

**Where's the nitrogen?
The timing and impacts of internal nitrogen cycling on switchgrass (*Panicum virgatum*
L.) yield and biofuel quality**

by

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CHAPTER 1. GENERAL INTRODUCTION

Introduction

Global concern

Forty years ago, Chapman et al. (1972) described that energy crisis as, “simply that the supplies of fuels and power are less than we want, or that they might cost much more in the future”. The same is true today, with the added onus of environmental sustainability, as energy independence and security concerns continue to push the U.S. towards domestic and renewable resources (EISA, 2007). In that spirit, the Energy Independence and Security Act (EISA) of 2007 was passed to provide clean, efficient biofuels and to reduce harmful greenhouse gas emissions by carbon capture and sequestration efforts. This, and supporting action by state and federal agencies and private industry, states that transportation fuels derived from petroleum can be replaced with biofuels, and that, with their use, we can address the imminent challenges posed by global climate change and the scarcity of petroleum supplies (Solomon, 2010). At the same time, increases in global population and affluence are placing greater pressure on agricultural productivity. A preponderance of scientific literature suggests that, with drastic and immediate action that includes deployment of biofuels derived from perennial crops, it should be possible to sustainably feed and fuel the world while adapting to global climate change (Georgescu et al., 2011; Heaton et al., 2008; McLaughlin and Walsh, 1998; Perlack et al., 2011; Popp et al., 2011; Sanderson and Adler, 2008; Sanderson et al., 2006; Schmer et al., 2008; Solomon, 2010; Tilman et al., 2011; Valentine et al., 2012).

In response, and as an explicit first step towards energy independence, EISA mandates that 36 billion gallons of renewable fuels be used per year by 2022, 21 billion of which must be advanced biofuels, i.e., made from non-food sources like agricultural residues and lignocellulosic feedstocks (EISA, 2007; Perlack et al., 2011). In order to meet this goal, considerable research is focused on dedicated bioenergy crops that can be used to make a variety of biofuels (Boateng et al., 2006; Somerville et al., 2010) including ethanol, butanol or direct petroleum substitutes, i.e., “drop-in fuels”. Numerous investigators have considered which plants are best suited for energy cropping particularly on lands marginal for food cropping and found switchgrass (*Panicum virgatum* L.) to be a leading candidate species (Beringer et al., 2011; Georgescu et al., 2011; Heaton et al., 2008; Jones, 2011; Jorgensen, 2011; Nijsen et al., 2012; Popp et al., 2011; Sang, 2011; Sang and Zhu, 2011; Tilman et al., 2011; Valentine et al., 2012).

Switchgrass as a bioenergy crop

Characteristics

Switchgrass is a warm-season, C₄ perennial grass native to the tallgrass prairie regions of North America (Moser and Vogel, 1995) that has great potential as a biofuel feedstock candidate (Fike et al., 2006a; Vogel, 1996). It has been planted on thousands of acres in the U.S. and is planned for planting on millions more. The U.S. Dept. of Energy and others have used it as a “model” crop for bioenergy (Perlack et al., 2011; Wright and Turhollow, 2010). Switchgrass has several characteristics that are desirable for biomass energy crops. Switchgrass stands pose little threat of invasion, have consistently high yields with minimal inputs, and are well suited to marginal lands and well-managed stands can be

expected to last at least 10 years (Evanylo et al., 2005; Fike et al., 2006b; Perlack et al., 2011).

Internal nitrogen cycling

Nitrogen (N) is needed in greater quantities, compared to other nutrients, for plant biomass generally and switchgrass production in particular (Lemus et al., 2008; McLaughlin and Kszos, 2005; Parrish and Fike, 2005). Conversely, high nitrogen concentration ([N]) is undesirable in the harvested biomass feedstocks for two reasons: 1) it is economically and environmentally expensive to replace N removed from cropping systems and 2) N reduces the conversion efficiency of biomass to biofuel, especially when using a thermochemical process (Boateng et al., 2006; Lewandowski and Kicherer, 1997; McKendry, 2002).

Immediate reductions in the [N] of the harvested portions of perennial feedstocks can be achieved by exploiting seasonal nutrient cycling (translocation). During plant senescence, nutrients, especially N, are moved from actively growing tissues to below-ground tissues where it can be stored and used for new growth (Beale and Long, 1997; Clark, 1977; Cooke and Weih, 2005; Hayes, 1985; Heaton et al., 2004; Van Heerwaarden et al., 2003). Allowing switchgrass to fully senesce and translocate nutrients to below-ground storage organs will improve feedstock quality, as well as minimize the nutrient requirements for maintaining a sustainable biomass production system in the long term (Fike et al., 2006a; Heggenstaller et al., 2009; McLaughlin and Kszos, 2005; Muir et al., 2001).

Timing of switchgrass harvest

Harvesting before complete translocation of N to below-ground storage organs can result in reduced stand density and biomass yields over time (Casler and Boe, 2003). Several investigations have found that in the Midwestern United States, maximum yields can be

achieved with a mid-August harvest (Adler et al., 2006; Casler and Boe, 2003; Vogel et al., 2002). Although maximum yields are achievable, the [N] in the harvestable portions remains high, [N] in the root storage is low, and harvest at this time results in maximum N removal from the field and minimum N stored for subsequent years growth (Heaton et al., 2009). Therefore, recommendations have been made to delay harvest until the fall and potentially until the spring (Adler et al., 2006; Casler and Boe, 2003) to reduce fertilizer requirements and maintain yields.

Thermochemical conversion

Biofuel production

To date, it is not clear what platforms or processes for converting crops to fuels will become dominant. Biochemical platforms focused on saccharification and fermentation processes are common in academia and industry, as are thermochemical platforms like gasification, combustion, and fast pyrolysis. Fast pyrolysis is particularly interesting in that it converts biomass to bio-oil, an analogue of crude oil, and is a promising and practical alternative to converting cellulosic biomass to alcohols (Boateng et al., 2006; Boateng et al., 2007; Mullen and Boateng, 2008).

Description of fast pyrolysis

Fast pyrolysis is a process wherein biomass is rapidly heated in the absence of oxygen to convert the feedstock to solid, liquid, and gas fractions (Bridgwater et al., 1999; Czernik and Bridgwater, 2004; Mohan et al., 2006). The liquid fraction, bio-oil, is the main product formed during fast pyrolysis and the solid and gaseous fractions, char and non-condensable gas, are considered co-products. Bio-oil is a dark brown liquid that physically resembles crude-oil and can be similarly upgraded to drop-in fuels to replace petroleum-based

transportation fuels (Bridgwater, 1994; Bridgwater, 1996; Czernik and Bridgwater, 2004; Huber et al., 2006).

The role of N in bio-oil upgrading

Although N is necessary for plant growth and biomass production, it has negative consequences in the process of upgrading bio-oil to a transportation fuel. Upgrading requires deoxygenation and can be achieved using two different routes: hydrotreating or catalytic vapor (zeolite) cracking (Bridgwater, 1994; Czernik and Bridgwater, 2004). Hydrotreating gives a greater maximum stoichiometric yield compared to catalytic vapor cracking (Bridgwater, 1994). It uses a catalyst and hydrogen to deoxygenate bio-oil compounds, producing liquid consisting largely of aliphatic and aromatic hydrocarbons (Czernik and Bridgwater, 2004). Hydrotreating was designed to remove N, sulfur, and other contaminants from petroleum as well as oxygen (Brown, 2011; Huber and Corma, 2007), but most of the commercially available catalysts do not handle N well. Maxted et al. (1951) found that N even in low concentrations could deactivate the catalysts. The target [N] of bio-oil is 0.2 % and is typical of woody feedstocks (Bridgwater, 1994; Bridgwater, 1996; Czernik and Bridgwater, 2004).

How to achieve low N content in biomass

The biology of switchgrass provides some opportunity to naturally manipulate the [N] of this biomass feedstock, but there is a trade-off between the quantity and quality of the feedstock depending on time of harvest. An early harvest results in increased biomass quantity, but a later harvest leads to increased biomass and biofuel quality (Adler et al., 2006; Lewandowski and Heinz, 2003; Lewandowski et al., 2003b; Sanderson and Adler, 2008) and may also require less N inputs (Adler et al., 2006; Heaton et al., 2009; Heggenstaller et al.,

2009; McLaughlin and Kszos, 2005; Muir et al., 2001; Parrish and Fike, 2005; Reynolds et al., 2000; Vogel et al., 2002). The research objectives of this project were to: 1) elucidate the spatial and temporal distribution of N in above- and below-ground tissues of field-grown switchgrass to determine its impacts on yield and N removal and 2) determine how switchgrass harvest date influences biofuel quality from fast pyrolysis.

Thesis Organization

The following thesis will be organized into two research papers. The first research paper, to be submitted to *Agronomy Journal*, outlines a field study conducted to determine how [N] changes in above- and below-ground biomass of switchgrass over the annual growing season and its effect on yields and N removal. The second research paper, in review at BioEnergy Research, outlines a study conducted to determine how harvest date of switchgrass over the annual growing season influences N and yield of biomass and pyrolysis products (bio-oil, char, and non-condensable gas). Following the two research papers is a general conclusions section that outlines results from both studies. References used throughout each chapter of this thesis will be cited at the end.

CHAPTER 2. WHERE'S THE NITROGEN? SEASONAL CHANGES IN SWITCHGRASS NITROGEN DISTRIBUTION

A paper to be submitted to Agronomy Journal

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Abstract

Nitrogen (N) is a critical nutrient for biomass production, but reductions in N removal from agroecosystems via harvested feedstocks are needed to minimize environmental and economic costs of energy cropping and to improve its quality for thermochemical conversion to fuel. Reducing the [N] of switchgrass can be done simply and easily through harvest management, which exploits seasonal nutrient cycling of N, but detailed knowledge of the temporal and spatial distribution of N in above- and below-ground tissues is currently lacking. This study investigated: 1) How does [N] in above- and below-ground biomass of switchgrass change over the growing season? 2) How does harvest time affect the yield? and 3) How is N partitioned between the shoot components and below-ground biomass? Above- and below-ground (to 1 m) switchgrass biomass was harvested at five dates from field plots in central Iowa. As expected, above-ground [N] decreased during the growing season, with no further change from post-frost to spring harvest. Meanwhile, the [N] of below-ground biomass increased significantly from July to post-frost in upper strata of the soil ($P=0.0040$). Harvesting at peak biomass, prior to senescence, lead to significant yield reductions the following season ($P<0.0001$), suggesting this harvest strategy is not sustainable in the long-

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term. Delaying harvest to post-frost reduced harvested biomass, but less N was removed with harvest and more was stored in below-ground tissues. Compared to a continuous corn with 50% stover removal system, a post-frost switchgrass harvest would remove 114 kg N ha⁻¹ compared to 1,050 kg N ha⁻¹ over a five year period.

Introduction

United States bioenergy demand

Tilman et al. (2011) estimated that there would be a 100-110 % increase in the global demand for crops from 2005 to 2050; an increase that would result in the expansion and intensification of agricultural land and greater nitrogen (N) inputs to increase yields with less land. At the same time that food demand is increasing, there is added pressure on agricultural lands to also produce energy. For example, United States energy legislation requires that 21 billion gallons of advanced biofuels be used per year by 2022 (EISA, 2007; Perlack et al., 2011). This is projected to: 1) increase our energy security by reducing our dependence on fossil fuels for energy and 2) reduce the dependence of global agriculture on fossil fuels (EISA, 2007; Perlack et al., 2011; Valentine et al., 2012). The use of dedicated ‘next-generation’ perennial crops has been promoted to meet our biofuel demands because they require low agronomic inputs, can be grown on lands marginal for food crop production, and they do not compete with food supplies (IPCC, 2007; Nijssen et al., 2012; Parrish and Fike, 2005; Sanderson and Adler, 2008; Sang, 2011; Sang and Zhu, 2011; Valentine et al., 2012).

Switchgrass production

Switchgrass (*Panicum virgatum* L.) is a warm-season, perennial grass native to the tallgrass prairie regions of North America (Parrish and Fike, 2005) and has a long history as

a biofuel feedstock candidate (Fike et al., 2006a; Vogel, 1996). Since switchgrass is native to the Midwest, it poses little threat for becoming invasive (Perlack et al., 2011), which gives it an advantage over other biomass crops. Switchgrass has been used by the U.S. Dept. of Energy as a “model” biomass crop for biofuel production (Sanderson et al., 2006; Vogel, 1996; Wright and Turhollow, 2010) and is a leading perennial biomass feedstock that is planted on thousands of acres and planned for millions more in the United States (Perlack et al., 2011). Several characteristics make switchgrass an ideal crop for bioenergy. It is high yielding and can be maintained for 10 years (Evanylo et al., 2005; Fike et al., 2006b; Perlack et al., 2011) and possibly 15-30 years (Valentine et al., 2012). It also requires minimal inputs and is well suited to marginal lands (Perlack et al., 2011; Wright and Turhollow, 2010), which minimizes competition with primary food production (Valentine et al., 2012). Although fertilizer inputs are necessary for optimum switchgrass yields and to replace nutrients removed with biomass harvest, agronomic management practices have the potential to reduce the amount of inputs needed, particularly those related to N (Mitchell et al., 2008; Perlack et al., 2011; Tilman et al., 2011; Vogel et al., 2002; Wright and Turhollow, 2010).

Internal N cycling

Manipulate the biology

Nitrogen is needed in greater quantities, compared to other nutrients, for plant biomass generally, and switchgrass production in particular (Lemus et al., 2008; McLaughlin and Kszos, 2005; Parrish and Fike, 2005). Conversely, high nitrogen concentration ([N]) is undesirable in the harvested biomass feedstocks for two reasons: 1) it is economically and environmentally expensive to replace N removed from cropping systems and 2) N reduces the conversion efficiency of biomass to biofuel, especially when using a thermochemical

process (Boateng et al., 2006; Lewandowski and Kicherer, 1997; McKendry, 2002). A simple management strategy can be used to minimize the amount of N harvested with perennial feedstocks. Immediate reductions in the [N] of the harvested portions of perennial feedstocks can be achieved by exploiting seasonal nutrient cycling (translocation). During plant senescence, nutrients, especially N, are moved from actively growing tissues to below-ground tissues where it can be stored and used for new growth (Beale and Long, 1997; Clark, 1977; Cooke and Weih, 2005; Hayes, 1985; Heaton et al., 2004; Parrish and Fike, 2005; Somerville et al., 2010; Van Heerwaarden et al., 2003).

Benefits of N cycling

Successfully providing both harvestable biomass and agroecosystem benefits by properly managing biomass cropping systems is necessary for their long-term future (Robertson et al., 2008; Tilman et al., 2009). Several investigations have found that maximum switchgrass yields can be achieved with a mid-August harvest in the Midwestern United States (Adler et al., 2006; Casler and Boe, 2003; Vogel et al., 2002). Although maximum yields are achievable, harvesting in mid-summer also removes N with the plant (McLaughlin and Kszos, 2005) depriving the plant of N that would otherwise be stored in the below-ground tissues at the end of the season for new crop growth. Casler and Boe (2003) found that harvesting when above-ground tissues had not fully senesced resulted in reduced stand density and biomass yields over time; consequently, recommendations in the Midwestern US have been to wait until after a killing frost (Adler et al., 2006; Casler and Boe, 2003). Allowing switchgrass to fully senesce and translocate nutrients to below-ground storage organs will improve feedstock quality, as well as minimize the nutrient requirements for a sustainable biomass production system (Fike et al., 2006a; Heggenstaller et al., 2009;

McLaughlin and Kszos, 2005; Muir et al., 2001; Parrish and Fike, 2005; Reynolds et al., 2000; Vogel et al., 2002), while improving the energy output to input ratio (Valentine et al., 2012).

By contrast, corn production requires large quantities of N; most of this N is supplied from chemical fertilizers and removed with the grain. Stover harvest, widely expected to provide the majority of cellulosic biomass in the Midwest and already in pilot testing in Iowa, removes even more N (Hoskinson et al., 2007). Nitrogen fertilizers are the most energy-consuming part of corn grain production and their inefficient use leads to loss from the system (Ma and Dwyer, 1998).

Feedstock quality

In the future, switchgrass may be converted to a fuel through a thermochemical process; e.g., pyrolysis, gasification or combustion. Feedstock quality requirements differ depending on the conversion process used; additionally, the quality of biomass affects biofuel quality (Adler et al., 2006; Sanderson and Adler, 2008) (Ch. 3). Investigations have found a later harvest leads to increased biofuel quality (Adler et al., 2006; Lewandowski and Heinz, 2003; Lewandowski et al., 2003b; Vogel et al., 2002) (Ch. 3). It is unclear when and how much N is recycled to below-ground tissues at the end of the growing season (Sanderson and Adler, 2008; Sanderson et al., 2006) and how much N is removed with the feedstock (Reynolds et al., 2000). Knowing when and how much N translocates in switchgrass will enable management that reduces the need for N fertilizer, minimizes N removal, increases stand longevity, and improves feedstock quality for thermochemical processes.

Objectives of research

This paper explores the temporal and spatial resolution of N translocation during the annual growing season to determine harvest time and guide crop management for improved feedstock quality. The following research questions were addressed:

- 1) How does the [N] in above- and below-ground biomass of switchgrass change over the annual growing season?
- 2) How does harvest date affect the yield of switchgrass?
- 3) How is N partitioned between the different shoot components and below-ground depths of switchgrass?

Materials & Methods

Study site

Field trials of switchgrass ('Cave-In-Rock') were established in spring 2008 at the Iowa State University South Reynoldson Farm in Boone County, IA (41° 55' N, 93° 44' W). The field was previously managed in a corn (*Zea mays* L.) and soybean (*Glycine max* L.) rotation with soybean grown in 2007. Soils at the experimental site were classified as Nicollet loam (fine-loamy, mixed, superactive, mesic Aquic Hapudolls), Webster silty clay loam (fine-loamy, mixed, superactive, mesic Typic Endoaquolls), and Clarion loam (fine-loamy, mixed, superactive mesic Typic Hapludolls). Mean monthly air temperature and total precipitation were measured approximately 15-km from the site at the ISU Agronomy and Agricultural Engineering Farm during 2009, 2010, and 2011, and were compiled from the Iowa Environmental Mesonet (2012) (Fig. 1, 2).

Experimental design and establishment

The experiment was a randomized complete block design with four replications ($n = 4$) and treatments were arranged in a split-plot design. It was conducted during the 2009 and 2010 growing seasons. Switchgrass and corn were the main plot treatments, (plot size: 18.3×27.4 m) and harvest date (June, July, September, post-frost, and spring of the next year) was the subplot treatment (plot size: 3.7×27.4 m) within switchgrass plots. Switchgrass was seeded in May 2008 with a 3.7-m wide drill that dropped the seed and cultipacked at a seeding rate of 5.45 kg pure live seed (pls) ha⁻¹, and was fertilized with 84 kg N ha⁻¹ of NH₄NO₃ (ammonium nitrate) in April 2009 and 2010. Weed control in 2009 consisted of hand weeding horseweed (*Conzya Canadensis* L.) from plots in June. Weed control in 2010 included a 2% and 5% solution of glyphosate (*N*-(phosphonomethyl)glycine) sponged on grass weeds and an application of 2-4,D (2, 4-dichlorophenoxyacetic acid) at a rate of 1.12 kg ha⁻¹ with crop oil on 7 June 2010. Switchgrass harvest dates were chosen to reflect both ends of the growing season in central Iowa (Heaton et al., 2009), (Table 1), and are hereafter referred to as June, July, September, post-frost, and spring harvest dates. Biomass from each growing season was allowed to stand over the winter and the spring harvest represents the end of the season. On 8 May 2009, corn main plots were seeded (Agrigold 6325 VT3-104day maturity) in 0.76-m rows at 82,500 seeds ha⁻¹ in 2009 with 84 kg N ha⁻¹ (32 % liquid urea ammonium nitrate) injected at planting and an additional 134 kg N ha⁻¹ injected between alternate rows on 17 June. On 6 May 2010, corn main plots were seeded (Agrigold 6325 VT3-104day maturity) in 0.76-m rows at 83,000 seeds ha⁻¹ with 87 kg N ha⁻¹ (32 % liquid urea ammonium nitrate) injected at planting and an additional 82 kg N ha⁻¹ injected between alternate rows on 17 June. Weed control in corn plots included an application of glyphosate

(*N*-(phosphonomethyl)glycine) at a rate of 1.12 and 0.84 kg ha⁻¹ (active ingredient) in 2009 and 2010, respectively.

Above-ground measurements

Switchgrass harvest

Above-ground switchgrass samples were hand cut with a hand clipper (Corona, Model no. BP6250) 5 cm above soil surface from two 1-m² quadrats randomly placed in the subplot with 1-m border from the edge of the subplot. Each sample from the 1-m² quadrats was weighed and tillers were counted. A subsample was taken, tillers counted, and separated into specific plant components (stems, viable leaves, e.g., >50 % green tissue, dead leaves, flowers, and litter) to determine the proportion of each represented to estimate subplot yield. Subsamples were then dried to a constant mass at 60 °C in a forced air oven. Component dry matter yield was calculated by multiplying its proportion of total dry subsample mass by the dry matter of the harvested 1-m². Following removal of hand harvested quadrats, the entire 3.7×27.4-m subplots were harvested with a self propelled forage harvester (John Deere Model 5830, John Deere Co., Moline, IL) with a direct cut 3.7-m head. Attached to the forage harvester was a Stan Hoist barge box weigh wagon equipped with a weigh scale (Model M640, Avery Weigh-Tronix, Fairmont, MN) used to collect the biomass from each field replicate at the specific harvest dates.

Corn grain and stover harvest

Samples of corn grain and stover were hand harvested at approximately 5-cm height from two 1-m² quadrats placed over two rows in the plot on 9 October 2009 and 27 September 2010. Biomass from each 1-m² quadrat was weighed and tillers counted. A subsample of 5 stalks was taken and separated into specific plant components (corn grain,

stalks with cobs, leaves with husks, flowers, and litter) to determine the proportion of each and estimate yield. Subsamples were dried to a constant mass at 60 °C in an air forced dryer. Component dry matter yield was calculated by multiplying its proportion of total dry subsample mass by the dry matter of the harvested 1-m².

Below-ground biomass measurements

Switchgrass and corn

Switchgrass and corn below-ground biomass samples were collected after removal of above-ground biomass by using a 41-mm diameter soil core attached to a truck-mounted hydraulic soil probe (Giddings Machine Co., Ft. Collins, CO). Four soil cores were taken from the same two quadrats in each subplot; two cores were taken directly over a plant and two taken between plants to a depth of 0.5-m in June, July, and September and to 1-m at the post-frost and spring harvests. After removal, each core was separated into depth increments (0-0.15, 0.15-0.30, 0.30-0.45, 0.45-0.60, 0.60-0.75, 0.75-0.90, 0.90-1.0 m) and stored in plastic bags at 5 °C until processed. Below-ground samples from the 0- to 15-cm depth included plant crowns and rhizomes, representing whole plant material (Dohleman et al., 2012; Heggenstaller et al., 2009). Following removal from cold storage, below-ground biomass was separated from bulk soil by washing in a soil elutriator (Wiles et al., 1996) for 2 h. Remaining soil and debris not removed by the elutriator was hand processed to ensure all soil particles and non-root material was removed from below-ground tissues using strainers (CIA Masters collection, model no. 23335). All below-ground biomass was rinsed with distilled water, oven dried at 60°C for 24 h, and weighed.

Analysis of biomass for total C and N content

Switchgrass and corn above-ground components were ground to 1-mm by using a Thomas-Wiley mill (Model 4, Arthur H. Thomas Co., Philadelphia, PA). To have sufficient sample quantity for analysis, the four cores from under and the four cores from between plants were pooled within blocks; similarly, biomass from 60- to 100-cm increments had to be combined for analysis. Switchgrass and corn below-ground biomass was ground to \approx 1-mm using a Cyclone UDY sample mill (UDY Corp., Fort Collins, CO). A 100- to 150-mg sample of plant components and below-ground biomass were weighed on an analytical balance (Mettler AE200, Mettler-Toledo Inc. Columbus, OH) and enclosed in an aluminum foil capsule to be analyzed for carbon, hydrogen, and N (CHN) (LECO TruSpec CHN, LECO Corp. St. Joseph, MI) at the Iowa State University Soil and Plant Analysis Laboratory and Center for Sustainable Environmental Technologies (CSET). The N content of above- and below-ground tissues was calculated by multiplying the [N] by the biomass yield.

Data analyses

Data were analyzed using a mixed model analysis of variance and protected mean separation (PROC GLIMMIX, SAS 9.2, SAS Institute Inc., Cary, NC, USA). Data that exhibited non-homogeneity of variance (above-ground corn and below-ground corn and switchgrass) were log transformed. Switchgrass harvest date, year, component, and their interactions were considered fixed effects and random effects were harvest date \times block, harvest date \times block \times year, and harvest date \times block \times year \times rep. Switchgrass harvest date, year, depth, and their interactions were considered fixed effects for switchgrass below-ground yield, [N], and N content. Random effects were harvest date \times block and core \times spacing (block \times harvest date \times rep \times year). Significant effects were determined using the F-

Statistic and $\alpha=0.05$. The differences between least-squared means were determined with a Tukey's adjustment.

Results & Discussion

This study investigated the temporal and spatial resolution of N in switchgrass over two growing seasons in central Iowa. Mean monthly air temperature (Fig. 1) during the duration of this experiment was average and conducive to switchgrass plant growth. Precipitation varied by year (Fig. 2); rainfall was greater than average (spring) and below average during the periods of active growth in 2009, and above average in 2010 (June, July, August, and September). Three specific questions were addressed and each is now considered.

Nitrogen concentration

1) How does the [N] in above- and below-ground biomass of switchgrass change over the annual growing season?

Above-ground [N]

As expected, the [N] in above-ground tissues decreased during the growing season (Fig. 3), a trend that was consistent over both years, as indicated by a non-significant interaction between harvest date and year (Table 2). This agrees with literature and other investigations of perennial grasses (Beale and Long, 1997; Heaton et al., 2009; Kering et al., 2012; Lewandowski et al., 2003a; Lewandowski et al., 2003b; Waramit et al., 2011). In central Iowa, rapid switchgrass vegetative growth occurs during June, followed by stem elongation and initiation of reproductive growth in July. During these months, a stand is predominantly comprised of stems and viable leaves. The latter are actively photosynthesizing, (Dohleman et al., 2009) and thus a major portion of plant N is a

component of photosynthetic enzymes (Cooke and Weih, 2005), as indicated by the consistently high concentrations of N in viable leaves during the early summer of both years of this experiment. The N concentration of viable leaves was always significantly higher than the [N] of stems ($P < 0.0001$, Fig. 3). As the growing season progressed, there was a shift in N allocation from leaves and elongating stems to flowers, as well as to senescing and shedding lower (dead) leaves, which still contained some N (Fig. 5). In both years, stems from both the post-frost and spring harvest had significantly lower [N] than did dead leaves ($P < 0.0001$ and $P = 0.0350$, respectively in 2009 and $P < 0.0001$ and $P = 0.0003$ in 2010, Fig. 3). Generally, delaying the harvest until spring did not lead to a significant decrease in the [N] of any measured plant component (Fig. 3). This disagrees with Adler et al. (2006) who found that leaving the switchgrass to stand over the winter season will further decrease [N], but agrees with Heaton et al. (2009) who found minimal reductions in biomass [N] from spring harvest compared to post-frost.

Below-ground [N]

Concentrations of N varied throughout the measured rooting profile, and this in turn varied by year, as indicated by significant harvest date \times year interactions within both the 0- to 45-cm and 45- to 100-cm depth classes ($P = 0.0011$ and $P = 0.0200$, respectively, Table 3). Generally, the seasonal trend in below-ground biomass [N] was reciprocal to that of the above-ground biomass, increasing later in the season and suggesting translocation from above- to below-ground tissues (Fig. 3). This pattern of translocation from senescing tissues to storage organs has been documented in switchgrass and other prairie grasses in response to both drought and cold (Adams and Wallace, 1985; Hayes, 1985; Heckathorn and DeLucia, 1994; Heckathorn and Delucia, 1996), but the amount of plant N conserved in living tissues

varies considerably by species (Adams and Wallace, 1985). The [N] of below-ground biomass also changed significantly with depth (Table 3) with the highest concentrations in the top layers of the profile (Fig. 3), which has not been well documented in switchgrass stands. Averaged across all harvest dates and both years, the [N] was highest in the 0- to 15-cm range of the rooting profile ($P < 0.0001$). Each year, the [N] in this layer was consistently lowest in July, then significantly increased by winter ($P = 0.0135$ and $P < 0.0001$ in 2009 and 2010, respectively, Fig. 3). During rapid vegetative growth in the early summer, N reserves in below-ground biomass are depleted as a flush of N goes to growing tissues (Hayes, 1985) and following this depletion of N, below-ground [N] increases as viable leaves start to senesce (Hayes, 1985; Lemus et al., 2008).

Biomass yields

2) How does harvest time affect the yield of switchgrass?

Above-ground yield

Generally, biomass yields in this study (Fig. 3) were similar to, or lower than, those for Cave-in-Rock in other Midwestern research trials (Dohleman et al., 2012; Heaton et al., 2004; Heaton et al., 2008) but greater than those from farm-scale trials (Lemus et al., 2002; Schmer et al., 2008). The amount of above-ground biomass produced at different harvest dates varied between years, as did the amount partitioned between plant components, as indicated by highly significant interactions of harvest date \times year and harvest date \times year \times component (Table 2). Total above-ground yields were significantly greater in 2009 compared to 2010 ($P < 0.0001$, Fig. 4). Most dramatically, the peak yields in July 2009 were followed by a 57% lower biomass yield from those subplots the following season in July 2010 ($P < 0.0001$, Fig. 4). In cool, temperate climates like the upper Midwest, harvesting

before switchgrass has completely senesced can impact long-term yields (Casler and Boe, 2003). Considering that the weather in both years was similarly conducive to plant growth (occasional moisture deficit in 2009 and excess in 2010, with average temperatures throughout), we hypothesize that removing vegetative biomass in 2009 reduced subsequent biomass yield in 2010, as has been shown in previous studies where switchgrass is harvested before senescence, and reviewed in Parrish and Fike (2005). Cave-in-Rock is an upland type selected from a humid location, but it is well suited for drier areas (David and Ragauskas, 2010; Lemus et al., 2002; Perlack et al., 2011). This may be why yields suffered in 2010, but conversely some suggest that it is suited for high humidity areas (Lewandowski et al., 2003a). Lee and Boe (2005) found in their Cave-in-Rock studies in South Dakota that increased precipitation during July to August increased biomass yields later in the season. Since this experiment encompassed only two years, further long-term yield studies would be necessary to determine the effects of environment vs. harvest management.

Even though we found peak yields in July 2009 (12.4 Mg ha^{-1}), they were not significantly different than those harvested in September 2009 ($P=0.8143$, Fig. 4). Lee and Boe (2005) also found Cave-in-Rock yields peaked in late July in South Dakota. Typically, in the central US, a one-cut switchgrass harvest occurs post-frost or in the spring after the stand has allowed to go dormant and effectively wash nutrients. However, yield reductions from tiller lodging, particularly in high snowfall regions, are always a concern. Delaying switchgrass harvest from peak yield in July 2009 to post-frost harvest resulted in a yield reduction of 54 % ($P=0.0217$), compared to the 10-20 % Adler et al. (2006) found. In 2009, the post-frost yield (10.0 Mg ha^{-1}) was significantly greater than the spring harvest (5.6 Mg ha^{-1}) ($P=0.0005$), but in 2010, there was no significant difference in total biomass yields (6.6

Mg ha⁻¹ and 5.7 Mg ha⁻¹, respectively; P=0.9209, Fig. 4). Adler et al. (2006) found an 11 % yield reduction with a spring harvest compared to a post-frost harvest compared to our 44 % yield reduction in 2009 and 13 % in 2010.

The amount of biomass harvested in different components varied by harvest date and by year. Significantly more stem biomass was harvested in September compared to post-frost in 2009 and 2010 (P<0.0001 and P<0.0001, respectively, Fig. 4), possibly reflecting a loss of leaf-sheath from the stem. The amount of biomass in stems at the post-frost harvest was significantly greater than that in dead leaves, the other major component of harvestable biomass at that point in the season, in both 2009 and 2010 (P<0.0001 and P<0.0001, respectively) (Fig. 4). Heaton et al. (2009) also found dead leaves still attached to switchgrass by the end of the season.

Below-ground yield

There was no significant harvest date × year interaction for below-ground biomass yields, although in the top 45-cm there was a significant harvest date effect and for 45- to 100-cm there was a significant year effect (Table 4) suggesting the majority of seasonal fluxes are occurring in upper layers of the rooting profile. Averaged over years, there was significantly less biomass between 0- to 45-cm in June than there was in July, September, and post frost (P=0.0372, P=0.0128, and P=0.0197, respectively) and these later dates were not significantly different from one another, again suggesting that resources are translocated from below-ground storage organs to actively growing shoots, then replaced as the season progresses. The main effect of depth was highly significant for biomass yields throughout the measured soil profile (Table 4). The top 15-cm had significantly more biomass than the next 30-cm (P<0.0001), which is similar to results reported by Frank et al. (2004), Ma et al.

(2000), and Jarchow and Liebman (*in press*). Also, we found a significant reduction in below-ground biomass with increasing depth, which agrees with Frank et al. (2004) and the general morphology of the switchgrass root system, especially when moisture is not limiting.

Nitrogen removal

3) How is N partitioned between the different shoot components and below-ground depths of switchgrass?

Above-ground N content

All main effects and interactions related to N content were highly significant (Table 2). Total above-ground N content was significantly greater in 2009 compared to 2010 ($P < 0.0001$, Table 2, Fig. 5), reflecting the greater above-ground biomass yields in 2009 compared to 2010 (Fig. 4). Switchgrass that was harvested at peak biomass in July 2009 (Fig. 4), and still had N-rich viable leaves (Fig. 3), resulted in N removal of 112 kg N ha^{-1} in July compared to 40 kg N ha^{-1} in spring when the [N] had decreased in above-ground tissues ($P < 0.0001$, Fig. 5). This agrees with work on other perennial grasses finding maximum N removal coincides with maximum biomass yields and minimum below-ground nutrient concentrations (Beale and Long, 1997; Dohleman et al., 2012). Additionally, Adler et al. (2006) found that harvesting during peak biomass removed 2 times more N, and subsequently reduced yields, compared to a post-frost harvest. We found 1.5 times more N removed in July 2009 compared to the post-frost harvest. Further, the total N content of biomass from the post-frost harvest in 2009 was significantly greater than that from the following spring harvest ($P = 0.0006$), but this was driven by reductions in harvestable yield rather than [N] as described earlier. No such changes were evident in 2010: the N content of biomass harvested post-frost 2010 was not significantly different than spring 2011

($P=0.8088$, Fig. 5). The only significant difference in above-ground N content in the second season was the September harvest that removed 69 kg N ha^{-1} compared to the spring harvest that removed 36 kg N ha^{-1} ($P=0.0034$, Fig. 5).

Viable leaves removed significantly more N compared to stems in June 2009 and 2010 (Fig. 5), which coincides with [N]. A large contributing component to N content at later harvest dates is litter harvested from the ground. There is significantly more N removed with litter compared to stems from post-frost biomass in 2009 and 2010 ($P<0.0001$ and $P<0.0001$, respectively) and from spring biomass ($P<0.0001$ and $P<0.0001$, respectively, Fig 5), indicating the importance of leaving litter on the field for long-term sustainability of switchgrass cropping systems. We recommend that harvest of switchgrass biomass crops avoid litter removal, thus the N in this material would be available for cycling through the ecosystem, with some further uptake by switchgrass expected in later years (Clark, 1977). If litter was left on the field at the post-frost and spring harvest dates, the N removal would be reduced by 50 % and 70 % in 2009 and by 62 % and 73 % in 2010. A post-frost harvest would remove between 18 and 39 kg N ha^{-1} , which, similar to above-ground yield, is within or slightly less than other reported values (Anderson et al., 1997; Beale and Long, 1997; Heaton et al., 2009; Heggenstaller et al., 2009; Prophet and Staggenborg, 2010; Reynolds et al., 2000; Vogel et al., 2002).

Below-ground N content

The amount of N stored in below-ground biomass changes significantly with depth throughout the top 100-cm (Table 5). The majority of N stored in the measured profile was in the top layer, reflecting the greater biomass production and [N] found at this depth ($P<0.0001$, Fig. 5). Clark (1977) also found that N content is concentrated in the upper 10-cm

of below-ground biomass. By the end of the growing season in both years, more N was stored in below-ground tissues than was removed at harvest, indicating reduced need for external N fertilizer in late-harvested cropping systems.

Corn yields, [N], and N content

Above-ground (grain and stover) and below-ground

Generally, grain and stover yields were comparable, although slightly greater in the first year (Table 6 and 8), to those found in other Midwestern trials (Anderson et al., 1997; Avila-Segura et al., 2011; Jarchow and Liebman, *in press*; Propheter and Staggenborg, 2010; Varvel et al., 2008). Below-ground biomass was 1.9 and 1.3 Mg ha⁻¹ in 2009 and 2010, respectively (Table 7 and 8), which is consistent with continuous corn treatments (Jarchow and Liebman, *in press*). Corn below-ground biomass is less than switchgrass below-ground biomass reported in this study and compared to other studies (Beale and Long, 1997; Bransby et al., 1998; Heggenstaller et al., 2009; Jarchow and Liebman, *in press*; Lemus et al., 2008; Ma and Dwyer, 1998; McLaughlin and Kszos, 2005; Zan et al., 1997). More than 88% of the total corn biomass was found in the above-ground components compared to roughly 50% for switchgrass, which is consistent with results from Jarchow and Liebman (*in press*). The [N] of above- and below-ground corn biomass was similar to switchgrass [N], but much greater N removal rates were found and are typical due to greater yields. Unlike switchgrass and other perennial crops, the [N] in the below-ground biomass of corn will not be recycled to new growth in the spring and instead, N inputs will be needed in greater quantities compared to switchgrass for the next season.

Impacts

We have reported measurements that provide spatial and temporal resolution of biomass and N distribution in above- and below-ground tissues of switchgrass. Based on this work, we found delaying switchgrass harvest overwinter does not improve feedstock quality as indicated by a reduction in [N], but does carry a significant yield penalty in some years, thus, we recommend harvesting post-frost, rather than during early spring, in central Iowa. Even if litter is left in the field, the biomass harvested post-frost will still remove at least 40 kg N ha⁻¹, or approximately 6-8 kg N per harvested Mg, and therefore some fertilizer would still be required in order to maintain stands in the long-term.

How do switchgrass yields and N content/removal compare to corn (grain + stover)? Overall, corn produced significantly more biomass than did switchgrass ($P < 0.0001$, Table 9), with concomitantly greater N content/removal ($P < 0.0001$). Neither switchgrass biomass yield nor N content varied significantly between years of this experiment at post-frost harvests ($P = 0.0981$ and $P = 0.3951$, respectively) despite variable weather conditions, emphasizing the stability of this perennial crop. Though this study was limited to one location over two growing seasons, post-frost switchgrass N removal rates were similar to other investigations (Anderson et al., 1997; Heaton et al., 2009; Heggenstaller et al., 2009; Jarchow and Liebman, *in press*; Lemus et al., 2008; Propheter and Staggenborg, 2010; Reynolds et al., 2000; Vogel et al., 2002) while corn N removal rates were slightly higher than those reported (Anderson et al., 1997; Avila-Segura et al., 2011; Heggenstaller et al., 2008; Jarchow and Liebman, *in press*; Propheter and Staggenborg, 2010). If these biomass cropping systems are harvested with the same yields and management for the next five years, there would be approximately 1,050 kg N ha⁻¹ removed with corn grain and 50% stover

harvest and 114 kg N ha⁻¹ removed with switchgrass. Nutrient removal has an economic and environmental cost that may not be supported by biomass produced in input-intensive annual cropping systems (Jarchow and Liebman, *in press*). Though corn did produce more biomass than switchgrass in both years, corn grain and stover yields declined between years of this study, and several studies have found that continuous corn production in a no-till system with stover removal reduces crop yields in subsequent years (Blanco-Canqui et al., 2006; Jarchow and Liebman, *in press*; Varvel et al., 2008; Wilhelm et al., 2004).

Conclusions

Switchgrass harvest time influences [N], yield, and therefore, N content/removal of the harvested portions of biomass. Significant yield reductions were seen the year following harvest of switchgrass during vegetative stages, i.e., before it could senesce and move N to below-ground tissues, mostly the upper 15-cm of rooting profile, for storage. There was no benefit to leaving the switchgrass stand over the winter; the [N] of the harvestable portions did not significantly decline any further and major yield reductions were seen. Harvest time manipulation of perennial biomass feedstocks is a simple and effective strategy to exploit internal N cycling to improve feedstock quality, minimize N inputs/removal, and increase long-term sustainability of cropping systems.

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Tables

Table 1. Harvest dates for above- and below-ground switchgrass biomass during the 2009 and 2010 growing season.

Harvest date	Growing season			
	2009		2010	
	Above-ground	Below-ground	Above-ground	Below-ground
June	11 June	17 June	21 June	02 July
July	20 July	24 July	20 July	06 August
September	01 September	04 September	30 August	01 September
Post-frost	13 October	16 October	08 November	03 December
Spring	20 April	06 May	04 April	04 April

Table 2. ANOVA of above-ground switchgrass biomass (flowers, viable leaves, dead leaves, stems, and litter) yield, nitrogen (N) concentration, and N content during two growing seasons (June 2009-April 2011) in Boone County, IA.

Analysis	Source of variation	Num DF [†]	Den DF	F value	P
Yield					
	Harvest date	4	15.00	48.80	<0.0001
	Year	1	15.00	113.63	<0.0001
	Year x harvest date	4	15.00	22.65	<0.0001
	Component	4	280.00	941.34	<0.0001
	Component x harvest date	16	280.00	147.97	<0.0001
	Component x year	4	280.00	23.26	<0.0001
	Component x harvest date x year	16	280.00	14.77	<0.0001
N concentration					
	Harvest date	4	14.99	12.73	0.0001
	Year	1	15.03	0.42	0.5284
	Year x harvest date	4	15.02	2.63	0.0760
	Component	4	317.20	37.97	<0.0001
	Component x harvest date	16	317.20	126.58	<0.0001
	Component x year	4	317.20	5.40	0.0003
	Component x harvest date x year	16	317.20	9.42	<0.0001
N content					
	Harvest date	4	29.82	24.12	<0.0001
	Year	1	29.82	84.39	<0.0001
	Year x harvest date	4	29.82	9.66	<0.0001
	Component	4	277.90	158.61	<0.0001
	Component x harvest date	16	277.90	165.85	<0.0001
	Component x year	4	277.90	10.08	<0.0001
	Component x harvest date x year	16	277.90	10.28	<0.0001

[†]DF=degrees of freedom

Table 3. ANOVA of switchgrass root nitrogen (N) concentration (g kg^{-1}) during two growing seasons (June 2009-April 2011) in Boone County, IA. The upper layers were sampled five times per season and the deeper layers sampled twice per season.

Analysis	Source of variation	Num DF [†]	Den DF	F value	P
N concentration (0-45 cm)					
	Harvest date	4	15	7.36	0.0017
	Year	1	54	26.31	<0.0001
	Harvest date x year	4	54	5.31	0.0011
	Depth	2	128	72.16	<0.0001
	Harvest date x depth	8	128	9.06	<0.0001
	Year x depth	2	128	6.75	0.0016
	Harvest date x year x depth	7	128	3.23	0.0022
N concentration (45-100 cm)					
	Harvest date	1	6	0	0.9955
	Year	1	19	13.54	0.0016
	Harvest date x year	1	19	6.45	0.0200
	Depth	1	21	4.95	0.0372
	Harvest date x depth	1	21	0.07	0.7967
	Year x depth	1	21	1.82	0.1912
	Harvest date x year x depth	1	21	1.94	0.1781

[†]DF=Degrees of freedom

Table 4. ANOVA of switchgrass root biomass yield during two growing seasons (June 2009-April 2011) in Boone County, IA. The upper layers were sampled five times per season and the deeper layers sampled twice per season.

Analysis	Source of variation	Num DF [†]	Den DF	F value	P
Biomass (0-45 cm)					
	Harvest Date	4	15	4.98	0.0093
	Year	1	295	1.64	0.2019
	Harvest Date x Year	4	295	0.09	0.9852
	Depth	2	616	591.80	<0.0001
	Harvest Date x Depth	8	616	1.51	0.1510
	Year x Depth	2	616	0.53	0.5904
	Harvest Date x Year x Depth	8	616	0.80	0.6053
Biomass (45-100 cm)					
	Harvest Date	1	6	1.15	0.3252
	Year	1	118	4.16	0.0437
	Harvest Date x Year	1	118	1.75	0.1880
	Depth	1	124	20.43	<0.0001
	Harvest Date x Depth	1	124	0.73	0.3943
	Year x Depth	1	124	2.93	0.0893
	Harvest Date x Year x Depth	1	124	1.58	0.2107

[†]DF=Degrees of freedom

Table 5. ANOVA of switchgrass root biomass nitrogen (N) content (kg ha^{-1}) during two growing seasons (June 2009-April 2011) in Boone County, IA. The upper layers were sampled five times per season and the deeper layers sampled twice per season.

Analysis	Source of variation	Num DF [†]	Den DF	F value	P
N content (0-45 cm)					
	Harvest date	4	15	2.21	0.1167
	Year	1	54	0.23	0.6334
	Harvest date x year	4	54	0.34	0.8507
	Depth	2	128	193.80	<0.0001
	Harvest date x depth	8	128	0.53	0.8332
	Year x depth	2	128	0.58	0.5608
	Harvest date x year x depth	7	128	0.52	0.8362
N content (45-100 cm)					
	Harvest date	1	6	2.60	0.1582
	Year	1	19	4.64	0.0442
	Harvest date x year	1	19	7.47	0.0132
	Depth	1	21	24.83	<0.0001
	Harvest date x depth	1	21	1.45	0.2413
	Year x depth	1	21	0.01	0.9383
	Harvest date x year x depth	1	21	5.85	0.0248

[†]DF=Degrees of freedom

Table 6. ANOVA of corn biomass (grain, flowers, stalks with cobs, leaves with husks, and litter) yield, nitrogen (N) concentration, and N content during two growing seasons (2009-2010) in Boone County, IA.

Analysis	Source of variation	Num DF [†]	Den DF	F value	P
Yield					
	Year	1	14.00	70.50	<0.0001
	Component	4	56.00	753.09	<0.0001
	Year x Component	4	56.00	3.98	0.0065
N concentration					
	Year	1	14.72	43.42	<0.0001
	Component	4	54.43	41.59	<0.0001
	Year x Component	4	54.43	33.63	<0.0001
N content					
	Year	1	14.60	94.09	<0.0001
	Component	4	54.38	400.01	<0.0001
	Year x Component	4	54.38	21.53	<0.0001

[†]DF=Degrees of freedom

Table 7. ANOVA of corn root biomass yield, nitrogen (N) concentration, and N content during two growing seasons (2009-2010) in Boone County, IA.

Analysis	Source of variation	Num DF [†]	Den DF	F value	P
Yield					
	Year	1	62.13	11.25	0.0014
	Depth	4	245.5	57.25	<0.0001
	Year x depth	4	245.5	1.41	0.2310
N concentration					
	Year	1	14	42.85	<0.0001
	Depth	4	26	1.08	0.3868
	Year x depth	4	26	1.062	0.1994
N content					
	Year	1	14	3.62	0.0777
	Depth	4	26	24.88	<0.0001
	Year x depth	4	26	2.78	0.0480

[†]DF=Degrees of freedom

Table 8. Above- and below-ground corn biomass yields, nitrogen (N) concentration, and N content for the 2009 and 2010 growing season in Boone County, IA.

Year	Component	Yields	N concentration	N content
		Mg ha ⁻¹	g kg ⁻¹	kg ha ⁻¹
2009		Above-ground		
	Grain	21.41a	10.84b	232.18a
	Stalks with cobs	8.68bc	4.75ef	41.47b
	Leaves with husks	3.34d	7.14cd	23.96bcd
	Litter	2.33d	9.07bc	21.22cd
	Flowers	0.17f	24.24a	4.25f
2010	Grain	13.9ab	9.37bc	131.1a
	Stalks with cobs	7.04c	4.2f	30.02bc
	Leaves with husks	2.24d	6.22cd	13.88de
	Litter	1.11e	8.92bc	9.94e
	Flowers	0.07g	4.91def	0.35g
2009		Below-ground		
	0-15 cm	1.19a	10.94a	9.61a
	15-30 cm	0.23bcd	11.82a	2.72abc
	30-45 cm	0.14de	12.34a	1.84bcd
	45-60 cm	0.11ef	12.01a	1.36bcd
	60-100 cm	0.26bc	9.78ab	2.55bcd
2010	0-15 cm	0.89ab	5.92bc	14.64ab
	15-30 cm	0.17cde	5.04bc	1.42cd
	30-45 cm	0.09ef	8.65abc	0.66abcd
	45-60 cm	0.05f	3.51c	0.14d
	60-100 cm	0.13e	6.0abc	0.46cd

† Entries in a column followed by the same letter are not significantly different (n=4, p <0.05)

Table 9. Total harvestable yields and N removal for corn (grain + stover) and switchgrass (post-frost harvest) for 2009 and 2010 growing season in Boone County, IA.

Crop	Year	Yield	N removal
		Mg ha ⁻¹	kg ha ⁻¹
Corn	2009	33.6a†	301.9a
	2010	23.3b	167.8b
Switchgrass	2009	6.7c	39.4c
	2010	4.0c	18.3c

† Entries in a column followed by the same letter are not significantly different (n=4, p <0.05)

Figures

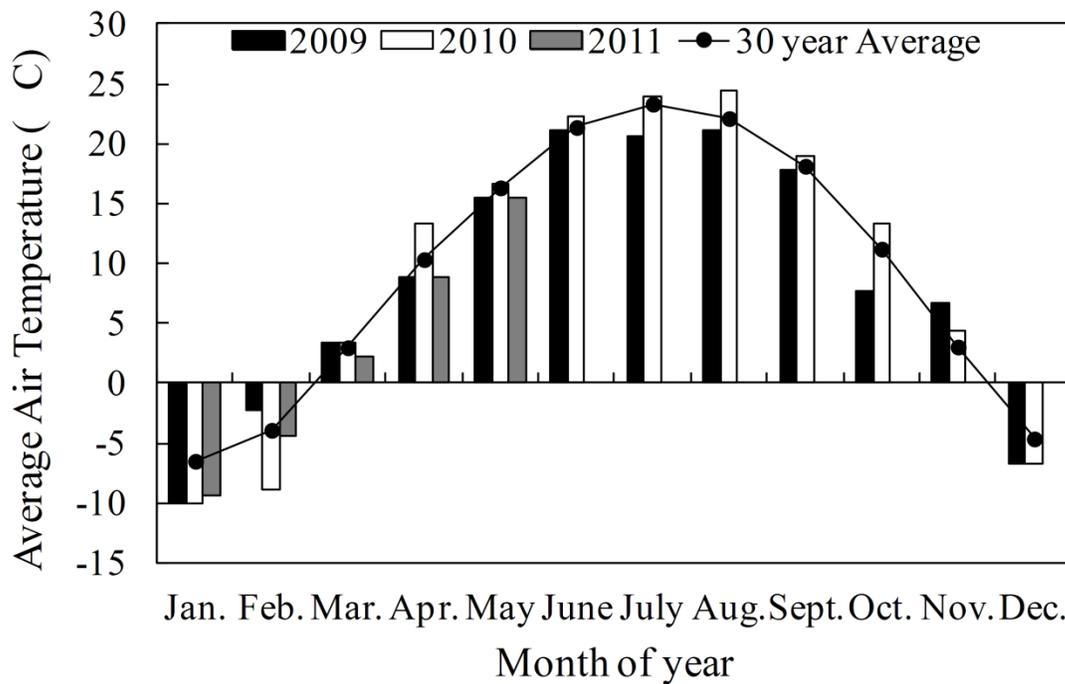


Figure 1. Mean monthly air temperature at the ISU Agronomy and Agricultural Engineering Farm in Boone County, Iowa during the duration of this experiment (bars) and the average over 30 years (line).

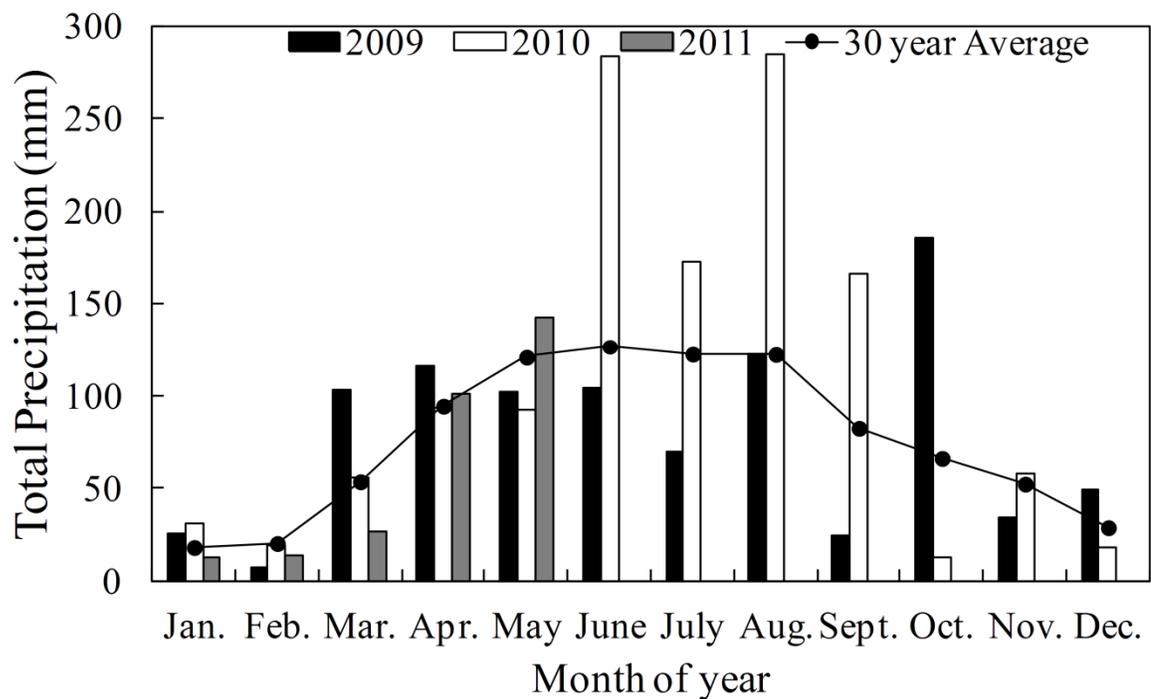


Figure 2. Total, monthly precipitation at the ISU Agronomy and Agricultural Engineering Farm in Boone County, Iowa during the duration of this experiment (bars) and the average over 30 years (line).

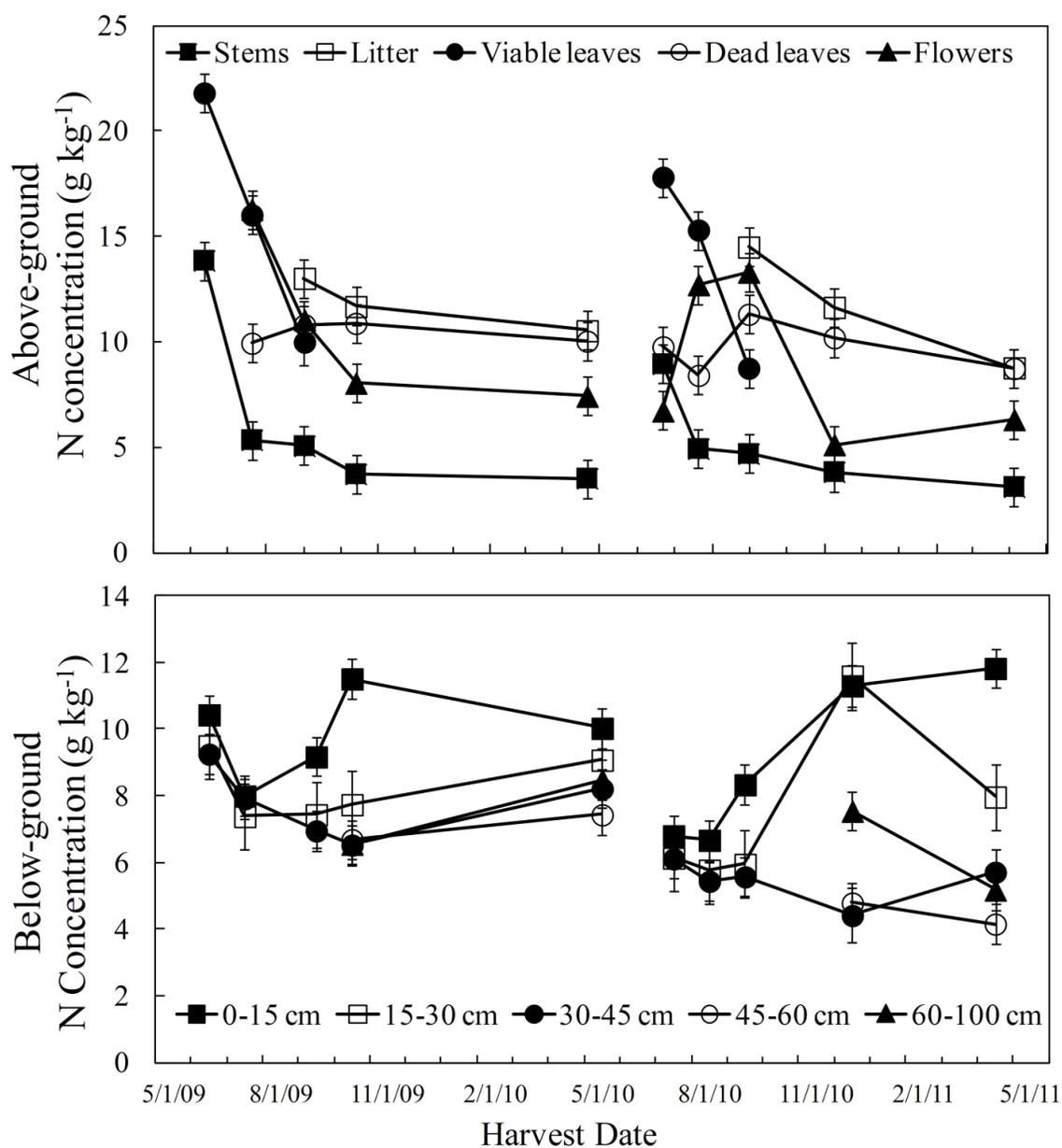


Figure 3. Switchgrass above- and below-ground biomass [N] (g kg⁻¹) for the 2009 and 2010 growing seasons in Boone County, Iowa. Least-squared means for above-ground and below-ground [N] were calculated from $n = 4$ biological blocks, and error bars indicate ± 1 standard error of the mean.

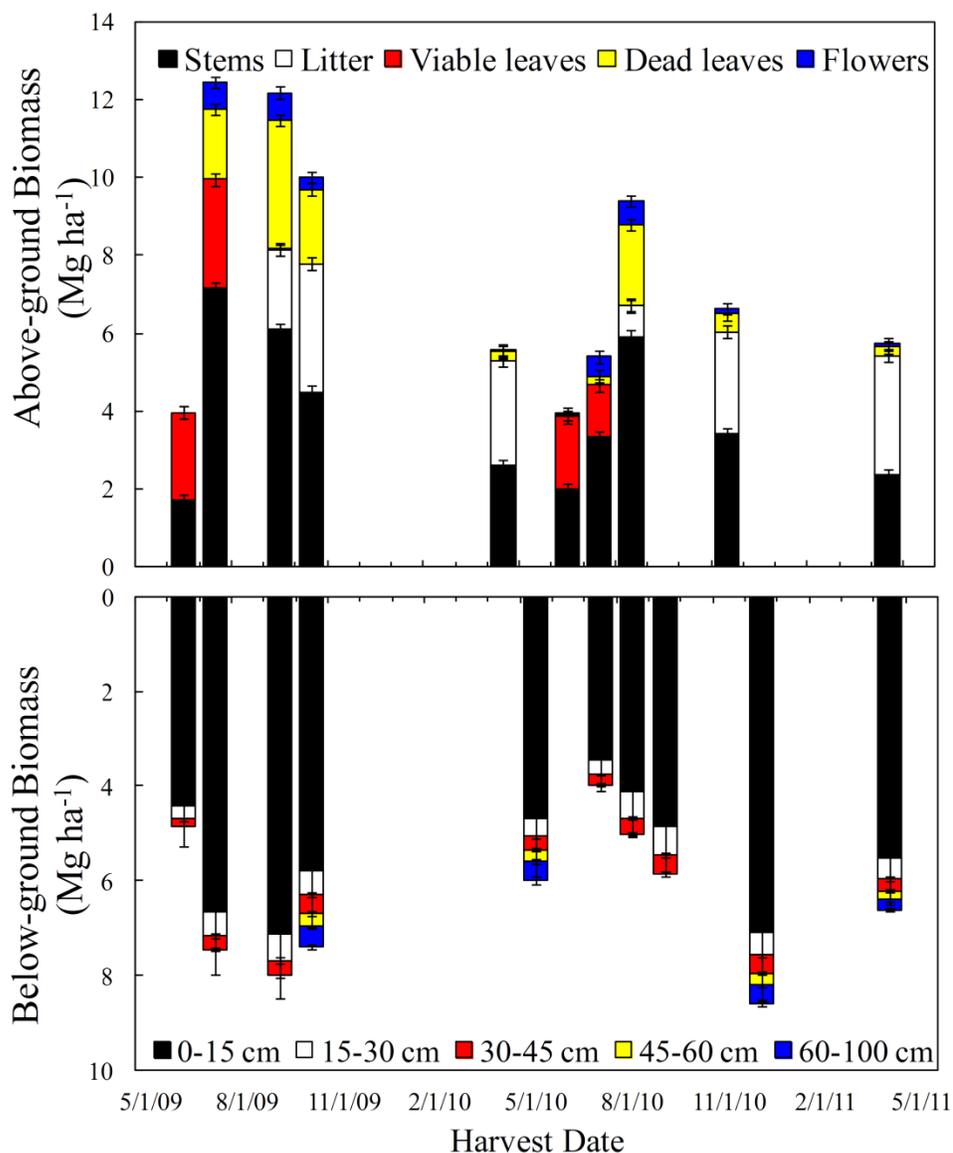


Figure 4. Switchgrass above- and below-ground biomass dry matter (DM) yields (Mg ha^{-1}) produced during the 2009 and 2010 growing seasons in Boone County, Iowa. Least-squared means were calculated from $n = 4$ biological blocks, and error bars indicate ± 1 standard error of the mean. Below-ground biomass means were calculated from $n = 4$ biological blocks, and error bars indicate ± 1 standard error of the mean.

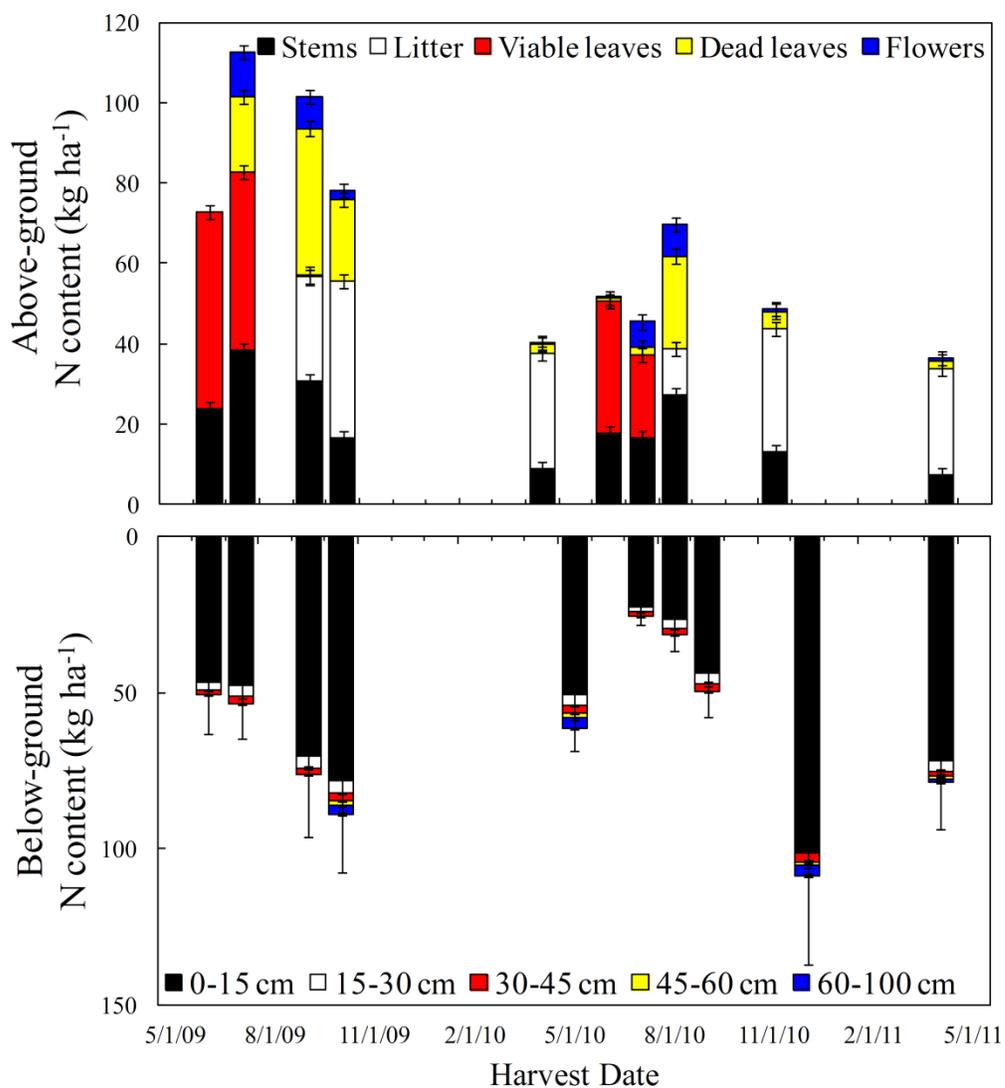


Figure 5. Switchgrass above- and below-ground biomass nitrogen (N) content/removal (kg ha⁻¹) during the 2009 and 2010 growing seasons in Boone County, Iowa. Least squared means for above- and below-ground (45-60 cm) N content were calculated from $n = 4$ biological blocks, and error bars indicate ± 1 standard error of the mean. Means for below-ground (0-45 cm) N content were calculated from $n = 4$ biological blocks, and error bars indicate ± 1 standard error of the mean.

CHAPTER 3. CROP MANAGEMENT IMPACTS BIOFUEL QUALITY: INFLUENCE OF SWITCHGRASS HARVEST TIME ON YIELD, NITROGEN, AND ASH OF FAST PYROLYSIS PRODUCTS

A paper in review at BioEnergy Research

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Abstract

Although upgrading bio-oil from fast pyrolysis of biomass is an attractive pathway for biofuels production, nitrogen (N) and mineral matter carried over from the feedstock to the bio-oil represents a serious contaminant in the process. Reducing the N and ash content of biomass feedstocks would improve process reliability and reduce production costs of pyrolytic biofuels. This study investigated: 1) How does switchgrass harvest date influence the yield, N concentration ([N]), and ash concentration of biomass and fast pyrolysis products? and 2) Is there a predictive relationship between [N] of switchgrass biomass and [N] of fast pyrolysis products? Switchgrass from five harvest dates and varying [N] from central Iowa were pyrolyzed using a free-fall reactor. Harvestable biomass peaked in August (8.6 Mg ha⁻¹), dropping significantly by November (6.7 Mg ha⁻¹, P=0.0027). Production of bio-oil per unit area mirrored that of harvested biomass at each harvest date; however, bio-oil

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yield per unit dry biomass increased from 46.6 % to 56.7 % during the season ($P < 0.0001$). Allowing switchgrass to senesce lowered biomass [N] dramatically, by as much as 68 % from June to November ($P < 0.0001$). Concurrently, bio-oil [N] declined from 0.51 % in June to 0.17 % by November ($P < 0.0001$). Significant reductions in ash concentration were also observed in biomass and char. Finally, we show for the first time that the [N] of switchgrass biomass is a strong predictor of the [N] of bio-oil, char, and non-condensable gas with R^2 values of 0.89, 0.94, and 0.88, respectively.

Introduction

Bioenergy demand

United States energy legislation mandates 21 billion gallons of advanced biofuels be used per year by 2022 (EISA, 2007; Perlack et al., 2011). In order to meet this goal, considerable research is focused on bioenergy crops that can be used to make advanced biofuels (Boateng et al., 2006; Somerville et al., 2010) like ethanol, butanol, or direct petroleum substitutes, i.e., “drop-in fuels”. Switchgrass (*Panicum virgatum* L.), a warm-season, perennial grass widely used as a model crop for bioenergy by the U.S. Dept. of Energy and others, has been planted on thousands of acres in the U.S. for this purpose and is planned for millions more (Perlack et al., 2011; Wright and Turhollow, 2010). The development of cost-effective processes to biologically convert cellulosic biomass to alcohols on a large scale still remains a roadblock, despite extensive research (Boateng et al., 2006), but a promising and practical alternative is fast pyrolysis, a thermochemical process that converts biomass to bio-oil (Boateng et al., 2006; Boateng et al., 2007; Mullen and Boateng, 2008), which can then be upgraded in a manner similar to petroleum refining to produce hydrocarbon fuels (Bridgwater, 1994; Bridgwater, 1996; Czernik and Bridgwater,

2004; Huber et al., 2006). This paper explores the possibility of improving pyrolysis-based biofuels by minimizing the amount of nitrogen (N) in the biomass feedstock, which is a primary contaminant in bio-oil upgrading.

Fast pyrolysis

Fast pyrolysis is a process wherein biomass is rapidly heated in the absence of oxygen (O) to convert biomass to solid, liquid, and gas fractions (Bridgwater et al., 1999; Czernik and Bridgwater, 2004; Mohan et al., 2006). The liquid fraction, bio-oil, is the main product formed during fast pyrolysis. Bio-oil is a dark brown liquid physically resembling petroleum (Czernik and Bridgwater, 2004), that is made up of a mixture of polar organic compounds and water (Bridgwater et al., 1999). Char and non-condensable (NC) gas are co-products, typically comprising 10-30 % and 20 %, respectively, of the total products on a feedstock weight basis (Brewer et al., *in press*; Laird, 2008). The char contains virtually all of the mineral (ash) content from the original feedstock (Brewer et al., *in press*). Char and NC gas can be used for heating the system thus reducing waste generated during pyrolysis and increasing overall thermodynamic efficiency (Bridgwater et al., 1999). In recent years, char has also gained attention for use as soil amendment to improve soil quality. Laird (2008) has proposed, as part of 'The Charcoal Vision', that char be used as a soil amendment for multiple benefits, including to: enhance soil quality, sequester carbon, return nutrients that were removed with biomass harvest, lower bulk density, increase drainage, aeration, and root penetration in clay soils, increase the ability of sandy soils to retain moisture and nutrients, and even act as a liming agent. The use of chars on agricultural soils supports a sustainable system for bioenergy production from biomass and helps to close nutrient cycles (Laird, 2008; Laird et al., 2010; Vanderbosch and Prins, 2011).

Ash content in pyrolysis

Some elements frequently present in biomass can reduce pyrolytic conversion efficiency. Alkali and other metals essential to plant growth promote undesirable decomposition reactions of plant polymers and influence ash content, which can cause corrosion, slagging, and fouling in thermochemical processes (Demirbas, 2004; Fahmi et al., 2007; McKendry, 2002). Ash content is of particular importance, since it has a dominant negative effect on the possible bio-oil yield from biomass; bio-oil yields decrease and char yields increase with increasing ash content (Lewandowski and Kicherer, 1997; Vanderbosch and Prins, 2011). Investigations have reported that the ash concentration in switchgrass decreases as the plant matures during the growing season and nutrients are translocated or leached from harvestable plant tissues (Adler et al., 2006; Sanderson and Wolf, 1995).

Deactivation of upgrading catalysts by N

Bio-oil from pyrolysis can be catalytically upgraded to a transportation fuel. Although N is necessary for plant growth and biomass production, it has negative consequences during bio-oil upgrading. Upgrading requires deoxygenation of the bio-oil, which can be achieved by either of two different routes: hydrotreating or catalytic vapor (zeolite) cracking (Bridgwater, 1994; Czernik and Bridgwater, 2004). Hydrotreating preserves most of the feedstock carbon as a fuel product, thus achieving a greater maximum stoichiometric yield compared to catalytic vapor cracking (Bridgwater, 1994). Hydrotreating uses a catalyst and hydrogen (H) at high temperature and high pressure to deoxygenate bio-oil compounds with the elimination of O as water and also hydrogenates many of the bio-oil compounds, producing liquid consisting largely of aliphatic and aromatic hydrocarbons (Czernik and Bridgwater, 2004).

Hydrotreating was designed to remove N, sulfur (S), and other contaminants from petroleum in addition to O (Brown, 2011; Huber and Corma, 2007), but most of the commercially available catalysts are readily poisoned by N in the feed stream. The catalysts used to hydrotreat bio-oil are similar to those used in petroleum refining and include sulfides of CoMo or NiMo supported on alumina (Czernik and Bridgwater, 2004). The nickel-based catalysts, in particular, are readily deactivated by N (Du et al., 2006; Hughes, 1984; Maxted, 1951). Other investigations have reported that elements from periodic table groups 15 (N, P, As, Sb) and 16 (O, S, Se, Te) can foul catalysts (Du et al., 2006; Maxted, 1951). Maxted et al. (1951) found that even in low concentrations, N could deactivate catalysts. The desired elemental analysis of bio-oil is approximately 0.2 % N and is typical of woody feedstocks (Bridgwater, 1994; Bridgwater, 1996; Czernik and Bridgwater, 2004); bio-oil derived from switchgrass may range from 0.33 % to 0.79 % N (Agblevor et al., 1995; Boateng et al., 2007; Mullen and Boateng, 2008; Tillman, 2000), well above the desired target.

Switchgrass harvest management to improve the quality of bio-oil

As described above, N is a critical nutrient for production of biomass feedstocks and typically the most limiting (Havlin, 2005; Lemus et al., 2008; Parrish and Fike, 2005; Snyder C.S. and Leep, 2007) while it is a contaminant in the pyrolytic conversion of biomass to fuels. A possible resolution to this quandary is to manipulate the biology of switchgrass to control the amount of N found in the harvested energy crop. As a perennial grass with the C₄ photosynthetic pathway, switchgrass is already inherently N use efficient but the way it is managed dictates the amount of N removed in harvested biomass (Heaton et al., 2009). Perenniality means the plant has evolved to go dormant during times unfavorable for growth. At the onset of winter or a major drought it will senesce, transporting nutrients, namely N,

from above-ground, actively growing tissues to storage tissues below-ground, and saving them for use during better times. The nutrient content of above-ground tissues (leaves and stems) can further be reduced if the crop stands during rain or snow events which leach N and mobile ions like potassium (K^+), effectively “washing” the biomass (Heaton et al., 2009; Heckathorn and DeLucia, 1994; Suzuki and Stuefer, 1999; Van Heerwaarden et al., 2003) and making it more suitable for fast pyrolysis.

Minimizing the N content of biomass removed from the field is also important to the sustainability of biomass cropping systems, given the high energetic, economic and carbon costs of N fertilizer. Heaton et al. (2009) found that the N concentration ([N]) in switchgrass biomass declined ~80 % over the course of the growing season in multi-year trials at multiple locations in Illinois, USA, from about 1.5 % to less than 0.5 %, on average. This decline, coupled with changes in harvestable biomass, meant that the amount of N removed from the field could vary from as much as 187 kg ha^{-1} to as little as 5 kg ha^{-1} depending on the date of harvest, with concomitant impacts on the carbon footprint of the system.

Objectives of research

The overall goal of this research is to establish the relationship between [N] of biomass feedstock and the [N] of bio-oil. Elucidating this relationship may allow a predictive understanding of crop management for improved fuel quality, thus improving the economic and environmental performance of biofuels.

Here we address the following research questions:

1) How does biomass harvest date influence the (a) yield, (b) [N], and (c) ash concentration of switchgrass biomass and fast pyrolysis products (bio-oil, char, and NC gas)?

2) Is there a predictive relationship between [N] of switchgrass biomass and [N] of fast pyrolysis products?

Materials and Methods

Field design

Switchgrass ('Cave-In-Rock') trials were established at the Iowa State University South Reynoldson Research Farm in Boone County, IA, USA (41° 55' N, 93° 44' W) in spring 2008. Switchgrass was seeded with a 3.7 m wide drill that dropped the seed and cultipacked at a seeding rate of 5.45 kg pure live seed (pls) ha⁻¹. The field was previously managed as a corn (*Zea mays* L.) and soybean (*Glycine max* L.) rotation (soybeans grown in 2007) and was fertilized with 84 kg N ha⁻¹ in the form of NH₄NO₃ annually beginning in 2009. The field trial was arranged as a randomized complete block design with four replications; blocks of switchgrass (18.3 × 27.4 m) were subdivided into harvest date plots (3.7 × 27.4 m). Harvest dates were chosen based on previous research to capture both ends of the growing season and times of active N movement within the switchgrass plant (Heaton et al., 2009). Switchgrass plots were sampled on 21 June, 20 July, 30 August, and 8 November in 2010 (following a killing frost) and 4 April in 2011. Biomass from the 2010 growing season was allowed to stand in the field over the winter and the April harvest date in 2011 represents the end of the season for potential harvest.

Biomass harvest methods

The plots were harvested with a self-propelled forage harvester (John Deere Model 5830, John Deere Co., Moline, IL) with a direct cut 3.8 m head. A 1.8 × 3.4 m Stan Hoist barge box weigh wagon equipped with a weigh scale (Model M640, Avery Weigh-Tronix, Fairmont, MN) was used to collect the biomass from each field replicate at the specific

harvest dates. Five subsamples were collected from the weigh wagon, mixed, placed in cloth bags and weighed. Subsamples were dried to a constant mass at 60 °C in a forced air drier. The final weight was recorded and dry matter content was calculated. Dried biomass samples were ground to 1 mm using a Thomas-Wiley mill (Model 4, Arthur H. Thomas Co., Philadelphia, PA). The milled biomass was sieved using a Ro-Tap® RX-29 sieve shaker (Laval Lab, Laval, Quebec, Canada) using three pans of 712, 500, and 212 µm screen size. Biomass which passed the 712 µm screen and did not pass the 212 µm screen was used for experiments on the reactor system.

Pyrolysis of biomass with free-fall reactor methods

Free-fall reactor methodology

A free-fall pyrolyzer (Table 1, Fig. 1) as described by Ellens and Brown (2012) was used for pyrolysis of the switchgrass samples. The free-fall pyrolyzer (Fig. 1f) was 183 cm in length with a 2.1 cm inner diameter, providing a residence time of approximately 1.5 s. The reactor was radiatively heated using ceramic heaters (Watlow Electric Manufacturing Company, St. Louis, MO) set to operate isothermally along the length of the reactor. Temperature and pressure were monitored across the reactor and the subsequently described bio-oil collection system using an automated data acquisition system (National Instruments, Austin, TX) and software (LabView®, National Instruments, Austin, TX). A volumetric feeder (Tecweigh® CR5, Tecnetics Industries Inc., St. Paul, MN) (Fig. 1e) was used to feed the ground and sieved switchgrass samples at 1 kg hr⁻¹ with the exception of three samples which were fed at 0.5 kg hr⁻¹ due to insufficient sample quantity. Nitrogen carrier gas was introduced at the top of the reactor at 5.1 Pa m³ s⁻¹ to purge vapors from the reactor tube and decrease residence time of the vapors in the reactor. Char was collected in a catch bin (Fig.

1g) at the bottom of the drop tube and the pyrolysate/carrier gas mixture was passed through a cyclone to remove any residual char. A vacuum pump was used to maintain a differential pressure of approximately 0.5-1.3 kPa above atmospheric pressure across the reactor system for each run. The composition of gas leaving the reactor was monitored and biomass feed began only after O had been purged. It took 45 to 60 minutes to pyrolyze each sample, depending on the amount of sample available.

Bio-oil collection

The bio-oil condensation system consisted of two condensers, followed by an electrostatic precipitator (ESP), and a stainless steel coil submerged in an ice bath to condense any remaining vapors. First, each condenser, operated at 60 °C and 10 °C wall temperature respectively, produced separate bio-oil fractions which are referred to as stage fraction 1 (SF1) and stage fraction 2 (SF2) (Fig. 1m, n). The pyrolysis stream then entered the ESP, designated as stage fraction 3 (SF3), where aerosols not caught in the previous stages were removed (Fig. 1o). Finally, any vapors or aerosols remaining after the ESP were condensed in a stainless steel coil submerged in an ice bath, which was referred to as stage fraction 4 (SF4) (Fig. 1p).

Mass balances

Mass balances of bio-oil and char were obtained by weighing the empty char catches (Fig. 1g, h) and each component of the bio-oil collection system (Fig. 1i-k, m-p) individually before each test followed by reweighing each component after the run to determine the amount of bio-oil or char that accumulated during the run. A drum type gas meter (Ritter® TG5/4-ER-1, Litre Meter Limited, Buckinghamshire, UK) with water as the packing liquid was used to measure total volumetric flow rate through the system. The average

concentration of each gaseous component and the overall volumetric flow through the reactor were used to calculate the mass of each compound that was calibrated on the micro-gas chromatograph as described below. Mass closures were then calculated based on the sum of the char, bio-oil, and calculated NC gas mass divided by the total mass of the biomass fed during the experiment.

NC gas compositional analysis

Permanent gases such as CO₂, CO, and light hydrocarbons are not condensed in the bio-oil collection and are therefore referred to as (NC) gases. A Varian® CP-4900 Micro-Gas Chromatograph (micro-GC) (Agilent Inc., Palo Alto, CA) interfaced with Galaxie® Chromatography 1.9 software (Agilent Inc., Palo Alto, CA) was used for NC gas analysis. A split line was taken from the exhaust outlet of the wet test meter where an air sampling pump (Model no. 224-PCXR4, SKC Inc., Eighty Four, PA) was used to supply the micro-GC with a constant flow of exhaust. The micro-GC was programmed to sample for 30 s followed by a 140 s run time. Sample line and injector temperatures were set to operate isothermally at 110 °C with a 40 ms injection time on channels one and two and an injector temperature of 80 °C with 80 ms injection time on channel three. A thermal conductivity detector was used for gas detection on each channel. Channel one was set up with a Varian® Molesieve 5Å column (Agilent Inc., Palo Alto, CA) with the oven operated at 100 °C and argon carrier gas at 151.7 kPa. Helium, H₂, O₂, N₂, CH₄, and CO₂ were calibrated on channel one. A Varian® PoraPLOT Q column (Agilent Inc., Palo Alto, CA) was set up in channel two with an oven temperature of 58 °C and helium carrier gas at 117.2 kPa. Channel two was calibrated to measure CO₂, ethylene, ethyne, and ethane. A Varian® AL2O3 column (Agilent Inc., Palo Alto, CA) was set up in channel three with an oven temperature of 60 °C and 55.2 kPa for

helium carrier gas. Channel three was calibrated to measure propane. The average volumetric concentration during steady state operation and total volumetric flow of gases were then used to calculate the mass yield of NC gases.

Analysis of biomass and pyrolysis products for total C and N content

Ultimate analysis was performed using a carbon, hydrogen, and nitrogen (CHN) analyzer (TruSpec, LECO Corp., St. Joseph, MI). Ethylenediamine-tetra-acetic acid (EDTA) was used as the standard for CHN determinations in bio-oil and biomass and phenylalanine was used for these determinations in char. Calibration lines were prepared using four different concentrations of each of the standards. Four trials were run of each concentration. The amount of N in NC gas was calculated using the difference of N in the bio-oil and char subtracted from the amount of N in the feedstock, known as calculation by difference.

Analysis of biomass and pyrolysis products for ash content

Proximate analysis was performed using a thermogravimetric analysis system (TGA/DSC 1 system, Mettler-Toledo International Inc., Columbus, OH) with STARe software version 10.0a (Mettler-Toledo International Inc., Columbus OH). The sample size range for the biomass was 10-20 mg; bio-oil 10-90 mg; and char 10-70 mg. The program was set to ramp from 25 °C to 105 °C at a rate of 10 °C min⁻¹ and held for 40 min to measure moisture content. The temperature was then ramped to 900 °C at a rate of 10 °C min⁻¹ and was held for 20 min to measure volatile content. Nitrogen purged the system at 100 ml min⁻¹. The N flow was then replaced with 100 ml min⁻¹ of air and the samples were held for 30 min at 900 °C where the remaining residue was considered ash content and the difference between the volatiles and ash content was considered fixed carbon.

Data analyses

Data were analyzed using a mixed model analysis of variance and protected mean separation (PROC GLIMMIX, SAS 9.2, SAS Institute Inc., Cary, NC, USA). Main effects considered fixed were switchgrass harvest date and pyrolysis product type (bio-oil, char, and NC gas). The interaction of harvest date \times pyrolysis product type was considered fixed and the interaction of harvest date \times block was considered random. Significant effects were determined using the F-statistic and $\alpha=0.05$. The differences between least-squared means were determined with a Tukey's adjustment.

Results and Discussion

This study investigated the relationship between [N] of switchgrass biomass feedstock and the yield and [N] of resultant pyrolysis products (bio-oil, char and NC gas). Analysis of the bio-oil is presented as whole bio-oil, i.e., the sum of all the bio-oil fractions collected from the four stage fractions on a weighted average. The [N] for NC gases are found by difference as described above. Two specific questions were addressed in this study and each is now considered. The first question, "How does biomass harvest date influence the (a) yield, (b) [N], and (c) ash concentration of switchgrass biomass and fast pyrolysis products (bio-oil, char, and NC gas)?" is split into three sections (1a-1c).

1a). How does harvest date influence the yield of switchgrass biomass and fast pyrolysis products (bio-oil, char, and NC gas)?

Switchgrass biomass yields

As expected, switchgrass biomass harvested during the 2010 growing season varied significantly with time of harvest (Table 2). Overall, switchgrass yields were comparable to typical yields for Cave-in-Rock in the Midwestern US (Heaton et al., 2008; Lemus et al.,

2002). Harvestable biomass peaked in August (8.6 Mg ha^{-1}), dropping significantly by November (6.7 Mg ha^{-1} ; $P=0.0027$, Fig. 2). This is consistent with Vogel et al. (2002) who found maximum switchgrass biomass yields occurred during a mid-August harvest in the Midwestern US. Other studies have suggested that the ideal time to harvest switchgrass and other perennial crops is following a killing frost, although delaying harvest until after a killing frost can reduce yields by 10-20% (Adler et al., 2006). We saw a 22% yield reduction from peak yields in August to a harvest after a killing frost in November. By delaying harvest over winter to April 2011, 37% more of the harvestable biomass was lost, dropping to 4.2 Mg ha^{-1} and was significantly different from a November harvest ($P=0.0002$, Fig. 2). This agrees with other studies that have also found the amount of harvestable dry matter declines as the feedstock stands in the field after senescence (Adler et al., 2006; Lewandowski et al., 2003a). For example, Adler et al. (2006) found a significant yield reduction of 32-43% from a fall to spring harvest, compared to our 37% yield reduction. Overwinter losses are especially common in temperate climates where snowfall can impact tiller lodging and make the stand difficult to harvest with conventional equipment.

Pyrolysis product yields

Harvest time influences pyrolysis product yield (Table 3, Fig. 2). Here we consider the yield, i.e., the mass, of bio-oil, char, and NC gas produced from both a unit of land and a unit of dry switchgrass biomass. The greatest cumulative amount of product per hectare came in August, matching the time of maximum biomass harvest and totaling 8.26 Mg ha^{-1} (Fig. 2). Mass balance data (Table 4) shows how the distributions of pyrolysis products change at each switchgrass harvest date. Pyrolysis product yields are comparable to other investigations that have reported bio-oil yields ranging from 60-80 wt % (Boateng et al.,

2007; Bridgwater et al., 1999; Czernik and Bridgwater, 2004; Laird et al., 2010; Mohan et al., 2006), char yields ranging from 10-30 wt % (Brewer et al., *in press*; Laird, 2008; Mohan et al., 2006), and NC gas yields ranging from 10-20 wt % (Brewer et al., *in press*; Laird et al., 2010; Mohan et al., 2006). Overall production of bio-oil per hectare mirrored that of harvested switchgrass biomass at each harvest date (Fig. 2); however, bio-oil yield per dry biomass increased from 46.6 % to 56.7 % throughout the growing season ($P < 0.0001$, Table 4) with no significant differences between November and April. The amount of bio-oil from an August harvest (4.6 Mg ha^{-1} bio-oil) was significantly greater than the amount of bio-oil produced at all other harvest dates ($P < 0.0001$, Fig. 2). Bio-oil yields reduced to 3.8 Mg ha^{-1} from November harvested biomass and further declined to 2.4 Mg ha^{-1} of bio-oil by April ($P < 0.0001$, Fig. 2). Significantly more char is produced from pyrolysis of biomass harvested during July and August compared to that in June ($P = 0.0486$, $P = 0.0015$ respectively, Fig 2). Although we saw an increase in bio-oil yields over the season, char yields were significantly greater from the spring harvested material (Table 4), which disagrees with Fahmi et al. (Fahmi et al., 2008) who found increases in bio-oil and NC gas yields at the expense of char yield. We found the opposite to be true: NC gas yields from pyrolysis of switchgrass harvested during the growing season decreased from June to April of the following year, 24.4 % to 10.6 % respectively ($P < 0.0001$, Table 4). This data is supported by Boateng et al. (2006) who found a decreasing trend in NC gas yield with increasing stage of grass maturity when pyrolyzed at temperatures below $750 \text{ }^\circ\text{C}$.

1b). How does harvest date influence the [N] of switchgrass biomass and pyrolysis products (bio-oil, char, and NC gas)?

Switchgrass biomass [N]

Switchgrass biomass [N] differed significantly with time of harvest ($P < 0.0001$, Table 2). Nitrogen concentration decreased steadily from a peak in June at $1.24 \text{ g N } 100 \text{ g}^{-1}$ dry biomass to a $0.39 \text{ g N } 100 \text{ g}^{-1}$ dry biomass in November with significant differences between each harvest date (Table 2, Fig. 3). Peak [N] occurred in June during the start of the growing season; similar results were observed in switchgrass and other perennial crops (Beale and Long, 1997; Heaton et al., 2009; Waramit et al., 2011). The [N] declined to $0.30 \text{ g N } 100 \text{ g}^{-1}$ dry biomass in April but was not significantly different from November ($P = 0.6572$, Table 5, Fig. 3). End of season [N] is less than that reported by Boateng et al. (2007) who found 0.50 % N in the ultimate analysis of switchgrass received at 2.65 % moisture. Although recommendations—which observations support (Adler et al., 2006; Heaton et al., 2009)—have been to delay harvest over the winter to reduce concentrations of contaminants, including N (Lewandowski and Heinz, 2003), the [N] in our spring harvested biomass did not significantly differ from the fall harvested biomass. By allowing switchgrass to senesce and recycle nutrients, specifically N, to root tissues, immediate reductions in [N] were apparent in the switchgrass biomass: as much as 68 % from June to November ($P < 0.0001$) with an additional 23 % reduction from November to April ($P = 0.6572$).

Pyrolysis product [N]

The [N] of fast pyrolysis products differed significantly with harvest date ($P < 0.0001$, Table 3). The [N] of fast pyrolysis products decreased from $1.23 \text{ g N } 100 \text{ g}^{-1}$ dry biomass in June to $0.39 \text{ g N } 100 \text{ g}^{-1}$ dry biomass in November ($P < 0.0001$, Fig. 3), mirroring that of the

[N] of switchgrass biomass. There was no significant interaction of [N] in bio-oil with time during the periods of active switchgrass growth in June, July, and August. But, the [N] in bio-oil changed significantly between these dates (June, July, and August) and a November harvest ($P < 0.0001$, $P = 0.0003$, $P = 0.0010$ respectively, Fig. 3). The N in bio-oil was 0.51 % in June and declined to 0.11 % by April ($P < 0.0001$), although an April harvest was not significantly different from a November harvest ($P = 0.7964$, Table 5). Ultimate analysis of bio-oil from switchgrass in the literature ranges from 0.33 % to 0.79 % N (Agblevor et al., 1995; Boateng et al., 2007; Mullen and Boateng, 2008; Tillman, 2000); the desired or maximum amount of N in bio-oil is 0.2 % and is typical of wood feedstocks (Bridgwater, 1994; Bridgwater, 1996; Czernik and Bridgwater, 2004). A novel finding of this work is that the [N] of bio-oil can be reduced below target levels simply by delaying harvest of the biomass. The amount of N in char also decreased significantly from 0.33 % in June to 0.16 % in April ($P < 0.0001$, Table 5).

1c). How does harvest date influence the ash concentration of switchgrass biomass and fast pyrolysis products (bio-oil, char, and NC gas)?

Switchgrass biomass ash

Switchgrass biomass ash concentration varied significantly with harvest date ($P < 0.0001$, Table 2). A July harvest contained the greatest concentration of ash with 7.1 g ash 100 g^{-1} dry biomass compared to April that contained 4.3 g ash 100 g^{-1} dry biomass ($P < 0.0001$, Fig. 4). November biomass was not significantly different from April biomass ($P = 1.0000$). McKendry (2002) reported very similar April biomass ash concentrations (4.5%) and generally these results are consistent with other studies showing that a later switchgrass harvest date leads to lower ash concentrations (Lewandowski et al., 2003b;

Sanderson and Wolf, 1995) . The reduced biomass ash concentration is a positive attribute for thermochemical conversion, indicating more of the fuel is usable energy.

Pyrolysis product ash

The vast majority of ash in pyrolysis products was found in the char. Char contained significantly greater ash than bio-oil ($P < 0.0001$, Table 3, Fig. 4). Others have shown small amounts of ash in bio-oil may be from char passing to downstream equipment due to cyclone inefficiencies (Boateng et al., 2007; Bridgwater et al., 1999) of approximately 0.1 % (Bridgwater et al., 1999). Char from the July harvest contained 6.6 g ash 100 g^{-1} dry biomass, which was significantly more than char from the November harvest that had 4.2 g ash 100 g^{-1} dry biomass ($P < 0.0001$, Fig. 4). As with other measured variables, November char ash concentration was not significantly different from April char ash concentration. Char ash concentrations at later harvest dates were less than the 7.63 % reported by Tillman (2000).

Because investigators have shown the importance of its uses on agricultural soils, char is considered a co-product of thermochemical conversion. The majority of plant macronutrients and micronutrients, along with nearly half of the N and S from the original biomass feedstock are partitioned into the char (Laird et al., 2010). Chars can be applied to agricultural soils to supply what was originally taken off with the harvested biomass. According to Bridgwater et al. (1999) and Lewandowski and Kicherer (1997), alkali metals from biomass ash are present in high concentrations in the char. Presence of these metals in biomass feedstock can increase amount of char produced and potentially decrease bio-oil yields in pyrolysis (Fahmi et al., 2008). Some of these metals, including potassium and sodium, will increase ash concentrations and may contribute to a catalytic effect on the

thermal degradation of biomass during pyrolysis (Fahmi et al., 2008). Here, a reduction in ash concentration during the growing season was accompanied by a concomitant increase in bio-oil yields (Table 4) which is similar to results found by Fahmi et al. (Fahmi et al., 2008).

2). Is there a predictive relationship between [N] of switchgrass biomass and [N] of fast pyrolysis products (bio-oil, char, and NC gas)?

Here we show for the first time that the [N] of switchgrass biomass is a strong predictor of the [N] of bio-oil, char, and NC gas with R^2 values of 0.89, 0.94, and 0.88, respectively (Fig. 5). All three pyrolysis products have a positive linear relationship; the greater the [N] in the biomass, the greater the [N] in the pyrolysis products (Fig. 5). Bio-oil [N] has a stronger positive linear relationship compared to char and NC gas and is predicted to have a y-intercept of nearly zero (1:1). Even though our data come from only one growing season of switchgrass biomass, they are supported by a detailed survey of the peer-reviewed literature. We were unable to find any dedicated studies examining this question, so we reviewed the peer-reviewed literature on bio-oil, found nine studies that met our criteria, (Agblevor et al., 1995; Boateng et al., 2009; Ferdi Gercei, 2002; Mullen and Boateng, 2008; Mullen et al., 2010; Putun, 2002; Schnitzer et al., 2007; Sensoz et al., 2006; Williams and Horne, 1994; Yorgun, 2003), extracted the [N] of various biomass feedstocks and regressed them against the [N] of their resultant bio-oil products (Fig. 6). Doing so revealed that our switchgrass-specific results are consistent with a strong overall trend. Even among diverse biomass feedstocks, the literature shows that biomass feedstock [N] is a strong predictor of bio-oil [N] ($R^2=0.85$) and has a strong positive linear relationship. Unlike our results, the literature values indicate that as biomass [N] approaches zero, bio-oil will still contain some

N. The difference in predicted y-intercepts may be skewed by biomass samples with greater [N] in the literature.

Impacts

We have shown here, for the first time, that the [N] of biomass feedstock is strongly correlated to the [N] of resultant bio-oil, char and NC gas from fast pyrolysis. With refinement, this relationship may enable screening of feedstock for quality, as is done with grain commodities, allowing refiners to minimize costs from N fouling of catalysts during bio-oil upgrading and farmers to receive premiums for low-N biomass. Improvements in bio-oil quality were realized not with expensive treatments to the bio-oil, but instead with a simple management strategy, i.e., delayed harvest, that has also been shown to also improve the economic and environmental sustainability of biofuels by minimizing external N fertilizer inputs (Heaton et al., 2009; Lemus et al., 2008).

Conclusions

Switchgrass harvest time influences pyrolysis products and bio-oil quality. By allowing switchgrass to senesce in the fall and harvesting after a killing frost, N and ash concentrations of pyrolysis products (bio-oil, char, and NC gas) were significantly reduced. Although biomass yields were also significantly reduced after August, a mass balance of pyrolysis products showed that significantly more bio-oil was produced per unit of dry biomass at later harvest dates. In this study, however, there seemed to be no advantage to leaving the crop in the field overwinter, as there were no further improvements in quality, but major reductions in harvestable biomass. Tailoring harvest management of perennial crops for improved fuel quality will directly benefit biomass producers by allowing them to

minimize N fertilizer inputs, while concomitantly allowing thermochemical facilities to reduce costs associated with upgrading bio-oil to a transportation fuel.

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Tables

Table 1. Fast pyrolysis reaction conditions.

Reactor Conditions	
Reactor configuration	Free-fall
Feedstock particle size	212 to 716 μm
Biomass feed rate ^z	1 kg hr ⁻¹
N ₂ flow rate	5.1 Pa m ³ s ⁻¹
Reactor temp.	550 °C
Vapor temp at outlet	500 °C
Cyclone/heat tracing	475 °C
Condenser 1 wall (SF1)	60 °C
Condenser 2 wall (SF2)	10 °C
ESP ^y wall (SF3)	Ambient (~22 °C)
Icebath coil (SF4)	0 °C

^zthree samples were fed at 0.5 kg hr⁻¹ due to insufficient sample size

^yESP= electrostatic precipitator

Table 2. ANOVA for switchgrass biomass at five switchgrass harvest dates during the 2010 growing season. Harvest date was considered a fixed effect.

Analysis	Source of Variation	Num DF ^z	Den DF	F Value	P
Yield	Harvest Date	4	15	46.96	<0.0001
N concentration	Harvest Date	4	15	71.23	<0.0001
Ash concentration	Harvest Date	4	15	20.58	<0.0001

^zDF = degrees of freedom

Table 3. ANOVA for total pyrolysis products (bio-oil, char, and non-condensable gas) at five switchgrass harvest dates during the 2010 growing season. Harvest date was considered a fixed effect.

Analysis	Source of Variation	Num DF ^z	Den DF	F Value	P
Yield					
	Harvest date	4	15	45.21	<0.0001
	Product type ^y	2	30	414.21	<0.0001
	Harvest date x product type	8	30	15.20	<0.0001
N concentration					
	Harvest date	4	15	71.23	<0.0001
	Product type ^{yx}	2	30	35.44	<0.0001
	Harvest date x product type	8	30	5.51	0.0003
Ash concentration					
	Harvest date	4	15	11.04	0.0002
	Product type ^w	1	15	1,198.15	<0.0001
	Harvest date x product type	4	15	13.58	<0.0001

^zDF = degrees of freedom

^yProduct type = bio-oil, char, and non-condensable gas

^xProduct type = non-condensable gas found by difference

^wProduct type = bio-oil and char

Table 4. Moisture-free mass balance for pyrolysis products (bio-oil, char, and non-condensable gas) at five switchgrass harvest dates during the 2010 growing season in Boone County, IA.

Harvest Date	Mass Balance			Mass Closure
	% Bio-oil	% Char	% NC Gas	
June	46.6 ^z	22.9b	24.4a	93.9
July	50.4bc	24.3b	23.4a	98.1
August	53.5ab	21.3b	20.9a	95.7
November	56.4a	21.4b	19.4a	97.1
April	56.7a	27.9a	10.6b	95.2

^zEntries in a column followed by the same letter are not significantly different (n=4, p <0.05)

Table 5. Moisture-free nitrogen concentration (%) of switchgrass biomass at five harvest dates in Boone County, IA in 2010 and its pyrolysis products (bio-oil, char and non-condensable gas).

Harvest Date	Biomass	Product		
		Bio-oil	Char	NC Gas ^z
% Nitrogen				
June	1.24a ^y	0.51a	0.33a	0.40a
July	0.98b	0.41a	0.29ab	0.28a
August	0.75c	0.39a	0.24b	0.12b
November	0.39d	0.17b	0.17c	0.05b
April	0.30d	0.11b	0.16c	0.03b

^zThe N concentration of non-condensable gas was found by difference.

^yEntries in a column followed by the same letter are not significantly different (n=4, p <0.05)

Figures

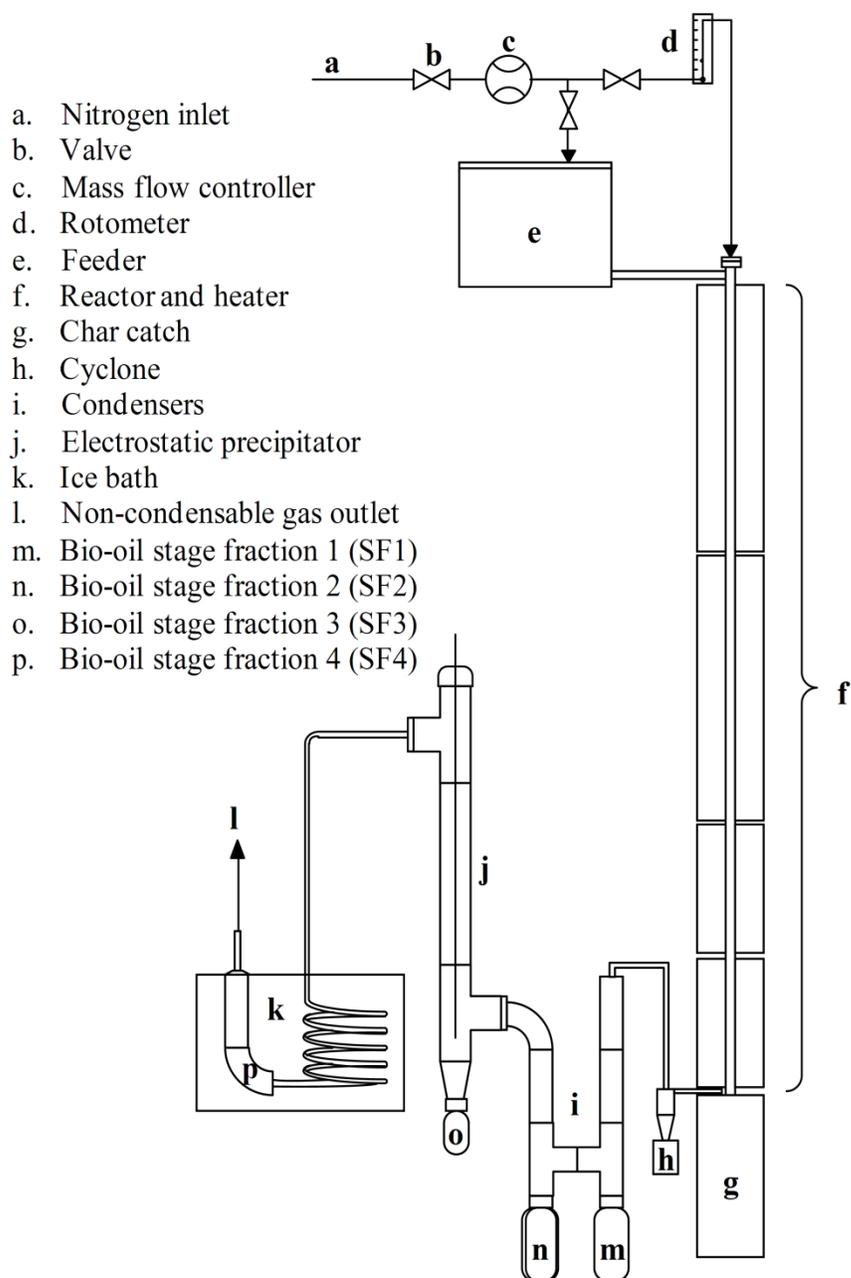


Fig. 1 Free-fall reactor schematic. Modified and used by permission of Cody Ellens (Ellens and Brown, 2012)

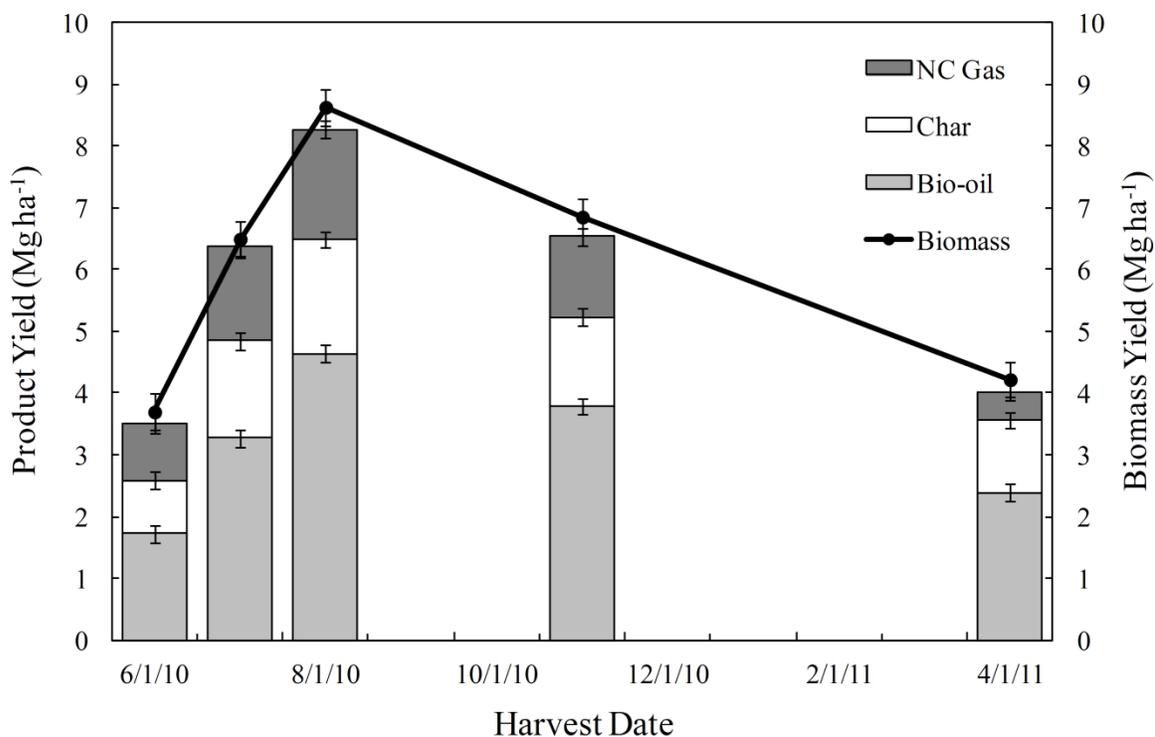


Fig. 2 Switchgrass dry matter yields (Mg ha^{-1}) from 2010 growing season at five harvest dates in Boone County, IA and yield (Mg ha^{-1}) of fast pyrolysis products (bio-oil, char and non-condensable gas) from a free-fall reactor. Bio-oil yield is normalized to moisture-free biomass. Least-squared means were calculated from $n = 4$ biological blocks, and error bars indicate ± 1 standard error of the mean

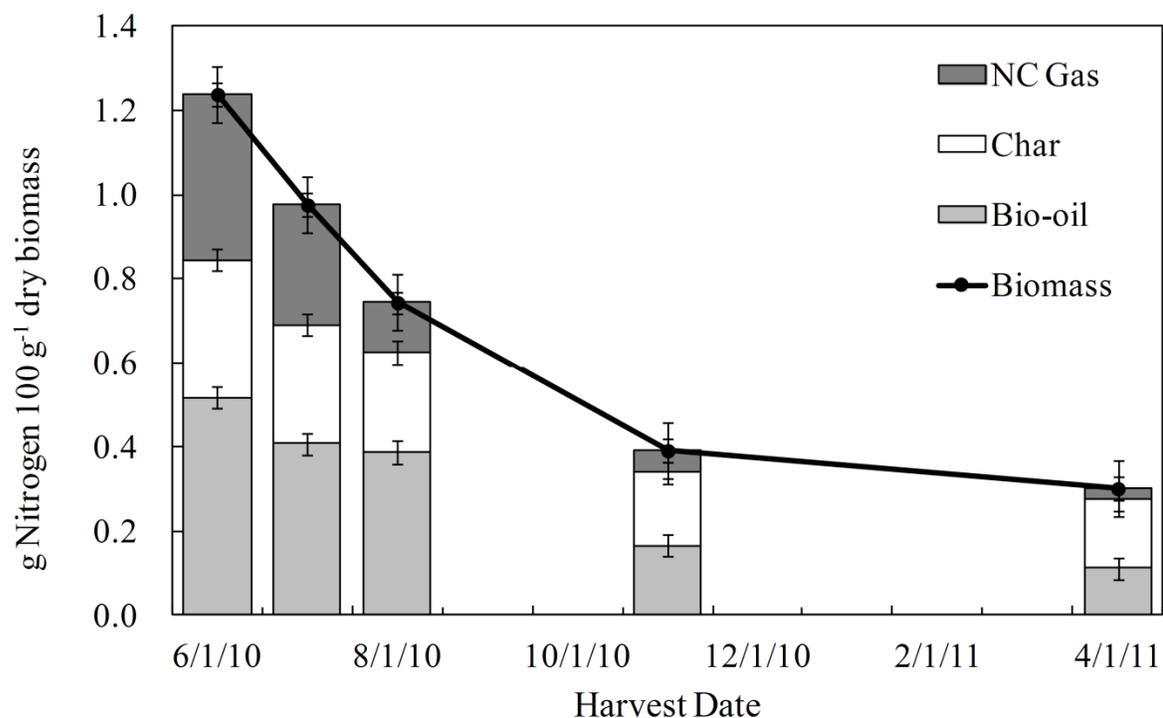


Fig. 3 Nitrogen concentration (g N 100 g⁻¹ dry biomass) of switchgrass biomass and fast pyrolysis products (bio-oil, char and non-condensable gas) at five harvest dates during the 2010 growing season in Boone County, IA. Bio-oil is normalized to moisture-free biomass. Least-squared means were calculated from $n = 4$ biological blocks, and error bars indicate ± 1 standard error of the mean

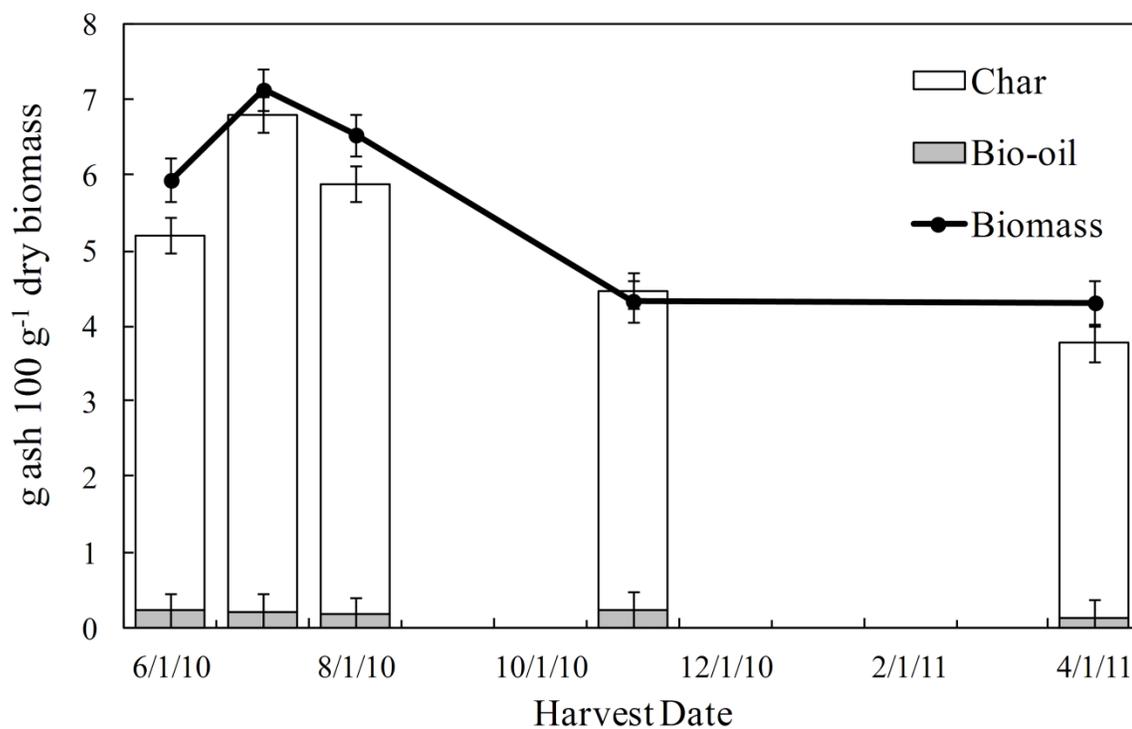


Fig. 4 Ash concentration (g ash 100 g⁻¹ dry biomass) of switchgrass biomass and fast pyrolysis products (bio-oil and char) at five harvest dates during the 2010 growing season in Boone County, IA. Bio-oil is normalized to moisture-free biomass. Least-squared means were calculated from $n = 4$ biological blocks, and error bars indicate ± 1 standard error of the mean

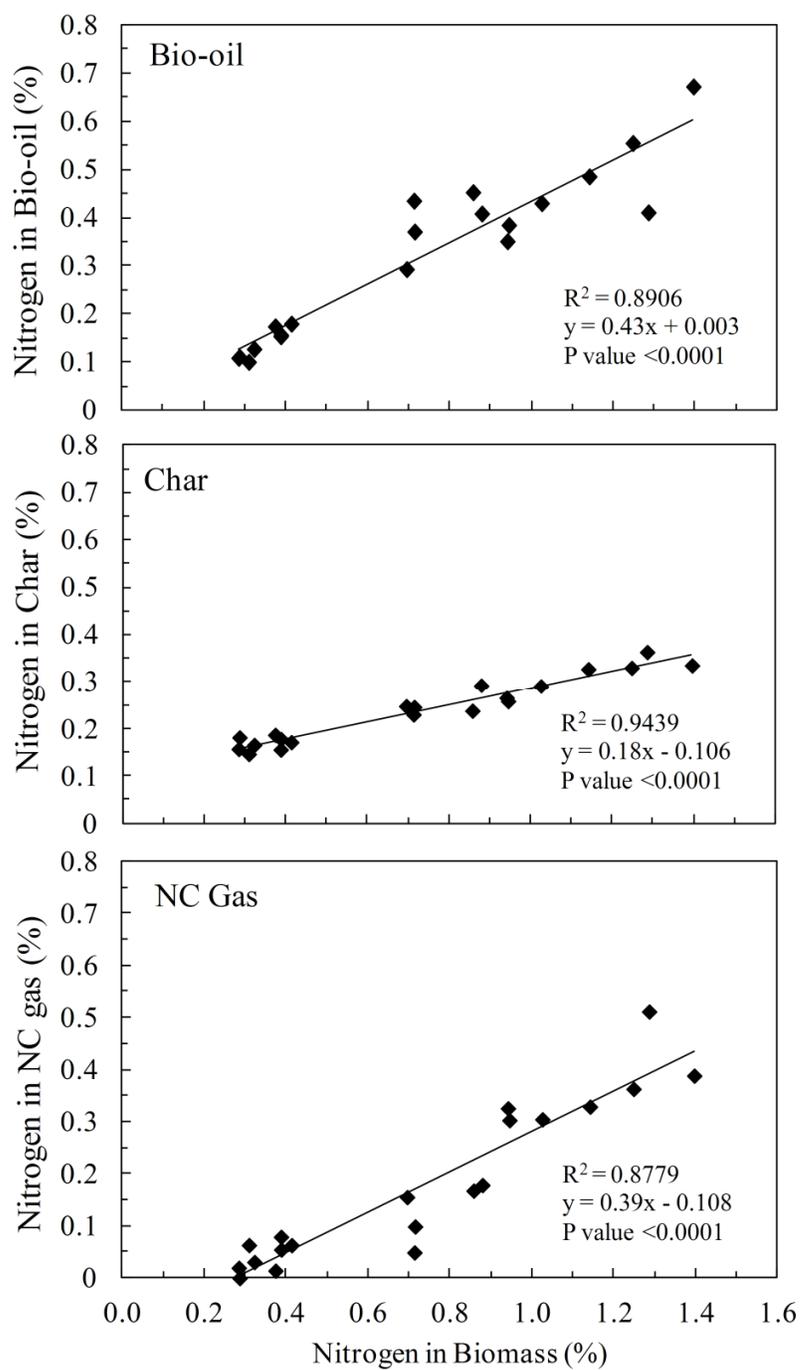


Fig. 5 Nitrogen concentration (%) of switchgrass biomass regressed against the N concentration (%) of their resultant pyrolysis products (bio-oil, char, and non-condensable gas)

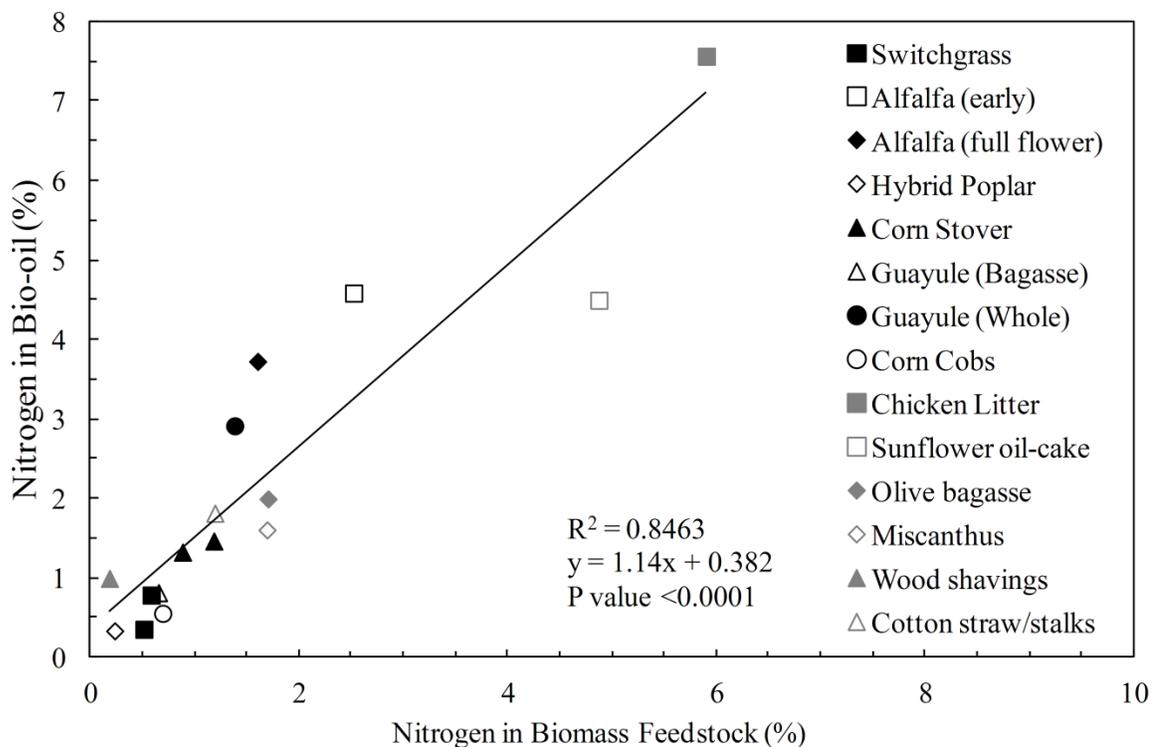


Fig. 6 Nitrogen concentration (%) of various biomass feedstocks, from peer-reviewed literature, regressed against the N concentration of their resultant bio-oil products (Aglevor et al., 1995; Boateng et al., 2009; Ferdi Gercei, 2002; Mullen and Boateng, 2008; Mullen et al., 2010; Putun, 2002; Schnitzer et al., 2007; Sensoz et al., 2006; Williams and Horne, 1994; Yorgun, 2003)

CHAPTER 4. GENERAL CONCLUSIONS

General Discussion

Production and management

The sustainability, long-term environmental effects, and quality of fuel from biomass cropping systems are all important aspects of bioenergy production (Georgescu et al., 2011; Heaton et al., 2008; Popp et al., 2011; Schmer et al., 2008; Solomon, 2010; Tilman et al., 2011; Valentine et al., 2012).

First, this research has suggested that N cycling occurs in switchgrass field studies. The [N] of the harvested portions of switchgrass biomass decrease during the annual growing season; concurrently, the [N] in the below-ground tissues decreases rapidly during rapid stem elongation and viable leaf growth and then increases until post-frost. Switchgrass yields are reduced the following season by harvesting prior to complete translocation of N during the middle of summer. By delaying switchgrass harvest to post-frost, yields are reduced but there is significantly less N removed with the harvested biomass compared to a late July harvest. The extensive root data shows that the [N], yield and therefore the N content are all concentrated in the upper 15 cm of the rooting profile. Additionally, compared to a conventional corn grain and 50 % stover harvest, switchgrass would remove significantly less N with harvest. If these two systems were harvested over the next 5 years, switchgrass would remove 114 kg N ha⁻¹ compared to 1,050 kg N ha⁻¹ for corn grain and 50 % stover removal. The long-term sustainability of switchgrass compared to conventional corn for bioenergy is much greater, given the reduced N removal, maintained yields over the course of the stand life, and reduced N inputs in order to maintain yields. Nitrogen cycling in

perennial crops doesn't completely dismiss the need for N inputs; some N will still be needed in order to maintain yields (Mitchell et al., 2008; Vogel et al., 2002) even when properly managed.

Management for improved feedstock quality

The quality of biomass is important for the quality of biofuels produced; the time of switchgrass harvest has impacted the quality of the biomass and can therefore impact biofuel quality (Adler et al., 2006). Next, this research has shown that the [N] of whole switchgrass biomass is a strong predictor of the [N] of bio-oil, char, and NC gas produced from fast pyrolysis. Bio-oil [N] had a stronger positive linear relationship compared to its co-products and its y-intercept was nearly zero (1:1). Our research is supported by the literature (Agblevor et al., 1995; Boateng et al., 2009; Ferdi Gercei, 2002; Mullen and Boateng, 2008; Mullen et al., 2010; Putun, 2002; Schnitzer et al., 2007; Sensoz et al., 2006; Williams and Horne, 1994; Yorgun, 2003) when we extracted the [N] of various feedstock and regressed them against their resultant bio-oil. The data suggest that a later harvest of switchgrass biomass provides higher quality bio-oil from fast pyrolysis and higher quality biomass in terms of low ash content. There was no added benefit to leaving the switchgrass stand over the winter, therefore a post-frost harvest is again recommended in order to maximize yields and still provide high quality biomass.

Future work

Further studies should attempt long-term (5-10 years) switchgrass field trials for above- and below-ground biomass at different harvest dates. This would help access the long-term effects of harvesting biomass at varying [N] to determine their yield potential and N removal with fertilizer requirements. In addition to field studies, more studies on the bio-

oil produced from biomass harvested at different times during the annual growing season would be beneficial to include hydrotreating with the use of catalysts to determine their effectiveness with the varying [N]. This would provide more details about the accepted [N] of our feedstocks targeted towards thermochemical facilities.

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