energy output for corn could be increased further, but unknowns regarding excess nutrient and organic matter removal by collecting the stover, as well as expensive equipment additions to the harvest, led to its exclusion in this study. Extending the length of this study could provide the longer-termed impacts of species and stand age on overall biomass production. Further analysis of different fertility inputs on both monoculture and polyculture grass stands could also determine the full potential of each system. In addition, it is clear that the energy produced by corn grain can be greater than the energy produced by perennial grass systems in some growing seasons, yet the opposite appears true under stress conditions and when soy is added to the rotation. The potential energy boost from fertilizing Miscanthus may also be a factor. Plant genetics and selection, location, soil type, equipment availability, climate, and cultural practices will all play a role in determining the optimum bioenergy production system.

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## Tables and Figures

**Table 4.1. Energy Farm (Urbana, IL) prairie planting species listing and seeding rates.**

| Scientific Name | Common Name | Seeding Rate ($kg/ha^{-1}$) |
|---|---|---|
| **C4 Grasses** | | |
| *Andropogon gerardii* | Big bluestem | 2.24 |
| *Schachyrium scoparium* | Little bluestem | 11.2 |
| *Sorghastrum nutans* | Indiangrass | 2.24 |
| **C3 Grasses/Grass-like** | | |
| *Carex bicknellii* | Copper-shouldered oval sedge | 0.28 |
| *Elymus canadensis* | Canada wild rye | 1.12 |
| **N-Fixers** | | |
| *Astragalus canadensis* | Canada Milk Vetch | 0.14 |
| *Baptisia leucantha* | White Wild Indigo | 0.28 |
| *Desmodium canadense* | Showy tick trefoil | 0.28 |
| *Lespedeza capitata* | Round Head Bush. | 0.14 |
| *Petalostemum purpureum* | Purple prairie clover | 1.12 |
| **Other Forbs** | | |
| *Aster novae-angliae* | New England Aster | 0.14 |
| *Coreopsis tripteris* | Tall coreopsis | 0.56 |
| *Echinacea pallida* | Pale purple coneflower | 1.12 |
| *Helianthus grosseserratus* | Sawtooth sunflower | 0.25 |
| *Heliopsis helianthoides* | Early Sunflower | 0.28 |
| *Monarda fistulosa* | Wild bergamot | 0.28 |
| *Parthenium integrifolium* | Wild quinine | 0.28 |
| *Penstemon digitalis* | Foxglove Beardtongue | 0.07 |
| *Pycnanthemum virginianum* | Common mountain mint | 0.28 |
| *Ratibida pinnata* | Yellow coneflower | 0.56 |
| *Rudbeckia subtomentosa* | Sweet Blackeyed Su. | 0.14 |
| *Silphium integrifolium* | Rosin weed | 0.28 |
| *Silphium laciniatum* | Compass plant | 0.28 |
| *Silphium perfoliatum* | Cupplant | 0.14 |
| *Solidago rigida* | Stiff goldenrod | 0.56 |
| *Veronicastrum virginicum* | Culver's root | 0.28 |

**Figure 4.1. Average yields of Miscanthus, switchgrass, and prairie mixture perennial grass systems harvested with commercial haying equipment in Urbana, 2009-2015.**

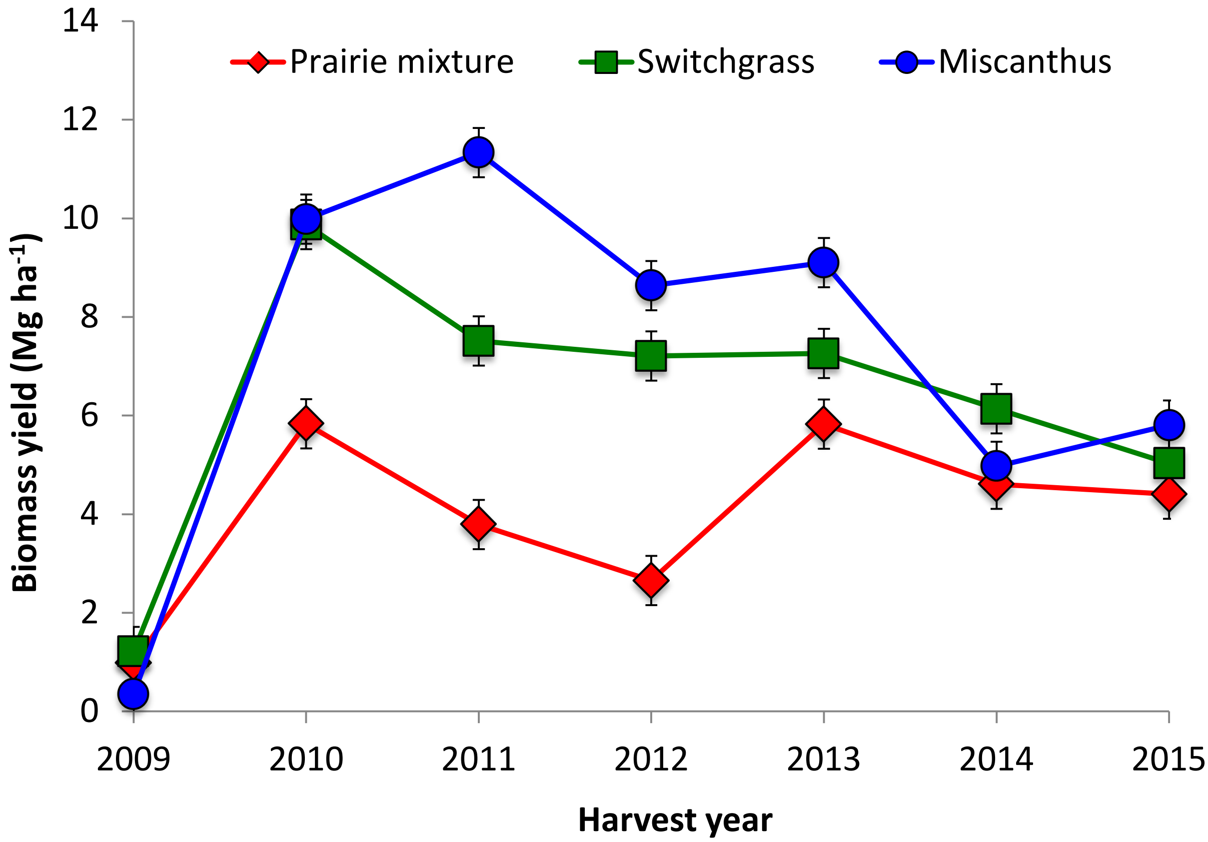


**Figure 4.2. Monthly 2012 precipitation rates and 20-year average rainfall for Champaign, IL (Illinois State Water Survey). The 2012 growing season was classified as an "extreme" drought due to below-average rainfall for the first half of the growing season.**

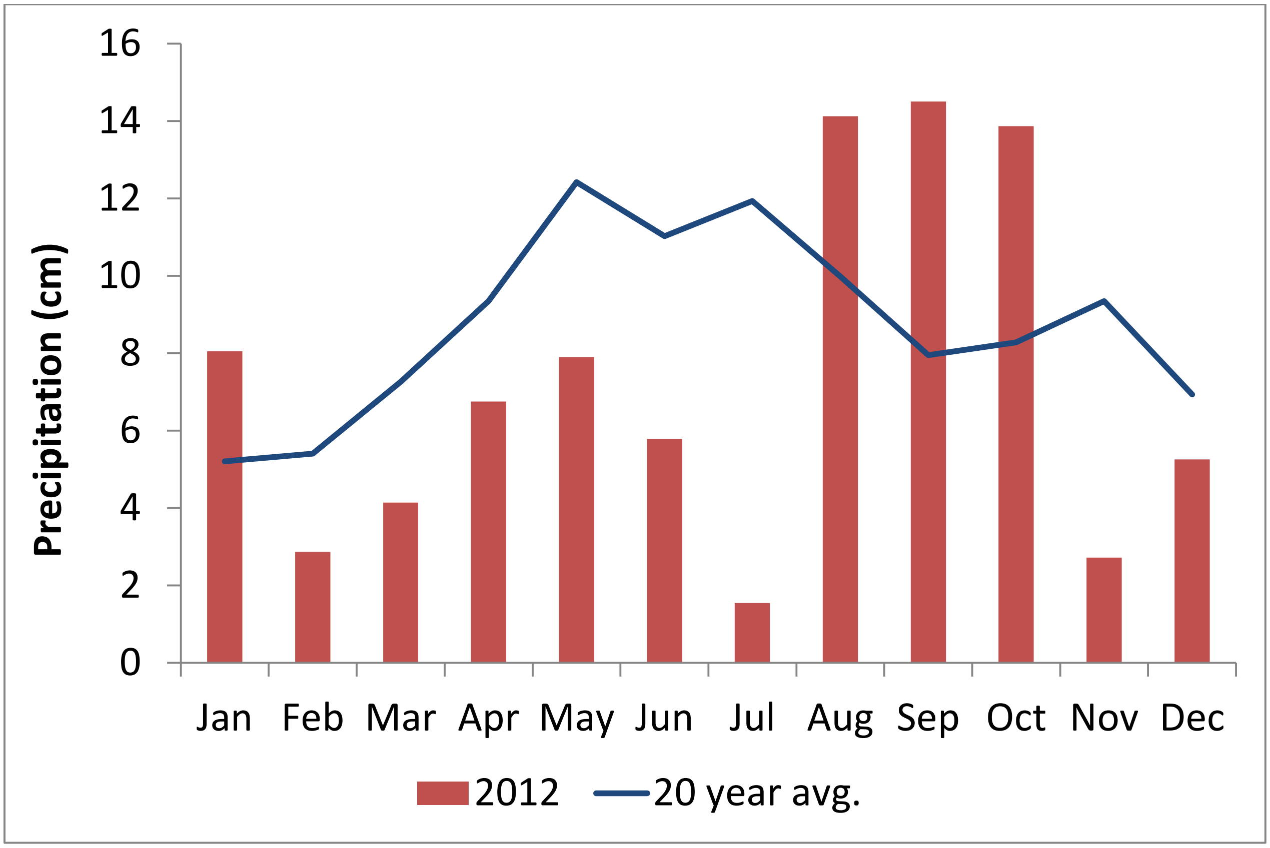


**Figure 4.3. Average yields of Miscanthus, switchgrass, and prairie mixture systems harvested with plot-scale harvesting equipment in Urbana, 2009-2015.**

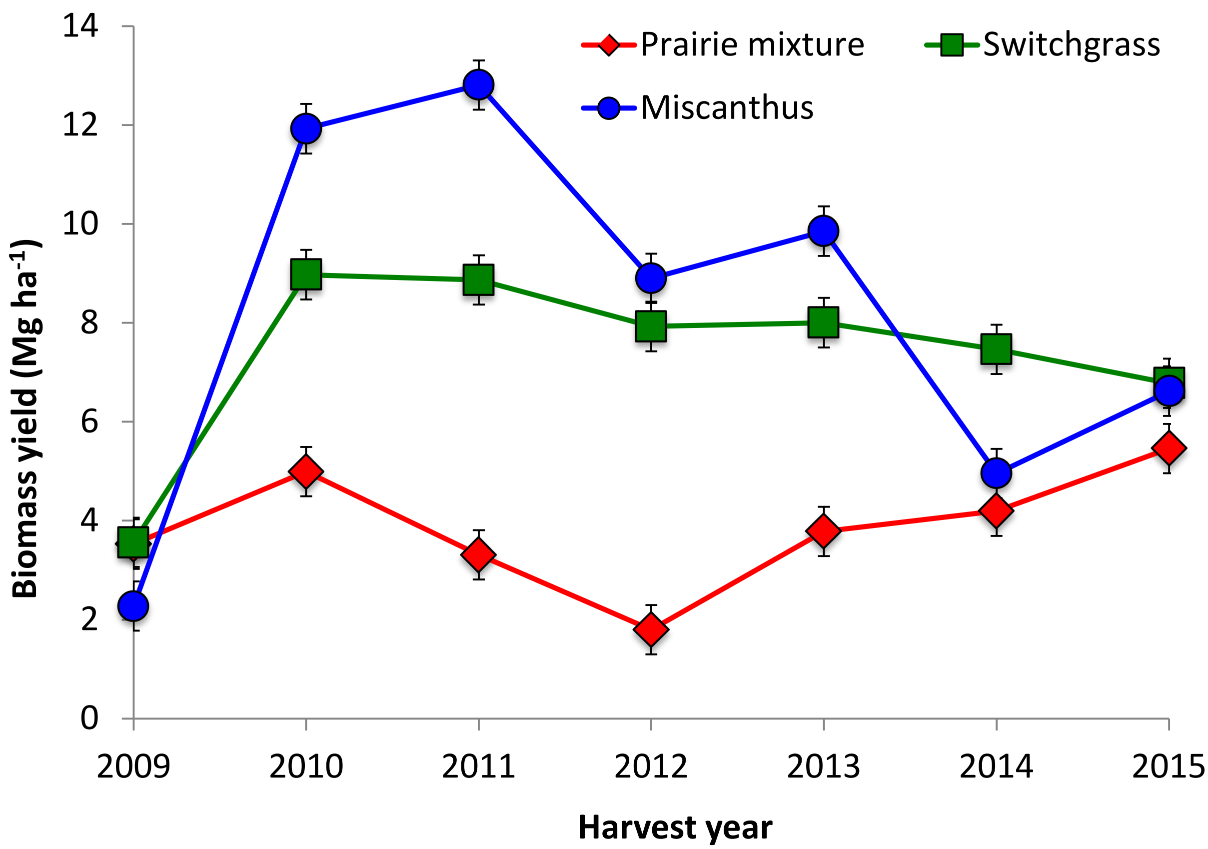


**Figure 4.4. Correlation between commercial and plot-scale harvest methods for measuring Miscanthus, switchgrass, and prairie mixture from 2010 to 2014 at Urbana, IL.**

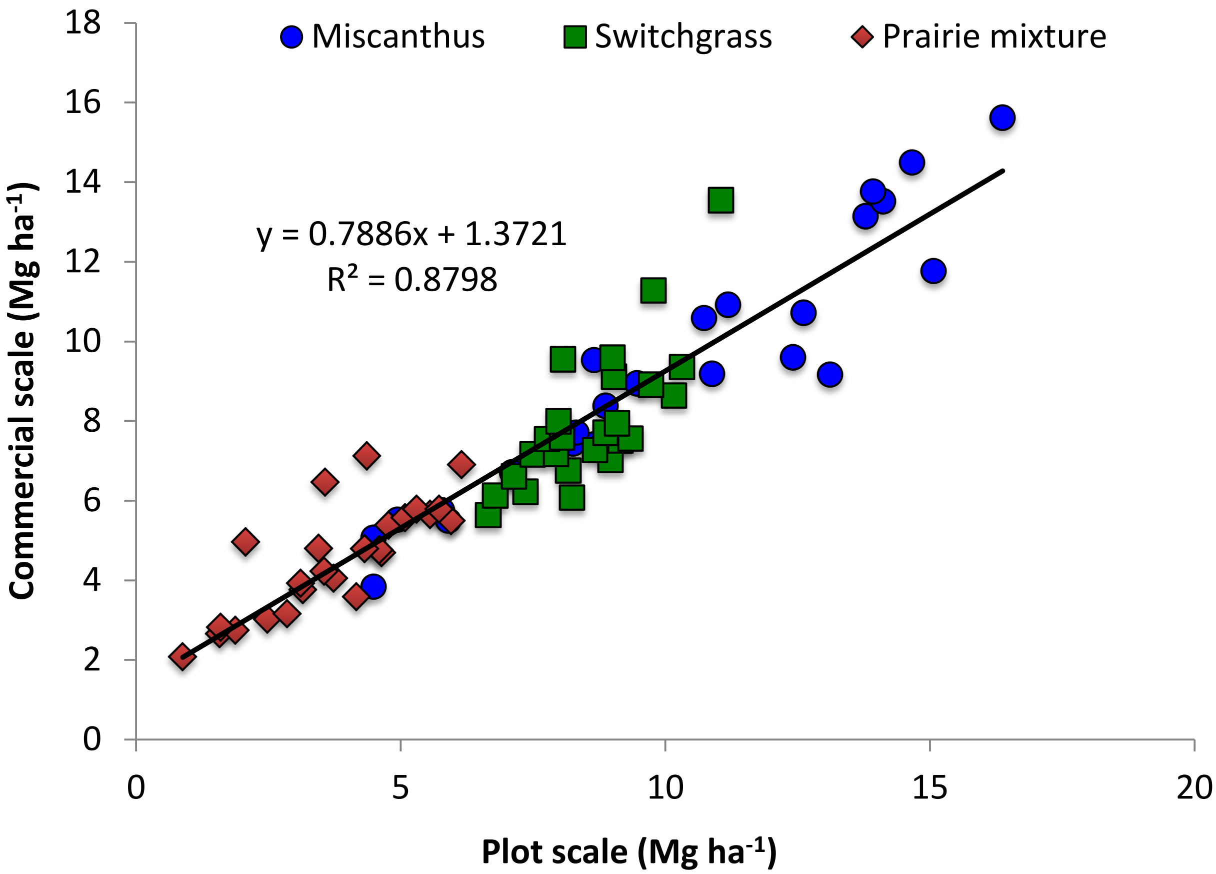


**Figure 4.5. Fertilized vs. unfertilized Miscanthus yields in 2014 and 2015 at Urbana. Plots were split and nitrogen was applied beginning in 2014 due to a noticeable decrease in yield, and due to other research and anecdotal evidence indicating the added nitrogen would increase yields.**

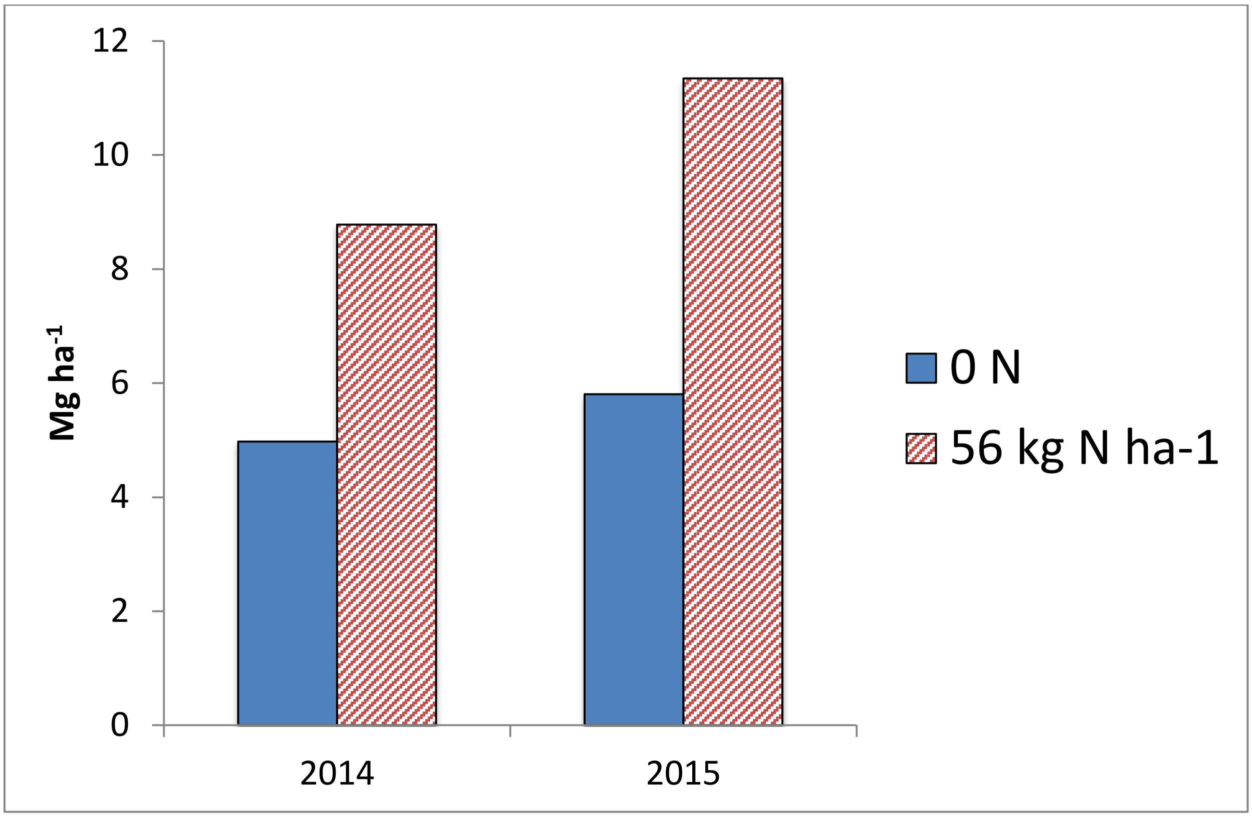


**Figure 4.6. Average annual energy output of four energy crop systems across all years of the study at Urbana, IL, 2010-2015. Harvested energy units were converted to Megajoules per hectare to create a common unit of measurement. The 2010 and 2013 growing seasons were soybean planting years in the corn/soy rotation.**

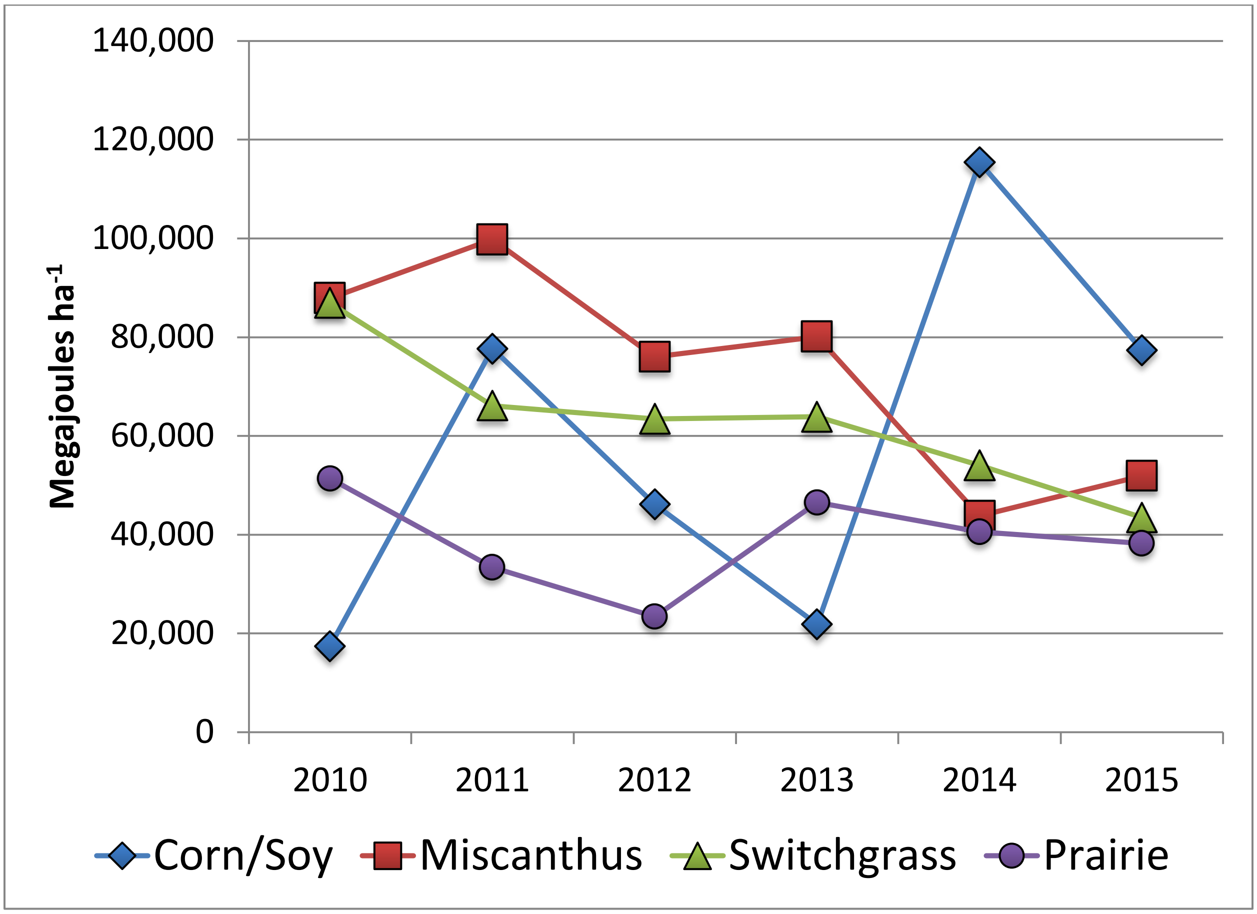
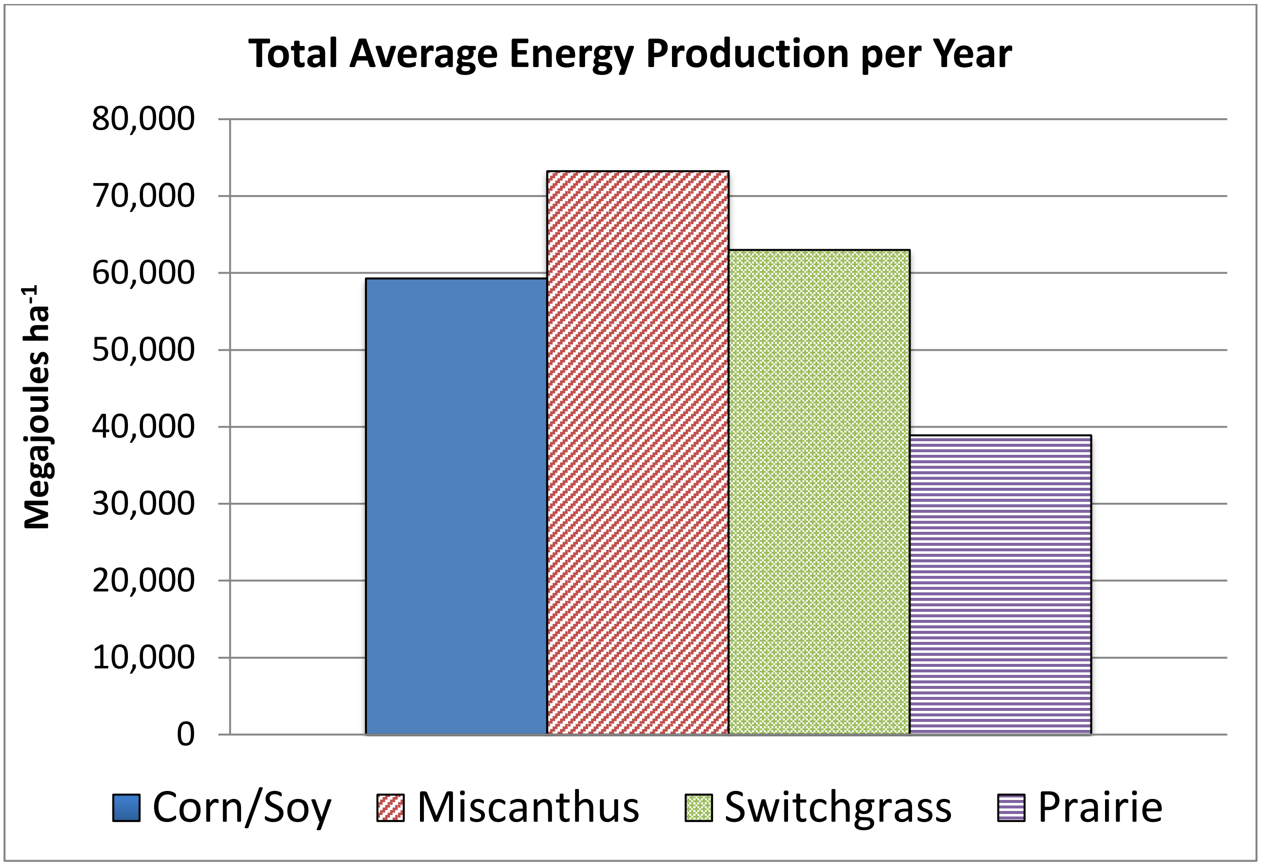


**Figure 4.7. Average total energy output from corn/soy, Miscanthus, switchgrass, and prairie cropping systems in Urbana, 2010-2015. Harvested units were converted to Megajoules per hectare to create a common energy unit for direct comparison. Energy output for each cropping system was averaged across all years.**



## Chapter V

## Learning from the Past and Potential for the Future

Compared to many other cropping systems, bioenergy crop research is still relatively new. Even though there have been several large projects funded to study these crops (e.g., the U.S. Department of Energy-funded North Central Sun Grant Feedstock Partnership and the BP-funded Energy Biosciences Institute), the volume of funding for traditional row crop agriculture dwarfs these sources. Therefore, much of the early bioenergy crop research has been fairly high-impact, since there is no other research to fall back on. For example, the first side-by-side Miscanthus (*Miscanthus* x *giganteus*) and switchgrass (*Panicum virgatum*) plots established throughout Illinois in 2002 and 2004 touted very high potential yields for both systems, while requiring little or no fertility inputs. A great many crop models and research projects that followed this initial work were based on this small subset of data, which benefitted many players in the fledgling bioenergy world. In the years that followed, especially after the oil price spike of 2008, many new studies were implemented across the country to further examine these perennial grass systems, including the studies described in this dissertation. The "buzz" surrounding bioenergy was palpable, and the future looked bright.

However, many of these new studies revealed bioenergy cropping-system limitations. Yields often declined as stands aged, and fertilization was, in fact, required in many settings to maintain desirable productivity. Moreover, producing millions of tons of biomass would provide numerous logistical challenges, and converting biomass to cellulosic ethanol has proved to be more difficult and complicated than previously believed. Prices for biomass needed to be high for it to compete with cash grain crops.  Declining oil prices and an increasing natural gas supply made bioenergy less attractive.  None of these revelations were a surprise to some, but it seems others may have been lost in the rush to be on the leading edge of saving the planet.

That's not to say that this later research that brought bioenergy back down to earth isn't valuable.  In fact, many of the second-wave studies that began around 2008, including the ones in this dissertation, may arguably be some of the most beneficial for the development of the bioenergy economy for the foreseeable future.  When more agronomic-scale sampling practices were employed, unlike early Miscanthus/switchgrass studies, yields were still found to be high, but not magically so.  As basic crop and soil sciences forecast, perennial grasses did respond to fertilizer applications in many soils over time.  Many new, highly productive switchgrass varieties can be grown in more regions than originally thought, potentially with yields that approach those of Miscanthus.  Other species, such as prairie cordgrass (*Spartina pectinatata* Link) have been found to be high yielding and adaptable to extreme environments.

Many of these recent revelations may disappoint those that initially saw bioenergy crops as "magic bullets" that could cure all of our energy and rural development woes, but many of these evaluations place biomass research on firm footing for the first time.  These studies provide solid results that can be a foundation for other fundamental studies going forward; the type of studies that have been performed on annual row crops for decades.  Fertility requirements for several species can be fine-tuned.  Many of the early bioenergy studies have been performed at research stations with good soils; not on the marginal soils where many believe bioenergy crops will ultimately be grown.  Further breeding efforts into improving biomass yields of fertile species such as switchgrass and prairie cordgrass should be carried out over a broader range of environments. Even the sterile hybrid, Miscanthus, can be improved by studying commercial propagation methods to make establishment more cost-effective moving forward. Decades of research on corn and soybean have produced large yield increases, so the same is possible for energy crops.

Chapter IV reports on the large-scale comparison of several cropping systems, and is the sort of long-term, quality research that offers the impact its founders anticipated. Tillman et al. (2006) suggested that high-diversity prairie systems could produce much more biomass than any monoculture system with far fewer inputs, even though the initial yields discussed were very low. Those familiar with how these systems grow knew this was not a likely outcome in the real world, but there was no proof to dispel this theory. Now, a long-term study on a fairly large scale has shown that diversity, even with its other benefits, does not necessarily equal productivity.

This is just one example within early bioenergy work that can be better understood with long-term research. In defense of the early research findings, there was limited information available that could be used for basing conclusions. There are undoubtedly a few scientists, particularly within the charged environment of climate change research, who preferred certain outcomes. But fortunately, the pool of knowledge on bioenergy crops has progressed to a level on which solid decisions about future research can be made.

With specific regard to these studies, there are only a few changes that perhaps could have been made. The first study, involving Miscanthus biomass and yield components, is the study that could have perhaps been improved the least. At planting, it was found that the site had a more sandy soil type than is typical in central Illinois. But this may actually have helped the Miscanthus exhibit greater differences due to added fertility than otherwise would have been seen, therefore improving the impact of the study. In the future, a greater number of sites with this layout and intense harvesting methods should be studied to further determine the yield impacts of added nitrogen, and eventually other nutrients, on Miscanthus yield.

In the second study in which Miscanthus and switchgrass productivity were compared at several locations across eastern North America, a few modifications could have been made. These sites were the first Miscanthus and switchgrass side-by-side plots planted in North America (probably in the World!). At that time, the switchgrass cultivar ‘Cave-in-Rock’ was planted because it was native to Illinois, there was seed available, there was local production knowledge, and it was relatively productive. As time went on, however, it was found that a few varieties of switchgrass, such as ‘Kanlow’, were much higher yielding across a broad area. Planting a high-yielding switchgrass cultivar at all locations makes for easier comparisons with the high-yielding Miscanthus clone, and allows for environmental differences to be identifiable. Further studies at these locations would be valuable, especially if the fertility requirements involving other nutrients can be examined.

The third study, involving the large-scale comparison of multiple species, might also be redesigned. First, a different mix of prairie species should be planted. The initial planting perhaps focused on diversity over productivity. Many species planted were beneficial to the larger prairie ecosystem, but did not contribute appreciably to overall yield. A mixture of species that flower later in the summer and produce more biomass could increase production from the prairie system. Second, another cultivar of switchgrass should have been planted. As mentioned above, ‘Cave-in-Rock’ was found to decline and produce lower yields than some other varieties, but primarily after this study began. It would have also been interesting to compare fertilized to unfertilized switchgrass. Finally, it is clear that at this location, Miscanthus does require added nitrogen to maximize its yield, something that was not clear until several years into the study. In 2014, the Miscanthus plots were split and half received added nitrogen, greatly increasing yields. If this split had occurred from the beginning, the expected yield decline could have been observed over multiple years, providing more valuable information. If possible, extending this study will be valuable in determining the longevity of these cropping systems.

In our current 2016 era of relatively low oil prices, it is my hope that quality scientists like my colleagues and friends at the University of Illinois and at several other universities across the country will continue to conduct outstanding research into the development of bioenergy cropping systems. Low fossil fuel prices will not last, whether it is due to the supply and price volatility of world governments or due to more and more regulations and taxes on carbon or fuels here in the United States. When prices inevitably rise again, bioenergy crop research will hopefully allow for solid agronomic bioenergy production practices on a large scale.

No matter where my life or my career may take me, energy will always impact my daily life. When energy markets ultimately change, it is good to know that the solid research basis developed these past few years will likely play a vital role in determining how energy impacts our lives well into the future.

**Literature Cited**

Tillman, D., J. Hill, and C. Lehman. 2006. Carbon-negative biofuels from low-input high-diversity grassland biomass. *Science*, **314**, 1598-1600.