YIELD RESPONSE TO NITROGEN FERTILIZATION AND HARVEST TIMING ON A MATURE MISCANTHUS X GIGANTEUS STAND

BY

ALLEN SCOTT PARRISH

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Crop Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2013

Urbana, Illinois

Master's Committee:

Assistant Professor DoKyoung Lee, Chair
Associate Professor Thomas Voigt, Co-chair
Assistant Professor Maria Villamil

ABSTRACT

The U.S. demand for renewable fuels has increased due to the concerns about the availability of nonrenewable fuels in the future and the impacts these fuels have on the environment. It is likely that *Miscanthus* x *giganteus* will be an important renewable energy crop in the U.S. due to great biomass production and low input requirements. This research focuses on the effects of nitrogen fertilization rates and harvest timings on yields and quality of a four-year-old crop of *M.* x *giganteus*. Research plots in Champaign, IL were harvested at five dates from August through March 2009, 2010, and 2011, and received five rates of nitrogen fertilizer (from 0 to 224 kg ha^{-1}) to determine biomass yields and quality over three growing seasons. From this study, we concluded that *M.* x *giganteus* production increased with nitrogen applications up to 112 kg ha^{-1}, and that harvesting following senescence is optimal for long-term biomass yield, increases carbon content and reduces nitrogen, moisture, and ash content in the harvested biomass. These results lay a foundation as agronomic researchers to develop best management practices for the emerging feedstock industry.

ACKNOWLEDGEMENTS

I would like to thank the invisible hand that brought me back to the University of Illinois in Champaign-Urbana. I want to thank the Department of Crop Sciences, Energy Biosciences Institute, faculty, staff, and undergraduates who have helped assist me throughout my graduate studies.

I would like to especially thank my advisor, Dr. DoKyoung Lee, for the opportunity to work for him. He has provided much assistance and guidance throughout my education and has contributed greatly to my professional development. I want to also thank Dr. Thomas Voigt for his support throughout this research project as well as helpful insight and comments on my thesis. I would also like to thank Dr. Maria Villamil for serving on my graduate committee and her encouragement throughout the completion of my thesis. It has been an honor and a privilege being mentored by my graduate committee. They have a great wealth of knowledge to share and have a passion for their students that fosters a learning environment.

I am thankful my family for their encouragement and assistance through out my education. I am especially appreciative of my wife, Lindsey, for her love in support and my children who inspire me to be a better man, husband, and father.

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INTRODUCTION

With the implementation of the Energy Policy Act of 2005, the Renewable Fuel Standards (RFS) mandates that 34 billion liters of renewable fuels be blended with gasoline by 2022 (USA EPA). The 2007 Energy Independence and Security Act (EISA) increased the 34-billion liter mandate to 136 billion liters by 2022, of which the RFS-2 requires no more than 57 billion liters be corn-based ethanol and the remaining 79 billion liters be advanced biofuels (USA EPA). While corn-based ethanol is not considered to be an advanced biofuel, advanced biofuels can originate from any other feedstock source (USA EPA). These renewable-fuel mandates have encouraged research that evaluates plant species and management practices to determine the potential of different crops.

Energy crop research and production using perennial grasses and fast-growing woody crops has been carried out in Europe for more than 30 years, and studying European activities will assist the U.S. as it moves into energy crop production. One of the crops that has been produced successfully in Europe is *Miscanthus* x *giganteus*. This high-yielding crop was found to have relatively low maintenance requirements, and it is likely that this grass will also be an important dedicated energy crop in the U.S. At present, studies to determine how location, fertilization, and harvest timing affect the yield and quality are needed in the US.

Members of the *Miscanthus* genus have a native range from tropical and subtropical Pacific islands in the south, throughout China to the Himalayas, and to the northern regions of Japan (Greef et al., 1993). This broad native range provides the genus with a wide spectrum of genetic diversity and adaptation to many climates. *Miscanthus* x *giganteus* is a perennial C4 sterile triploid (2n=3x=57) hybrid that was originally collected in Japan and has *M. sacchariflorus* (2n=2x=38 or 2n=4x=76) and *M. sinensis* (2n=2x=38) as parents (Linde-Laursen et al., 1993; Lafferty et al., 1994). Additional research has provided a better understanding of the relationship among the three grasses. For example, Greef et al. (1996) wrote that *M.* x *giganteus* is more closely related to *M. sacchariflorus* than to *M. sinensis*. Moreover, these genetic studies have lead to the discovery that some types of *M. sacchariflorus* have been misidentified and are actually selections of *M. sinensis* (Greef et al., 1996). One example, *M. sacchariflorus* ‘Hohenheim’ is a mislabeled botanical garden variety that is actually a *M. sinensis* (Greef et al., 1996).

Miscanthus x *giganteus* has been widely used as a landscape ornamental plant and has received attention as a biomass feedstock, first in Europe and then in North America, because of its great biomass production (Heaton et al., 2008) and low-maintenance requirements (Kering et al., 2011). The biomass productivity of *M.* x *giganteus* has been attributed to its low input requirements (Heaton et al., 2004a) and also to its ability to utilize a large percent of available solar radiation throughout the growing season; its growth starts in April and ends in November in temperate climates (Beale et al., 1995; Dohleman et al., 2009). In the Midwestern US, *M.* x *giganteus* begins growth in the spring as temperatures warm, grows vegetatively through the spring and summer, flowers in late September or early October, and begins to senesce with hard frosts in mid-to-late autumn (Heaton et al., 2008). Peak standing biomass was found to occur during the late summer or August, (Heaton et al., 2008) or in early fall or September, (Schwarz et al., 1994; Himken et al., 1997). Naidu et al., (2003) compared corn (*Zea maize*) and *M.* x *giganteus* photosynthetic rates and found that both species had an optimum temperature range of 30-35^{o} C, and when grown at 14^{o}/11^{o} C day/night temperatures, *M.* x *giganteus* was able to maintain its high level of photosynthesis CO_2 uptake, while *Z. maize* had an 80% drop in productivity (Naidu et al., 2003).

Perennial crops have several important characteristics that allow them to provide the sustainability required for biofuels. Perennial crops reduce the use of fossil-fuel consumption because they only need to be planted once and then grow back each year. Fuel used annually for tillage and planting are not consumed, thus saving producers time and reducing equipment wear. *Miscanthus* x *giganteus* can also translocate nutrients from aboveground biomass and dying rhizomes to belowground biomass during senescence for use in the next growing season (Kahle et al, 2001). Stored nitrogen, potassium, phosphorus, and magnesium, as well as carbohydrates, provide the energy needed to begin growth when conditions are favorable (Kahle et al., 2001), and translocation also aids in removal of nutrients that contribute to high ash content (Lewandowski et al., 1997). Monti et al. (2009) found that 90% of *M.* x *giganteus* roots were in the top 35 cm of soil in 1.2 m deep cores, accounting for 4.2 Mg ha^{-1} of belowground biomass, and because of its relatively shallow root system, translocated minerals are important to maintaining its low input capabilities.

Production - Miscanthus x *giganteus* studies report a range of productivity, primarily due to differences in the ages of the grass. Commonly, the first year growth of *M.* x *giganteus* is not harvested because there is insufficient biomass produced (Himken et al., 1997). Because the grass has been planted often on meter spacing (10,000 plants ha^{-1}), studies conducted on young stands are commonly not fully filled in (Huisman et al., 1994; Himken et al., 1997). Since *M.* x *giganteus*, a sterile hybrid, is planted using rhizomes, plugs, plants generated through micropropagation, or embryoid plants (Lewandowski, 1998). Lewandowski (1998) also wrote that in early growing seasons, plugs were higher yielding, but as the stands matured, differences between the planting methods were not significant. It can take up to five years for these stands to reach full maturity, and makes long-term studies more rare to find (Miguez et al, 2008; Lewandowski et al, 2000). Lewandowski et al. (2000) found that in studies that took place throughout Europe, *M.* x *giganteus* can yield up to 25 Mg ha^{-1} annually which makes it one of the highest yielding biofuel crop feedstocks.

Since long-term studies are not frequently undertaken for a wide range of locations, modeling scenarios to determine optimum production for bioenergy crops will be useful for *M.* x *giganteus* production. Clifton-Brown et al. (2004) modeled *M.* x *giganteus* yield potential using leaf-area index, radiation-use efficiency, and the length of the growing season, and their findings were validated using weather data and yield data from European trials. The model accurately predicted yields within 10%, in Sweden, Denmark, Germany, and Portugal, but overestimated yields in England by 27% (Clifton-Brown et al., 2004). Heaton et al. (2004b) attempted to predict Illinois yields using the model described in Clifton-Brown et al. (2000). Using that model, *M.* x *giganteus* yield predictions ranged from 27 to 44 Mg ha^{-1} (Heaton et al, 2004b) compared to countries across Europe where *M.* x *giganteus* averaged 25.8 Mg ha^{-1} in Belgium to 13.0 Mg ha^{-1} in Finland and Sweden (Clifton-Brown et al., 2004). Since models were based on a three-year old stand as the standard for biomass yield, they were unable to predict yields over long time periods (Clifton-Brown et al., 2004).

Long-term studies are often expensive to conduct or are time prohibitive, yet are crucial to future producers. Christian et al. (2008) evaluated the quality of a *M.* x *giganteus* stand over 10-years (1993 to 2002), and found that yields declined from an initial average of 32.2 Mg ha^{-1} to 9.2 Mg ha^{-1} by the end of the study. This 71% reduction in yield does not reflect a sustainable crop, especially if a biorefinery is built based on yields higher than 9.2 Mg ha^{-1}. As more long-term studies are completed, explanations into the decline in yield may be uncovered and future research may find methods to maintain high yields of *M.* x *giganteus*.

Harvesting - Miscanthus x *giganteus* is typically harvested during the winter months before new growth occurs in the spring (Strullu et al., 2011; Lewandowski et al., 2003a; Smith et al., 2011). For many plant species, as winter progresses, biomass yields decrease due to leaf loss and stem drying, while feedstock qualities increase because of leaf loss and mineral leaching from stems (Lewandowski et al., 2003a; Burvall, 1997; Adler et al., 2006). Thus, these two traits are inversely related with smaller biomass yields occurring as biomass quality increases.

Because biorefineries will be operating year-round, they will likely have reduced stockpiles of biomass toward the end of the summer prior to the next harvest period (Zhu et al., 2011; Ebadian et al., 2011), forcing increased feedstock prices in order to keep the biorefineries running at capacity. Biomass producers may be tempted to harvest biomass prior to a killing frost because yields will be highest during the late summer and early fall (Heaton et al., 2004a; Beale et al., 1997). Mos et al. (2013) wrote that harvesting in the early fall (September) produces bio-oil of the same quality as that produced from biomass harvested during the winter months (February). This allows for a longer harvest window, yet Mos et al. (2013) observed that significant levels of beneficial nutrients, primarily nitrogen, were removed in the green biomass. Mos et al. (2013) also hypothesized that harvesting during the fall before senescence may impact sequential stand yields. Reynolds et al. (2000) conducted a six-year study on early and late maturing cultivars of another C4 grass, switchgrass (*Panicum virgatum*), and concluded that for all varieties under optimum growing conditions, a two-cut system was more productive than a single cut system. Vogel et al. (2002) conducted a two-year switchgrass study with eight different harvest dates and a two-cut system, and found that that peak biomass yields were achieved after the panicles had fully emerged. Vogel et al. (2002) also reported that the second cut contributed less to the overall yield than the first cut and did not document any negative effects of early harvesting. Casler et al. (2003) conducted switchgrass research and found that harvesting during August reduced ground cover to 33%, and ground cover following September and October harvests were 47% and 44%, respectively, by the fifth year of the study. Tahir et al. (2011) conducted a multi-year and multi-location study using reed canarygrass (*Phalaris arundinacea*), a C3 grass, indicating that multi-cut systems, up to three cuts, yielded higher than single cut systems.

These studies were conducted in Midwestern US areas where it's likely that *M.* x *giganteus* will be produced taking place over a wide geographic region with varied weather conditions. Brookings, SD (44^o N) was the northern-most site in a study (Casler et al., 2003), while Knoxville, TN (36^o N) was the Southern-most (Reynolds et al., 2000). These studies were carried out over wide ranging geography and climates and with Urbana, IL (40^o N) in between. This may make varying *M.* x *giganteus* harvest times possible. In order to prevent growers from harvesting too early in order to get two-cuttings, it will be beneficial to evaluate various *M.* x *giganteus* harvest timings, especially at peak yield.

Few studies have focused on harvesting *M.* x *giganteus* during the growing season. Of the studies conducted in which harvest occurred prior to a killing frost, peak standing biomass occurred during the late summer or early fall (Schwarz et al., 1994; Heaton et al., 2008; Himken et al., 1997). Yields from these studies were determined by hand harvesting individual plants, and the long-term effects of early harvesting prior to a killing frost might not have been evident. Most studies recommend harvesting from mid-autumn through early spring, that is, following a killing frost through late March or early April, with the highest yields occurring in early winter before too much leaf material is lost (Lewandowski et al., 2003a; Strullu et al., 2011).

Fertilization - Many *M.* x *giganteus* nitrogen fertilization studies have been carried out with the goal of determining the effects of N on biomass productivity. In a literature review by Heaton et al. (2004a), 21 articles were analyzed to determine critical yield factors and concluded that nitrogen was not a significant factor (P =.08, $\alpha = 0.05$). Strullu et al. (2011) conducted a study with two harvest dates, October and February, and two nitrogen rates, 0 and 120 kg N ha^{-1}, and concluded that harvest date, not fertilization, was the significant yield factor. The October harvest had a peak yield of 25 Mg ha^{-1} while the February had a yield of 19 Mg ha^{-1} (Strullu et al., 2011). Himken et al. (1997) conducted a study where biomass samples were collected while the plants were actively growing from April through the following February using three nitrogen fertilizer rates, 0, 90, 180 kg N ha^{-1}, and found no significant yield effects of fertilizer rates at peak stand when 30 Mg ha^{-1} was produced in September and again in February when 16-18 Mg ha^{-1} was produced. They also found that with nitrogen fertilization, *M.* x *giganteus* maintained high yields due to leaf retention through the fall and early winter compared to the unfertilized plots, but at the end of the season there was no significant differences between the yields of the treated and untreated plots (Himken et al., 1997). One of the longest *M.* x *giganteus* studies was carried out by Christian et al. (2008) in which they found no significant differences between plots fertilized with nitrogen and unfertilized plots. In their study, plots were harvested once and received three fertilizer rates, 0, 60, and 120 kg N ha^{-1} (Christian et al., 2008). The highest yields occurred in 1993, the first harvest year, averaging 32.2 Mg ha^{-1}, but by the end of the study in 2002, the average had fallen to 9.2 Mg ha^{-1} (Christian et al., 2008). While the effects of nitrogen fertilization on biomass production was not significant, the yield from the study's start to end was. Further research will be important to determine methods for maintaining high yields over time.

Some studies have found *M.* x *giganteus* nonresponsive to nitrogen fertilization, while others have found a response. A review of European *M.* x *giganteus* productivity literature conducted by Lewandowski et al. (2000) found that the grass was less responsive to nitrogen fertilization once it reached maturity (older than three years) and concluded that the optimal level for nitrogen fertilization was 60 kg N ha^{-1} and that nitrogen fertilization might be needed on soils with low fertility. In Italy, Ercoli et al., (1999) conducted a study of irrigated and rainfed *M.* x *giganteus* plots receiving three nitrogen rates, 0, 100, and 200 kg ha^{-1}. All plots were harvested at anthesis (October), and the greatest yields, 37.5 Mg ha^{-1}, came in the third year from irrigated plots receiving 200 kg N ha^{-1} (Ercoli et al., 1999). In the 0 kg N ha^{-1} control plots, irrigation and rainfed plots did not differ producing approximately 17.5 Mg ha^{-1}, averaged over the course of the study, but N applications did increase the yields in the rainfed plots to approximately 24.5 Mg ha^{-1} (Ercoli et al., 1999). The greater response to nitrogen under the irrigated plots makes sense due to N being a water-soluble nutrient that is most readily available to a plant when there is moisture to aid in uptake. Boehmel et al., (2008) compared various energy crop species including *M.* x *giganteus* fertilized at three nitrogen rates, 0, 40, and 80 kg N ha^{-1}. The highest yielding perennial crop, *M.* x *giganteus* fertilized at the 80 N kg ha^{-1}, produced 18.1 Mg ha^{-1} of biomass, while the 0 N kg ha^{-1} plots produced significantly less at approximately 15 Mg ha^{-1}.

Research Justification - While *M.* x *giganteus* productivity, harvest timing, and fertilizer applications have been widely studied, there has been few long-term research that combines these factors over an extended harvesting window on the same plot. This lack of research poses several questions that are to be answered in this research. First, how does harvesting prior to senescence impact future harvest yields and biomass quality? Second, will applications of nitrogen fertilizer compensate for harvesting prior to senescence? Finally, what are the optimal nitrogen fertilization rates when harvesting prior to senescence? Developing fertilizer and harvest recommendations will be essential to the sustainability of biofuel feedstocks, as well as providing best management practices for growers in the United States.

MATERIALS AND METHODS

Site Conditions and Plot History - This research was carried out at the SoyFACE research farm (N 40.041382, W -88.224506), Champaign, Illinois, on a Drummer silty clay loam (Fine-silty, mixed, superactive, mesic Typic Endoaquolls). Prior to planting, the fields were in a continuous rotation of corn (*Zea maize*) and soybean (*Glycine max*) research. The *M.* x *giganteus* plants were transplanted in the spring of 2005 from pots at 1 plant m^{-2}, and the stand was considered at full maturity having little space between clumps and few missing plants in each subplot when this study began. Prior to this study, the aboveground biomass was cleared off annually with conventional mowing and baling equipment. No fertilizer has been applied to the stands until this study began. Baseline 30-cm soil samples were taken and divided into two depths, 0-15 cm and 15-30 cm prior to fertilizer application in the spring of 2009. Soil pH, organic matter content, nitrate-N, phosphorus, and potassium levels were determined. Precipitation (Figure 1a) and temperature data (Figure 1b) were collected from nearby stations through the Illinois Climate Network (Atkins et al., 2012). Weed competition was minimal throughout the experiment, and thus, no herbicides were used to control weeds.

Treatments – The experiment was arranged as a randomized complete block with split plots and four replications. Harvest timing was assigned to main plots and N rates were assigned to the 2.13 m x 5.57 m subplots. Within each harvest treatment, plots were fertilized by hand broadcast in Spring 2009, 2010, and 2011, using granular urea (46-0-0) at rates of 0, 56, 112, 156, 224 kg N ha^{-1} on the dates shown in Table 1. Monthly harvests were planned for August to February. Due to precipitation in 2009, the initial harvest schedule was delayed because the ground was too wet for machinery, and because of snowfall in the winter of 2009/2010, the January and February harvests were delayed until March and the January and February harvests were combined into a March harvest. Thus, there were five harvests (August, September, November, December and March) in each year of the study (Table 1). In all years, to mimic a situation similar to commercial production, the standing biomass around the plots was removed after the March harvest, and the leaf litter was left on the plots. Prior to plot harvest, alleys between blocks were mowed to produce the plot length of 3.35 m, which were harvested using a plot forage harvester having a 1.22 m swath (Wintersteiger Cibus S, Ames, IA). Subsamples of approximately 1 kg of harvested biomass were used to determine moisture and nutrient content by weighing, drying at 60^{o} C for 5-7 days, and reweighing.

Chemical Analysis - Nutrient content and ash were analyzed using the subsamples used for moisture content. The samples were ground to pass through a 1 mm screen using a cutting mill (Retsch SM2000, Haan, Germany). Samples were stored in airtight plastic bags until analysis for carbon and nitrogen content could be completed using a Costech ECS 4010 CHNSO analyzer (Costech Analytical Technologies, INC Valencia, CA). Ash concentrations were determined using the methods described by Undersander et al., (1993).

Statistical Analysis – Biomass yield and quality data were subjected to analysis of variance (ANOVA) using SAS JMP (SAS Institute INC, Cary, NC) using the Fit Model. A Student's t-test was used to separate means when the F-test was significant (α=0.05). Nitrogen rates, harvest timings, and harvest years were analyzed as fixed effects and replication was treated as a random effect.

RESULTS

Soil Conditions – The average organic matter level was 40.07 g kg^{-1}, the pH was 5.97, the phosphorus level was 19.97 g kg^{-1}, the potassium level was 143.33 g kg^{-1}, and the nitrate-N level was 2.33 g kg^{-1} in the soil samples collected from 0-15 cm. The average organic matter level was 30.50 g kg^{-1}, the pH was 6.3, the phosphorus level was 4.00 g kg^{-1}, the potassium level was 143.67 g kg^{-1}, and the nitrate-N level was 1.00 g kg^{-1} in the soil samples collected from 15-30 cm.

Weather – Precipitation and average air temperature during the experiment and the 30-year averages are summarized in Figure 1a and 1b. The 2009 growing season was cooler and wetter than the 30-year average. The 2010 growing season was warmer and drier than average, except in June when rainfall was significantly higher than the 30-year average. The 2011 growing season was also warmer than average with abundant rainfall in the spring months and exceptionally low rainfall during the summer months. The dates of the first killing frost (the first day in the fall or winter when temperatures fell below -2.22^{o} C) were 3 December 2009, 29 October 2010, and 11 November 2011. In 2009 temperatures fell below 0^{o} C several times throughout November, but it was not until December 3 that temperatures fell below -2.22^{o} C, which was the latest killing frost for the three years. In 2010, the killing frost occurred the earliest for the three years on 29 October.

Productivity – Prior to this study, recorded annual harvests were conducted on 21 January 2008 and 14 January 2009 for the 2007 and 2008 growing season, respectively and biomass yields were 18.16 Mg ha^{-1} and 19.05 Mg ha^{-1}, respectively (unpublished). Analysis of the yield data indicates that all effects, N rate, harvest timing, and harvest year, were significant (Table 2). The greatest yearly harvests across all fertility treatments were 30.3 Mg ha^{-1} in September 2009, 28.1 Mg ha^{-1} in November 2010, and 22.8 Mg ha^{-1} in November 2011. The lowest yearly yields across all nitrogen rates were 20.2 Mg ha^{-1} in December 2009, 19.1 Mg ha^{-1} in August 2010, and 14.7 Mg ha^{-1} in August 2011 (Table 3). The harvests for 2009 and 2010 were not significantly different from each other, but 2011 yields were significantly lower than the other two years. This is a 9% decline from 2009 to 2010 and a 30% decline from 2009 to 2011.

The yields from the August harvests were significantly different from the yields of the other harvest timings when all years were combined (Figure 2). September and November were not significantly different from each other, nor were the December and March harvests significantly different from each other, especially under lower N rates (Figure 2). When the harvest schedule was separated into year and harvest timing, the difference in yield becomes more significant (Figure 3). The March harvests were 22.7 Mg ha^{-1} in 2009, 23.2 Mg ha^{-1} in 2010, and 20.0 Mg ha^{-1} in 2011, across all fertility treatments. However, the August harvests were significantly decreased over time and were 27.3 Mg ha^{-1} in 2009, 19.1 Mg ha^{-1} in 2010, and 14.5 Mg ha^{-1} in 2011, across all fertility treatments. When analysis of the interaction of harvest timing and nitrogen fertilization are investigated, the significance each of these factors is uncovered. For the March harvests, the 0 kg N ha^{-1} plots averaged 17.5, 17.4, and 15.8 Mg ha^{-1} for 2009, 2010, and 2011, respectively while the 168 kg N ha^{-1} plots averaged 24.2, 23.6 and 20.2 Mg ha^{-1} in 2009, 2010, and 2011, respectively. This is a decline of less than 1% from 2009 to 2010 and 10% from 2009 to 2011 for the unfertilized plot, while the fertilized plot had declines of 2% and 17% for the same period. The yield increases between the 0 kg N ha^{-1} and the 168 kg N ha^{-1} plots was 38% in 2009, 36% in 2010, and 28% in 2011. The yield declines from the 0 kg N ha^{-1} rates to the 168 kg ha^{-1} N rates for the September harvests was even greater, with 26.6, 19.2, 13.2 Mg ha^{-1} for the 0 kg N ha^{-1} plots and 32.3, 29.4, and 24.5 Mg ha^{-1} for the 168 kg N ha^{-1} plots in 2009, 2010, and 2011, respectively. These yields declined 28% from 2009 to 2010 and 50% from 2009 to 2011 for the unfertilized plots, while the fertilized plot decline was 9% from 2009 to 2010 and 24% from 2009 to 2011. These declines were less drastic for harvests after the plants had senesced and transitioning to dormancy.

Both the 0 and 56 kg N ha^{-1} rates were significantly different from all other fertility rates across all years, while the 112, 168, and 224 kg N ha^{-1} rates did not differ significantly from one another (Figure 4). When comparing biomass productivity, the plots receiving 168 kg N ha^{-1} were significantly more productive in 2009 and 2011, yet the highest three N rates were not significantly different from each other in 2010.

Biomass Quality - Carbon concentration was significant for harvest timing and fertility rate (Figure 5). The carbon concentration increased from early harvest to later harvests in each year of the study. The increase in carbon concentration in harvested biomass from August to March was 431 to 461 g kg^{-1} in 2009, 425 to 466 g kg^{-1} in 2010, and 406 to 470 g kg^{-1} in 2011. There was a linear correlation ($r^2 = 0.7821$ and $P = 0.0140$) between increasing N rates and biomass carbon concentration, yet the range from 0 to 224 kg N ha^{-1} was 445 - 451 g kg-1 (data not shown).

Nitrogen concentration was significant for fertility rate, year, harvest timing, and the interaction between year and harvest timing (Table 2). There was a general N concentration decrease from early-to-late harvests with 5.68 g kg^{-1} in August and 2.08 g kg^{-1} in March (Figure 6a). Across all harvest treatments and years, the range of N was the lowest at the 0 kg N ha^{-1} application rate at 3.25 g kg^{-1} and highest at the 224 kg N ha^{-1} application rate at 3.86 g kg^{-1} (Figure 6b). The N concentration was highest during 2011 with an average of 3.73 g kg^{-1} while 2009 and 2010 were not significantly different from each other with 3.34 g kg^{-1} and 3.41 g kg^{-1}, respectively (data not shown).

Ash content was similar to nitrogen and was significant across all treatments (Table 2). Ash content was highest at the August harvest at 5.8% and lowest in March at 2.7% (Figure 7a), and decreased as fertility rates increased ranging from 4.0% for 224 kg N ha^{-1} to 4.7% for the 0 kg N ha^{-1} rate across all years and harvest dates (Figure 7b).

Dry Matter - Dry matter content increased from the August to March harvests for all years (Figure 8). The only harvest that had a distinct change in moisture was November 2010, when dry matter significantly increased from the September 2010 harvest. The amounts of harvested dry biomass were similar for the November and December harvests and were lowest in March 2009 and 2011.

DISCUSSION

Production - The harvest timing for *M.* x *giganteus* is critical to stand longevity. The highest yielding month for all fertility treatments in 2009 was September with 30.3 Mg ha^{-1}, yet declined to 27.6 Mg ha^{-1} in 2010 and 21.2 Mg ha^{-1} in 2011. This decline across harvest years for plants that are still actively growing confirms the hypothesis that Mos et al. (2013) predicted about September harvests harming stands by early harvesting. Biomass yield declines were observed the later the plots were harvested, thus finding a harvest timing that is early enough to maximize yield, as well as stand health, is important. This research identified November or December (late fall/early winter) as the optimum time to harvest because of high yield and the lowest levels of negative feedstock quality components (ash and nitrogen). Additionally, this research confirms that delayed harvest until March provides comparable biomass yield as described in previous research (Strulle et al., 2011; Zub et al. 2011). This research found that application of N fertilizer does not compensate for the detrimental effects of harvesting *M.* x *giganteus* prior to senescence. In Austria, however, Schwarz et al. (1994) found that harvesting from June through December while the plant was still growing did not negatively affect yield under all fertilizer treatments from 0 to 180 kg ha^{-1}. This finding is most likely due to the study's small sample size in which the effects of early harvesting could have been overlooked when the most representative samples were collected. In some instances, growers may be tempted to harvest early due to contract obligations, the needs of a processing plant, or to take advantage high, early-season prices. This is not advisable because an early harvest may require additional inputs and cause a reduction in yield for following years. The finding of Himken et al. (1997) were that nitrogen fertilization was able to delay the yield loss from senescence and leaf fall, was not confirmed by this research. No leaf litter was collected to determine the amount of yield loss from leaf drop. Harvest systems that are comprised of a mow, rake, and bale can collect some of the biomass that has fallen, and leaf retention may be of less importance to some producers. Further research that quantifies the amount of biomass lost from leaf drop will be useful in determining how nitrogen helps retain leaves after senescence. Nitrogen fertilization that prevents leaf fall and ensures high yields may be a risk management practice in the future if producers know they will have time or environmental constraints preventing them to harvest in a timely manner. Producers looking to eradicate a field of *M.* x *giganteus*, could also use early harvesting as a way to weaken their stand before rotating into another crop.

Application of nitrogen fertilization is an effective way to increase yield on a variety of crops. The 0 N fertilizer rate yielded the lowest for all harvest timing for all years, indicating that *M.* x *giganteus* responded to nitrogen fertilization under certain growing conditions (Table 3). *Miscanthus* x *giganteus* responded to nitrogen fertilization up to 112 kg N ha^{-1}, with highest rates of increase at the 0 and 56 kg N ha^{-1} levels (Figure 4). Even though 168 kg N ha^{-1} did yield higher than the 112 kg N ha^{-1}, these two rates were not significantly different from each other. This trial was the first time the plot was fertilized. The response to nitrogen may be considerably less drastic with annual or alternate years of fertilization at lower rates. Annual or biennial soil fertility tests could have aided in determining the importance of N fertilizer applications to yield increase. This was also a mature stand and the space between plant clumps was minimal. Considering the plants were transplanted at 1-m^2 spacing, these plants would had a large area to mine nutrients before they filled in and began competing with adjacent plants for nutrients. Fertilizer may only need applied only when the soil fertility levels became low which could be identified through soil testing.

There may have been other constraints on nitrogen availability. The growing conditions for this study were atypical growing seasons. The first season was cooler and wetter while the other two were considerably warmer and drier. *Miscanthus x giganteus* is an efficient plant, yet still requires a lot of water to facilitate its biomass production (Lewandowski et al., 2000; Heaton et al., 2004a). Due to the lack of rainfall during the late growing stages of 2010 and 2011, nitrogen might have been lacking at this critical time of the year. Besides low soil moisture, mineralization, other microbes or plants competing for these same nutrients could have caused nitrogen availability to be limiting. The first two years had abundant spring rains, which could have cause nitrate leaching from the spring fertilizer applications. The use of slow release fertilizers may be another option in order to provide nitrogen throughout the growing season (Anderson et al., 2013).

Miscanthus x *giganteus* demonstrates a symbiosis with N fixing bacteria (Davis et al., 2010), yet these bacteria were not able to meet the nitrogen requirements of the plant after it had been harvested prior to senescence. No scouting for any of these bacteria was conducted, so they might not be present or were altered with the application of nitrogen fertilizer (Mao et al., 2011). Additionally, soil fertility should be monitored and maintenance fertilizer should be applied as needed. Himken et al (1997) stated that the soil nitrogen levels were high, 185 kg N ha^{-1}, and may be a reason for why there was no significant yield response to nitrogen. This study only observed nitrogen, and additional studies should be conducted to better understand how macronutrients and micronutrients affect yield and biomass quality.

Biomass Quality - This study found that the best overall quality and harvestable yields occurs from late November to early January after the grass has senesced and a killing frost has occurred. This harvest timing is a common recommendation so that stands continue to translocate nutrients to the rhizomes for winter survival and spring emergence (Zub et al., 2011).

Nitrogen concentrations in the biomass were highest when the plants were actively growing and steadily decreased throughout the autumn and winter months (Figure 6a). Similar findings were observed by Strullu et al. (2011) and Heaton et al. (2009) in studies in which N concentrations decreased the longer the plants remained in the field. The application of nitrogen fertilizer had an increasing linear effect ($r^2 = 0.8794$ and $P < 0.0001$) on nitrogen concentration, which was a similar to the results of Schwarz et al. (1994) where they found an $r^2 = 0.8649$ where the 0 N rate was 8 g kg^{-1} and the 180 N rate was 9 g kg^{-1} across all harvest timings. March was the only harvest timing where N fertilization at all levels was not significantly different from each other.

Ash is also an important component of feedstock quality, and understanding the effects of nitrogen fertilization is vital to bioenergy production efficiency. Nitrogen applications increased biomass yield and the percentage of ash could have been diluted by the increase in other usable biomass components, which was a similar observation by Lewandowski et al. (1997) and Lewandowski et al. (2003). Results from this study conflict with the results observed by Baxter et al. (2012) where increasing nitrogen rates increased ash content. When comparing the ash content among harvest timing and fertility rates, the increase in fertilizer rates are significant for the August. By the March harvest there is no significance between any of the nitrogen treatments. Baxter et al. (2012) does confirm this finding, and harvest timing becomes the significant factor in determining ash consentration (Figure 7).

Dry matter - Moisture content is an important aspect of the storability and the transportation of herbaceous feedstocks. Materials that are not compressed or are high in moisture result in higher transportation costs due to material that is unused or nonessential, i.e. air or water. Moisture content higher than 70% in corn stover greatly reduced the efficiency of energy production for thermal and ethanol production (Wang et al., 2009). Thus, allowable thresholds of moisture will need to be established in order to prevent complications during the conversion process.

Other considerations besides moisture will be determined from the harvest timing. Le Ngoc Huyen et al. (2010) did not conduct their study with fresh material so the extent of moisture content effects on conversion cannot be assessed, yet the saccharification process does use water and some moisture may not be detrimental to the process. The early harvested, November, *M.* x *giganteus* was able to produce higher saccharification yields when given a pretreatment than that of later harvested, February, *M.* x *giganteus* regardless of the age of the crop (Le Ngoc Huyen et al., 2010). The conversion process of biomass to biofuels is complex and many factors will need to be controlled for biofuel production. In order for biofuels to achieve competitive prices as conventional fuel sources, exploiting these quality differences from harvest timing may be crucial to increase conversion efficiency.

Not all biofuels will be converted to liquid fuels, and some will be used for combustion as a replacement or supplement for coal or natural gas. The logistical problems identified in Ebadian et al. (2011) were primarily associated with transportation and storage. One solution to this problem would to be to locate a densifying plant close to the biomass production fields. These bales could then be converted into pellets more efficiently through the economy of scales and be transported farther distances at lower costs by the use of rail lines. For this study the latest harvests had moistures ranging from 8.4-12% for all years. Ideal moisture content for wood pellets is between 11-13% moisture and lower moisture content resulted in higher bulk density of pellets (Samuelsson et al., 2012). This moisture range indicates that *M.* x *giganteus* would have adequate moisture needed for processing into pellets or combustion later in the season. Torrefication is a process that uses low heat temperatures (200-300^{o} C) and the absence of oxygen to produce low moisture energy dense materials with improved grinding properties (Larsson et al., 2013). For torrefication of woody materials for pelletization, moisture content can be greatly reduced to approximately 2% and the addition of moisture can be problematic to pellet making (Larsson et al., 2013). Larsson et al. (2013) was able to conclude that increasing the die temperature could help improve the pelletizing efficiency and alternative bonding chemicals need to be investigated. The quality of biomass changes over time and as the biomass composition changes so could the end use. Early harvested materials could be used primarily for ethanol production and later harvest materials could be use for other thermochemical conversions. This would potentially give producers alternative markets for their products.

IF I HAD IT TO DO OVER AGAIN AND FUTURE RESEARCH DIRECTIONS

Miscanthus x *giganteus* is a long-lived perennial feedstock, and because long-termed studies in the US have not been conducted, it is difficult to anticipate yields from year to year. This research provides evidence that consistently harvesting *M.* x *giganteus* prior to senescence will likely reduce its long-termed yields. Nitrogen fertilizer applications improved yields, but did not compensate for the yield losses in years 2 and 3 resulting from early harvests (August and September) and nitrogen removed with the biomass.

A long-termed study to determine the effects of early harvesting in one year, followed by winter harvests in successive years should be researched. This will determine if alternating the harvest timing periods allows the grass to recover from the early harvest and provide a source of biomass early in one year, without impacting future yields. Late harvests could be conducted over different numbers of winters to determine the time it takes for a field to recover.

Moreover, this study only evaluated nitrogen applications and did not address applications of other nutrients. In order to test total nutrient removal ash content could have been analyzed to determine if there were other factors besides nitrogen affecting yield. Additional characteristics of a *M.* x *giganteus* may also change. Christian (1994) found that stem weights increased with higher nitrogen rates. This increase in carbon with increasing fertilization may be explained by changes within the stem structure causing thicker stem cells where the majority of the carbon of a plant is accumulated versus the less dense pith. Physiological research should be combined with future research to determine how a plant reacts to varying fertilizers.

The effect of harvesting early caused both a decline in stand height and density. A nondestructive method could have been used prior to harvesting to determine if these characteristics changed across harvest timing as well as fertility rate. Additional soil testing each year could be done to determine if there was an accumulation of nitrate-N in the soil. *Miscanthus* x *giganteus* roots play an important part in nutrient storage and mining. The effects of age on rooting depth, as well as soil carbon accumulation, would be invaluable information for producers looking to apply the nutrients where they need to be since leaf litter may prohibit these nutrients from coming in contact with the soil. Understanding the nutrient recycling process through leaf litter accumulation, decomposition, and their affects on soil fertility will be beneficial to future feedstock producers.

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TABLES AND FIGURES

Table 1. Nitrogen fertilizer application and biomass harvest dates for *Miscanthus* x *giganteus* study in Urbana, IL, 2009-2011.

Treatment		Year		
		2009	2010	2011
Fertilization		Apr 15	Apr 13	Apr 13
Harvest	AUG	Sep 1	Aug 18	Aug 22
	SEP	Sep 29	Sep 15	Sep 29
	NOV	Nov 13	Nov 12	Nov 11
	DEC	Dec 17	Jan 4*	Dec 8
	MAR	Mar 17*	Mar 19*	Marc 14*

*Biomass was harvested in the following year of growing season.

Table 2. Analysis of variance (ANOVA) and probability values for *Miscanthus* x *giganteus* biomass yield, dry matter, and feedstock composition affected by nitrogen rate, harvest timing, and year at Urbana, IL, 2009-2011.

			Biomass			
Source of variation	DF	Dry matter	Yield	Carbon	Nitrogen	Ash
Harvest timing (HT)	4	<0.0001	<0.0001	0.0004	<0.0001	<0.0001
N rate (NR)	4	<0.0001	<0.0001	0.0140	<0.0001	<0.0001
HT*NR	16	0.0008	0.0002	0.1258	0.6117	0.0005
Year (YR)	2	<0.0001	<0.0001	0.1927	0.0008	<0.0001
HT*YR	8	<0.0001	<0.0001	0.9921	<0.0001	<0.0001
NR*YR	8	0.2195	0.0010	0.1765	0.7448	<0.0001
HT*NR*YR	32	0.8275	0.8981	0.3293	0.2370	0.0989

Table 3. Biomass yield across all harvest timings, nitrogen fertilizer rates, and years for *Miscanthus* x *giganteus* study located at Urbana, IL, 2009-2011.

Harvest Timing	Nitrogen Rate (kg ha^{-1})	Biomass Yield (Mg ha^{-1})		
		2009	2010	2011
August	0	22.98 (3.47)	11.41 (1.50)	7.88 (1.20)
	56	27.21 (1.61)	16.94 (1.83)	12.36 (1.70)
	112	24.86 (1.83)	20.23 (1.26)	17.56 (1.11)
	168	29.96 (1.67)	22.96 (1.06)	18.01 (1.44)
	224	31.41 (2.95)	24.11 (1.41)	17.68 (1.78)
September	0	26.58 (1.90)	19.17 (3.21)	13.23 (2.12)
	56	28.44 (2.94)	23.97 (2.08)	17.97 (2.64)
	112	33.19 (2.97)	31.20 (1.72)	24.85 (0.69)
	168	32.34 (1.08)	29.43 (1.79)	24.49 (1.15)
	224	30.86 (1.56)	32.49 (0.90)	25.58 (1.79)
November	0	19.42 (2.03)	18.57 (0.95)	13.89 (1.04)
	56	25.99 (1.97)	25.55 (1.99)	22.33 (1.18)
	112	23.42 (.77)	33.05 (3.72)	25.46 (1.32)
	168	28.47 (2.16)	33.25 (2.08)	28.41 (1.02)
	224	21.91 (0.43)	30.02 (0.81)	24.15 (1.67)
December	0	16.89 (1.53)	19.48 (1.22)	15.65 (2.32)
	56	20.29 (1.35)	25.44 (1.04)	20.07 (1.05)
	112	21.16 (.92)	30.53 (1.35)	22.07 (1.52)
	168	22.16 (1.55)	31.49 (2.32)	24.55 (1.46)
	224	20.42 (1.08)	30.85 (1.20)	25.00 (0.71)
March	0	17.52 (1.23)	17.39 (0.67)	15.83 (2.25)
	56	23.74 (1.21)	25.00 (0.59)	21.30 (0.93)
	112	25.96 (1.89)	25.67 (1.03)	21.25 (1.10)
	168	24.19 (1.32)	23.64 (1.66)	20.18 (1.09)
	224	22.09 (1.64)	24.21 (1.57)	21.29 (0.96)

Figure 1. Monthly average temperature (a) and monthly precipitation (b) for 2009 though 2011 and 30-yr average at Urbana, IL.

Figure 2. Yield response to nitrogen fertilization for each harvest timing (a) and yield response to nitrogen fertilization levels at harvest timings (b) for all years on *Miscanthus* x *giganteus* at Urbana, IL.

Figure 3. Yield response over sequential harvest timings for each year (a) and effects of consecutive harvests on yield for each harvest timing (b) for all nitrogen rates on *Miscanthus* x *giganteus* at Urbana, IL.

Figure 4. Yield response over sequential years for different nitrogen levels (a) and yield response to nitrogen fertilizer rates for each year (b) for all harvest timings on *Miscanthus* x *giganteus* at Urbana, IL.

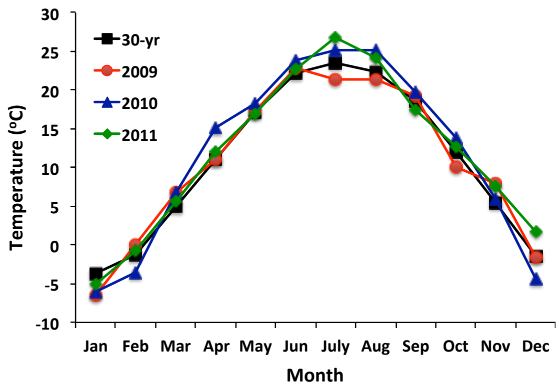
Figure 5. Effect of harvest timing on biomass carbon concentration and yield on *Miscanthus* x *giganteus* for all years and nitrogen fertilizer rates at Urbana, IL.

Figure 6. Effect of harvest timing for all nitrogen fertilizer rates (a) and N rate for all harvest timings (b) on N concentration in *Miscanthus* x *giganteus* biomass for all years at Urbana, IL.

Figure 7. Ash response to harvest timing and all nitrogen fertility for each year (a) and ash response to nitrogen rate for all years and harvest timings (b) in *Miscanthus* x *giganteus* biomass at Urbana, IL.

Figure 8. Dry matter concentration over sequential harvest timing for all nitrogen fertility rates for each year for *Miscanthus* x *giganteus* at Urbana, IL.

Figure 1.


30-yr
2009
2010
2011
Temperature (ºC)
Jan Feb Mar Apr May Jun July Aug Sep Oct Nov Dec
Month

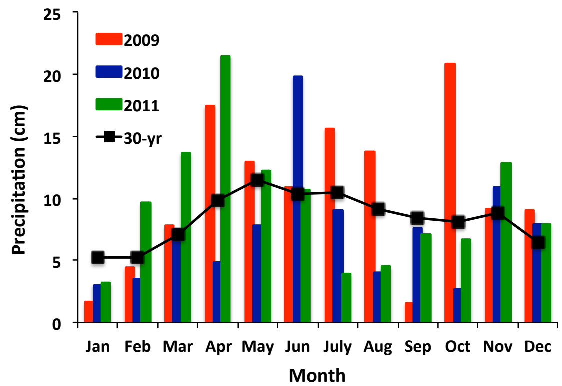

2009
2010
2011
30-yr
Precipitation (cm)
Jan Feb Mar Apr May Jun July Aug Sep Oct Nov Dec
Month

Figure 2.

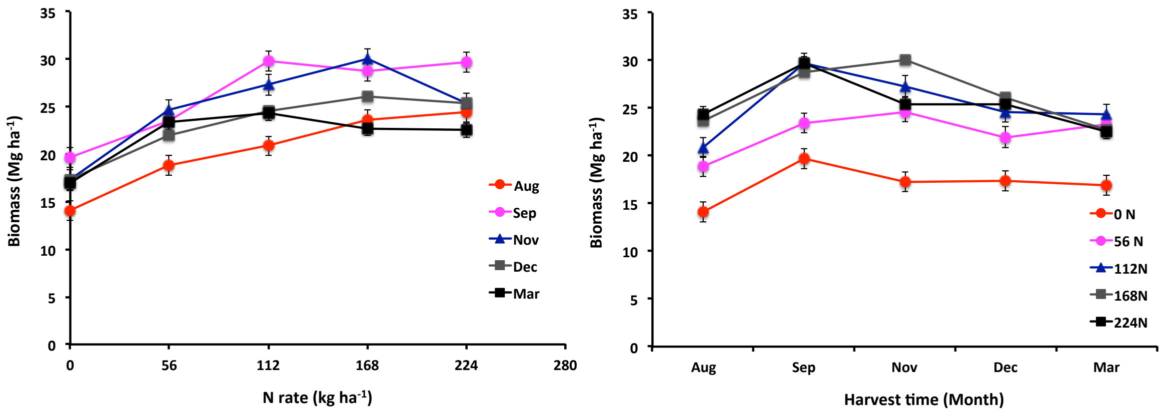

Biomass (Mg ha-1)
N rate (kg ha-1)
Aug
Sep
Nov
Dec
Mar
Harvest time (Month)
0 N
56 N
112N
168N
224N

Figure 3.

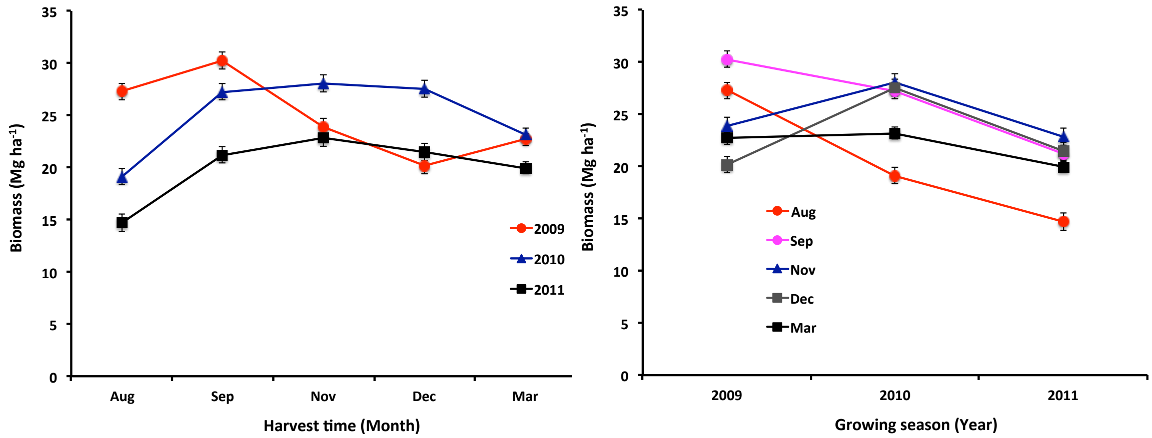
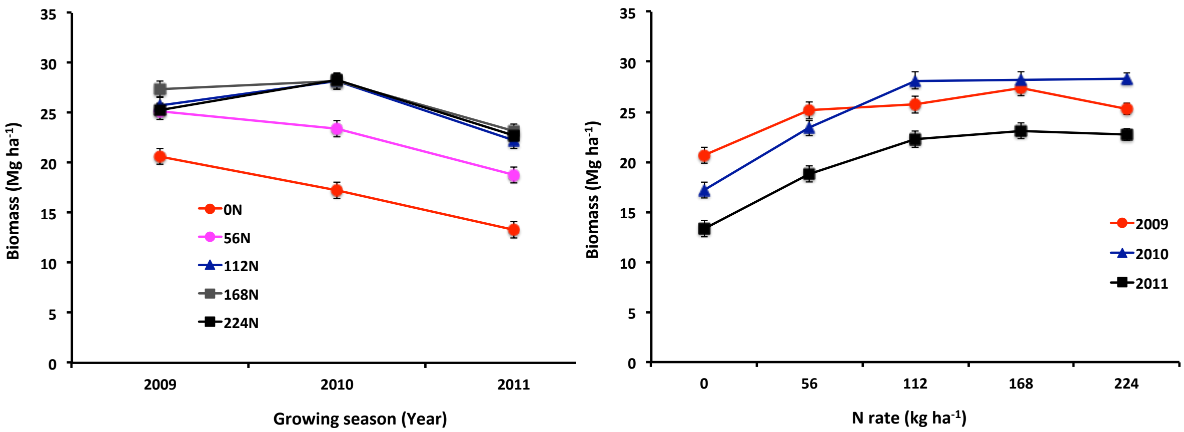

Biomass (Mg ha-1)
35
30
25
20
15
10
5
0
Aug
Sep
Nov
Dec
Mar
Harvest time (Month)
2009
2010
2011
Biomass (Mg ha-1)
2009
2010
2011
Growing season (Year)
Aug
Sep
Nov
Dec
Mar

Figure 4.



Biomass (Mg ha-1)
0N
56N
112N
168N
224N
2009
2010
2011
Growing season (Year)
Biomass (Mg ha-1)
2009
2010
2011
0
56
112
168
224
N rate (kg ha-1)

Figure 5.

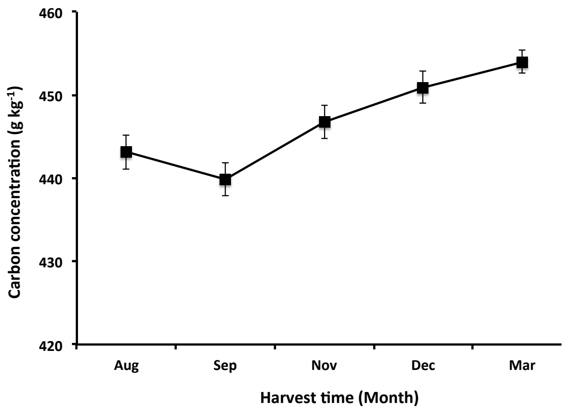
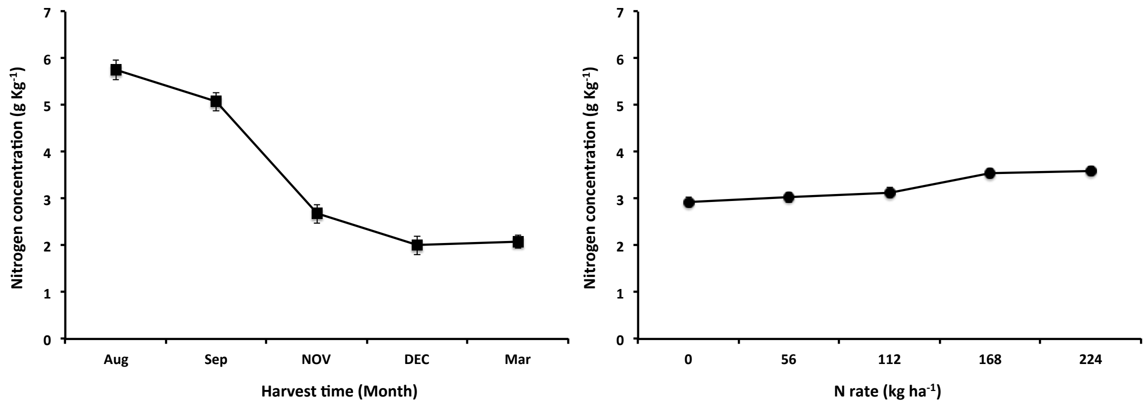

460
450
440
430
420
Carbon concentration (g kg-1)
Aug
Sep
Nov
Dec
Mar
Harvest time (Month)

Figure 6.



Nitrogen concentration (g Kg-1)
7
6
5
4
3
2
1
0
Aug
Sep
NOV
DEC
Mar
Harvest time (Month)
Nitrogen concentration (g Kg-1)
0
56
112
168
224
N rate (kg ha-1)

Figure 7.

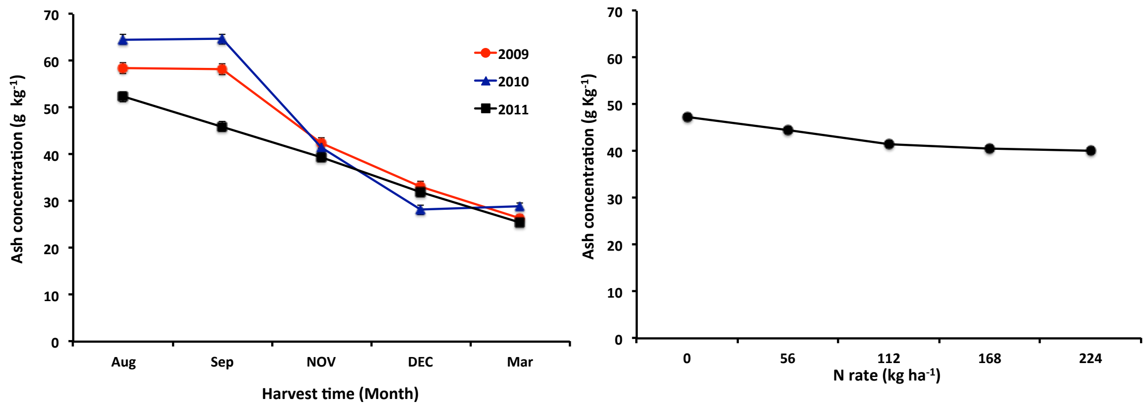
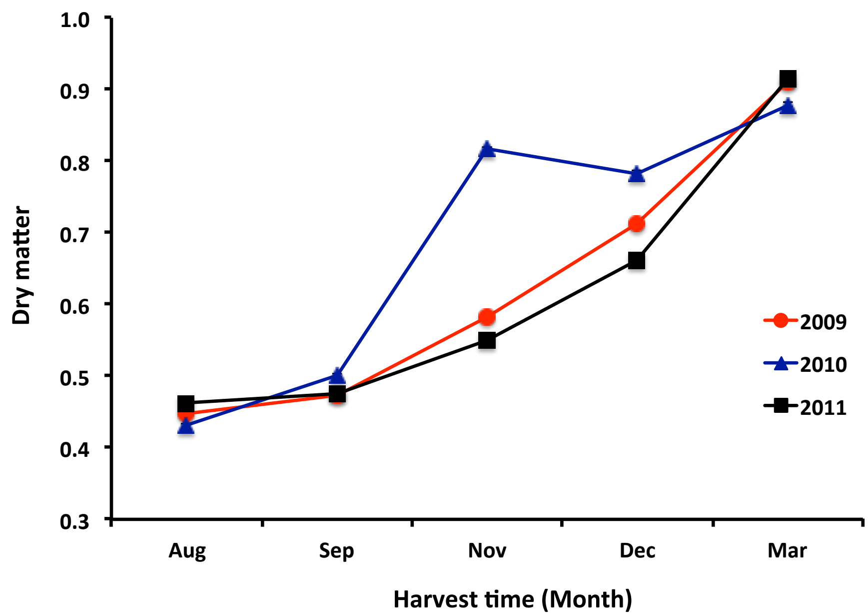

70
60
50
40
30
20
10
0
Ash concentration (g kg-1)
2009
2010
2011
Aug
Sep
NOV
DEC
Mar
Harvest time (Month)
70
60
50
40
30
20
10
0
Ash concentration (g Kg-1)
0
56
112
168
224
N rate (kg ha-1)

Figure 8.


1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
Dry matter
2009
2010
2011
Aug
Sep
Nov
Dec
Mar
Harvest time (Month)