NITROGEN AND HARVEST IMPACT ON WARM-SEASON GRASS BIOMASS YIELD AND FEEDSTOCK QUALITY

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by

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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

NITROGEN AND HARVEST IMPACT ON WARM-SEASON GRASS BIOMASS YIELD AND FEEDSTOCK QUALITY

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and hereby certify that, in their opinion, it is worthy of acceptance.

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CHAPTER 1: INTRODUCTION

Incr1easing global demand for energy, along with the raised awareness of sustainability related issues of existing sources of energy elevates the necessity to search for alternative sources and technologies. Current world energy supply heavily depends on fossil fuel sources including petroleum, coal, and natural gas. However, these energy sources have led to numerous negative environmental, economic, and political issues faced by every country in the present world. Global warming, price volatility, and uncertainties of future supply are among them (Goldemberg, 2007; Bassam, 2010). As a result of these issues and continuous search for alternative energy sources, biomass has gained a growing interest as a feedstock for bioenergy production. Biomass is a form of renewable source of energy that is capable of mitigating negative environmental concerns associated with use and refining of fossil fuels while contributing towards rural economic growth (Goldemberg et al., 2004; Bardhan et al., 2015; Williams 2015).

Ethanol is a biofuel that has the potential of being one of the dominating sources of biofuel and already has been introduced in Brazil, the US, and some European countries. Furthermore, ethanol is well known for its favorable fuel characteristics including higher octane number, heat of vaporization, and flammability temperature. Therefore, it is being considered as the most suitable fuel for internal combustion engines (Gray et al., 2006; Koç et al., 2009). In addition, ethanol can be produced from renewable sources such as sugar, cane, cassava, corn, barley, and many types of waste biomass materials. Corn, and sorghum g1rain/starch is the major feedstock for ethanol production. However, due to the limitations in supply, current focus has directed towards producing a significant portion of fuel ethanol from the feedstocks other than corn grain (Gray et al., 2006). Agricultural residues, wood municipal solid waste, and dedicated energy crops are among those feedstocks. For example, the US government has established a goal to produce 30% of transportation fuel needs with domestically grown and refined biofuels by 2030. The amount of ethanol required for achieving this goal is about 60 billion gallons. Furthermore, two thirds of this ethanol requirement is projected to be produced from lignocellulosic-based feedstock (Hahn-Hägerdal et al., 2006).

Warm-season grasses including switchgrass (*Panicum virgatum* L.), big bluestem (*Andropogon geradii Vitman*), and indiangrass (*Sorghastrum nutans* L.), as a feedstock for lignocellulosic ethanol production have demonstrated multiple advantageous characteristics in both environmental and production points of view (U.S. Department of Energy, 2011; Waramit et al., 2011). These species are native to northern US, are highly productive even with minimal annual inputs and under marginal conditions, offer multiple environmental benefits (including improving water quality, C sequestration, soil erosion prevention, wildlife habitat), have favorable lignocellulosic properties, and have net energy balances (McLaughlin and Walsh; 1998, McLaughlin et al., 2002; Lee et al., 2003; Simpson et al., 2008; Bonin et al., 2014).

However, successful implementation of proper agronomic management practices affects the sustainability and longevity of bioenergy feedstock production systems. Nitrogen fertility and harvesting management are crucial and interdependent agronomic management practices for warm-season grass biomass feedstock production systems. For example, greater nutrient removal and concentrations were observed when switchgrass was harvested twice per year suggesting higher annual fertilizer requirements compared to practicing single harvest per year (Guretzky; Seepaul). In addition, N and harvest management operations consume significant proportion of energy out of total energy involved in biomass energy production systems and thereby cause potential net energy balance implications (Tilman et al; 2006).

Similar to any cropping system in the world, N fertilizer use has both economic and environmental implications related to warm-season grass biomass production (Tilman et al., 2002). Nitrogen fertilization improves productivity and profitability of most cropping systems (Ceotto and Candilo, 2010). Biomass yields of warm-season grasses have generally responded positively to incremental N applications (Vogel et al., 2002; Thomason et al., 2005; Wilson et al., 2013; Seepaul et al., 2014). Furthermore, N application rates affected the composition of the warm-season grass harvest, nutrient removal or concentrations, and nutrient and biomass partitioning between shoots and roots, depending on the location, harvest practices, and plant species (Heggenstaller et al., 2009; Guretzky et al., 2011; Waramit et al., 2011).

Harvest management practices for warm-season bioenergy grasses affect biomass yield, feedstock quality/composition, nutrient removal, stand longevity, and wildlife benefits (Adler et al., 2006; Sanderson et al., 2006; Waramit et al., 2010; Guretzky et al., 2011; Hong et al., 2012 Sadeghpour 2014; Seepaul et al., 2014). Timing and frequency are the critical management considerations in warm-season biomass grass harvesting management practices. However, timing and frequency aspects of warm-season grass harvesting practices depend upon species, cultivar, location, and biofuel conversion system (Adler et al., 2006; Sanderson et al., 2006). For example, Fike et al. (2006) reported based on their work in southeastern US, the highest biomass yields of switchgrass with a single late fall harvest for lowland cultivars while upland cultivars gave greater yields when harvested twice yearly. Harvest management practices that include late season harvests are considered favorable in the terms of sustainability and quality of the biomass due to efficient recycling of the nutrients within the plants and higher biomass energy contents, and lower moisture contents (Casler and Boe, 2003; Adler et al., 2006; Seepaul et al., 2014).

Even with all this background research, few studies have examined the interactions of N management and harvest timing on biomass and energy yield. The core objective of this research was to evaluate the impacts of N and harvest management and their interactions on warm-season biomass yield and feedstock quality. Moreover, there were two specific objectives of this research: 1) to evaluate the impact of harvest and N management strategies on the biomass yield of warm-season grasses; and 2) to quantify the effects of N and harvest management strategies and their interactions on feedstock quality of warm-season biomass grasses. These two objectives are addressed in Chapters 3 and 4 of this thesis.

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CHAPTER 2: REVIEW OF LITERATURE

World Primary Energy Consumption and the Sources

According to the world energy statistics, by the end of 2011, the global energy consumption is 548 EJ. The major sources of this consumed energy are petroleum (oil), coal, natural gas, renewable electricity, and nuclear and the contribution of these are 34, 29, 23, 8, and 5 percent respectively. In addition, the there is an exponential growth of global energy demand due to the exponential world population growth (Demirbaş, 2001). For example, World Energy Outlook 2015 projected over 30% growth in the world energy demand between 2013 and 2040 (IEA, 2015).

Summary of U.S. Energy Supply

Similar to the global energy supply, petroleum, natural gas, coal, nuclear, and renewable energy are the major sources of energy being consumed in the U.S. According to the contribution of each source for the national energy consumption only 10% is coming from the renewable sources (Figure 2.1). Furthermore, United States is the second among top five energy consuming countries and accounts for 19% of global energy consumption and 17% of global energy – related carbon dioxide (CO_2) emissions. In addition, it is responsible for the greatest per capita consumption (313 million Btu) (EIA, 2014).

The share of total U.S. energy used by the transportation sector was 28% in 2015 and 92% of that came from petroleum products while biofuels including ethanol and biodiesel contributed to about 5% (EIA, 2016).

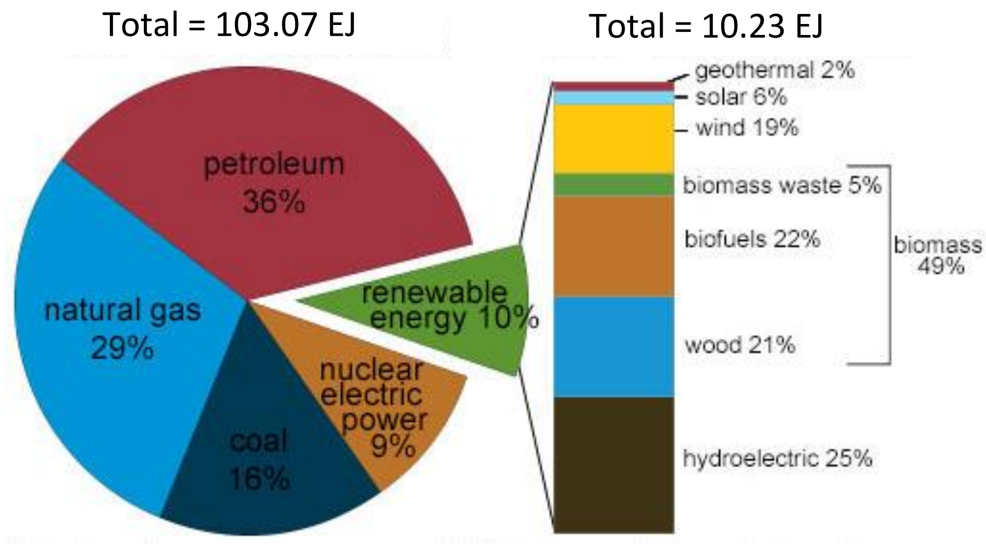

Figure 2.0.1. Energy consumption in the U.S. by source in 2015

Source: U.S. Energy Information Administration

Moreover, at present U.S. energy supply is significantly dependent upon the imported foreign petroleum based and other liquid fuels. For example, in 2012, imported petroleum and other liquid fuels as a share of total U.S. use reached 40% and it is projected to be 32% by 2040 (AEO, 2014). This high dependency on foreign fuel imports has numerous concerns including global economic disruptions due to the finite nature of fossil fuel reserves and the negative environmental externalities associated with the exploitation process and the use (Parish and Fike, 2007; Fike et al., 2013).Therefore, energy coming from renewable sources drew growing attention as an alternative that is capable of alleviating environmental concerns of fossil fuel usage.

Biomass as an Energy source

All organic materials derived from plants, in a broad sense can be named as biomass. Photosynthesis is the process in which carbohydrates; the precursor of the biomass is being produced in green plants (including algae, trees and crops), through the reaction between atmospheric CO_2 and water with use of sunlight as the energy source. In other words, solar energy is being stored as chemical energy in the chemical bonds present in the biomass. Therefore, biomass is considered as a renewable source of energy. Biomass, as an energy source accounts for more than 10% of the global energy supply by releasing chemical energy stored in the biomass (McKendry, 2002a). Cooking and heating spaces were the major uses of biomass and since the 18th century heat, steam, and power for work processes were derived using biomass (Williams, 2015). Biomass has drawn a growing interest as a renewable energy source globally and locally within the last few decades. Energy independence and security, rural economic development, and environmental concerns including mitigation of greenhouse gas emissions are among major advantages of biomass fuels over the fossil fuels that led to this significant attention on biomass as a source of energy (Demirbas, 2009). This growing demand for biomass energy has resulted in search for alternative sources such as agroforestry, conservation lands, and algae in addition to the current sources including forests, agriculture, and wastes (Williams, 2015).

Terms “bioenergy” and “biofuels” are used for energy and fuels derived using recently living or currently harvested biomass as a feedstock. Furthermore, biofuel can be described as liquid and gas fuels used for transportation and industrial processes (Singh, 2013; Williams, 2015). Depending on the source or the feedstock used in the refinery process biofuels are divided in to three broad categories. They are first, second, and third generation-biofuels. Among these three, plants, plant products, or plant based residues are the source or the feedstock for first and second-generation biofuels while third-generation biofuels are produced from algae (John and Anisha, 2011; Williams, 2015).

Moreover, food crops those are rich of oils, sugars, and starches are being used as feedstocks for production of first-generation biofuels while second generation biofuels are derived using nonfood/dedicated bioenergy crops and food crop residues that are rich of lignocellulosic material including perennial grasses, woody materials, and food crop residues (Williams, 2015).

Related to the sourcing of feedstock and production process issues, second-generation biofuels exhibit numerous advantages over first-generation biofuels. Production of feedstocks for first-generation biofuels may have negative impacts on food supply, biodiversity, and soil and water quality due to the requirements for intensive management practices including nutrient management and cultivation practices compared to the perennial plant species. Perennial species used for production of second generation biofuels include warm-season grasses and short rotation woody plants (Blanco-Canqui, 2010; Naik et al., 2010). In addition, second-generation biofuels production systems have a neutral or even negative C balance (Naik et al., 2010). Furthermore, Schmer et al. (2008) reported an average net energy yield of 60 GJ ha^{-1} when switchgrass was managed for production of cellulosic ethanol.

Lin and Tanaka (2005) divided biomass resources in the world into four broad categories and arranged based on the abundance as follows.

1. Wood residues: This is the most abundant source of biomass for energy production. These are the by-products of wood product industry.
2. Municipal solid waste.
3. Agricultural residues.

4. Dedicated energy crops: These include both woody crops and herbaceous crops including tall grasses.

Perennial Warm-Season Grasses as a Biomass Feedstock

Perennial warm-season grasses including switchgrass (*Panicum virgatum* L.), big bluestem (*Andropogon gerardii* Vitman), and indiangrass (*Sorghastrum nutrans* L. Nash) are the dominant grass species in the North American tallgrass prairie (Weaver, 1968). Furthermore, these grass species played a significant role as an alternative source of animal forage during warm and dry months when typical pastures of cool-season grasses (C3), are unproductive (Anderson, 2004). High productive capacity of warm-season grasses due to efficient use of water, nutrients (especially N), and solar radiation, capacity to sequester C, and prevent soil erosion have made these ideal candidates as feedstocks for biofuel production (Muir et al., 2001; McLaughlin and Kszos, 2005; Lewandowski and Schmidt, 2006). However, among these grasses, switchgrass has drawn a growing interest as a bioenergy feedstock in the US.

Switchgrass is a warm-season perennial grass species native to North America which has promising advantageous effects on carbon (C) and nitrogen (N) balances (Bransby et al., 1998, Wullschleger et al., 2010). Reduced nutrient (nitrate nitrogen (NO_3-N)) loss and increased C sequestration associated with deep rooting system and large yielding perennial nature of switchgrass are responsible for associated soil health benefits of switchgrass bioenergy systems apart from the production of cleaner burning fuels (Ma et al., 2000).

After a long run screening program using more than 30 herbaceous crops species, by the Bioenergy Feedstock Development Program (BFDP) at Oak Ridge National Laboratory switchgrass has been selected as the model herbaceous biomass crop in the U.S. in 1991 (Sanderson et al., 1996, McLaughlin et al., 1999, McLaughlin et al., 2002; Parrish and Fike, 2005). After that, numerous research efforts have been made throughout the U.S. focusing on varietal development, establishing agronomic practices which maximize the biomass yield and quality and conversion processes into the biofuels such as cellulosic ethanol.

Maximizing Biomass Yield of Warm-season Grasses

Nitrogen (N) fertilizer management and harvesting management are critical and interconnected agronomic management practices that lead to the biomass yield, quality, and sustainability of warm-season bioenergy cropping systems (Sanderson et al., 1999; Muir et al., 2001; Vogel et al., 2002 Anderson et al., 2013). For example, greater N removals have been reported when practicing multi-harvests per growing season compared to single harvest for warm-season grasses due to the removal of N-rich immature biomass form the system (Reynolds et al., 2000; Parrish and Fike, 2005; McLaughlin and Kszos, 2005).

Nitrogen Fertility Management for Switchgrass

Nitrogen fertilizer inputs are important for optimizing the biomass yield and sustainable stands and this fertilizer requirement depends upon factors like cultivar and the productivity of the site (Vogel et al., 2002, Mitchell et al., 2010). Furthermore, N management is also significant due to its impact on financial and environmental costs as a contributor to cost of production and air, groundwater, and stream water pollution (McLaughlin and Adams Kszos, 2005).

Research on N fertility management for switchgrass for biomass energy production is still emerging. According to Muir et al. (2001) efficient use of water and N in switchgrass are crucial factors since they are the principal sources which limit the productivity of warm season grasses. Furthermore, Boyer et al. (2012) stated that, annual N fertilizer applications are necessary to produce higher yields that make switchgrass production for lignocellulosic biomass economically viable.

Pedroso et al. (2013) concluded that significant N fertilizer input is necessary for sustaining greater switchgrass yields in intensively managed multi-harvest systems. They also reported that after the first year, the switchgrass yields have been increased linearly with the increase of N fertilization up to 300 kg N ha^{-1} yr^{-1}. However, agronomic N use efficiency declined with higher N rates. Vogel et al. (2002) have conducted research at two locations in Midwest US (Ames, IA and Mead, NE) using Cave-in-Rock switchgrass and the N source has been ammonium nitrate (NH_4NO_3). They reported that the biomass yield responses were different between the two locations and this difference was attributed to initial soil NO_3-N concentration and the precipitation. Higher initial soil NO_3-N at Mead has contributed to higher biomass yield at zero N rate and lower response to increasing N rates in comparison to Ames.

Muir et al. (2001) reported increases in biomass yields with the increase of N rate at two Texas sites. At one site, the increment has been linearly increased up to 168 kg N ha^{-1} and leveled off. At same site, both tiller density and mass increased. Furthermore, at with no N treatment, a reduction in biomass yields has been shown over the time indicating that initial N fertilization is necessary to sustain production. This result also has shown a positive correlation of biomass yield to the growing-season rainfall. Conclusions of the research were that the N fertilization and rainfall controlled switchgrass production in the south-central USA, and a single, spring application, around 168 kg N ha^{-1} maximized the biomass. They further reported that N rates of 56 to 112 kg ha^{-1} produced the highest apparent N recovery efficiency values.

Harvest Management for Switchgrass

Limited research information is available on harvest schedules for switchgrass managed as a bioenergy crop (Vogel et al., 2002). Adler et al. (2006) reported switchgrass biomass production is influenced by the season of the harvest. They observed reductions in biomass yield when harvesting was delayed over winter until spring. Furthermore, Anderson et al. (2013) reported that the timing of harvest is a critical factor for switchgrass management as a bioenergy feedstock.

Sadeghpour et al. (2014) reported important research findings on numerous parameters related to switchgrass (Cave-in-Rock) from research conducted in Massachusetts from 2009 to 2012. Biomass yields were found to decline when harvesting was delayed from fall to spring. At the same time, a reduction of ash content was reported due to the delayed harvesting thereby increasing the energy content of the biomass for combustion. They concluded that delayed harvesting over winter until spring led to the highest quality switchgrass biomass yield.

According to Anderson et al. (2013), the harvest timing impacts on energy quality depends whether the biomass is fermented to biofuels or thermo-chemically processed.

Late-fall harvesting was found more favorable for fermentation and early spring harvest more favorable for thermo-chemical processing, with the latter addressing moisture content issues that lead to storage and transportation concerns.

According to the findings at two sites by Vogel et al. (2002), the highest biomass yield was when the first harvest was at the late physiological maturity of the plants. Furthermore, greater regrowth yields have been reported when first harvesting was earlier. In addition, harvests after killing frost was reported to be lower than harvests made after peduncles were fully elongated.

Conversion of Biomass to Energy

Heat generation and transportation fuels are the two energy-related products of biomass conversion process. Warm-season grasses including switchgrass can be converted to both of these energy products (Larson et al., 2009). Thermochemical and biochemical processing techniques are the two major categories of biomass to energy conversion pathways, which are in use depending on the type/quality and quantity of the feedstock, desired end use of the product, environmental standards, and economic conditions. Direct combustion, gasification, and pyrolysis techniques fall under thermochemical processing of biomass. Heat, syngas, and bio-oils are the major products of these biomass energy conversion processes. Considering biochemical conversion of biomass, fermentation (Simultaneous Saccharification and Fermentation (SSF)) and anaerobic digestion are the two major processes. Furthermore, ethanol and biogas (mainly methane) are the major products of biochemical conversion technologies (McKendry, 2002b). However, recent warm-season grass research is more concentrated on producing ethanol using the SSF process due to its favorable lignocellulosic properties or biomass quality in combination with environmental and economic benefits.

Conversion of lignocellulosic biomass to ethanol

Major constituents of lignocellulosic biomass are cellulose, hemicellulose, and lignin (Öhgren et al., 2007; Kumar et al., 2009). For example, percent contents of each of these constituents in switchgrass are, 28-37%, 23-27%, and 15-18%, respectively (Keshwani and Cheng, 2009). Cellulose and hemicellulose (sugar polymers) are a carbohydrate fraction of lignocellulosic biomass that can be fermented to ethanol after hydrolysis/breaking down into simple monomeric sugars including hexoses and pentoses using microbes (Öhgren et al., 2007; Keshwani and Cheng, 2009). The combined process of hydrolysis and fermentation of biomass is called simultaneous saccharification and fermentation (SSF) (Figure 2.2) (Öhgren et al., 2007).

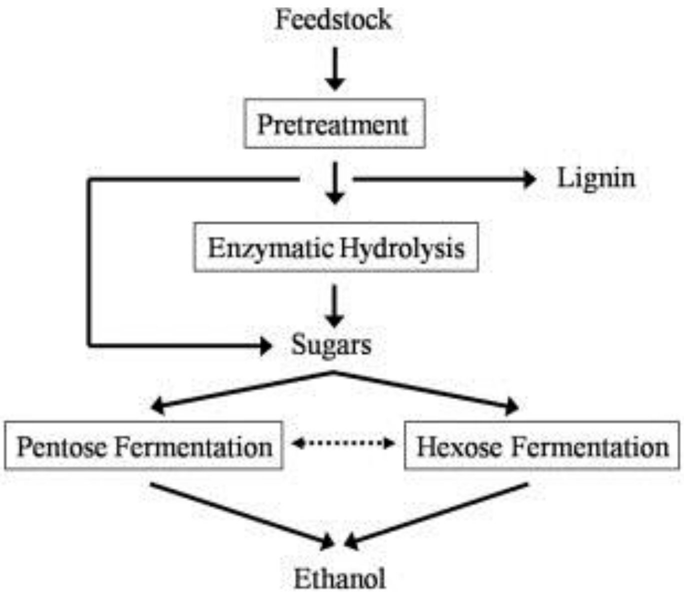

Figure 2.0.2. Schematic diagram of biomass ethanol production (Keshwani and Cheng, 2009)

Biomass feedstock quality of warm-season grasses for ethanol production

The efficiency of the ethanol production depends upon multiple factors as SSF is an integration of multiple processes. However, being the raw material for the process, biomass feedstock affects the sustainability of the whole ethanol conversion system, especially the composition/quality of biomass. Genetics and environmental conditions, stage of maturity of the grasses at harvest, and soil nutrients, especially N, have impacts on not only the yield of biomass grasses, but also the composition of biomass harvest (Vogel et al., 2002; Adler et al., 2006). Therefore, improvements in genetics and agronomy of warm-season grasses along with the conversion process are necessary for establishing feasible and sustainable biofuel production systems (Keshwani and Cheng, 2009).

Numerous research reported the effects of N and harvest management practices on fiber composition (acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid detergent lignin (ADL)) that related to the concentrations of cellulose, hemicellulose, and lignin of warm-season biomass grasses (Mulkey et al., 2006; Waramit et al., 2011; Guretzky et al., 2011; Hong et al., 2012; Seepaul et al., 2014). All these research reported increases in cell wall constitutes with maturity or late harvesting for the three grass species of switchgrass, big bluestem, and indiangrass. However, among three grass species, switchgrass exhibited lower cellulose compared to other two species. Furthermore, Hong et al. (2012) reported that growing of two-way or three-way mixes of these species helped in improving overall cellulose content of the harvested biomass. However, location and weather, especially precipitation, affected composition of the grass biomass (Guretzky et al., 2011; Hong et al., 2012)

Only a limited number of research studies have examined the effects of above agronomic management practices on ethanol yield (a function of composition of biomass (L Mg^{-1} biomass)) and energy production from warm season grass species (a function of ethanol yield and biomass yield (L ha^{-1})) (Schmer et al., 2012; Seepaul et al., 2014). Furthermore, Seepaul et al. (2014) reported a positive correlation between ethanol yield and production. However, ethanol yield was negatively correlated with ADF, NDF, lignin, and cellulose concentrations. Schmer et al. (2012) reported that observed differences in theoretical ethanol yield and production were due to the harvest years. According to Seepaul et al. (2014), higher harvest frequencies greater than two harvests per year resulted in poor feedstock quality, biomass yields, and greater nutrient removals from the system. Furthermore, they reported five year means of theoretical ethanol yield and production of 380-430 L Mg^{-1} biomass and 1750-3690 L ha^{-1} depending on the location or the weather condition of the sites. Therefore, maximizing the biomass feedstock quality and ethanol production is realistic with agronomic management practices that improve yields with minimal nutrient removals, genetic improvements of grass species optimizing the feedstock quality, and improved processing or refining facilities with optimal recovery of ethanol.

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CHAPTER 3: NITROGEN AND HARVEST IMPACT ON WARM-SEASON GRASSES BIOMASS YIELD

Abstract

Perennial warm-season grasses including switchgrass (*Panicum virgatum* L.), big bluestem (*Andropogon geradii Vitman*), and Indiangrass (*Sorghastrum nutans* L.) have drawn interest as bioenergy feedstocks due to their high yielding capacity with minimal amounts of inputs under a wide range of environments, and their capability to produce multiple environmental benefits. Nitrogen (N) fertility and harvest timing are considered as critical management practices when optimizing biomass yield and the feedstock quality of these grasses. The objective of this investigation was to quantify the impact of N fertilizer rate, N timing and harvest date on warm season biomass dry matter yield. Research was conducted in 2014 and 2015 on a total of four field-plot locations situated in central and west-central Missouri. Nitrogen fertilizer was applied using dry ammonium nitrate at the rates of 0, 34, 67, and 101 kg ha^{-1} at two application times, all N early spring and split N (early spring and following 1st harvest). Harvest treatments were as follows: 1) one cut in September; 2) one cut in November; 3) one cut in June and a second in September; and 4) one cut in June and a second in November. Treatments were arranged in a split-plot design with N rate as the main plot and harvest as the sub-plot in a randomized complete block design. Both N and harvest date and their interactions impacted biomass yield at all four locations. Delaying harvesting until late fall or killing frost increased yield. November harvest in combination with N rates $\geq$ 67 kg ha^{-1} year^{-1} produced higher yields compared to the control and 34 kg ha^{-1}N treatments and other harvest timing strategies. Although N was needed to optimize yield, partial factor productivity (PFP) of applied N was flat when N applied was > 34 kg ha^{-1}. Nitrogen fertilization at 67 kg ha^{-1} per growing season provided an opportunity to maintain a balance between both yield and efficiency of N inputs. Results of this research highlight the interactions of N fertilization and harvest management have when optimizing yield of warm-season grasses grown as bioenergy feedstocks.

List of acronyms: N, Nitrogen; PFP, partial factor productivity

Introduction

Deriving energy from plant biomass through burning and transformation to liquid or gaseous forms is a promising energy alternative that is capable of reducing the great reliance on the fossil fuels and greenhouse gas emissions and improving rural economies. (McLaughlin et al., 2002; Parrish and Fike, 2005; Simpson et al., 2008). Volatile price, uncertainties of supply, and energy security and environmental concerns associated with fossil fuels led to significant legislations and investments towards the use of biomass energy (U.S. Department of Energy, 2011). The Energy Independence and Security Act of 2007 and its predecessor, the Energy Policy Act of 2005 are examples of such legislation (Hochman et al., 2008). Increased attention on native perennial warm-season grass species including switchgrass (*Panicum virgatum* L.), big bluestem (*Andropogon geradii Vitman*), and Indiangrass (*Sorghastrum nutans* L.) as bioenergy feedstocks is one of the significant results of programs such as the Herbaceous Energy Crops Research Program (HECP) launched by the U.S. Department of Energy (DOE). (Wright, 1994; McLaughlin et al., 2002; McLaughlin and Kszos, 2005). Bioenergy production systems using these species are carbon (C) negative while producing net energy balances due to their high yielding capacity under diverse growing conditions. They even adapt well when grown on marginal landscapes which are not suitable for row crop production, have high water and nutrient use efficiency, and require minimal fertilizer and agrichemical inputs (Tilman et al., 2006).

Perennial warm-season grasses produce multiple environmental benefits in addition to providing biomass feedstock. These benefits include improving the water quality, soil conservation, and C sequestration, as well as providing wildlife habitat (McLaughlin and Walsh; 1998, McLaughlin et al., 2002; Lee et al., 2003; Simpson et al., 2008; Bonin et al., 2014). For example, below ground C inputs by deep, extensive root systems of switchgrass and reduced erosion led to increased soil organic C levels when grown as a bioenergy crop (Liebig et al., 2008).

Agronomic management practices play a significant role in managing warm-season grasses as a bioenergy feedstock by affecting the yield and composition of the harvested biomass, nutrient removal from the system, longevity of the plant stand, and anticipated environmental benefits (Waramit et al., 2011; Sadeghpour et al., 2013; Seepaul et al., 2014). Among these management practices, N fertilizer and harvest timing are considered interconnected critical factors in managing switchgrass as a bioenergy feedstock. Furthermore, these are the two management practices which have been studied in a significant portion of research related to biomass feedstock production using switchgrass. However, the interaction of these two management factors are less studied.

Frequency and timing are the critical components of harvest management strategies when managing warm-season grasses, such as switchgrass, as a bioenergy crop. Harvest strategies with one or two harvests per year have generally produced optimum yields while allowing extended stand life in most of the systems (Thomason et al., 2005). Furthermore, two harvests per year systems with adequate nutrient supplies allow switchgrass to be used as a dual-purpose species by providing early-season animal forage and late-season biomass feedstock (Sanderson et al., 1999; Guretzky et al., 2011; Seepaul et al., 2014). However, when these grasses are grown as bioenergy feedstocks, harvesting the biomass after a killing frost has shown to be more acceptable since it provides high quality feedstocks while allowing the plants to recycle nutrients in a sustainable manner (Casler and Boe, 2003; Adler et al., 2006; Seepaul et al., 2014).

Nitrogen fertilization is a crucial agronomic practice which improves productivity and profitability in most food or bioenergy cropping systems (Ceotto and Candilo, 2010). To date, numerous studies evaluated the effects of N fertilization for switchgrass production. Switchgrass was responsive to incremental N application despite the disparities existing between the results of these studies (Vogel et al., 2002; Thomason et al., 2005; Wilson et al., 2013; Seepaul et al., 2014). Investigations show that switchgrass N fertilizer requirements depend upon the yield potential of the site, productivity of the cultivar, and harvesting schedule (McLaughlin et al., 1999, Mitchell et al., 2008, Anderson et al., 2013). Removal of a significantly greater portion of applied N in the two-cut system compared to single cut system (McLaughlin et al., 1999) is an example of the impact of harvesting regime on the N fertilizer requirement.

Although numerous studies have been conducted toward investigating the individual impacts of N fertilizer management and harvest time regime on switchgrass biomass yield, only a few studies viewed these factors simultaneously. Therefore, the objective of this study was to evaluate the impact of harvest and N management strategies on the biomass yield of warm-season grasses. From this, optimum combination of management practices can be determined.

Materials and Methods

Study Sites

The research was conducted during the years 2014 and 2015 in Missouri, USA. The study was conducted at four field sites located in Gallatin, De Witt, Strasburg, and Green Ridge in 2014. In 2015, five sites were used with two sites in both De Witt and Strasburg and one site in Green Ridge (Table 3.1 and Figure. 3.1). The sites in Gallatin and Strasburg consisted with monocultures of big bluestem and switchgrass, respectively and there were mixtures of warm-season grasses in De Witt and Green Ridge. Moreover, all of these sites have been categorized as lands that require moderate or special conservation practices due to the limitation associated with poor drainage or high risk for erosion. Thirty-year average precipitation and monthly values received at each site during the experimental period were obtained from the nearest National Weather Service station to the site from a weather database maintained by the Utah State University (2016), and are graphically represented in Figure. 3.2.

Experimental Design and Management Practices

Treatments were arranged as split-plots in a randomized complete block design with three replicates, with N as the main plot and harvest as sub-plot treatments. Ammonium nitrate (NH_4NO_3) was used as the N source and applied in three rates, which were applied in May at 34, 67, and 101 kg ha^{-1} year^{-1}. Furthermore, 67, and 101 kg ha^{-1} year^{-1} treatments were also applied as split applications (67 kg ha^{-1} year^{-1}, 0.5 May + 0.5 June split application; 101 kg ha^{-1} year^{-1}, 0.67 May + 0.33 June split application, and 0.33 May + 0.67 June split application) (Table 3.2). Nitrogen fertilizer was pre-weighed and hand-broadcasted. There were two zero N fertilizer treatments. One of them was planted to native legumes as an N source for the warm season grass, and the other was considered as the overall N control (Table 3.2). There were four harvest timing treatments (Table 3.2) with two two-cut and two one-cut harvests which include both timing and frequency aspects of harvesting management. Both two-cut harvest treatments consisted with the first cut in mid- to late June. The second cut was either September or November.

Sampling and Data Collection

For harvesting, a 0.7-m swath of grass was harvested from each plot using a sickle-bar mower (BCS model 710, BCS America, Portland, OR) leaving a 12 ± 3 cm stubble height. The wet biomass weight of the harvested grass was measured and a representative subsample was collected from each plot. Subsamples were measured for the fresh weight and dried in a forced air oven at 65°C for 72 hours for dry matter determination.

Nitrogen Use Metrics

Agronomic efficiency and partial factor productivity (AE and PFP) of applied N were estimated using the following equations presented by Dobermann (2007).

$$\text{Partial Factor Productivity (PFP)} = \frac{\text{kg biomass ha}^{-1}}{\text{kg total applied N ha}^{-1}}$$

$$\text{Agronomic efficiency (AE)} = \frac{(\text{Yield at N}_x - \text{Yield at N}_0)}{\text{kilograms of N applied}}$$

Where, N_x = N rate > 0, and N_0 = control or no N application.

Statistical Analysis

Data were analyzed using the GLIMMIX procedure of SAS (SAS Institute, 2011) to determine significant (α=0.05) treatment effects. Fixed effects were N, harvest treatments, and their interactions. Block, block by N treatments, and block by harvest treatment interactions were treated as random effects. Tukey's honest significant difference (HSD) test was used for mean separations when the fixed effects were significant at $\alpha \leq 0.05$ significance level.

Results and Discussion

Biomass Yield

The impact of N and harvest timing management, as well as their interactions, varied by site and year (Table 3.3). Generally, warm-season grass yield almost always increased with N fertilization (exception Gallatin 2014). Likewise, harvest timing management always impacted yield. Sites resulting in N rate by harvest timing interactions are discussed separately below.

Nitrogen

Nitrogen fertilization positively impacted grass biomass yields both years (Figure 3.3). Four of five of the N responsive sites had yield increases of at least 3 Mg ha^{-1}. A leveling off of biomass yields above N levels of 67 kg ha^{-1} was observed at De Witt 1 and Green Ridge in 2014 and Strasburg 2 in 2015, while yield leveled after 34 kg ha^{-1} at both sites in De Witt in 2015. Furthermore, at all the N responsive sites application of N at 67 or 101 kg ha^{-1} produced superior yields (5.8-11.1 Mg ha^{-1}) compared to 34 kg N ha^{-1} and non-fertilized plots (3.5-8.6 Mg ha^{-1}). Except for the two sites in De Witt in 2015, this observation is in line with an indication of a yield plateau of switchgrass biomass yield with an application of N at 67 kg ha^{-1} as reported by Haque et al. (2009).

Though not statistically tested, biomass yields at De Witt 1 were generally 2-3 Mg ha^{-1} more in 2014 than 2015. For example, the range of biomass yields in 2014 and 2015 were 5.9-9.9 Mg ha^{-1} and 3.5-6.9 Mg ha^{-1} respectively. This variation of biomass yield in two years can be attributed to the excessive precipitation received in the months of May and July for 2015 (141 mm and 219 mm, respectively) relative to that received in 2014 (8 mm and 95 mm) (Figure. 3.2). In addition, soil of the De Witt 1 site was poorly drained, and developed along a small stream (Udifluvent). As such in growing seasons with frequent and high precipitation there is great potential for N to being lost through denitrification rather than leaching due to poor soil drainage conditions (Table 3.1) at the site (Cameron et al., 2013). Apart from the losses of applied N, plant stress induced by flooded conditions in the field may have negatively affected the biomass yield (Scott and Sallam, 1987; Sharma et al., 1990; Moraghan and Smith, 1996; Alam, 1999). Furthermore, in 2014 at De Witt 1, 101 and 67+34 kg ha^{-1} gave significantly higher biomass yields compare to non-fertilized (control and legume) and 34 kg ha^{-1} treatments. In 2015 at De Witt1, 67+34 kg ha^{-1} produced biomass yield significantly greater compared to 34+34 kg ha^{-1} and non-fertilized plots while the yields from both 101 and 34+67 kg ha^{-1} were greater compared to control and legume. In contrast, the De Witt 2 was an upland site and located on a back-slope of the landscape with soils that are better drained. Here biomass yields in 2015 were generally higher (not statistically tested) under each N treatment compared to De Witt 1 site (4.6-8.6 vs.3.5-6.9 Mg ha^{-1}). This difference can be attributed to the differences in soil drainage conditions (well drained at De Witt 2 vs. poorly drained at De Witt 1) and landscape characteristics (> 5% slope at De Witt 2 vs. <1% slope at De Witt 1). When considering the biomass yield performance under each N management at De Witt 2 in 2015, 34+34, 101 and 67+34 kg ha^{-1} dominated over non-fertilized.

At Green Ridge in 2014, 67+34, 101, 34+67, and 67 kg ha^{-1} performed better compared to control, legume, and 34 kg ha^{-1} N treatments (Figure. 3.3). In addition, for 2015 at Strasburg 2, 67 and 101 kg ha^{-1} were significantly higher than the control, legume, and 34 kg ha^{-1}. However, for these two sites, there were no significant differences in biomass yields when comparing one-time and split N application strategies when fertilizing at N rates of 67 and 101 kg ha^{-1}.

Since there were no significant differences in grass biomass yields among different N treatments with same amount of total N applied, N treatments were pooled within the same total amount of N applied and statistically re-analyzed (Table 3.4 and Figure. 3.4). Yields increased with only 34 kg N ha^{-1} at the 2015 De Witt sites and up to 67 kg N ha^{-1} at the other sites.

Harvest timing effect

Harvest timing influenced biomass yield at all the sites (Table 3.3 and Figure. 3.5). At each site in both years, biomass yield variation with harvest timing exhibited a common pattern. Greater biomass yields were associated with harvesting that generally included a late fall November harvest (N and J/N) (Figure. 3.5). In contrast, harvest timing strategies which typically consisted with early fall (S and J/S) produced lower biomass yields than the late fall harvest management. This variation of biomass yields can be attributed to the extended biomass growth that occurred during fall months. Late fall harvest strategies can be much more sustainable due to both higher biomass yields and reduced annual N inputs as a result of remobilization of considerable N from aboveground to the belowground root structures (Vogel et al., 2002).

On a site-by-site basis when comparing the two single cut harvest strategies (S and N), yield was significantly greater with the November harvest timing than the September harvest, except at the Gallatin site where overall productivity was the lowest of all the sites (Figure. 3.5). Over all sites, delaying harvest to November resulted in a 28-51% increase in yield compared to the September harvest. Similarly, when contrasting the 2-cut harvest management strategies, the J/N system always outyielded the J/S system. Averaged over all sites this was 50-200% increase over the J/S harvest timing. It is apparent the difference in biomass yields between these harvest systems can be attributed to the contribution of biomass coming from the fall growth between September and November. The benefit of N recycling back into roots would also be more realized with the J/N practice.

In 2015, De Witt 2 was the only site where biomass yields were greater with the 1-cut N harvest management over the 2-cut J/N harvest management (Figure. 3.5). With all sites except De Witt 1 in 2014, an early summer harvest (J) along with a September harvest resulted in lowest biomass yields compared to the other management strategies. Thus, harvesting feedstock for animals in June necessitates allowing the warm season grass to grow throughout the entire fall until after cold dormancy has been initiated (November harvest) without causing a growing-season yield drag.

Interactions of Nitrogen and harvest timing

Interaction of Nov and harvesting timing influenced dry matter yield of grasses at Green Ridge in 2015 and at Strasburg 1 in both years [Table 3.3 (with N timing included), Table 3.4 (with N timing excluded), Figure. 3.6 (with N timing included), and Figure. 3.7 (with N timing excluded)]. Early harvesting of biomass for animal forage suppressed response to N when the second harvest was not delayed until post maturity. However, at Green Ridge supplementing N at 101 kg ha^{-1} as a single application with June+Sep harvest timing regime produced the highest dry matter yield (6.2 Mg ha^{-1}) and it was significantly greater than the control (3.3 Mg ha^{-1}). With harvest timing strategies June+Nov, a continuous increase of dry matter yields was observed with increasing N supplies at each site. Conversely with single harvest in September (Sep), biomass yields tended to level off at 67 kg ha^{-1}. Furthermore, at each site across all the N management strategies, a single harvest in November (Nov) produced significantly higher dry matter yields compared to June+Sep harvest timing strategy. Especially with both 67+34 and 34+67 kg ha^{-1} application strategies, both harvest regimes June+Nov and N out yielded June+Sep. Moreover, same harvest timing strategies produced significantly higher biomass yields compared to single Sep harvest strategy, except in 2015 at Strasburg 1. This variation of biomass yields can be attributed to the prolonged and sustained uptake of applied N associated with June+Nov and Nov harvest strategies and thereby minimizing the risk of environmental losses of N via leaching and runoff that potentially occurs after September in both June+Sep and Sep harvest practices.

The ratio of regrowth to the first cut yields in double harvest strategies were 0.3-1.4 and 0.5-2.9 for June+Sep and June+Nov, respectively. Additionally, relative to June+Nov harvesting for 2014 at Strasburg 1, the control, legume, and 34 kg ha^{-1}, N management strategies had regrowth yield ratios relative to the first cut of less than 1.0 (0.5, 0.7, and 0.9, respectively). The same ratio with each split N application strategy ranged between 0.4-1.4 and 1.4-2.9 with June+Sep and June+Nov harvest strategies. This result implies that late fall harvest of two-cut harvest systems was taking advantage of split applied N.

Under each harvest strategy, split application of N at both rates of 67 and 101 kg ha^{-1} per growing season did not improve biomass yields compared to single application strategies. Although not statistically significant, there was a slight decline in biomass yields when N was applied as split applications. Moreover, N fertilizer application and harvesting of biomass are among most energy and time consuming operations in cellulosic biomass feedstock production. Therefore, split application of N while practicing two harvests per growing season may not be energy efficient (Vogel et al., 2002; Vogel et al., 2011). In addition, these management decisions should be made based on the demand of these biomass grasses in both animal forage and bioenergy feedstock markets.

Nitrogen Use Metrics

Partial factor productivity

Nitrogen fertility management impacted PFP at all the sites during both 2014 and 2015 (Figure. 3.8). Furthermore, the variation of PFP relative to the N management followed a similar trend at all the sites in both years. Highest PFP (90-250 kg biomass kg^{-1} N) was associated with application of 34 kg N ha^{-1} and the lowest (30-110 kg biomass kg^{-1} N) with 101 kg N ha^{-1}. At almost all the sites, PFP with application of N in split applications at the rates of 67 kg ha^{-1} (34+34 kg ha^{-1}) and 101 kg N ha^{-1} (67+34 and 34+67 kg ha^{-1}) was slightly reduced compared to one-time application, but this was never statistically different. With De Witt 1 and Green Ridge sites, numerically lower PFP values were observed in 2015 compared to 2014. This can be attributed to the greater amounts of precipitation received during 2015 (Figure. 3.2), which likely caused losses of N via denitrification. In addition, noticeably lower PFP values were observed at Gallatin in 2014 under each N management strategy. Lower plant density giving overall lower production along with and the steep terrain at this site, potentially with increased risk of losing of applied N fertilizer with surface runoff, are potential reasons for lower PFP values.

Agronomic efficiency

Agronomic efficiency of applied N was affected by N fertilization at both sites at De Witt in 2015 (Figure. 3.9). The highest AE values were associated with 34 kg N ha^{-1} at both the sites (68 and 95 kg biomass kg^{-1} N). Furthermore, at De Witt 1 the AE with 34 kg N ha^{-1} was significantly greater than all other N treatments, while at De Witt 2 there was no significant difference between 34 kg N ha^{-1} (95 kg biomass kg^{-1} N) and split application of N at 67 kg ha^{-1} (60 kg biomass kg^{-1} N). However, at De Witt 2, AE with 34 kg N ha^{-1} was significantly greater than the one-time application of N at 67 kg ha^{-1} and all 101 kg ha^{-1}. Furthermore, there were no significant differences in AE among 67 and 101 kg N ha^{-1} treatments.

The range of PFP and AE of applied N from this research varied between 30-250 kg biomass kg^{-1} N and 24-95 kg biomass kg^{-1} N, respectively, depending on the site and N rate (Figure. 3.8). This range is wider than the 35-99 kg biomass kg^{-1} N for PFP and 14-33 kg biomass kg^{-1} N for AE as reported by Sadeghpour et al. (2014). This is, in part, because of the lower N rates used in this research compared to theirs (34-101 kg N ha^{-1} vs. 67-134 kg N ha^{-1}). Furthermore, numerically lowered PFP and AE values associated with each site during 2015, the wetter year of the two of this research, are consistent with lower PFP findings highlighted by Sadeghpour et al. (2014) as a result of wet conditions. In addition, AE values for the two sites at De Witt in 2015 (Figure. 3.9) confirm the above statement by exhibiting comparatively lower values in relation to the wetter/poorly drained site and vice versa. Overall, results related to N use metrics from this study agree with diminishing returns with increased N inputs highlighted by Lemus et al. (2008).

Conclusions

Dry matter yield of warm-season grasses increased with increasing N for eight of nine sites, and reached a plateau after 34 kg N ha^{-1} or 67 kg ha^{-1}. Although higher N rates caused yield increases, AE and PFP of applied N tended to decrease with increasing N supply. However, supplementation of N at 67 kg ha^{-1} per growing season provides an opportunity to maintain a balance between both yield and efficiency of N inputs.

Therefore, it is important to take both dry matter yield and N use metrics such as AE and PFP in to account when producing these biomass grass species on a commercial scale in order to achieve economic and environmental sustainability of the system.

Delayed harvest timing until November or a killing frost in both one harvest and two harvests per growing season sustained greater biomass yields. Such harvesting strategies are well known for improving the longevity of the grass stands by facilitating nutrient recycling between aboveground components and belowground components. In contrast, harvest timing strategies with an early fall (September) harvest resulted in lower biomass yields. Since, harvesting of biomass is one of the operations that consume significant amounts of time and energy, decisions on harvesting frequency needs to be made based on energy balances and availability of labor and machinery within the growing season. However, effective implementation of combinations of N and harvest strategies is highly important to achieve long term sustainability of warm-season biomass energy feedstock production systems.

Finally, characterization of field sites in relation to both spatial and temporal variability in soil, plant populations, and weather conditions helps lead to meaningful response to management for a given year as well as for the long-term sustainability of the bioenergy grass production systems. Most importantly performing a life-cycle analysis, which uses data coming from field experiments similar to this study, will be helpful for policy makers, scientists, and landowners/farmers in gaining a greater perspective on environmental and economic sustainability.

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Table 3.1. Field site locations, years of operation, soil classification, and grass composition descriptions.

Site	Year	Soil	Landscape Position, slope, and Capability Class	Grass Composition (%)
De Witt-1 (39° 22' N, 93° 17' W)	2014/2015	Nodaway silt loam (Fine-silty, mixed, superactive, nonacid, mesic Mollic Udifluvents)	Foot slope, 0-5%, 3-w	Indian grass (60), Big bluestem (40)
De Witt-2 (39° 22' N, 93° 17' W)	2015	Wakenda silt loam (Fine-silty, mixed, superactive, mesic Typic Argiudolls)	Summit, 2-9%, 3-e	Indian grass(70), Big bluestem (30)
Gallatin (39° 51' N, 93° 58' W)	2014	Mandeville silt loam (Fine-loamy, mixed, superactive, mesic Typic Hapludalfs)	Side slope, 2-30%, 3-e	Big bluestem (100)
Green Ridge (38° 36' N, 93° 21' W)	2014/2015	Hartwell silt loam (Fine, mixed, active, thermic Typic Argialbolls)	Summit, 0-5%, 2-w	Switchgrass (50), Indian grass (50)
Strasburg-1 (38° 45' N, 94° 9' W)	2014/2015	Haig silt loam (Fine, smectitic, mesic Vertic Argiaquolls)	Summit, 0-2%, 2-w	Switchgrass (100)
Strasburg-2 (38° 45' N, 94° 9' W)	2015	Sampsel silty clay loam (Fine, smectitic, mesic Vertic Argiaquolls)	Side slope, 2-14%, 3-e	Switchgrass (100)

Table 3.2. Summary of the N and harvest treatments applied in the research.

N/main-plot treatments				Harvest/sub-plot treatments		
	Time of application				Time of harvest	
N trt ID	May	June	Total inorganic N/year	Harvest trt ID	First cut	Second cut
	------------------- kg N ha^{-1} -------------------					
0	0	0	0	J/S	June	September
				S	September	-
				J/N	June	November
				N	November	-
L[†]	0	0	0	J/S	June	September
				S	September	-
				J/N	June	November
				N	November	-
34	34	0	34	J/S	June	September
				S	September	-
				J/N	June	November
				N	November	-
67	67	0	67	J/S	June	September
				S	September	-
				J/N	June	November
				N	November	-
34+34	34	34	67	J/S	June	September
				S	September	-
				J/N	June	November
				N	November	-
101	101	0	101	J/S	June	September
				S	September	-
				J/N	June	November
				N	November	-
67+34	67	34	101	J/S	June	September
				S	September	-
				J/N	June	November
				N	November	-
34+67	34	67	101	J/S	June	September
				S	September	-
				J/N	June	November
				N	November	-

[†] Native legumes (Patridge pea (*Chamaecrista fasciculate*) and Illinois bundleflower (*Desmanthus illinoensis*) seeds were sown in May 2014).

Table 3.3. Fixed effects (P<F) of nitrogen rate and timing combinations, harvest timing, and their interactions on dry matter (DM) yield at each site in each year.

Year	Site	Source of Variation		
		N	Harvesting	N × Harvesting
2014	De Witt 1	0.04	<0.0001	0.28
	Gallatin	0.24	<0.0001	0.28
	Green Ridge	<0.0001	<0.0001	0.15
	Strasburg 1	<0.0001	<0.0001	<0.0001
2015	De Witt 1	<0.0001	<0.0001	0.15
	De Witt 2	<0.01	<0.0001	0.76
	Green Ridge	<0.0001	<0.0001	<0.01
	Strasburg 1	<0.0001	<0.0001	0.04
	Strasburg 2	<0.01	<0.0001	0.34

Table 3.4. Fixed effects (P<F) of nitrogen rate, harvest timing, and their interactions on dry matter (DM) yield at each site in each year.

Year	Site	Source of Variation		
		N rate	Harvesting	N x Harvesting
2014	De Witt 1	0.04	<0.0001	0.11
	Gallatin	0.45	<0.0001	0.36
	Green Ridge	<0.0001	<0.0001	0.03
	Strasburg 1	0.003	<0.0001	<0.0001
2015	De Witt 1	0.0003	<0.0001	0.09
	De Witt 2	0.02	<0.0001	0.81
	Green Ridge	0.0001	<0.0001	0.0004
	Strasburg 1	0.0005	<0.0001	0.005
	Strasburg 2	0.007	<0.0001	0.29

Table 3.5. Fixed effects (P<F) of nitrogen rate nitrogen use metrics at each site in each year.

Year	Site	Source of Variation
		N
------------------------Agronomic Efficiency------------------------		
2014	De Witt 1	0.0007
	Gallatin	<0.0001
	Green Ridge	<0.0001
	Strasburg 1	<0.0001
2015	De Witt 1	<0.0001
	De Witt 2	<0.0001
	Green Ridge	<0.0001
	Strasburg 1	<0.0001
	Strasburg 2	<0.0001
------------------------Partial Factor Productivity------------------------		
2014	De Witt 1	0.91
	Gallatin	0.1
	Green Ridge	0.18
	Strasburg 1	0.67
2015	De Witt 1	0.005
	De Witt 2	0.03
	Green Ridge	0.18
	Strasburg 1	0.11
	Strasburg 2	0.3

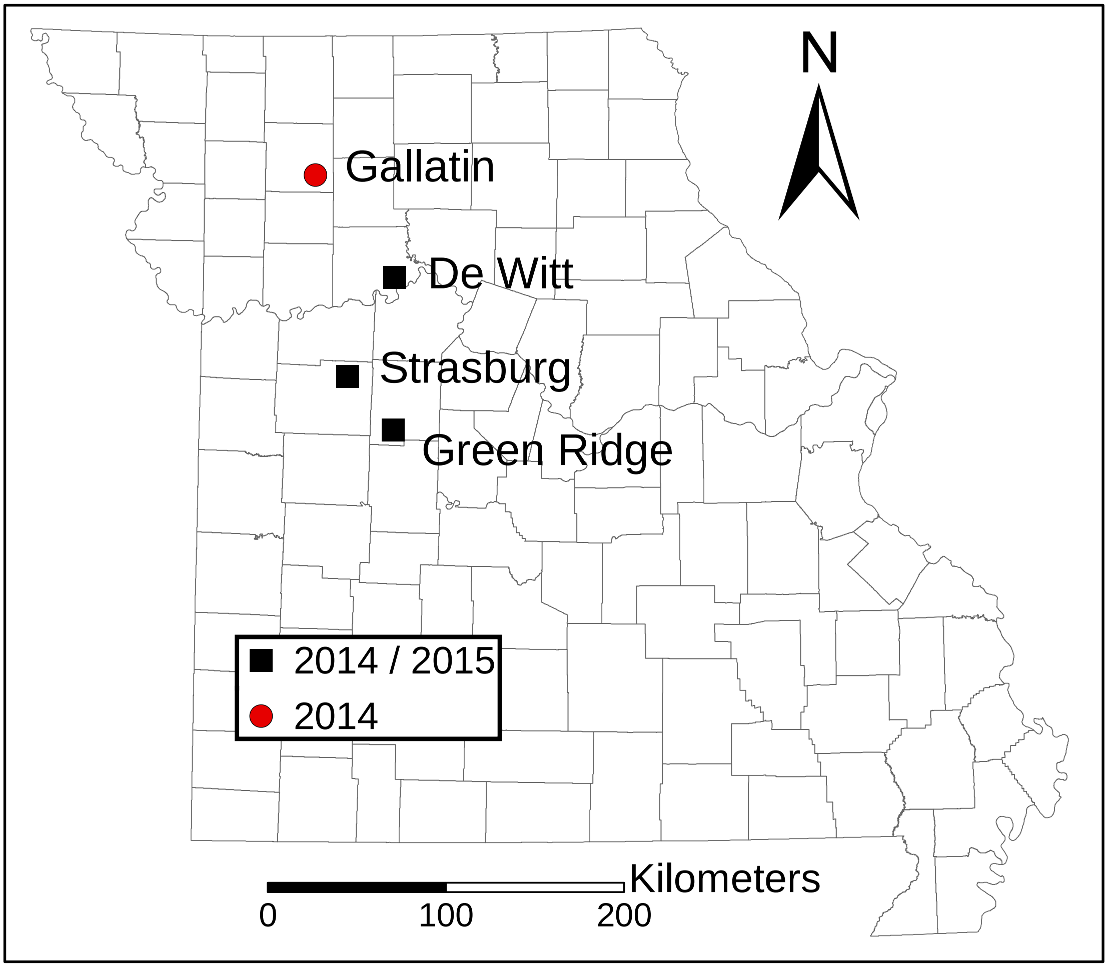

Figure 3.1 Location of the field sites and the years of operation.

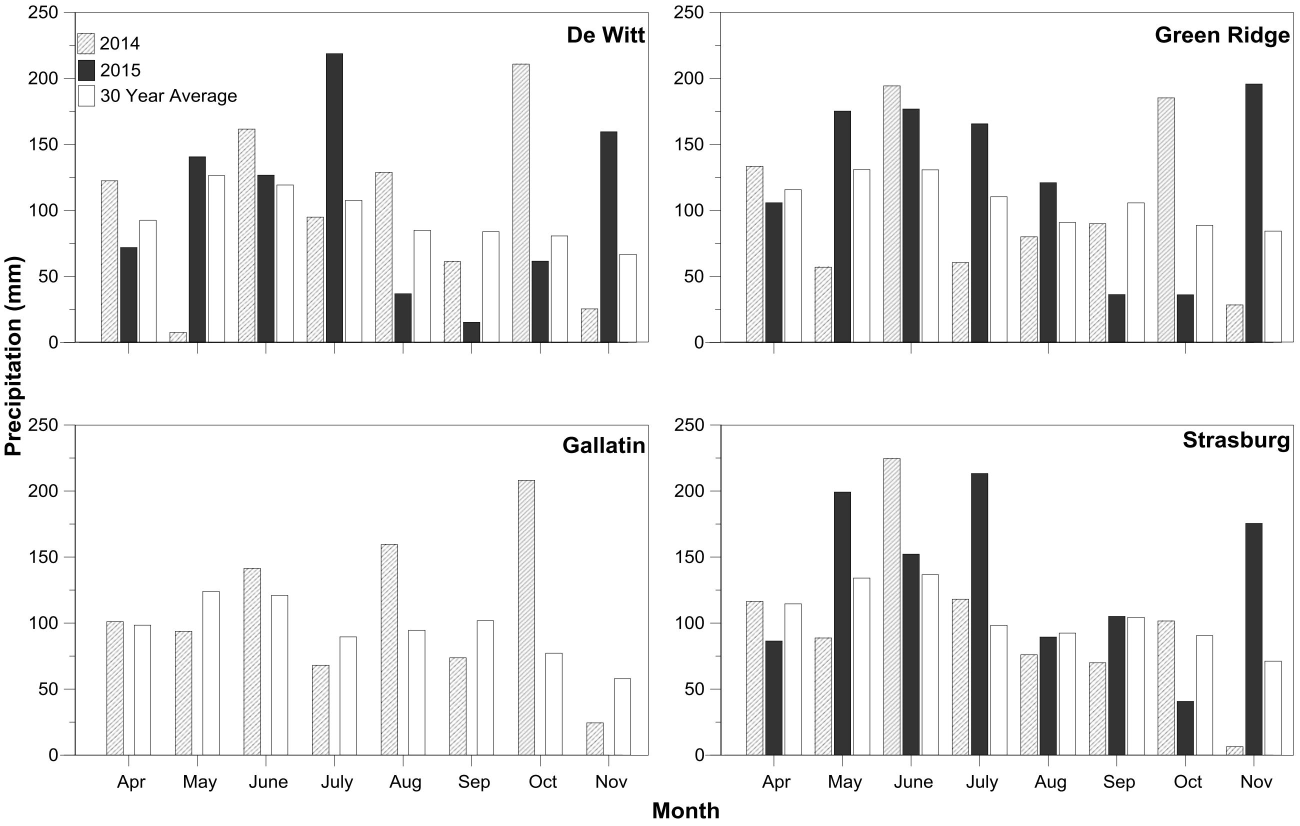

Figure 3.2 Monthly precipitation for study locations in 2014 and 2015 and 30-year long term average.

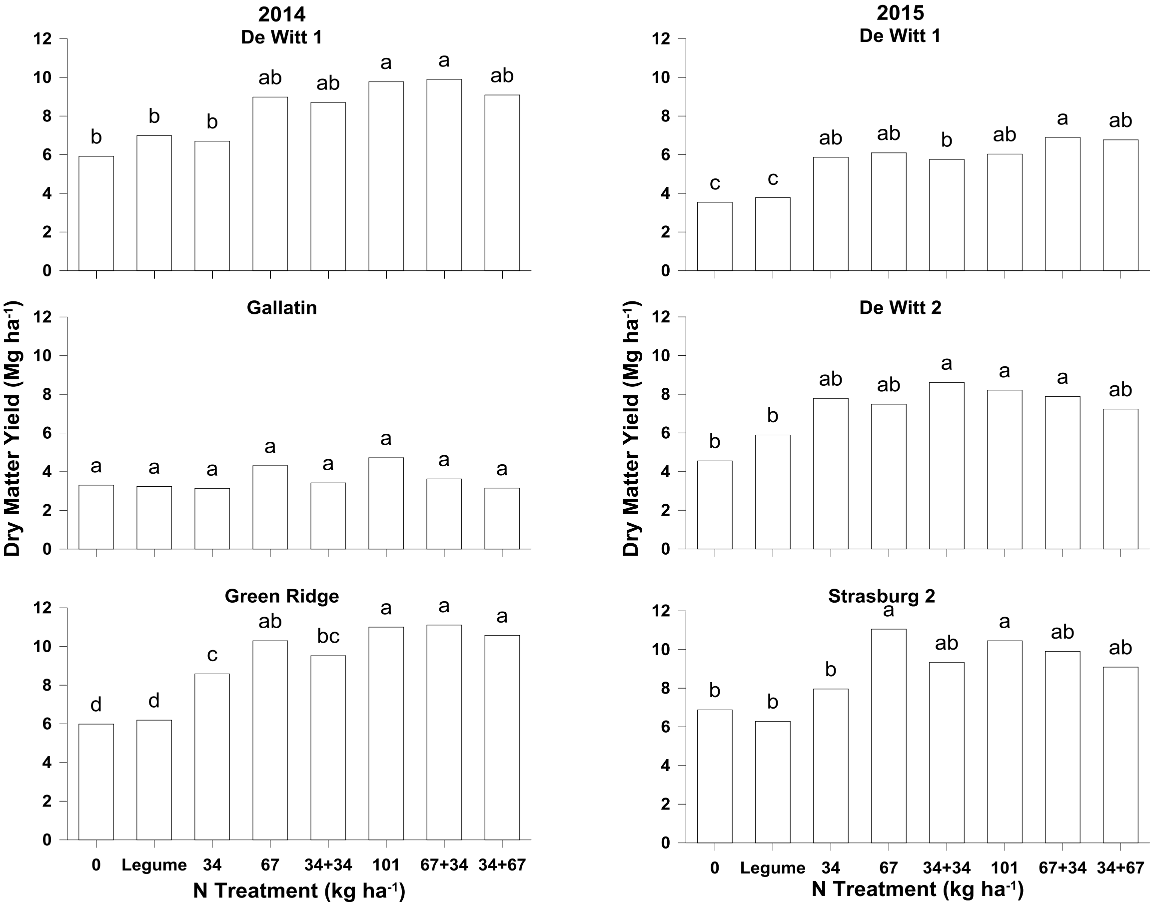

Figure 3.3 Main effect of N on the dry matter yield at each site in 2014 and 2015. Columns with the same letter are not significantly different at $P < 0.05$.

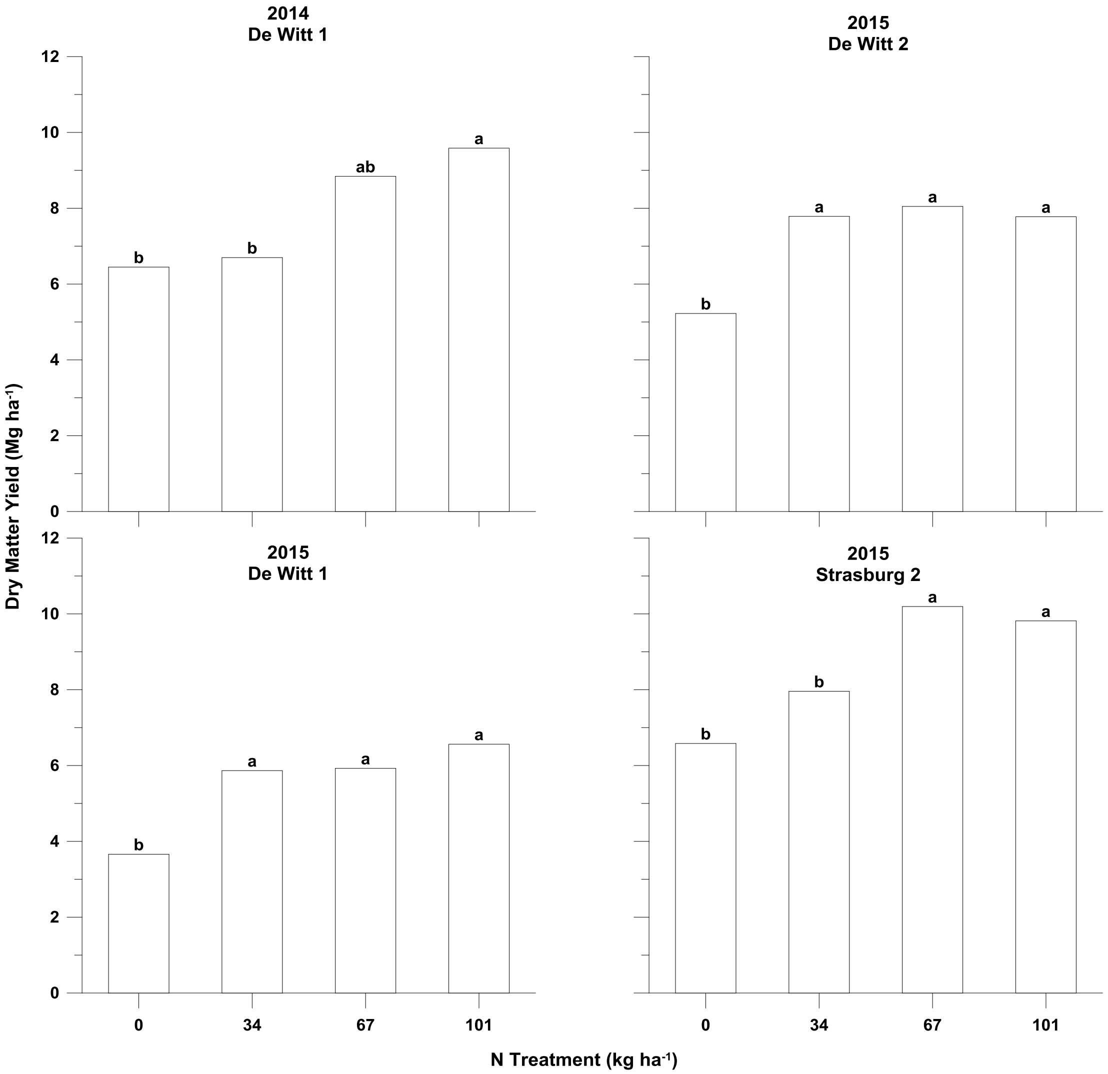

Figure 3.4 Main effects of N rates on biomass yield (averaged across split and one time N application treatments with the same amount of total N applied). Columns with the same letter are not significantly different at $P < 0.05$.

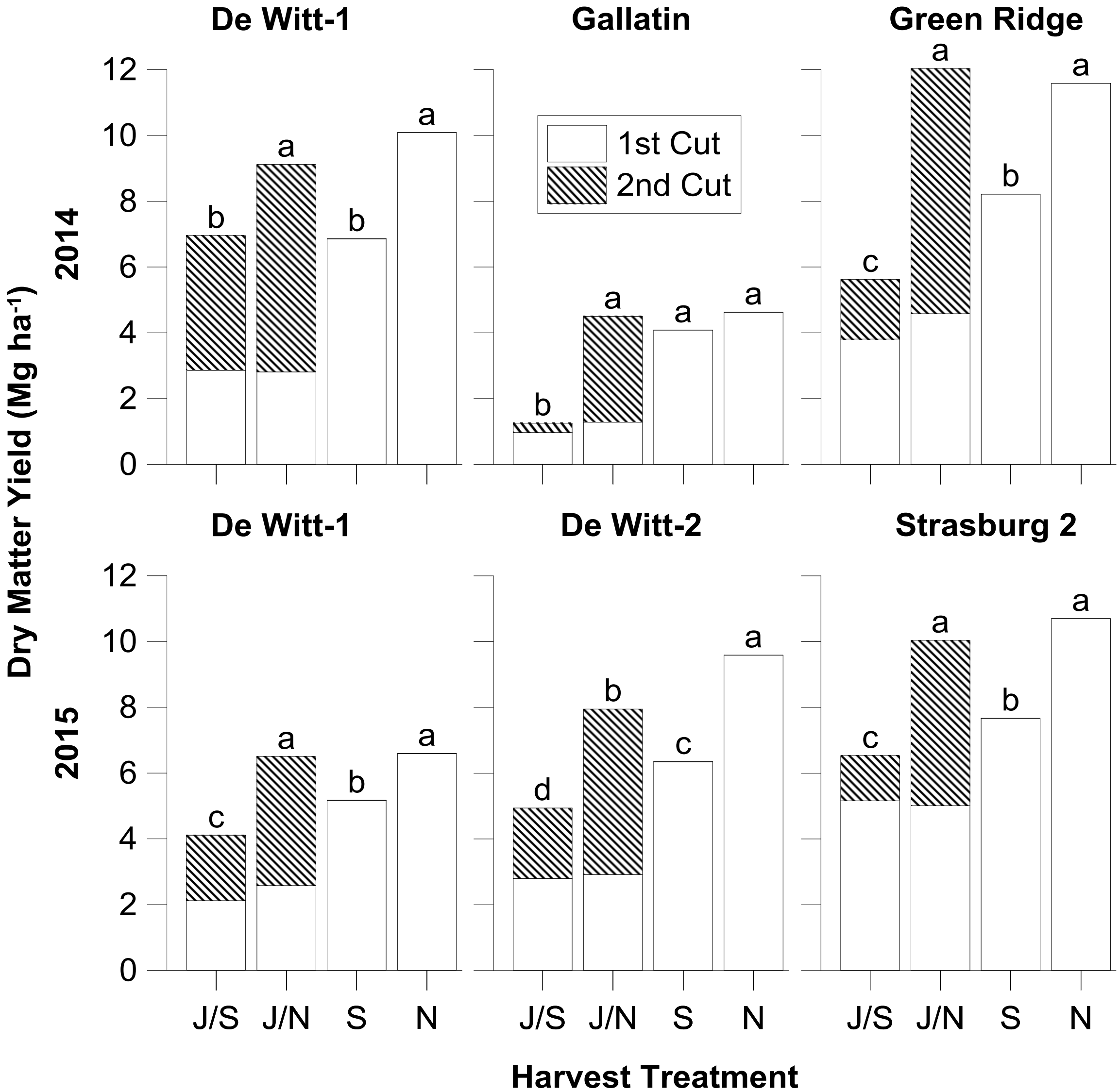

Figure 3.5 Main effects of harvest treatments at each site in 2014 and 2015. Harvest treatments included: J/S-June+Sep harvest; J/N-June+Nov harvest; S-Sep harvest; and N-Nov harvest. Columns with the same letter are not significantly different at $P < 0.05$.

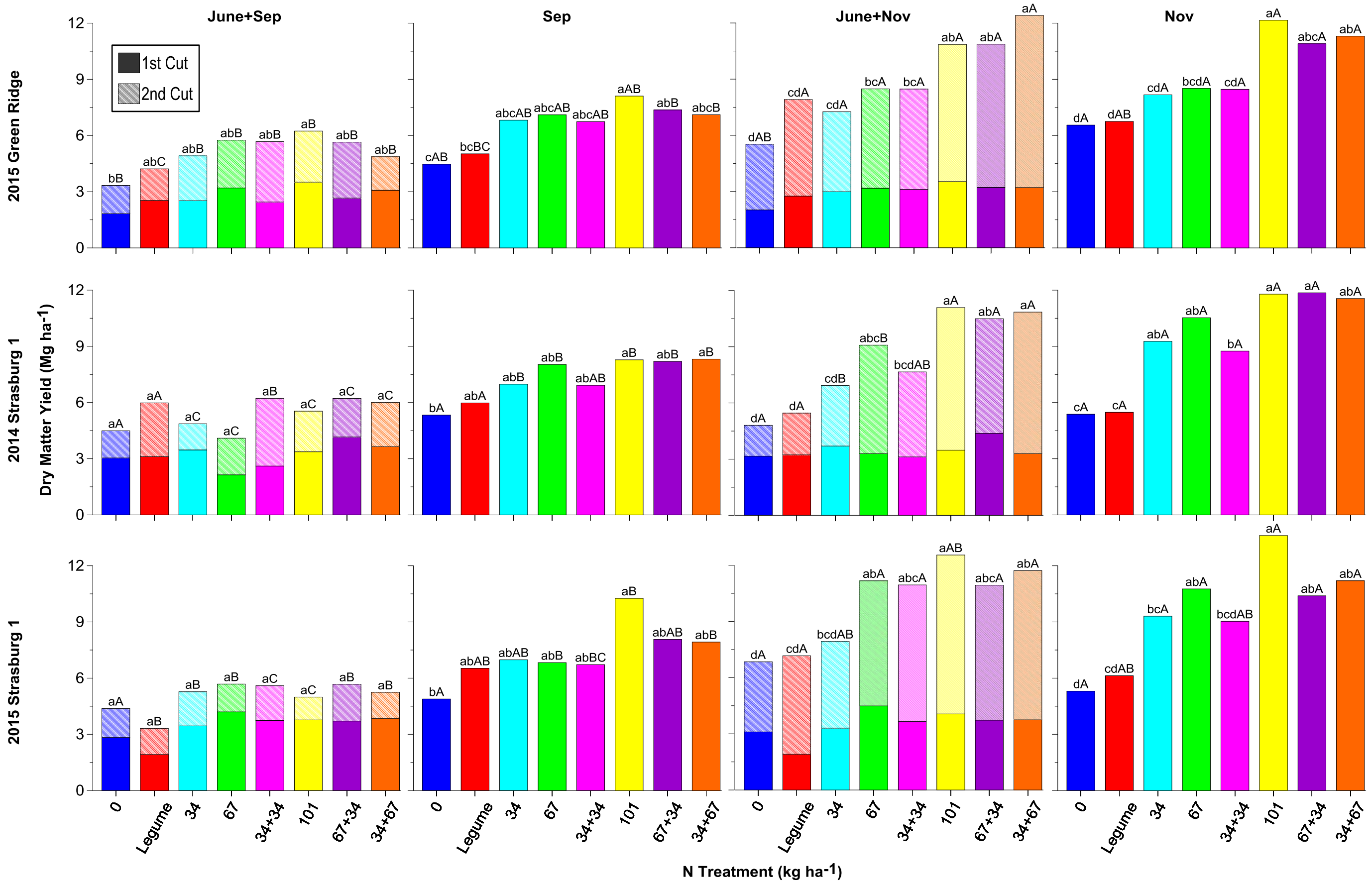

Figure 3.6 Nitrogen x harvest interaction effects on dry matter yield at Green Ridge in 2015 and at Strasburg 1 in 2014 and 2015. Columns with the same letter are not significantly different at $P < 0.05$. Lowercase letters denote differences between each N treatment within each harvest strategy and uppercase letters denote differences between each harvest strategy within each N treatment.

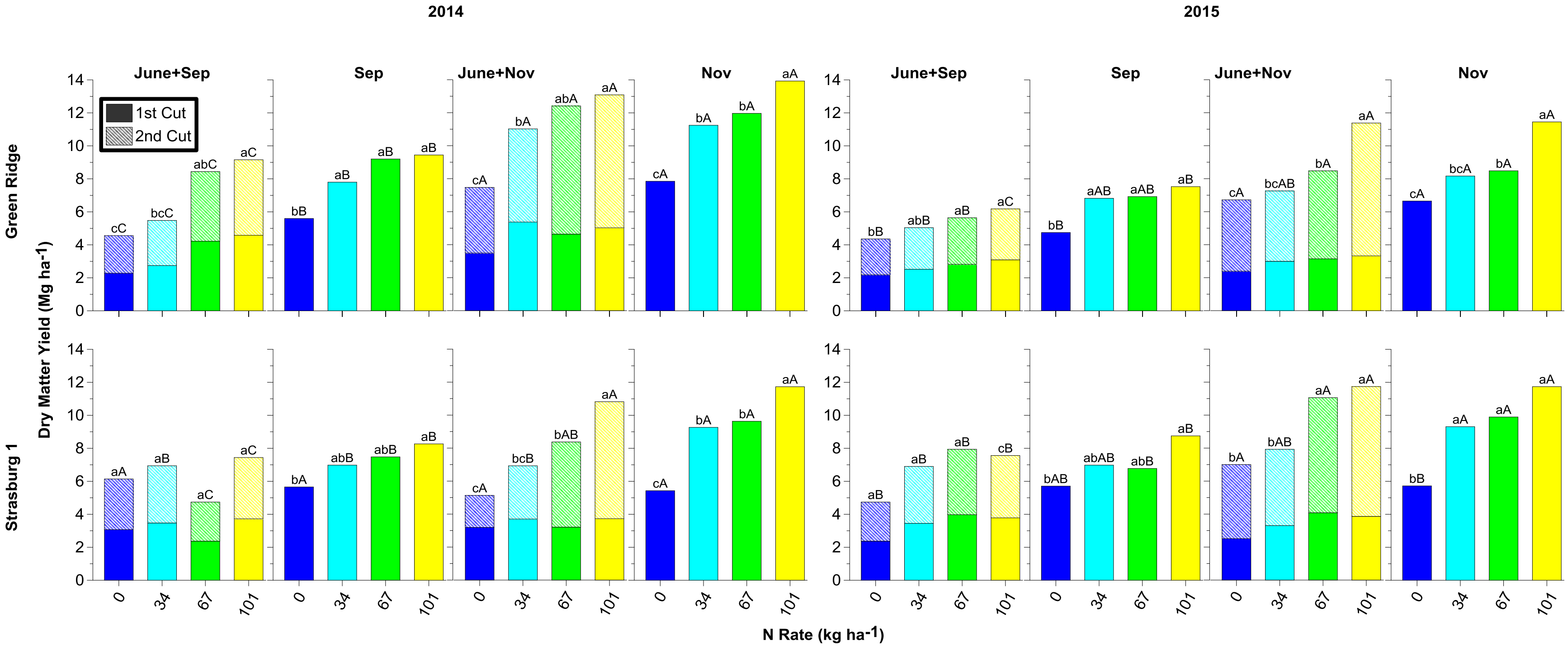

Figure 3.7 Effects of harvesting by N rate on biomass yield (averaged across split and one time N application treatments with the same amount of total N applied) interaction at Green Ridge and Strasburg 1 in 2014 and 2015. Bars with the same letter in each column are not significantly different at $P < 0.05$. Lowercase letters denote differences between each N rate within each harvest strategy and uppercase letters denote differences between each harvest strategy within each N rate.

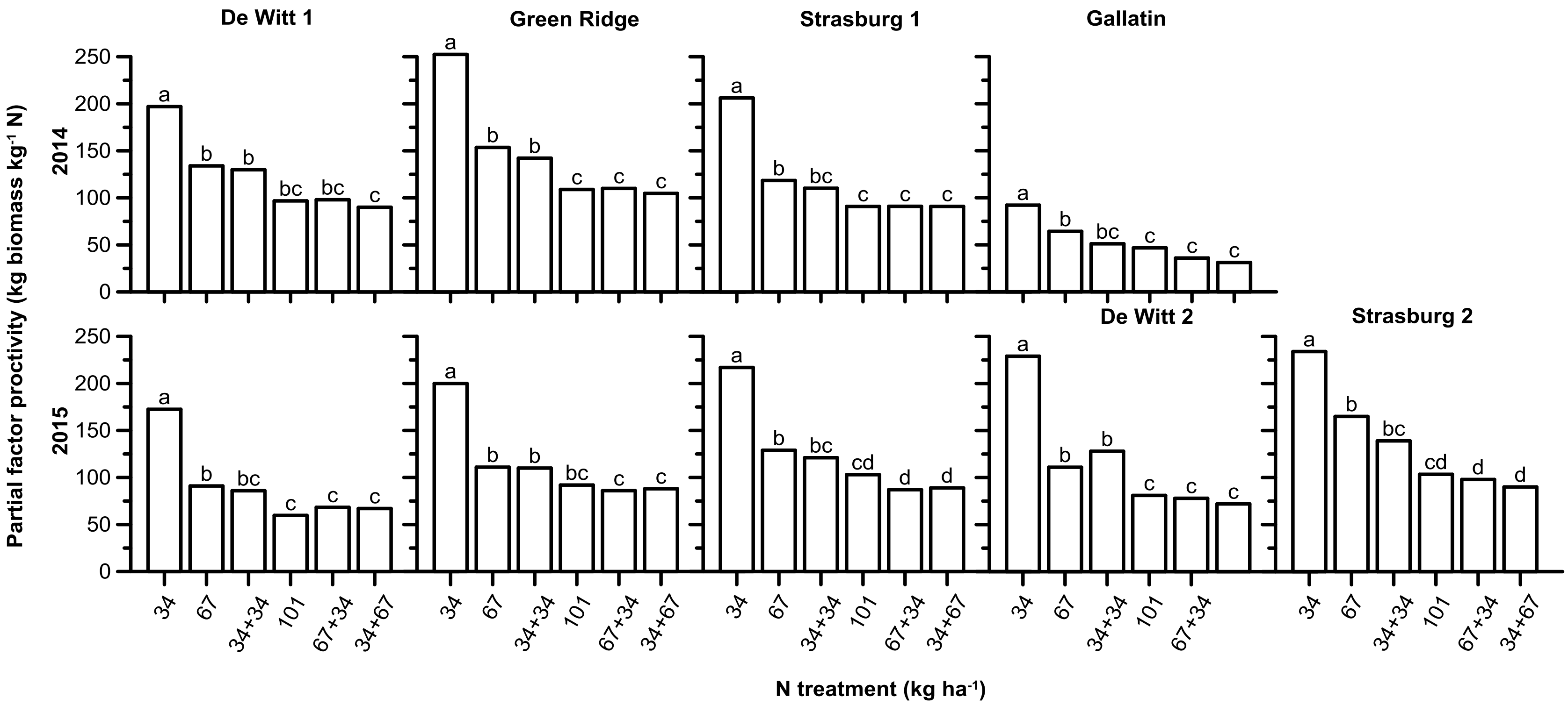

Figure 3.8 Partial factor productivity under N treatments for each site during the experimental period. Bars with the same letter in each column are not significantly different at $P < 0.05$.

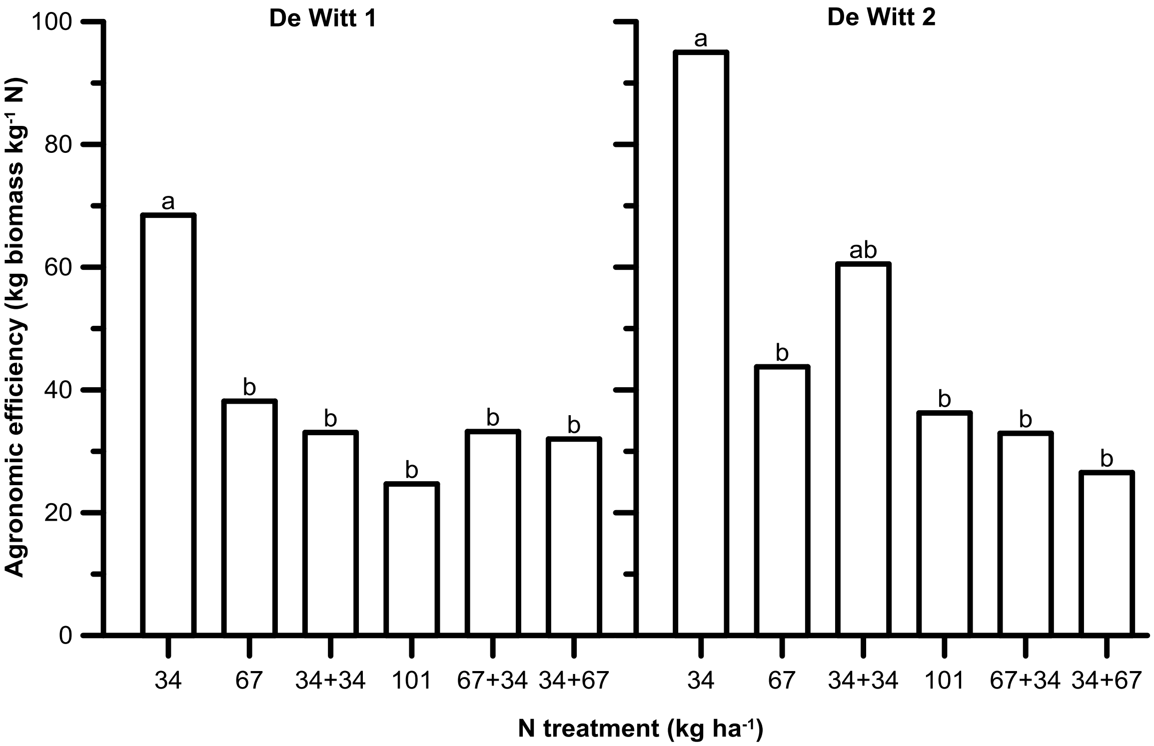

Figure 3.9 Agronomic efficiency under each N treatment at De Witt 1 and 2 in 2015. Columns with the same letter are not significantly different at $P < 0.05$.

CHAPTER 4: NITROGEN AND HARVEST IMPACT ON WARM-SEASON GRASS BIOENERGY QUALITY

Abstract

Producing energy using plant biomass is a viable alternative that can help alleviate negative environmental externalities and promote independence from fossil fuels. Switchgrass (*Panicum virgatum* L.), big bluestem (*Andropogon geradii Vitman*), and Indiangrass (*Sorghastrum nutans* L.) are native perennial warm-season grasses that that have been considered for bioenergy feedstocks. Nitrogen (N) fertility and harvest timing decisions for these grasses are critical agronomic management practices that affect biomass yield, feedstock quality, and overall sustainability. The objective of this research was to quantify the effects of N fertilizer rate and timing and harvest management strategies on warm-season grass biomass feedstock quality. This study was conducted in 2014 and 2015 on a total of four field-plot locations located in central and west-central Missouri. Nitrogen fertilizer was applied using dry ammonium nitrate at the rates of 0, 34, 67, and 101 kg ha^{-1} at two application times, all N early spring and split N (early spring and following 1st harvest). Harvest treatments were as follows: 1) one cut in September; 2) one cut in November; and 3) one cut in June and a second in November. A randomized complete bock design with a split-plot treatment arrangement was used with N rate and timing as the main plot and harvest strategy as the sub-plot. A broad-based near infrared reflectance spectroscopy (NIRS) calibration was used to measure the characteristics of the biomass feedstock quality. Delaying harvesting until November resulted in overall high quality biomass with higher energy concentration, ethanol yield, and lower total N and ash contents compared to harvesting in June and September. Two harvests and one harvest per year harvest regimes in combination with higher N inputs

greater than 67 kg ha^{-1} $year^{-1}$ resulted in higher energy yields and ethanol productions per unit land area at each site compared to single harvest in September. Results of this study exhibit the significance of interactions of N fertility and harvest timing and frequency on the quality of the biomass and the quantity of the energy and ethanol yield per unit area of land.

List of acronyms: N, Nitrogen; NIRS, near infrared reflectance spectroscopy

Introduction

Humans have used biomass an energy source since early history. However, at present, biomass has been also used as the raw material/feedstock for bioenergy, a promising energy alternative that is capable of lowering negative environmental externalities of the use of fossil fuels while contributing towards economic growth of rural communities (Goldemberg 2000; Goldemberg et al., 2004; Bardhan et al., 2015; Williams 2015). Forests, agriculture, and organic-based wastes are the major sources of bioenergy feedstocks and these feedstocks are consist of the following main three types of biomass constituents; lipids, sugars/starches, and cellulose/lignocellulose (Williams 2015). Sugar and thermochemical conversion approaches are the two basic processes in which biomass is being converted to energy or energy sources. In short, sugar conversion mainly uses feedstocks including cereal grains and herbaceous lignocellulosic materials (grasses and crop residues) for ethanol production via saccharification and fermentation processes. Direct combustion and thermal degradation/pyrolysis of biomass for obtaining energy and bio-oils fall under thermal conversion (Sanderson et al., 2006).

Each of these biofuel conversion approaches has different feedstock quality requirements (Adler et al., 2006). For example, high biomass mineral concentration is detrimental to thermal conversion systems since it is potent of causing corrosion, slagging, and fouling of boilers (Demirbas, 2004; Fahmi et al., 2007). Furthermore, in sugar conversion systems, the composition of long-chain and complex C compounds affect ethanol yield from biomass (Weimer et al., 2005; Dien et al., 2006). Development of rapid, inexpensive, and high accuracy methods such as near infrared reflectance spectroscopy (NIRS) for assessing feedstock quality is vital for agronomists, plant breeders, commercial feedstock producers, and biorefinery facility professionals (Adler et al., 2006; Vogel et al., 2011) since such methods provide an opportunity to quickly evaluate the feedstock in order to optimize the process of biomass processing.

The United States government made significant legislative and investment efforts for bioenergy starting in the early 2000's, attempting to overcome some major issues associated with fossil fuels. Price volatility, uncertainties of supply driven by finite resources, and issues related to energy security were some of the stated justifications (U.S. Department of Energy, 2011). National goals included a mandate of 21 billion gallons of second generation biofuels per year as established by the Energy Independence and Security Act of 2007 (U.S. Department of Energy, 2011, Wilson et al. 2013) and setting up a national goal for biomass to supply 25% of the national transportation fuels by 2030 by the Biomass Research and Development Technical Advisory Committee (Sanderson et al., 2006).

Native perennial warm-season grasses including switchgrass (*Panicum virgatum* L.), Indian grass (*Sorghastrum nutans* L.), and big bluestem (*Andropogon geradii Vitman*) offer multiple benefits over other plant species that have been tested as dedicated bioenergy crops (McLaughlin et al., 2002; Roth et al., 2005). Among the benefits of these bioenergy crops include reliable productivity without competing for arable lands used for food and fiber production, minimal use of agricultural inputs, water quality improvement, soil carbon (C) sequestration, soil conservation, and provision of wildlife habitat (McLaughlin and Walsh, 1998; McLaughlin et al., 2002; Lee et al., 2003; Simpson et al., 2008; Margeot et al., 2009).

Nitrogen fertility and harvest strategy management decisions are among the most significant agronomic practices affecting yield and biomass quality in warm-season grass bioenergy feedstock production systems. These practices affect the productivity, longevity, and both economic and environmental sustainability of these systems (Waramit et al., 2011; Sadeghpour et al., 2013; Seepaul et al., 2014). For example, in addition to the yield potential and productivity of the cultivar, harvest schedule is a determinant of annual N fertilizer requirements in switchgrass biomass feedstock production systems (McLaughlin et al., 1999, Mitchell et al., 2008, Anderson et al., 2013). Moreover, lower harvest frequencies resulted in improved feedstock quality due to increased cell wall constituent concentrations while reducing nutrient removal (Seepaul et al., 2014).

To date, only a few research studies have been conducted to investigate the simultaneous effects of N fertilizer rates and timing and harvesting management in terms of timing and frequency on warm-season grass biomass feedstock quantity and quality. Therefore, the objective of this research was to quantify the effects of N and harvest management strategies and their interactions on feedstock quality of warm-season biomass grasses.

Materials and Methods

Study Sites

The research was conducted during the years 2014 and 2015 in Missouri, USA. In 2014, the study was conducted at four field sites located in Gallatin, De Witt, Strasburg, and Green Ridge. In 2015, five sites were used with two sites in both De Witt and Strasburg and one site in Green Ridge (Figure 4.1). All these sites have been considered as marginal lands due to the poor drainage and high risk for soil erosion (Table 4.1). The effect of site on this study is confounded by differences in both soil and grass specie composition, as shown in Table 4.1. Thirty-year average precipitation and monthly values received during the experimental period were obtained from the nearest National Weather Service station to represent each site from the Utah State University (2016), and are graphically represented in Figure 4.2.

Experimental Design and Management Practices

Treatments were arranged as split-plots within a randomized complete block design with three replicates, with N as the main plot and harvest strategy as sub-plot treatments. Ammonium nitrate (NH_4NO_3) was used as the N source and applied in three rates, which were applied in May at 34, 67, and 101 kg ha^{-1} year^{-1}. Furthermore, 67, and

101 kg ha^{-1} $year^{-1}$ treatments were also applied as split applications (67 kg ha^{-1} $year^{-1}$, 0.5 May + 0.5 June split application; 101 kg ha^{-1} $year^{-1}$, 0.67 May + 0.33 June split application, and 0.33 May + 0.67 June split application) (Table 2). Nitrogen fertilizer was pre-weighed and hand-broadcasted uniformly on the soil surface. One treatment had no N fertilizer and was considered the N control (Table 4.2).

There were three harvest timing treatments (Table 4.2) with one two-cut and two one-cut harvests which include both timing and frequency aspects of harvesting management. Two-cut harvest treatment consisted with the first cut in mid- to late June and the second cut in November.

Sampling and Data Collection

A 0.7-m swath of grass was harvested from each plot using a sickle-bar mower (BCS model 710, BCS America, Portland, OR) leaving a 12 ± 3 cm stubble height. Then biomass samples were dried in forced-air ovens at 65°C for 72 hours. Dried samples were ground in a Wiley mill to pass a 1-mm screen. Ground samples were scanned using a Model 6500 near-infrared (NIR) spectrometer (FOSS NIRSystems, Inc., Laurel, MD) for biomass energy content, forage quality composition (NDF, ADF, ADL, N, and ash) biomass carbohydrates, and actual and potential ethanol yield predictions using the broad-based calibrations developed by Vogel et al. (2011). The references for traditional analytical methods for each of these biomass feedstock quality parameters are given in Table 4.3.

Calculations and Statistical Analysis

The list of cell wall constituents, ethanol yield and production parameters with the equations used for calculating those parameters are given below. Statistical analysis of data was performed using GLIMMIX procedure of SAS (SAS Institute, 2011) to determine significant (α=0.05) treatment effects. Fixed effects were Site (i.e., soil and grass specie composition), N (rate and timing), harvest timing, and their interactions. Block, block by N treatments, and block by harvest treatment interactions were treated as random effects. Tukey's honest significant difference (HSD) test was used for mean separations when the fixed effects were significant at $\alpha \leq 0.05$ significance level. The SLICE option was used to break down the interactions when the effects of interactions of independent variables were significant.

List of cell wall constituents, ethanol yield and production parameters and equations developed by Vogel et al. (2011) for calculating them are given below.

1. Biomass cellulose content (mg g^{-1}):

$$\text{Cellulose (mg g}^{-1}) = \text{ADF} - \text{ADL}$$

2. Biomass hemicellulose content (mg g^{-1}):

$$\text{Hemiellulose (mg g}^{-1}) = \text{NDF} - \text{ADF}$$

3. Ethanol yield from simultaneous saccharification and fermentation (SSF) released glucose from biomass:

$$\text{ETOHL (L Mg}^{-1}) = \text{ETOH} \times 1.267$$

4. Theoretical ethanol yield from all biomass hexoses:

$$\mathrm{HEXEL\ (L\ Mg^{-1})} = \Big(\big((\mathrm{MAN} + \mathrm{GAL} + \mathrm{GLC} + \mathit{S}\mathrm{TA}) \times 0.57\big) + \big((\mathrm{GLCS} + \mathrm{FRU}) \times 0.51\big) + (\mathit{S}\mathrm{UC} \times 0.537)\Big) \times 1.267$$

Assumption: Conversion of sugars is 100%.

5. Ethanol yield from SSF released pentose sugars:

$$\mathrm{PENTEL\ (L\ Mg^{-1})} = \mathrm{PENT} \times 0.51 \times 1.267 \times 0.8$$

Assumption: Conversion of sugars is 80%.

6. Theoretical ethanol yield from pentose sugars:

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

7. Total ethanol yield (ETOHTL (L Mg^{-1})) from SSF:

$$\mathrm{ETOHTL\ (L\ Mg^{-1})} = \mathrm{ETOHL} + \mathrm{PENTEL}$$

8. Total theoretical ethanol yield (ETOHTLT (L Mg^{-1})) from all biomass sugars:

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

9. Total ethanol production (ETOHTLH (L ha^{-1})) from SSF:

$$\mathrm{ETOHTLH\ (L\ ha^{-1})} = \mathrm{ETOHTL} \times \mathrm{biomass\ production\ (Mg\ ha^{-1})}$$

10. Total theoretical ethanol production (ETOHTLTH (L ha^{-1})) from all biomass sugars:

$$\mathrm{ETOHTLTH\ (L\ ha^{-1})} = \mathrm{ETOHTLT} \times \mathrm{biomass\ production\ (Mg\ ha^{-1})}$$

Results and Discussion

All the biomass feedstock quality parameters were affected by the site, N fertility management, and harvest timing practices (Table 4.4-4.7). Harvest timing and site impacted all the feedstock quality parameters in both years. Biomass hemicellulose content was the only biomass feedstock quality parameter that was not influenced by their interactions. Furthermore, N fertility management influenced the majority of these feedstock quality parameters as an individual factor, as well as interacting with harvest timing strategies and sites.

Energy Content of Biomass and Energy Yield

Energy content (High Heating Value (HHV)) of biomass is a reflection of the maximum amount of energy potentially recoverable from a unit weight of given biomass source (McKendry, 2002). Energy yield (GJ ha^{-1}) is calculated by multiplying HHV of biomass by the dry matter yield of the feedstock per unit land area.

Both biomass energy content/concentration per unit mass of biomass and energy yield per unit land area were influenced by site and N and harvest management strategies and their interactions (Table 4.4). Nitrogen interactions with harvest and site affected biomass energy content in 2015 (Figure 4.3). In both 2014 and 2015, harvest timing by site interaction influenced energy content of the biomass (Figure 4.4; Figure 4.5). However, related to energy yield per unit land area, it was influenced by N by harvest interaction in 2015 (Figure 4.6) and N by site and harvest by site interactions in 2014 and 2015 (Figure 4.7; Figure 4.8).

Energy content

Biomass energy content did not exhibit much variability under different N management strategies when biomass was harvested in June, September, and November as the second harvest of two-cut system (Figure 4.3). However, the highest energy concentration (17.35 MJ kg^{-1}) was observed with one-cut November with split application of N as 67+34 kg ha^{-1} and it was significantly greater compared to both N control (17.26 MJ kg^{-1}) and one-time application of 67 kg N ha^{-1} (17.25 MJ kg^{-1}). Furthermore, harvesting of biomass once a year in November gave higher biomass energy content values (17.25-17.35 MJ kg^{-1}) under each N management strategy. The lowest range of energy content values (17.17-17.22 MJ kg^{-1}) was reported with September harvested biomass. Energy concentration of biomass also responded differently to N by site in 2015 (Figure 4.4). Only the two De Witt sites showed biomass energy content differences among N management strategies. For De Witt 1 and De Witt 2 the lowest biomass energy concentrations (17.15 and 17.17 MJ kg^{-1}) was observed with 34 kg N ha^{-1} and 101 kg N ha^{-1} N strategies, respectively. The highest biomass energy content values (17.25 and 17.26 MJ kg^{-1}) for these same two sites were observed with 67+34 and 34 kg N ha^{-1}, respectively. Overall these two sites exhibited lower biomass energy content values under each N management strategy.

Biomass energy content was affected by the interactive effects of harvest timing and site in both years. In 2014 at each site, biomass harvested early in the growing season gave higher biomass energy content values while the lowest values were observed with second harvest biomass of the two-cut harvest system. In contrast, in 2015 late harvested biomass produced higher energy content values at two sites in De Witt and Green Ridge where the stand was composed of a mix of grass species (Table 4.1). In contrast, at two sites in Strasburg where switchgrass was as a monoculture the biomass harvested early in the growing season produced higher energy content values.

Overall, the energy content values obtained in this research fell within the range of previously reported values (17-17.4 MJ kg^{-1}; McKendry, 2002; Lewandowski et al.,

2003) According to the results of this study, no clear patterns in the variation of biomass energy content values in response to the N and harvest interactions were observed. Further studies would be needed to identify such variations. The energy content variations observed under the effects of interactions between harvest timing and site can in part be attributed to the plant compositions at each site (Table 1), which specie composition will impact major cell wall constituents, as well as the weather conditions that prevailed at each site (Figure 4.2). For example, Waramit et al, (2011) reported that big bluestem had higher contents of cellulose and lower contents of ash compared to switchgrass which contained higher lignin contents in biomass which potentially affect biomass energy contents.

Energy yield

In 2015 the impact of N management on energy yield of warm-season grasses differed by harvest strategy (Figure 4.6). Increasing of energy yield was observed with incremental N inputs under each harvest strategy. In both years, energy yield was impacted by N differently by site (Figure 4.7). Further, energy yield over both years was not the same by harvest strategy by site (Figure 4.8).

In general, over most N management, harvest yield increased in the order of September < June+November ~ November, however exceptions occurred. For example, November harvest with 101 kg ha^{-1} at planting out-yielded the same N application with the June+Nov harvest. Under two-harvests per year harvest strategy, all N application strategies that supplement N at 101 kg ha^{-1} gave significantly higher energy yields (175-180 GJ ha^{-1}) compared to both control and 34 kg N ha^{-1} strategies (97 and136 GJ ha^{-1} respectively). That is over 80% increase in energy yield compared to control.

Furthermore, in this two-cut harvest strategy, percent contribution of regrowth harvest towards the total energy yield was 53-66%. The highest energy yield value (204 GJ ha^{-1}) was recorded with one-time application of N at 101 kg ha^{-1} when biomass was harvested one time in November. It was greater compared to energy yield recorded under no N (105 GJ ha^{-1}), 34 kg ha^{-1} (156 GJ ha^{-1}), and 34+34 Kg ha-1 (163 GJ ha^{-1}). The minimum percent increase in energy yield with N supplementation was 33%. Application of N at 67 kg ha^{-1} or beyond produced significantly greater energy yields compared to control and the percent energy yield increase was ≥53%.

Harvesting biomass in September resulted in lower energy yield values under each N management strategy compared to both two-cut and November harvest strategies (Figure 4.6). In addition, the energy yields were improved by at least by 21% under each N management strategy by delaying harvesting until late growing season. The maximum percent increase (48%) due to delayed harvesting until a killing frost or November from September was recorded when N was applied at 101 kg ha^{-1}. The interpretation of these results would suggest that optimal bioenergy yields will be obtained when harvesting in November alone or with June + November, with a minimum of 67 kg N ha^{-1}, and that split N application does not improve energy yield.

Supplementing N resulted in improved biomass energy yields at each site during both years (except Gallatin in 2014) (Figure 4.7). However, in 2014 De Witt 1 was the only site at which there was no significant improvement in energy yield between N rates 0 and 34 kg N ha^{-1}. In 2015, Green Ridge and both sites in Strasburg didn’t show significant energy yield improvements when comparing the N control with 34 kg N ha^{-1}. In 2014 Gallatin site produced the lowest energy yields (64-91 GJ ha^{-1}) under each N management strategy. De Witt 1 exhibited lower energy yield values (66-130 GJ ha^{-1}) under each N strategy compared to other sites in 2015.

In both years the two-cut harvest and November harvest strategies produced higher energy yield compared to September harvesting (Exception Gallatin in 2014) (Figure 4.8). Furthermore, harvesting biomass as a single harvest in November resulted in significantly greater energy yields compared to two-harvests per year harvesting strategy at De Witt 1 in 2014 and De Witt 2 in 2015. The contribution of the regrowth harvest towards the total energy yield was 59-72% in 2014 and 61-67% in 2015. Under each harvest management strategy in 2014 and 2015, Gallatin and De Witt 1 respectively gave the lowest energy yields.

Since energy yield is a function of biomass energy concentration/high heating value and biomass yield, higher energy yields were observed especially with N and harvest management strategies which gave both higher energy concentrations and biomass yields. However, the amount of energy consumed by each N and harvest management scenarios have to be taken into account for maximizing the net energy output of warm-season grass bioenergy production systems. Therefore, one-time application of N at the rates between 67-101 kg ha^{-1} while harvesting the biomass as one harvest per growing season will be viable in the means of energy balances since significant amounts of energy should be spent on split application of fertilizer inputs and harvesting biomass twice per growing season.

Total Ethanol Yield from SSF

Total ethanol yield from SSF (ETOHTL) was affected by N, time of harvest, site, and time of harvest by site interactions in both years (Table 4.5). Furthermore, around

70% of ETOHTL consisted of ETOHL and the remainder with PENTEL (Figure 4.9; Figure 4.10). In both years the highest value of ETOHTL (146 L Mg^{-1} biomass in 2014 and 138 L Mg^{-1} biomass) was observed with control N treatment. Furthermore, in 2014, 101 and 67+34 kg N ha^{-1} N management strategies gave significantly lower ETOHTL values (137 and 136 L Mg^{-1} biomass respectively). In 2015, application of N at the rates equal or greater than 67 kg ha^{-1} per growing season gave significantly lower ETOHTL values (130-132 L Mg^{-1} biomass). This observation of lower ETOHTL with the increasing N inputs is likely attributable to the increased lignin content in the biomass which limits the biodegradability of biomass in the biochemical processing (SSF) (McKendry, 2002).

When considering time of harvest by site interactions in 2014 and 2015, the variation of ETOHTL followed the same trend across each site (Fig. 4.10). June harvested biomass gave the highest ETOHTL while November harvested biomass gave the lowest. This variation of ETOHTL can be attributed to the higher availability of soluble sugars and carbohydrates in early harvested biomass compared to late harvested biomass in which the cell wall concentrations are greater (Vogel et al., 2002; Vogel et al., 2011). In spite of this trend, in 2015 at Strasburg-2, June, September, and regrowth harvest biomass gave ETOHTL values that were not statistically significant to each other.

Total Ethanol Production from SSF

Total ethanol production (ETOHTLH) was impacted by N and harvest timing management, site, and the interactions of site with N and harvest strategies. Furthermore, in 2015, total ethanol production was influenced by N by harvest interactions (Table 4.6).

Higher ETOHTLH values were observed with two-cut harvest strategy at each site in each year (Figure 4.11). The regrowth harvest of two-harvests per year strategy contributed by more than 50% to the total ethanol production. Moreover, harvesting biomass in November gave the next competitive ETOHTLH while September harvest gave the lowest. All the sites except Gallatin gave ethanol production ≥ 750 L ha^{-1} under each harvest strategy, with the majority producing ≥ 1000 L ha^{-1} during each year.

Total ethanol production (ETOHTLH) values at each site (except Gallatin) were higher with increasing N inputs (Figure 4.12). Double harvests per growing season generally gave higher ETOHTLH values under each N management strategy (820-1470 L ha^{-1}). A single harvest in November with 101 kg N ha^{-1} exhibited ETOHTLH values comparable to double harvest system, while single harvest in September gave lower values (620-1060 L ha^{-1}) (Figure 4.13).

Total Nitrogen

Total N content of biomass was affected by N fertilizer rate, time of harvest, harvest by N, and harvest by site interactions (Table 4.7; Figure 4.14; Figure 4.15). Related to N by harvest (Figure 4.14) and harvest by site (Figure 4.15), highest and lowest biomass N contents were observed with June and November harvested biomass respectively.

Variability of biomass N content in both years was only apparent when the biomass was harvested in June (Figure 4.14). Among different N fertilizer management strategies, the highest biomass N contents were observed when N was applied at 101 kg ha^{-1} in both years and the lowest with the control. The lower values and less variability of biomass N concentrations associated with late harvested biomass can be attributed mainly to the dilution of plant N by greater amounts of C fixed within the plants in the form of cell wall concentration (Vogel et al., 2002). In addition, Vogel et al. (2002) and Adler at al. (2006) suggested that translocation of significant amounts of N from above ground components to belowground structures of the plants during the late growing season may also resulted in lower biomass N concentrations in late harvested biomass allowing efficient recycling of N during subsequent growing seasons and thereby reducing annual N input requirements.

Considering how harvest timing in 2015 differed by site, both sites in De Witt exhibited higher biomass N concentrations with June harvest compared to other sites (Figure 4.15). However, N contents in regrowth harvest of two-cut system and September harvested biomass in two sites in De Witt were significantly lower compared to other three sites. In addition, De Witt 1 exhibited the lowest biomass N concentration when biomass was harvested in November. Guretzky et al. (2011) reported location effects on switchgrass biomass N concentrations and they attributed the effects of location on biomass N concentration partly to the differences in total rainfall and inherent physical and chemical characteristics of soils. In addition to these factors, biomass N concentrations was likely affected by grass species composition differences by site, as previously described.

Ash Content

Time of harvest of biomass, site, and time of harvest by site interactions affected ash content of biomass in both years. Further, interactions of N by time of harvest affected biomass ash content in 2015 (Table 4.8; Figure 4.16; Figure 4.17). For most sites in 2014 regrowth harvest of the two-harvest strategy generally gave the highest ash content, while the June harvest often produced the lowest ash content. However, in 2015 for the De Witt sites, the highest and lowest ash contents were observed with June and November harvested biomass respectively. Furthermore, in 2015 biomass from regrowth harvest and September harvested biomass exhibited highest and lowest ash contents, respectively, at the remaining three sites. Similarly, according to the N by harvest interactions effects in 2015, biomass from the regrowth harvest exhibited higher ash contents compared to both September and November harvested biomass.

Conclusions

Nitrogen fertility and harvest timing management strategies impacted warm season biomass feedstock quality parameters individually and in interactions. In addition, different grass species compositions of the experimental sites interacted with both N and harvest timing management practices and thereby caused significant differences in feedstock quality parameters. Better feedstock quality parameter values including lower nutrient contents (N and ash), higher energy contents and yields, and higher ethanol yields and production were observed when switchgrass was present in the grass mixtures.

Single late harvest after a killing frost resulted in better quality grass biomass under each N management and site conditions. In short, the biomass harvested late in the growing season had higher values of favorable quality characteristics including energy contents, cell wall constituents, and ethanol yield parameters. At the same time, late harvested biomass exhibited lower biomass N and ash contents which can negatively affect sustainability and longevity of biomass production and biomass energy conversion systems. Among harvest frequency practices, both single harvest and double harvests per growing season with a November harvest, in combination with annual N inputs which are equal or greater than 67 kg ha^{-1} gave higher energy yield and ethanol production per unit area of land.

However, overall sustainability of the biomass production and bioenergy conversion systems depends upon the net balances of carbon, energy, and economic returns. Therefore, conducting life cycle analysis studies is necessary in determining ideal combinations of agronomic management practices including N fertility and harvesting. Use of NIRS techniques for biomass feedstock quality analyses offer significant information that can be used in such life cycle analyses.

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Table 4.1. Field site locations, years of operation, soil classification, and grass composition descriptions.

Site	Year	Soil	Landscape Position, slope, and Capability Class	Grass Composition
De Witt-1 (39° 22' N, 93° 17' W)	2014/2015	Nodaway silt loam (Fine-silty, mixed, superactive, nonacid, mesic Mollic Udifluvents)	Foot slope, 0-5%, 3-w	Indian grass/ Big bluestem
De Witt-2 (39° 22' N, 93° 17' W)	2015	Wakenda silt loam (Fine-silty, mixed, superactive, mesic Typic Argiudolls)	Summit, 2-9%, 3-e	Indian grass/ Big bluestem/ Switchgrass
Gallatin (39° 51' N, 93° 58' W)	2014	Mandeville silt loam (Fine-loamy, mixed, superactive, mesic Typic Hapludalfs)	Side slope, 2-30%, 3-e	Big bluestem
Green Ridge (38° 36' N, 93° 21' W)	2014/2015	Hartwell silt loam (Fine, mixed, active, thermic Typic Argialbolls)	Summit, 0-5%, 2-w	Switchgrass/ Indian grass
Strasburg-1 (38° 45' N, 94° 9' W)	2014/2015	Haig silt loam (Fine, smectitic, mesic Vertic Argiaquolls)	Summit, 0-2%, 2-w	Switchgrass
Strasburg-2 (38° 45' N, 94° 9' W)	2015	Sampsel silty clay loam (Fine, smectitic, mesic Vertic Argiaquolls)	Side slope, 2-14%, 3-e	Switchgrass

Table 4.2. Summary of the N and harvest treatments implemented in the research.

N/main-plot treatments				Harvest/sub-plot treatments		
	Time of application				Time of harvest	
N trt ID	May	June	Total inorganic N/year	Harvest trt ID	First cut	Second cut
	------------------ kg N ha^{-1} ------------------					
0	0	0	0	S	September	-
				J/N	June	November
				N	November	-
34	34	0	34	S	September	-
				J/N	June	November
				N	November	-
67	67	0	67	S	September	-
				J/N	June	November
				N	November	-
34+34	34	34	67	S	September	-
				J/N	June	November
				N	November	-
101	101	0	101	S	September	-
				J/N	June	November
				N	November	-
67+34	67	34	101	S	September	-
				J/N	June	November
				N	November	-
34+67	34	67	101	S	September	-
				J/N	June	November
				N	November	-

Table 4.3. Biomass composition (forage quality, carbohydrates) and ethanol yield traits used in calculation of feedstock quality parameters and references for the traditional analytical procedures.

Variable	Abbreviation	Reference
----------------------- Forage quality composition (g kg^{-1}) -----------------------		
Neutral detergent fiber	NDF	Vogel et al. (1999)
Acid detergent fiber	ADF	Vogel et al. (1999)
Acid detergent lignin	ADL	ANKOM Technology-9/99
Nitrogen	N	Watson and Isaac (1990)
Minerals (Total ash)	-	450 ^{0}C muffle furnace for 6 hours.
----------------------- Biomass carbohydrates (g kg^{-1}) -----------------------		
Arabinose	ARA	Theander et al. (1995)
Xylose	XYL	Theander et al. (1995)
Mannose	MAN	Theander et al. (1995)
Galactose	GAL	Theander et al. (1995)
Glucose	GLC	Theander et al. (1995)
Soluble glucose	GLCS	Dien et al. (2006)
Fructose	FRU	Dien et al. (2006)
Sucrose	SUC	Dien et al. (2006)
----------------------- Actual and potential ethanol (g kg^{-1}) -----------------------		
Ethanol/g dry forage	ETOH	Dowe and McMillan (2001) and Dien et al. (2004)
Pentose sugars released/g dry forage	PENT	Dowe and McMillan (2001) and Dien et al. (2004)

Table 4.4. Fixed effects (P<F) of nitrogen, harvest timing, site, and their interactions on biomass energy concentration (High heating value (HHV)) and energy yield.

Source of Variation	Year 2014	 2015
--------------------High heating value (MJ kg^{-1} biomass) --------------------		
N	0.17	0.2367
Harvest	<0.0001	0.0007
Site	<0.0001	<0.0001
N x Harvest	0.93	0.02
N x Site	0.24	0.04
Harvest x Site	<0.0001	<0.0001
N x Harvest x Site	0.99	0.31
--------------------Energy yield (GJ ha^{-1}) --------------------		
N	<0.0001	<0.0001
Harvest	0.002	0.0006
Site	<0.0001	<0.0001
N x Harvest	0.12	0.04
N x Site	0.002	0.0004
Harvest x Site	0.0004	0.013
N x Harvest x Site	0.67	0.96

Table 4.5. Fixed effects (P<F) of nitrogen, harvest, site, and their interactions on total ethanol yield from simultaneous saccharification and fermentation (ETOHTL).

	Year	
Source of Variation	2014	2015
--------------------ETOHTL (L Mg^{-1} biomass) --------------------		
N	0.007	0.002
Harvest	<0.0001	<0.0001
Site	<0.0001	<0.0001
N x Harvest	0.56	0.15
N x Site	0.76	0.14
Harvest x Site	<0.0001	<0.0001
N x Harvest x Site	0.13	0.51

Table 4.6. Fixed effects (P<F) of nitrogen, harvest, site, and their interactions on total ethanol production from simultaneous saccharification and fermentation (ETOHTLH).

	Year	
Source of Variation	2014	2015
--------------------ETOHTLH (L ha^{-1} biomass) --------------------		
N	<0.0001	<0.0001
Harvest	<0.0001	<0.0001
Site	<0.0001	<0.0001
N x Harvest	0.47	0.03
N x Site	0.005	0.02
Harvest x Site	0.009	0.03
N x Harvest x Site	0.46	0.99

Table 4.7. Fixed effects (P<F) of nitrogen, harvest, site, and their interactions on total Nitrogen content.

Source of Variation	Year 2014	 2015
--------------------Total N (mg g^{-1} biomass) --------------------		
N	0.008	0.001
Harvest	<0.0001	<0.0001
Site	0.003	<0.0001
N x Harvest	<0.0001	<0.0001
N x Site	0.27	0.06
Harvest x Site	<0.0001	<0.0001
N x Harvest x Site	0.66	0.84

Table 4.8. Fixed effects (P<F) of nitrogen, harvest, site, and their interactions on total ash content.

Source of Variation	Year 2014	 2015
--------------------Ash (mg g^{-1} biomass) --------------------		
N	0.77	0.58
Harvest	<0.0001	<0.0001
Site	<0.0001	<0.0001
N x Harvest	0.77	0.03
N x Site	0.23	0.79
Harvest x Site	<0.0001	<0.0001
N x Harvest x Site	0.96	0.47

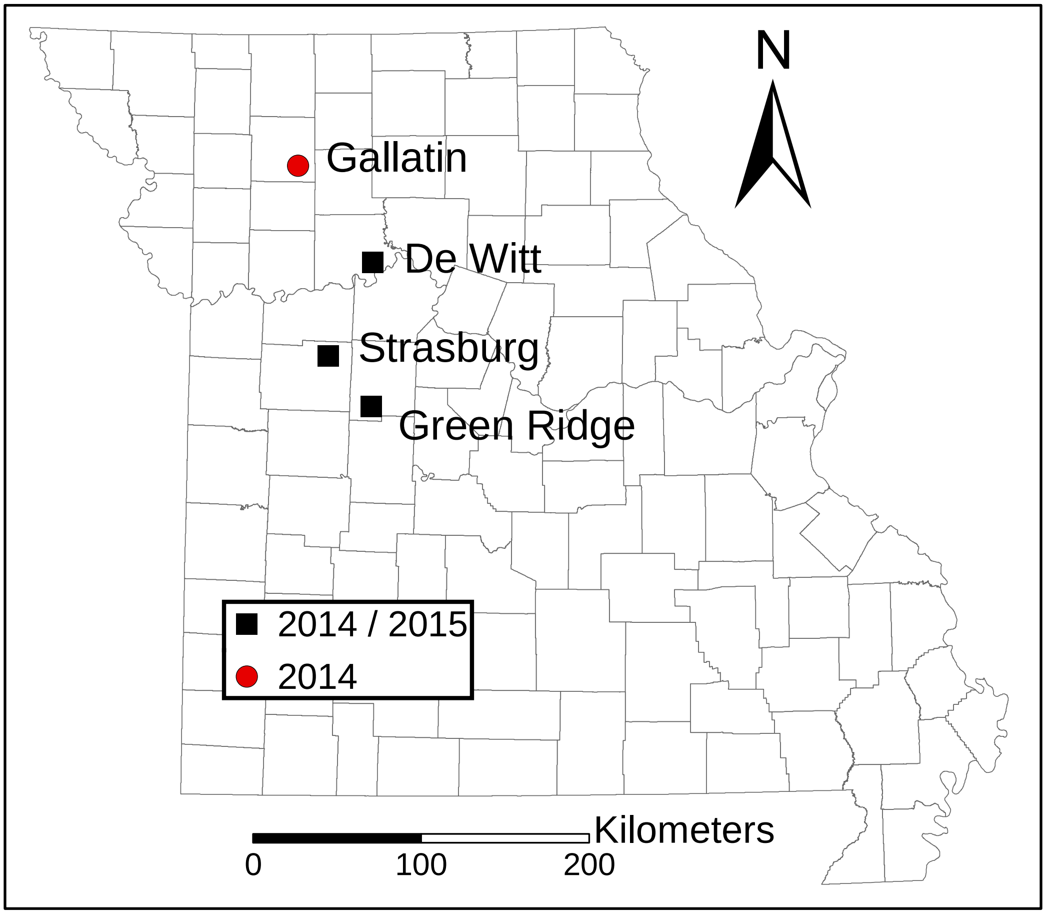


Figure 4.1 Location of the field sites and the years of operation.

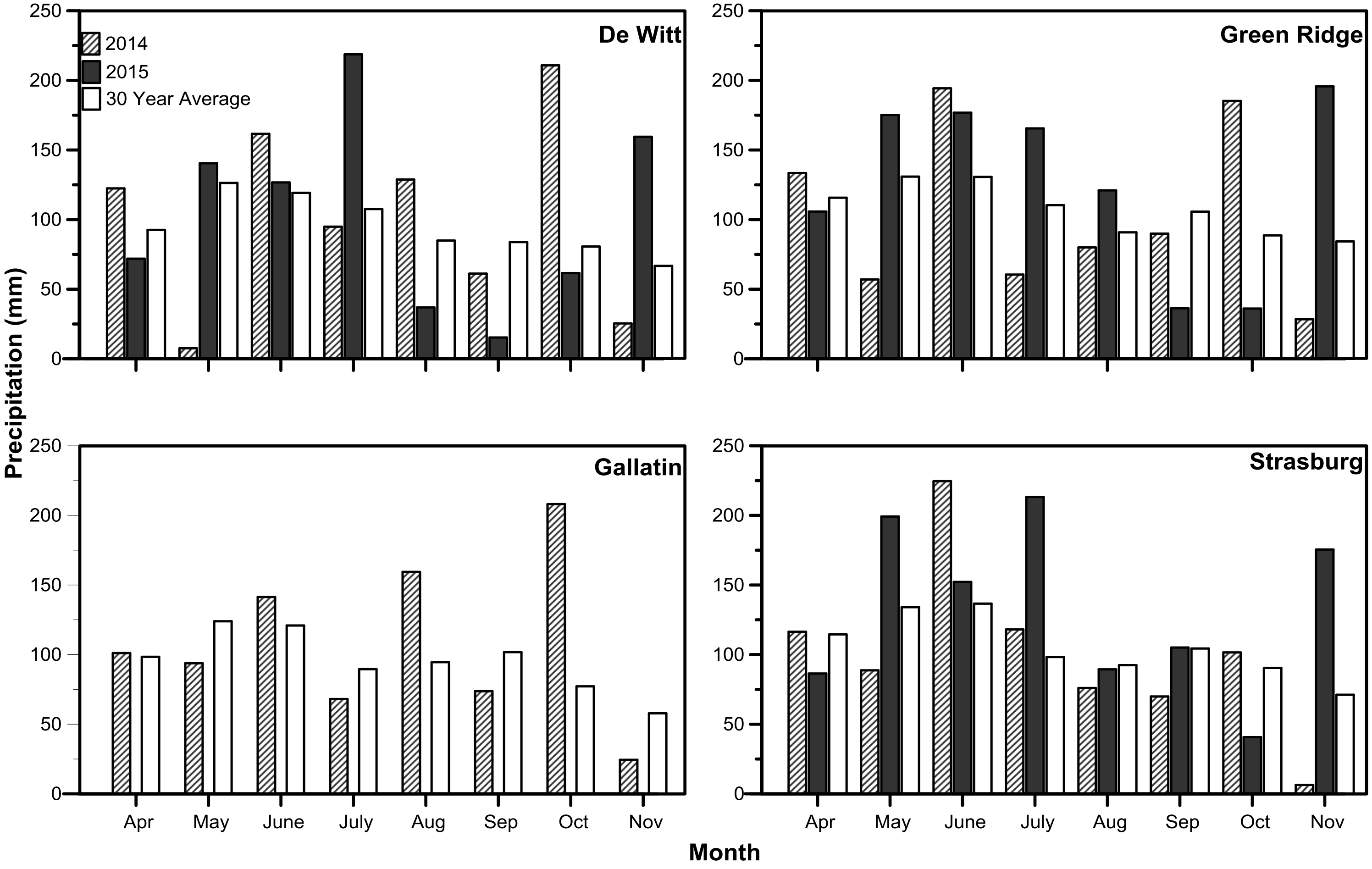


Figure 4.2 Monthly precipitation for study locations in 2014 and 2015 and 30-year long term average.

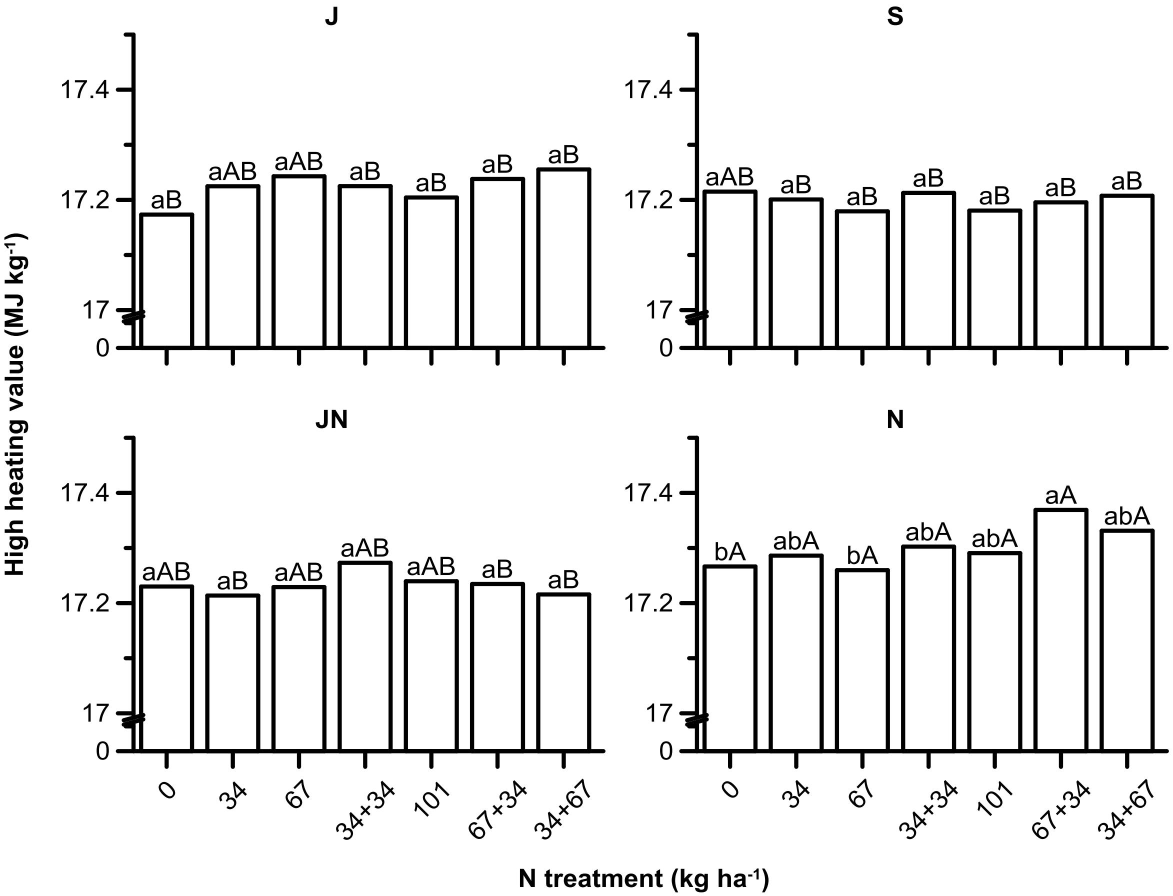


Figure 4.3 Interactive effects of harvest timing and nitrogen (N) on biomass energy content (high heating value (HHV)) in 2015. Harvest timing treatments included: J-June (first harvest of June+Nov harvest strategy); S-September; JN-regrowth harvest of June+Nov harvest strategy; and N-November. Columns with the same letter are not significantly different at $P < 0.05$. Lowercase letters denote differences between N treatments within each harvest timing strategy and uppercase letters denote differences between harvest timing treatments within each N treatment.

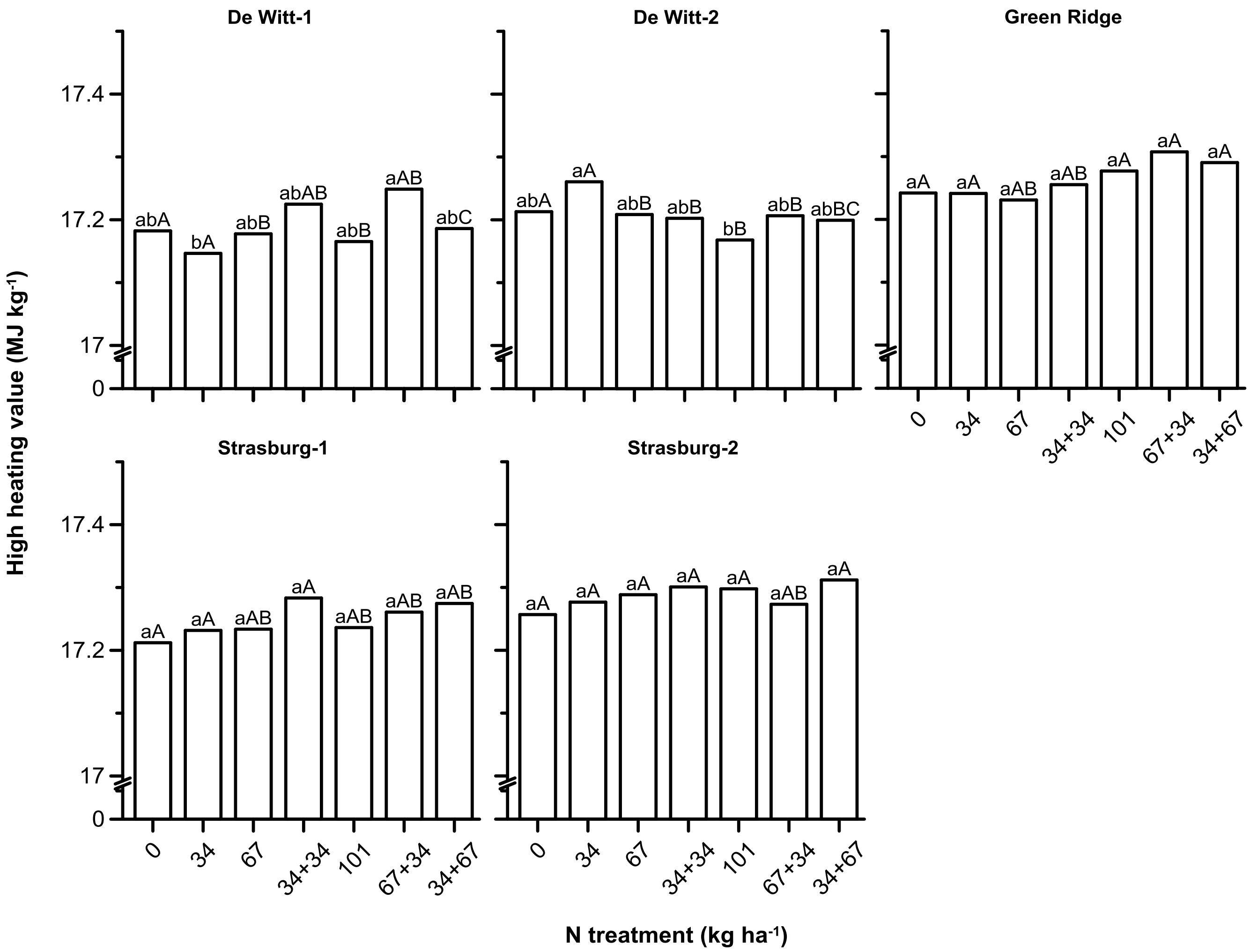


Figure 4.4 Interactive effects of nitrogen (N) management and site on biomass energy content in 2015. Columns with the same letter are not significantly different at $P < 0.05$. Lowercase letters denote differences between N treatments within each site and uppercase letters denote differences between sites within each N treatment.

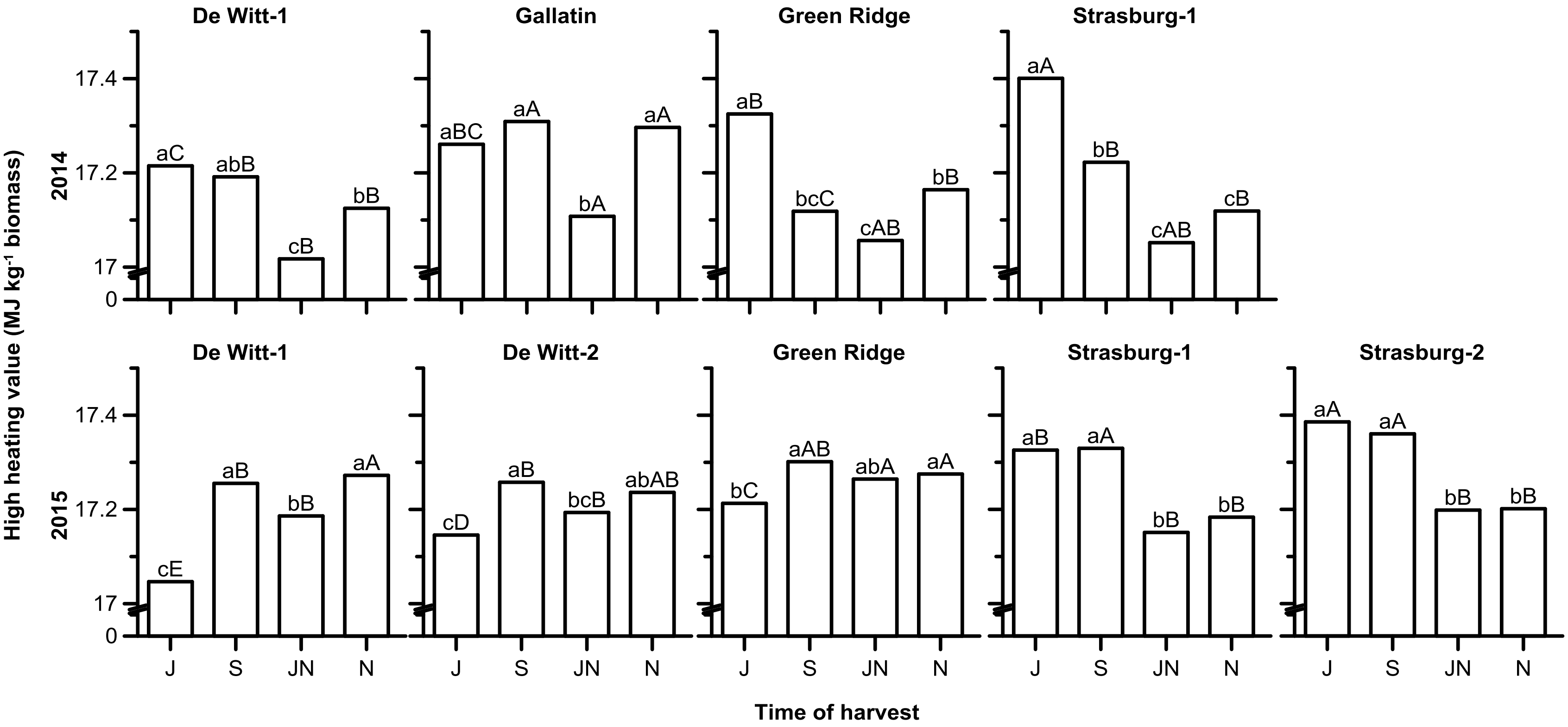


Figure 4.5 Interactive effects of harvest timing and site on biomass energy content in 2014 and 2015. Harvest timing treatments included: J-June (first harvest of June+Nov harvest strategy); S-September; JN-regrowth harvest of June+Nov harvest strategy; and N-November. Columns with the same letter are not significantly different at $P < 0.05$. Lowercase letters denote differences between harvest timing treatments within each site and uppercase letters denote differences between sites within each harvest timing treatment.

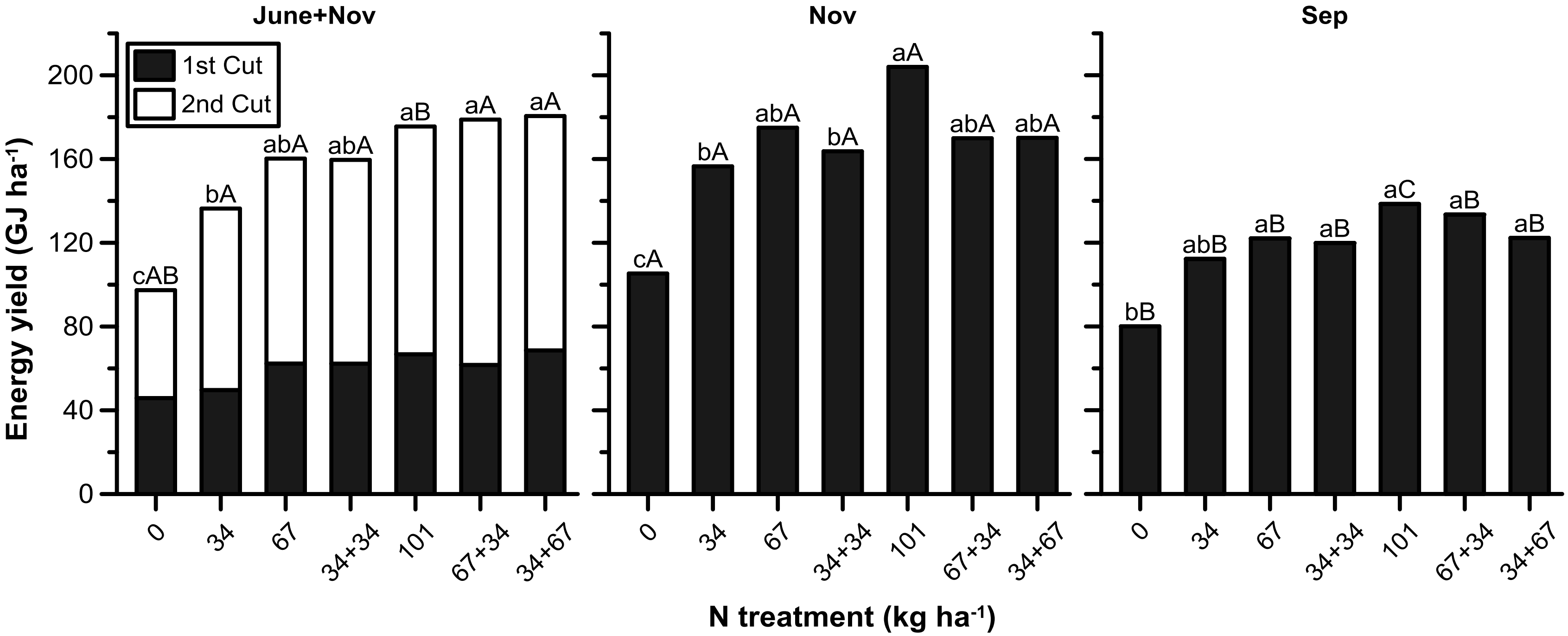


Figure 4.6 Interactive effects of harvest strategy and nitrogen (N) on energy yield in 2015. Columns with the same letter are not significantly different at P < 0.05. Lowercase letters denote differences between N treatments within each harvest strategy and uppercase letters denote differences between harvest strategies within each N treatment.

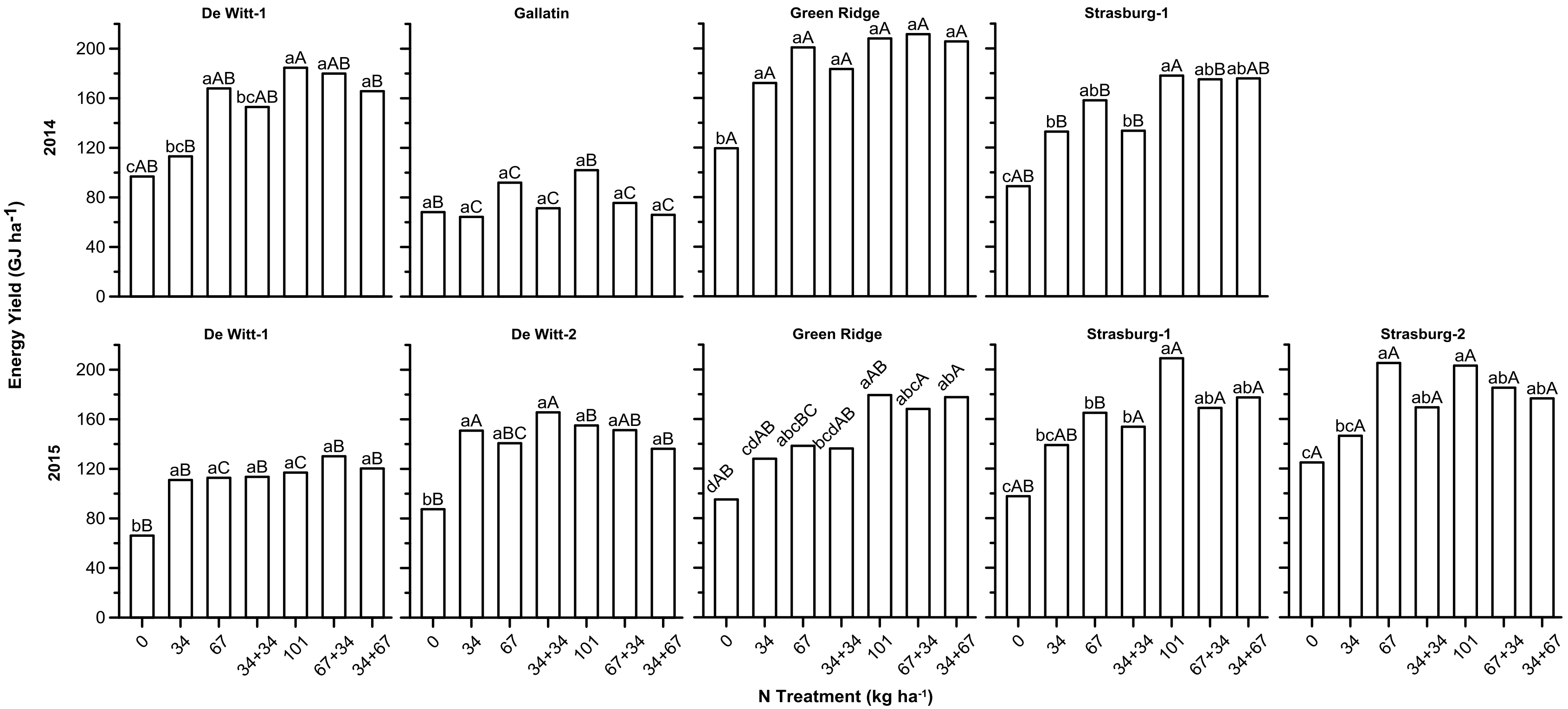


Figure 4.7 Interactive effects of nitrogen (N) management and site on energy yield in 2014 and 2015. Columns with the same letter are not significantly different at $P < 0.05$. Lowercase letters denote differences between N treatments within each site and uppercase letters denote differences between sites within each N treatment.

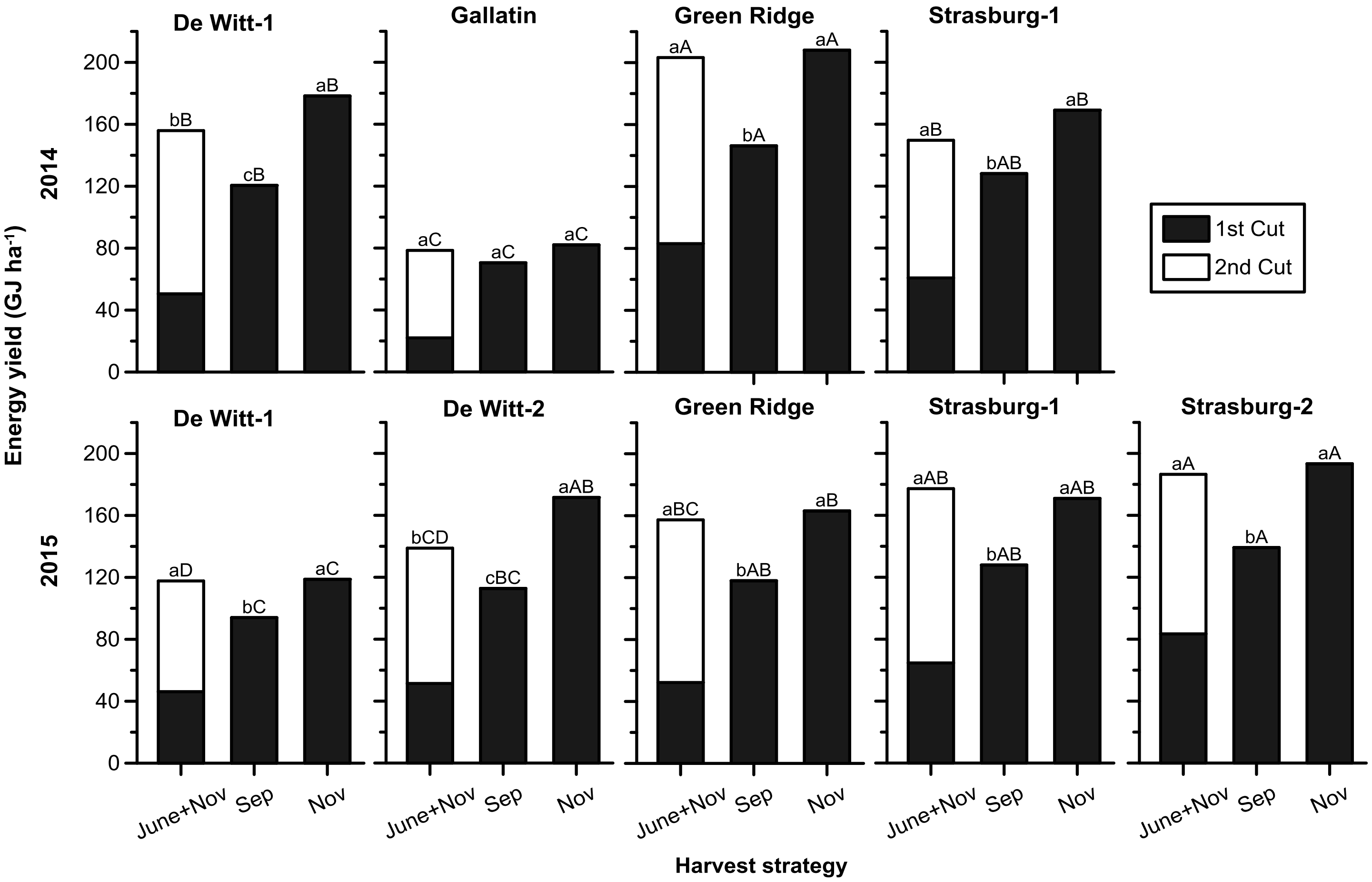


Figure 4.8 Interactive effects of harvest strategy and site on energy yield in 2014 and 2015. Columns with the same letter are not significantly different at $P < 0.05$. Lowercase letters denote differences between harvest strategies within each site and uppercase letters denote differences between sites within each harvest strategy.

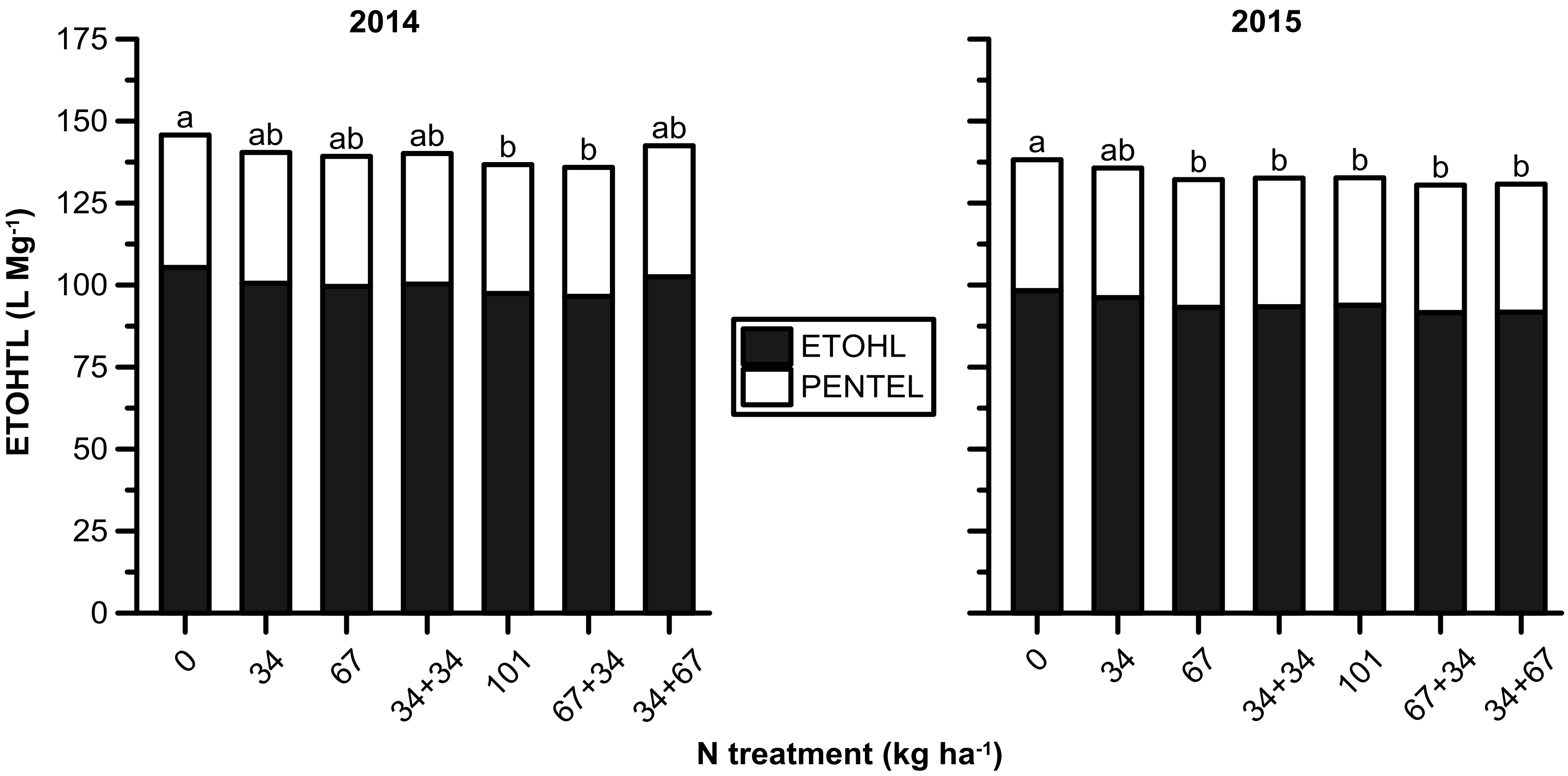


Figure 4.9 Effects of nitrogen management on total ethanol yield from simultaneous saccharification and fermentation in 2014 and 2015. Columns with the same letter are not significantly different at $P < 0.05$.

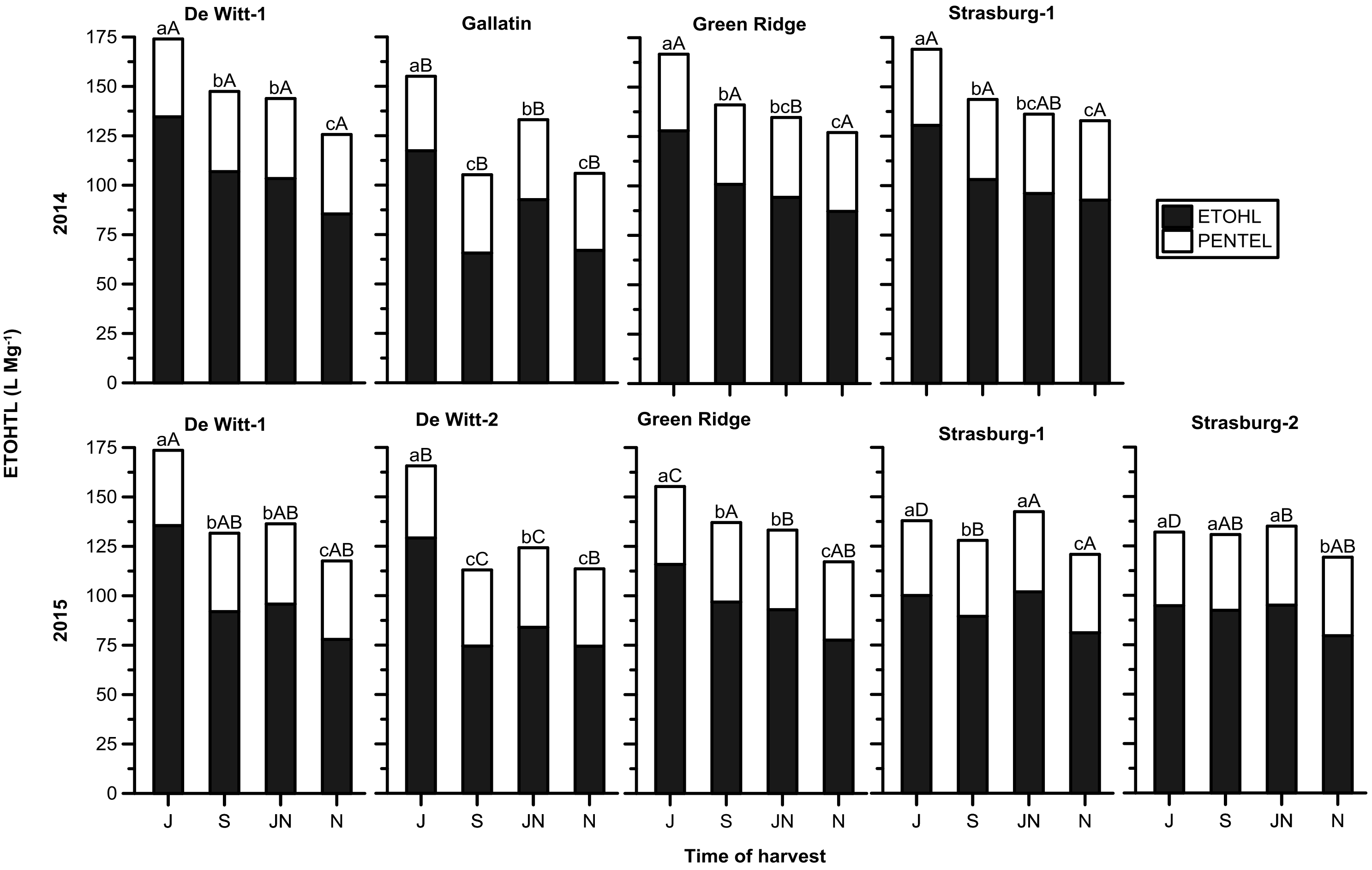


Figure 4.10 Interactive effects of harvest timing and site on total ethanol yield from simultaneous saccharification and fermentation in 2014 and 2015. Harvest timing treatments included: J-June (first harvest of June+Nov harvest strategy); S-September; JN-regrowth harvest of June+Nov harvest strategy; and N-November. Columns with the same letter are not significantly different at P < 0.05. Lowercase letters denote differences between harvest timing treatments within each site and uppercase letters denote differences between sites within each harvest timing treatment.

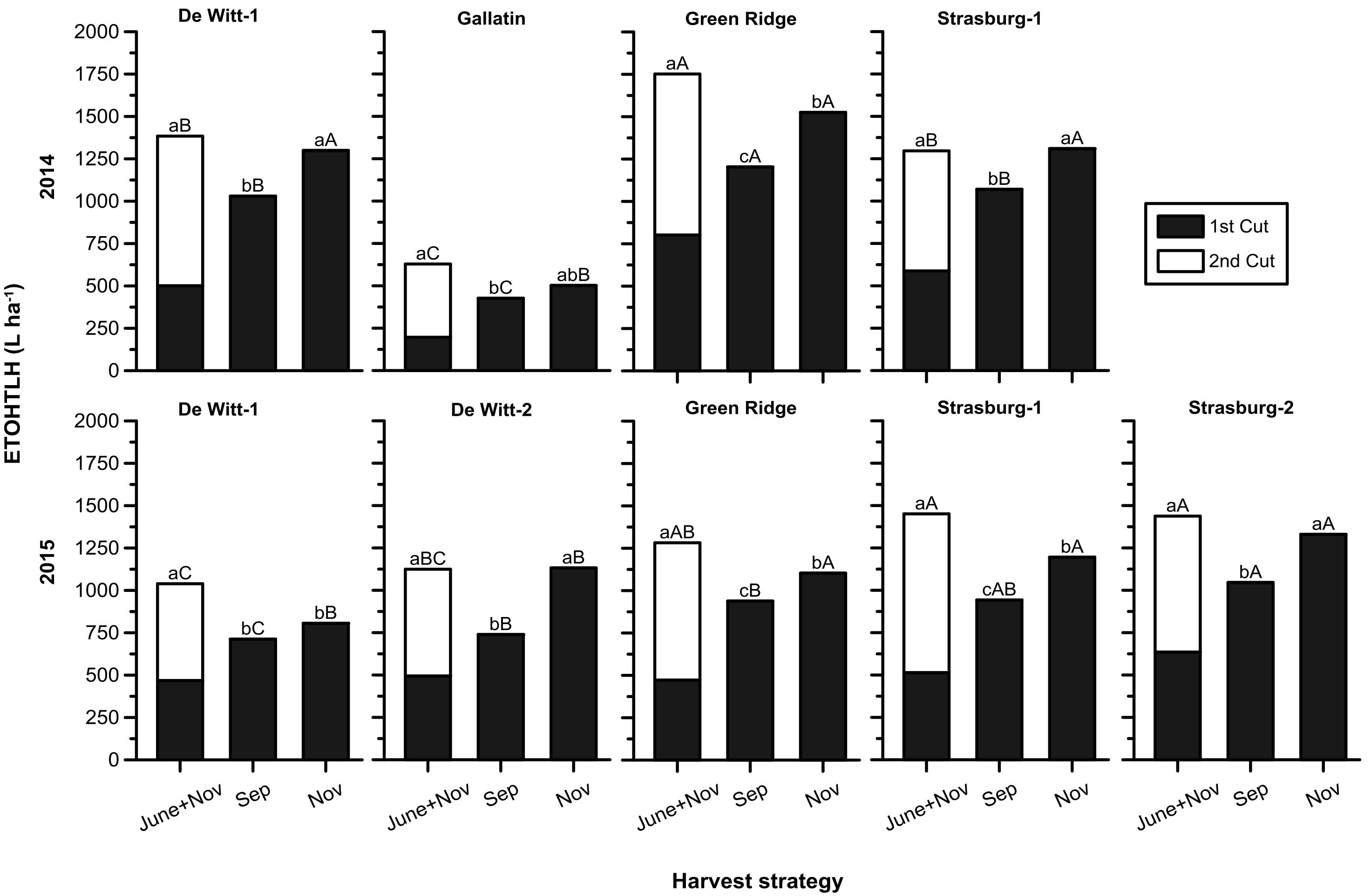


Figure 4.11 Interactive effects of harvest strategy and site on total ethanol production from simultaneous saccharification and fermentation. Columns with the same letter are not significantly different at $P < 0.05$. Lowercase letters denote differences between harvest strategies within each site and uppercase letters denote differences between sites within each harvest strategy.

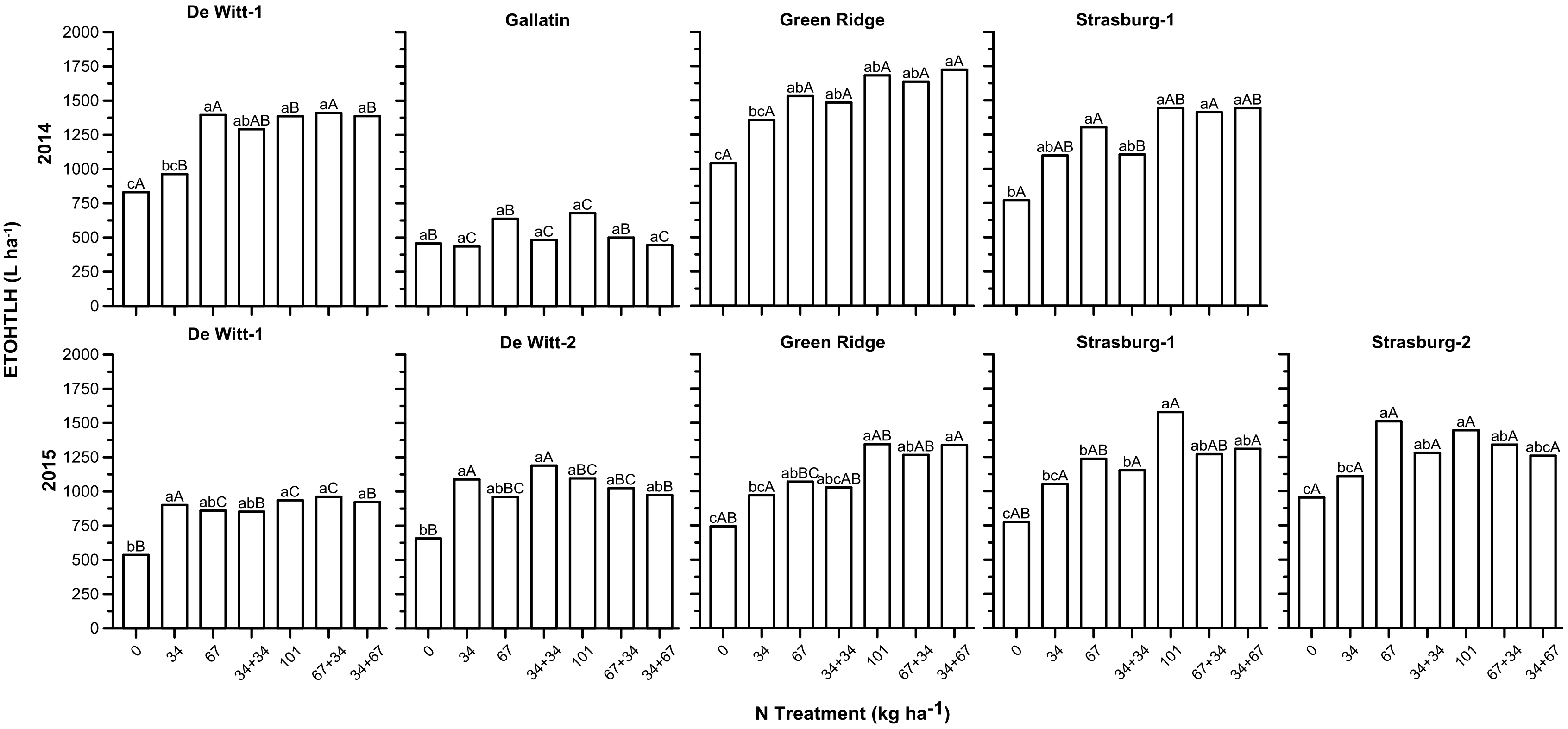


Figure 4.12 Interactive effects of nitrogen management and site on total ethanol production from simultaneous saccharification and fermentation. Columns with the same letter are not significantly different at $P < 0.05$. Lowercase letters denote differences between nitrogen treatments within each site and uppercase letters denote differences between sites within each nitrogen management strategy.

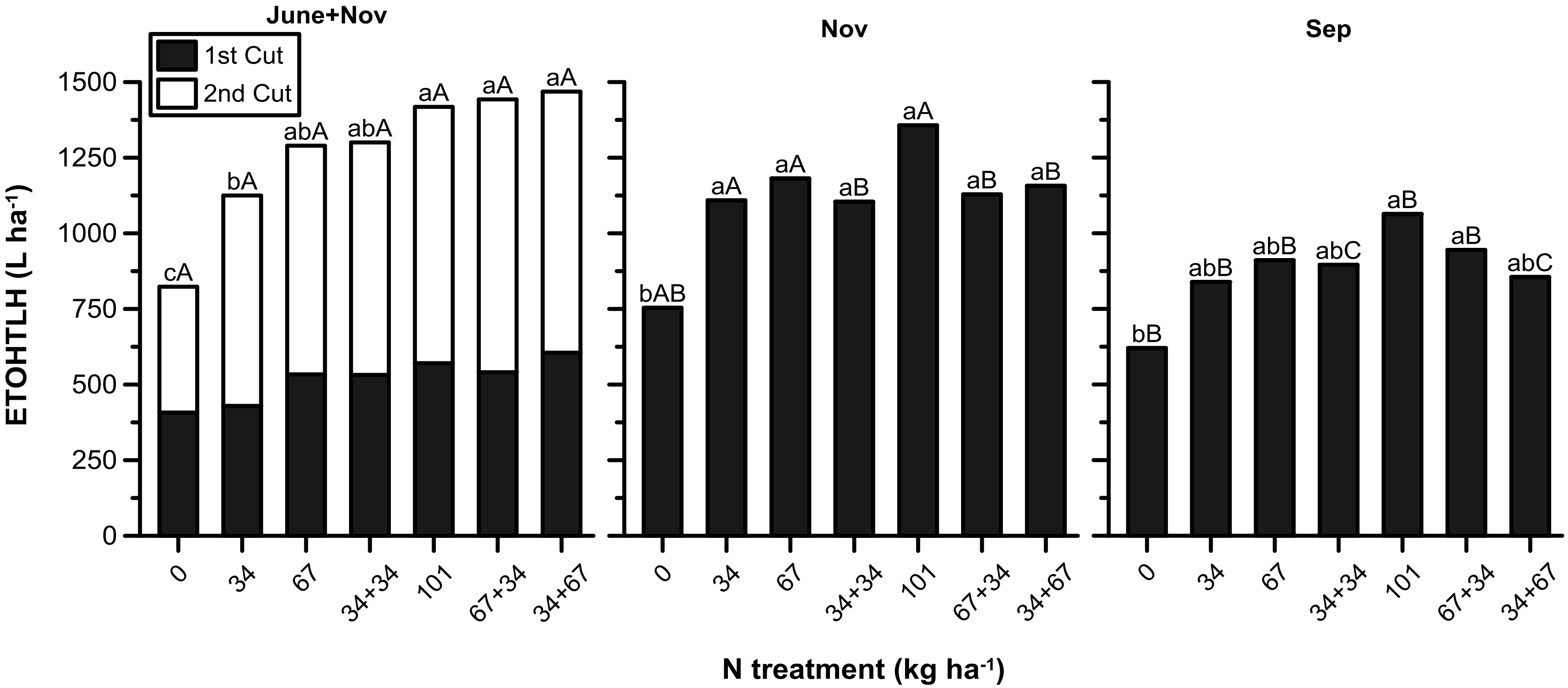


Figure 4.13 Interactive effects of nitrogen management and harvest strategy on total ethanol production from simultaneous saccharification and fermentation in 2015. Columns with the same letter are not significantly different at $P < 0.05$. Lowercase letters denote differences between nitrogen management strategies within each harvest strategy and uppercase letters denote differences between harvest strategies within each nitrogen management strategy.

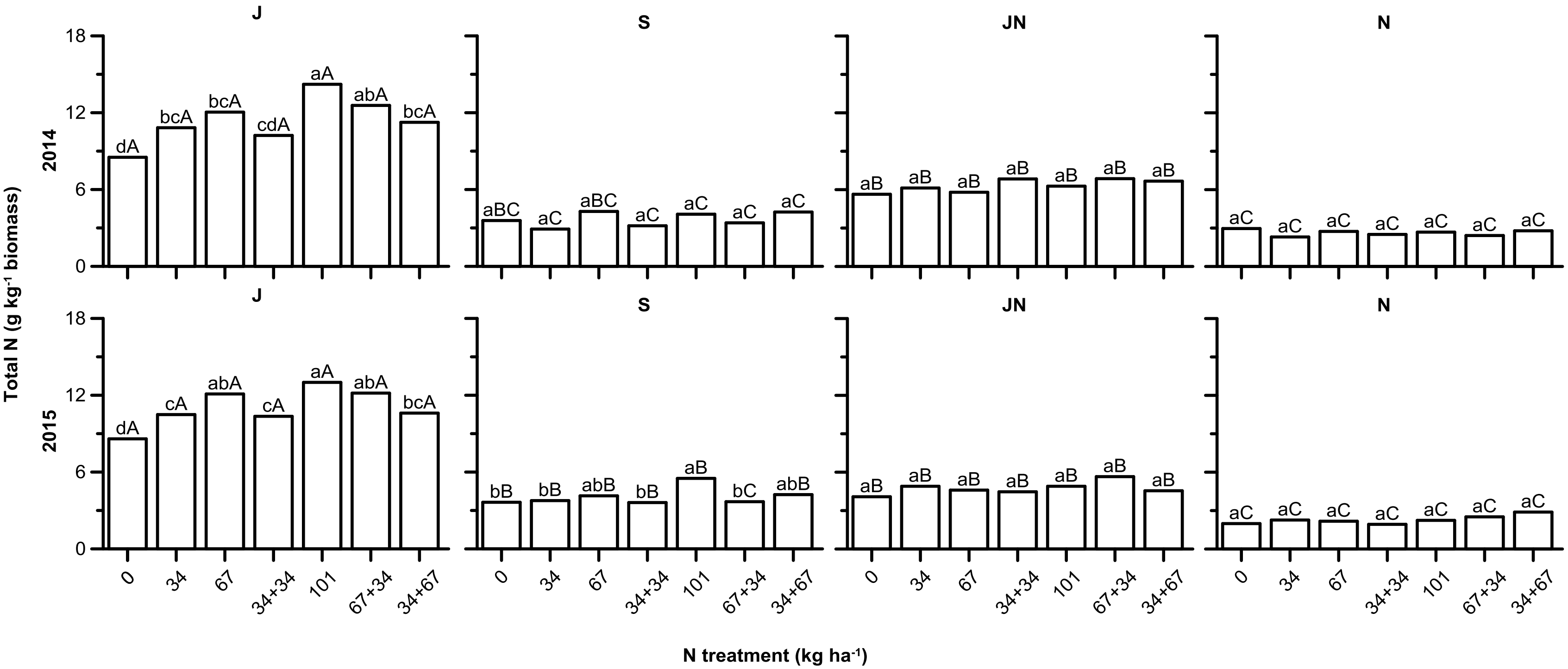


Figure 4.14 Interactive effects of harvest timing and nitrogen on total nitrogen content of biomass in 2014 and 2015. Harvest timing treatments included: J-June (first harvest of June+Nov harvest strategy); S-September; JN-regrowth harvest of June+Nov harvest strategy; and N-November. Columns with the same letter are not significantly different at $P < 0.05$. Lowercase letters denote differences between N treatments within each harvest timing strategy and uppercase letters denote differences between harvest timing treatments within each N treatment.

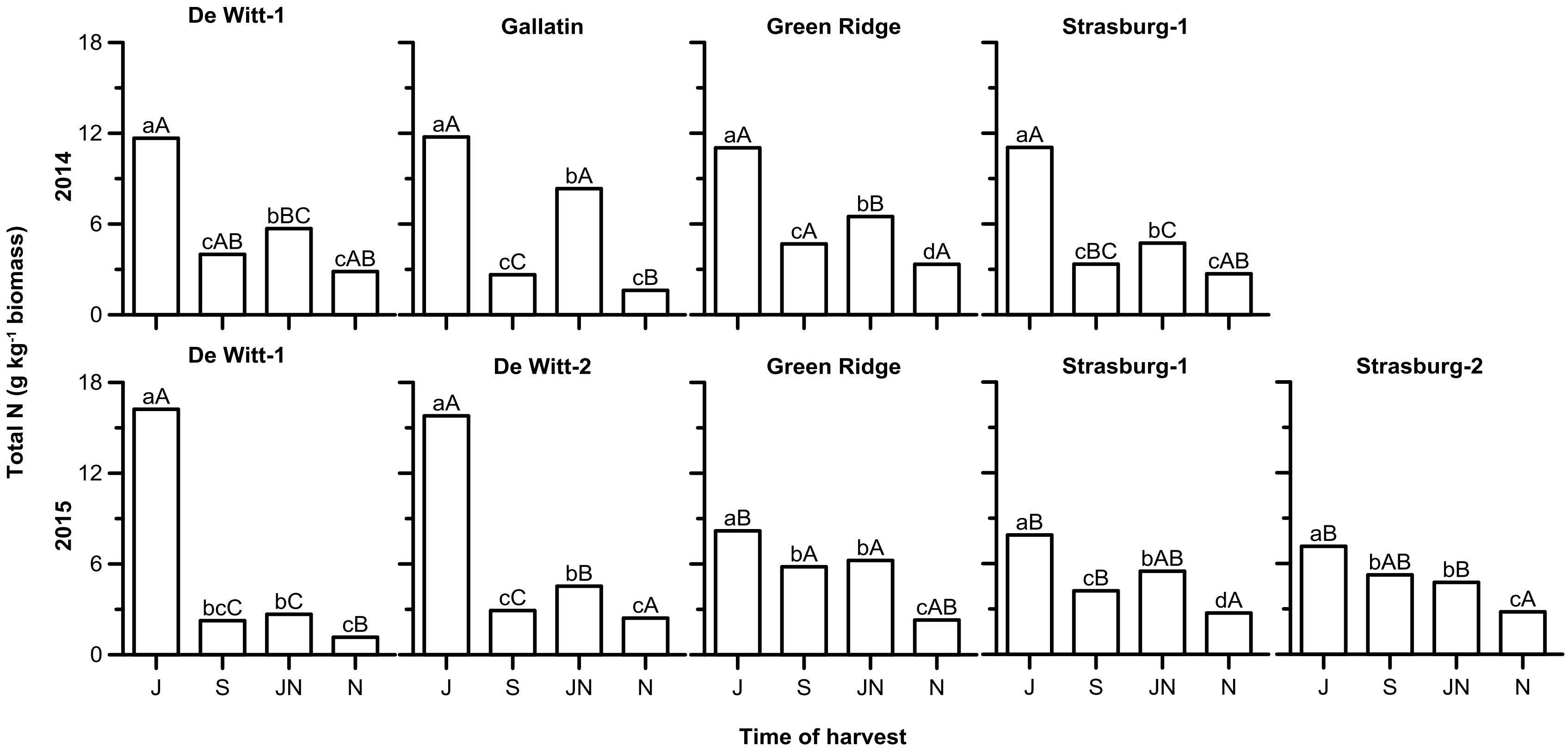


Figure 4.15 Interactive effects of harvest timing and site on total nitrogen content of biomass in 2014 and 2015. Harvest timing treatments included: J-June (first harvest of June+Nov harvest strategy); S-September; JN-regrowth harvest of June+Nov harvest strategy; and N-November. Columns with the same letter are not significantly different at $P < 0.05$. Lowercase letters denote differences between harvest timing treatments within each site and uppercase letters denote differences between sites within each harvest timing treatment.

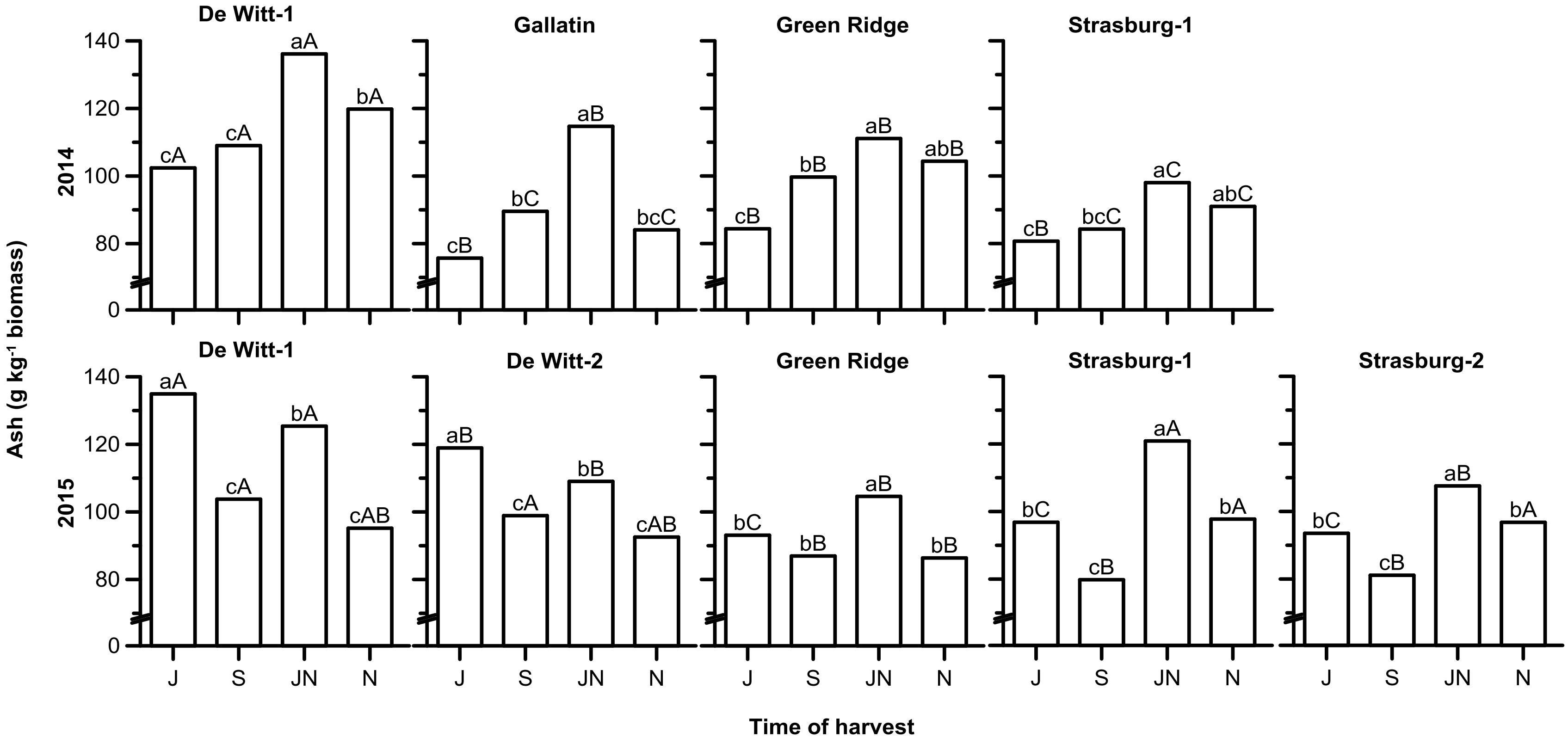


Figure 4.16 Interactive effects of harvest timing and site on total ash content of biomass in 2014 and 2015. Harvest timing treatments included: J-June (first harvest of June+Nov harvest strategy); S-September; JN-regrowth harvest of June+Nov harvest strategy; and N-November. Columns with the same letter are not significantly different at $P < 0.05$. Lowercase letters denote differences between harvest timing treatments within each site and uppercase letters denote differences between sites within each harvest timing treatment.

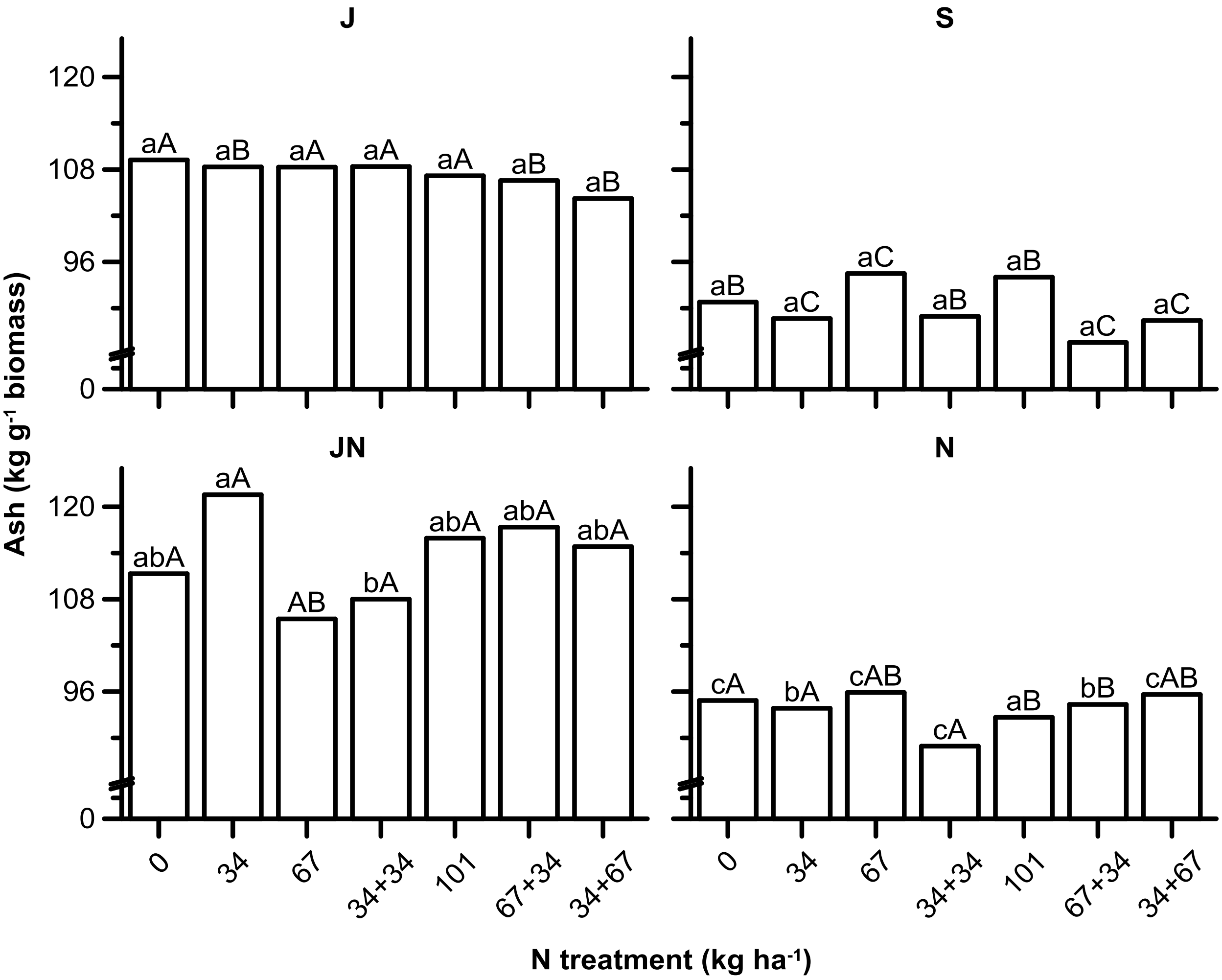


Figure 4.17 Interactive effects of nitrogen and harvest timing on total ash content of biomass in 2015. Harvest timing treatments included: J-June (first harvest of June+Nov harvest strategy); S-September; JN-regrowth harvest of June+Nov harvest strategy; and N-November. Columns with the same letter are not significantly different at $P < 0.05$. Lowercase letters denote differences between N treatments within each harvest timing strategy and uppercase letters denote differences between harvest timing treatments within each N treatment.

CHAPTER 5: CONCLUSIONS

The results of this study demonstrated that N and harvest timing management strategies and their interactions have significant impacts on both yield and feedstock quality of the warm-season grasses. There were some clearly identifiable patterns of biomass yield and feedstock quality observations in response to the tested N and harvest management practices. At the same time, there were substantial differences in observed responses of biomass yields and feedstock quality parameters to N and harvest management practices between two years and among sites. Conditions including weather, soil, plant density and composition, etc. unique to each year and site can be considered as potential causal factors for such differences.

Incremental annual N applications while practicing double or single annual harvest that include a late fall harvest sustained significantly higher biomass yield and ethanol production values per unit land area at each site. Biomass yields tend to increase with incremental N supplies, while agronomic efficiency and partial factor productivity values become lower. Therefore, implementation of annual N application at 67 kg ha^{-1} would be helpful in maintain the balance between efficiency of N inputs and both biomass yields and ethanol production values. As split application of N fertilizer cause significant impacts on biomass yields and ethanol production, one spring N application will provide an opportunity to become energy and C efficient due to the least fossil fuel usage for machinery.

Nutrient removal, especially N and minerals/ash, associated with two harvests per year systems was higher compared to one late season harvest. However, biomass harvested during early growing season in two-cut systems exhibits favorable forage quality characteristics. Therefore, two harvests per year regimes offers flexibility in market decision making for farmers/commercial producers to target more profitable option available based on future prices of biofuel feedstock and forage markets.

Simultaneous consideration of N and harvest management practices and their impacts on both the quantity and quality of biomass in this research study helped in acquiring valuable data. Therefore, conducting similar studies in the long run while studying the effects of spatial and temporal variations in environmental, weather, and site conditions will provide an opportunity to generate data that can be used in life cycle accounting studies to determine the overall sustainability of feedstock production systems for future bioenergy economies.

APPENDIX Tables and Figures

Appendix Table1. Fixed effects (P<F) of nitrogen, harvest, site, and their interactions on total theoretical ethanol yield from all biomass sugars (ETOHTLT).

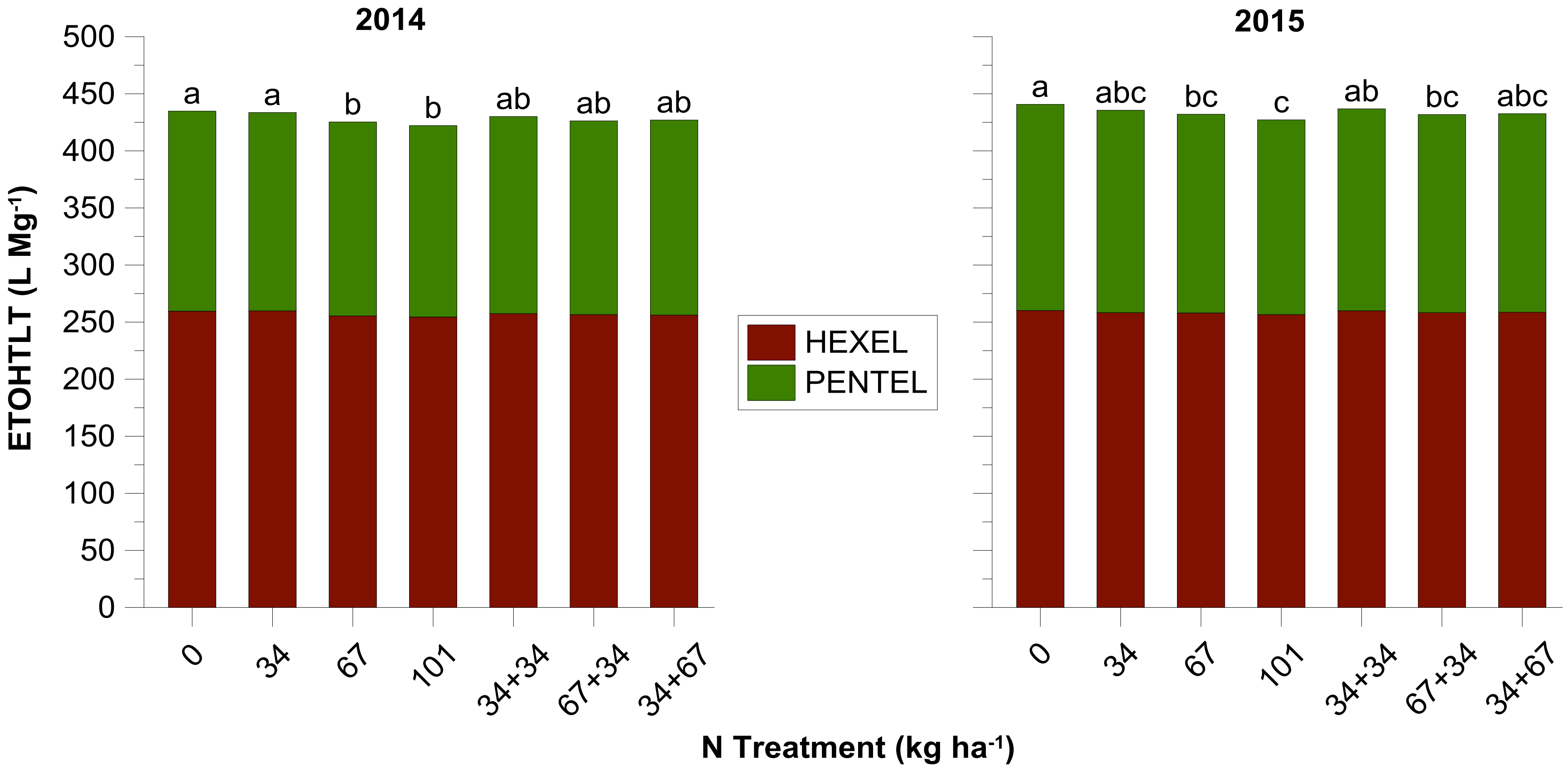
Source of Variation	Year	
	2014	2015
--------------------ETOHTLT (L Mg^{-1} biomass) --------------------		
N	0.04	0.003
Harvest	<0.0001	<0.0001
Site	<0.0001	<0.0001
N x Harvest	0.06	0.10
N x Site	0.08	0.72
Harvest x Site	<0.0001	<0.0001
N x Harvest x Site	0.72	0.98

Appendix Table 2. Fixed effects (P<F) of nitrogen, harvest, site, and their interactions on total theoretical ethanol production (ETOHTLTH).

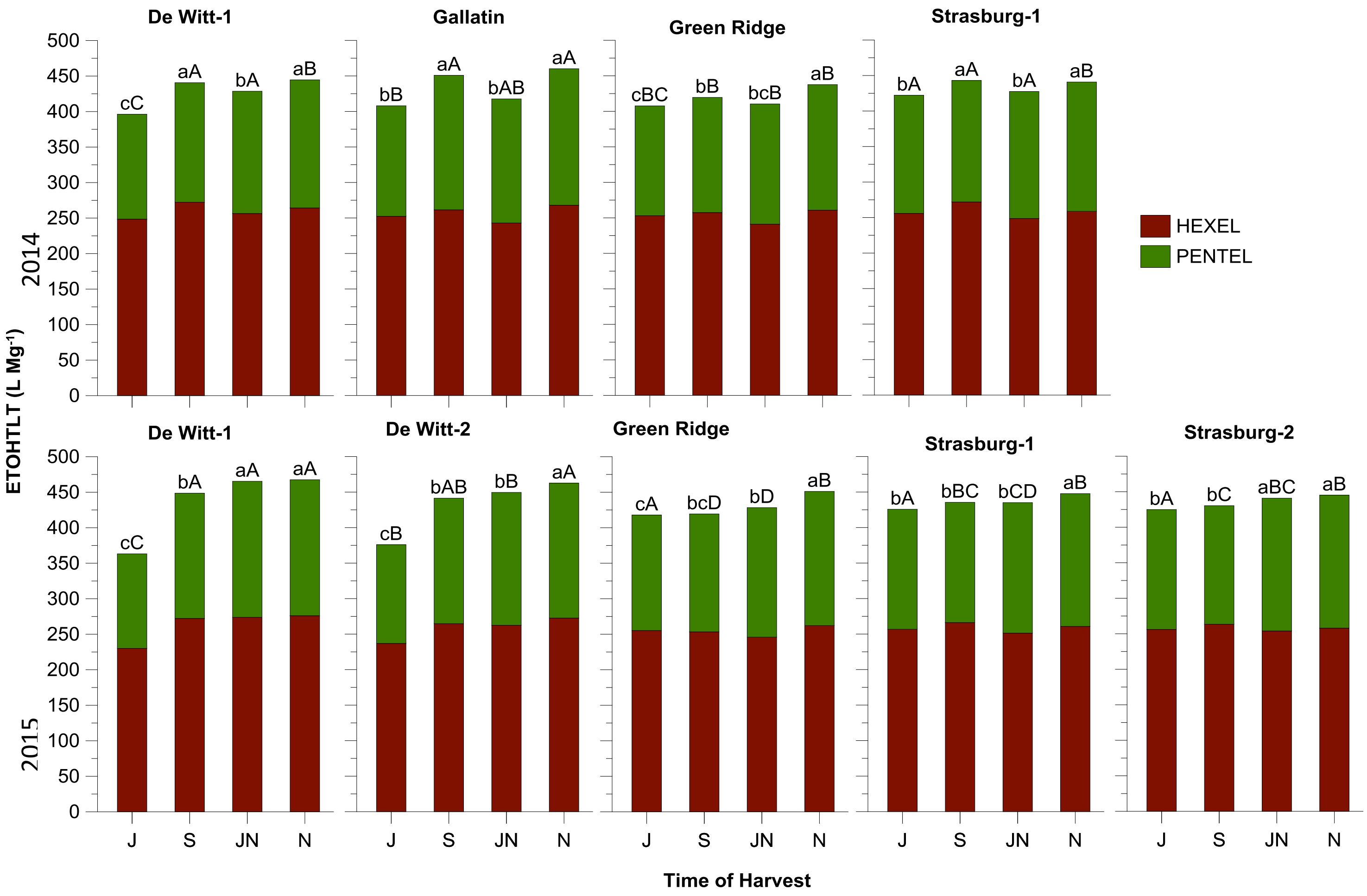
Source of Variation	Year	
	2014	2015
--------------------ETOHTLTH (L ha^{-1} biomass) --------------------		
N	<0.0001	<0.0001
Harvest	<0.0001	<0.0001
Site	<0.0001	<0.0001
N x Harvest	0.12	0.06
N x Site	0.001	0.0005
Harvest x Site	0.0004	0.002
N x Harvest x Site	0.69	0.95

Appendix Table 3. Fixed effects (P<F) of nitrogen, harvest timing, site, and their interactions on major cell wall constituents; Cellulose, hemicellulose, and acid detergent lignin.

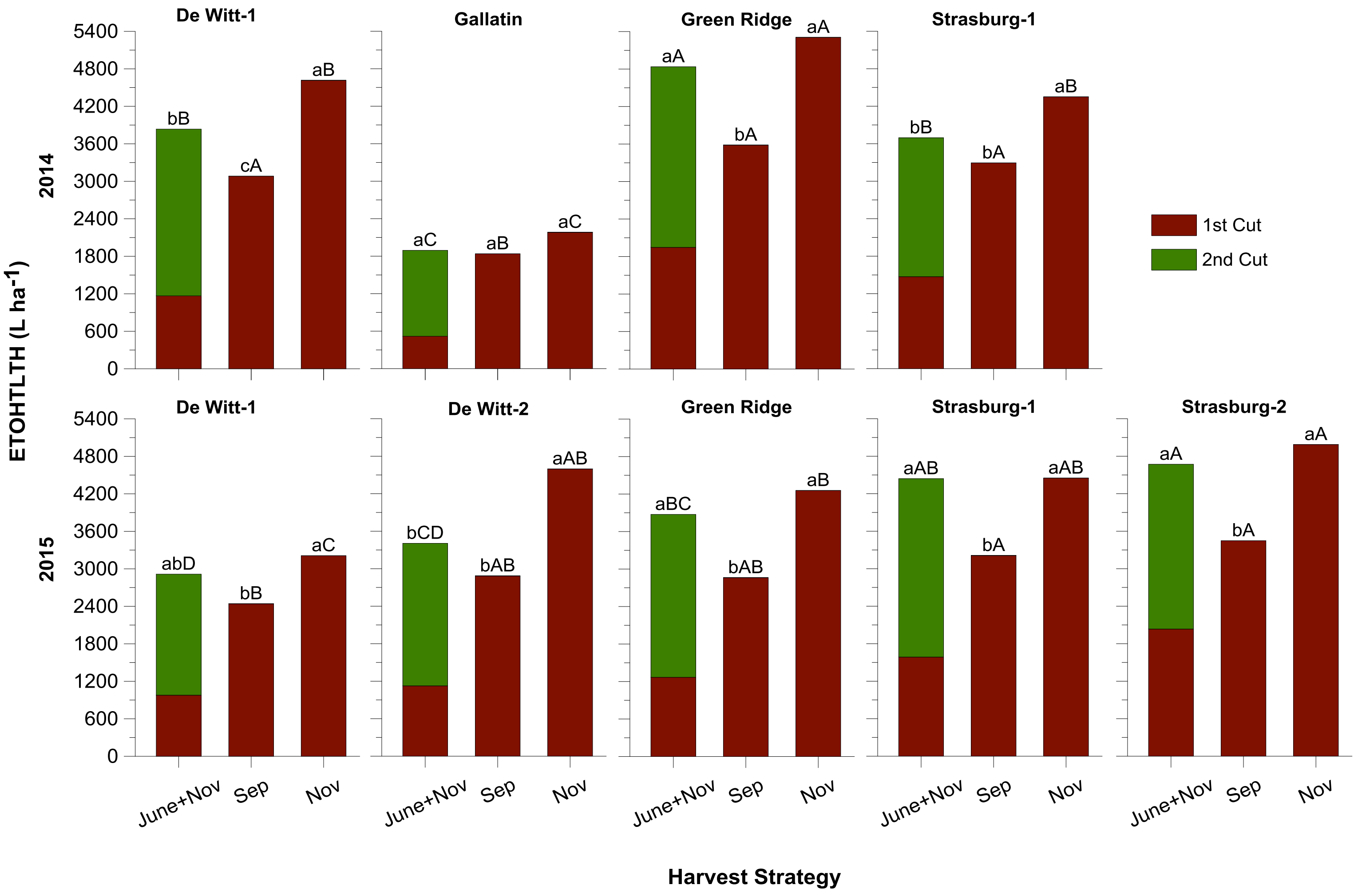
	Year	
Source of Variation	2014	2015
--------------------Cellulose (mg g^{-1} biomass) --------------------		
N	0.22	0.05
Harvest	<0.0001	<0.0001
Site	0.02	<0.0001
N x Harvest	0.28	0.037
N x Site	0.48	0.96
Harvest x Site	<0.0001	<0.0001
N x Harvest x Site	0.39	0.48
--------------------Hemicellulose (mg g^{-1} biomass) --------------------		
N	0.12	0.21
Harvest	<0.0001	<0.0001
Site	<0.0001	<0.0001
N x Harvest	0.65	0.10
N x Site	0.66	0.72
Harvest x Site	0.06	0.06
N x Harvest x Site	0.98	0.64
--------------------ADL (mg g^{-1} biomass) --------------------		
N	0.20	0.10
Harvest	<0.0001	<0.0001
Site	<0.0001	<0.0001
N x Harvest	0.28	<0.0001
N x Site	0.41	0.20
Harvest x Site	0.004	<0.0001
N x Harvest x Site	0.35	0.30



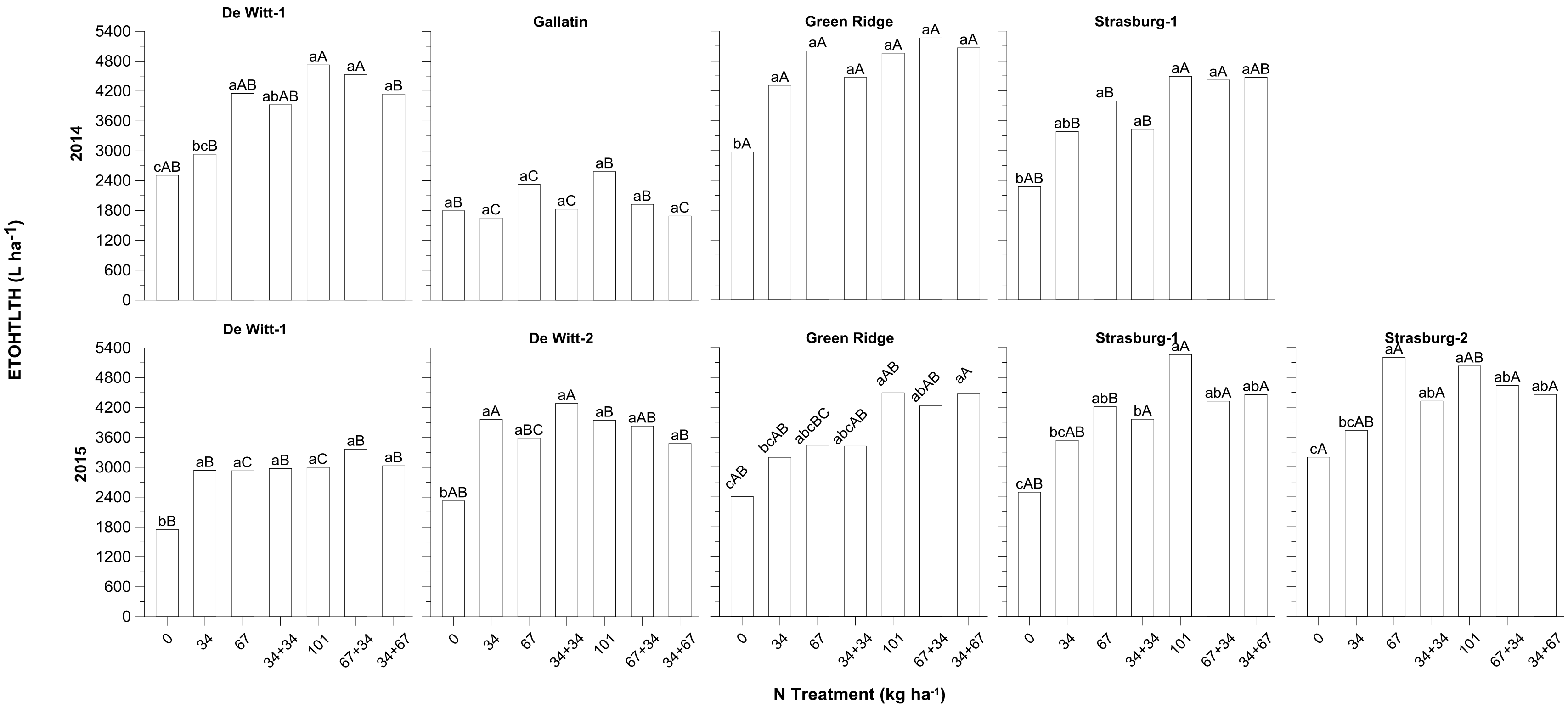
Appendix Figure 1. Effects of nitrogen management on total theoretical ethanol yield from all biomass sugars in 2014 and 2015. Columns with the same letter are not significantly different at $P < 0.05$.



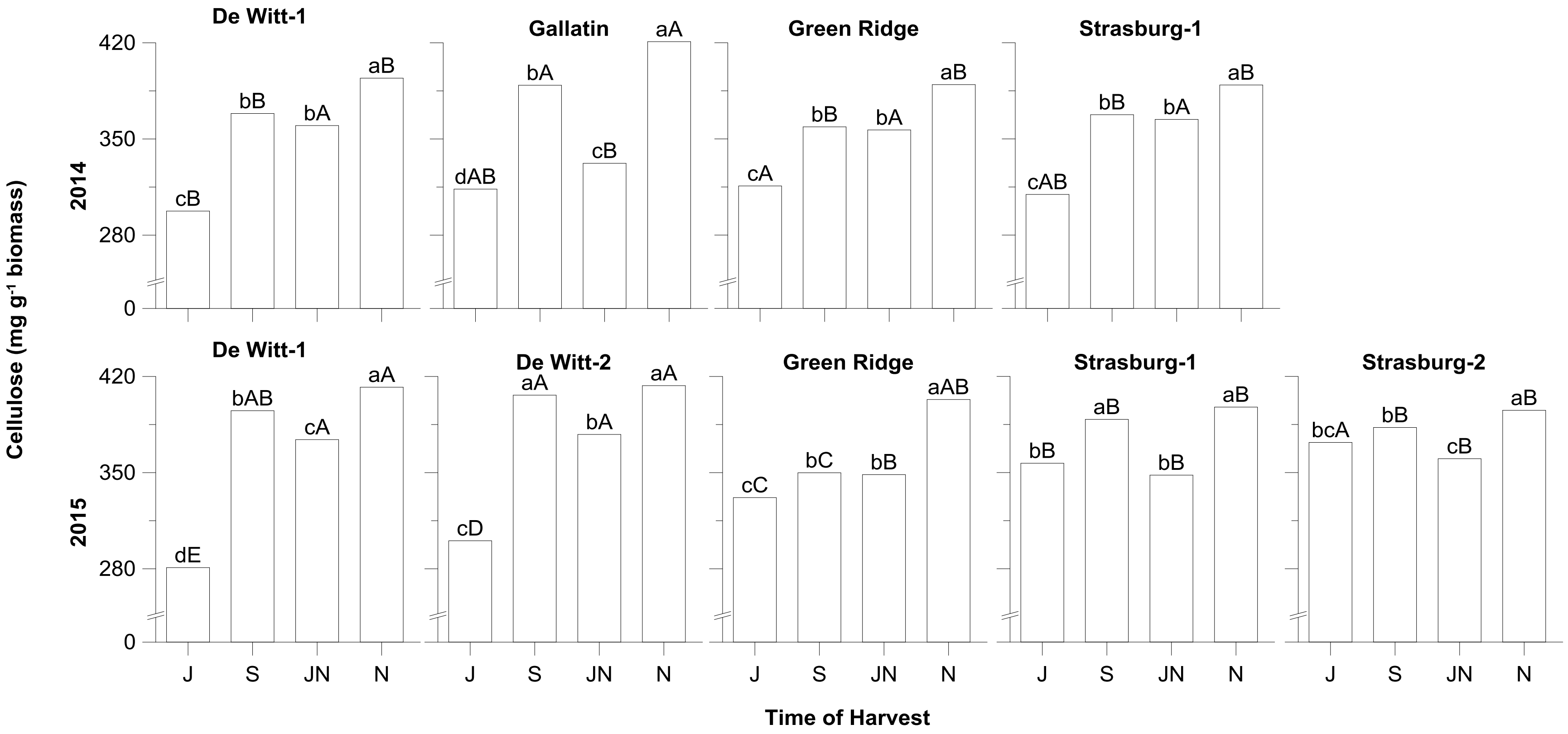
Appendix Figure 2. Interactive effects of harvest timing on total ethanol yield from all biomass sugars in 2014 and 2015. Columns with the same letter are not significantly different at $P < 0.05$. Lowercase letters denote differences between harvest timing treatments within each site and uppercase letters denote differences between sites within each harvest timing treatment.



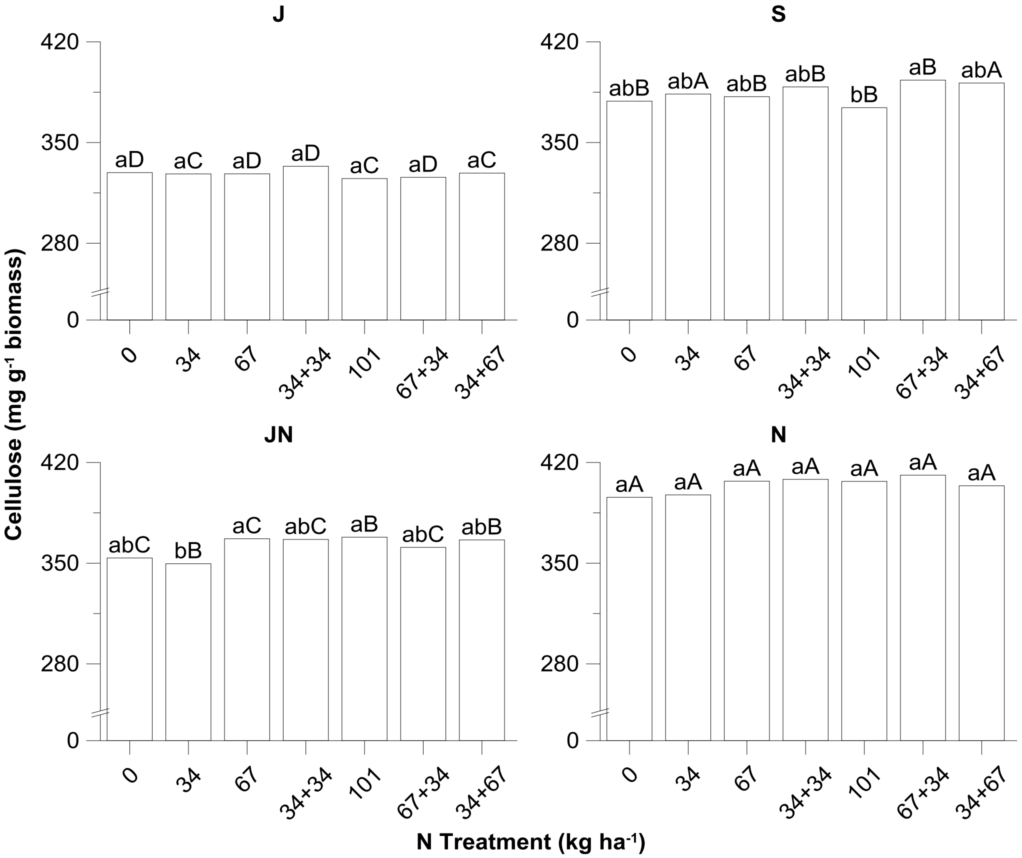
Appendix Figure 3. Interactive effects of harvest strategy and site on total theoretical ethanol production from all biomass sugars. Columns with the same letter are not significantly different at $P < 0.05$. Lowercase letters denote differences between harvest strategies within each site and uppercase letters denote differences between sites within each harvest strategy.



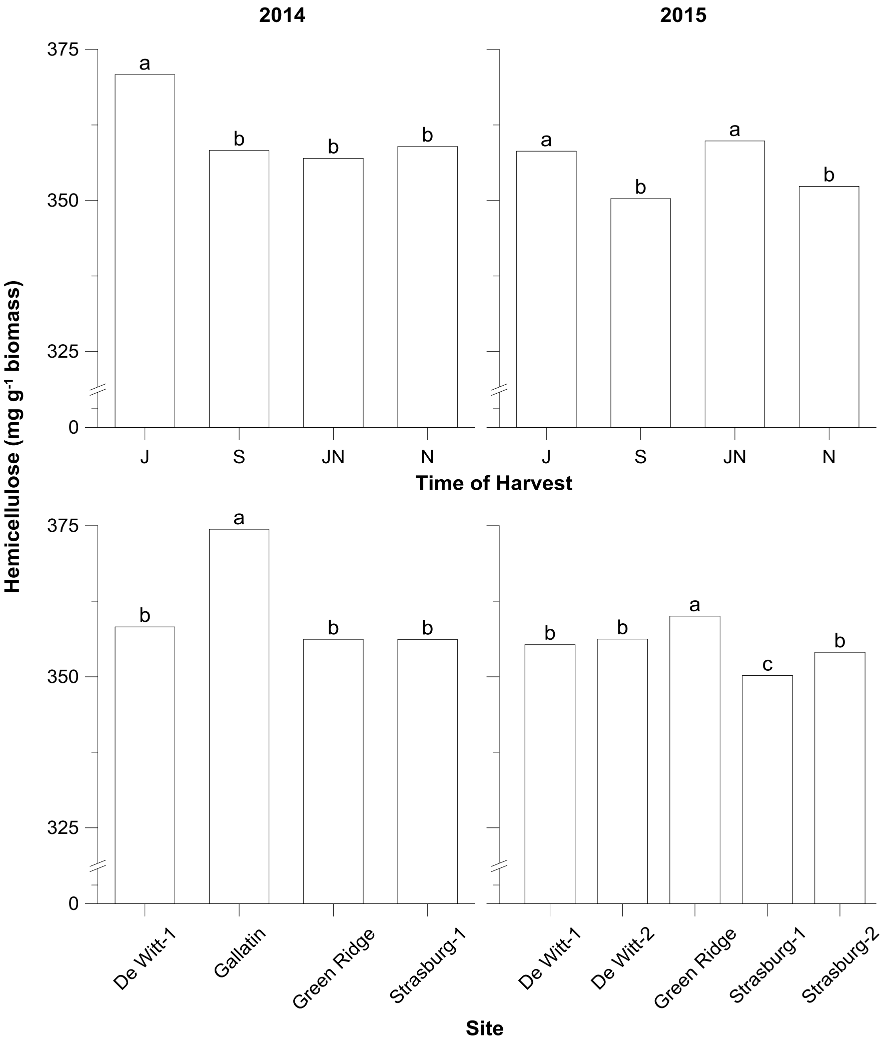
Appendix Figure 4. Interactive effects of nitrogen management strategy and site on total theoretical ethanol production from all biomass sugars. Columns with the same letter are not significantly different at $P < 0.05$. Lowercase letters denote differences between nitrogen management strategies within each site and uppercase letters denote differences between sites within each nitrogen management strategy.



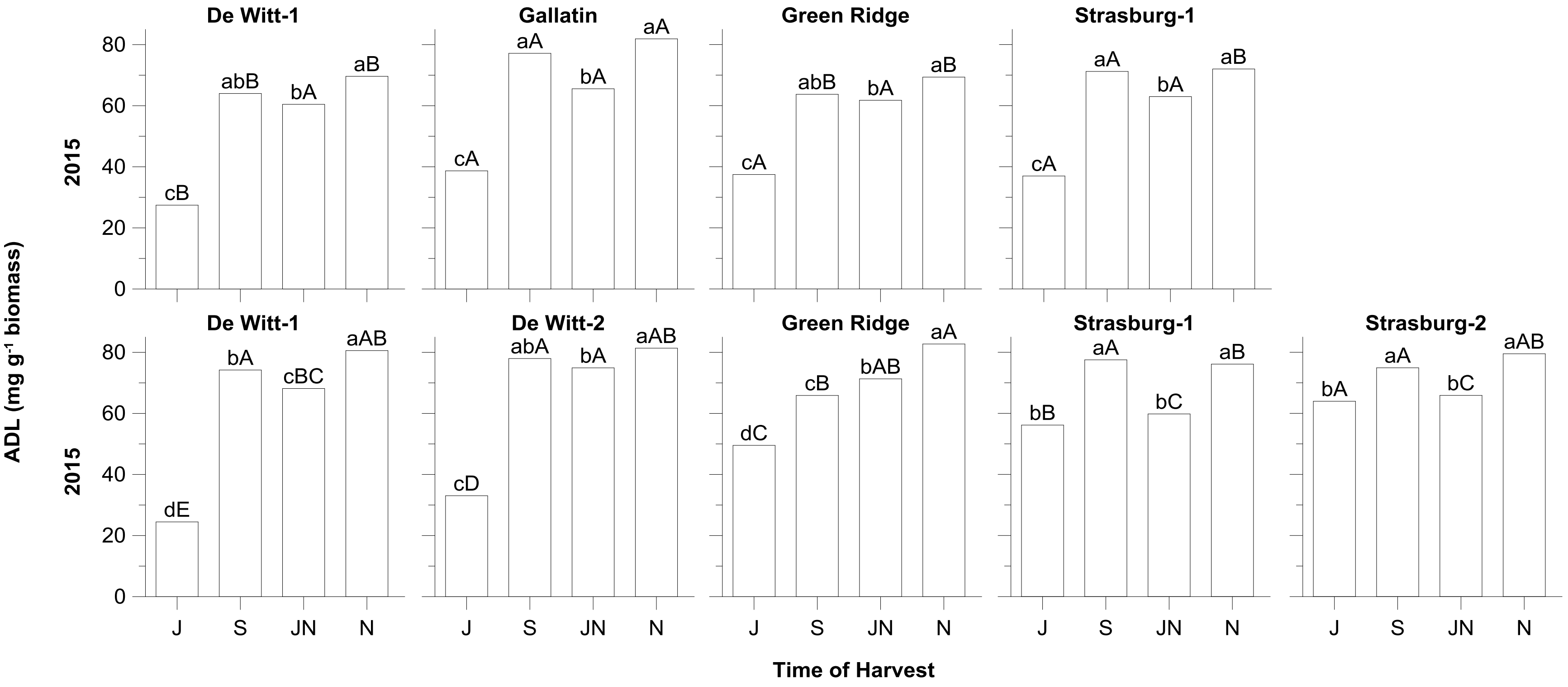
Appendix Figure 5. Interactive effects of time of harvest and site effects on biomass cellulose content in 2014 and 2015. Columns with the same letter are not significantly different at $P < 0.05$. Lowercase letters denote differences between harvest timing treatments within each site and uppercase letters denote differences between sites within each harvest timing treatment.



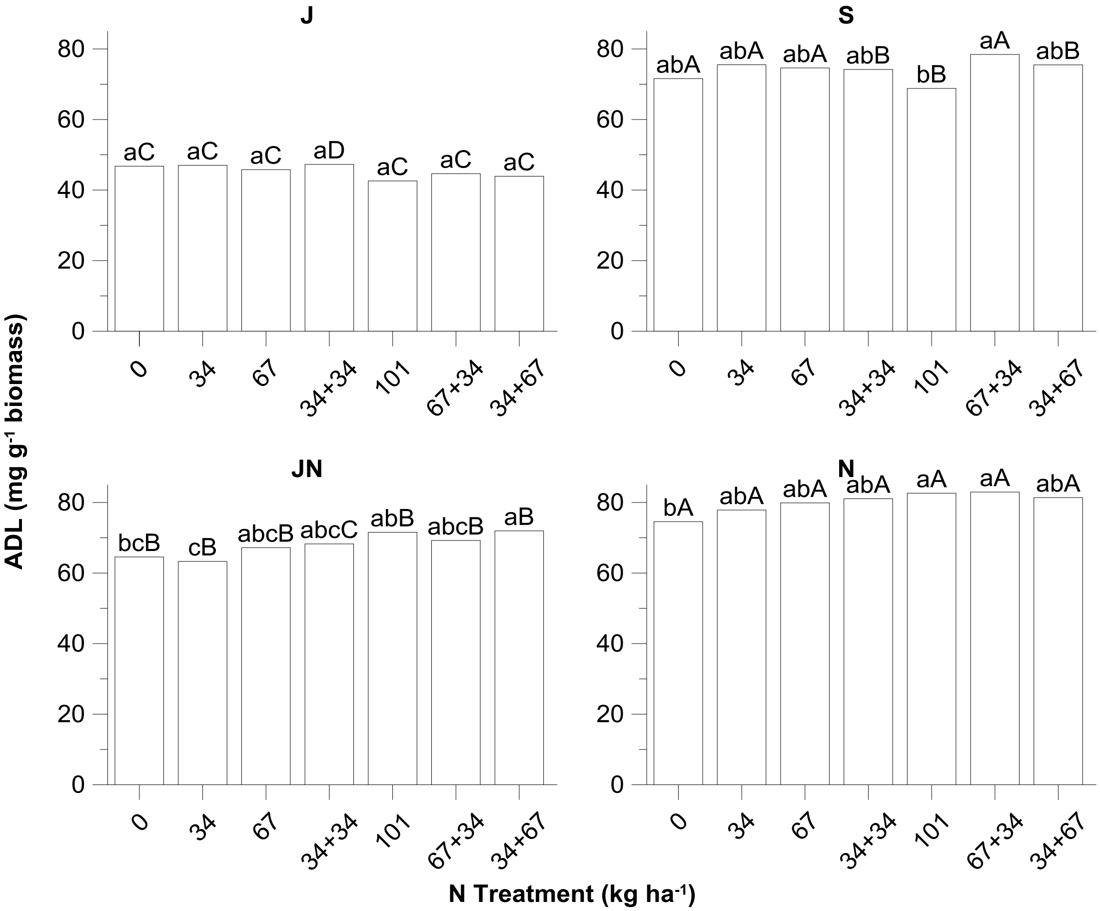
Appendix Figure 6. Interactive effects of nitrogen and harvest timing on biomass cellulose content in 2015. Columns with the same letter are not significantly different at $P < 0.05$. Lowercase letters denote differences between N treatments within each harvest timing strategy and uppercase letters denote differences between harvest timing treatments within each N treatment.



Appendix Figure 7. Main effects of time of harvest timing and site on biomass hemicellulose content in 2014 and 2015. Columns with the same letter are not significantly different at $P < 0.05$.



Appendix Figure 8. Interactive effects of harvest timing and site on biomass acid detergent lignin content in 2014 and 2015. Columns with the same letter are not significantly different at $P < 0.05$. Lowercase letters denote differences between harvest timing treatments within each site and uppercase letters denote differences between sites within each harvest timing treatment.



Appendix Figure 9. Interactive effects of nitrogen and harvest timing on biomass acid detergent lignin content in 2015. Columns with the same letter are not significantly different at $P < 0.05$. Lowercase letters denote differences between N treatments within each harvest timing strategy and uppercase letters denote differences between harvest timing treatments within each N treatment.

Descriptions for Appendices

Total theoretical ethanol yield

Similar to ETOHTL, total theoretical ethanol yield (ETOHTLT) in response to N management generally resulted in better yields with low N or with the two-cut harvest strategy (Fig. 1). HEXEL contributed to around 60% of the ETOHTLT and was generally stable across N rates. Biomass with the lowest ETOLHTLT values produced from June harvested biomass (363-426 L Mg^{-1} biomass), while the greatest came from November harvested biomass (445-467 L Mg^{-1} biomass). These higher ETOHTLT values associated with late season harvested biomass can be attributed to the higher amounts of cell wall constituents; especially cellulose and hemicellulose concentrations (Seepaul et al., 2014).

Total theoretical ethanol production

Similar to ETOHTLH, high ETOHLTH values were observed with high N inputs greater than 34 kg ha^{-1} while interacting with site (Figure 2; Figure 3; Figure 4). However, single harvest in November gave greater ETOHTLTH values compared to double harvest strategy with response to harvest by site interactions.

Major cell wall constituents

Biomass cellulose, hemicellulose, and ADL contents were affected by time of harvest and site (Table 3; Figure 5; Figure; 6; Figure7; Figure 8 Figure 9). Especially, time of harvest and site impacted hemicellulose content of harvested biomass during both 2014 and 2015. However, time of harvest, site and their interactions affected both biomass cellulose and ADL contents in both years. Furthermore, interactions of N and time of harvest impacted biomass cellulose and ADL contents in 2015.

Variation of both cellulose and ADL contents followed similar patterns in terms of time of harvest by site interactions in two years and N by time of harvest interactions in 2015. At each site, biomass harvested in September and November had greater contents of both cellulose and ADL compared to June and the regrowth harvest of two-cut harvest system. Furthermore, the highest and lower values of cellulose and ADL contents were associated with N and June harvested biomass respectively, at each site in both years. Moreover, related to the N by time of harvest interaction, greater cellulose and ADL values were observed in late-season harvested biomass (N and S). Cellulose contents of biomass harvested in June ranged between 280-370 mg g^{-1} across two years. For biomass harvested in November across two years, cellulose contents ranged between 390-420 mg g^{-1}. In addition, the ranges of ADL contents of biomass harvested in June and November were 24-64 mg g^{-1} and 69-82 mg g^{-1}. These results are in line with the findings of Mulkey et al. (2006) who reported higher values of cellulose and lignin contents associated with maturity and is physiologically a result of decreased leaf to stem ratio.

Variation of hemicellulose content of biomass harvested in two years as responsive to time of harvest had different trends (Fig. 11). In 2014, biomass harvested in June exhibited the highest hemicellulose content (370 mg g^{-1} biomass). In contrast, in 2015 higher hemicellulose contents were observed with the biomass coming from second cut of J/N harvest strategy and November harvested biomass compared to June and September harvested biomass. Related to the effects of site on biomass hemicellulose content, in 2014 and 2015, highest values were observed at Gallatin and Green Ridge (374 and 360 mg g^{-1}) respectively.

Contents of major cell wall constituents tend to increase when biomass is being harvested late in the growing season due to the physiological maturity of the plants (Mulkey et al., 2006; Waramit et al., 2011; Seepaul et al., 2014). Effects of site and its interactions on cellulose, hemicellulose, and ADL contents in harvested biomass can be attributed to the differences in grass species composition (Waramit et al., 2011), soil, and weather conditions prevailing at each site (Guretzky et al., 2011).