

**Native warm-season grasses: Species, nitrogen fertilization, and harvest
date effects on biomass yield and composition**

by

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ABSTRACT

For traditional forage use, native warm-season grasses are highly productive in the summer months and are suited for a complementary role with cool-season forages in full-season forage systems of the Midwest. For renewable energy sources, these grasses can be used to produce biofuels, an alternative energy source in the future for fossil fuel which is becoming less available. Farmers may integrate forage and biomass cropping for flexibility and diversity in their farming systems. For dual-purpose crops (forage or biomass), the production of warm-season grasses would provide farmers some protection against the market failure of a single purpose. The goals for the production of warm-season grasses as biomass differ from those when used as forage. The goal for biomass production is to maximize the concentration of lignocellulose in the feedstock and minimize the concentration of N and minerals. To achieve the production potential, optimal management practices are required.

The purposes of this research were to determine in some detail the yield and quality of four native warm-season grasses as influenced by nitrogen application and harvest timing for (i) forage and (ii) biomass production.

In the first study (Chapter 3), we investigated the nutritive quality of native warm-season grasses as influenced by N fertilization rate and harvest timing. Forage quality of native warm-season grasses decreases as the plants mature. For all species, harvesting at early maturity increased forage quality including increased IVDMD and crude protein, and decreased NDF concentrations, whereas allowing forage to reach later maturity reduced forage quality with declines in IVDMD and crude protein and increases in NDF

concentration. There were variations in NDF and IVDMD concentration among warm-season grass species in response to N application rates, but none was observed for crude protein. The concentration of crude protein for four warm-season grass species increased as higher rates of N fertilization. Therefore, although there were variations in the effect of N fertilization on NDF and IVDMD making drawing conclusions difficult, N fertilization could improve forage quality for warm-season grasses through increasing the concentration of crude protein in warm-season grasses. Across big bluestem, switchgrass, and indiangrass, quadratic regression equations used for predicting the changes in forage quality parameters from mean stage count (MSC) had high coefficients of determination ≥ 0.96 for IVDMD, 0.88 for NDF, and 0.88 for CP, respectively. However, predicting forage quality changes based on MSC did not provide reasonable estimates for eastern gamagrass.

In the second study (Chapter 4), we determined optimum N fertilization rates and harvest timing for the biomass production of native warm-season grasses and investigated if traits of canopy architecture including mean stage by count and tiller demographics of native warm-season grasses can explain observed yields of warm-season grasses with varied harvest dates and N fertilization rates. Biomass yields increased with advancing maturity but differently among species before declining during senescence. Eastern gamagrass obtained the maximum yield at the highest mean stage count (MSC; 1.6 and 2.2) when the largest seed ripening tillers are present. Big bluestem, switchgrass, and indiangrass are more determinate in their growth patterns. Their maximum yields occurred at MSC 3.5, 3.9, and 2.9, respectively when the largest reproductive tillers are present. Delaying harvest to late fall may improve biomass quality, but it decreased yield due to a large proportion of senesced tillers and increased litter. In terms of a biomass supply strategy, eastern gamagrass may be

used as a feedstock in early summer, while big bluestem and switchgrass may be used between mid-and late summer, and indiangrass in early fall. Nitrogen fertilization increased yield by increasing the proportion of elongating, reproductive, seed tillers for determinate grasses (big bluestem, indiangrass, and switchgrass) and increasing tiller density for indeterminate grass (eastern gamagrass).

In the third study (chapter 5), we determined biomass quality of four warm-season grasses as influenced by nitrogen fertilization rates and harvest timing. In both years, delaying harvest dates increased cellulose, lignin, and C concentrations, but decreased ash and N concentrations, however, the magnitude of these effects varied among species. The concentration of N, C, cellulose, and lignin increased, whereas ash content declined with increasing rates of N fertilization, but also varied among species. Our results indicated that the warm-season grasses supplied with N fertilizer at 140 kg ha^{-1} and delayed to harvest until fall provide the optimal composition for biomass feedstock production.

Therefore, the four native warm-season grasses in this study could serve dual purposes as forage when harvested at early maturity, and as biomass feedstocks when harvested during late maturity. Optimal N fertilization could provide economically feasible alternative uses of these warm-season grasses by improving both total yields and quality of forage and biomass.

CHAPTER 1. GENERAL INTRODUCTION

Background

In the Midwest, the summer slump in cool-season forage production prompted livestock growers to look for alternative crops in forage-livestock systems. With the C_4 photosynthetic pathway providing more drought tolerance and better growth in summer, native warm-season grasses have been used to supplement cool-season forages. Cool-season grasses produce most of their forage during the spring and early summer providing 60 to 70 percent of forage production before June. These grasses are normally semi-dormant during the hot summer months. In contrast, the growth of warm-season grasses starts later in spring, but increases throughout the summer and into early fall. Warm-season grasses are highly productive between June and August. By combining the productivity of perennial warm-season grasses during summer season with that typically available from cool-season grasses during the spring and fall seasons, the grazing system in these regions can be managed for more uniform supply of forage throughout the entire growing season.

Native, perennial warm-season grasses also have been used for other uses. In addition to use as livestock feed, they have great potential for soil conservation, waste nutrient management, water quality protection, and wildlife habitat. In recent years, a renewed interest in the potential role for native warm-season grasses as herbaceous energy crops (HEC) has developed as the U.S. biofuel policy goals were set up by the USDA and DOE (Perlack et al., 2005). The development of domestic and renewable alternatives to current transportation fuels can overcome a number of challenges in terms of energy while reducing national dependency on imported oil, and developing the nation's agricultural potential for

rural economic growth, and improving environmental quality (Brown, 2003). Biofuels including ethanol, biodiesel and butanol are an attractive alternative to current petroleum-based products (Dhugga, 2007). They are produced from biomass feedstocks as raw materials in biochemical and thermochemical conversion (Ortiz-Canavate, 1994). Of all biofuels, ethanol production is receiving increasing government support resulting from the perception associated with the development of a bioeconomy. However, large scale ethanol production from corn grain, a predominant feedstock for ethanol production in the U.S., has raised concerns. In the world's growing economies, corn-based ethanol suffers from their reliance on a feedstock with a high value for food and feed applications (Cassman and Liska, 2007; Trostle, 2008; Abbot et al., 2008). It has been suggested that the contribution of food crops for ethanol may soon reach its limit soon. More intensive cultivation for foods, feeds, and biofuels with annual tillage and pesticide application can threaten soil and water resources (Burke et al., 1989; Keeney et al., 1993; Schilling and Libra, 2000). Moreover, the indirect effect of land use change can increase greenhouse gas (GHG) emissions (Fargione et al., 2008; Searchinger et al., 2008).

In this respect, the sustainable production of bioethanol and other biofuels from lignocellulosic feedstocks is therefore expected to become one of the most credible alternatives within a few years, and to overcome the challenges associated with corn-based ethanol (McLaughlin, 1998; McLaughlin, 2002). Although lignocellulosic biorefineries can use crop residues (such as corn stover), wood residues, and other waste products as feedstock, dedicated energy crops will be required to achieve the U.S. biofuels policy goals (Perlack et al., 2005). Research in the USA has determined that many species and varieties of warm-season grasses have potential as biomass feedstock crops. Of all these crops,

switchgrass was selected on a wide range of 30 sites across seven states as a model species (Wright, 2007).

Livestock growers may be more interested in seeding and managing their pasturelands to potential lignocellulosic grasses, which would offer an alternative use and provide some protection against the market failure of a single commodity. To effectively incorporate warm-season perennial grasses into the Midwestern forage-livestock systems and biomass production, management practices must be developed and evaluated. Appropriate management practices will offer optimum production of quality herbage which can meet the needs of dual purposes (forage and biomass).

A number of factors affect forage yield and quality of warm-season grasses including species, soil fertility, soil moisture, harvest timing, and environmental conditions. Across the Midwest, big bluestem, eastern gamagrass, indiagrass, and switchgrass are widely recommended for pasture and forage production. These warm-season grasses, however, potentially function as one component in a multipurpose cropping system including livestock feed, wildlife habitat, and cash hay. Currently, switchgrass is the most promising species for biomass production in the area, but the other three grass species have been evaluated for biomass qualities and adaptability (Nelson et al., 1994). However, the presence of some given limitations such as insufficient biomass quality or the need for adaptation to certain ecological/climate zones for those grasses may be overcome by effective crop management (Lewandowski et al., 2003).

Warm-season grasses for biomass can be planted and harvested like a traditional forage crop (Raneses et al., 1998), but management strategies differ considerably between biomass and hay or pasture production because of differences in quality requirements

between the two systems (Lowenberg-DeBoer and Cherney, 1989). Biomass production is primarily concerned with total dry matter yield, whereas forage production is also concerned with nutritive value. Nitrogen fertilization and harvest management are key management tools for economically viable production of dual-purpose crops.

Appropriate harvest management is required for optimizing production for livestock and biofuel refinery system (Sanderson et al., 1999). Forage quality of perennial warm-season grasses can be manipulated by harvest management which requires a compromise between forage yield, quality, and plant persistence. Harvesting less mature forage may reduce total season yield, while allowing forage to reach later maturity to increase yield will decrease forage quality. Harvesting native tallgrasses for hay is recommended when they are at least 45 to 60 cm tall and before the boot stage as a compromise between forage yield and quality (Moser and Vogel, 1994). Increased harvest frequency (two, three, or four harvests per season) may produce forage with higher quality, but this may reduce total seasonal forage yields (Brejda et al., 1996; Forwood and Magai, 1992). However, harvest management for biomass production may be different from hay production because the goal is to produce a high yield of lignocellulose and digestibility is not a consideration. Therefore, a single late-season harvest may be most suitable for biomass fuel cropping. Although previous studies have reported that perennial, native warm-season grasses cut once between July and September each year provided the greatest forage yields (Sanderson et al., 1995; Dwyer et al., 1963), more understanding relative to harvest timing would be desirable for these grasses managed for biomass production.

Nitrogen is often the most limiting element needed to meet yield goals in warm-season grass production (Woodhouse and Griffith, 1974; Berg, 1995). Proper N fertilization

is an important management tool for improving stand establishment and increasing forage production and quality of native warm-season grasses. Due to high nitrogen-use efficiency, nitrogen requirements for native warm-season grasses are commonly less than cool-season grasses. Ideally, optimum N fertilizer rates should be specific for the grass species, the yield potential of the grass sward for the given soil and climate, end-uses for grazing, hay or biomass production and the economic return per unit of N applied. Fertilization with a wrong rate is economically inefficient (Ziadi et al., 2000) may cause stand degradation through weed invasion, and can lead to environmental degradation through contamination of surface and groundwater (Brejda, 2000). Information on N requirements is needed for producers interested in using native warm-season grasses for biomass production. Developing economically optimum N fertilizer rates for each purpose requires a consideration of yield goals, N requirements of the stand, residual soil N availability, and N concentration in plant tissues (Baloch, 1998; Brejda, 2000).

A number of studies on harvest regimes and N requirements in warm-season grasses managed as hay or grazing have been reviewed by Brejda (2000). Recent studies for biomass production have been focused mostly on a model energy crop, switchgrass (Ma et al., 2001; Muir et al., 2001; Thomson et al., 2004; Lemus et al., 2008), but there are risks associated with relying on a single species for this use. Different species have evolved to take advantage of different combinations of environmental factors, especially soil and climatic conditions, and development of additional biomass species could increase the range and profitability of biofuel production systems (USDA, 1992). Less is known about big bluestem, eastern gamagrass, and indiangrass biomass yield and quality response to N and harvest timing. Moreover, information on the interaction between N rates and harvest time

for each grass managed to integrate forage and biomass cropping for flexibility and diversity in the farming operation is not available.

Objectives

Three studies were conducted to investigate N and harvest management of four warm-season grasses. The first experiment was designed to determine the effect of N fertilization rate on the nutritive quality of four warm-season grasses and examine the nutritive quality of four warm-season grasses as influenced by harvest timing.

The second experiment was conducted to determine optimum N fertilization rates and harvest timing for the biomass production of warm-season grasses and to investigate if traits of canopy architecture including mean stage by count and tiller demographics of four warm-season grasses can explain observed yields of warm-season grasses with varied harvest dates and N fertilization rates.

The third experiment was conducted to determine biomass quality of four warm-season grasses as influenced by nitrogen application rates and harvest timing.

Dissertation Organization

This dissertation is organized into six chapters. Following this chapter is an in-depth literature review of concepts related to the studies. Following the literature review chapter, the investigations of the productivity and quality of four warm-season grasses influenced by N fertilization and harvest timing managed as forage and biomass production is presented in three chapters in the form of manuscripts. In the final chapter, results from the three experiments are generally summarized.

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CHAPTER 2. LITERATURE REVIEW

Warm-Season Grass Overview

Warm-season grasses belonging to the Poaceae (Gramineae) family are characterized by having the C₄ photosynthetic system (Kellogg, 2001). These grasses grow well and yield well under high temperatures and typically have better NUE and WUE than cool season grasses (Long, 1999; Moore et al. 2004; Wedin, 2004). They play a key role in the forage-livestock system in the tropical and subtropical areas of the world. Most of them are found between 30° N lat and 30° S latitude of the equator (Sage et al., 1999). In addition, both annual and perennial, warm-season grasses are well adapted in many warm environments in temperate areas of the world where the warm period is rather long during the summer (Barbour and Christensen, 1993). These grasses become dormant in the winter and re-grow in the spring, producing new green tillers from the crown of the plant when soil temperatures increase. In the tallgrass prairie region of the North America, including the Midwest and Great Plains states, warm-season grasses were once quite widespread before the arrival and establishment of European settlers. They supplied food and shelter for native wildlife such as the American bison, elk, whitetail deer, small mammals and numerous bird species in these regions. Also, the settlers used these grasses as pasture for their livestock (Massengale, 2000; Moser et al., 2004).

Most of the original Tallgrass Prairie has been converted to cropland and cool-season pastures introduced by incoming settlers from other countries. Despite the relatively low area of native prairie remaining, the genetic diversity of these species is preserved in

remnants scattered throughout the extent of the region. Today, native warm-season grasses exist in rangelands, unbroken prairie areas, or on lands under government agricultural programs such as the Soil Bank or Conservation Reserve (Massengale, 2000).

Over the last two decades, interest has increased in native, perennial, warm-season grasses for multiple uses including hay and grazing, soil conservation, waste nutrient management, water quality protection, wildlife habitat, and alternative energy, due to their ability to produce high yields under hot, dry conditions even on poorer soils. This literature review intends to summarize the importance and possibility of utilizing warm-season grasses for the production of forage and lignocellulosic biomass feedstock.

Characteristics of the C₄ Warm-Season Grasses

Warm-season grasses are characterized by having the C₄ photosynthetic system. The C₄ photosynthetic system obtains its name because the first products produced from carbon fixation are four-carbon acids (malate and aspartate) instead of the three-carbon product (3-phosphoglyceric acid; PGA) produced by the C₃ photosynthetic system. Phosphoenolpyruvate carboxylase (PEP) is the first enzyme of carboxylation for C₄ grasses. C₄ plants are classified into three different types according to the decarboxylating enzyme in the bundle sheath cells, chloroplast structure and arrangement (Kellogg, 1999). The NADP-malic enzyme (NADP-ME) type including the important crop plants maize and sugarcane, is most abundant in more humid areas. The NAD-malic enzyme (NAD-ME) type and the phosphoenolpyruvate carboxykinase (PCK) types are more prevalent in arid areas and

include millet and the fast-growing tropical grasses used as forage crops, respectively (Heldt, 2005).

All C₄ grasses have a distinct leaf structure referred to as Kranz anatomy (Dengler and Nelson, 1999). This special leaf anatomy is characterized by having prominent bundle sheath cells surrounding the leaf vascular bundles and closely arranged mesophyll cells (Crookston and Moss, 1974). Chloroplasts contained in the bundle sheath cells exhibit large starch granules and unstacked thylakoids membranes, whereas chloroplasts randomly arranged in mesophyll cells have stacked thylakoids and little or no starch. The importance of Kranz anatomy to the C₄ pathway is determined by the functional specialization of mesophyll and parenchyma bundle sheath cells, found in the C₄ leaves. In mesophyll cells of C₄ grasses, CO₂ forming bicarbonate (HCO₃⁻) in water is initially fixed in mesophyll with phosphoenolpyruvate (PEP) as four-carbon intermediates. This process is catalyzed by the enzyme PEP carboxylase. The four-carbon compound is then transported to the bundle sheaths where they are decarboxylated leading to the release of CO₂. High concentrations of CO₂ accumulated in the bundle sheath cells are refixed with the 5-carbon sugar ribulose-1,5-bisphosphate (RuBP) by rubisco (ribulose-1,5-bisphosphate carboxylase) yielding carbohydrates. Similar to C₃ plants, the rubisco in bundle sheath of C₄ plant has oxygenase activity, catalyzing the reaction between O₂ and RuBP producing one 3-phosphoglycerate and a 2-carbon acid 2-phosphoglycolate. Further metabolism of 2-phosphoglycolate leads to the loss of one CO₂ from the plant termed photorespiration. The process of CO₂ accumulation increases the concentration of CO₂ in bundle sheath cells shifting the competition ratio, CO₂/O₂, by nearly 100-fold at the reaction site of rubisco. Therefore, two

types of photosynthetic cells function together to inhibit oxygenase activity and to overcome the negative impact of photorespiration (Kanai and Edwards, 1999). Grasses with the C_4 photosynthetic pathway are well adapted to hot and arid climates and exhibit higher photosynthetic efficiency than cool-season grasses. This is a reason why C_4 grasses are commonly defined as warm-season plants.

Water Use Efficiency (WUE)

The C_4 pathway in the warm-season grasses exhibits improved N and water-use efficiency (Brown, 1999) compared to cool-season grasses. Instantaneous water-use efficiency is expressed as the ratio of photosynthesis to transpiration. At high temperatures, water loss during CO_2 assimilation is a very serious problem for grasses. C_4 species evolved in a way to avoid this problem. Due to the C_4 mechanism, water use efficiency of warm-season grasses is nearly twofold higher than that of C_3 grasses. At the leaf-level and for whole canopies, the photosynthetic rates are much higher in C_4 species than C_3 species at nearly the same daily water use or at the same rate of transpiration (Hay and Walker, 1989). The higher water use efficiency physically results from the ability of C_4 cycle to concentrate CO_2 . Assuming all things are equal, the higher water use efficiency in C_4 grasses occurs because the relative stomatal aperture is regulated at a lower value in C_4 than C_3 grasses having the same CO_2 assimilation capacity. Under this condition, the key factor for the maintenance of CO_2 assimilation in C_4 grasses is the presence of a pumping mechanism elevating the concentration of CO_2 at the carboxylation site. Because of increased CO_2 concentration at the carboxylation site in C_4 mechanism, the oxygenase reaction is eliminated

to a great extent and the loss of energy connected with the photorespiratory pathway is largely decreased. The principle by which the C₄ grasses save water can be demonstrated by doubling the stomatal resistance prevailing in C₃ grasses and reducing the diffusive efflux of water vapor by 50%. In warm-climate C₃ grasses, stomatal closure for water conservation combines with thermal enhancement of photorespiration to give a synergistic reduction in CO₂ assimilation capacity. In C₄ grasses, rising temperatures stimulate the activity of phosphoenolpyruvate carboxylase (PEPCase) and the other enzymes of the C₄ pump. Thus, the ability to supply rubisco with high concentrations of CO₂ is increased, even at the lower intercellular CO₂ levels. In C₃ grasses, the oxygenase activity of rubisco increases more rapidly than the carboxylase activity at higher temperature. This is why in warm climates C₄ grasses, not only with their decreased water requirement, but also with their suppression of photorespiration, have an advantage over C₃ grasses. C₃ species that are conservative in their water use may compete with C₄ species that have similar water demands, but twice the CO₂ acquisition potential and therefore a much greater growth capacity in dry conditions. This greater growth potential may enable the C₄ species to produce roots and leaves faster, thereby capturing the remaining water, nutrient, and light resources in the environment (Moore et al., 2004; Heldt, 2005).

Nitrogen Use Efficiency (NUE)

Warm-season grasses with the C₄ photosynthetic pathway produce high biomass yields per unit of N uptake, and have low N concentrations in foliar tissues, resulting in high nitrogen-use efficiency (NUE). The higher NUE also derives from the C₄ mechanism,

offering high assimilation with lower rubisco concentrations and at relatively low leaf N concentrations (Brown, 1978; Sage and Pearcy, 1987a; 1987b). C_4 species allocate a much lower fraction of leaf soluble protein to rubisco whereas C_3 species invest 30 to 50% of their soluble protein in rubisco (Ku et al., 1979; Brown, 1994). The C_4 species operate with much lower rubisco and lower total protein because of their CO_2 concentrating mechanism leading to CO_2 saturation of rubisco enzymes restricted to bundle sheath cells. The result is that less of this enzyme is required for high rates of photosynthesis in C_4 than C_3 grasses.

Consequently, maximum yield potential occurs at a lower N concentration for C_4 than for C_3 species. Additionally, C_4 grasses require lower leaf N concentrations for the saturation of % maximum relative growth rate (RGR) (Brown, 1978) and for new leaf production than do C_3 grasses (Wilson and Brown, 1983). In C_4 species, the combination between this feature and the relatively high photosynthesis at low N concentrations provides great biomass production even when grown on soil with low N availability (Brown, 1978). Although the C_4 mechanism allows grasses a considerable yield production at low N concentrations, this negatively affects forage quality. The high NUE unfortunately leads to a decrease in protein concentration for C_4 grasses. In addition, the low digestibility of C_4 grasses appears to be in part a consequence of the suberized bundle-sheath cells (Mullahey et al., 1992). Kiniry et al. (1999) reported that the percentage of protein for most C_4 grasses decreases with advancing maturity and increasing biomass when not harvested.

Species Description

Eastern Gamagrass

Eastern gamagrass [*Tripsacum dactyloides* L.] is a New World, native, perennial, warm-season (C₄), bunchgrass that produces short, thick rhizomes near the soil surface. The surface rhizomes spread outward and form large circular clumps over time. It is believed to be one of the progenitors of corn (*Zea mays* L.). Leaves of this grass emerging from the basal nodes may range from 30 to 95 mm long and from 1 to 6 mm wide. This grass reaches a height of 0.3 m or more, with the seedheads extending to 3 m. It is monoecious with both male and female spikelets on racemes which are 30-50 mm long. The top three quarters of racemes are made up of male spikelets and the bottom quarter of female spikelets (Springer and Dewald, 2004). This tall grass is native to eastern half of the US found from central Texas to southeastern Nebraska and central Iowa and eastward to the Atlantic Ocean (Hitchcock, 1951).

Eastern gamagrass is highly productive with high photosynthetic rates and water-use efficiency. It has long been recognized among native warm-season grasses as having high forage quality (Redfearn and Nelson, 2003). It provides forage of high digestibility and palatability, but is vulnerable to close a frequent grazing which leads to deteriorated native stand (Kindiger and Dewald, 1997). Eastern gamagrass is used for grazing, stored forage, and soil amelioration and conservation (Springer and Dewald, 2004). The average yield of eastern gamagrass grown for commercial forage production is 13.5 t ha⁻¹ with crude protein averaging 150 g kg⁻¹ (Eaheart, 1992). Additionally, this grass can be a good alternative to corn for making silage (Dickerson and van der Grinten, 1990; Brejda et al., 1994a) which is

equal to or higher than corn silage for quality characteristics (Brejda et al., 1994a), except for in vitro dry matter digestibility which averages 150 to 200 g kg⁻¹ lower than corn. However, most previous research has focused on eastern gamagrass as a grazing forage.

Eastern gamagrass has been evaluated as a potential energy crop (Pfeifer et al., 1990). With low nitrogen and phosphorus concentration (Esquivel et al., 2000), it would be a suitable biomass feedstock in direct combustion or pyrolysis, compared to other warm-season grasses (Anderson et al., 2008). The production cost is a major factor determining the economic feasibility of biofuel crops. Using eastern gamagrass in the Conservation Reserve Program for biocrude had the total cost associated with conversion into bioenergy (million Btu) averaging from \$4.50 to \$9.50 GJ⁻¹, which is similar to using big bluestem or indiangrass (Nelson et al., 1994). Thus, the cost of biomass production of eastern gamagrass was competitive with current prices and future projections for other fuel sources such as natural gas (\$7.32 GJ⁻¹) and fuel oil (\$ 15.60 GJ⁻¹) (Dale, 2007).

One significant barrier to using this grass as forage or bioenergy feedstock is difficulty in stand establishment which is caused by poor seed production, seed quality (partly due to sterility), and seed dormancy (Hitchcock and Clothier, 1899; Springer et al., 2004). Research by Ahring and Frank (1968) demonstrated that removal of empty seed units can improve seed quality. Studies have been conducted to develop methods to stimulate the germination of intact capsules (Anderson, 1985; Tian et al., 2003). Tian et al. (2002) suggested that complete germination of caryopses occurred when the pericarp near the embryo was scarified. USDA-NRCS (2006) suggested that eastern gamagrass seed dormancy can be broken by either moist scarification, removing the cupule, or applying

hydrogen peroxide. Planting caryopses or wet-chilling treated seed has been recommended for improved stand establishment, but specialized equipment needed to hull gamagrass without significantly damaging the caryopsis has not been developed. Eastern gamagrass has been genetically improved to a limited extent for use as forage. With recent advances in breeding mechanisms, eastern gamagrass has great potential to be improved for use as a bioenergy feedstock (Anderson et al., 2008).

Big Bluestem

Big bluestem (*Andropogon gerardii* Vitman) is a native perennial warm-season grass with short, tough rhizomes. The presence of fine silky hairs dispersed near the base of the upper leaf surface is often used to identify this grass before flowering (Harper et al., 2004). Big bluestem belongs to the subfamily Panicoideae and tribe Androgoneae. This native grass once dominated the tall-grass prairie of North America (Weaver, 1968; Gould and Shaw, 1983), with a mix of other warm-season grasses. The original range of big bluestem is reported to be similar to switchgrass extending from central Mexico north to Canada (Gould and Shaw, 1983).

Big bluestem is a bunch-type grass that reaches 1-3 m tall depending on water and nutrient availability (Stubbendieck et al., 1991). With deep roots extending up to 2.7 m, big bluestem can grow on a wide variety of soils and is extremely drought-tolerant. It adapts well in moderately to excessively well drained, clay loam soils, but tends to dominate rich, sandy soils (Weaver, 1968). The seedhead consists of two or three racemes arising from a common joint of the seedstalk, looking like a turkey's foot (Vogel, 2000). Seed are relatively

dark and hairy. The onset and cessation of growth and flowering are controlled by photoperiod. Considered a short-day plant, flowering of big bluestem is initiated by reducing day length in late summer (Newell, 1968; Waller and Lewis, 1979).

Just as most native warm-season grasses, seedlings are very slow to establish from seed, and it can be propagated with crown divisions. Seedling vigor is commonly weak (Redfearn and Nelson, 2003) and seedling are often overlooked in a field during establishment due to the dominance of annual grassy weeds (Hsu et al., 1985; DeLucia et al., 1992). Enhanced weed control is an important management practice for the establishment of big bluestem. A slightly delayed planting to obtain optimum soil temperature at 25° C coupled with the application of timely broad spectrum herbicide is desirable. In addition, burning the stubble of a previous crop and the existing stand of weeds in the early spring may be of advantage in increasing subsequent growth and biomass yield (Mitchell et al., 1994). Although big bluestem grows best on moist sites in the true prairie, it will tolerate long periods of drought due to its deeper root system and greater energy storage in rhizomes (Weaver, 1954). It has been widely accepted that use of big bluestem for forage and biomass feedstock is limited due to the weak seedling vigor and difficulty in establishment (Balasko and Nelson, 2003). Proper fertilization is a critical management practice for improving stand establishment. The application of fertilizers at the wrong time, especially during the establishment year leads to stand degradation through invasion of weedy grasses (Brejda, 2000; Mitchell and Britton, 2000).

Big bluestem is one of the most palatable warm-season grasses and has good forage quality when harvested or grazed early. Thus, primary use of this grass is pasture or hay for

livestock, but it has relatively poor grazing tolerance (Balasko and Nelson, 2003). It is considered nutritious early in the growing season when the grass sward is at a vegetative stage and composed mostly of leaves (Redfearn and Nelson, 2003). However, when stem elongation occurs during the onset of flowering during late summer, its digestibility decreases. In addition, it is a top choice for erosion control, because of its rapid growth pattern over a wide range of climates and soils (Boe et al., 2004). Big bluestem also provides excellent wildlife habitat. It has been planted for grassland birds and mammals to use for nesting and escape cover throughout the year (Sanderson et al., 2004).

Big bluestem is one of the potential bioenergy crops which have been screened by the US herbaceous energy crops research program (Cherney et al., 1990). Because this species offered greater in vitro fermentability than other warm-season grasses species (Jung and Vogel, 1992), potential for ethanol production and value-added chemicals through a direct fermentation process may provide this species a distinct advantage over acid hydrolysis, saccharification and fermentation of switchgrass (Weimer and Springer, 2007). These authors also suggested that big bluestem is a superior feedstock, and may offer accelerated development in the biofuel production, based on preliminary research utilizing consolidated bioprocessing. Although switchgrass was faster to establish, big bluestem became the most productive species by the second year. Additionally, it produced more bio-oil from pyrolysis than switchgrass. Consequently, cost of processing for bio-oil from big bluestem was cheaper than that from switchgrass. Tiffany et al. (2006) reported that big bluestem offered US\$8.91 Mg⁻¹ greater returns on investment of processing for bio-oil than switchgrass. However, cost of production of big bluestem in Iowa was more expensive than that of

switchgrass. This indicated that cost of biomass production can vary by location (Hallam et al., 2001).

Indiangrass

Indiangrass [*Sorghastrum nutans* (L.) Nash] is an erect, bunch-type, native, perennial, warm-season grass with short, stout creeping rhizomes. It is classified into subfamily Panicoideae, supertribe Andropogonodae, tribe Andropogoneae, and subtribe Andropogoninae (Watson and Dallwitz, 1992). The name *Sorghastrum* coming from *Sorghum* and the Latin suffix *astrum* (a poor imitation of), indicates the resemblance to *Sorghum* (Gould, 1975). *Sorghastrums* are likely to be phylogenetically allied to sorghum species in which the cyanogenic glycoside, dhurrin [(S)-p-hydroxymandelonitrile β -D-glucopyranoside] has been found (Gorz et al., 1979, 1981; Haskins et al., 1979). Indiangrass is native to the Americas and distributed from east-central Canada south to southern Mexico (Stubbendieck et al., 1997). It was one of the primary grass components of the Tallgrass Prairie in the central USA, being a companion with big bluestem, little bluestem, and switchgrass (Gould, 1978). A sod of indiangrass is produced originally in prairies, open woods, and dry slopes, reaching from 1-to 2.5-m tall (Weaver, 1954; Hitchcock, 1971). Indiangrass produces a deep root system reaching 1.6 m deep and is quite drought-tolerant. It is adapted to a wide variety of soil textures with a pH range from 5.6 to 7.1 (Duke, 1978), and withstands occasional flooding and moderate amounts of salt (Stubbendieck et al., 1997). The growth and flowering of indiangrass cultivars and strains are governed by photoperiod. Both biomass yield and winter survival also are affected by photoperiod responses. When

southern ecotypes are moved far north they flower late compared with those from northern latitudes, and do not survive the winters (McMillan, 1959). Moving northern ecotypes south commonly decreases biomass yield (Cathey, 1990). Mitchell and Vogel (2004) reported that most cultivars of indiangrass grown in the USA initially were developed in the Great Plains from germplasm collected in that region. 'Newberry,' 'Osage' and 'Rumsey' are cultivars best-suited for the Mid-South (Harper et al., 2004).

Indiangrass seed is chaffy and is high in dormancy, especially newly harvested seed (Fullbright, 1988). Seed dormancy can be broken by chilling imbibed seed (cold stratification) for 2 to 4 wk (Geng and Barnett, 1969), exposing the germinating seeds to 10 h of daylight or 2 h of red light day⁻¹ (Emal and Conard, 1973). The amount of seed dormancy decreases with storage time. Optimum time for planting indiangrass in the USA is the same time as corn in the spring. When delayed planting is later than 4 wk after the completion of corn planting, the possibility for establishment failure increases (Anderson, 1989). Previously, establishment difficulties of indiangrass were caused by its seed and the competition from weeds due to its lack of seedling atrazine tolerance (Martin et al. 1982; Bahler et al., 1984). Recently, these problems have been solved. Development of new herbicide technology for managing weed competition with seedlings offers greater seed survival and increased opportunity to include indiangrass in more C₄ grass mixtures. Processing seed with a de-awner to remove most of pubescence and awns enhances flow of indiangrass seed in grass drills (Brown et al., 1981). These improved management practices will likely increase the use of indiangrass both for forage and biomass production in the Great Plains and Corn Belt of the USA.

Indiangrass provides quality wildlife habitat, and quality forage for livestock.

Indiangrass stands are primarily used as pasture or hay. It has greater forage quality than switchgrass and big bluestem, because it flowers and matures later in the summer than those two species (Perry and Baltensperger, 1979). Thus, it offers higher quality forage for grazing or hay in late summer and early autumn than other warm-season grasses. Krueger and Curtis (1979) demonstrated that steers (*Bos Taurus*) grazing pure stands of indiangrass gained $1.08 \text{ kg head}^{-1} \text{ day}^{-1}$, which was 54% greater than big bluestem, and 16% greater than switchgrass. But indiangrass gave 111 grazing day ha^{-1} fewer than big bluestem providing 199 grazing day ha^{-1} . Additionally, beef production on indiangrass pastures was less than that produced on switchgrass and big bluestem.

Since the concentration of hydrocyanic acid (prussic acid) is high for indiangrass early in the growing season, pure stands of indiangrass should not be grazed during that period of time (Haskins et al., 1979). However, the hydrocyanic acid potential (HCN-p) of the indiangrass stands declines with advancing maturity and increased plant height (Vogel et al., 1987) and can be diluted by growing a mixture of species (Gorz et al., 1981).

Data is limited or sometimes nonexistent on the production of indiangrass for bioenergy production in Iowa. In addition to switchgrass, indiangrass is a native warm-season grass of the Great Plains and western Corn Belt of the USA and is well suited to use because it grows well on a variety of soils with minimal inputs, responds well to fertilization, is water-use efficient, and provides good biomass yield. These factors are desirable for a bioenergy crop and will likely increase indiangrass use for bioenergy production in these regions. Thus, it is possible to promote indiangrass as a warm-season grass to take advantage

of the later maturation of the species. Incorporating indiangrass into either forage-livestock systems or biomass feedstock production as an additional warm-season option in these regions will prolong the number of harvesting days in the season beyond that of other C₄ grasses.

Switchgrass

Switchgrass (*Panicum virgatum* L.) is an erect, perennial C₄ grass native to the prairies, open woodlands, brackish marches, and pinewoods (*Pinus* spp.) of North America (Hitchcock, 1951; Stubbendieck et al., 1991). Along with indiangrass and big bluestem, switchgrass is one of “big three” grasses making up of the predominant species found in tall-grass prairies. In much of the prairie and grasslands in North America, switchgrass and other native grasses once occupied were converted into cropland. However, switchgrass still exists in remnant prairie sites which are an invaluable as a germplasm resource (Hopkins et al., 1995b). Switchgrass was seeded and managed as native range or as a component of conservation reserve program (CRP) lands, and is grown to use as highly managed pasture or hay.

Most switchgrass genotypes are caespitose growing in small dense clumps, and have short rhizomes allowing it to form sod over time. The seedling root system belongs to Panicoid type (Newman and Moser, 1988; Tischler and Voigt, 1993) and roots of established plants can reach 3 m in depth (Weaver, 1954). Switchgrass can reach 0.5-to3-m in height (Moser and Vogel, 1995; Vogel, 2004). The inflorescence of switchgrass is a diffuse panicle 15 to 55 cm long with two-flowered spikelets disarticulating below the glumes. Leaves can

reach 10 to 60 cm long, with fringed-membrane ligules. The number of leaves per tiller depends on genotype and environment (Redfearn et al., 1997). Switchgrass is a polymorphic species with two predominant ecotypes including upland and lowland (Brunken and Estes, 1975; Porter, 1966; Vogel, 2004). The upland ecotype is shorter in height than lowland ecotypes and provides lower biomass yield. This ecotype is found commonly in drier areas with colder temperatures, which are not subject to flooding. In contrast, lowland ecotypes are taller, coarser plants, with more bunch-type growth and more rapid growth with higher biomass yields than upland ecotypes. This ecotype group is commonly found on flood plains and other areas subject to inundation with mild winter temperatures. Switchgrass is photoperiod sensitive requiring short days to flower (Benedict, 1941). In North America, decreasing day length during early summer induces switchgrass to flower. Consequently, the development of switchgrass is location dependent. When southern ecotypes are moved north, they remain vegetative longer and produce more forage than northern ecotypes moved south. But staying vegetative too late in the fall can lead to stand losses from winter injury varying with cultivar. Early maturity and flowering with short stature occurs when northern ecotypes are moved south providing them a shorter than normal daylength (Newell, 1968). Additionally, the physiological development of switchgrass in the Great Plains of the USA is highly correlated to growing degree days (GDD) measuring accumulated heat or photosynthesis energy (Mitchell et al., 1997; 2001).

Switchgrass stands can be established by seeds, tillers, and rhizomes. Optimum seed germination was obtained at 25° C (Hanson and Johnson, 2005). Soil temperatures < 20° C decrease the germination and growth of switchgrass seedling (Hsu et al., 1985). However,

planting in early spring in cold soil may help with breaking dormancy (cold stratification) of dormant seeds (Vassey et al., 1985). In addition to cold treatment, simple dormancy will be broken if the seed is aged long enough (Zheng-Xing et al., 2001). The recommended seeding dates for switchgrass correspond to those for corn. Switchgrass adapts to a wide range of soil conditions from sands to clay loam soils with low moisture content and poor drainage, and tolerates soil with pH values ranging from 3.9 to 7.6 (Duke, 1978; Hopkins and Taliaferro, 1977). In the establishment year, the growth of switchgrass depends on soil fertility, soil moisture, and infestation by weeds. Stand failures occur when planting is too late because seedlings do not have time to establish and develop the root reserves essential to survive winter. Brejda et al. (1998) reported that a symbiotic relationship exists between switchgrass roots and arbuscular mycorrhizal fungi (AMF) and this symbiosis is important for stand establishment and persistence. Rhizosphere microflora in native prairies, old seeded stands of switchgrass and inoculation can enhance the growth of switchgrass seedlings and increase switchgrass production (Brejda et al., 1998; Brejda, 1996).

Due to its array of desirable characteristics, switchgrass has been used for livestock production, conservation, and biomass production (Vogel, 2004). In the past 20 years, switchgrass has been seeded primarily for pasture and hay production in the Great Plains. It normally matures earlier than other warm-season grasses. Thus, it should be managed as a monoculture in pastures. Yet no more than 20% of switchgrass seed planted in a mixture can provide good pastures to support beef cattle herds in the summer months. Switchgrass cut at the beginning of the seedhead stage will be able to provide an optimum combination of yield and quality for hay production (Newell and Moline, 1978). Depending on time of harvest,

cultivar, fertilization, and location, switchgrass forage yield ranges from 1.5 to >20 Mg ha⁻¹ (Vogel, 2004). Vogel (2004) also reported that average daily gain of beef cattle grazing on switchgrass pasture ranged from 0.5 to 1.1 kg d⁻¹. However, horses (*Equus caballus*) or sheep (*Ovis aries*) exhibit hepatogenous photosensitization (an inflammatory condition) caused by changes in the serum concentration of acute phase proteins. Animals may die when grazing on pure stands of switchgrass (Pouli et al., 1992; Lee et al., 2001). In addition to use for forage-livestock systems, switchgrass is used for soil and water conservation plantings such as waterways, highway rights-of-way, buffer strips, and for wildlife plantings (Sanderson et al., 2004). In the Midwest and the Great Plains, switchgrass was one of native warm-season grasses used in the Conservation Reserve Program that was designed to take marginal cropland out of production and reduce soil erosion. Planting switchgrass for buffer strips and hedges has been accepted as an economical and efficient method for reducing soil erosion and improving water quality from agricultural fields (Dewald et al., 1996; Eghball et al., 2000; Gilley et al., 2000).

Due to its high yield, perennial nature, its soil and wildlife enhancing ability, ability to be established from seed, its status as a native grass, and its adaptability to poor soils and marginal cropland, switchgrass has been identified as a promising species for development into an herbaceous biomass fuel crop in the USA (Vogel, 1996). The Bioenergy Research Development Program (BRDP) began at the DOE's Oak Ridge National Laboratory in 1978 and supported a significant research effort to develop switchgrass as a cellulosic biomass feedstock with breeding and management research including improving available cultivars with high yield on a regional basis, optimizing harvest methods and timing and minimizing N

fertilization. These projects have led to a reduction of its projected production costs by 25% (McLaughlin and Kszos, 2005). Existing cultivars of switchgrass have provided 14 Mg ha⁻¹ in the Midwest (Hopkins et al., 1995a) and up to 20 Mg ha⁻¹ biomass yield in the Southeastern USA (McLaughlin et al., 1999). One of key characteristics by which switchgrass was chosen as the model herbaceous biomass crop is its ability to be established in poor soils although lower yield may be obtained. Schmer et al. (2008) reported that biomass yield of switchgrass grown on marginal croplands in North and South Dakota and Nebraska ranged from 5.2 to 11.1 Mg ha⁻¹ varying with both precipitation and temperature. However, switchgrass provided 93% more biomass yield and equivalent net energy relative to human-made prairies, offered 540% more renewable energy than used to produce it, and produced 94% lower estimated greenhouse gas emissions than that of gasoline. It was estimated that 1 Mg biomass can produce 330 L ethanol assuming 75% extraction efficiency (Turhollow et al., 1988; Dobbins et al., 1990). In addition to fermentation for ethanol, switchgrass can be used in combustion processes to produce heat, steam, or electricity or it can be gasified to produce a syn-gas and used in variety of end point processes (McLaughlin et al., 1999). Therefore, switchgrass is considered a model energy crop today.

Forage Quality

When animals having production potential are fed only with forage, forage quality is defined as the potential of forage to provide the desired animal response such as milk production, and weight gain (Collins and Fritz, 2003; Marten et al., 1988; Coleman et al., 2004). Forage quality is sometimes used synonymously with forage nutritive value.

However, the latter does not include the efficiency of nutrient and energy utilization.

Collectively, forage quality encompasses nutritive value and includes also a function of voluntary intake and the effects of any antiquality constituents (Collins and Fritz, 2003; Coleman et al., 2004). In forage quality, the nutritive value is responsible for approximate 30% of the variation, whereas voluntary intake is responsible for the remainder.

Combining intake and digestibility with utilization of the digested nutrients provides means of evaluating the feeding value of forages more effectively than evaluating either alone, and becomes a concept similar to the definition of forage quality (Heaney, 1970; Mott and Moore, 1970). Practically, intake is more difficult to estimate from forage characteristics than is digestibility, primarily because digestibility is an intrinsic property of forage, while intake is a function of the properties of forage fed to animals (Orskov, 1996).

The warm-season (C_4) grasses provide excellent forage quality early in the growing season. However, these grasses grow and mature rapidly. The quality of warm-season grasses decreases with advancing maturity similar to cool-season grasses. These C_4 grasses may not meet maintenance requirements for medium or high yielding dairy cows, if they are not managed properly (Laksessvela and Said, 1978).

Digestibility

Of all components of forage quality, digestibility (energy value) and crude protein are two of the most important aspects of nutritive value. The amount of digestible energy (DE) determines primarily the nutritive value of warm-season grasses (Moore, 1980; Gillet et al., 1985). Digestibility is the difference between the amount of a nutrient eaten over a period of

time, and that voided in the feces as a proportion of the total amount consumed. Forages with higher digestibility provide more energy to the animal per unit of forage dry matter consumed than do less digestible forages. In contrast to C₃ cool-season grasses, C₄ warm-season grasses contain more cell wall constituents and less cell contents. The relatively high concentration and low digestibility of fiber in warm-season grasses restrict DE intake (Abrams et al., 1983; Mertens, 1987, 1993). Most nutrients are lost from forages via the feces. Therefore, the digestibility of the nutritional components is very important. Coleman et al. (2004) noted that digestibility is often used a proxy of nutritive value, but it is more specially the loss of dietary constituents upon consumption and passage through the animal.

Van Soest (1994) proposed that dry matter digestibility (DMD) is defined as microbial fermentation in the rumen by which the fibrous or cell wall proportion of forages is degraded partially, followed by digestion of feed residues and microbial biomass by enzymes in the abomasum and small intestine. This ruminal fermentation offers a conversion of plant fiber into valuable products, commonly not available for humans. On average, C₄ grasses have about 444-670 g kg⁻¹ digestibility (Moore et al., 1996), and are on average less than 130 g kg⁻¹ dry matter digestible do C₃ grasses (Wilson and Minson, 1980). Differences in anatomical structure of tissues associated with the different photosynthetic pathways and higher temperature at which they are commonly grown result in relatively lower digestibility of C₄ grasses (Wilson and Kennedy, 1996). Although C₄ grasses have a greater percentage of cell-wall material potentially digestible than do C₃ grasses, the rate of fermentation in C₄ grasses is relatively slower because of its chemical composition and physical structure (Coleman and Popham, 1996).

Forage Intake

Under unrestricted feeding conditions, voluntary intake is the intake of forage that is determined considerably by the behavior of forage in the digestive tract (Reid, 1994). Daily herbage intake is evaluated by the function of feeding time and intake rate. Intake rate is function of intake per bite and biting rate. Warm-season grasses may restrict intake rate by grazing ruminants because leaf density is not enough to support suitable intake per bite (Stobbs, 1973). The interacting factors are involved in intake regulation, including feed bulk density, ease of consumption, ease of comminution, palatability, and ease of digestion and passage once in the rumen (Weston, 1982). When energy is scarce, then voluntary consumption and rate of digesta removal from the rumen may vary with changing the intensity of signals to the central nervous system regulating feeding (Weston, 1982;1996). The reciprocal NDF concentration of the forage can be used to determine constraints of both fill and energy deficit. This single variable helps resolve some of the interacting factors involved in intake regulation (Mertens, 1985). NDF and its relationship to digestibility could describe both fill and energy. But it may be a poor predictor of intake across several forage types, especially C₄ warm-season perennials, when forages are sole component of the diet (Moore et al., 1996). On average, forage intake of C₄ grasses is less than that of C₃ grasses (Minson, 1990). The less forage intake is primarily caused by the greater proportion of cell-wall materials in C₄ grasses, simultaneously reduced rate and extent of digestibility, decreased rate of passage of undigested residue, and extended rumination. However, C₄ grasses may provide a greater intake than do C₃ grasses at the same digestibility (Moore et al., 1996).

Lignocellulosic Biomass Feedstocks

Lignocellulosic biomass refers to all organic material composed of three major components including cellulose, hemicelluloses, and lignin, representing the primary structural components of plant cell wall. Lignocellulosic materials are the most abundant organic compounds on earth (Heldt, 2005) derived from four sources, including 1) agricultural residues such as corn stover, wheat straw, and bagasse, 2) wood and forest residues such as sawdust, tinnings, and mill wastes, 3) dedicated energy crops including woody crops (such as poplar and willow) and grasses/herbaceous crops (such as switchgrass, miscanthus), 4) solid wastes from industrial and municipal sources such as paper waste, and food waste (Gupta and Demirbas, 2010; Zhu and Yang, 2010). Lignocellulosic materials from these sources provide a low-cost and abundant resource for the production of biofuel and chemicals.

Plant materials are produced from the conversion air, water, and sunlight, by photosynthesis, into carbohydrates forming the building blocks of biomass. Photosynthesis is driven by the solar energy stored in the chemical bonds of the structural components of biomass. The stored, chemical energy is released when the bonds between adjacent carbon, hydrogen and oxygen molecules are broken by various conversion processes. If the conversion of biomass into energy is efficiently processed, the carbon is oxidized to produce CO₂ and water. The CO₂ released from combustion system is used by the photosynthetic pathway to renew biomass. Therefore, bioenergy production from biomass is cyclical and renewable (McKendry, 2002). Based on the carbon cycle of the production and use of biomass, fossil fuels derived from the fossilized remains of plants, are excluded from the

biomass definition, mainly because the combustion of fossil fuels increases CO₂ concentration in the atmosphere (Gubta and Demirbas, 2010), causing global warming.

To meet the goal of displacing 30% of petroleum usage by 2030, the USDA and DOE have estimated that one billion dry tons of harvestable biomass and 60 billion gallons of ethanol would need to be supplied each year. Among a wide variety of biomass sources in the US, perennial grasses accounted for the majority available biomass (Perlack et al., 2005). Thus, high yield perennial grasses are of interest for biofuel production. Sanderson and Adler (2008) reported that the biomass yields of warm-season grasses including switchgrass, miscanthus, and eastern gamagrass with N fertilization between 0 and 212 kg N ha⁻¹ ranged from 5 to 11, 1.4 to 18.2, and 6.5 to 15.9 ton ha⁻¹, which can be converted into energy at 134, 150, and 185 GJ ha⁻¹, respectively. Nonetheless, perennial grasses such as switchgrass, require less input including fertilizer, water, and pesticide. These lignocellulosic biomass feedstocks do not compete with the food supply, and can grow in low-value marginal land (Gubta and Demirbas, 2010).

Herbaceous biomass feedstocks including perennial warm-season grasses can be converted into energy by a number of methods including fermentation of carbohydrates to produce transport fuels (McKendry, 2002; Hamelinck et al., 2005) and thermochemical processes (e.g., combustion, pyrolysis, and gasification) to produce electrical/heat energy and chemical feedstocks (McKendry, 2002). The efficiency and end product of the various conversion processes of biomass to energy depend upon the feedstock characteristics and composition, pretreatment processes, and the fermentation technologies that are used (McKendry, 2002; Hamelinck et al., 2005; Cheng, 2010).

Biomass Quality

Lignocellulosic biomass can be converted thermochemically through pyrolysis, gasification, and pyrolysis (Ortiz-Canavate, 1994) or direct combustion to produce steam and generate electricity (McKendry, 2002). Different biochemical processes such as fermentation and anaerobic digestion can be used to convert biomass to ethanol and methane (Hayn et al., 1993). Particular material properties become important depending on the energy conversion processes.

Forage quality of native warm-season grasses has been extensively studied for use as livestock feed (Moore and Buxton, 2000) but to a limited extent for the conversion to energy. In contrast to forage quality, the goal of biofuel crops production is to maximize the concentration of lignocelluloses in the feedstock, minimize N and mineral concentrations and limit water concentration (Lewandowski and Kicherer, 1997; McKendry, 2002).

The key chemical composition for biomass can be quantified in terms of moisture content, ash content, heating value, lignocellulose content (lignin, hemicelluloses, and cellulose), and elemental composition. Moisture, total energy, ash, and alkali metal content are main characters of biomass determining the suitability of energy crops for thermochemical processes, while lignocellulosic content are of prime concern for biochemical conversion of biomass into biofuel (McKendry, 2002; Gupta and Demirbas, 2010).

Moisture

Moisture content in biomass is stored within and between cells. Normally, the stem contains the lowest moisture and the moisture content increases toward the roots and the crown (Gupta and Demirbas, 2010). Moisture has two forms including intrinsic and extrinsic moisture. Intrinsic moisture is the moisture content of material that is not related to weather at harvest. It can be investigated only under laboratory conditions. Extrinsic moisture is influenced by prevailing weather conditions during harvesting on the overall biomass moisture content. Higher moisture content in biomass at the time of harvest increases the cost of transportation and handling, and adversely affects overall energy balance due to energy requirement for moisture vaporization during the combustion process (Lewandowski and Kicherer, 1997). The harvested biomass of switchgrass with moisture concentration of 13-15% at baling time recovers net energy yield of 18.0 Gj that reduces from 18.4 Gj on a dry weight basis (McLaughlin et al., 1996). While thermal conversion (dry processing technique) requires feedstock with low moisture content, typically less than 50%, bio-conversion such as fermentation of biomass into ethanol can use high moisture feedstocks. In terms of recoverable energy from harvested biomass, thin-stemmed grasses such as switchgrass with their lower moisture content, however, has a slightly higher level of recoverable energy than wood, because less energy for drying is required. In addition, the harvested biomass can be contaminated with ash and alkali metals from soil due to high moisture content as a result of the prevailing weather conditions at harvest (McKendry, 2002), leading to lower net energy yield for combustion (Agblevor et al., 1992; Nordin, 1994). To decrease danger of self-ignition during storage and improve efficient combustion,

the desired moisture concentration of switchgrass should be less than 230 g kg^{-1} (Lewandowski and Kicherer, 1997) or even less. Standard recommendations for hay storage are $150\text{-}180 \text{ g kg}^{-1}$. With wet fuels burning less cleanly and requiring a supplemental fuel source to support combustion, the upper limit for self-supporting combustion of biomass is $650 \text{ g moisture kg}^{-1}$ (Jenkins et al., 1998). Therefore, the desired plant biomass should be dry. McLaughlin et al. (1996) reported that the thin-stemmed, herbaceous plants such as switchgrass and miscanthus had a much lower moisture content than woody plant (moisture content of 13-15% for switchgrass; 11.5% for miscanthus; 45% for poplar; 60% for willow). Delaying the harvest until late fall–early spring decreases moisture concentration in reed canarygrass (Burvall, 1997), *Miscanthus* sp. (Lewandowski and Kicherer, 1997), and switchgrass (Adler et al., 2006).

The biochemical conversion involves biologically mediated reactions under wet processing techniques. Thus, the relationship between appropriate biochemical conversion technology and biomass moisture is essentially straightforward. In contrast to thermochemical conversion, high moisture content biomass does not affect on or may be more suited to wet conversion via biologically mediated reactions, such as fermentation for ethanol (McKendry, 2002).

Energy content

Energy is stored in the chemical bonds of the structural components of biomass of which about 80% of the plant dry weight is the cell wall fraction (cellulose, hemicelluloses, and lignin) (McLaughlin et al., 1996). The energy content can be expressed as calorific value

or heat value achieved from combustion. The higher heating value (HHV) is often expressed as the gross calorific value (CV) representing the maximum amount of energy potentially recoverable from a given biomass source with its moisture content. McKendry (2002) reported that the energy content (HHV) of plant materials ranged from 17-21 MJ kg⁻¹, slightly less than coal ranging from 21-28 MJ kg⁻¹ in combustion processes. With much lower moisture content, C₄ warm-season grasses had nearly the same energy content as hard wood (heat value of 17.4 MJ kg⁻¹ for switchgrass; 18.5 MJ kg⁻¹ for miscanthus; 18.5 MJ kg⁻¹ for poplar; 20 MJ kg⁻¹ for willow) (McKendry, 2002).

Ash content

Ash is produced by burning a solid and is the residue remaining when a biomass fuel is produced by either thermochemical (i.e. combustion) or bio-chemical processes (i.e. fermentation). The ash concentration in biomass negatively impacts both handling and processing costs of the overall biomass energy conversion. Miles et al. (1993) reported that the chemical composition of the ash can present operational problems in a thermochemical conversion process. In combustion processes, ash content in feedstock can contribute to slagging of internal boiler surfaces resulting in formation of deposits that decrease boiler efficiency and increase maintenance costs. The available energy of the fuel decreased proportionately depending on the amount of ash in feedstock. Cassida et al. (2005) demonstrated that every 1% increase in ash concentration in a feedstock decreases the heating value in a combustion process by 0.2 MJ kg⁻¹. Thus, ash content forms a standard measurement parameter for solid and liquid fuels.

Mineral content

Mineral composition or inorganic elements (C, N, Al, Ca, Cl, Fe, K, Mg, Na, P, S, Si) are important for any thermochemical conversion processes. Blander (1997) suggested that the direct combustion and gasification of biomass with these minerals often forms molten salts and silicates, which are the main cause of fouling, slagging, and corrosion. The degree of serious problems caused by ash content in biomass feedstock to power plants is promoted with the reaction of alkali metals with silica present in the ash (McLaughlin et al., 1996). The products produced from this reaction are a sticky, mobile liquid phase, resulting in blockages of airways in the furnace and boiler plant (McKendry, 2002). Chemically, Si has a high melting point (1,700 °C) that would not impact a combustion plant. However, when K or Ca is present, Si easily reacts with these alkalis forming alkali silicates of much lower melting points (about 700 °C) (Baxter et al., 1998).

In addition, high N concentration causes reduction in hydrocarbon yields during thermochemical conversion (Agblevor et al., 1992; Agblevor et al., 1995) and leads to high NO_x emissions in unstaged combustion. The NO_x emissions can be lowered by choosing the proper combustion technologies such as staged combustion and steam gas recirculation (Lewandowski and Kicherer, 1997). To ensure low emissions and corrosion risks in conventional boilers, they also reported that critical limit for N concentration in biomass is 10 g kg⁻¹ DM (Lewandowski and Kicherer, 1997). Nitrogen removed along with biomass from the cropping system must also be replaced to support production for the following season so that input cost is raised.

Therefore, harvested biomass with low inorganic composition is more desirable for combustion processes (Miles et al., 1996). Proper management practices can reduce the concentration of these inorganic elements in biomass feedstocks. Lewandowski and Kicherer (1997) and Adler et al. (2006) reported that delaying a single harvest to fall generally decreased the concentration of N, and alkali in warm-season grasses. In addition, nitrogen fertilization has positive impacts on the concentration of mineral elements in biomass feedstocks (Lemus et al., 2008).

Lignocellulose

Lignocellulose represents the primary structural component of the plant cell wall fraction which consists of three main components including cellulose, hemicelluloses, and lignin. The relative proportion of these polymers is dependent on the species, the cell type and the developmental stage. On average, the grass cell wall is made up of 25-50% cellulose, 15-30% hemicellulose, 15-25% lignin, and 5-10% proteins (Vermerris, 2008; Keshwani, 2010).

The most abundant organic material is cellulose, which is a main structural element in plant cell walls and makes up approximately a third of all plant matter. Cellulose $(C_6H_{10}O_5)_n$ is a pure glucose polymer, containing linear chains of (1, 4)-D-gluco-pyranose units. These units are linked by β -(1, 4)-glycosidic bonds, with an average molecular weight of about 10,000. Depending on the source, each cellulose chain can contain 500 to 5000 glucose units. Linear cellulose chains are linked by strong intra and intermolecular hydrogen bonding which forms a crystalline structure (McKendry, 2002; Keshwani, 2010; Gupta and

Demirbas, 2010). The bundles of linear cellulose chains are aggregated to form a microfibril. Bundles of microfibrils constitute cellulose fibrils in the cell wall structure (Fratzl, 2003). Although each hydrogen bond is weak, a crystalline structure of cellulose molecules is difficult to break down by acid and enzymatic hydrolysis. But ruminants can break down cellulose into sugars using β -glycosidase enzymes produced by intestinal microorganism (Gupta and Demirbas, 2010).

Hemicellulose is referred to as all major polysaccharides in plant cell walls except cellulose and pectin. It is a mixture of different polysaccharides with a typical molecular weight of 30,000 or less. In contrast to cellulose, hemicelluloses are heterogeneous branched polysaccharides, primarily composed of pentoses (arabinose, rhamnose, and xylose), hexoses (galactose, glucose, and mannose), and uronic acids (galacturonic, glucuronic, and methylglucuronic acids). They bind tightly to surface of each cellulose microfibril via hydrogen bonds and form covalent bonds with functional groups in lignin. Of these polysaccharides, xylose ($C_5O_{10}H_5$) is the most important sugar which constitutes the xylan backbone. Hemicellulose is amorphous in structure, which is more susceptible to enzymatic hydrolysis compared with crystalline cellulose (Goldstein, 1981; McKendry, 2002; Keshwani, 2010; Gupta and Demirbas, 2010).

When cellulose and hemicellulose are combined, they are referred to as holocellulose (Gupta and Demirbas, 2010). With microbiological or chemical processes, cellulose and hemicelluloses can be converted into sugars which can be used in fermentation for ethanol or butanol production (Hayn et al., 1993; Luukkonen et al., 2001; McKendry, 2002; Chandra et

al., 2007). Thus, a biomass feedstock with high cellulose and hemicellulose is desirable to provide a high biofuel yield.

Lignin is an important component of the cell walls of higher plants which plays a key role in vascular transport and provides structural support and resistance to microbial attack. With a typical molecular weight of more than 10,000, lignin is a highly cross-linked aromatic polymer of phenylpropane units including p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. As the fundamental units of lignin, these phenylpropane compounds are referred to as monolignols, varying with the number of methoxy (OCH_3) groups attached to the phenolic unit (McKendry, 2002; Keshwani, 2010). In grasses, lignin is typically composed of all three monolignol units. Lignin content represents a potentially large energy source of about 26.1 G Mg^{-1} , an energy value similar to coal as a result of the carbon-carbon linkages that can be oxidized (Johnson et al., 1995; Vermerris, 2008). Biomass feedstock with high lignin content is desirable for thermochemical conversion. Lignin produced as a coproduct in the Simultaneous Saccharification and Fermentation (SSF) process plays a key role in producing heat for the production process. In addition, lignin can also be reacted with all other constituents into carbon monoxide and hydrogen used in synthetic pathways to produce a wide variety of useful end products during thermochemical conversion (McLaughlin et al., 1996). However, current conversion techniques through hydrolysis or enzymatic systems cannot directly convert the lignin into ethanol (McLaughlin et al., 1996; McKendry, 2002).

Lignin interacts with cellulose and hemicellulose in the cell wall. This structure of lignocellulose is very difficult to biodegrade (McKendry, 2002). Pretreatments are necessary to loosen its structure and remove lignin from the lignocellulosic material, so the cellulose

and hemicellulose are accessible for hydrolysis to produce fermentable sugars (Dale et al., 1996; Laxman and Lachke, 2008). Dien et al. (2006) has observed a negative relationship between Klason lignin concentration and efficiency of glucose recovery after dilute-acid pretreatment and enzymatic saccharification, similar to the negative impact of liginification on digestibility of forages by ruminants (Jung and Deetz, 1993). These complex processes for the current conversion technology of lignocellulosic materials to ethanol make it too expensive for commercial bioethanol production. However, the cellulose-platform bioethanol production has great potential because the lignocellulosic materials are so abundant in the world and the conversion technologies are advancing very rapidly to lower the processing costs (Cheng, 2010).

Nitrogen

Nitrogen Functions and Requirements in Grass Production

Nitrogen is an essential macronutrient required by C₄ grasses and grazing ruminants. Nitrogen is a constituent of amino acids, proteins, and nucleic acids playing a key role in plant and animal nutrition (McDowell, 1992, Marschner, 1995). Mathews et al. (2004) reported that warm-season grasses and grazing ruminants have a minimal requirement of about 20 and 15-20 g kg⁻¹ of N, respectively. Just about 25% of the warm-season grasses sampled around the world would routinely meet this requirement.

Nitrogen is often considered as the most limiting nutrient in grass production grown both for forage and biomass (Ziadi et al., 2000 and Barker and Collins, 2003). This is commonly attributed to large N use in grass growth (up to 6% of dry matter), high solubility

of plant-absorbable forms (NH_4^+ , NO_3^-), making it subject to leaching, and numerous pathways for loss of N from pasture compared with other nutrients. Nitrogen is only provided indirectly by the soil, with the predominant source being the atmosphere. Nitrogen input of soil significantly is supplied via fixation by a legume (Barker and Collins, 2003).

Sustainability of warm-season grass pastures is deteriorated by losses of N due to biomass removal, erosion, leaching, or denitrification (Miles and Manson, 1992; Boddey et al., 1996) and by immobilization in relatively stable pools of grass-derived soil organic matter (Robbins et al., 1986; Myers et al., 1987).

Nitrogen Fertilization

Fertilization of warm-season grasses is an important method to meet both plant and animal mineral requirements. Modeling yield response of warm-season grasses to N fertilization is an effective way of determining the range of application rates and converging to optimum values at low and high nutrient input (Overman, 1995). Just as any other forage species, native warm-season grasses, therefore, require proper fertilization for improving stand establishment and increasing forage production and quality.

The N requirement is defined as the minimum amount of N in the aboveground biomass associated with maximum production (Standford and Legg, 1984). In general, C_4 plants have greater photosynthetic nitrogen utilization efficiency (PNUE) than C_3 plants (Anten et al., 1995). Therefore, N requirements for native warm-season grasses are less than cool-season grasses. Previous studies reported that the growth rate of warm-season grasses is higher than that of cool-season grasses across a range of plant N concentrations (Wilson,

1975; Wilson and Brown, 1983; Brown, 1985). The optimum growth rate for warm-season grasses occurs at lower plant N concentrations than cool-season grasses (Brown, 1978; Wilson and Brown, 1983). This is likely due to the relatively smaller proportion of N invested in ribulose biphosphate carboxylase (rubisco) for C₄ plants compared with C₃ plants (Brown, 1978). Significant genetic variation in efficiency of C₄ photosynthesis can be found even within C₄ genera (Meinzer and Zhu, 1998). However, there is a penalty in terms of reduced herbage N concentrations in C₄ grasses compared with C₃ grasses, legumes, or forbs, due to greater PNUE and a lower amount of N invested in rubisco.

Understanding the N requirement is needed to determine N application rate for a crop. However, N requirement may confound N application rate. Only some portion of N fertilizer applied will be taken up by the crop to use for yield components. Some of the N in the yield may be obtained from sources other than the applied N fertilizer while some portion of the applied N will be lost from the system.

The application of N fertilizer at the wrong time or rate is economically inefficient, leading to stand degradation via invasion of undesirable plant species, and environmental degradation via contamination of surface and groundwater (Brejda, 2000). Craswell and Godwin (1984) suggested that the optimum N fertilization will be able to increase nitrogen use efficiency (NUE), and economic return per unit of N applied, and reduce leaching of N into surface or groundwater.

Nitrogen Recommendations for Warm-Season Grasses

The response of C₄ grasses to applied N can reach 100 kg DM kg⁻¹ N (Vicente-Chandler et al., 1974), yielding 8 to 24 Mg DM ha⁻¹ yr⁻¹ or more (Tamimi, 1972; Wilkinson and Langdale, 1974). However, the application rate of N varies with grass species, yield potential of the stand for the given soil and climate, management practices being used, whether the stand will be used for grazing, hay, or biomass production and the economic return per unit of N applied. Of these factors, the economic return for N fertilization is often the most important factor for decisions on how much N should be applied. For forage production, maximum DM yield increases has been reported by Brejda (2000). The practical starting point of economic N application is to compensate the N loss in the harvested feedstock. The amount of protein for warm-season grass hay will be specific for species, cultivar, and stage of maturity at harvest. Nitrogen recommendations for pasture and hay are 67 to 100 kg N ha⁻¹ yr⁻¹ and 85 to 110 kg N ha⁻¹ yr⁻¹, respectively, in southeast Nebraska growing season precipitation where is the highest.

N application Rate for Different Species

Brejda (2000) suggested that the N requirement of native warm-season grasses used for forages largely depends on the yield potential of the site, productivity of grass species, and management practices being used. In Midwest states and the Central Great Plains of the USA, he reported that significant fertilizer N inputs are required to optimize biomass production by warm-season grasses when managed as forage crops. The primary N fertilizers for pasture include urea [(NH₂)₂CO], ammonium nitrate (NH₄NO₃), (NH₂)₂CO-

NH_4NO_3 solutions, and ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4]$. The N requirement of grasses varies with the ability of soils to supply N to grasses. In general, it ranges from 40 to 120 $\text{kg ha}^{-1} \text{yr}^{-1}$ (Blue, 1970; Tamimi, 1972; Vicente-Chandler et al., 1974). In contrast to P and K fertilization, N fertilization is based on crop needs, not on soil tests because the current reliable methods of analysis to predict the rather limited concentration of inorganic N are difficult. However, most soils provide grasses with N through mineralization of organic N (Mathews et al., 2004).

Optimum N rates for maximum dry matter yield of eastern gamagrass ranged from about 56 to 224 kg ha^{-1} while of switchgrass ranged about 50 to 120 kg ha^{-1} . Maximum dry yield of big bluestem and indiangrass occurred when N fertilizer was applied at from 45 to 90 kg ha^{-1} (Brejda, 2000).

Only few studies have assessed the effect of N fertilization on yield of perennial, warm-season grasses managed as bioenergy feedstocks in the central United States. Most of them were conducted on switchgrass, a model crop for bioenergy feedstock production (Ma et al., 2001; Muir et al., 2001; Thomason et al., 2004; Lemus et al., 2008) while information on the optimum N fertilization rate for other warm-season grasses managed for biomass feedstocks other than switchgrass is limited. McLaughlin and Walsh (1998) reported that N application rates applied annually for switchgrass are of 70-100 kg ha^{-1} , about half of those applied in corn (138-154 kg ha^{-1}). Similar to forage management, the N requirement of warm-season grasses grown as bioenergy feedstocks depends largely on the yield potential of the site, productivity of grass species/cultivar, and management practices being used. In previous studies in IA and NE, the optimum biomass yield was achieved when switchgrass

was fertilized with 120 kg ha⁻¹ (Vogel et al., 2002). In Texas, the optimum total ‘Alamo’ switchgrass yields were obtained when the N fertilizer was applied at 168 kg ha⁻¹ (Muir et al., 2001). In forage management, the optimum combination of N fertilization rate and harvest management will depend on the relative needs for yield and quality. Optimum forage yields with improved quality achieved with multiple harvests lead to greater N removal as result of harvest of immature, N-rich biomass. In contrast, N fertilization requirements might be decreased for single-harvest feedstock management systems compared to multiharvest forage systems (Parrish and Fike, 2005; McLaughlin and Kszos, 2005).

Harvest Management

Harvest management is essential to maximize the economic yield of high-quality while ensuring stand persistence. Seasonal time of harvest significantly affects yields (Madakadze et al., 1999; Sanderson et al., 1999; Vogel et al., 2002) and forage quality (Anderson and Matches, 1983; Sanderson and Wolf, 1995). Harvesting grasses later in maturity may increase yield while forage quality is reduced. In contrast, harvesting less mature forage may reduce total season yield while forage quality is increased (Sanderson, 2000). Previous studies conducted in Iowa demonstrated that switchgrass harvested early would be suitable for forage livestock programs by improving quality forage in late spring or early summer, and providing good yields of moderate quality forage in late summer regrowth (Anderson and Matches, 1983; Sanderson, 2000).

Increased harvest frequency provides a higher forage quality (Brejda et al., 1996) because grasses are harvested at younger stages, whereas harvesting less frequently generally

results in greater yields and increased stand longevity because grasses can restore reserves between cutting to maintain vigor (Brink and Marten, 1989). Response of C₄ perennial grasses to defoliation varies with species.

Defoliation-sensitive species such as big bluestem were less flexible in allocating C between below ground organs (root and crown) and regrowth (new leaf blades). These species maintained more C in roots than in new leaf growth after cutting than did a defoliation-tolerant species. *Bouteloua rigidisetata* was more tolerant of defoliation and more flexible in allocating C to above ground growth. The defoliation-tolerant species allocate more C to new leaf growth than to root growth after clipping, allowing them to quickly reestablish photosynthesizing leaf area (Briske et al., 1996). Therefore, the frequency at which grasses are cut should depend on the nutrient needs of the livestock consuming the forage, as well as the desired longevity of stand.

Although reserve carbohydrates in roots, rhizomes, and shoot bases of grasses play an important role in regrowth after cutting (Danckwerts, 1993), the size of the pool of active meristems remaining after defoliation may be more important (Richards and Caldwell, 1985). George and Oberman (1989) reported that switchgrass is very sensitive to frequent or intensive defoliation. Because switchgrass elevates the growing points well above ground during early vegetative growth stage, they are susceptible to removal by defoliation (Sanderson and Wolf, 1995a). The stand loss of switchgrass occurred because the growing points (crown buds and aerial auxiliary meristems) elevated well above ground during early vegetative growth stage were removed (Brejda et al., 1994; Haferkamp and Copeland, 1984). Moreover, switchgrass has a high ratio of reproductive to vegetative tillers. To maintain the

desired longevity of stand, a 15 to 20- cm stubble height remaining after cutting is, therefore, recommended for big bluestem, indiangrass, and switchgrass while a 20-cm stubble height should be left for eastern gamagrass (USDA-NRCS, 1991).

Harvest management of native warm-season grasses as biofuel crops, however, may be different from forage production for livestock. Because the objective of harvest management for biomass production is to achieve as high accumulation of lignocellulose materials as possible while the forage production is concerned with nutritive value (Sanderson et al., 1996; Sanderson et al., 1999). Proper time of harvest is important for reaching high biomass yields and for maintaining grass stands.

As a compromise between forage yield and quality in forage-livestock systems, multiple harvests are recommended for the native tallgrasses (Forwood and Magai, 1992; Moser and Vogel, 1995). However, increased harvest frequency for these grasses may not benefit biomass production. Yields of perennial grasses increased as higher N fertilization rates when clipping frequencies were reduced (Sibma and Alberda, 1980). A previous study conducted in Oklahoma showed that harvesting monoculture stands of switchgrass, big bluestem, little bluestem, indiangrass, and sideoats grama [*Bouteloua curtipendula* (Michx.) Torr.] at 30-or 60-day intervals for 6 years provided lower biomass yields compared with cutting once in July each year because root production was generally reduced as clipping intensity and frequency increased (Dwyer et al., 1963), resulting in limited water and nutrient uptake. In Florida, biomass yields of eastern gamagrass decreased after the second year of frequent cuttings (Kalmbacher et al., 1990). Sanderson et al. (1995) suggested that increased harvest frequency decreased total biomass yields of switchgrass compared with a

single harvest in September in Texas. In the Midwest, Vogel et al. (2002) reported that harvesting switchgrass stands at the R3 to R5 stage of maturity (panicle fully emerged from boot to anthesis) was recommended to maximize biomass yields. In years with enough precipitation, regrowth may be harvested after a killing frost. For a study in South Dakota, optimum switchgrass yields were achieved when a single harvest was taken after a killing frost provided higher total yields and improved switchgrass persistence compared with anthesis harvests (Mulkey et al., 2006). Thus, a single late-season harvest may be the most appropriate harvest management for biomass fuel cropping (Sanderson et al., 1999; Sanderson et al., 1996). Harvesting once late in the growing season can also improve the biomass fuel quality due to increased lignocellulose concentrations and reduced mineral content of the harvested biomass feedstocks. This is the result of a decrease in the leaf-to-stem ratio (Griffin and Jung, 1983; Twidwell et al., 1988) and the translocation of nutrients from the above ground biomass to the roots after a killing frost (Parrish et al., 1996; Vogel et al., 2002).

However, delaying harvest too long may cause lower biomass yields. In Texas, the greatest biomass yield was obtained when Alamo switchgrass was harvested once in September. A study conducted in Texas by Walker et al. (1995) demonstrated that delaying the fall harvest past September reduced biomass yields of Alamo switchgrass. Although biofuel quality of reed canarygrass (*Phalaris arundinacea* L.) (Burvall, 1997) and *Miscanthus* sp. (Lewandowski et al., 2003) increased due to a reduction in the mineral content when harvest was further delayed until spring, yields decreased. Parrish and Wolf (1993) suggested that a dry matter yield reduction of approximately 20% was caused by the

remobilization and translocation of nutrients. Some of the yield loss of switchgrass when fall harvest was delayed from September to November was caused by leaf loss (Sanderson et al., 1996; Sanderson et al., 1999). Adler et al. (2006) also found that switchgrass yield generally decreased almost 40% (from about 7-4.4 Mg ha⁻¹) when harvest was delayed from fall to spring. They noted that the decrease in biomass yield resulted from 1) biomass not picked up by the baler either because it was not cut due to lodging or it was cut but not picked up by the baler, and 2) a decrease in standing tiller weight due to loss of leaves and panicles during winter.

Therefore, efficient harvest management of native perennial warm-season grasses requires an understanding of their developmental morphology and response to defoliation (Sanderson, 2000). This would allow producers to integrate forage and biomass cropping for more flexibility and diversity in their management systems and may provide a more efficient response to potential fluctuations in feedstock markets (Sanderson et al., 1999; Mitchell and Moser, 2000).

Developmental Morphology of Warm-Season Grasses

Increase in dry weight of grasses is the result of expansion of leaves, stems, and reproductive structures referred to as growth (Frank, 1996). With positive correlation with growth, the developmental morphology refers to the predictable series of changes in structure and arrangement of plant components associated with plant maturity (Esau, 1960). Plant maturity is the primary factor affecting the morphological development and forage quality within a species (Kalu and Fick, 1983; Nelson and Moser, 1994). Changes in plant

morphology occurring during primary growth can be important determinants of potential productivity and quality in perennial forage grasses (Redfearn et al., 1997; Frank et al., 1993). Therefore, understanding changes in the developmental morphology of grasses allows producers to make management practice decisions properly such as timing of initial harvest and fertilizer application (Moore et al., 1991; Frank et al., 1993; Mitchell and Moser, 2000). Briske (1991) reported that developmental morphology is generally similar among grass species with only minor variations separating growth forms. However, each warm-season grass has a particular pattern of developmental morphology and tiller recruitment. The rate of morphological development in warm-season grasses can be affected by environmental factors. Among these factors, air temperature and photoperiod are the main environmental factors determining the rate of developmental morphology (Briske, 1991; Gillen and Ewing, 1992; Frank, 1996). Developmental morphology among grass species has strong linear relations to day of the year, partially attributed to the process of floral induction (Mitchell et al., 1997; Briske, 1991). Many warm-season grasses require a specific photoperiod for floral induction (Loch et al., 2004; Moore et al., 2004). Allard and Evans (1941) reported that big bluestem and switchgrass require shortening day length for floral induction, whereas indiangrass is intermediate in its flowering behavior. An improved understanding on the differences in pattern of developmental morphology among native warm-season grass species will improve management procedures.

Quantifying Developmental Morphology

Quantification of the developmental morphology of tiller populations indicates the architectural changes in the grass sward. Many systems have been developed to quantify the growth and development of grasses (Vanderlip, 1972; Haun, 1973; Zadoks et al., 1974; Simon and Park, 1983; Moore et al., 1991; Sanderson, 1992). No single system for quantifying developmental morphology of grasses has been widely accepted (Sanderson, 1992). Some systems can be used with populations of annual grasses, but not with populations of perennial grasses representing numerous related genotypes rather than a pure line due to the open-pollinated nature of most perennial grasses (Moore and Moser, 1995). Additionally, many of these systems were difficult to apply in the field. However, the system for quantifying morphological development of plants developed by Moore et al. (1991) is applicable to most annual and perennial grass swards, and is easily applied in the field. Based on mean stage count (MSC) and mean stage weight (MSW), this system used four primary growth stages for quantifying developmental morphology of established perennial grasses including vegetative, elongation, reproductive, and seed development and ripening (Table 1) (Moore et al., 1991).

With the Moore et al. (1991) classification system, Hendrickson (1992) and Moore and Moser (1995) found that there was the close relationship between developmental morphology of sand bluestem [*Andropogon gerardii* var. *paucipilus* (Nash) Fern.] and prairie sandreed [*Calamovilfa longifolia* (Hook.) Scribn.] tiller populations with day of the year as to GDD. However, the seasonal variability in developmental morphology within tiller populations may make developmental morphology difficult to predict from one growing

season to the next. Mitchell et al. (1997) reported that switchgrass and big bluestem developmental morphology quantified with the Moore et al. (1991) system increased linearly with day of the year across six environments in Nebraska and Kansas. Switchgrass MSC advanced linearly with accumulated GDD, while big bluestem MSC increased quadratically with accumulated GDD in all six environments. Based on the results, they suggested that morphological development of big bluestem was affected more by non-photoperiodic environmental variations than switchgrass. Morphological development of switchgrass is highly predictable, and switchgrass is more photoperiod responsive than big bluestem. More sensitivity of switchgrass to photoperiod may be a reason why switchgrass matures earlier than big bluestem.

Tiller Development and Demographics

Tiller initiation in perennial grasses is stimulated with releasing axillary buds from hormonal inhibition by auxin referred as apical dominance. This mechanism of physiological control is associated with tiller stimulation by cytokinin, a growth regulator produced primarily in the roots (Nelson, 1996). The synthesis or use of cytokinin for axillary bud growth is inhibited by auxin produced in shoot apex. Defoliation releases axillary buds from hormonal inhibition by auxin (Murphy and Briske, 1992). Nelson (1996) also suggested that the auxin/cytokinin ratio in the grass plant may affect the developmental morphology of warm-season grasses.

Canopy architecture is important for describing many grass canopy processes influenced by the interaction between plants and the environment (Welles and Norman,

1991). Canopy architecture of the grass swards results from continual changes and are a function of the tiller morphology at different growth stages and the growth stage distribution within the tiller population (Moore and Moser, 1995). Canopy architecture affects plant physiology and can be related to relative light interception, forage productivity and quality, forage availability, and forage accessibility to grazing livestock (Nelson and Moser, 1994). A key trait of canopy architecture is tiller density representing the pool of meristematic tissues. Growth of grasses occurs from these meristematic tissues associated with the basis for potential productivity (Murphy and Briske, 1992). High yielding grass species are associated with accumulation of a large number of reproductive tillers. Kalmbacher (1983) suggested that most of the dry matter was in the nodes and internodes of tillers, with leaf blades contributing the smallest proportion to dry matter production. However, Twidwell et al. (1988) found that forage quality and rate of fiber digestion declined primarily as a result of increased stem proportion. The seasonality of tiller recruitment determines the number of live tillers related to tiller longevity (Briske, 1991). The rate of recruitment of new tillers and the mortality of existing tillers affects tiller density (Langer et al., 1964; Briske, 1991). Asexual reproduction of new tillers or rhizomes from axillary buds causes perennation of established grass swards (Waller et al., 1985).

Generally, a population of tillers referred to as the grass sward responds to environmental stimuli. Changes in structure of tiller population with time reflect the seasonal variability in developmental morphology of individual tillers (Moore and Moser, 1995). With respect to time, principles of plant demography can be used to describe the variation in developmental morphology within tiller populations. Tiller demographic analyses of grasses

are displayed with plotting the number of tillers per square meter in the vegetative, elongating, reproductive (seedhead development), and seed ripening primary growth stages with respect to time. Tiller density of some warm-season grasses decreases as morphological development increased. Mitchell et al. (1997) reported that maximum tiller density for switchgrass and big bluestem occurred on Day 157 of year and reduced by an average of 9.4 and 5.1 tillers $\text{m}^{-2} \text{d}^{-1}$ for switchgrass and big bluestem respectively. Tiller demographic analyses of switchgrass and big bluestem tiller populations in their study indicated that initial grazing should be begun at Day 157 of year when a large proportion of the tillers are vegetative with MSC of 1.59 for switchgrass and 1.32 for big bluestem to maximize tiller regrowth, tiller recruitment, and livestock use. Delayed harvest of switchgrass after Day 172 of year when the large proportion of elongating, reproductive, and seed ripening tillers present would result in low animal performance and may be detrimental to stand persistence due to removed apical meristems by defoliation (Anderson and Matches, 1983; Waller et al., 1985). It was also determined that big bluestem matures later in the growing season than switchgrass. The greater proportion of vegetative tillers before Day 206 illustrated why big bluestem is better suited to mid-summer grazing than switchgrass in the central Great Plains (Moser and Vogel, 1995). Therefore, taking into consideration the differences in developmental morphology within tiller populations occurring from interactions between plants and the environment will aid in improving management (Welles and Norman, 1991).

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Table 2.1. Primary and secondary growth stages and their numerical indices and descriptions for staging and development of perennial grasses (Moore et al., 1991)

Stage	Index	Description
Germination		
G0	0.0	Dry seed
G1	0.1	Imbibition
G2	0.3	Radicle emergence
G3	0.5	Coleoptiles emergence
G4	0.7	Mesocotyl and/or coleoptiles elongation
G5	0.9	Coleoptile emergence from soil
Vegetative-Leaf Development		
VE or V0	1.0	Emergence of first leaf
V1	$(1/N)+0.9$	First leaf collared
V2	$(2/N)+0.9$	Second leaf collared
Vn	$(n/N)+0.9$	Nth leaf collared
Elongation-Stem Elongation		
E0	2.0	Onset of stem elongation
E1	$(1/N)+1.9$	First node palpable/visible
E2	$(2/N)+1.9$	Second node palpable/visible
En	$(n/N)+1.9$	N th node palpable/visible
Elongation-Floral Development		
R0	3.0	Boot stage
R1	3.1	Inflorescence emergence/1 st spikelet visible
R2	3.3	Spikelets fully emerged/peduncle not emerged
R3	3.5	Inflorescence emerged/peduncle fully elongated
R4	3.7	Anther emerged/anthesis
R5	3.9	Post-anthesis/fertilization
Seed Development and Ripening		
S0	4.0	Caryopsis visible
S1	4.1	Milk
S2	4.3	Soft dough
S3	4.5	Hard dough
S4	4.7	Endosperm hard/physiological maturity
S5	4.9	Endosperm dry/seed ripe

CHAPTER 3. FORAGE QUALITY OF NATIVE WARM-SEASON-GRASSES IN RESPONSE TO NITROGEN FERTILIZATION AND HARVEST DATE

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Abstract

Native warm-season grasses have great potential as summer pasture grasses, and are appropriate for a complementary role with cool-season forage grasses in full-season forage systems throughout much of the Corn Belt and Great Plains. Although they generally are found to be lower in forage quality than cool-season (C_3) grasses, their quality could be improved by proper management practices. The objectives of this research were to: 1) determine the effect of N levels on the nutritive quality of four warm-season grasses, 2) examine the effect of plant maturity on quality of different warm-season grass species, and 3) evaluate relationships between forage quality and morphological development. The field study was conducted at the Sorenson Research Farm near Ames, IA, during 2006 and 2007. The experimental design was a split-split plot in time with four replications. Big bluestem (*Andropogon gerardii* Vitman), eastern gamagrass (*Tripsacum dactyloides* L.), indiangrass (*Sorghastrum nutans* L. Nash), and switchgrass (*Panicum virgatum* L.) were main plots,

seeded in a randomized complete block design. Across grass species, three nitrogen application rates (0, 65, and 140 kg ha⁻¹) were subplots, and ten harvest dates were sub-subplots, randomly assigned within each subplot. For all species, harvesting at early maturity increased forage quality including increased IVDMD and crude protein, and decreases in NDF concentrations, whereas allowing forage to reach later maturity reduced forage quality with declines in IVDMD and crude protein and increases in NDF concentration. The NDF concentration in warm-season grasses with the exception of big bluestem increased with N fertilization at 65 kg ha⁻¹, while N fertilization at 140 kg ha⁻¹ had no consistent effect on NDF concentration of the four grass species across both years. Nitrogen fertilization increased the IVDMD value across four grass species in 2006, whereas it decreased the IVDMD value for switchgrass and did not affect the IVDMD value for the other three species in 2007. The concentration of crude protein for all four warm-season grass species increased at higher rates of N fertilization. Therefore, although there were variations in the effect of N fertilization on NDF and IVDMD making drawing conclusions difficult, N fertilization could improve forage quality for warm-season grasses through increasing the concentration of crude protein. For big bluestem, switchgrass, and indiangrass, forage quality parameters showed a quadratic relationship to MSC, with coefficients of determination ≥ 0.96 for IVDMD, 0.88 for NDF, and 0.88 for CP, respectively. However, predicting forage quality changes based on MSC did not provide reasonable estimates for eastern gamagrass.

Introduction

Traditionally, native warm-season grasses have played a key role in livestock production as summer forages in the US. In the Midwest and eastern Great Plains, most pasture species consumed by beef cattle and sheep are cool-season grasses introduced from other parts of the world, including Europe, Asia and North Africa. They are well adapted to the local environment and provide high quality pasture, range, hay and silage for ruminant livestock in these regions (Massengale, 2000; Nelson et al., 2003). Many introduced cool-season grasses, however, have low productivity during the summer (Anderson et al., 1976; Jung et al., 1978), limited by reduced photosynthetic rates at warmer temperatures than optimum for C₃ photosynthesis (Long, 1999).

For this reason, native warm-season grasses (C₄) have been promoted as alternative species to fill the deficiency in forage supply during warm and dry months (Moser and Vogel, 1995). Using warm-season grasses in pastures during the June-August time period, combined with cool-season pastures during the spring and fall months has efficiently improved livestock production by providing a more uniform supply of forage during the entire year (Redfearn and Nelson, 2003).

Switchgrass (*Panicum virgatum* L.), big bluestem (*Andropogon gerardii* Vitman), and indiangrass (*Sorghastrum nutans* L. Nash) have the most potential as pasture in the central and eastern USA (Massengale, 2000). They are perennial warm-season grasses of the North American Tallgrass Prairie (Weaver, 1968), naturally occurring throughout the Great Plains and the western Corn Belt (Hitchcock, 1971; Stubbendieck et al., 1991). Steer (*Bos taurus*) gains in indiangrass, switchgrass and big bluestem grazing trials averaged 1.08 (Krueger and Curtis, 1979), 0.65 (Barnhardt and Wedin 1984) and 1.22 (Michell et al., 2005)

kg hd⁻¹, respectively. In addition to those native prairie grasses, another highly productive perennial warm-season grass, which has become increasingly interesting as a promising pasture grass, is eastern gamagrass (*Tripsacum dactyloides* L.). It is probably native to Mexico and is widely spread from Massachusetts to Nebraska and southward to upper South America. Eastern gamagrass is highly productive, very palatable, and of good quality when grown under proper management. Dry matter yield of eastern gamagrass ranged from 5,000 to more than 10,000 kg ha⁻¹ varying with management and area of production (Springer and Dowald, 2004). It produced forage of high quality, with IVDMD ranging from 400 to 773 g kg⁻¹ (Voigt and Sharp, 1995), and crude protein concentrations averaging 150 g kg⁻¹ (Eaheart, 1992). Yield and quality varied with N fertilization, age of regrowth, and rainfall. Because of its high potential quality, eastern gamagrass can be a good alternative to corn silage (Dickerson and van der Grinten 1990; Brejda et al., 1994).

However, forage quality of warm-season grasses varies significantly with genetics and environmental factors. Maturity and soil nutrients, especially nitrogen, during growth are primary factors affecting forage quality of pastures. Similar to C₃ grasses, protein concentrations in warm-season grasses varies with species, season, and maturity of growth (Poppi and McLennan, 1995). Although the quality of C₄ grasses declines rapidly with maturity, it can be quite excellent if the grasses are managed properly (Sanderson, 2000; Brejda, 2000).

The overall objectives of this research were to 1) determine the effect of N levels on the nutritive quality of four warm-season grasses, 2) examine the effect of plant maturity on quality of different warm-season grass species, and 3) evaluate potential interactions among these factors on nutritive quality relationships.

Materials and Methods

Plant establishment

Field experiments were conducted during 2006 and 2007 at the Iowa State University Sorenson Farm, near Ames, IA (42°0'40" N, 93°44'46" W) on a Canisteo silty clay loam (fine-loamy, mixed, superactive, calcareous, mesic, Typic Endoaquoll), Webster silty clay loam (fine-loamy, mixed, superactive, mesic, Typic Endoaquoll), and Clarion silty clay loam (fine-loamy, mixed, superactive, mesic, Typic Hapludolls) soil. Weather data were compiled from the Iowa Environmental Mesonet and collected from the climate station located approximately 4.8 km from the research site (Fig. 5.1a and 5.1b). Grass plots were established in a fallow field managed in a corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] rotation. Four warm-season grass species, big bluestem ('Roundtree'), indiangrass ('Rumsey'), eastern gamagrass ('Pete'), and switchgrass ('Cave-In-Rock'), were seeded at 3.6, 3.6, 4.5, and 2.3 kg pure live seed ha⁻¹, respectively, in the fall of 2003 in a randomized complete block with four replications. Big bluestem, indiangrass, and switchgrass were seeded in 20-cm rows using a 10-row small grain drill (Tye model 2007, AGCO Co., Lockney, TX) while eastern gamagrass was seeded in 76-cm rows using a 2-row corn planter (John Deere model 71 Flexi Planter, John Deere Co., Moline, IL). Big bluestem and indiangrass plots were overseeded to increase plant density in the spring of 2005.

The experimental design was a split-split plot in time. The four different warm-season grass species were whole plots that measured 3 m x 42.8 m. Three nitrogen application rates (0, 65 and 140 kg N ha⁻¹) were subplots (3 m x 10.7 m) and the ten harvest dates were sub-sub plots that were randomly assigned. Harvest dates in each year were treated as repeated measures in the analysis of variance.

Before initiation of spring growth each year, imazapic [(RS)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-methylnicotinic acid] was applied at a rate of 140 g a.i. ha⁻¹ to indiangrass and atrazine [6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5,-triazine-2,4-diamine] was applied at a rate of 2.3 kg a.i. ha⁻¹ to all other species for weed control. Eastern gamagrass plots received a single inter-row cultivation in June 2004 and 2005. Standing dead material was annually mowed to a 5-cm stubble height, and the residue was removed from plots using a self-propelled forage harvester (John Deere model 5480, John Deere Co., Moline, IL). To reduce weed density and to remove the vast majority of accumulated aboveground material before the initiation of N treatments, all grass plots were burned in April 2006 (Moser and Vogel, 1995; Mitchell and Britton, 2000). In early May of 2004 and 2005, nitrogen fertilizer was applied at a rate of 85 kg N ha⁻¹.

Nitrogen treatments

Ammonium nitrate (NH₄NO₃: 0, 65, and 140 kg N ha⁻¹) was preweighed and applied with 1.5-m-wide drop spreader (Model 6500, Gandy Co., Owatonna, MN) on each individual subplot nitrogen treatment on 8 May 2006 and 12 May 2007. Based on soil test results no other fertilizer was needed.

Forage harvest treatment

Biomass samples were harvested on ten dates between 16 May and 3 October in 2006, and 22 May and 8 October in 2007. For the first seven harvest dates (16 May, 31 May, 13 June, 27 June, 11 July, 25 July, and 8 August in 2006; 22 May, 4 June, 18 June, 2 July, 16 July, 30 July, and 13 August in 2007), the samples were collected at approximately 2-week

intervals and at approximately 3-week intervals for last three harvest dates (22 August, 12 September, and 3 October in 2006; 27 August, 17 September, and 8 October in 2007).

Depending on spring growth in each year, the first harvest occurred at early vegetative growth stage (Table 3.1). At each harvest, the developmental stage of the warm-season grass stands were visually scored using the index system of Moore et al. (1991).

At each sampling date, two 0.19-m² samples were harvested at ground level from each subplot at each harvest date. The samples were dried at 60°C in a forced-air oven for approximately 72 h and reweighed. Dried materials were ground sequentially using a Wiley Mill model 4 (Thomas Manufacturing, Philadelphia, PA) to pass an 8-mm screen and reground to uniformity with a 1-mm screen. Subsamples of homogenized tissue were stored in plastic vials at room temperature until quality analyses were performed.

Forage quality

The samples were equilibrated with the environment for approximately 48 h by opening the plastic storage vials before quality analysis. Samples were analyzed sequentially for neutral detergent fiber (NDF) using an ANKOM 200 Fiber Analyzer (ANKOM Technology Corp., Fairport, NY). Total nitrogen was quantified with a LECO CHN-2000 instrument (LECO Corp., St. Joseph, MI). The CP concentration was calculated with $\text{g kg}^{-1} \text{N} \times 6.25$ (AOAC, 1990). The IVDMD value was determined using the inoculation and direct acidification procedure (Marten and Barnes, 1980). Rumen fluid was collected from a fistulated steer (*Bos taurus*) and mixed with a Kansas State buffer in a 4:1 ratio (vol. /vol.) of buffer to rumen.

Statistical analysis

Data were analyzed with the SAS MIXED procedure (Littell et al., 1996), with grass species and nitrogen application rates considered as fixed effects, and block and its interactions considered as random effects. Harvest dates were treated as repeated measures. Least squares means for species, nitrogen application rates, harvest dates, and interactions were separated by the SAS PDIFF option. Quadratic regression equations were fit with MSC as the independent variable and the forage quality parameters as dependent variables of the pooled data set for each species from both years using the PROC REG procedure (SAS Inst., 1985). All differences were considered significant at the 0.05 probability level.

Results and Discussion

Forage quality

There were significant two-way interactions between treatments for forage quality parameters (NDF, IVDMD, and CP), except for IVDMD in 2006 and for CP in both study years (Table 3.2). These interactions indicated that the response to each main treatment effect cannot be evaluated independently of the others.

Variability in forage quality due to a three-way interaction of species x nitrogen rate x harvest date (S x N x H) occurred for IVDMD in both years and CP in 2006 (Table 3.2). However, these interactions accounted for only a very small although significant, portion of the total variation in forage quality. These interaction effects have little or no agronomic importance and will not be discussed.

Neutral detergent fiber (NDF). The responses of NDF concentration to treatments were similar in both years (Table 3.2). The species x harvest date interactions indicated that the concentration of NDF increased differently among species with grass maturity (Fig. 3.1a and 3.2a). The increase in NDF concentration for eastern gamagrass, big bluestem, indiangrass, and switchgrass ranged from 630 to 760, 539 to 776, 521 to 747, and 523 to 715 g kg⁻¹ in 2006, respectively, and from 629 to 767, 558 to 822, 543 to 753, and 552 to 756 g kg⁻¹, respectively, in 2007. The NDF concentration increased rapidly early in the season and the rate of change in NDF concentration began to decline between the mid-season and the end of harvest (Fig. 3.1a and 3.2a). From the first harvest (DOY 136 in 2006; DOY 142 in 2007) to third harvest (DOY 164 in 2006; DOY 169 in 2007) for both years, eastern gamagrass was remarkably higher in NDF concentration than other species averaged across nitrogen levels and replications (Fig. 3.2a and 3.3a). Yet they became more similar in NDF concentration during mid of season. In both years, the trends of NDF concentration for switchgrass and eastern gamagrass leveled off earlier than any other species and dropped slightly at later harvest dates. In 2006, the maximum NDF concentration for eastern gamagrass, big bluestem, and indiangrass occurred at the last harvest, while that for switchgrass was observed initially at harvest 5 (DOY 192). Among these four species, big bluestem had highest fiber concentration at the late-season harvest for both years. This is most likely because of the differences in morphological development (leaf/stem ratio) among species (Buxton and Fales, 1994; Buxton et al. 1995). Griffin and Jung (1983) and Twidwell et al. (1988) observed that the proportion of leaf tissue was generally higher in switchgrass than big bluestem at all sampling dates. The concentrations of fiber in leaves changed little

as grasses mature, while it increased rapidly in stems. Therefore, grasses with lower leaf:stem ratios at later harvest have greater fiber concentration.

The interactions of species x N rate indicated that warm-season grass NDF increased with N fertilizer application rate, differently among species for both years (Fig. 3.3a and 3.4a). In both years, the application of 65 kg N ha⁻¹ increased NDF concentration approximately 8 g kg⁻¹ for both eastern gamagrass and indiangrass while that of 140 kg N ha⁻¹ did not increase, relative to the zero N rate for 2006 and was not different from the 65 kg N ha⁻¹ for 2007 (Fig. 3.3a and 3.4a). Switchgrass NDF concentration increased linearly with N application rate for both years. However, big bluestem NDF concentration did not respond to N fertilization.

Wilson (1982) suggested that N fertilization positively affected dry matter yield for grasses, by increasing stem development with greater NDF concentration. For this current study, the harvest date x N rate interaction for NDF concentration indicated that the increases in NDF for warm-season grasses receiving 65 and 140 kg N ha⁻¹ occurred between fifth (DOY 192) and seventh harvest (DOY 220) in 2006, and between fourth (DOY 183) and ninth (DOY 260) harvest in 2007 (Fig. 3.5a and 3.6a), when grass swards developed the largest proportion of stems. At any other harvest date, the concentrations of NDF were not different among N application rates.

***In vitro* dry matter digestibility (IVDMD).** *In vitro* dry matter digestibility (IVDMD) in both years was affected by species, N rate, harvest date and their interactions, except the species x N rate interaction in 2006 (Table 3.2).

The interaction of species x harvest date for IVDMD for 2006 and 2007 suggested that the value of IVDMD decreased with each successive harvest although differently among species (Fig. 3.1b and 3.2b). Throughout the growing season, the decreases in IVDMD values for eastern gamagrass, big bluestem, indiangrass, and switchgrass ranged from 676 to 342, 696 to 278, 693 to 359, and 727 to 298 g kg⁻¹ in 2006, respectively, and 640 to 348, 653 to 244, 674 to 358, and 672 to 279 g kg⁻¹ in 2007, respectively. The evident differences in IVDMD among species were found between the middle (DOY 178 in 2006; DOY 183 in 2007) and the end season (DOY 276 in 2006; 281 in 2007). During those growth stages, IVDMD values for big bluestem and switchgrass decreased more rapidly than eastern gamagrass and indiangrass in both years. For eastern gamagrass, IVDMD appeared to level off as the growing season progressed after the 5th harvest (DOY 192) in 2006 and the 6th harvest (DOY 211) in 2007. The differences in IVDMD value among warm-season grasses reported by Roundtree et al. (1974) were that IVDMD was higher for indiangrass and switchgrass than for caucasian bluestem [*Bothriochloa caucasica* (Trin.) C.E. Hubb] based on whole-plant samples. In vitro dry matter digestibility of switchgrass and caucasian bluestem decreased significantly with maturity. These differences were likely to due to differences in morphological development among the species. Coleman et al. (2004) noted that quality of C₄ grasses declines primarily with the beginning of stem elongation. The digestibility for switchgrass hay harvested at 14-d intervals declined markedly during a 28-d period when the plants advanced from vegetative culm elongation to nearly early-boot stage (Burns et al., 1997). Dabo et al. (1987) inferred that forage quality decreases with increasing maturity caused by reduced growth rate as the tillers mature. The proportion of new growth

(high quality) was diluted with older growth (low quality), in addition to increased production of culms and their maturation.

Among treatment effects, N rate was a minor source of variation in IVDMD relative to harvest date and species (Table 3.2). In 2006, there was no species x N rate interaction suggesting that the IVDMD value across four species was increased approximately 176 and 256 g kg⁻¹ dry matter, when nitrogen fertilizer was applied at 65 and 140 kg N ha⁻¹, respectively (Table 3.2 and Fig. 3.3b). In contrast, the species x N rate interaction in 2007 indicated that the differences in IVDMD value among species varied with N rate (Fig. 3.4b) (Table 3.2 and Fig. 3.4b). The digestibility value for switchgrass was slightly decreased with both rates of N whereas IVDMD values for eastern gammagrass, big bluestem, and indiangrass did not respond to N fertilization (Fig. 3.4b). The decreases in IVDMD for switchgrass with N fertilization were consistent with the increase in NDF concentration (Fig. 3.4a) and may result in slightly greater decrease in IVDMD for grasses receiving N fertilization during late season (Fig. 3.6b). Although it was not clear why N fertilization at 140 kg ha⁻¹ did not increase the IVDMD value from that at 65 kg ha⁻¹, IVDMD values for warm-season grasses except big bluestem tend to increase with N fertilizer application rate in 2006 (Fig. 3.3b). In contrast to 2007, the value of IVDMD for all species in 2006 slightly increased with higher N fertilization during the early vegetative stages, while there was no difference during later growth stages (Fig. 3.5b).

Our results indicate that there was variation in response of IVDMD for warm-season grasses to N fertilization. Similarly, Minson (1973) reported a variable response from an increase of 6.9 to a depression of 5.2 percentage units in dry matter digestibility of three tropical grasses applied with N fertilizer. Puoli et al. (1991) reported that the application of

78 kg N ha⁻¹ to warm-season grasses increased crude protein and voluntary intake by 1.1 and 11 %, respectively, but digestibility for switchgrass and big bluestem was not affected. In addition, a lack of effect of N fertilization on digestibility was observed in temperate grasses (Reid et al., 1966). However, Hall (1978) reported that digestibility of warm season grasses seemed to increase in a linear manner with N fertilization. Therefore, there was no consistent pattern in DMD response due to N fertilization (Minson, 1990). The response of dry matter digestibility to N fertilization was variable and the causes were complex (Wilson, 1982). The application of nitrogen fertilizer results in either a positive effect by increasing the production of new tissue or a negative effect by increasing the rate of senescence. The most likely negative effect would be due to increased stem development (Coleman et al., 2004).

Crude Protein (CP). Forage crude protein for warm-season grasses in both 2006 and 2007 was affected significantly by N application rate, harvest date, and interaction effect of species x harvest date, and N rate x harvest date, while species effect in 2006 and species x N rate interaction in both years did not contribute (Table 3.2).

The species x harvest date interaction indicated that the concentration of CP decreased with grass maturity, differently among species. The highest concentration of CP for all species occurred at first harvest and rapidly decreased between the first four harvest dates and then decreased gradually throughout the rest of the growing season (Fig. 3.1c and 3.2c). Responses of forage CP to harvest date in this study agree with previous studies reporting that CP concentration generally declined as the growing season progressed (Allen et al. 1976; Anderson et al. 1976; Ross and Krueger, 1976; and Hall, 1978). In the current study, the decreases in CP concentration for eastern gamagrass, big bluestem, indiagrass,

and switchgrass ranged from 198 to 43, 231 to 37, 219 to 38, and 221 to 36 g kg⁻¹, respectively, in 2006 and 151 to 46, 166 to 28, 169 to 32, and 141 to 36 g kg⁻¹, respectively, in 2007. Remarkable differences in CP concentration among species occurred at early season and later season while no evident change in CP concentration among species was observed at other harvest dates (Fig. 3.1c and 3.2c). With the exception of eastern gamagrass, the rate of decrease in CP concentration for warm-season grasses was similar. The concentration of CP for eastern gamagrass began to level off at the fifth harvest (DOY 192 in 2006; DOY 197 in 2007) earlier than that for any other species in both years. These changes likely accounted for the major variation of the interaction effect of species x harvest date.

Fertilizing warm-season grasses with N increased CP concentration for both years (Fig. 3.3c and 3.4c). Due to no species x N interaction, response of forage CP concentration among grass species to N fertilization can be evaluated independently. In 2006, the CP concentration across four species was increased approximately 18 and 29 g kg⁻¹, when nitrogen fertilizer was applied at 65 and 140 kg N ha⁻¹, respectively (Fig. 3.3c). In 2007, the CP concentration across four species receiving 65 and 140 kg N/ha increased approximately 10 and 25 g kg⁻¹, respectively (Fig. 3.4c).

The interaction of N rate x harvest date illustrated that the increase in the concentration of herbage CP across four species receiving N fertilizer at both rates, relative to zero N treatment, occurred during early harvests both years (Fig. 3.5c and 3.6c). Between the first (DOY 136) and the third harvest (DOY 164) in 2006, warm-season grasses applied with both N application rates contained similar forage CP concentration, and then leveled off toward a similar CP value to no N fertilization during late harvests. Between the sixth (DOY 211) and tenth (DOY 281) harvest in 2007, the concentration of CP across four species

receiving 65 kg N ha^{-1} were not significantly different from that with no N fertilization (Fig. 3.6c). Balasko and Smith (1971) reported that increasing temperature that normally occurs during season can decrease herbage nitrogen concentration leading to the decrease in CP. Moreover, plots with no N fertilization (the check) and a low N rate (65 kg N ha^{-1}) had smaller amounts of soil available N left as the season progressed, compared with the plots treated with a high rate of N fertilizer (140 kg N ha^{-1}). In addition to a lower proportion of leaves with relatively more N contents, N decrease with advanced maturity is likely attributed to N translocation from aboveground biomass to belowground organs between the time of anthesis and after a killing frost (Vogel et al., 2002). When harvest was delayed to late July and early August, the amount of tiller N (aboveground) in big bluestem and indiangrass decreased while N increased in rhizomes (belowground). Plants use this translocated N for producing new growth the following next spring (McKendrick et al., 1975).

Predicting forage qualities with MSC quadratic regression equation

The regression equations for predicting the forage quality parameters for warm-season grasses from the morphological development as quantified by mean stage count (MSC), had high coefficients of determination and low root mean square error (RMSE) (Mitchell et al., 2001). The calibration data of forage quality parameters for those grasses could be reasonably predicted by a quadratic MSC model.

In the current study, calibrations of IVDMD for big bluestem, switchgrass, and indiangrass predicted by a quadratic MSC model, accounted for approximately 98% of the

variation in response, in 2006 (Fig. 3.7a). But the MSC regression equations were not able to predict the IVDMD concentration for eastern gamagrass.

Similar to 2006, the calibration data of big bluestem, switchgrass, and indiangrass IVDMD in 2007 predicted using a quadratic MSC accounted for $\geq 96\%$ of the variation, but was not significant for eastern gamagrass. The decline in IVDMD concentration for the former three grasses with higher MSC was consistent with previous research in the central Great Plains (Mitchell et al., 2001). In both years, predicted IVDMD indicated that the decrease in the IVDMD leveled off between MSC 4.0 and 4.3 for big bluestem, 4.5 and 4.6 for switchgrass, 3.1 and 3.2 for indiangrass.

The levels of IVDMD in switchgrass were generally greater than those observed for big bluestem and indiangrass with a similar MSC. The decline in IVDMD concentration for indiangrass was similar to that for big bluestem, but indiangrass had greater IVDMD concentration than big bluestem, as it reached mid-reproductive stage (MSC 3.5) at the late season (Fig. 3.7a and 3.7b).

The calibration data for NDF of big bluestem, switchgrass, and indiangrass which was predicted by a quadratic MSC model accounted for $\geq 88\%$ of the variation in 2006 (Fig. 3.8a) and $\geq 91\%$ in 2007 (Fig. 3.8b), respectively. The MSC regression equation was not able to predict the IVDMD concentration for eastern gamagrass. With the exception of eastern gamagrass, the concentration of NDF for warm-season grasses increased as the growing season progressed. This confirms results of a previous study by Mitchell et al. (2001). The increase in the concentration of NDF leveled off between MSC 3.8 and 4.3 for big bluestem, 3.7 and 4.0 for switchgrass, 2.7 and 2.8 for indiangrass. When grass swards were at early vegetative-leaf development ($\text{MSC} \leq 2$), the concentrations of NDF in eastern

gamagrass were greater than those for other species in both years. Indiangrass had greater NDF concentration than big bluestem and switchgrass, when grass swards were in stem elongation stages (Fig. 3.8a and 3.8b).

The CP calibration data for big bluestem, switchgrass, and indiangrass predicted by a quadratic MSC model, accounted for $\geq 88\%$ of the variability in the CP (Fig. 3.9a and 3.9b). Similar to IVDMD and NDF, the regression equation for predicting the eastern gamagrass CP concentration from MSC was not significant, in both 2006 and 2007. Previous studies by Griffin and Jung (1983) and Mitchell et al. (2001) have shown similar decreases in CP concentration for warm-season grasses with advancing maturity. The decrease in the concentration of CP leveled off between MSC 3.6 and 3.8 for big bluestem, 4.0 and 4.1 for switchgrass, and 2.6 and 2.7 for indiangrass.

Among the four species, switchgrass had the greatest CP concentration followed by big bluestem, indiangrass, and eastern gamagrass at common values of MSC (Fig. 3.9a and 3.9b). This result would explain why switchgrass had generally lower CP concentration than big bluestem at specific DOYs (Griffin and Jung, 1983), because switchgrass matured earlier (greater MSC on common DOYs) than other species. However, at the final harvest, eastern gamagrass (with MSC 1.5) had a greater CP concentration than big bluestem (with MSC 4.6-4.9), switchgrass (with MSC 4.5-4.7), and indiangrass (with MSC 3.3) (Fig. 3.1c, 3.2c, 3.9a, and 3.9b).

Based on the results above, MSC was an excellent predictor for the IVDMD, CP and NDF of warm-season grasses, with the exception of eastern gamagrass (Fig. 3.7, 3.8, and 3.9). In contrast to these other three species, eastern gamagrass is indeterminate with respect to reproductive growth, with appearance of spikes on the same plant occurring over a

considerable time period (Jackson and Dewald, 1994). Different generations of eastern gamagrass tillers in the field grows as population of connected monocarpic shoots (tillers) that remain vegetative in the season in which they are initiated and typically become reproductive in the second or third season of growth, apparently after achieving some minimum size (Dewald and Louthan, 1979). For these reasons, the relationship between MSC and forage quality parameters was not observed in the eastern gamagrass swards.

It has been reported that indiangrass had higher NDF, and lower IVDMD and CP concentration than big bluestem and switchgrass between early and mid season (Fig. 3.1a-c and 3.2a-c), when grass swards were at $MSC \leq 3$ (Table 3.1). However, the interaction effect between species and harvest date indicated that the forage qualities of indiangrass were greater than those of big bluestem and switchgrass on many harvest dates. Indiangrass generally matured later than big bluestem and switchgrass (Table 3.1). Therefore, for similar MSCs, indiangrass swards were quantified on later harvest dates than did those two grass swards (Table 3.1). At the final harvest, indiangrass sward and reached only floral development (MSC 3.3) whereas big bluestem and switchgrass swards were at hard dough stage or more ($MSC \geq 4.5$). However, these results indicated that only the MSC variable was not able to consistently predict the IVDMD, CP, and NDF across all warm-season grasses studied. Previous studies by Hill et al. (1995) and Mitchell et al. (2001) have reported that forage quality parameters also varied with the accumulated growing degree day (GDD) or day of the year (DOY). It is possible because the DOY adequately predicted the forage quality parameters for warm-season grasses due primarily to the photoperiod sensitivity and the general predictability of weather pattern (i.e. rainfall and temperature). The cell wall component of grasses generally increases with increasing temperature (Van Soest, 1982).

But variations in the photoperiod each year and environmental factors would make forage quality estimation using a DOY model difficult among species. In those cases, GDD models may be more likely than DOY models to be better predictors of the forage quality. The incorporation of additional environmental variables such as temperature, hours of above-horizon sunlight, hours of sunlight, or deviation between pan evaporation and precipitation to GDD may be required for the accurate forage quality prediction across several environments (Onstad and Fick, 1983; Hill et al., 1995; Mitchell et al., 2001). Therefore, although the morphological development occurring in a very predictable manner provided reasonable predictions for several forage quality parameters, it can be affected by species and environmental factors.

Conclusions

Although native warm-season grasses generally are found to be lower in forage quality, i.e. fiber digestion, IVDMD or crude protein concentration, than cool-season (C_3) grasses, their quality could be improved by harvest management and nitrogen fertilization. In our study, time of harvest accounted for much of the differences in forage quality observed among species. Forage quality of native warm-season grasses decreases as the plants mature. For all species, harvesting at early maturity increases forage quality including increased IVDMD and crude protein, and decreased NDF concentration, whereas allowing forage to reach later maturity reduces forage quality with declines in IVDMD and crude protein and increases in NDF concentration. Predicting forage quality changes based on developmental morphology provides valuable information for making decisions in forage-livestock systems. The regression equations for predicting NDF, IVDMD, and CP values from quadratic MSC

model in warm-season grasses had high coefficients of determination for grasses with determinate growth patterns including big bluestem, indiangrass, and switchgrass, but it was not significant for eastern gamagrass with an indeterminate growth habit. Predicting forage quality parameters with a quadratic model suggested that harvesting before the anthesis stage (MSC 3.7) in big bluestem and switchgrass, and harvesting before the onset of the 6th node at stem elongation stage (MSC 2.6) in indiangrass can increase forage quality. The DOY or GDD models incorporated with environmental variables may provide more reasonable forage quality predictions in eastern gamagrass. Forage quality for eastern gamagrass could be considerably increased when it was cut before late July.

Responses of forage quality to N fertilization were affected by species and harvest timing. The NDF concentration for warm-season grasses except big bluestem increased as they received 65 kg N ha⁻¹. Nitrogen fertilization at 140 kg ha⁻¹ has shown no consistent effect on increases in NDF concentration of four grass species across both years. The effect of N application at both rates on IVDMD for all four species is highly variable among grass species and across study years. Although these variations make drawing conclusions difficult, we can conclude that N fertilization could improve forage quality for warm-season grasses, in particular for crude protein. Similar for four warm-season grass species, the concentration of crude protein increased as higher rates of N fertilization. However, the optimal cutting date and N fertilization rate for these grasses often require a compromise between quality, forage yield, and plant persistence (Sanderson, 2000).

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Table 3.1. Initial harvest date and numerical index for each growth stage of warm-season grass for ten harvest treatments in 2006 and 2007 at Ames, IA.

Harvest schedule	Harvest date	MSC [†] for 2006				Harvest date	MSC [†] for 2007			
		EG [‡]	BB	IG	SW		EG	BB	IG	SW
1	May 16	1.4 e [§]	1.3 j	1.2 j	1.3 j	May 22	1.3 b	1.3 g	1.3 c	1.4 i
2	May 31	1.5 de	1.5 i	1.4 i	1.9 i	June 4	1.5 ab	1.4 g	1.4 bc	1.9 h
3	June 13	1.9 c	1.8 h	1.6 h	2.2 h	June 18	1.5 a	1.6 f	1.4 ab	2.2 g
4	June 27	2.0 bc	1.8 g	1.8 g	2.5 g	July 2	1.5 a	1.9 e	1.7 b	2.6 f
5	July 11	2.2 a	2.2 f	1.9 f	2.8 f	July 16	1.5 a	2.1 e	1.7 b	2.8 e
6	July 25	2.2 a	2.4 e	2.0 e	3.0 e	July 30	1.6 a	2.5 d	1.8 b	3.0 d
7	Aug 8	2.1 ab	2.6 d	2.1 d	3.3 d	Aug 13	1.5 ab	2.1 e	2.1 a	3.1 c
8	Aug 22	2.1 ab	2.7 c	2.3 c	3.6 c	Aug 27	1.6 a	3.1 c	2.2 a	3.5 b
9	Sep 12	1.6 d	3.2 b	2.6 b	4.2 b	Sep 17	1.4 ab	4.3 b	2.6 a	4.4 a
10	Oct 3	1.5 de	4.6 a	3.3 a	4.7 a	Oct 8	1.5 a	4.9 a	3.3 a	4.5 a

[†] MSC, mean stage count (Moore et al., 1991). 1.1-1.9, vegetative stage (first-ninth leaf collared); 2.0, stem elongation (onset of stem elongation); 2.1-2.5, stem elongation (first-fifth node palpable); 3.0, boot stage; 3.1, inflorescence emergence (first spikelet visible); 3.3, spikelets fully emerged (peduncle not emerged); 3.5, inflorescence emerged (peduncle fully elongated); 4.3, soft dough; 4.5, hard dough; 4.7, endosperm hard (physiological maturity); 4.9, endosperm dry (seed ripe).

[‡] EG=eastern gamagrass, BB=big bluestem, IG=indiangrass, and SW=switchgrass

[§] For index score means, values in the same column followed by a common letter are not significantly different ($P \leq 0.05$).

Table 3.2. Neutral detergent fiber (NDF), in vitro dry matter digestibility (IVDMD), and crude protein (CP) *F*-value and significance for main effect and interaction for 2006 and 2007.

Effect	NDF		IVDMD		CP	
	2006	2007	2006	2007	2006	2007
Species(S)	158.73**	120.63 **	123.54 **	134.22 **	0.47ns	8.38**
N rate (N)	6.98**	32.68 **	16.10 **	11.21 **	279.08**	129.14**
S*N	3.71**	6.22 **	1.85 ns	9.51 **	2.11 ns	0.54 ns
Harvest (H)	904.71**	864.14 **	2188.97 **	2407.87 **	1450.00 **	756.46**
S*H	39.37**	32.94 **	26.10 **	35.80 **	10.51 **	18.10**
N*H	3.09**	3.91 **	1.72 *	4.40 **	8.00**	14.50**
S*N*H	1.06ns	1.20 ns	1.42 *	1.63 **	2.05**	1.50 ns

* significant at the 0.05 probability level.

** significant at the 0.01 probability level.

ns non-significant

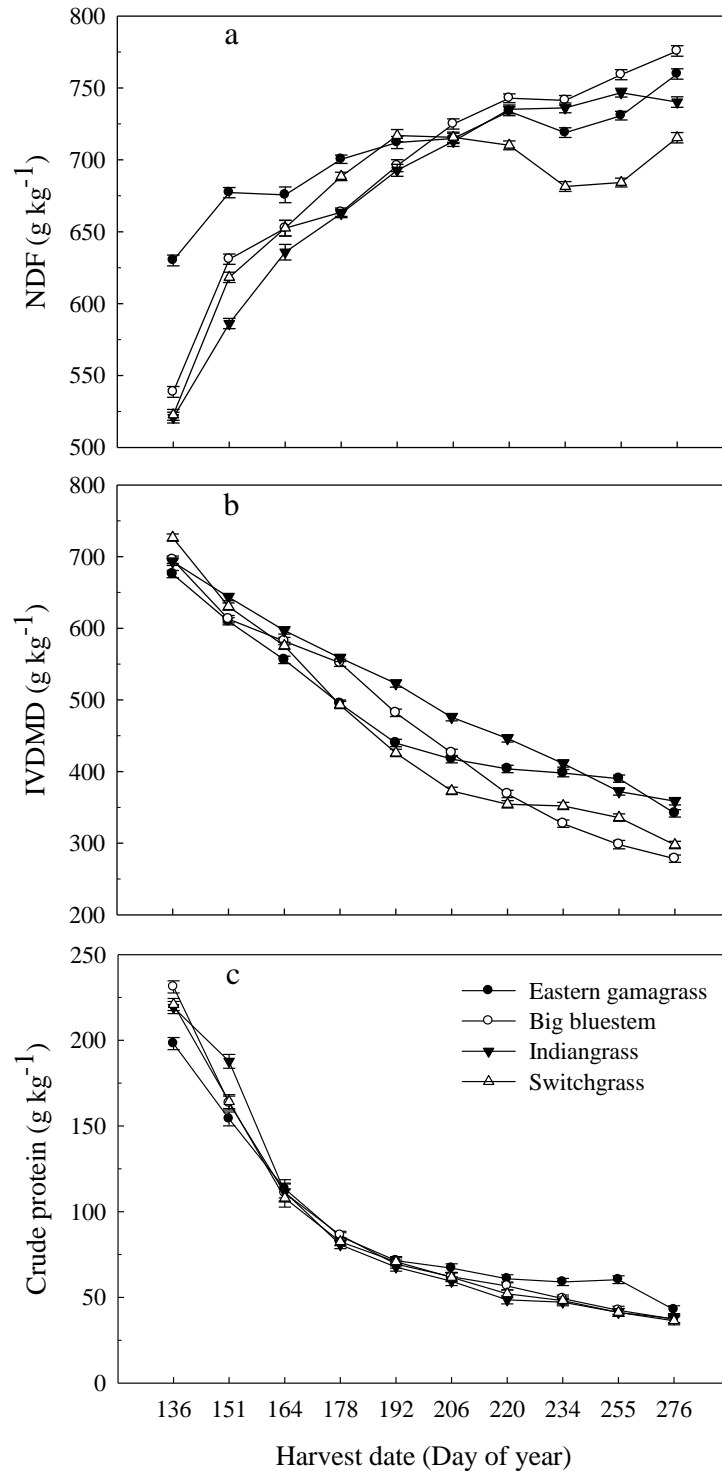


Figure 3.1. Mean forage NDF (a), IVDMD (b), and crude protein (c) concentration as influenced by eastern gamagrass (EG), big bluestem (BB), indiagrass (ID), and switchgrass SW) and ten harvest dates. Data are averaged over four replications and three nitrogen rates, in 2006, at Ames, IA. Standard error bars are partially covered by graph symbols.

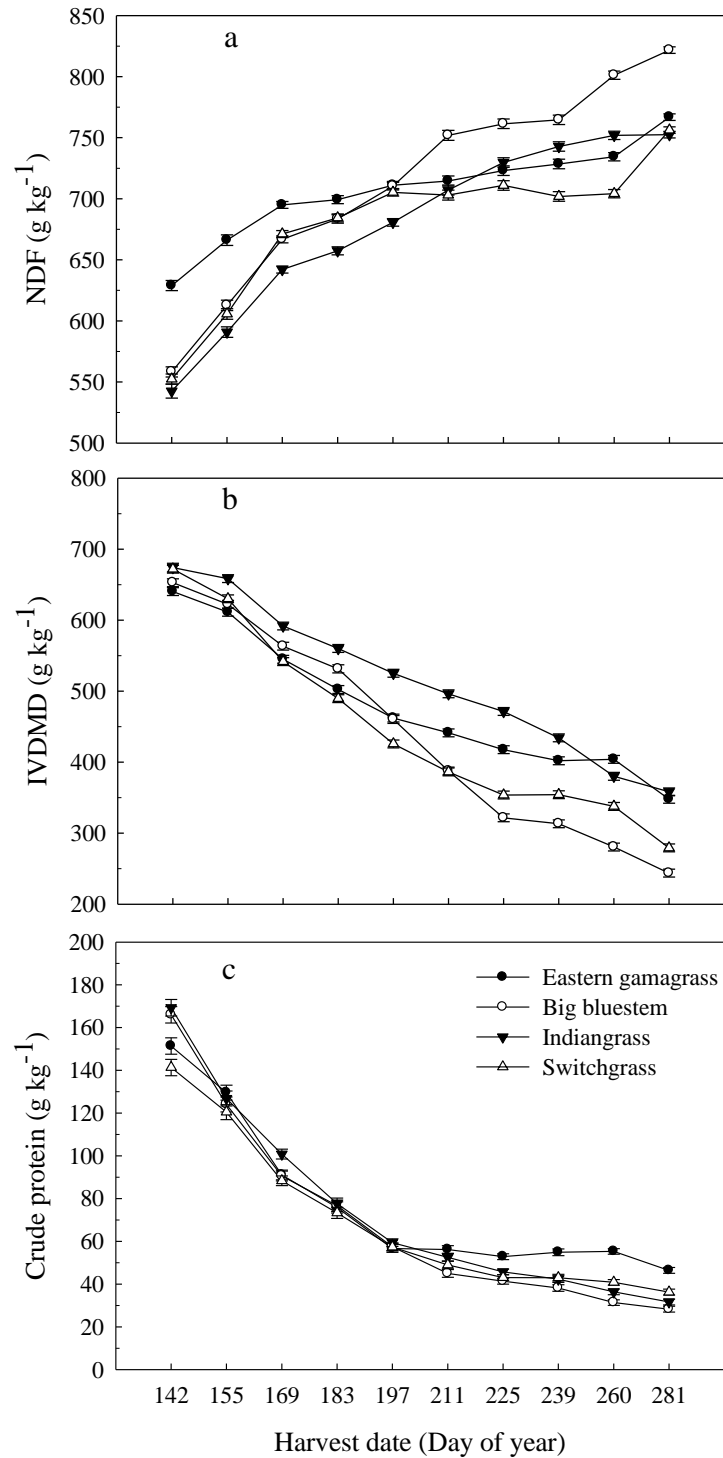


Figure 3.2. Mean forage NDF (a), IVDMD (b), and crude protein (c) concentration as influenced by eastern gamagrass (EG), big bluestem (BB), indiangrass (ID), and switchgrass (SW) and ten harvest dates. Data are averaged over four replications and three nitrogen rates, in 2007, at Ames, IA. Standard error bars are partially covered by graph symbols.

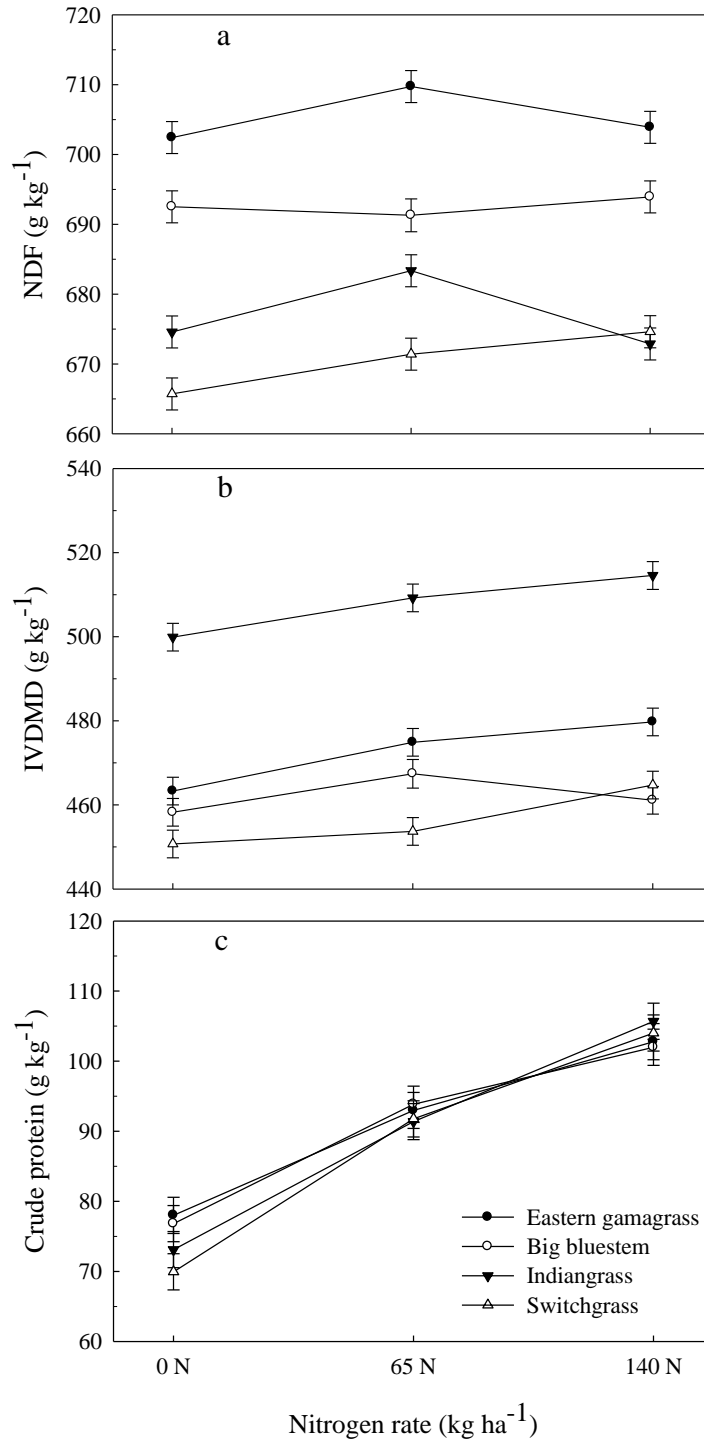


Figure 3.3. Mean forage NDF (a), IVDMD (b), and crude protein (c) concentration as influenced by eastern gamagrass (EG), big bluestem (BB), indiagrass (ID), and switchgrass (SW) and three nitrogen rates at 0 (0N), 65 (65N), and 140 (140N) kg N ha^{-1} . Data are averaged over four replications and ten harvest dates in 2006, at Ames, IA. Standard error bars are partially covered by graph symbols.

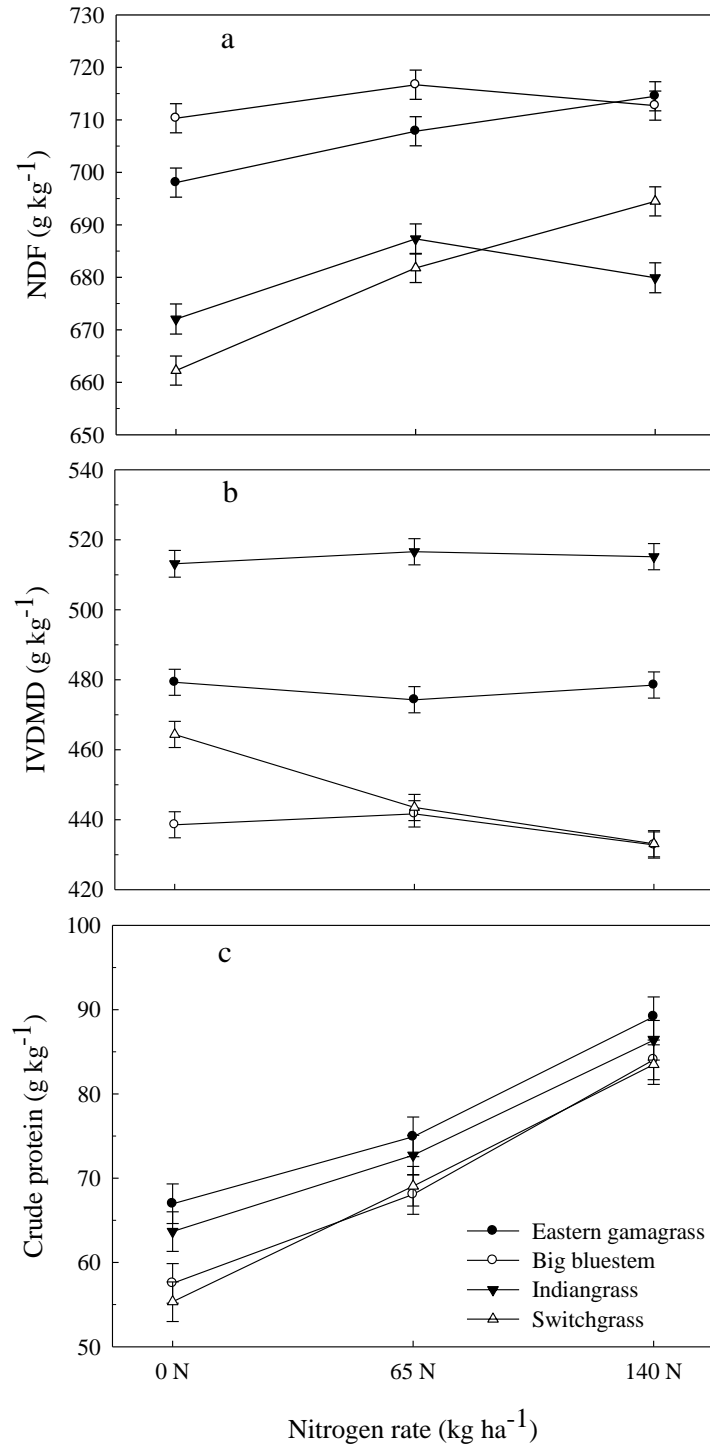


Figure 3.4. Mean forage NDF (a), IVDMD (b), and crude protein (c) concentration as influenced by eastern gamagrass (EG), big bluestem (BB), indiangrass (ID), and switchgrass (SW) and three nitrogen rates at 0 (0N), 65 (65N), and 140 (140N) kg N ha⁻¹. Data are averaged over four replications and ten harvest dates in 2007, at Ames, IA. Standard error bars are partially covered by graph symbols.

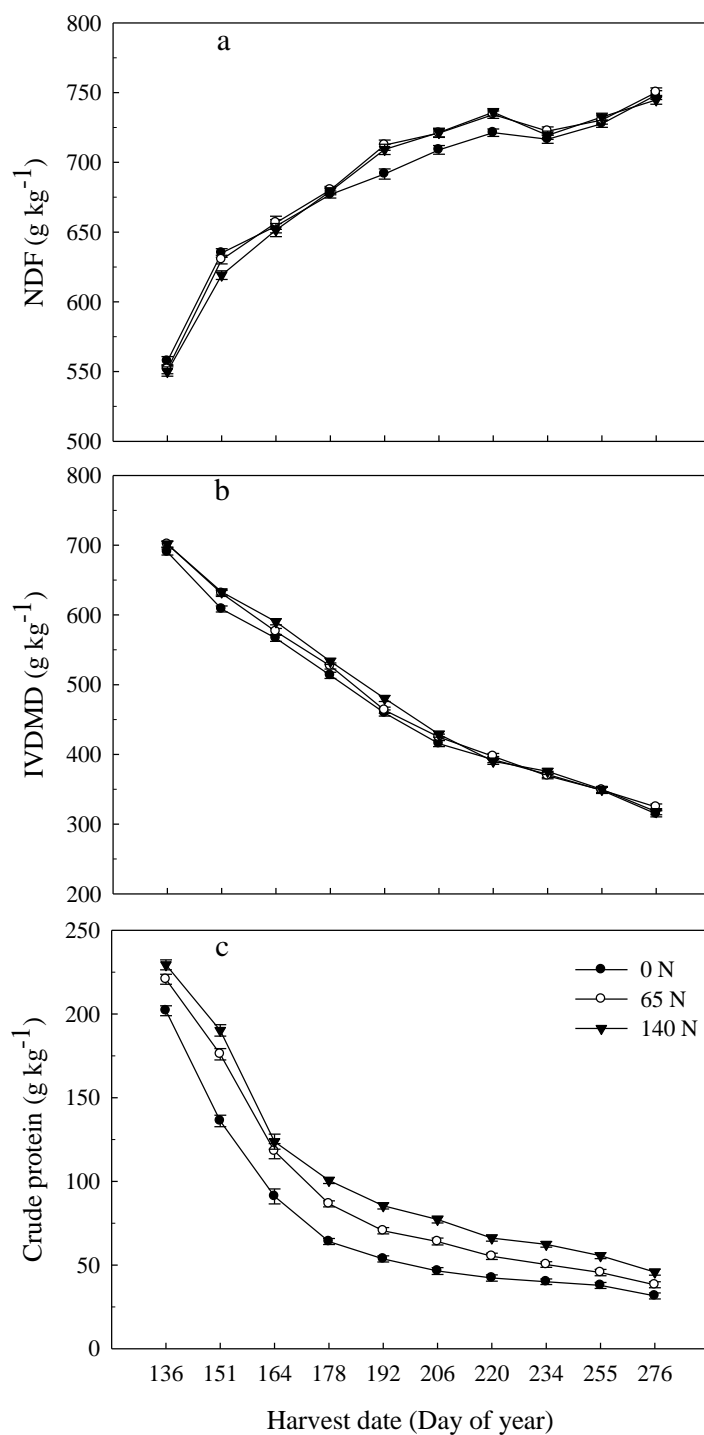


Figure 3.5. Mean biomass NDF (a), IVDMD (b), and crude protein (c) concentration as influenced by three nitrogen rates at 0 (0 N), 65 (65 N), and 140 (140 N) kg N ha^{-1} and ten harvest dates. Data are averaged over four replications and four species, in 2006, at Ames, IA. Standard error bars are partially covered by graph symbols.

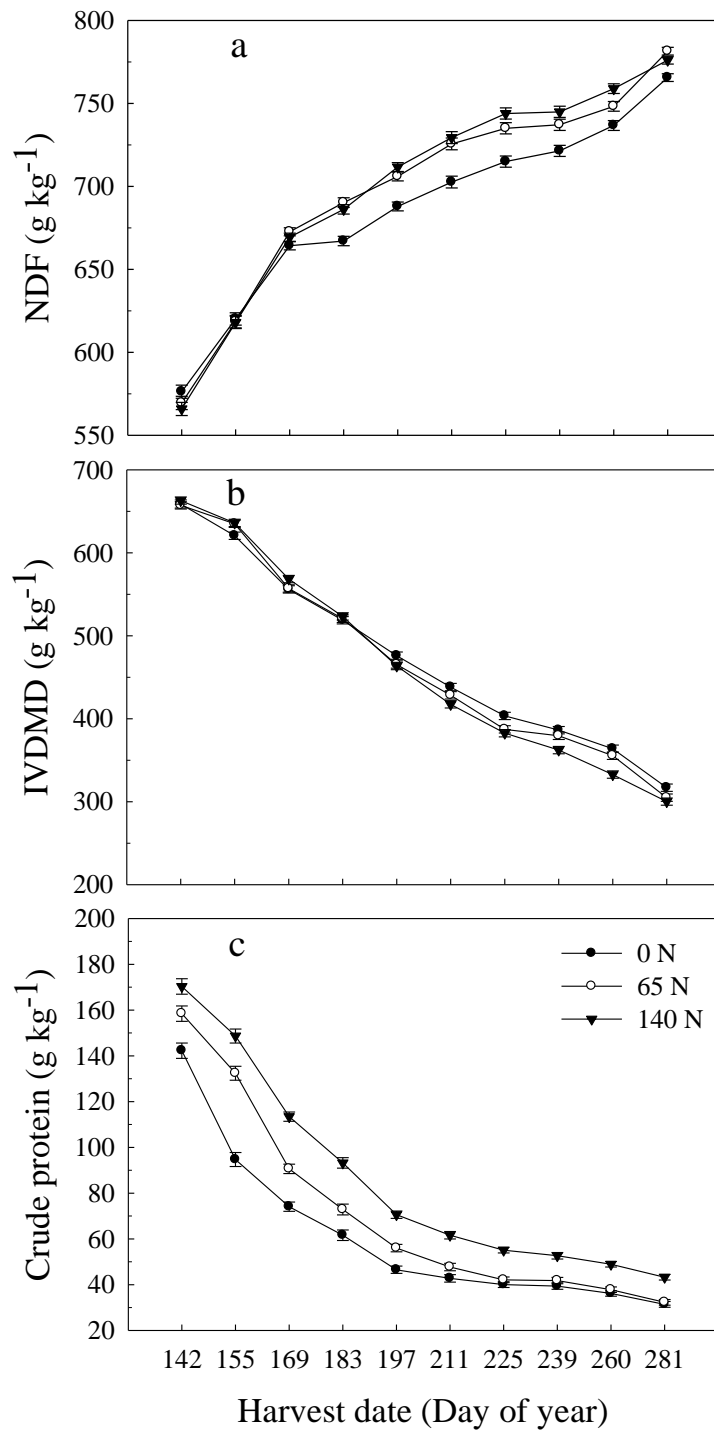


Figure 3.6. Mean biomass NDF (a), IVDMD (b), and crude protein (c) concentration as influenced by three nitrogen rates at 0 (0 N), 65 (65 N), and 140 (140 N) kg N ha⁻¹ and ten harvest dates. Data are averaged over four replications and four species, in 2007, at Ames, IA. Standard error bars are partially covered by graph symbols.

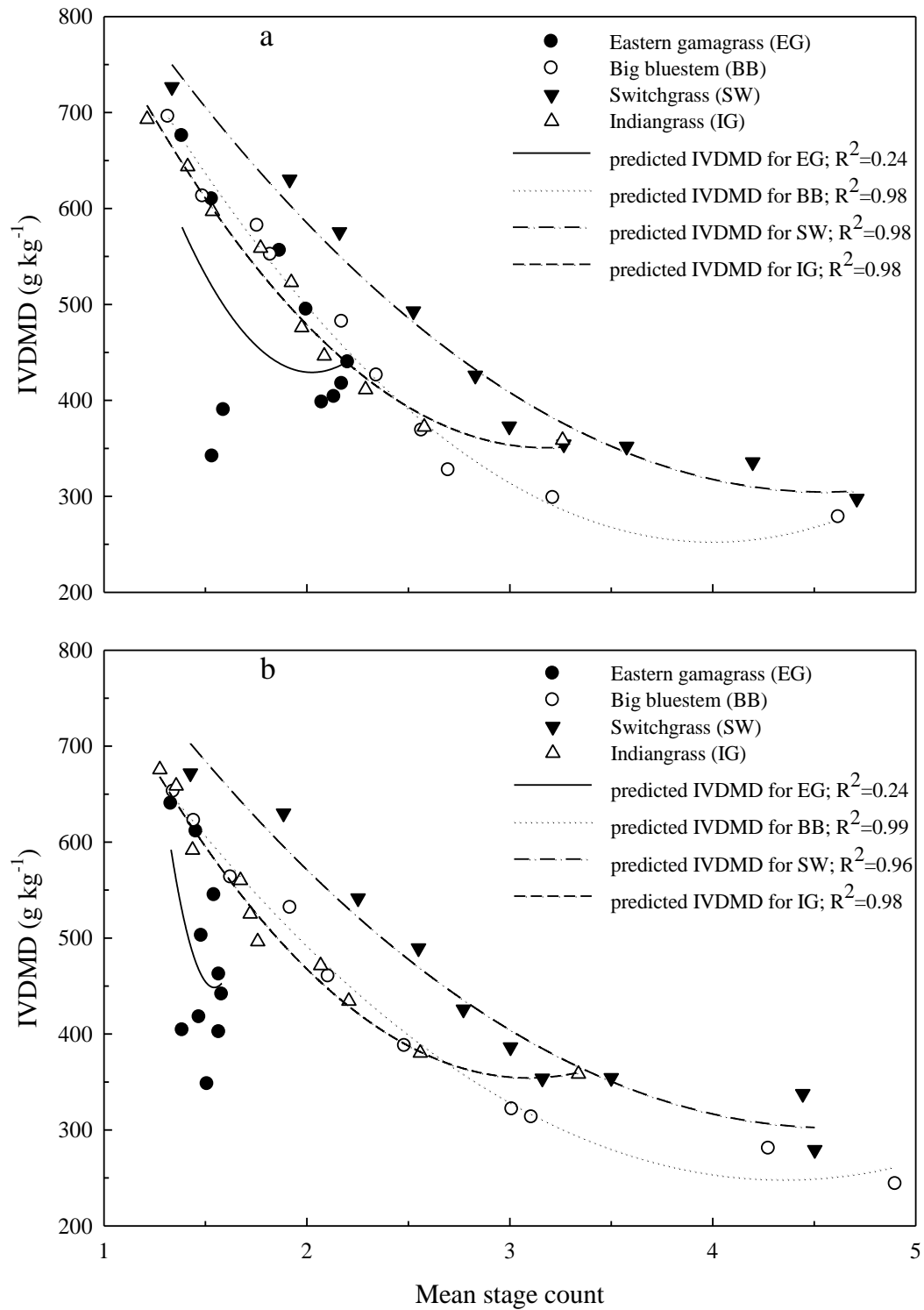


Figure 3.7. Mean stage count (MSC) and mean in vitro dry matter digestibility (IVDMD) relationships for eastern gamagrass, big bluestem, switchgrass, and indiagrass grown near Ames, IA in 2006 (a) and 2007 (b).

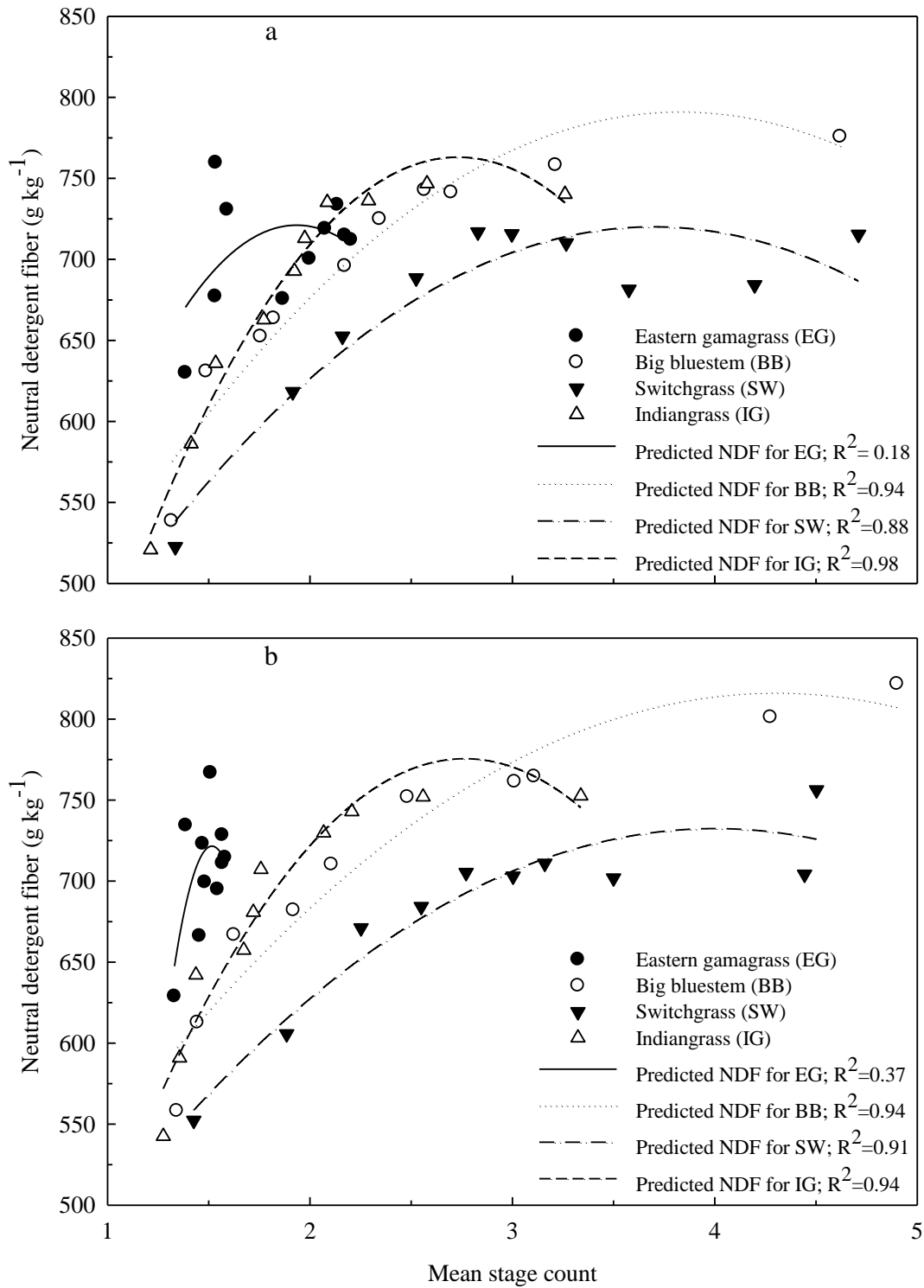


Figure 3.8. Mean stage count (MSC) and mean neutral detergent fiber (NDF) relationships for eastern gamagrass, big bluestem, switchgrass, and indiangrass grown near Ames, IA in 2006 (a) and 2007 (b).

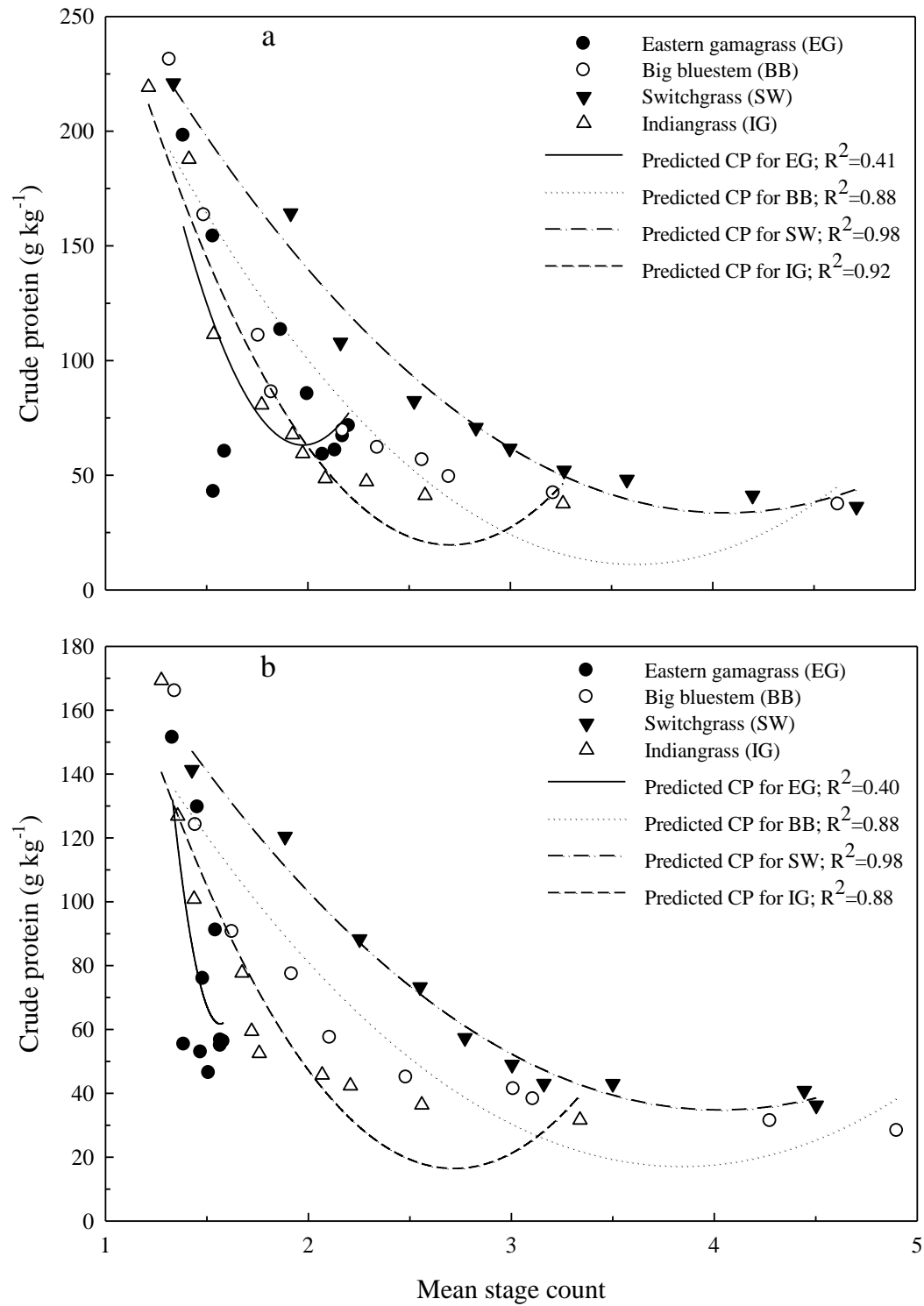


Figure 3.9. Mean stage count (MSC) and mean crude protein (CP) relationships for eastern gamagrass, big bluestem, switchgrass, and indiangrass grown near Ames, IA in 2006 (a) and 2007 (b).

CHAPTER 4. RELATIONSHIP BETWEEN BIOMASS YIELD AND DEVELOPMENTAL MORPHOLOGY OF WARM-SEASON GRASSES

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Abstract

In the Midwest, native warm-season grasses are often used to fill summer deficiency in forage supply. Recently, warm-season grasses have also been evaluated and promoted as a potential biofuel feedstock. However, information on the growth and development of warm-season grasses in response to management is required to use them successfully as a biomass crop. Our objectives were to i) determine optimum harvest periods and N fertilization rates for the production of four warm-season grasses as biomass crops in the Midwest, and ii) to investigate if traits of canopy architecture can explain observed yields of warm-season grasses with varying harvest dates and N fertilization rates. A field study was conducted at Sorenson Farm near Ames, IA, during 2006 and 2007. The experimental design was a split-split plot in time with four replications. Big bluestem (*Andropogon gerardii* Vitman) eastern gamagrass (*Tripsacum dactyloides* L.), indiangrass (*Sorghastrum nutans* L. Nash), and switchgrass (*Panicum virgatum* L.) were main plots, seeded in a randomized complete blocks. Across grass species, three nitrogen application rates (0, 65, and 140 kg/ha) were

subplots, and ten harvest dates were sub-sub plots, all randomly assigned. Harvest dates were treated as repeated measures. Biomass yields increased with advanced maturity but differently among species. Eastern gamagrass obtained the maximum yield at the highest mean stage count (MSC; 1.6 and 2.2) when the largest seed ripening tillers were present. Big bluestem, switchgrass, and indiangrass growth were more determinate. Their maximum yields occurred at MSC 3.5, 3.9, and 2.9, respectively when the largest reproductive tillers were present. Delaying harvest to late fall may improve biomass quality, but it decreased yield due to a large proportion of senescent tillers and increased litter. In terms of a biomass supply strategy, eastern gamagrass may be used as a feedstock in early summer, while big bluestem and switchgrass may be best used between mid-and late summer, and indiangrass in early fall. Nitrogen fertilization increased biomass yield by increasing the proportion of elongating, reproductive, seed tillers for determinate grasses (big bluestem, indiangrass, and switchgrass) and increasing tiller density for indeterminate grass (eastern gamagrass). Optimum biomass yields were obtained later in the season when they were fertilized with 140 kg ha⁻¹.

Introduction

Across the Midwest and Great Plains of the USA, native prairie grasses including switchgrass (*Panicum virgatum* L.), big bluestem (*Andropogon gerardii* Vitman.), indiangrass (*Sorghastrum nutans* Nash.) and eastern gamagrass [*Trisacum dactyloides* (L.) L.] have been used to supplement the uneven distribution of forage production throughout the grazing season when cool-season grasses are relatively unproductive during the hot summer months (Burns and Bagley, 1996; Massengale, 2000). In many parts of the world, these C₄

grasses have recently attracted considerable interest as a source plant biomass to produce energy (Lewandowski et al., 2003). Excellent yield potential and efficient use of resources, especially nitrogen, are important traits which make these grasses desirable for biomass energy production (McKendry, 2002; Heaton et al., 2004).

The quantity of dry matter biomass produced by warm-season grasses per unit area of production, determines the potential energy production capacity, or yield, of the available land area. The energy yield of cultivated crops can be obtained from combining between dry matter yield and the high heating value (HHV) of the biomass (McKendry, 2002). Therefore, the more biomass yield produced, the more energy yield is generated.

Native warm-season grasses can provide excellent yield during the summer months, but to reach full production potential, crop management practices must be optimized. To maximize dry matter yield and optimize biofuel quality, nitrogen fertilization and harvest management are important considerations that can reduce the major costs of producing biomass (Lemus et al., 2008; Keeney and DeLuca, 1992; Vogel et al., 2002), and improve biomass quality by minimizing the concentration of minerals (McKendry, 2002). In Midwest states and the Central Great Plains of the USA, most studies have reported that significant fertilizer N inputs are required to optimize biomass production by warm-season grasses when managed as forage crops (reviewed by Brejda, 2000). Of the few studies that have assessed the effect of N fertilization on yield of a perennial, warm-season grass managed for bioenergy, most were conducted on switchgrass (Ma et al., 2001; Muir et al., 2001; Thomason et al., 2004; Lemus et al., 2008), a model crop for bioenergy feedstock production (Sanderson et al., 1996). According to a review by Parrish and Fike (2005), there is still no clear consensus on best management of N fertility in switchgrass. For example, the

optimum biomass yield was achieved in IA and NE when switchgrass was fertilized with 120 kg ha⁻¹ (Vogel et al., 2002), but 168 kg ha⁻¹ was required in Texas (Muir et al., 2001). In addition, information on the optimum N fertilization rate for other warm-season grasses managed for biomass production is limited or non-existent.

Similar to N fertility, many studies on harvest management of warm-season grasses have been conducted for forage production (Sanderson, 2000), but limited research information is available on harvest schedules for biomass production. As a compromise between forage yield, quality, and plant persistence, harvesting when warm-season grasses are at least 45 to 60 cm tall and before the boot stage typically provided optimum yield for hay production of native tall grasses (Moser and Vogel, 1994). Greater forage quality is obtained when native warm-season grasses are manipulated by cutting management (Sanderson, 2000) and increasing harvest frequency (Sanderson et al., 1995; Brejda et al., 1996). However, harvest management of native warm-season grasses for biomass production may be different from forage production because the objectives of the producer are different. In biomass production, the objective is to obtain high lignocellulose yield with a low mineral concentration. In contrast to forage production, a single late-season harvest may work best for biomass fuel cropping (Sanderson, 2000). In the Midwest, Vogel et al. (2002) demonstrated that maximum first-cut yields were obtained at the 3.3 (R3) to 3.5 (R5) stage of maturity (panicles fully emerged to postanthesis), and, depending on the year, sufficient regrowth may be obtained for a second harvest after a killing frost. They suggested that whether or not a second harvest is made will depend on biomass yield and price and cost of harvesting.

The yield or quantity of perennial grass produced is determined by size, architecture, and developmental status of tiller populations within the sward. Increase in dry weight of grasses results from expansion of leaves, stems, and reproductive structures referred to as growth (Frank, 1996). Changes in plant morphology occurring during primary growth can be important determinants of potential productivity and quality in perennial forage grasses (Frank et al., 1993; Redfearn et al., 1997). Thus, changes in the developmental morphology of grasses will influence management practices such as timing of initial harvest and fertilizer application (Moore et al., 1991; Frank et al., 1993).

Canopy architecture is important for describing many grass canopy processes influenced by the interaction between plants and the environment (Welles and Norman, 1991). Tiller density is an important trait of canopy architecture, related to relative grass productivity and quality. Redfearn et al. (1997) reported that reduced yields of switchgrass were expected to occur as a result of low plant densities. Accumulation of a large number of reproductive tillers provided greater yield for forage species compared with forage species with less reproductive tillers.

Quantification of the developmental morphology of tiller populations indicates the architectural changes in the grass sward. Many systems have been developed to quantify the growth and development of grasses (Vanderlip, 1972; Haun, 1973; Zadoks et al., 1974; Simon and Park, 1983; Moore et al., 1991; Sanderson, 1992). Of these systems, the system for quantifying morphological development of grasses developed by Moore et al. (1991) is applicable to most annual and perennial grass swards, and is easily applied in the field.

Little is known about developmental morphology for native warm-season swards in relation to tiller and canopy architecture as affected by the interaction of harvest date and N

fertilization. Elementary information on the growth and development of the canopy structure response to management is required to use native warm-season grasses successfully as a biomass crop. Therefore, the objectives of this research were: 1) to determine optimum harvest periods and N fertilization rates for the production of warm-season grasses as a biomass crop in the Midwest and 2) to investigate if traits of canopy architecture can explain observed yields of warm-season grasses with varied harvest dates and N fertilization rates.

Materials and Methods

Plant establishment

Field experiments were conducted during 2006 and 2007 at the Iowa State University Sorenson Farm, near Ames, IA (42°0'40" N, 93°44'46" W) on Canisteo silty clay loam (fine-loamy, mixed, superactive, calcareous, mesic, Typic Endoaquoll), Webster silty clay loam (fine-loamy, mixed, superactive, mesic, Typic Endoaquoll), and Clarion loam (fine-loamy, mixed, superactive, mesic, Typic Hapludoll). Weather data were compiled from the Iowa Environmental Mesonet and were collected from a climate station located approximately 4.8 km from the research site (Fig. 5.1a and 5.1b). The experiment was laid out as a split-split plot design with hierarchical classification in the sub-plots in a completely randomized block with four replications. Four warm-season grass species, big bluestem ('Roundtree'), indiangrass ('Rumsey'), eastern gamagrass ('Pete'), and switchgrass ('Cave-In-Rock'), were main plots measuring 3 x 42.8 m. Three N application rates (0, 65, and 140 kg N ha⁻¹) were subplots (3x10.7 m) and the 10 harvest dates were sub-sub plots, all randomly assigned. Harvest dates in each year were treated as repeated measures.

Grass plots were established in a fallow managed in a corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] rotation. Four warm-season grasses, big bluestem, indiangrass, switchgrass, and eastern gamagrass were seeded at 3.6, 3.6, 2.3, and 4.5 kg pure live seed ha⁻¹, respectively, in the fall of 2003. The three former species were seeded in 20-cm rows using a 10-row small grain drill (Tye model 2007, AGCO Co., Lockney, TX) while eastern gamagrass was seeded in 76-cm rows using a 2-row corn planter (John Deere model 71 Flexi Planter, John Deere Co., Moline, IL). Big bluestem and indiangrass plots were overseeded to increase plant density in the spring of 2005.

Before initiation of spring growth each year, imazapic [(RS)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-methylnicotinic acid] was applied at a rate of 1.17 oz of a.i. ha⁻¹ to indiangrass and atrazine [6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5,-triazine-2,4-diamine] was applied at a rate of 2.24 kg of a.i. ha⁻¹ to all other species for weed control. In early May of 2004 and 2005, nitrogen fertilizer was applied at a rate of 85 kg N ha⁻¹. Eastern gamagrass plots received a single inter-row cultivation in June 2004 and 2005. Standing dead material was annually mowed to a 5-cm stubble height, and the residue was removed from plots using a self-propelled forage harvester (John Deere model 5480, John Deere Co., Moline, IL). To reduce weed density and to remove the vast majority of accumulated above-ground material before the initiation of N treatments, all grass plots were burned in April 2006 (Moser and Vogel, 1995; Mitchell and Britton, 2000).

Nitrogen treatments

Ammonium nitrate (NH₄NO₃: 0, 65, and 140 kg N ha⁻¹) was preweighed and applied with 1.5-m-wide drop spreader (Model 6500, Gandy Co., Owatonna, MN) on each individual

subplot N treatment on 8 May 2006 and 12 May 2007. Based on soil test results in a companion research project conducted by Heggenstaller et al. (2009), no other fertilizer was needed.

Forage harvest

Biomass samples were harvested on 10 dates between 16 May and 3 October in 2006, and 22 May and 8 October in 2007. The first harvest occurred at an early vegetative growth stage and depended on spring growth in each year. For the first seven harvest dates (at day 136, 151, 164, 178, 192, 206, 220 for 2006; day 142, 155, 169, 183, 197, 211, and 225 for 2007), the samples were collected at approximately 2-week intervals and at approximately 3-week intervals for last three harvest dates (day 234, 255, and 276 for 2006; day 239, 260, and 281 for 2007). At each harvest, the developmental stage of the warm-season grass stands were visually scored using the system of Moore et al. (1991).

At each sampling date, two 0.19-m² samples were harvested by a grass clipper at ground level from a different portion within each subplot at each harvest date. After recording fresh weights, the samples were dried at 60°C in a forced-air oven for approximately 72 h and reweighed to determine dry-matter yield.

Quantifying developmental morphology

Before drying to dry-matter yield, samples at each harvest date were examined to determine the morphological development of the tiller populations using the mnemonic scale developed by Moore et al. (1991). The life cycle of individual grass tillers was divided into four primary growth stages including: (i) vegetative, (ii) elongation, (iii) reproductive, and

(iv) seed ripening. Substages within each primary growth stage describe specific morphological events occurring in most grasses. To quantify the developmental morphology of a population of tillers, the mean stage by count (MSC) was calculated by determining the stages of each tiller in the sample, using the following equation:

$$\text{Mean Stage by Count (MSC)} = \frac{\sum_{i=0}^{4.9} S_i N_i}{C}$$

Where S_i = growth stage, $i = 0$ to maximum growth stage (4.9), N_i = number of tillers in stage S_i , C = total number of tillers. Quantifying morphological development of the tiller population was reported as a decimal value of a primary growth stage. A more morphologically advanced tiller population was indicated by a higher MSC value.

Statistical analysis

Data were analyzed with the SAS MIXED procedure (Littell et al., 1996), with grass species and nitrogen application rates considered as fixed effects, and block and interactions with blocks considered as random effects. Harvest dates were treated as repeated measures. Least squares means for species, nitrogen application rates, harvest dates, and interactions were separated by the SAS PDIFF option. Pearson correlation coefficients between MSC and harvest date, MSC and dry matter yield, and harvest date and dry matter yield were calculated independently for each species. All differences were considered significant at the 0.05 probability level.

Results and Discussion

Mean Stage Count (MSC)

The MSC of all four warm-season grasses was influenced by the interaction of species x N rate, species x harvest date, in both years, and species x N rate x harvest date in 2007 (Table 4.1).

The MSC of big bluestem, indiangrass, and switchgrass were correlated positively with harvest date, ranging from 0.89, 0.89 and 0.96, respectively, in 2006, and 0.94, 0.85 and 0.95, respectively, in 2007 (Table 4.2). These correlations were illustrated by the species x harvest date interaction suggesting that the MSC for big bluestem, indiangrass, and switchgrass increased linearly until the final harvest in 2006 and 2007 (Fig. 4.1a and 4.1b). The MSC for switchgrass was always greater than that for other species on common harvest dates, illustrating that switchgrass matured more rapidly than other species. Big bluestem and switchgrass had a larger proportion of tillers developing to the seed production stage late in the season than did other two species (Fig 4.7 and 4.8), resulting in the greater MSC for big bluestem and switchgrass in both years (Fig.4.1a and 4.1b). Few indiangrass tillers reached reproductive maturity before the completion of harvest causing a lower MSC, as indicated by tiller demographics (Fig. 4.7 and 4.8). In contrast, the MSC of eastern gamagrass was not correlated with harvest date in either year (Table 4.2). The MSC for eastern gamagrass increased gradually from the first harvest to the fifth harvest (from day 136 to day 192 for 2006; from day 142 to day 197 for 2007) and decreased until the final harvest (Fig. 4.1a and 4.1b) due to the appearance of new tillers across the season (Fig. 4.7a and 4.8a). Dewald and Louthan (1979) and Jackson and Dewald (1994) reported that eastern gamagrass is indeterminate with respect to reproductive growth. Appearance of new

vegetative tillers and spikes on the same plant occur over a considerable time period in the season. This makes each plant a multiaged population of vegetative and reproductive tillers.

In this study, morphological development of warm-season grasses varied with harvest date, with potentially important implications for crop management. Improper timing of warm-season grass cutting results in low quality and low yield, and may be detrimental to stand persistence. Waller et al. (1985) suggested that if grazing was delayed until day 183 of year or until at least 90% of elongating tillers had appeared, then regrowth would be limited following defoliation. Lemus et al. (2002) suggested that a lower leaf to stem ratio may improve biomass quality because stems have a higher fiber content. Thus, the proportion of stem tissue could be one of determinants of the biofuel quality of grasses. In this current study, the large proportion of elongating, reproductive, and seed ripening tillers after the fourth harvest could reinforce the importance of harvesting warm-season grasses for forage in early summer, but suggests harvesting at a later stage increasing lignocellulose and decreasing minerals in biomass (Jung and Vogel, 1992 Madakadze et al., 1999; Mitchell et al., 2001; Adler et al, 2006; Mulkey et al., 2006) may be beneficial for bioenergy production.

The species x N rate interaction indicated the MSC of big bluestem, indiangrass, and switchgrass slightly increased with N fertilization (Fig. 4.3a and 4.3b). Larger changes in tiller demographics for those three species resulted in larger changes in MSC than for eastern gamagrass. Fertilization with N did not affect the morphological development of eastern gamagrass. The application of N increased the proportion of elongating, reproductive, and seed ripening tillers after the fifth harvest (day 192 and 197 for 2006 and 2007, respectively) causing a larger MSC in the other three species (data was not shown). The N rate x harvest date interaction indicated the increase in MSC of grasses receiving N fertilization advanced

their maturity, in contrast to eastern gamagrass late in the season of 2006 (Fig. 4.2a), and between mid and late in the season of 2007 (Fig. 4.2b). These results are similar to those of Hill and Loch (1993) who found that application of N can increase inflorescence density per unit area. Also, Harlan and Kneebone (1953), George and Reigh (1987), and Masters et al. (1993) demonstrated that N application significantly increased the density of reproductive tillers and seed produced for big bluestem and switchgrass. This is in contrast to the trend found in the present study for eastern gamagrass, in which MSC decreased when N was applied at 140 g ha^{-1} in 2006. This is likely to due to increased vegetative growth from excessive N addition (Lemke et al., 2003). Light penetration into the crown area of the plants can be decreased by excessive vegetative growth from N application, leading to decreased inflorescence formation. The reproductive stem density in grasses was increased as light penetration into the canopy increased. Removing plant debris substantially increased reproductive stem density in big bluestem by improving the light environment of emerging shoots (Knapp, 1984).

A three-way interaction of species x N rate x harvest date for MSC occurred in 2007 (Table 4.1). This is likely because the MSC of eastern gamagrass did not increase with advanced maturity between mid and final harvests while for other species did. However, the interaction of species x N rate, N rate x harvest date, and species x N rate x harvest date contributed little variability when compared with the main effects in this study. Therefore, grass species and harvest dates appear to be more important to the MSC index of a biomass crop than the N rate.

Dry matter yield

Dry matter yield of the warm-season grasses was influenced by the interaction of species x harvest date in both years, and N rate x harvest date in 2006 (Table 4.1). The species x harvest date interaction indicated that dry matter yield of eastern gamagrass, big bluestem, indiangrass, and switchgrass increased to their maximum as the growing season progressed and then biomass yield was reduced during senescence (Fig. 4.4). Among these four species, dry matter accumulation of eastern gamagrass was the greatest, ranging from 24,357 to 27,838 kg ha⁻¹ in 2006 and ranging from 17,057 to 20,848 kg ha⁻¹ in 2007. Maximum dry matter yield of eastern gamagrass occurred between the fifth harvest (day 192; MSC=2.2) and eighth harvest (day 234; MSC=2.1) in 2006, when grass swards were at early elongation stage (Fig. 4.4a), and between the fifth (day 197; MSC=1.6) and ninth harvest (day 260; MSC=1.4) in 2007, when grass swards were at mid-vegetative stage (Fig. 4.4b). Maximum dry matter of big bluestem (21,885 kg ha⁻¹) occurred at the eighth harvest (day 234) when the grass sward was at late elongation stage (MSC=2.7) in 2006. Similar to eastern gamagrass for 2007, maximum dry matter accumulation of big bluestem ranged from 18,043 to 20,471 kg ha⁻¹ occurring between the sixth (day 211) and ninth harvest (day 260) when grass swards were between mid-elongation (MSC=2.5) and soft dough stages (MSC=4.3). Maximum dry matter of switchgrass occurred between the sixth (day 206) and ninth harvest (day 255) in 2006 when grass swards were between boot stage (MSC=3.0) and milk/soft dough stage (MSC=4.2), ranging from 15,689 to 19,009 kg ha⁻¹ in 2006, and between the fifth (day 211) and final harvest (day 281) when grass swards were between boot stage (MSC=3.0) and hard dough stage (MSC=4.5), ranging from 11,653 to 15,006 kg ha⁻¹ in 2007. These results are similar to a previous study by Vogel et al. (2002) reporting that

maximum yields of switchgrass grown in the Midwestern USA occurred in mid-August at full panicle emergence to postanthesis. However, harvesting during mid-August periods may decrease the long-term stand densities as observed in the north-central USA by Casler and Boe (2003) and result in lower yields. In both years, dry matter yield for indiangrass peaked later in the season than did other species. Maximum dry matter accumulation of indiangrass ranged from 15,610 to 19,598 kg ha⁻¹ in 2006 and from 12,248 to 14,699 kg ha⁻¹ in 2007, between the eighth (in September) and final harvest (in October) when grass swards were between early elongation (MSC=2.2-2.3) and early reproductive stage (MSC=3.3). Notably, yields for big bluestem, indiangrass, and switchgrass peaked when swards had the largest proportion of reproductive tillers before the onset of seed development (Fig. 4.4, 4.5, 4.7, and 4.8). Switchgrass, big bluestem, and indiangrass are determinate in growth habit. With inflorescence development, most vegetative growth of switchgrass, big bluestem, and indiangrass terminates. When the tillers advance to the seed ripening stages, growth stops, and tiller senescence occurs (Dahl and Hyder, 1977). Therefore, maximum biomass yield occurs at the initial seed development stage in these grasses when dry matter accumulation in vegetative parts stops (Fig. 4.7 and 4.8). In contrast to determinate grasses, eastern gamagrass seed maturity is indeterminate in growth habit with the earlier appearance of new reproductive tillers between mid-and late growing season. Maximum dry matter yield of eastern gamagrass, therefore, occurred at harvest periods with the highest MSC index. In these periods, the largest proportion of seed tillers was present within swards (Fig. 4.7 and 4.8).

After peaking, yields of switchgrass, indiangrass, big bluestem, and eastern gamagrass decreased up to 19%, 20%, 38%, and 61%, respectively, at the final harvest in

2006 ,and up to 16%, 6%, 30%, and 54%, respectively, at the final harvest in 2007 (Fig. 4.4a and 4.4b). The decrease in dry matter yield from maximum-yield harvests to final harvest occurred as senescence caused the loss of leaves and stems, and seed shattered. Vogel et al. (2002) reported that switchgrass yields in the Midwestern USA decreased 10 to 20% with harvests after a killing frost in October. Frank et al. (2004) reported overall, stems contributed 56% to 60% for switchgrass total aboveground biomass on the peak-yielding harvest, but stems accounted for 42% to 48% of total biomass at the final harvest. Leaf biomass decreased 4% to 11% for switchgrass at the final harvest (day 255 of year). Senesced biomass increased from 14% to 19% for switchgrass on the peak-yielding harvest date to 37% to 49% at the final harvest. In the present study, the large proportion of senesced biomass and litter were left in the field as residue, and not picked up during sampling.

Decreased yields for warm-season grasses were consistent with lower tiller density except in eastern gamagrass which senesced late in the season (Fig.4.7 and 4.8). Adler et al. (2006) demonstrated that more than twice as much residue was not picked up by the baler when harvest was delayed from fall to spring. Biomass was not picked up by the baler, either because it was not cut due to lodging or it was cut but not picked up by the baler. In addition, the decrease in biomass yield occurred from a lower standing tiller weight due to loss of leaves and panicles. Lemke et al. (2003) observed visually during the course of their study that only about 10% of the tillers on a grass advance to reproductive growth, whereas the rest remain vegetative. The lack of a species x nitrogen rate interaction for biomass dry matter yield indicates the response of each species to N fertilization is approximately the same (Table 4.1). Total biomass yields of all four species increased with increased application rate of N in both years. Nitrogen fertilization at 65 and 140 kg ha⁻¹ increased

total biomass across four species by 6.5% and 24%, respectively, in 2006 (Fig. 4.5a) and by 26% and 49%, respectively, in 2007 (Fig. 4.5b). In both study years, the N rate x harvest date suggested that the increase in biomass yields with N fertilization occurred between mid-growing season and later season (Fig. 4.5), when grass swards had a great proportion of elongated tillers. This is likely because N application in warm-season grasses increases stem development (Brejda et al., 1994). These results are consistent with earlier studies that found that warm-season grasses produce typically higher yields with N application rates ranging from 50 to 120 kg N ha⁻¹ in the Central Plains and Midwest states (Balasko and Smith, 1971; George et al., 1995; Vogel et al., 2002; Brejda, 2000). Yield of switchgrass supplied with 90 kg N ha⁻¹ increased 61% from May to June. However, warm-season grasses grown at locations where soil organic carbon concentration is very high, do not respond to N due to N released from mineralization of soil organic carbon (Mulkey et al., 2006).

Harvest date was correlated positively with dry matter yield for big bluestem, eastern gamagrass, indiangrass, and switchgrass ranging from 0.71, 0.44, 0.82, and 0.75, in 2006 and from 0.64, 0.45, 0.83, and 0.73, in 2007 (Table 4.2). The MSC of all species was correlated positively with dry matter yield and averaged between 0.53, 0.71, 0.77, and 0.72 in 2006, and 0.52, 0.45, 0.82, and 0.73, respectively, in 2007. The low correlation between biomass yields and MSC for eastern gamagrass was likely due to relatively constant MSC index across the growing season in 2007. The low correlation for big bluestem was likely due to the relatively decreased biomass yield that occurred from loss of standing tiller weight after the maximum yield while MSC considerably increased.

Conclusions

Biomass yield of warm-season grasses increases with advanced maturity before declining during senescence. There is a difference in optimal time to harvest for biomass yields among the warm-season grasses studied. Eastern gamagrass has an indeterminate growth habit in which vegetative growth, and concomitant biomass accumulation, still occurs after seed development. Maximum biomass yield for this grass is obtained at harvest dates with highest MSC index (2.1 to 2.2; first to second node palpable) when the largest proportions of reproductive tillers within sward are present. In contrast, reproductive development of big bluestem, indiangrass, and switchgrass is determinate. The optimal times to harvest these grasses for biomass yields are at 2.5 (E5; fifth node palpable) to 2.7 (E7; seventh node palpable), 3.0 (R0; boot stage) to 3.2 (R2; spike fully emerged), and 2.6 (E6; sixth node palpable) stage of maturity, respectively. At these morphological stages, a large proportion of reproductive tillers are present, but seeds are not yet visually observed. Although harvesting after these peak-yielding harvest dates could improve biomass quality, it may provide relatively less biomass yield resulting from a large proportion of senesced tillers and litter.

In terms of a biomass supply strategy, eastern gamagrass could be used as a feedstock in early summer, while big bluestem and switchgrass could be used between mid-and late summer and indiangrass in the early fall. However, this approach must be balanced with the biomass quality required and costs of production (Nelson et al., 1994; Vogel et al., 2002; Tiffany et al., 2006).

Biomass yield and mean stage count index for big bluestem, switchgrass, and indiangrass increases with higher rates of N fertilization. This is attributed to an increase in

the proportion of elongating, reproductive, and seed tillers, and an increase in total number of tillers for eastern gamagrass, leading to higher yields for these grasses. Higher grass yields are associated with accumulation of a large number of reproductive tillers. The nodes and internodes contribute most dry matter while leaf blades contribute the smallest proportion to dry matter production (Kalmbacher, 1983). But the larger number of vegetative tillers in eastern gamagrass receiving high N rates may contain high concentrations of N, thus decreasing biomass quality (Waramit et al., 2010).

Another potential harvest date for biomass yield would be after maximum-yielding periods. Harvesting delayed to late season may decrease biomass yield, but it could provide improved biomass quality. Vogel et al. (2002) suggested that significant amounts of N are remobilized from the above-ground biomass to underground organs of switchgrass that is harvested after a killing frost. The nitrogen fertilization requirement for the next season would be reduced with this harvest scheme leading to reducing a biomass production cost. They also suggested that the economic value of the yield loss with delayed harvest would be compensated for by the value of decreased fertilizer and application cost. Additionally, the concentration of N and other minerals of warm-season grasses that negatively affect conversion and combustion systems decrease as they mature during the growing season, leading to increased biofuel quality (Sanderson and Wolf, 1995; Vogel et al., 2002; Adler et al., 2006). Thus, optimum biomass yields with improved quality might obtain later in the season other than at maximum-yielding harvest dates when they were fertilized with 140 kg ha⁻¹.

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Table 4.1. Mean stage counts (MSC) and dry matter *F*-values and significances in response to four warm-season grass species, three nitrogen rates and ten harvest treatments during the growing season of 2006 and 2007 at Ames, IA.

Effect	2006	2007
<u>MSC</u>		
Species (S)	231.45 **	540.86 **
N rate (N)	23.07 **	47.96 **
S*N	8.79 **	5.58 **
Harvest (H)	547.58 **	786.11 **
S*H	83.44 **	103.84 **
N*H	2.86 **	3.50 **
S*N*H	1.02 ns	1.73 **
<u>Dry matter</u>		
S	56.52 **	9.42**
N	14.94 **	14.81**
SxN	2.02 ns	1.30 ns
H	339.76 **	60.39 **
SxH	9.77 **	3.65 **
NxH	2.71 **	1.17 ns
SxNxH	0.77 ns	1.21 ns

* significant at the 0.05 probability level.

** significant at the 0.01 probability level.

ns non-significant

Table 4.2 Pearson correlation coefficients (r) and P values (P) for mean stage count (MSC), dry weight and harvest date of big bluestem, eastern gamagrass, indiangrass, and switchgrass given N fertilization and harvested on ten dates during 2006 and 2007 near Ames, IA.

	Big bluestem			Eastern gamagrass			Indiangrass			Switchgrass		
	r	P		r	P		r	P		r	P	
2006												
MSC x H [†]	89.02(118 [§])	<0.0001		16.39(120)	0.074		89.16(120)	<0.0001		96.44(119)	<0.0001	
MSC x DM [‡]	53.42(113)	<0.0001		70.86(120)	<0.0001		77.41(120)	<0.0001		71.58(119)	<0.0001	
H x DM	70.66(113)	<0.0001		43.93(120)	<0.0001		81.61(119)	<0.0001		75.03(119)	<0.0001	
2007												
MSC x H	93.84(118)	<0.0001		14.45(120)	0.0051		82.92(120)	<0.0001		95.39(120)	<0.0001	
MSC x DM	51.98(112)	<0.0001		55.50(120)	<0.0001		81.75(119)	<0.0001		72.75(120)	<0.0001	
H x DM	63.52(116)	<0.0001		44.61(120)	<0.0001		82.86(119)	<0.0001		73.34(120)	<0.0001	

[†] H, harvest date.

[‡] DM, dry matter.

[§] Observation numbers

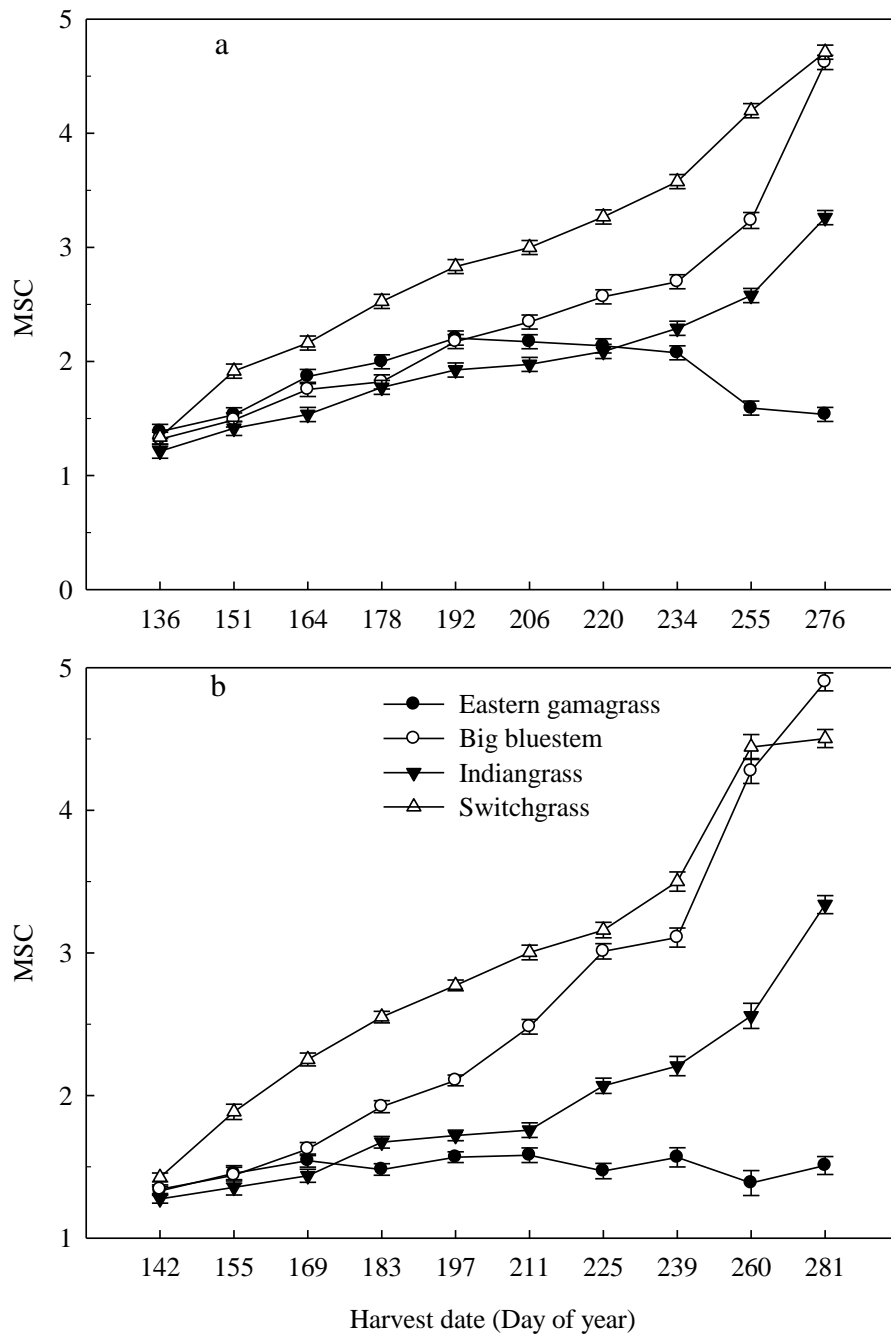


Figure 4.1. Mean stage count as influenced by eastern gamagrass (EG), big bluestem (BB), indiangrass (ID), and switchgrass (SW) and ten harvest dates. Data are averaged over four replications and three nitrogen rates, in (a) 2006 and (b) 2007, at Ames, IA. Standard error bars are partially covered by graph symbols.

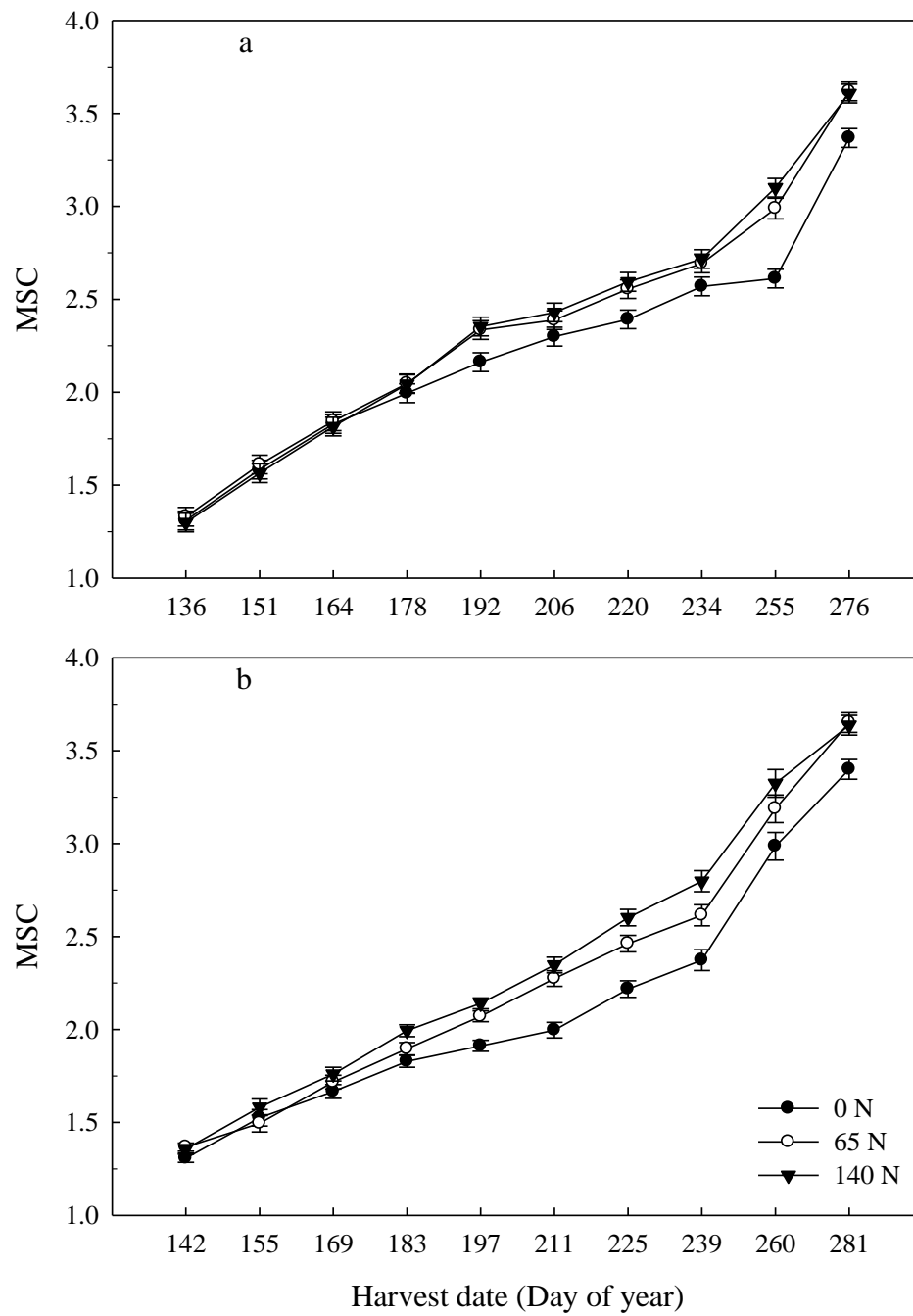


Figure 4.2. Mean stage count as influenced by three nitrogen rates at 0 (0 N), 65 (65 N), and 140 (140 N) kg N ha⁻¹ and ten harvest dates. Data are averaged over four replications and four species in (a) 2006 and (b) 2007, at Ames, IA. Standard error bars are partially covered by graph symbols.

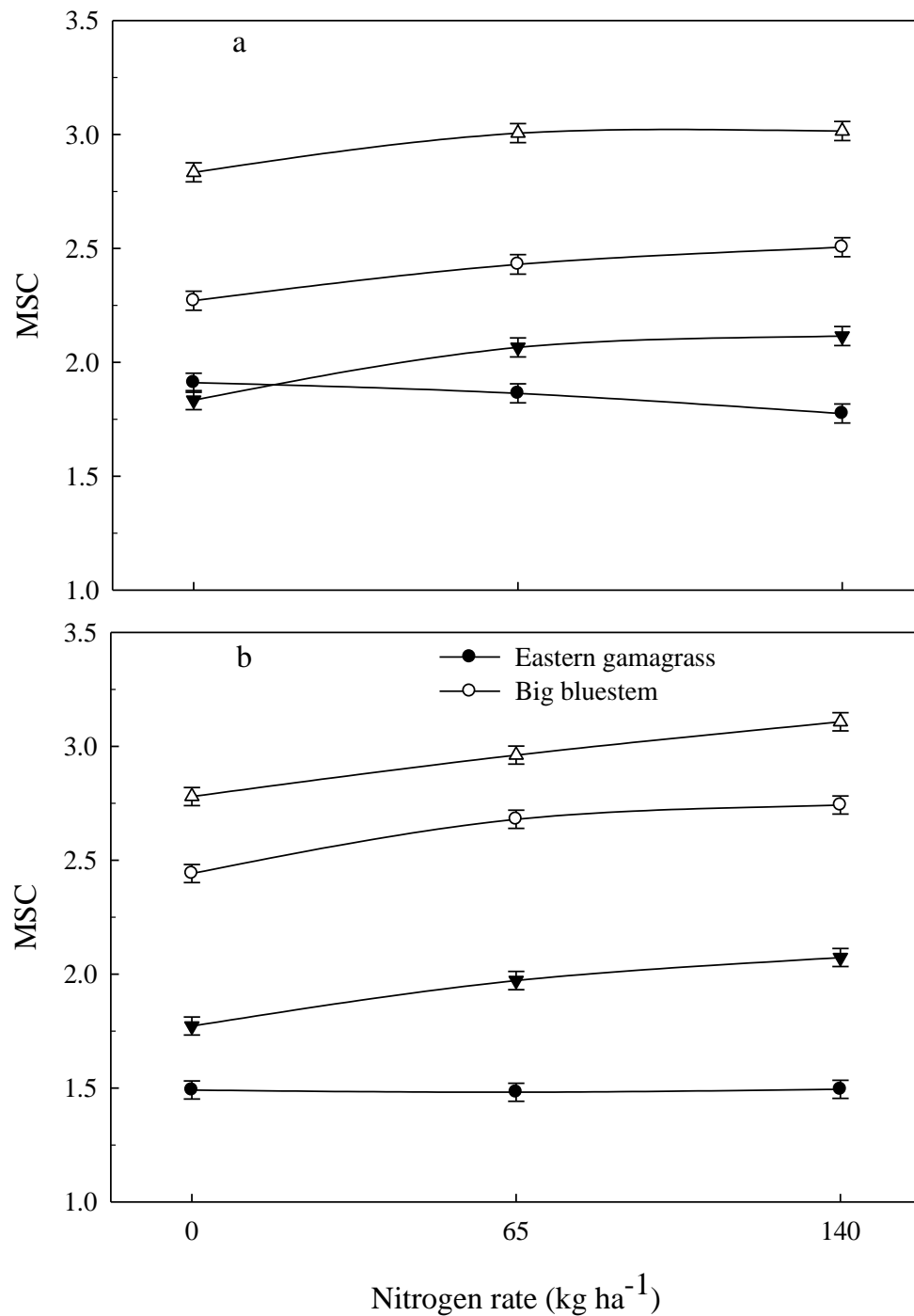


Figure 4.3. Mean stage count of eastern gamagrass (EG), big bluestem (BB), indiagrass (ID), and switchgrass (SW) as influenced by nitrogen application rate. Data are averaged over four replications and ten harvest dates, in (a) 2006 and (b) 2007, at Ames, IA. Standard error bars are partially covered by graph symbols.

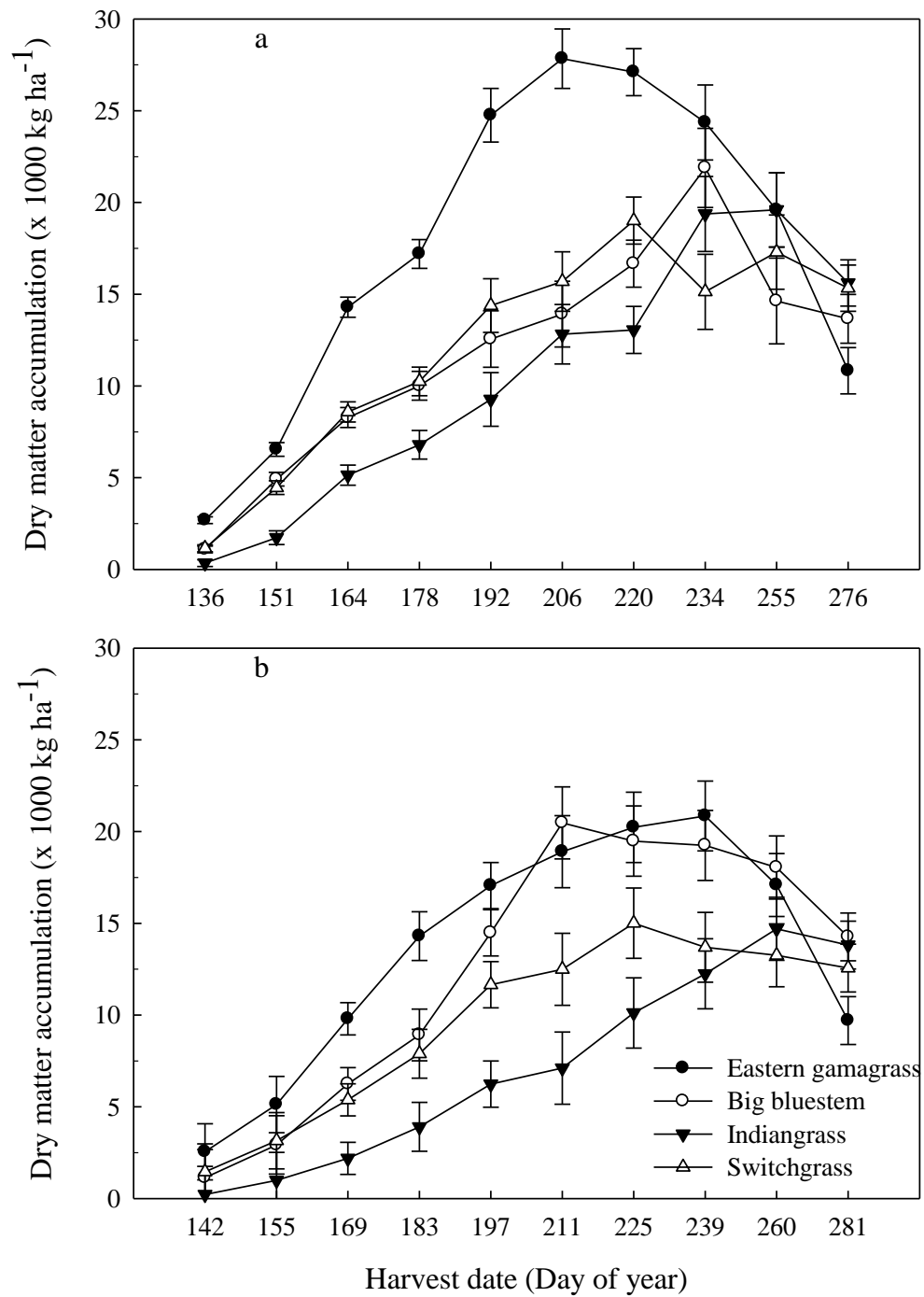


Figure 4.4. Mean dry matter accumulation as influenced by eastern gamagrass (EG), big bluestem (BB), indiangrass (ID), and switchgrass (SW) and ten harvest dates. Data are averaged over four replications and three N rates, in (a) 2006 and (b) 2007, at Ames, IA. Standard error bars are partially covered by graph symbols.

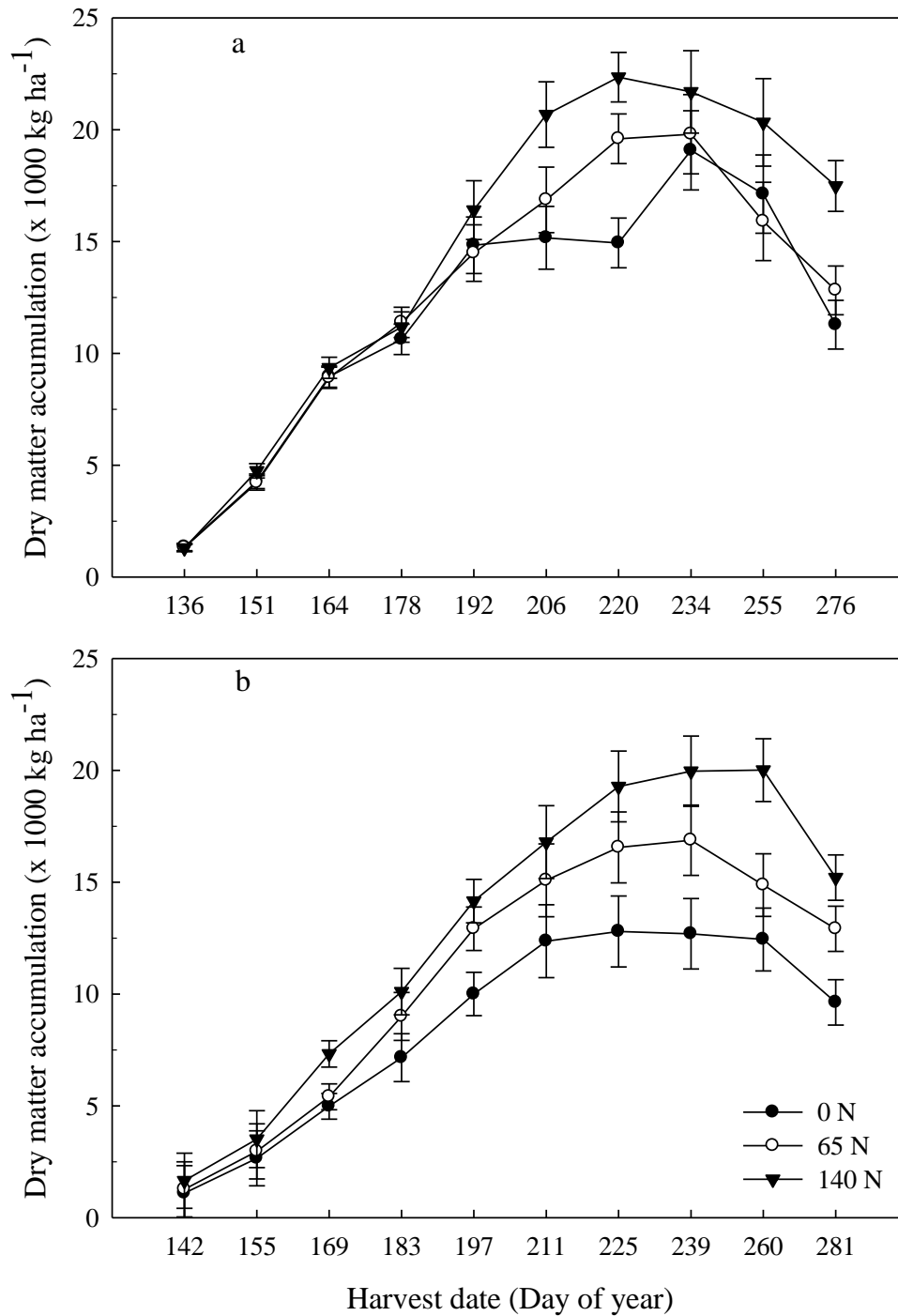


Figure 4.5. Mean dry matter accumulation as influenced by three nitrogen rates at 0 (0 N), 65 (65 N), and 140 (140 N) kg N ha⁻¹ and ten harvest dates. Data are averaged over four replications and four species, in (a) 2006 and (b) 2007, at Ames, IA. Standard error bars are partially covered by graph symbols.

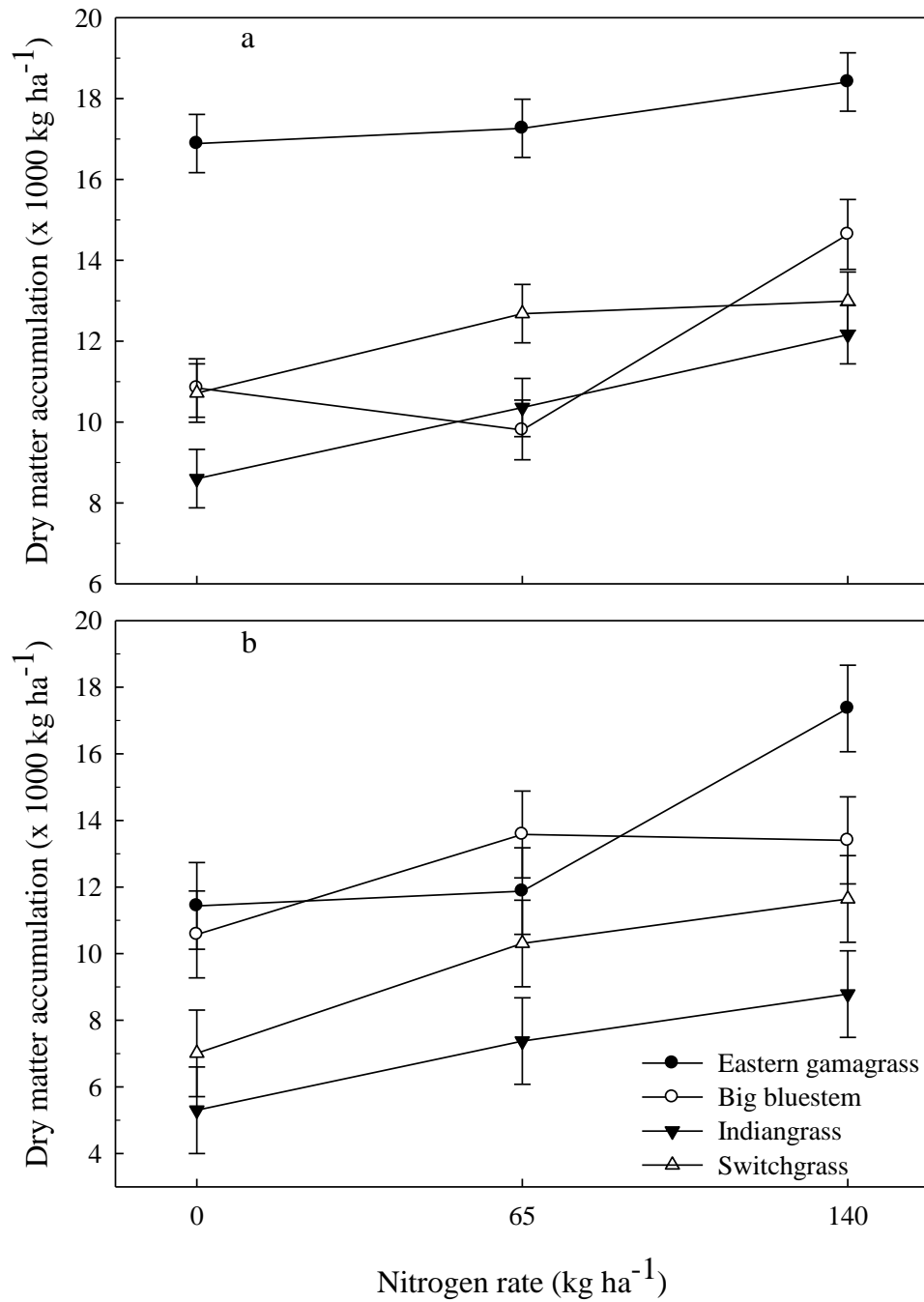


Figure 4.6. Mean dry matter accumulation as influenced by eastern gamagrass (EG), big bluestem (BB), indiangrass (ID), and switchgrass (SW) and three nitrogen rates at 0 (0 N), 65 (65 N), and 140 (140 N) kg N ha⁻¹ and ten harvest dates. Data are averaged over four replications and ten harvest dates in (a) 2006 and (b) 2007, at Ames, IA. Standard error bars are partially covered by graph symbols.

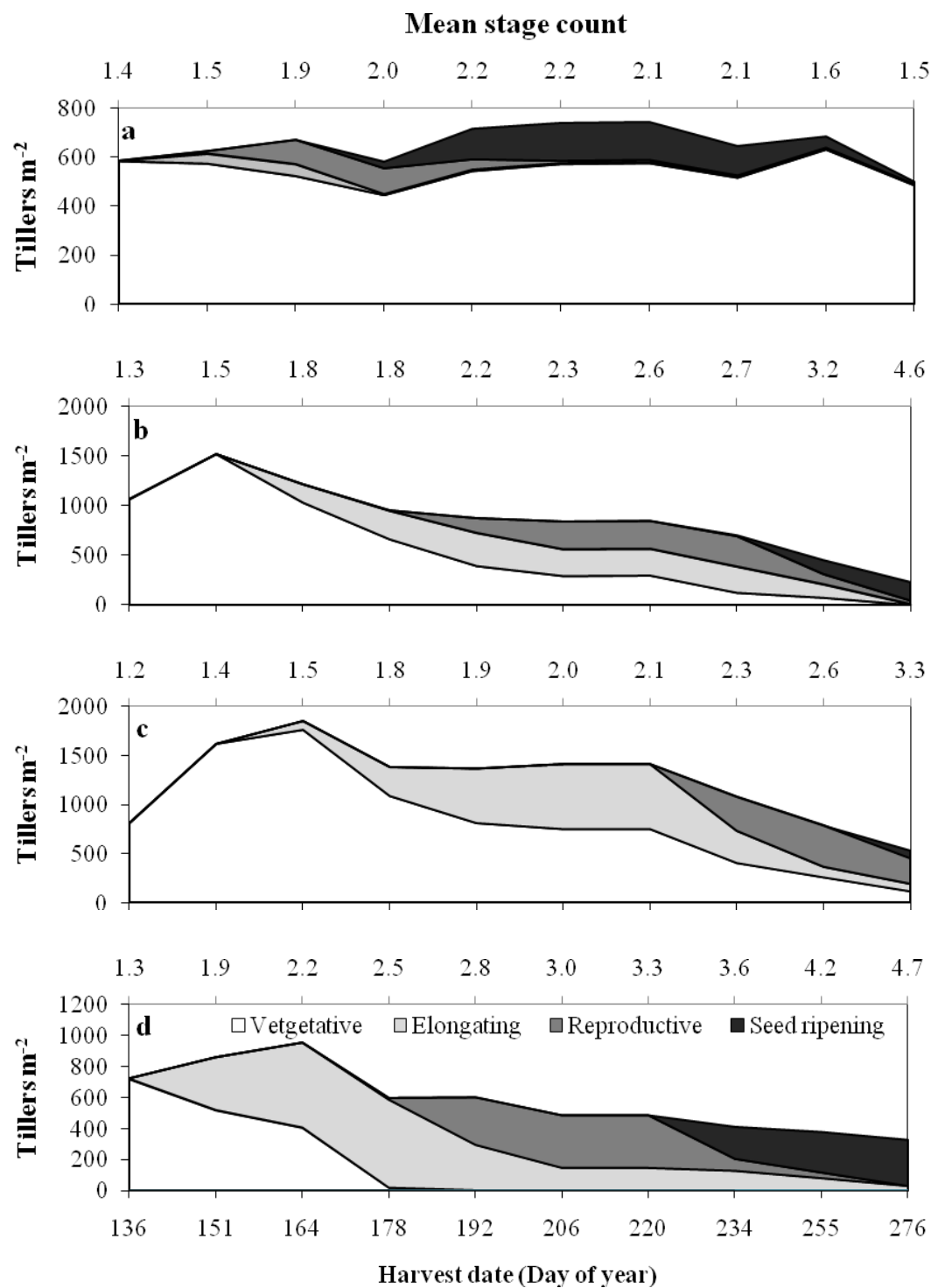


Figure 4.7. Tiller demographics and mean stage counts for eastern gamagrass (a), big bluestem (b), indiangrass (c), and switchgrass (d) grown near Ames, IA, in 2006.

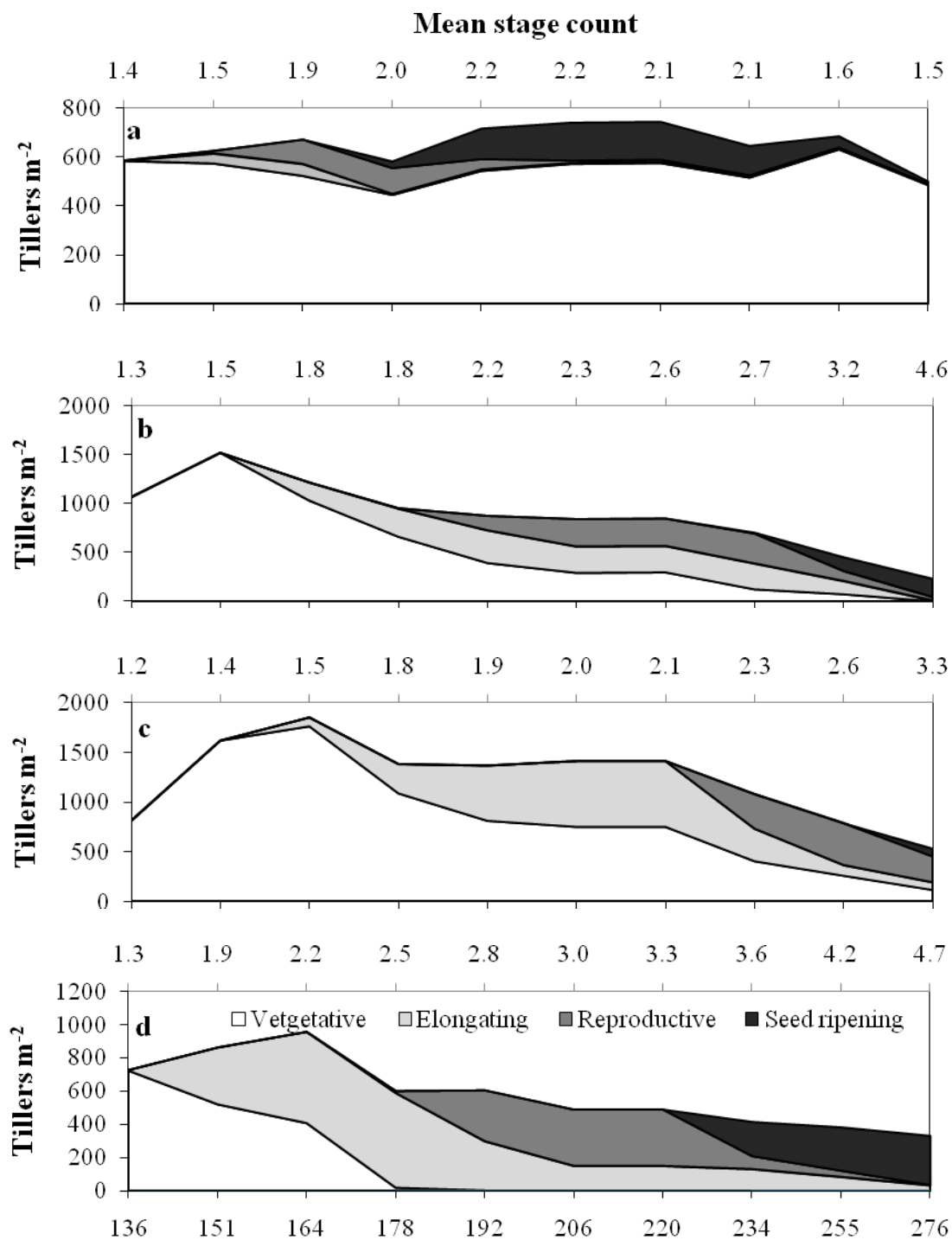


Figure 4.8. Tiller demographics and mean stage counts for eastern gamagrass (a), big bluestem (b), indiangrass (c), and switchgrass (d) grown near Ames, IA, in 2007.

CHAPTER 5. COMPOSITION OF NATIVE WARM-SEASON GRASSES FOR BIOENERGY PRODUCTION IN RESPONSE TO NITROGEN FERTILIZATION RATE AND HARVEST DATE

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Abstract

Information about the interaction of harvesting and N fertilization on composition of warm-season grasses grown as biofuel feedstocks is limited. Our objective was to determine composition of warm-season grasses as influenced by N fertilization rates and harvest dates. A field study was conducted near Ames, IA, during 2006 and 2007. The experimental design was a split-split plot arranged in a randomized complete block with four replications. Big bluestem (*Andropogon gerardii* Vitman), eastern gamagrass (*Tripsacum dactyloides* L.), indiangrass (*Sorghastrum nutans* L. Nash), and switchgrass (*Panicum virgatum* L.) were main plots. Three N application rates (0, 65, and 140 kg ha⁻¹) were subplots, and 10 harvest dates were sub-sub plots. In both years, delaying harvest increased cellulose, lignin, and C concentrations, but decreased ash and N concentrations, however, the magnitude of these effects varied among species. On average, big bluestem had the highest cellulose (430.5

g kg⁻¹) and C concentration (450.5 g kg⁻¹) while switchgrass had the highest lignin concentration (58.5 g kg⁻¹) among four species. Big bluestem had the lowest ash concentration (47.5 g kg⁻¹) whereas indiangrass had the lowest N concentration (5.5 g kg⁻¹). With increasing rates of N, average N, C, cellulose, and lignin concentrations increased 19 to 29%, 0.6 to 2.2%, 0.7 to 5.7%, and 5.6 to 23.9%, respectively, whereas ash concentration declined 1.8 to 18.4%, varied among species. Our results indicated that in general, warm-season grasses supplied with N fertilizer at 140 kg ha⁻¹ and harvest delayed until fall have the optimal composition for biomass feedstock production.

Abbreviations: ADF, acid detergent fiber; ADL, acid detergent lignin; DOY, day of year; NDF, neutral detergent fiber.

Introduction

With the C₄ photosynthetic system, warm-season grasses normally grow well under high temperatures in tropical and subtropical regions of the world where they play an important role in ruminant animal production, conservation, and as turf. Many of these species evolved in and are adapted to temperate regions with relatively long warm periods during summer. In the U.S., warm-season grasses dominate the North American tallgrass prairie (Weaver, 1968), which occurs naturally throughout the Great Plains and the Corn Belt (Stubbendieck et al., 1991; Moore and Anderson, 2000). Among these warm-season grass species, eastern gamagrass, big bluestem, switchgrass, and indiangrass have been widely used as pasture rangelands, or monocultures in agricultural systems. They are used to fill the deficiency in forage supply during warm and dry months in the central and eastern U.S. when

typical pastures of cool-season grasses (C_3), are unproductive (Moser and Vogel, 1995; Martin, 2000; Anderson, 2004).

Recently, warm-season grasses have gained prominent attention as renewable sources of biomass for energy and industrial raw materials. A renewable biofuel economy is projected as a pathway to reduce reliance on fossil fuels, reduce greenhouse gas emissions, and enhance rural economies (McLaughlin et al., 2002). Lignocellulosic feedstocks, such as mixtures of prairie grasses, are alternatives for current biofuel sources. These feedstocks from perennial grasses require fewer agricultural inputs than annual crops and can be grown on agriculturally marginal lands (McLaughlin et al., 2002). The dry matter constituents of the biomass influence their quality as a bioenergy feedstock, although different conversion systems have specific biomass quality requirements. The most important dry matter constituents for herbaceous biofuel feedstocks are lignocellulose primarily made up of cellulose, hemicellulose, and lignin; and mineral elements including nitrogen, and ash. High concentrations of lignocellulose are desirable for chemical and biofuel production (Trebbi, 1993), while high concentrations of nitrogen and/or ash reduce the effectiveness and chemical output of thermochemical conversion systems (Agblevor et al., 1992). Biomass composition of warm-season grasses for bioenergy production vary significantly due to genetics and environmental factors. Grass maturity stages and soil nutrients during growth, especially N, are primary factors which affect not only biomass yield, but total fiber, N, and ash concentration in grasses as well (Vogel et al., 2002; Adler et al., 2006). To achieve their production potential for conversion to biofuels, time of harvest and N application rate must be optimized.

Information regarding the effects of N application and harvest time on forage quality in the U.S. is available. However, information for warm-season grasses grown and managed as bioenergy crops is limited. Most of the previous research has been conducted on fertilizer requirements of native warm-season grasses, including switchgrass, when managed for hay or grazing. In the central Great Plains and Midwestern states, optimum N application rates for a model lignocellulosic crop, switchgrass, managed for pasture or hay range from about 50 to 120 kg ha⁻¹ (Brejda, 2000). In Texas, the optimum N fertilization rate for ‘Alamo’ switchgrass managed for biomass production was 168 kg ha⁻¹ (Muir et al., 2001). In southern Iowa, Lemus et al. (2008) found that N fertilization was used most efficiently by the switchgrass plant at levels between 56 and 115 kg N ha⁻¹, which substantially increased the biomass of switchgrass fields over time without negatively affecting the quality of the feedstock. In their study, concentrations of cellulose, lignin, and N increased while hemicellulose and ash declined, as nitrogen fertilization rate increased. These changes are desirable, given the greater energy density in cellulose and lignin compared to hemicelluloses and the potentially detrimental effects of ash to the power plant. However, the real significance of these results for overall biomass quality was inconsistent across both years, due likely to differences in harvest date. Understanding the interaction of N fertilization and harvest time on biomass composition in other warm-season species is limited at present. Therefore, the main objective of this research was to determine biomass chemical composition of four warm-season grasses as influenced by nitrogen application rate and harvest date.

Materials and Methods

Plant establishment

Field experiments were conducted during 2006 and 2007 at the Iowa State University Sorenson Farm, near Ames, IA (42°0'40" N, 93°44'46" W) on Canisteo silty clay loam (fine-loamy, mixed, superactive, calcareous, mesic, Typic Endoaquolls), Webster silty clay loam (fine-loamy, mixed, superactive, mesic, Typic Endoaquolls), and Clarion silty clay loam (fine-loamy, mixed, superactive, mesic, Typic Hapludolls). Weather data were compiled from the Iowa Environmental Mesonet and were collected from a climate station located approximately 4.8 km from the research site (Fig. 5.1a and 5.1b). The experiment was laid out as a split-split plot design with hierarchical classification in the sub-plots in a completely randomized block with four replicates. Four warm-season grass species were main plots that measured 3 x 42.8 m. Three N application rates (0, 65 and 140 kg N ha⁻¹) were subplots (3 x 10.7 m) and the 10 harvest dates were sub-sub plots that were randomly assigned. Harvest dates in each year were treated as repeated measures.

Grass plots were established in a fallow field managed in a corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] rotation. Four warm-season grass species, big bluestem ('Roundtree'), indiangrass ('Rumsey'), eastern gamagrass ('Pete'), and switchgrass ('Cave-In-Rock'), were seeded at 3.6, 3.6, 4.5, and 2.3 kg pure live seed ha⁻¹, respectively, in the fall of 2003. Big bluestem, indiangrass, and switchgrass were seeded in 20-cm rows using a 10-row small grain drill (Tye model 2007, AGCO Co., Lockney, TX) while eastern gamagrass was seeded in 76-cm rows using a 2-row corn planter (John Deere model 71 Flexi Planter, John Deere Co., Moline, IL). Big bluestem and indiangrass plots were overseeded at 3.6 kg pure live seed ha⁻¹ with this grain drill to increase plant density in the spring of 2005.

Before initiation of spring growth each year, imazapic [(RS)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-methylnicotinic acid] was applied at a rate of 140 g a.i. ha⁻¹ to indiangrass and atrazine [6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine] was applied at a rate of 2.3 kg a.i. ha⁻¹ to all other species for weed control. Eastern gamagrass plots received a single inter-row cultivation in June 2004 and 2005. Standing dead material was annually mowed to a 5-cm stubble height, and the residue was removed from plots using a self-propelled forage harvester (John Deere model 5480, John Deere Co., Moline, IL). In early May of 2004 and 2005, nitrogen fertilizer was applied at a rate of 85 kg N ha⁻¹. To reduce weed density and to remove the majority of accumulated aboveground material before the initiation of N treatments, all grass plots were burned in April 2006 (Moser and Vogel, 1995; Mitchell and Britton, 2000).

Nitrogen treatments

Ammonium nitrate (NH₄NO₃; 0, 65, and 140 kg N ha⁻¹) was preweighed and applied with 1.5 m wide drop spreader (Model 6500, Gandy Co., Owatonna, MN) on each individual subplot nitrogen treatment on 8 May 2006 and 12 May 2007. Based on soil test results in a companion research project conducted by Heggenstaller et al. (2009), no other fertilizer was needed.

Forage harvest treatment

Biomass samples were harvested on 10 dates between 16 May and 3 October 2006, and 22 May and 8 October 2007. For the first seven harvest dates (16 May, 31 May, 13 June, 27 June, 11 July, 25 July, and 3 August in 2006; 22 May, 4 June, 18 June, 2 July, 16 July, 30

July, and 13 August in 2007), the samples were collected at approximately 2-week intervals and at approximately 3-week intervals for last three harvest dates (22 August, 12 September, and 30 October in 2006; 27 August, 17 September, and 8 October in 2007). Depending on spring growth in each year, the first harvest occurred at the early-vegetative growth stage.

At each sampling date, two 0.19-m² samples were harvested by a hand clipper at ground level from a different portion within each subplot at each harvest date. The samples were dried at 60°C in a forced-air oven for approximately 72 h. Dried whole plant materials were ground sequentially using a Wiley Mill model 4 (Thomas Manufacturing, Philadelphia, PA) to pass an 8-mm screen and reground to uniformity with a 1-mm screen. Subsamples of homogenized tissue (mixed whole plant materials) were equilibrated with ambient humidity for approximately 48 h and stored in sealed plastic vials at room temperature until quality analyses were performed.

Biomass composition

Ground samples were mixed uniformly inside plastic vials and analyzed sequentially for neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) using an ANKOM 200 Fiber Analyzer (ANKOM Technology Corp., Fairport, NY). Ash concentrations were determined using the methods described by Undersander et al. (1993). Hemicellulose and cellulose were calculated as the difference between NDF and ADF, and the difference between ADF and ADL, corrected for ash, respectively. Total nitrogen and carbon were quantified with a LECO CHN-2000 instrument (LECO Corp., St. Joseph, MI).

Statistical analysis

Data were analyzed with the SAS MIXED procedure, with grass species and nitrogen application rates considered as fixed effects, and replications and interactions with replications considered as random effects. Harvest dates were treated as repeated measures and tested using a split-plot in time approach (Littell et al., 1996). Least squares means for species, nitrogen application rates, harvest dates, and interactions were separated by the SAS PDIFF option. Differences were considered significant at the 0.05 probability level.

Results

Due to the differences in the response of chemical composition to nitrogen application dates, harvest dates, and variable climatic conditions from year to year (Fig. 5.1a and 5.1b), data for each year were analyzed separately. There were interactions among variables indicating the response to each main treatment effect could not be evaluated independently of the others (Table 5.1).

Cellulose

The concentrations of cellulose for all grass species increased with advanced maturity in both years (Fig. 5.2a and 5.3a). The concentrations rapidly increased throughout the early and mid season, albeit slowly, after those initial periods of increase. The species x harvest date interaction indicated that clear separation among grass species in cellulose concentration occurred during the mid-to-late season. The amount of cellulose in big bluestem and indiagrass was greater than that of eastern gamagrass and switchgrass between mid season and late season harvest dates (Fig. 5.2a and 5.3a). Maximal cellulose concentration for

eastern gamagrass, big bluestem, and indiangrass occurred at the final harvest (DOY 276), while that for switchgrass occurred at the 6th harvest (DOY 206). Across harvest dates in 2006, the cellulose concentrations of eastern gamagrass, big bluestem, indiangrass, and switchgrass increased from 257 to 383, 244 to 420, 240 to 420, and 219 to 359 g kg⁻¹, respectively (Fig. 5.2a). In 2007, the cellulose concentrations of eastern gamagrass, big bluestem, indiangrass, and switchgrass increased to the maximum levels at the late-season harvest, where values ranged from 279 to 372, 264 to 441, 252 to 416, and 241 to 372 g kg⁻¹, respectively (Fig. 5.3a). The species x N rate interaction for cellulose showed there were differences in the increase in amount of cellulose among grass species that received N fertilization. The concentration of cellulose for indiangrass and switchgrass was increased 2.6 and 2.1% , respectively, in 2006 (Fig. 5.4a) and 3.5 and 5.3%, respectively, in 2007 (Fig. 5.5a) when they were supplied with N at 65 kg ha⁻¹. For switchgrass, the cellulose concentration was greater at 140 kg N ha⁻¹ than at 65 kg N ha⁻¹ in 2007. No differences in the cellulose concentration were detected between 65 kg N ha⁻¹ and 140 kg N ha⁻¹ for other species. The cellulose concentration of switchgrass was similar to that of eastern gamagrass, when N was applied at 140 kg ha⁻¹ (Fig. 5.5a), but neither rate of N fertilization increased the cellulose concentration of eastern gamagrass or big bluestem in either year (Fig. 5.4a and 5.5a). Although, there was an N rate x harvest date interaction in 2006, differences among harvest dates in the cellulose concentration with respect to N fertilization were not consistent (Fig. 5.6a).

Hemicellulose

The species x harvest date interaction for hemicellulose concentration in 2006 and 2007 showed that the changes in amount of hemicellulose were inconsistent among species throughout growing season (Fig. 5.2b and 5.3b). During early growth stages and late growth stages, eastern gamagrass had 8.8%, 9.2%, and 11.5% in 2006, and 11.7%, 9.8%, and 12.5%, in 2007, greater amount of hemicellulose than big bluestem, switchgrass, and indiagrass, respectively (Fig. 5.2b and 5.3b). There were no differences in hemicellulose concentration among N application rates across grass species in either year (Table 5.1).

Carbon

In both years, the amount of C in four grasses increased with advancing maturity (Fig. 5.2c and 5.3c). There was a species x harvest date interaction for C concentration (Table 5.1), indicating that there were differences in C trends with harvest date among grass species (Fig. 5.2c and 5.3c). The C concentrations of eastern gamagrass and switchgrass were slightly less than that of big bluestem between mid and late growth stages. Of all species, indiagrass contained the least carbon during vegetative stages in both years while the maximum carbon concentration observed each year was recorded for big bluestem (2006, 445 g kg⁻¹; 2007, 456 g kg⁻¹) at the final harvest. In 2006, the concentrations of C for eastern gamagrass and switchgrass increased from 421 and 420 g kg⁻¹, respectively, at the 1st harvest (DOY 136) to a maximum approximately 436 and 438 g kg⁻¹ respectively, at the 8th harvest (DOY 234). The C concentration of big bluestem and indiagrass increased from 413 and 406 g kg⁻¹, respectively, at the 1st harvest to a maximum at the 9th harvest (DOY 255) approximately 445 and 426 g kg⁻¹, respectively (Fig. 5.2c). In 2007, carbon concentration for all species

increased with progressing harvests and was greatest at the end of season (DOY 281). The increase in carbon concentrations from the 1st harvest to the maximum for eastern gamagrass, big bluestem, indiangrass, and switchgrass ranged from 423 to 443, 414 to 456, 402 to 441, and 420 to 453 g kg⁻¹, respectively (Fig. 5.3c). No interaction effect of species x N rate was detected for carbon concentration in 2006 showing consistency of species effect over N fertilization rates. Consequently, the C concentration across the four grass species and 10 harvest dates increased 1.0 % and 2.0% when N was applied at 65 and 140 kg ha⁻¹, respectively. The N rate x harvest date interaction for carbon concentration in 2006 suggested that perennial, warm-season grasses receiving N fertilization had a greater carbon concentration during the middle of the growing season than those not treated with N. (Fig. 5.6b). In contrast to 2006, the species x N rate interaction for carbon concentration in 2007 showed that the increase in carbon concentration for eastern gamagrass and indiangrass receiving 65 kg N ha⁻¹ was greater than that for switchgrass and big bluestem (Fig. 5.5b). Eastern gamagrass fertilized with 65 and 140 kg N ha⁻¹ had a similar carbon concentration as big bluestem and switchgrass in 2007 (Fig. 5.5b).

Nitrogen

The N concentration of all warm-season grass species decreased curvilinearly with delayed harvest in both years (Fig. 5.2d and 5.3d). Nitrogen concentrations decreased rapidly across the first four harvest dates and slower at later harvest dates. In 2006, the decrease in N concentrations of eastern gamagrass, big bluestem, indiangrass, and switchgrass between the first (DOY 136) and the final harvest (DOY 276) decreased from 32 to 7, 37 to 6, 35 to 6, and 35 to 6 g kg⁻¹, respectively (Fig. 5.2d). Similar to 2006, the N concentrations of eastern

gamagrass, big bluestem, indiangrass, and switchgrass decreased to the lowest levels at the final harvest (DOY 281) where the values ranged from 24 to 7, 27 to 5, 27 to 5, and 23 to 6 g kg⁻¹, respectively (Fig. 5.3d). In both years, the species x harvest date interaction for biomass N concentration indicated that the declines in N concentration during early vegetative stages were different and that after the 7th harvest (DOY 215 in 2006; DOY 225 in 2007) was less for eastern gamagrass than other species (Fig. 5.2d and 5.3d). There were no species x N rate interactions for N concentration suggesting that the concentration of N across four species receiving 65 and 140 kg N ha⁻¹ increased 19.0% and 29.0%, respectively, when averaged over both years. The N rate x harvest date interaction for N concentration of both years indicated that N concentration was greater with N fertilization during vegetative growth stages but similar regardless of fertilization at the end of season (Fig. 5.6c and 5.7a).

Lignin

There was a species x harvest date interaction for the concentration of lignin indicating that the lignin concentration of all four grass species increased with advanced maturity, but rates of increase were different among species in both years (Fig. 5.2e and 5.3e). The increase in lignin concentration was less pronounced and began to level off earlier for eastern gamagrass compared to other species. In 2006, the maximum lignin concentrations of all species were obtained between the 9th (DOY 255) and 10th (DOY 276) harvest. The lignin concentrations increased from 16 to 35 g kg⁻¹ for eastern gamagrass, 14 to 54 g kg⁻¹ for big bluestem, 10 to 39 g kg⁻¹ for indiangrass, and 15 to 59 g kg⁻¹ for switchgrass (Fig. 5.2e). Similar to 2006, the maximum lignin concentration for grasses obtained between the 9th and 10th harvest in 2007 (Fig. 5.3e). The lignin concentrations increased from 14 to 30

g kg⁻¹ for eastern gamagrass, 13 to 53 g kg⁻¹ for big bluestem, 9 to 37 g kg⁻¹ for indiagrass, and 15 to 58 g kg⁻¹ for switchgrass. Eastern gamagrass supplied with N-fertilizer did not increase lignin concentration while all other species did in 2006 (Fig. 5.4c). The N rate x harvest date interaction indicated that the lignin concentration in grasses receiving N fertilization increased relatively more between mid and late season (Fig. 5.6d and 5.7b).

Ash

In both years, significant effects were detected for the species x harvest date interaction for ash concentration indicating that the curvilinear decline in the ash concentration with delayed harvest was less pronounced in eastern gamagrass than the other species and tended even to increase slightly at the end of season (Fig. 5.2f and 5.3f). In 2006, the lowest ash concentrations were recorded at the 5th harvest (DOY 192) for eastern gamagrass, and at the 9th harvest (DOY 255) for big bluestem, indiagrass, and switchgrass. The ash values decreased with time from 105 to 61 g kg⁻¹ for eastern gamagrass, 120 to 52 g kg⁻¹ for big bluestem, 135 to 79 g kg⁻¹ for indiagrass, and 100 to 55 g kg⁻¹ for switchgrass (Fig. 5.2f). In 2007, the ash concentration of eastern gamagrass, big bluestem, indiagrass, and switchgrass decreased curvilinearly to the lowest level at the 5th harvest for eastern gamagrass, and at the 9th harvest (DOY 197) for big bluestem, indiagrass, and switchgrass in 2007 (Fig. 5.3f), consistent with changes in 2006. The ash concentration decreased from 93 to 62 g kg⁻¹ for eastern gamagrass, 111 to 43 g kg⁻¹ for big bluestem, 134 to 76 g kg⁻¹ for indiagrass, and 91 to 56 g kg⁻¹ for switchgrass. The species x N rate interaction indicated that when N fertilizer was given, ash concentrations in big bluestem and switchgrass did not decline in 2006 (Fig. 5.4d) and only slightly declined in 2007 (Fig. 5.5c) as that in eastern

gamagrass and indiangrass greatly decreased. The N rate x harvest date interaction indicated that ash concentration in these grasses was most responsive to N fertilization during mid season with slight decreases in ash concentration for warm-season grasses with N fertilization (Fig. 5.6e).

Discussion

The desirable composition of biomass for biofuels is dependent on the conversion processes used. In contrast to forage production, maximized concentrations of cell wall constituents (cellulose, hemicellulose, and lignin) and minimized level of N and ash in biomass feedstocks are goals for chemical and biofuel production (Trebbi, 1993).

Delaying harvest of warm-season grasses to later maturity stages commonly increases these cell wall constituents (Jung and Vogel, 1992; Madakadze et al., 1999; Mitchell et al., 2001; Adler et al., 2006; Mulkey et al., 2006) and decreases N and ash content in grasses (Madakadze et al., 1999). These findings were consistent with the results in this study. The concentrations of lignocellulose (especially, cellulose and lignin) increased, while the concentrations of ash and N declined, when harvesting all four species was delayed until fall. Changes in these chemical constituents as warm-season grasses mature are likely attributable to changes in their morphology, especially the leaf: stem ratio (Twidwell et al., 1988; Mulkey et al., 2006). Madakadze et al. (1999) reported for switchgrass that the proportion of stems relative to leaves for switchgrass increased with maturity.

Griffin and Jung (1983) found that fiber contents increased with respect to maturity due to greater concentrations of NDF in grass stems compared to leaves. In general, stems of most grasses contain greater concentrations of fiber constituents than do leaves (Jung and

Vogel, 1992). However, this study has demonstrated that changes in hemicellulose concentration with maturity for all four species were not likely due to similarity in hemicellulose concentration among plant parts (Twidwell et al., 1988). For thermochemical conversion systems, higher lignin concentration is desirable by contributing more energy to the conversion process (Hayn et al., 1993). But it has a detrimental effect on biochemical conversion process by reducing the availability of cellulose and hemicellulose resulting in reduced ethanol yield (Sun and Cheng, 2002).

The decrease in N concentration of warm-season grasses with advanced maturity is consistent with previous studies. Madakadze et al. (1999) showed that the concentrations of N decreased curvilinearly from approximately 25 to 6 g kg⁻¹. In addition to a lower proportion of leaves with relatively more N contents, N decrease with advanced maturity is likely attributed to N translocation from aboveground biomass to belowground organs between the time of anthesis and a killing frost (Vogel et al., 2002). McKendrick et al. (1975) demonstrated that when harvest was delayed to late July and early August, the amount of tiller N (aboveground) in big bluestem and indiangrass decreased while N increased in rhizomes (belowground). Plants use the translocated N for producing new growth the following spring. Also, this indicates that N-fertilizer requirements could be reduced in warm-season grasses harvested for biomass at the end of season.

The occurrence of ash has significant implications for an herbaceous feedstock. Agblevor et al. (1992) noted that high level of ash negatively affects thermochemical processing by reducing hydrocarbon yield. Components of ash negatively interfere with the conversion processes when combining with sulfur to produce corrosive sulfates. These components result in slag deposits on biomass combustion, reducing efficiency and

increasing maintenance costs (McLaughlin et al., 1996). Decreases in ash concentration for all four grasses with advanced maturity in this study is consistent to a previous study by Mulkey et al. (2006). They found that ash concentration of switchgrass decreased from the time of anthesis to the killing-frost harvest, presumably because the greater proportion of grass stems at late season contains less silica, a major component of ash, than do leaves.

Moreover, the reductions in nitrogen and ash concentration, and the increases in cellulose, carbon, and lignin concentration for most grasses are respective to their maximum and minimum at no N fertilization, respectively. Mulkey et al. (2006) reported that ash concentration for switchgrass tended to decrease with increasing N. Similarly, our current study demonstrated that decreases in ash and N concentration, and increases in lignocellulose concentration for grasses receiving greater rates of N were varied with species and harvest dates. This may be closely related to changes in leaf:stem ratio for different species and harvest dates, due to N inputs. Nitrogen fertilization increased tiller density and stem development for switchgrass (Bredja et al., 1994). Also, N fertilization increases grass leaf and stem weights and the greater effect on stem weight results in lower leaf:stem mass ratios (Perry et al., 1979; George et al, 1989).

Regardless of yield potential, these changes in chemical composition during later maturity stages could imply the conversion efficiency for different grasses. With higher lignin and lower ash content, switchgrass and big bluestem would be able to provide greater potential in thermochemical conversion than eastern gamagrass and indiagrass. Conversely, the latter two may be greater in biochemically conversion efficiency than the former two due to a detrimental effect of relatively higher ash contents on thermochemical processes.

Conclusions

Based on our results, we conclude that delaying harvest until fall could maximize the concentration of lignocellulose and minimize the N and ash concentration in native warm-season grasses grown as herbaceous energy crop in Iowa. In contrast, grasses grown as forage crops have low quality when the harvest is late (Sanderson et al., 1999). Application of N to warm-season grasses could increase biomass quality depending on species and harvest date. In practice, the decisions regarding harvest dates and N rates are made as a compromise among biomass quality, yield, and production costs in order to achieve the optimal harvest date and N rate for biomass energy production from warm-season grasses. However, biomass harvested at that end of season provides high lignin concentrations which can negatively impact both the efficiency of glucose recovery in lignocellulosic ethanol production (Dien et al., 2006) and the digestibility of forages by ruminants (Jung and Deetz, 1993), but would be a positive attribute for thermochemical processing (Wyman, 1994).

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Table 5.1 Cellulose, hemicellulose, carbon, nitrogen, lignin, and ash content *F*-values and significances in response to four warm-season grass species, three nitrogen rates and ten harvest dates during the growing seasons of 2006 and 2007 at Ames, IA.

Effect	Cellulose		Hemicellulose		Carbon		Nitrogen		Lignin		Ash	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
S [†]	106.9**	118.0**	135.2**	141.7**	192.1**	106.9**	0.5	8.4**	791.6**	647.7**	141.0**	124.7**
N [‡]	3.3	18.7**	0.6	0.2	33.0**	44.3**	279.1**	205.8**	48.2**	92.8**	12.7**	46.4**
SxN	5.9**	7.6**	3.7	1.9	2.4	2.6*	2.1	0.9	4.0**	2.8	3.2**	4.1**
H [§]	1401.5**	704.2**	28.8**	54.7**	290.7**	499.7**	1450.0**	929.1**	1058.1**	1454.6**	367.3**	232.3**
SxH	45.5**	30.8**	24.4**	11.0**	12.9**	14.4**	10.5**	14.3**	53.8**	81.8**	10.4**	22.1**
NxH	4.8**	3.3	1.1	1.1	2.0**	1.7	8.0**	15.3**	5.3**	10.8**	1.9*	1.2
SxNxH	0.9	1.4	1.2	1.2	1.6	1.4	2.1**	1.3	1.2	1.3	1.3	1.4

[†] S, species effect.
[‡] N, nitrogen-rate effect.
[§] H, harvest-date effect.
* significant at $P \leq 0.05$.
** significant at ≤ 0.01

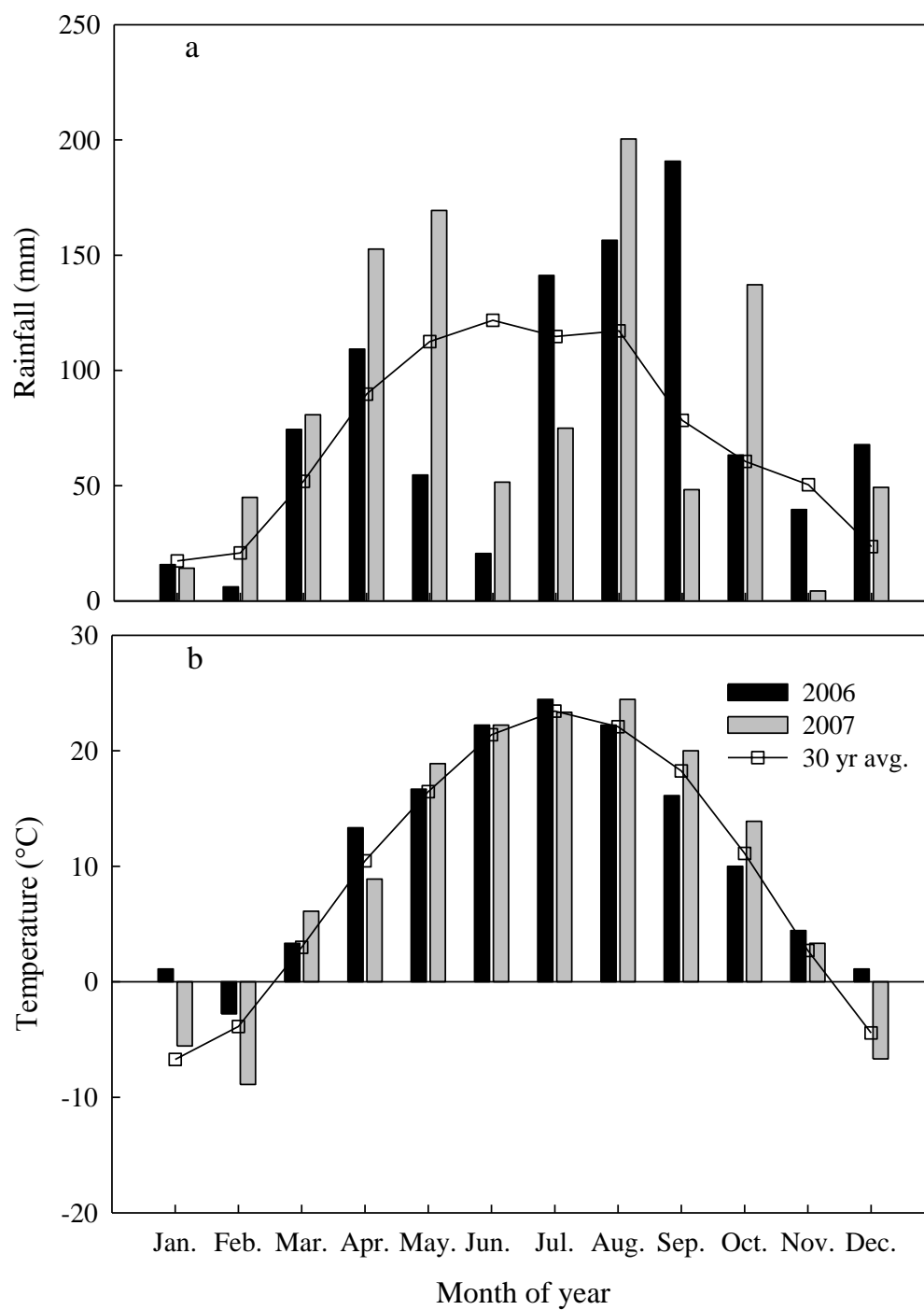


Figure 5.1. Mean total rainfall (a) and air temperature (b) for 2006, 2007, and 30-year average at Ames, IA.

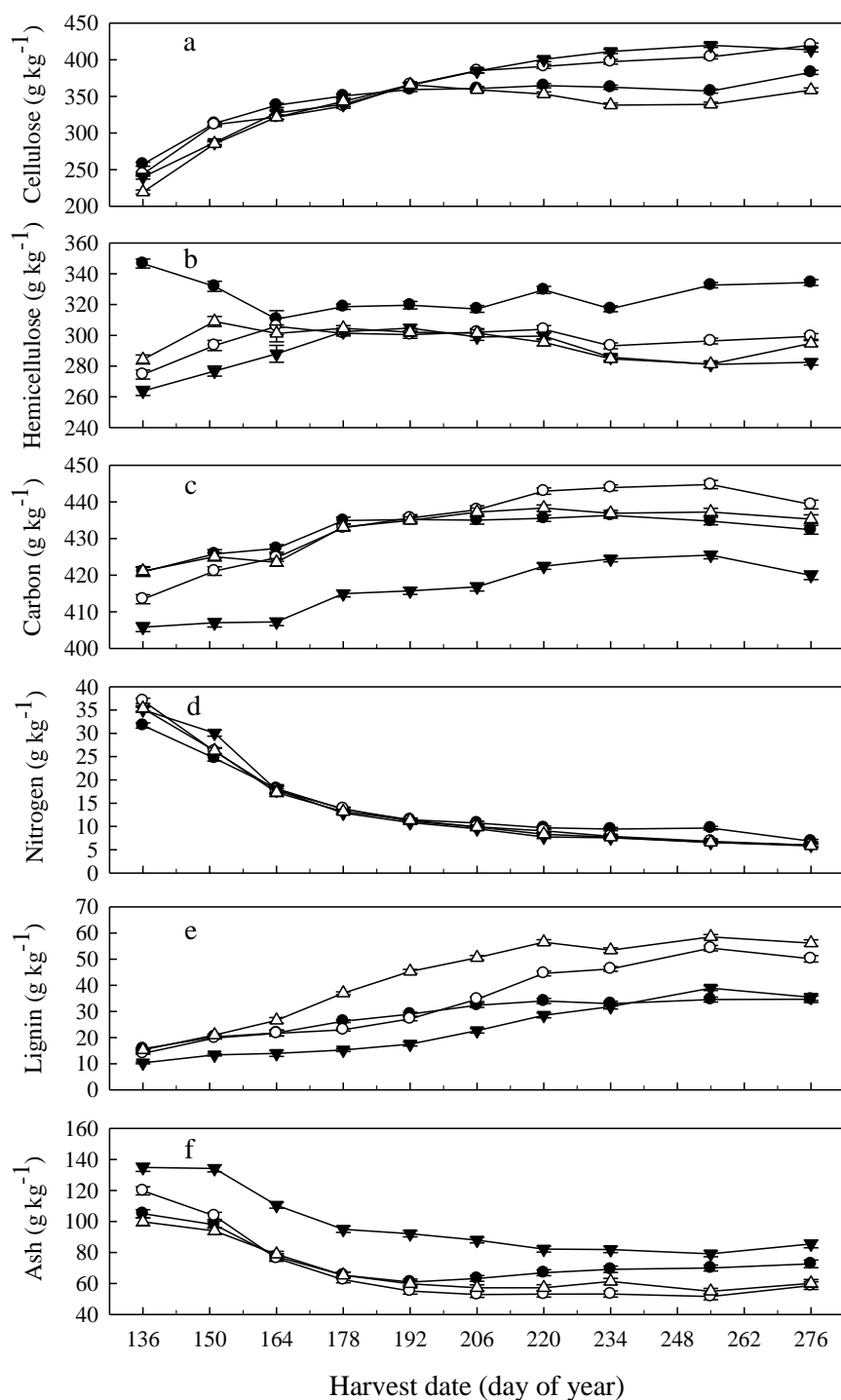


Figure 5.2. Mean biomass cellulose (a), hemicellulose (b), carbon (c), nitrogen (d), lignin (e), and ash (f) concentration as influenced by eastern gamagrass, big bluestem, indiagrass, and switchgrass and ten harvest dates. Data are averaged over four replications and three nitrogen rates, in 2006, at Ames, IA. Standard error bars are partially covered by graph symbols.

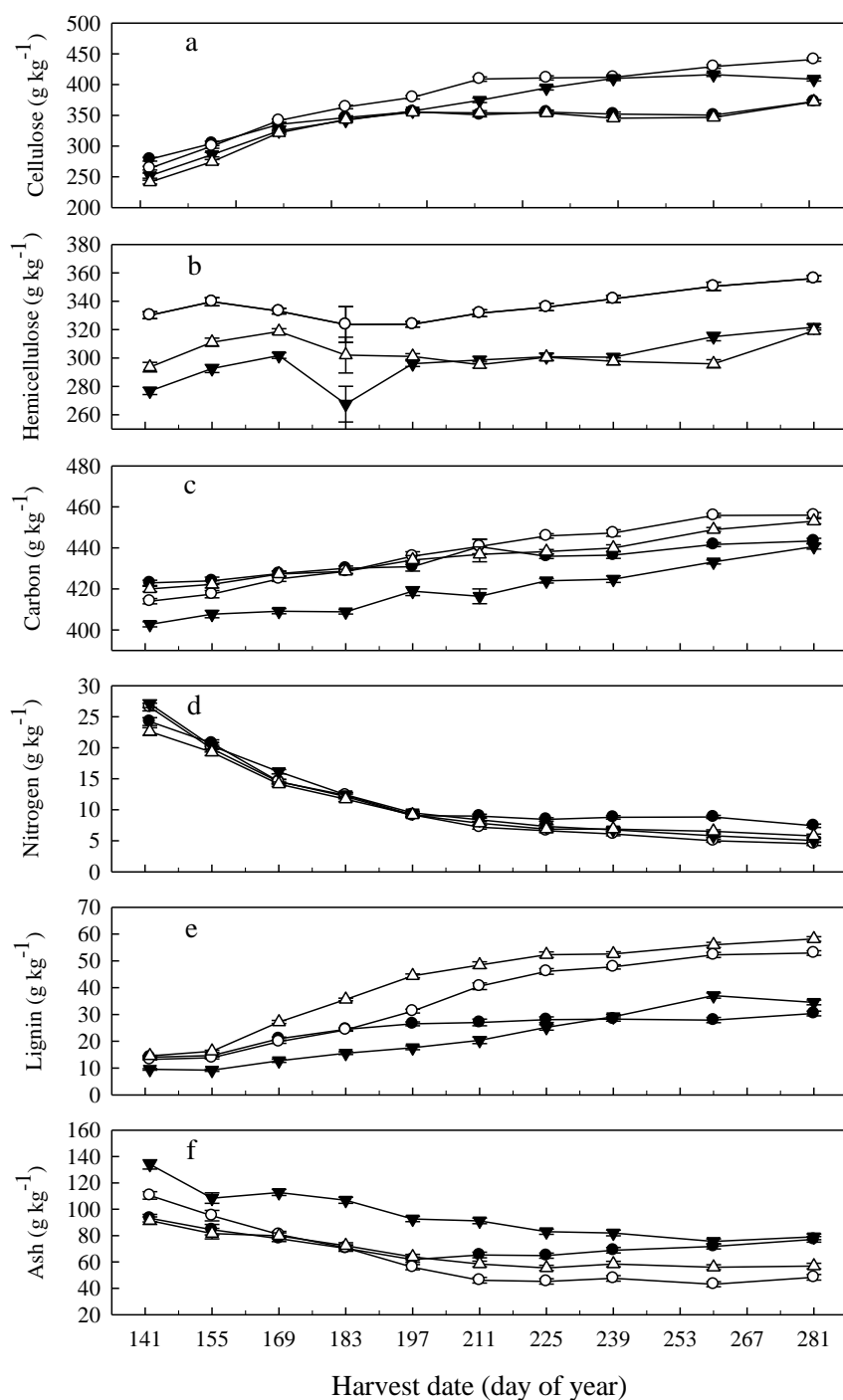


Figure 5.3. Mean biomass cellulose (a), hemicellulose (b), carbon (c), nitrogen (d), lignin (e), and ash (f) concentration as influenced by eastern gamagrass, big bluestem, indiagrass, and switchgrass and ten harvest dates. Data are averaged over four replications and three nitrogen rates, in 2007, at Ames, IA. Standard error bars are partially covered by graph symbols.

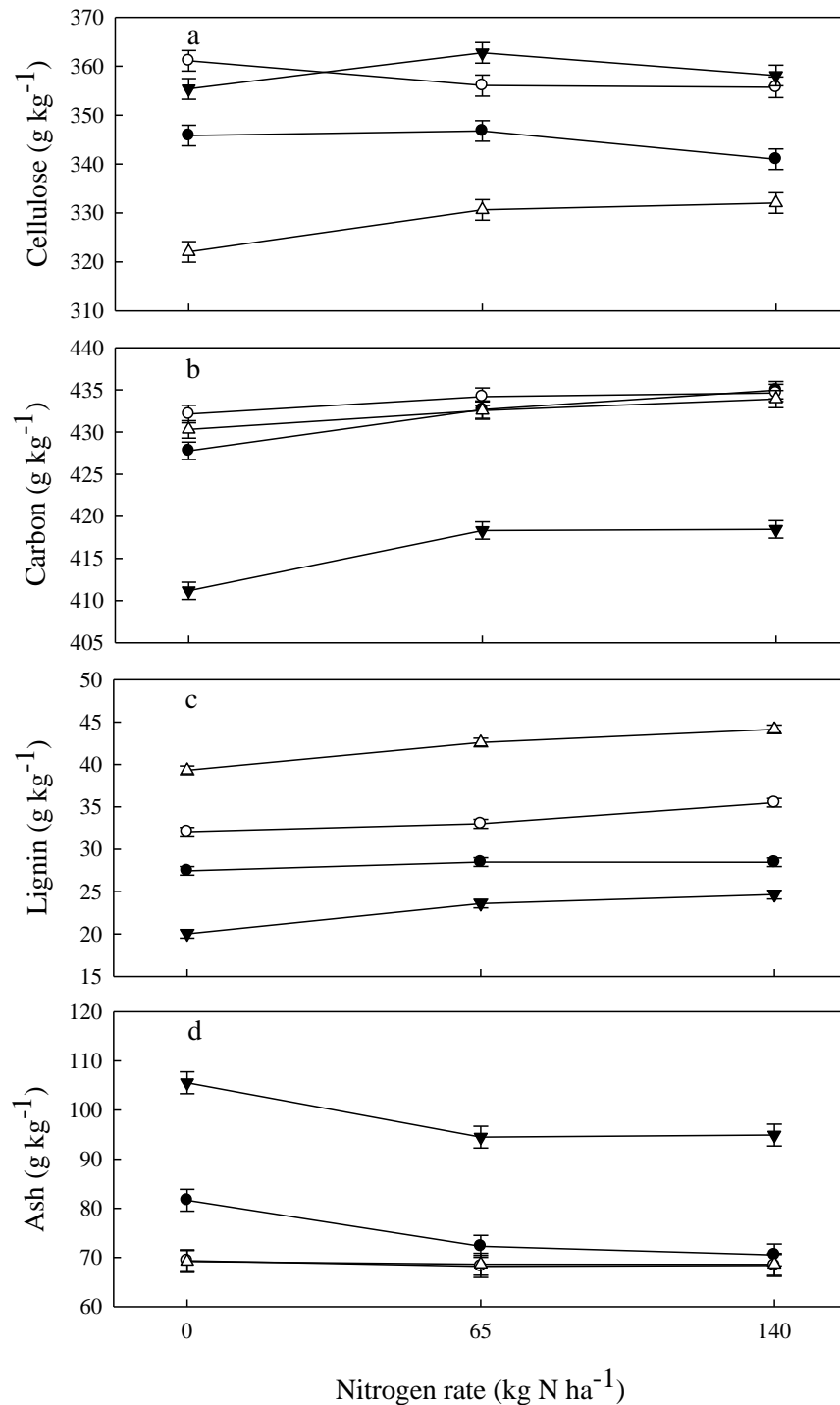


Figure 5.4. Mean biomass cellulose (a), carbon (b), lignin (c), and ash (d) concentration as influenced by eastern gamagrass, big bluestem, indiangrass, and switchgrass and three nitrogen rates at 0, 65, and 140 kg N ha⁻¹. Data are averaged over four replications and ten harvest dates in 2006, at Ames, IA. Standard error bars are partially covered by graph symbols.

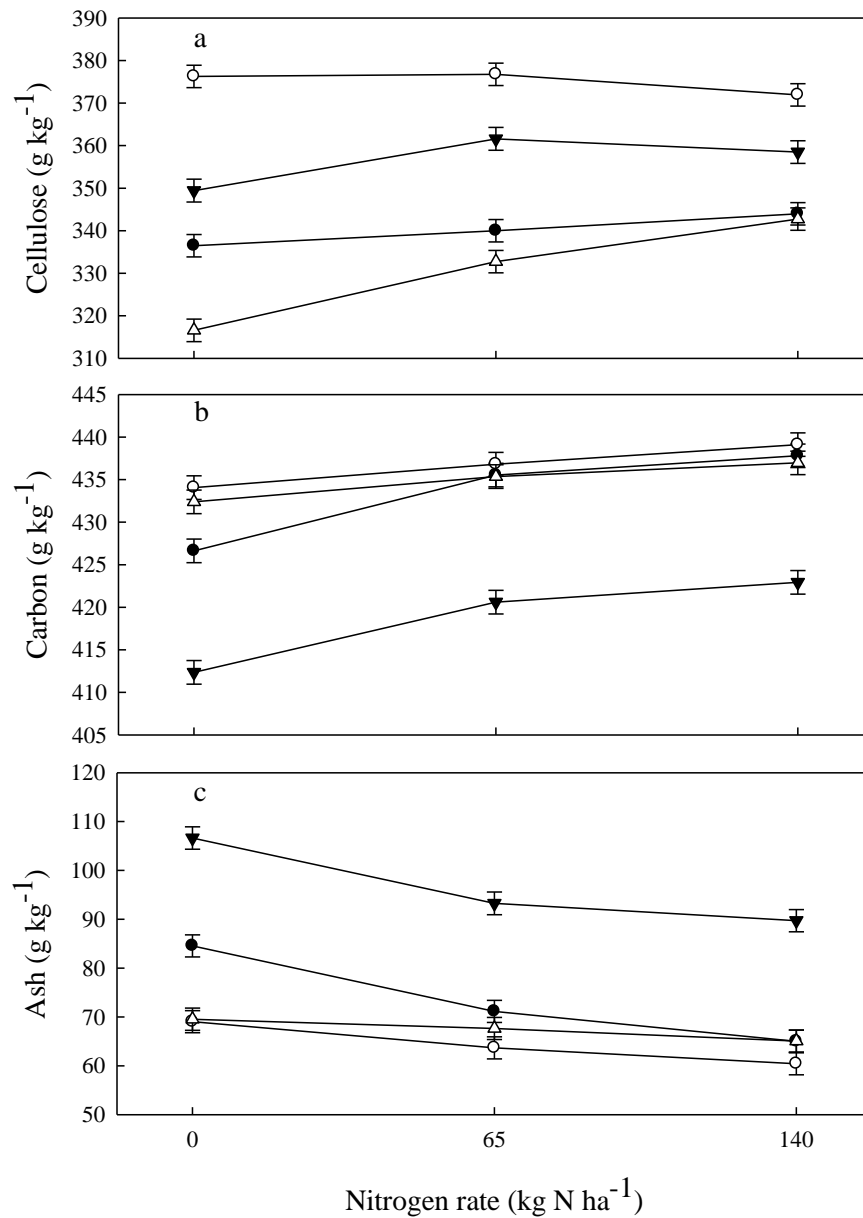


Figure 5.5. Mean biomass cellulose (a), carbon (b), and ash (c) means as influenced by eastern gamagrass, big bluestem, indiagrass, and switchgrass and three nitrogen rates at 0, 65, and 140 kg N ha⁻¹. Data are averaged over four replications and ten harvest dates in a) 2007, at Ames, IA. Standard error bars are partially covered by graph symbols.

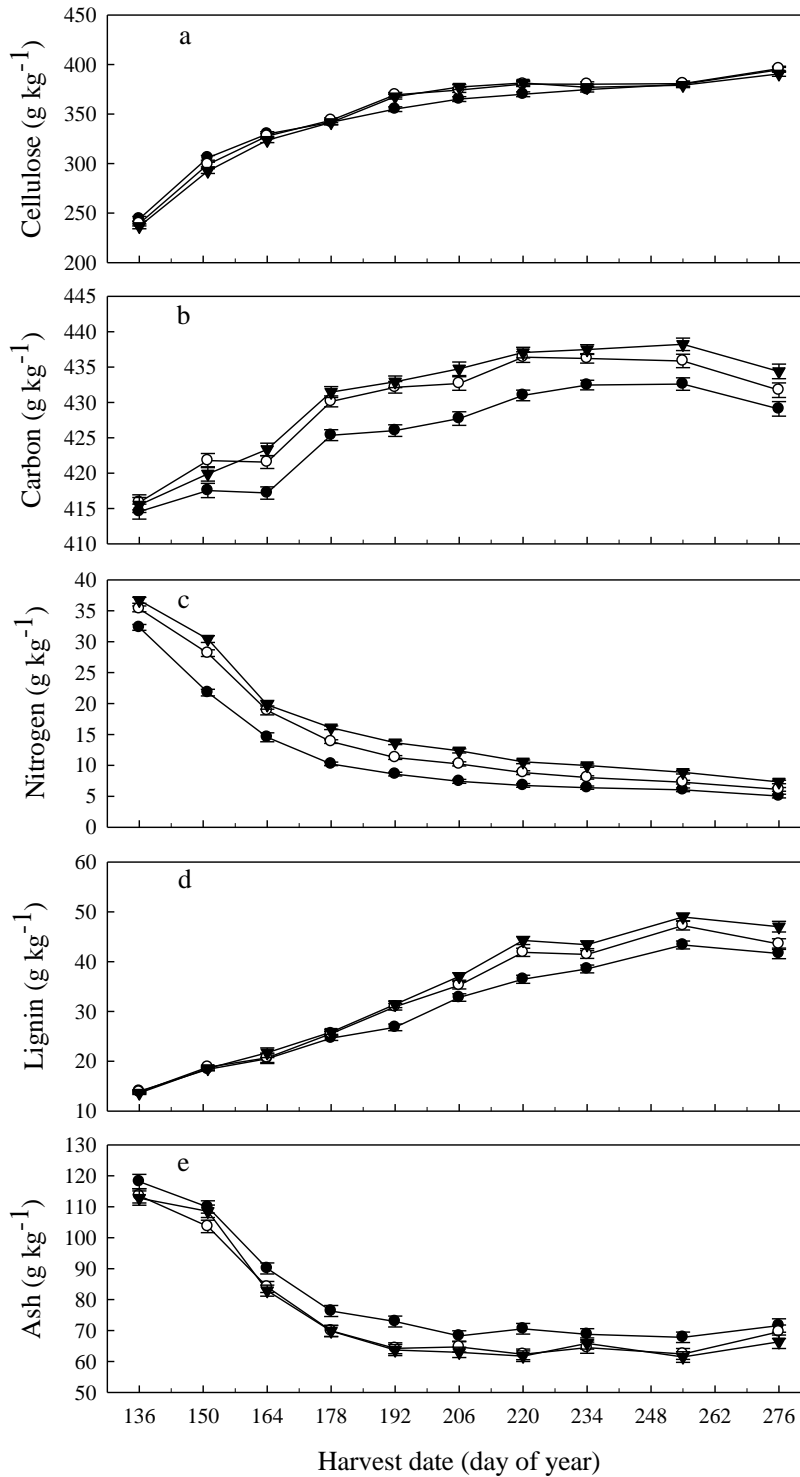


Figure 5.6. Mean biomass cellulose (a), carbon (b), nitrogen (c), lignin (d) and ash (e) concentration as influenced by three nitrogen rates at 0 (0 N), 65 (65 N), and 140 (140 N) kg N ha⁻¹ and ten harvest dates. Data are averaged over four replications and four species, in 2006, at Ames, IA. Standard error bars are partially covered by graph symbols.

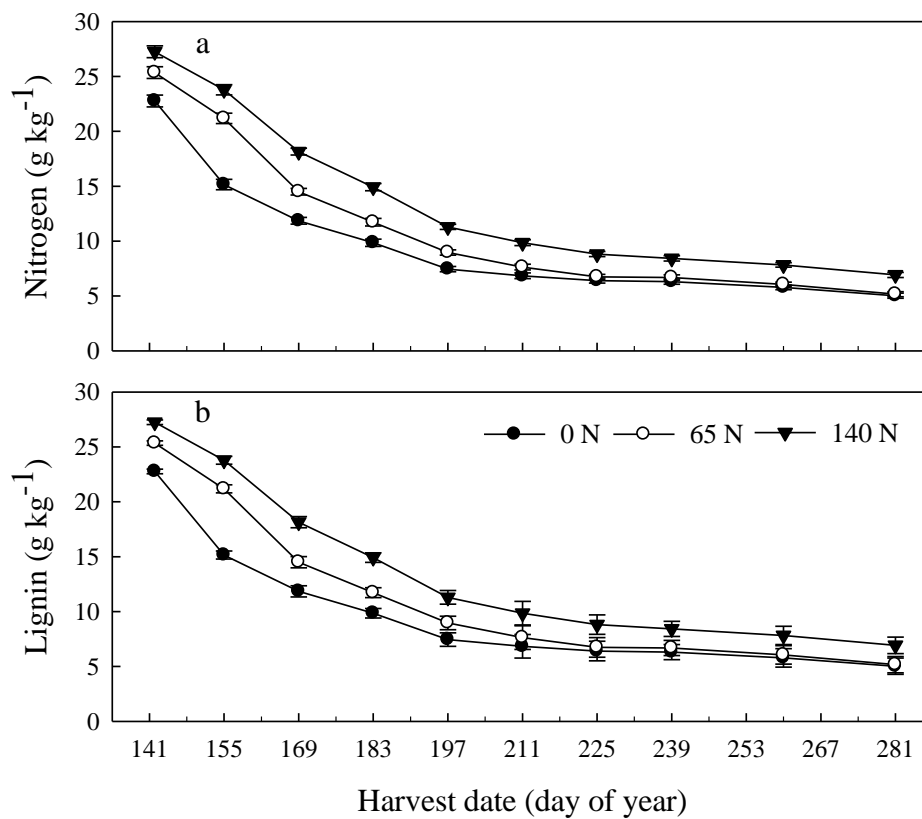


Figure 5.7. Mean biomass nitrogen (a) and lignin (b) as influenced by three nitrogen rates at 0 (0 N), 65 (65 N), and 140 (140 N) kg N ha⁻¹ and ten harvest dates. Data were averaged over four replications and four species, in 2007, at Ames, IA. Standard error bars are partially covered by graph symbols.

CHAPTER 6. GENERAL CONCLUSIONS

Native warm-season grasses are versatile biomass crops in the warm months of the Midwest, when cool-season grasses are relatively unproductive. They are more efficient than cool-season grasses in the use of water, and N and grow well in marginal soils with low fertility. These unique characteristics provide excellent potential for alternative uses of warm-season grasses including bioenergy cropping, soil conservation, waste nutrient management, water quality protection, and wildlife habitat (Sanderson et al., 2004).

For traditional forage use, warm-season grasses are highly productive in the summer months and are suited for a complementary role with cool-season forages in full-season forage systems of the Midwest. For renewable energy sources, warm-season grasses can be used to produce biofuels, an alternative energy source in the future for fossil fuel which is becoming less available. Farmers may integrate forage and biomass cropping for flexibility and diversity in their farming systems. For dual-purpose crops (forage or biomass), the production of warm-season grasses would provide farmers some protection against the market failure of a single purpose. The goal for the production of warm-season grasses as biomass differs from that when used as forage. The goal for biomass production is to maximize the concentration of lignocellulose in the feedstock and minimize the concentration of N and minerals. Based on the difference in desired quality traits between forage and biomass, the results from our study indicate that there is the possibility of using warm-season grasses as a dual-purpose crop. The early growth of these warm-season grasses, which has high forage quality, can supply nutrients for forage-livestock systems. Later growth can be allowed to mature and be harvested as a biomass crop.

An array of native warm-season grasses including eastern gamagrass, big bluestem, indiangrass, and switchgrass are well adaptable to Iowa. They have mainly been used for forage. In most of the recent research, only switchgrass has been evaluated as an herbaceous energy crop. Since the other three grass species are also productive in this region and have been evaluated for forage qualities (Nelson et al., 1994), they could reduce risks from relying on only a single species and extend the range and profitability of biofuel production systems. To optimize their productivity and quality for both forage and biomass at a regional level, management practices for different species have to be determined.

Harvest date greatly influences forage quality of native warm-season grasses. Generally, forage quality decreases with the maturation of grasses. The later the grasses are harvested, the lower the forage quality. Harvesting them early provides relatively high IVDMD value and crude protein, and low concentration of NDF, but forage yields will be relatively low. Conversely, allowing grasses to reach maturity decreases IVDMD value and crude protein and increases the concentration of NDF, but forage yields will be relatively higher until senescence.

Grass species differ in how forage quality varies with harvest time. Evident differences in forage quality exist among grass species during early season and late season. During late spring, eastern gamagrass had the greatest NDF followed by big bluestem and switchgrass, while indiangrass had the lowest NDF. During fall, big bluestem had the greatest NDF followed by indiangrass and eastern gamagrass, whereas switchgrass had the lowest NDF. The IVDMD values among the four species were quite similar during late spring. During summer, the IVDMD values in indiangrass were the highest among the four species and similar to eastern gamagrass during fall. All four species were similar in the

concentration of crude protein between spring and summer, but eastern gamagrass had a greater crude protein concentration than other species during fall.

To compromise between forage yield, quality, and persistence for optimum harvest time, delaying harvest to increase forage yields for all warm-season grasses should not be later than mid of July, otherwise the concentration of NDF is greatly increased leading to lower voluntary forage intake, and lower crude protein concentration than the lower limit for livestock requirements (<60 g crude protein kg^{-1}). In addition, harvesting warm-season grasses during these periods of time is unlikely to detrimentally affect plant persistence because reserve carbohydrates are able to be replenished by late summer and there will be enough active meristems remaining from cutting (Anderson et al., 1989).

Based on the results of this study, N fertilization is another important method which can improve forage quality for warm-season grasses. Although some variation in response of NDF and IVDMD concentration to N application rate among different species makes drawing conclusions difficult, N fertilization can increase forage quality in warm-season grasses through raised crude protein levels. Therefore, forage quality of warm-season grasses can be improved by choosing the optimum harvest time and N fertilization rate.

Variation in the environmental variables and adapted species make the prediction of forage quality with DOY difficult. The prediction of NDF, IVDMD, and CP values using regression equations from a quadratic MSC model for warm-season grasses which have a determinate growth pattern (i.e. big bluestem, indiangrass, and switchgrass) were acceptable. However, composition of eastern gamagrass with an indeterminate growth habit could not be predicted by this model. Incorporating DOY or GDD models with environmental variables may provide more reasonable forage quality predictions for this species. Predicted forage

quality parameters of these grasses suggests that harvest of big bluestem and switchgrass should occur before the anthesis stage (MSC 3.7) and indiangrass before the onset of the 6th node at stem elongation stage (MSC 2.6) can provide relatively higher forage quality.

The goal for biomass production differs from that for forage production. Management of warm-season perennial grasses for forage often requires a compromise between herbage yield and forage quality while that for biomass would emphasize yield and persistence. High N and mineral concentrations in grasses are desirable for livestock production, but feedstocks with low concentrations of these elements (K, Ca, Cl, and ash) are more desirable for combustion systems (Agblevor et al., 1992; Miles et al., 1996).

For animal productivity, cell wall content in forages often negatively affects dry matter digestibility. Animals consuming grass with high total fiber may not meet their requirements for energy and other nutrients (Collins and Fritz, 2003). However, high concentrations of cellulose and other structural polysaccharides in grass cell walls are required for maximizing ethanol yield via biochemical conversion (Sun and Cheng, 2002).

Native warm-season grasses require different management practices from forage use to optimize different attributes of quality for biomass feedstock use. The results of this study have shown that the quality of all species grown for biomass feedstocks can be improved when crops are harvested later in the season. During these periods of time, there are differences in biomass quality among species. Big bluestem and indiangrass had greater cellulose concentration than eastern gamagrass and switchgrass. Eastern gamagrass was greater in hemicellulose concentration than other species. Indiangrass was lower in C concentration than other species. Switchgrass and big bluestem had greater lignin and lower ash concentration than eastern gamagrass and indiangrass. Our results suggest that big

bluestem and switchgrass may be more suited for different energy or chemical end products by thermochemical conversion compared with eastern gamagrass and indiangrass. However, the latter two species may be more desirable for biochemical conversion for ethanol production than big bluestem and switchgrass. Nitrogen application at optimum rates could improve biomass feedstock quality depending on species and harvest time. This study has suggested that N fertilization at 65 kg ha^{-1} resulted in higher cellulose for indiangrass and switchgrass, while lower ash concentration for eastern gamagrass and indiangrass. The higher N application rate of 140 kg ha^{-1} may improve biomass feedstock quality depending on species. In addition, N fertilization provided higher lignin concentration for warm-season grasses, which does not have a detrimental effect on thermochemical product yield but may reduce ethanol yield by reducing the availability of cellulose and hemicellulose in biochemical conversion (Sun and Cheng, 2002).

High productivity of warm-season grasses with relatively low water and nutrients requirements is a primary attribute desirable for bioenergy cropping. Biomass yield of native warm-season grasses increases differently among species with maturity before declining during senescence. These results have indicated that there is a difference in optimum harvest time for biomass yield among warm-season grasses. Eastern gamagrass with an indeterminate growth habit had the maximum yield at DOY 192-260 with the highest MSC ranging from 1.4 to 2.2 (mid-vegetative stage to second node palpable), when the largest proportions of reproductive tillers within the sward were present. In contrast, the maximum yield of big bluestem, indiangrass, and switchgrass with a determinate growth habit were found between DOY 211 and 260 (MSC 2.5-2.7; fifth-seventh node palpable), 234 and 281 (MSC 3.0-3.2; boot stage-spike fully emerged), and 206-281 (MSC 2.6; sixth node

palpable), respectively. At these harvest dates for these three species, a large proportion of reproductive tillers were present, but seeds were not yet visually observed. Thus, optimum time to harvest can be determined by combining these results with changes in biomass quality with maturity. In terms of a biomass supply strategy, this study indicates that eastern gamagrass could be used as a biomass feedstock in early summer, while big bluestem and switchgrass could supply biomass between mid-and late summer and indiangrass in the early fall. Although there were variations in response of biomass quality to N application rates, biomass yield of warm-season grasses could be increased by N application due to increased stem development and tiller density. However, the application of N at a higher rate increased the concentration of N in biomass feedstock, leading to reduced biomass quality. However, this study has indicated that when harvest was delayed to late summer, the concentration of N in grasses with N fertilization declined to levels similar to grasses without N fertilization.

Thus, drawing on these results it can be concluded that the four warm-season grasses in this study could serve dual purposes as forage when harvested at early maturity, and as biomass feedstocks when harvested during late maturity. Optimal N fertilization could provide economically feasible alternative uses of these warm-season grasses by improving both total yields of forage/biomass and forage quality, without adversely affecting biomass quality.

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