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Response of Three Cool-Season Grass Species to Nitrogen Rate and Harvest Interval in North Central Mississippi

Jonathan Daniel Richwine

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By

Jonathan Daniel Richwine

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By

Jonathan Daniel Richwine

Approved:

Brian S. Baldwin
(Major Professor)

Rocky W. Lemus
(Committee Member)

J. Brett Rushing
(Committee Member)

Stephanie H. Ward
(Committee Member)

Michael S. Cox
(Graduate Coordinator)

J. Mike Phillips
(Department Head)

George M. Hopper
Dean
College of Agriculture and Life Sciences

Name: Jonathan Daniel Richwine

Date of Degree: May 6, 2016

Institution: Mississippi State University

Major Field: Plant and Soil Sciences

Major Professor: Brian S. Baldwin

Title of Study: Response of three cool-season grass species to nitrogen rate and harvest interval in North Central Mississippi

Pages in Study 150

Candidate for Degree of Master of Science

Nitrogen (N) use continues to be an important aspect of forage production. Experimentation was conducted to understand the combined effects of N application and harvest regime on three cool-season grasses: orchardgrass, southeastern wildrye, and tall fescue. Tests were established at Starkville and Brooksville, MS, in fall 2013 and 2014, respectively. Plots were fertilized with 0, 134, 202, or 269 kg N ha^{-1} yr^{-1} and harvested one, two, three, or four times during the 112-day season. Variables measured included: cumulative dry matter yield, relative forage quality, crude protein percentage, normalized difference vegetation index, nitrogen use efficiency, and persistence. Persistence was only recorded for southeastern wildrye. Species, N application, and harvests were significant in combination with one another (either two or all three) for all variables except persistence. Only harvest frequency was significant for persistence. Further research should be conducted to evaluate cutting height when incorporating N and multiple harvest events.

DEDICATION

First and foremost I would like to dedicate this work to my Lord and Savior Jesus Christ. His grace, mercy, and provisions allowed this research to be accomplished. Next I would like to dedicate my thesis to those closest to me: Aundrea (my fiancée or wife depending on how soon this document is published), Frances and Jim (my mom and dad), Kadie, Jeremy, and Maddie Claire (my sister, brother-in-law, and niece) as well as family and close friends. All of you have loved, encouraged, and supported me through this endeavor to which I cannot THANK YOU enough. Last but certainly not least I would like to dedicate this document to those who will read and/or use it to aid in their research. If I could give you any piece of advice it would be to contemplate how you are going run the statistics for your experiment prior to breaking ground. If you are confident in how to analyze your data then go ahead and conquer your study. Otherwise get over yourself and just use a randomized complete block design. Godspeed! Proverbs 16:9

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CHAPTER I

INTRODUCTION

The importance of grass as a plant for mankind cannot be over emphasized. The significance of vegetation as food or feedstuff, specifically grass, can be traced back more than 2400 years ago. In the book of Genesis in The King James Bible, it says: "And God said, Let the earth bring forth grass, the herb yielding seed, and the fruit tree yielding fruit after his kind, whose seed is in itself, upon the earth: and it was so (Gen. 1:11, KJV)." According to Webster's New World Dictionary, the word "grass" is defined as "a plant with long, narrow leaves, jointed stems, and seed-like fruit, as wheat [*Triticum aestivum* L.] or rye [*Secale cereal* L.]." This broad definition encompasses all types of grasses. They range from: common turf and forage grasses, such as bermudagrass [*Cynodon dactylon* (L.) Pers] and bahiagrass [*Paspalum notatum* Flueggé] to agricultural row crop production grasses such as corn [*Zea mays* L.] and sorghum [*Sorghum bicolor* (L.) Moench], as well as the aforementioned grains.

Grass is agriculturally important as surface cover to protect the soil from erosion, and as the major component of an ungulate's diet. A special type of ungulate, ruminants, have significant stomach characteristics that enable them to process vegetation, predominantly grasses, into usable energy.

With over 240,000 hectares devoted to hay production in Mississippi to feed such animals, responsible and profitable agricultural management decisions are vital for forage production. With the cost of grass production continually increasing due to factors such as rising fertilizer costs, more economically and sustainable practices for managing production needs to be reached. Nitrogen (N) is the element most often limited and most actively used by grass to sustain optimum yields. Nitrogen fertilizer is also the most expensive (on an elemental basis) and largest carbon input in a forage production system. With the price of N fertilizer continually on the rise (until recently), efficient N management strategies must be determined for each forage crop to optimize production. In any forage-based production system, it is imperative to produce feedstuffs efficiently with fewer input costs while maintaining the quality of feed.

In the Southeast, warm-season grasses (WSG) are the backbone of the forage production system. These grasses produce most of their growth in the summer months. When the atmospheric temperature declines and day length shortens, WSG become dormant, forcing producers to choose between several options in providing feed for the cooler parts of the year. One option is the incorporation of cool-season grasses (CSG) into the livestock's grazing regime which will help fill the forage gaps between seasons and aid in cutting feeding and supplementation costs. The longer animals can graze pastures, the less feedstuffs have to be utilized to sustain that animal until new pasture growth occurs. Cool-season grasses are generally higher in nutritional quality than WSG, which is another benefit of having them in a forage system.

Perennial cool-season grasses are not widely utilized across the southern United States. Drought potential and high temperatures associated with the region affect stand persistence. Southeastern wildrye [*Elymus glabriflorus* (Vasey ex L.H. Dewey) Scribn. & C.R. Ball] is a newly researched CSG native to the southeastern U.S. Preliminary research suggests there is potential for this species to be used as a forage crop. However, there is very little information available on the production and management of southeastern wildrye as a forage crop.

Other perennial grasses have been used to fill the role for cool-season production, but were introduced to North America. Tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.] and orchardgrass [*Dactylis glomerata* L.] are two commonly used CSG in the northern U.S. and in the Transition Zone between cool-temperate and subtropical zones but are relatively new to the Deep South (less than 100 years of traditional ag use). Through centuries of natural and applied selection, these two European grasses have proven to be strong forage species and are the basis for animal agriculture systems for the northern U.S. Therefore, other CSG have been overlooked as being suitable for filling the same role, and in the South tall fescue is the only reliable perennial CSG.

The research presented in this document is designed to compare basic agronomic production management practices (i.e. harvest frequency and N application) of southeastern wildrye, tall fescue, and orchardgrass in north central Mississippi. In doing so, specific questions can be answered: How does southeastern wildrye compare with tall fescue and orchardgrass with regard to N use efficiency? What is the optimal harvest regime that balances overall quality with N application for all three species? What effects will the various harvest regimes have on cumulative dry matter yield of all three CSG and persistence in southeastern wildrye?

CHAPTER II

OVERVIEW OF COOL-SEASON GRASSES

Cool-season grasses (CSG) are those members of the Poaceae family whose majority of growth occurs during the cooler daytime temperatures (18-27°C) of the year (Moser and Hoveland, 1996). The majority of the growth of these grasses occurs during the spring months (Riesterer et al., 2000) in Mississippi. These grasses function as the main feed source for grazing, ruminant animals (those mammals of the suborder Ruminantia that have a stomach consisting of four compartments) in areas where these temperatures dominate (Moser and Hoveland, 1996). Dairy farms in the northeastern United States rely heavily on these grasses as the basis for their forage production systems (Hall et al., 2003). Perennial CSGs are routinely utilized for hay in Northern Plains and Great Basin regions as well (Gillen and Berg, 2005). Cool-season grasses are grown not only for pasture and hay but also to aid in soil conservation and as wildlife habitat (Moser and Hoveland, 1996).

The majority of CSG are found north of 30° N and south of 30° S latitude (Moser and Hoveland, 1996). This is where the most favorable temperatures for growing CSG occur. The optimal temperature for growth of CSG is from 20 to 25°C. When temperatures drop below 10°C and transcend 25°C, cool-season plant growth slows drastically. At 30 to 35°C growth is greatly decreased and possibly stops (Moser and Hoveland, 1996). When temperatures rise above 35°C and heat and drought stresses accumulate, CSG begin to go dormant (Moser and Hoveland, 1996; Redfearn and Nelson, 2003). This period of time is known as the "summer slump" (Riesterer et al., 2000).

A major goal of many livestock producers is to possess forages that contain CSG that prove to be persistent and productive (Hopkins and Alison, 2006). Many CSG used in forage production systems in the northern U.S. are perennial species (Moser and Hoveland, 1996). Perennial plants regenerate from rootstock each year, whereas annuals have to be reestablished each growing season. Lowering input costs associated with reestablishment makes perennial crops more advantageous for producers than annuals (Gillen and Berg, 2005). Perennial CSG consist of several grass species such as wildrye, fescue, orchardgrass, and wheatgrass [*Agropyron* spp Gaertn.]. Southeastern wildrye is a relatively newly researched native CSG. Tall fescue and orchardgrass are two introduced perennial CSG most often used in the Southeast.

Southeastern wildrye

Origin

Southeastern wildrye [*Elymus glabriflorus* (Vasey ex L.H. Dewey) Scribn. & C.R. Ball] is a member of the *Elymus* genus, which is the largest and most diverse genus within the Triticeae tribe of the Poaceae family. Triticeae perennial grasses, such as those of *Elymus* L., are the most valuable forage crops worldwide (Jensen et al., 1990). Triticeae is comprised of 400-500 species with approximately 250 species being perennial grasses and 150 of these grasses being in the *Elymus* species (Dewey, 1984). Asia is home to the most *Elymus* species with approximately 53% of them. North

America is home to the second largest percentage of *Elymus* species with approximately 30% (McMillan and Sun, 2004).

Elymus species are prevalent across the north temperate region of the world; however, *Elymus* is not limited to this area. *Elymus* occupies land in five of the seven continents (North and South America, Asia, Australia, and Europe) (Mott et al., 2011). *Elymus* is found in both the northern and southern hemispheres ranging from the Arctic and temperate climates to subtropical areas (McMillan and Sun, 2004; Mott et al., 2011). *Elymus* can be found on terrain varying from grassland to semi-desert to mountain slope to forest (McMillan and Sun, 2004). These allopolyploids are comprised of chromosome numbers ranging from 2n=4x=28 to 2n=8x=56 (Jensen and Wang, 1997). The variation among *Elymus* comes from the arrangement of five genomes, S (St), H, Y, P, and W, within the chromosome sets (Jensen et al., 1990; Mason-Gamer, 2001). The S (St) genome is derived from *Pseudoroegneria spicata* [Pursh] A. Löve, H from *Hordeum* L., Y from an unknown diploid species, P from *Agropyron* Gaertn., and W from *Australopyrum* [Tzvelev] Löve (Dewey, 1971; Lewis et al., 1996; Mott et al., 2011). *Elymus* species that originated in North America have the S (St) genome joined with the H genome (StStHH) (Mason-Gamer, 2001).

Elymus grasses are widely adapted to various environmental conditions. They are able to withstand many abiotic stresses and are useful for forage and wildlife habitat (Asay and Jensen, 1996). Virginia wildrye (*Elymus virginicus* L.) is one species that has been researched and evaluated for forage nutritive value and for wildlife benefits in the northeastern US (Sanderson et. al, 2004). Basin wildrye (*Elymus cinereus)* was a primary forage grass of cattle in the Intermountain west prior to the turn of the century (Young et al., 1975; Evans and Young, 1983). *Elymus* has also been documented to be beneficial in helping control erosion and for soil stabilization (Jones and Larson, 2005).

Characteristics

Southeastern wildrye is a native, perennial, cool-season bunchgrass. This hardy, North American native is well adapted to various soil types, ranging from well-drained sandy soils to waterlogged clays, found within the southeastern portion of the United States. Southeastern wildrye has been shown to have high sun tolerance (Barkworth et al., 2007) and is often found throughout the perimeter of wooded areas, open woods, meadows, along roads and sometimes in open fields (Barkworth et al., 2007; Belt et al., 2013). Other notable characteristics of southeastern wildrye include high seed germination rates and long-term seed viability (Belt et al., 2013).

Southeastern wildrye is a short-lived perennial that stands 122 cm tall at flowering. The dull green leaves are 8-17 mm in width and its base consists of a fibrous root system which expands horizontally via tillers (Belt et al., 2013). Southeastern wildrye has shown to possess a high nutritive value when managed for forage. Rushing and Baldwin (2013) reported that stands of southeastern wildrye grown in Mississippi and harvested prior to seed production contained 11-18% crude protein (CP), 47-59% neutral detergent fiber (NDF), and 24-35% acid detergent fiber (ADF). Rushing and Baldwin (2013) also reported stand thinning within plots. Some plants persisted, however, causing them to hypothesize that these individual plants contained the innate ability to withstand multiple defoliation events.

Endophyte

Southeastern wildrye contains a systemic fungal endophyte known as *Epichloë elym*. Endophytes within the *Epichloë* genus use CSG as a host plant. Signs of infection of *Epichloë* can only be seen via microscope when the grass begins to flower. During this reproductive stage, the endophyte may cause some or all inflorescences to abort, also referred to as grass-choke disease (Craven et al., 2001). Further research on *Epichloë* infected Canada wildrye and Virginia wildrye needs to be conducted in order to understand the impact on the health of animals grazing wildrye (Saha et al., 2009).

Tall fescue

Origin

Tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.] is an introduced, perennial, cool-season grass (Ball et al., 2007). It originated in Europe and was introduced to the United States via European exploration and settlement. Germplasm was first collected for breeding and improvement in the United States on the William M. Suiter farm in Menifee County, KY. Dr. E.N. Fergus, a professor at the University of Kentucky, collected seed from the farm site in 1931. After multiple years of experimentation with the grass, in 1943 it was released to the public as the variety Kentucky-31 (KY-31). Between the mid-1940s and the 1960s tall fescue covered the majority of the forage production acreage of the Mid-South region, and by the 1970s it was the most cultivated forage grass in the United States (Harper et al., 2007). Tall fescue has been estimated to cover more than 14 million hectares of the east-central portion of the United States, the area of the country commonly referred to as the "Fescue Belt" (Ball et al., 2007; Harper et al., 2007). Even though tall fescue has been mainly developed for increased herbage production (Griffiths et al., 1980), it is also an essential grass for the turf industry, roadside vegetation, and conservation practices such as erosion control in the United States (Young III et al., 1999).

Characteristics

Tall fescue is a long-lived, perennial bunchgrass that stands 60 to 122 cm tall. The leaves are shiny, with a dark green hue and outstanding veins and abrasive textured sides. Tall fescue produces short rhizomes and expands via erect tillers. The deep, fibrous root system of tall fescue aids in controlling soil erosion. High levels of crude protein (CP), digestible dry matter (DDM), and minerals qualify tall fescue as a high quality forage species (Ball et al., 2007). Rushing and Baldwin (2013) found that CP, NDF, and ADF in 'Kentucky-31' tall fescue grown in northeast Mississippi ranged from 9.3-13.1%, 47.8-58.7%, and 24.3-35.2%, respectively.

Endophyte

The wild-type endophyte found in KY-31 tall fescue is associated with various health disorders that occur with livestock and wildlife. Tall fescue is naturally a host to a symbiotic fungal endophyte (*Neotyphodium coenophialum*) that manufactures secondary compounds, among them are harmful ergot alkaloids. This endophyte possesses various characteristics that benefit tall fescue by aiding in withstanding both physical (Malinowski et al., 2005) and biological (Popay and Bonos, 2005) stresses, which allows for increased longevity (Read and Camp, 1986). However, the ergo peptide by-products that are produced pose a threat to livestock. Poor weight gains, complications with reproduction, inability to endure hot temperatures, the incapability to shed winter coat, and loss of appendages (ears, hooves, and tails) are all indications of tall fescue toxicity that cattle (*Bos primigenius taurus*) display (Ball et al., 2007; Harper et al., 2007). Horses (*Equus ferus caballus*) exhibit difficulty foaling, abortion, carrying foals longer than term, and decreased milk production as a result of tall fescue toxicosis (Ball et al., 2007; Harper et al., 2007). Wildlife such as white-tailed deer (*Odocoileus virginianus*), eastern cottontail rabbits (*Sylvilagus floridanus*), and northern bobwhite quail (*Colinus virginianus*) have displayed health complications from consuming endophyte infected tall fescue as well (Harper et al., 2007).

Orchardgrass

Origin

Orchardgrass [*Dactylis glomerata* L.] is an introduced, perennial, cool-season, bunchgrass which originated from western and central Europe. It can be found living on every continent, including some of the Antarctic islands (van Santen and Sleper, 1996). It has been a domesticated species in North America since the 1750's (Bush et al., 2012). In Europe orchardgrass is known as cocksfoot, but it received the common name orchardgrass when it was discovered growing in an orchard in Virginia (van Santen and Sleper, 1996). This grass is of great importance in the intensive rotational grazing systems practiced in the northeastern United States (Bush et al., 2012). It is best adapted to the northeastern area of the United States (van Santen and Sleper, 1996) but is also adapted to southern portions of the United States like northern Alabama (Hoveland et al., 1981).

Characteristics

Orchardgrass is characterized by a blue-green leaf coloration, and an erect growth habit, standing 50 to 120 cm tall (Bush et al., 2012) with an open panicle seed head, flattened leaf sheath and a tall ligule (Ball et al., 2007). The leaf length ranges from 20-30 cm and average 2-8 mm in width (Bush et al., 2012). Orchardgrass requires a higher pH and better drainage than tall fescue (Hoveland et al., 1981).

Orchardgrass is used for multiple purposes. It is mainly utilized for forage production, whether for grazing or hay production (Ogle et al., 2011), but it is also used for erosion control and wildlife habitat (Bush et al., 2012). This grass has a high forage quality (Ball et al., 2007) and is highly palatable for a wide range of livestock animals (Bush et al., 2012). Rushing and Baldwin (2013) reported that 'Potomac' orchardgrass grown in north central Mississippi contained CP, NDF, and ADF ranging from 11.4-14.6%, 46.9-58.7%, and 24.6-38.7%, respectively. Harris et al. (1972) observed an average gain of 352 kg ha^{-1} yr^{-1} with cattle grazing orchardgrass for eight years in northern Alabama. Hoveland and others (1981) reported that orchardgrass yielded 8336 kg ha^{-1} yr^{-1} in the first year of variety trial assessment in northern Alabama.

Endophyte

All plant species contain an endophyte or endophytes (Promputtha et al., 2007). Marquez et al. (2007) identified many endophytes living inside various orchardgrass plants collected across Spain. An average of 2.63 species were observed within the 120 field-sampled plants. Farr et al. (1989) identified endophytes within *Dactylis* as well. Ten genera observed by Farr et al. (1989) were also found by Marquez et al. (2007): *Epichloë*, *Phaeosphaeria*, *Drechslera*, *Fusarium*, *Periconia*, *Asochyta*, *Colletotrichum*, *Phoma*, *Stagonospora*, and *Ustilago*. Orchardgrass is not associated with animal toxicity problems like those of endophyte infected tall fescue (Hoveland et al., 1981).

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CHAPTER III

EFFECT OF NITROGEN APPLICATION AND HARVEST INTERVAL ON YIELD AND NUTRITIVE VALUE OF THREE COOL-SEASON GRASS SPECIES

Abstract

In grass production systems, fertilizer application and harvest timing drive hay With over 240,000 hectares devoted to hay production in Mississippi, responsible and profitable agricultural management decisions are vital to ecological health and local and state economies yield and quality. A split, strip-plot study was established in Starkville and Brooksville, MS, in October 2013 and 2014, respectively, to better understand the combinatory effects of N fertility and harvest frequency on three cool-season grasses. Treatments were three forage species (orchardgrass [*Dactylis glomerata* L.], southeastern wildrye [*Elymus glabriflorus* (Vasey ex L.H. Dewey) Scribn. & C.R. Ball], and tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.]), three N application rates (0, 134, 202, and 269 kg N ha^{-1} yr^{-1} as 33-0-0 S [50% urea and 50% ammonium sulfate commercial available mix]), and four harvest regimes (one, two, three, or four times during the 112 day season). All plots were fertilized in split applications on a 28-day cycle. Dry weight was recorded, subsamples were ground, and forage analysis was conducted using NIRS. Relative forage quality (RFQ) was calculated using ADF and aNDF percentages. Environments impacted yield to the greatest degree. Southeastern wildrye yielded greater mean cumulative dry matter in multiple treatment combinations

but did not have a greater RFQ value than tall fescue. The lowest RFQ values were observed at the lowest harvest frequency. Similarly, CP percentages were significantly greater for tall fescue and orchardgrass than southeastern wildrye. Crude protein percentages were the lowest when no supplemental N was applied. Breeding efforts need to be implemented for increased forage quality in southeastern wildrye for it to compete with tall fescue for pasture hectarage in Mississippi.

Introduction

The main goal of fertilization in any forage production system is to produce high-quality herbage that will meet the nutritional requirements of livestock while yielding an amount adequate to minimize the need for supplemental feed (Snyder and Leep, 2007). Proper fertilizer utilization, appropriate application time and optimum quantity, as well as suitable harvesting practices (i.e. height, frequency, and intensity) can be highly profitable for cool-season grass (CSG) forage production.

The primary nutrients [nitrogen (N), phosphorus (P), and potassium (K)] play an integral role in the life cycle of CSG, with N being the most influential in above ground biomass production. To maximize growth N is needed in the largest quantity and considered least readily available (Snyder and Leep, 2007). According to Ball et al. (2007), N is the essential macro-nutrient that has the greatest effect on overall plant development. Nitrogen is an essential element in chlorophyll construction and thus aids in photosynthesis (Ball et al., 2007).

Nitrogen plays an integral part in the development of forage grasses, serving in a fundamental role in the formation of amino acids and proteins. It is also essential in photosynthesis by being a primary component of chlorophyll. Ample plant N levels produce dark green leaf coloration while N deficiency contributes to stunted growth and yellowing of leaf tissue (Snyder and Leep, 2007).

Nitrogen fertilizer application, whether in solid or liquid form, is the prominent source of N for managed grasses. Forage plants require large amounts of N for growth, and considering the potential for loss of N, through leaching, etc., more than one application should be applied during the growing season (Ball et al., 2007). To help avoid N loss, fertilizers should be applied immediately prior to, or during, active forage growth (Snyder and Leep, 2007).

Cool-season grasses do not have the potential to produce as much vegetation as WSG and, as such, usually do not require as much N (Snyder and Leep, 2007). According to van Santen and Sleper (1996) orchardgrass has been found to be one of the most responsive CSG to N fertilization due to the considerably higher dry matter yields produced when fertilized with N. Reynolds et al. (1969) studied orchardgrass in Tennessee and found that applying 224 kg N ha^{-1} combined with three, four, and six week harvest intervals produced greater dry matter yield than using 112 kg N ha^{-1} with the same harvest frequency. Studies by Wedin (1974) on CSG fertility showed yields three to six times higher than normal with N as the primary nutrient supplied.

Tall fescue is tolerant of low amounts of nutrients within the soil but reacts exceptionally well to N fertilization (Ball et al., 2007). Balasko (1977) reported tall fescue yields in West Virginia to be two to three times greater when fertilized with N as compared to no N supplementation. Belesky et al. (1982) observed greater fresh and dry matter yields when using 200 kg N ha^{-1} on tall fescue in Georgia than when not applying N.

Harvesting is a useful management practice to help optimize forage quality (Fales and Fritz, 2007). The stage of maturity of grass crops at harvest is considered the most influential factor affecting the quality of the forage, and, as such, plays a crucial role in choosing the proper time or times to harvest (Collins and Fritz, 2003). Reynolds et al. (1969) observed greater dry matter yield at lower harvest frequency (two harvests rather than four) on orchardgrass when using 224 kg N ha^{-1}. In general, more frequent harvesting results in lower forage yield (Volenec and Nelson, 1983).

Sanderson and others (2004) found that orchardgrass had significantly higher concentrations of NDF than Virginia wildrye [*Elymus virginicus* L.] when harvested at the same time. This significant effect was hypothesized to be attributed to maturity differences of the two CSG at the time of harvest. Orchardgrass was at least to inflorescence emergence as opposed to Virginia wildrye being at the sheath and internode elongation phases. Sanderson and others (2004) found that when Virginia wildrye and orchardgrass were harvested at the same time of the year, Virginia wildrye had significantly higher CP concentrations. This significant difference was hypothesized to be attributed to the difference in maturity of the grasses at harvest.

Our objective was to evaluate orchardgrass, southeastern wildrye, and tall fescue to determine the differences among above ground biomass yield and nutritive value as affected by N applications and harvesting frequency.

Materials and Methods

A field trial was established at two locations: Henry H. Leveck Animal Research Center (South Farm) at Mississippi State University near Starkville, MS (33°26'15.63" N, 88°47'50.51" W) and at the Black Belt Branch Experiment Station of Mississippi

State University near Brooksville, MS (33°15’38.72” N, 88°32’26.64” W). The soil type at the Starkville location was a Catalpa silty clay loam (fine, smectic, thermic, Fluvaquentic Hapludolls), moderately well drained with a pH of 5.6. The soil type at the Brooksville location was a Brooksville silty clay (fine, smectic, thermic, Aquic Hapludert), somewhat poorly drained with a pH of 7.2. Temperature and precipitation data for both locations can be found in Table D.1 and D.2. An initial soil test was taken prior to planting for each location. Fertilization, with the exception of N, was administered based on a soil test with recommendations for perennial cool-season forage grasses (Mississippi State University Soil Testing Lab). Pelletized lime ($CaCO_3$) was applied at a rate of 2.24 Mg ha^{-1} in Starkville 2013 prior to planting bringing soil pH to 6.2. No lime was applied at Brooksville.

Pre-plant burndown for both locations was achieved by applying Eraser™ A/P® glyphosate (N-[phosphonomethyl] glycine, isopropyl-amine salt; 41%) at 2.76 kg ae ha^{-1} once prior to tillage and again following tillage. An application of Banvel® [dimethylamine salt of dicamba (3,6-dichloro-o-anisic acid; 40%) 48.2%] was applied at 0.56 kg ae ha^{-1} for control of annual broadleaf weeds following seedling emergence.

Three CSG species were established at Starkville (Year 1) on October 7, 2013, in a prepared seed bed. A stand failure at Brooksville in 2013 necessitated establishment of additional plantings on March 27, 2014, at both Starkville and Brooksville. Neither of these spring plantings established successfully. Species were established at both Starkville and Brooksville on October 17 and 28, 2014, respectively. The Starkville site was abandoned due to excessive weed pressure. Supplemental sprinkler irrigation was used once after planting at Brooksville because of unusually dry conditions. The

Starkville 2013 site was harvested in the spring of 2014 (Year 1/Establishment Year) and again in the spring of 2015 (Year 2). Brooksville 2014 was harvested in the spring of 2015 (Year 1/Establishment Year).

The three CSG species used were: southeastern wildrye (Foundation Seed, Mississippi State, MS), 'Potomac' orchardgrass (Ernst Conservation Seeds Inc., Meadville, PA), and 'Kentucky-31' tall fescue purchased from Oktibbeha Co. Farmers' Cooperative (Starkville, MS). Seed were drilled into a prepared seed bed using an Almaco® (Almaco, Nevada, IA) 8-row light duty grain drill at a depth of 0.6 cm. Planting rate was based on a pure live seed (PLS) rate of 16.8 kg ha^{-1} corresponding to a bulk seed rate of 56.3, 17.2, and 20.0 kg ha^{-1} for southeastern wildrye, orchardgrass, and tall fescue, respectively. Seeding rates correspond with those used by Rushing and Baldwin (2013) for southeastern wildrye, Bates (1999) for orchardgrass, and the Mississippi Cool-Season Forage Variety Trial Testing Program for tall fescue (White et al., 2013).

The study design consisted of a split plot in strips, with three treatments: CSG species, N application, and harvest regime. Each block was first split by species. Each species plot was superimposed by N application and harvest regime. Each block was randomized and replicated four times across the field. Individual plots were 1.8 m x 3.0 m with eight drilled rows per plot with 25.8 cm spacing. Nitrogen was applied using a Gandy® (Gandy Co., Owatonna, MN) 1.8 m drop spreader. Plots received 0, 134, 202, and 269 kg $ha^{-1}yr^{-1}$ N of 33-0-0 S (ammonium sulfate & urea) in four split applications per season per specified plot every 28 days. An unfertilized control was also included. Plots were harvested one, two, three, or four times throughout the 112-day growing season. A Ferris® (Ferris, Munnsville, NY) zero-turn mower equipped with a bagging system and a 132.1 cm cutting width was used to harvest the center of each plot at a 10 cm stubble height (Brink and Casler, 2009, 2012; White et al., 2013). First harvest was conducted when 75% of the plots were ≥ 38 cm in height for both years, spring 2014 and spring 2015. In fall 2014, prior to second-year harvest for Starkville 2013, above ground biomass of deceased summer annuals were removed by hand to allow for cool-season grass growth.

Data collected for each plot included: visual canopy cover ratings, normalized difference vegetation index (NDVI), average plant height of plot, cumulative dry matter yield, and relative forage quality (RFQ). Canopy cover ratings for each plot were based on a scale ranging from one (poor; less than 20% of total plot coverage) to five (excellent; greater than 80% of total plot coverage) (Table A.8). Normalized difference vegetation index was measured using a handheld GreenSeeker® (Trimble®, Sunnyvale, CA). Average plant height of the plot was measured using Filip's® electronic folding pasture plate meter (Jenquip Co., Feilding, New Zealand). Fresh, aboveground biomass was removed by clipping the center 132.1 cm of biomass above 10 cm. For dry matter determination, a biomass subsample was collected from each plot, weighed, dried at 50°C until no further weight change was observed, and weighed again to record loss of moisture. Subsamples were then ground to pass a 1 mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) for forage analysis. Nutritive value measurements of acid detergent fiber (ADF), amylase neutral detergent fiber (aNDF), and crude protein (CP) were obtained from near infrared reflectance spectroscopy using a Foss 6500C® (Foss North America, Eden Prairie, MN) using the grass hay equation (NIR Forage and Feed

Testing Consortium, Hillsboro, WI). Due to the complexity of interactions among these parameters of nutritive value, relative forage quality (RFQ) was used as the characteristic of interest. Relative forage quality was calculated using the following equation:

$$\text{Calculation: RFQ} = \text{TDN} \times \text{DMI} / 1.23 \qquad (3.1)$$

Where: TDN (% of dry matter) = 96.35 – (%ADF x 1.15)

DMI (% of body weight) = 120 / %NDF

Statistical analysis was conducted using PROC MIXED using SAS® software, Version 9.4 (SAS Institute, Cary, NC, 2013). Mean separations were based on Tukey's protected least significant difference (LSD) and considered significant at $\alpha = 0.05$. Since there is a difference in year x location, year x location will be referred to in this document as environment.

Results and Discussion

Year 1 Results for Starkville 2013 and Brooksville 2014 Environments

Mean Cumulative Dry Matter Yield

For mean cumulative dry matter yield the interactions of environment x species (P = 0.0005), environment x harvests (P = 0.0260), and species x harvests (P = 0.0181) were significant, as well as the independent treatment of N application (P < 0.0001) (Table A.2). Since there was a significant interaction between environment x species and environment x harvests, environments results were not pooled. The interaction among environment x species x N application rate x harvests was not significant (P = 0.1469) (Table A.2), but Table 3.1 lists all mean cumulative dry matter yields for Year 1.

Analyzing environment x species and environment x harvests interactions, for Year 1 Starkville 2013, no yield difference was observed among species (Figure 3.1). For Year 1 Brooksville 2014, southeastern wildrye (3804 kg ha^{-1}) was greater than tall fescue (2745 kg ha^{-1}) which was greater than orchardgrass (2067 kg ha^{-1}). Heights prior to harvest are found in Table 3.2.

An assessment of the effect of harvest regime on mean cumulative dry matter yield for Year 1 Starkville 2013 indicate harvesting the CSG species twice (4549 kg ha^{-1}) was significantly greater than once, thrice, or four times (3745, 3934, and 3753 kg ha^{-1}, respectively) (Figure 3.2). One, three, and four harvests were not significantly different from one another. There was no effect due to cutting frequency for Year 1 Brooksville 2014.

Since the three way interaction for environment x species x harvests was not significant, when analyzing species x harvests, environments data were pooled. Southeastern wildrye harvested two times (4492 kg ha^{-1}) was significantly greater than all other species x harvests treatment combinations except southeastern wildrye harvested one time (4001 kg ha^{-1}) with respect to mean cumulative dry matter yield (Figure 3.3). More than half of the vegetation within these two harvests were southeastern wildrye with the one harvest having the most vegetation of the desired species (Table A.7). Orchardgrass at one harvest (2786 kg ha^{-1}) was the lowest with respect to mean cumulative dry matter yield, but was grouped with tall fescue at one, tall fescue at two, orchardgrass at three, and orchardgrass at four harvests (3032, 3246, 2918, and 3078 kg ha^{-1}, respectively).

When analyzing N application separately, 269 kg N ha^{-1} yr^{-1} had greater dry matter accumulation (4097 kg ha^{-1}) than all other N application treatments (Figure 3.4). The unfertilized control, 0 kg N ha^{-1} yr^{-1} was the lowest yielding treatment (2415 kg ha^{-1}). The N treatments of 134 kg N ha^{-1} yr^{-1} and 202 kg N ha^{-1} yr^{-1} were intermediate (3536 and 3687 kg ha^{-1}, respectively) and not different from one another.

Normalized Difference Vegetation Index (NDVI)

Normalized DVI is an index of plant "greenness". It is suggestive of photosynthetic activity and leaf greeness. Ratings for NDVI were taken prior to each harvest. Analysis of the NDVI data indicated a three-way interaction of environment x species x N application ($P = 0.0008$) and the two-way interactions of environment x harvests ($P < 0.0001$), and species x harvests ($P = 0.0053$) (Table A.3). Since there was a significant interaction between environments x species x N application and environment x harvests, environment results could not be pooled.

For Year 1 Starkville 2013 greatest "greenness" was for tall fescue at 269 kg N ha^{-1} yr^{-1} (0.54) was greater than all other environment x species x N application except for orchardgrass at 134, 202, and 269 kg N ha^{-1} yr^{-1} (0.52, 0.52, and 0.53, respectively) (Figure 3.5). The NDVI for southeastern wildrye had the lowest index for all N treatments that received supplementation. Southeastern wildrye (0.39) was equal to tall fescue (0.38) for the unfertilized control (0 kg N ha^{-1} yr^{-1}), but greater than the index for orchardgrass (0.34).

For Year 1 Brooksville 2014 greatest "greenness" was observed with tall fescue at 269 kg N ha^{-1} yr^{-1} (0.52), but this was the same as tall fescue at 134 and 202 kg N ha^{-1} yr^{-1} (0.50 and 0.50, respectively) (Figure 3.6). Orchardgrass with 0 kg N ha^{-1} yr^{-1} had the lowest index (0.35). Data from this experiment would suggest that there is a distinct species difference because at each N supplementation (excluding 0 kg N ha^{-1} yr^{-1}), tall fescue was the highest index, followed by southeastern wildrye, and then orchardgrass. Each species was visibly a different shade of green and each may reflect differently, so comparison between species may not be ideal but was required for analysis of variance (ANOVA).

Number of harvests also affects NDVI. Plots undergoing two or three harvests (0.49 and 0.50, respectively) for Year 1 Starkville 2013 had higher NDVI than a single harvest (0.43) or four harvests (0.45) per season (Figure 3.7). For Year 1 Brooksville 2014 the two and four harvest regimes (0.49 and 0.48, respectively) had the greatest index, followed by three harvests (0.44) and the one harvest (0.33). Data from Starkville would suggest "brown" from necrotic material in the plots since this data was taken prior to harvesting in mid-July, and these are CSG. The four harvests also had barren ground due to the high frequency of harvest. "Brown" may be attributed to that as well. However, the Brooksville data shows the four harvest regime equal to the two harvest.

Since the three way interaction for environment x species x harvests was not significant, when analyzing species x harvests, environments data were pooled. Greatest index was observed with tall fescue harvested two times (0.50), which grouped with tall fescue at three and four harvests, southeastern wildrye at two, three, and four harvests, and orchardgrass at two harvests (0.50, 0.50, 0.49, 0.48, 0.46, and 0.48, respectively) (Figure 3.8). The single harvest regime for southeastern wildrye is reading "brown" probably due to the senescing plant. The whole southeastern wildrye plant senesces in June/July after seeding, unlike orchardgrass and tall fescue. Repeated harvests keep plots green, either by compensatory growth or perhaps allowing summer annual weed growth (Table A.7).

Relative Forage Quality

In order to simplify the large volume of data on nutritive value, focus was placed on RFQ as a representative characteristic of the forages' overall nutritive value. Mean RFQ was significant for a three way interaction of environment x N application rate x harvests ($P = 0.0141$) and two way interactions of environment x species ($P = 0.0150$) and species x N application ($P = 0.0445$) (Table A.5).

Separated by environments, assessment of mean RFQ for Year 1 Starkville 2013 showed at 0 kg N ha^{-1} yr^{-1} under four harvests RFQ (98.8) was greater than all other treatments except for 134 kg N ha^{-1} yr^{-1} under four harvests, 269 kg N ha^{-1} yr^{-1} under four harvests, and 134 kg N ha^{-1} yr^{-1} with three harvests (96.3, 95.9, and 95.1, respectively) (Figure 3.9). Mean RFQ for all N application rates at one harvest were lower than all other N application rates x harvests treatments. The lowest mean RFQ at the one harvest was 0 kg N ha^{-1} yr^{-1} (74.2) with 134 and 269 kg N ha^{-1} yr^{-1} (76.6 and 76.4, respectively) not being significantly different.

At Brooksville (2014) mean RFQ for Year 1 was calculated to be 102.1, 101.0, and 100.6 corresponding to 269, 134, and 202 kg N ha^{-1} yr^{-1}, respectively, at four harvests. These were greater than all other treatment combinations (Figure 3.10). All N application rates under one harvest had a lower RFQ than all other N application rate x harvest treatment combinations. All N application rates under one harvest were less than the other N application rate x harvest treatment combinations in Starkville, too, indicating results were consistent for both environments. However, all N application treatments were equal to one another at Year 1 Brooksville 2014, which was not the case at Year 1 Starkville 2013.

After calculating mean RFQ, the environment x species interaction was significant. In both Year 1 environments, tall fescue was greater than orchardgrass and southeastern wildrye (Figure 3.11). For Starkville orchardgrass (87.7) and southeastern wildrye (86.7) were not significantly different from one another. For Brooksville orchardgrass (92.1) was significantly greater than southeastern wildrye (85.4).

Crude Protein (CP)

Crude protein was significant at the four way interaction of environment x species x N application x harvest (P = 0.0059). For Year 1 Starkville 2013 the greatest CP percentages were observed to be in treatments that had either the highest N application rate (269 kg N ha^{-1} yr^{-1}) or the most frequent harvest regime (four harvests) (Table 3.3). Only four treatment combinations did not follow this trend: orchardgrass with 134 kg N ha^{-1} yr^{-1} harvested three times (15.7%), orchardgrass with 202 kg N ha^{-1} yr^{-1} harvested one time (15.7%), tall fescue with 202 kg N ha^{-1} yr^{-1} harvested two times (15.4%), and orchardgrass with 134 kg N ha^{-1} yr^{-1} harvested two times (15.1%). Inversely three out of the four lowest CP percentages were those to have the lowest combination of both of these treatments (0 kg N ha^{-1} yr^{-1} harvested once). For Year 1 Brookville 2014 the greatest CP percentages were observed when harvesting was conducted either two or three times with any N application. The lowest CP percentages, except for one, were observed when harvested four times. The two trends among environments do not mimic one another indicating that another factor hindered the results such as lack of rainfall (Tables D.1 and D.2) or summer annual weed encroachment.

Year 2 Results for Starkville 2013 Environment

Mean Cumulative Dry Matter Yield

Evaluation of mean cumulative dry matter yield continued for the first year of forage production (first year following establishment year). The data for mean cumulative dry matter yield indicated a significant three way interaction of species x N application x harvests ($P = 0.0153$) (Table A.9). All species produced greater mean cumulative dry matter yield when applying at least 202 kg N ha^{-1} yr^{-1} as compared to 0 kg N ha^{-1} yr^{-1}. These results are consistent with Belesky et al. (1969). They observed greater dry matter yield in tall fescue grown in Georgia when applying 200 kg N ha^{-1} as compared to 0 kg N ha^{-1}. Species were separated to better understand the proper agronomic management scheme for each individual CSG.

Orchardgrass showed a trend of yielding the greatest cumulative mean dry matter by increasing the N application rate (Figure 3.12). The same results were found with Brink and Casler (2009) when applying N in four increasing amounts from 67 kg N ha^{-1} yr^{-1} to 269 kg N $ha^{-1}yr^{-1}$. Yield increased linearly as N application rate increased. Under each harvest regime yield increased as the amount of N applied increased with the highest yield occurring under the two harvest system combined with 269 kg N ha^{-1} yr^{-1} (5221 kg ha^{-1}) (Table 3.4). The greatest mean height was also observed in plots with the highest N rate per harvest (Table 3.5). Lowest yield was observed with 0 kg N ha^{-1} yr^{-1} under three harvests (1436 kg ha^{-1}), but that was grouped with all other harvest treatments at 0 kg N ha^{-1} yr^{-1}. For all harvest treatments supplemented with N (134, 202, and 269 kg N ha^{-1} yr^{-1}) (Figure 3.12) there was a decline in productivity (mean cumulative dry matter yield) as harvests exceed two per season. This indicates that under the environmental conditions of Starkville two harvests for orchardgrass is the upper limit for maximum yield.

Southeastern wildrye showed the same trend as orchardgrass. The greatest yields were produced when the highest N rate was applied (Figure 3.13). The greatest yield was attained when combining one harvest with the 269 kg N ha^{-1} yr^{-1} treatment (6461 kg ha^{-1}) (Table 3.4; Figure 3.13). It should be noted, based on weed ratings (Table A.15), the majority of the plot (> 70%) was the desired species, southeastern wildrye. The greatest mean height of southeastern wildrye was observed in plots under the 269 kg N ha^{-1} yr^{-1} treatment for each harvest regime, the same trend observed for orchardgrass. There was a definite decline in yield at each N level as harvest regimes increases (Figure 3.13). Southeastern wildrye is not a domestic species and has yet to undergo the genetic modification that will allow it to tolerate repeated (and close) harvest pressure.

Our results for southeastern wildrye were similar to those of Reynolds et al. (1969) working with orchardgrass in Tennessee with respect to harvest interval. They reported that within each N treatment (112 kg ha^{-1} and 224 kg ha^{-1}) as the length between harvests increased so did dry matter yields. In our study, the single harvest (following 112 days) produced significantly greater mean cumulative dry matter yields than all other harvests with respect to N application rates. Our study is also consistent with Reynolds et al. (1969) since southeastern wildrye had the greatest mean cumulative yields at the highest N application rate with respect to harvest frequency.

Tall fescue followed the same trend as the other two CSG. The greater the N applied, the greater the yield (Figure 3.14). In the case of 0, 134, and 202 kg N ha^{-1} yr^{-1} there is a decline in productivity (mean cumulative dry matter yield) as tall fescue gets harvested four times. Indicating that tall fescue can tolerate up to three harvests under these N rates, but incorporating a fourth harvest negatively impacts yield. The fourth harvest also occurred in July which is well into the dormancy period for tall fescue in Mississippi.

Normalized Difference Vegetation Index (NDVI)

Analyses of NDVI data for Year 2 Starkville 2013 indicated a significant interaction for species x N application x harvests ($P = 0.0190$) when measuring "greenness" of each plot. Like Year 1 Starkville 2013, each species was separated to compare "greenness" or shade of green reflectance amongst itself. Mean NDVI for orchardgrass was 0.55 at 269 kg N ha^{-1} yr^{-1} with three harvests (Figure 3.15). However, this was equal to 202 and 269 kg N ha^{-1} yr^{-1} under four harvests, 134 and 202 kg N ha^{-1} yr^{-1} under three harvests, and 202 and 269 kg N ha^{-1} yr^{-1} under two harvests (0.48, 0.52, 0.51, 0.50, 0.51, and 0.50, respectively). The unfertilized plots were lower than all other treatments at each harvest. Southeastern wildrye had the greatest index value at 0.60 under 269 kg N ha^{-1} yr^{-1} with three harvests (Figure 3.16), the same as orchardgrass (0.54; Figure 3.15). However, this was not different from southeastern wildrye at 134 and 202 kg N ha^{-1} yr^{-1} under three harvests or 134 and 269 kg N ha^{-1} yr^{-1} under two harvests (0.57, 0.56, 0.55, and 0.55, respectively; Figure 3.16). Again, the unfertilized control had the lowest value for the two, three, and four harvest regimes (0.42, 0.42, and 0.34, respectively). Tall fescue had the greatest NDVI value, 0.61, at 269 kg N ha^{-1} yr^{-1} with three harvests like the other two CSG (Figure 3.17). This was no different from 269 kg N ha^{-1} yr^{-1} at four harvests or 134 and 202 kg N ha^{-1} yr^{-1} at three harvests (0.56, 0.57, and 0.57, respectively). The 0 kg N ha^{-1} yr^{-1} was the lowest value in each harvest which was consistent with results found for orchardgrass but not southeastern wildrye. These results indicate that southeastern wildrye does not require as much nitrogen for greeness as orchardgrass and tall fescue. Under one harvest the control N treatment (0 kg N ha^{-1} yr^{-1}) was not different than 134 and 269 kg N ha^{-1} yr^{-1} (0.24, 0.22, and 0.24, respectively) for mean NDVI for southeastern wildrye.

Relative Forage Quality (RFQ)

Relative forage quality for the data obtained for Year 2 Starkville 2013 was significant at the three way interaction for species x N application x harvests (P = 0.0165), just like mean cumulative dry matter yield and NDVI for the same data set (Table A.13). In order to simplify the large volume of data on RFQ (Table 3.6), each species was separated to determine the optimum agronomic practices for combining N application and harvest frequency to maximize RFQ per species. Across all species the three and four harvests were greater than the one and two harvests for mean RFQ. Orchardgrass fertilized with 269 and 134 kg N ha^{-1} yr^{-1} and harvested three times had greater mean RFQ (102.6 and 102.1, respectively) than all other treatment combinations except for 134, 202, and 269 kg N ha^{-1} yr^{-1} at four harvests or 202 kg N ha^{-1} yr^{-1} at three harvests (100.7, 101.9, 99.6, and 98.7, respectively) (Figure 3.18). All supplemental N treatments at one harvest were lower than the other supplemental N applications for two, three, and four harvests.

Greatest mean RFQ for southeastern wildrye was observed for 269 kg N ha^{-1} yr^{-1} under four harvests (102.0) (Figure 3.19). However, this RFQ was not greater than 134 and 202 kg N ha^{-1} yr^{-1} at four harvests or 134 kg N ha^{-1} yr^{-1} at three harvests (99.8, 98.2, and 99.1, respectively). Relative forage quality for all N treatments at a single harvest were less than all other N rate x harvests treatments. Relative forage quality increases significantly at each harvest when using 202 or 269 kg N ha^{-1} yr^{-1}. While this trend follows the paradigm these data could be skewed due to the abundance of summer annual weed growth (> 65%) in the more frequently harvested southeastern wildrye plots (Table A.15).

Tall fescue had the greatest mean RFQ when fertilized with 134, 202, or 269 kg N ha^{-1} yr^{-1} and harvested four times (97.9, 98.2, and 97.8, respectively) (Figure 3.20). However, these results were not different from any N application at three harvests. The mean RFQ was 0 kg N ha^{-1} yr^{-1} with one harvest (69.6) was the least.

Crude Protein (CP)

No interaction was observed for CP data analysis for Starkville 2014. Mean CP was significant for species (P = 0.0110), N application (P < 0.0001), and harvests (P < 0.0001) independent from one another (Table A.14). Among the three CSG, orchardgrass (14.9%) and tall fescue (13.6%) were greater in mean CP percentage than southeastern wildrye (12.0%) (Figure 3.21). These results are not consistent with Sanderson et al. (2004) working with Virginia wildrye and orchardgrass. When harvesting Virginia wildrye and orchardgrass at the same time of the year, they observed higher CP percentages in Virginia wildrye than orchardgrass. They hypothesized that the difference was due to the maturity of the grasses at harvest. Maturity of southeastern wildrye and orchardgrass was not quantified or recorded prior to harvest in our study. However, as stated before, following heading in June/July the whole southeastern wildrye plant senesces, unlike orchardgrass and tall fescue. This is significant because as grasses mature, nutritive values such as CP percentage decline (Buxton and Marten, 1989; Collins and Casler, 1990; Cherney et al., 1993).

Crude protein percentages across all N application rates, 134, 202, and 269 kg N ha^{-1} yr^{-1} (13.5, 14.2, and 14.4%, respectively) were equal, but greater than the unfertilized control (11.9%) for mean CP percentage (Figure 3.22). Brink and Casler (2009) also found that applying N increased CP percentage which ranged from 12-16% at 0 kg N ha^{-1} yr^{-1} to 18-24% with 269 kg N ha^{-1} yr^{-1} applied. Collins and Balasko (1981) observed a linear increase in CP as N treatments on tall fescue increased from 0 to 60 to 120 to 180 kg N ha^{-1}. Our results did not follow the same pattern since all supplemental N treatments were not significantly different from one another. Across all harvest regimes, the two more rigorous regimes, three and four harvests per season, were greater for mean CP percentage than one and two harvests. (Figure 3.23).

Summary

Each treatment impacted mean cumulative dry matter yield, NDVI, RFQ, and CP differently. Environment influenced results the most across Year 1. If the influence of environment is discarded, each species displayed contradictory data when comparing cumulative dry matter yield and relative forage quality. Southeastern wildrye yielded as much or more than tall fescue in several instances but did not have as great a RFQ as tall fescue. The greatest RFQ values in Year 1 were found when 269 kg N ha^{-1} yr^{-1} was combined with four harvests. However, southeastern wildrye cannot withstand the more frequent harvest regimes (three or four harvests) like orchardgrass and tall fescue. For each species 269 kg N ha^{-1} yr^{-1} yielded the greatest mean cumulative dry matter yield at each harvest, and 269 kg N ha^{-1} yr^{-1} also resulted in the greatest CP percentages. Tall fescue and orchardgrass had higher CP percentages than southeastern wildrye.

Management schemes would have to be adjusted to support implementing southeastern wildrye into a grass production system. Harvesting two times during the establishment year would be the most conducted to obtain maximum yields. Increasing the harvest height from 10 cm to 20 cm could allow for more than two harvests, but this could also lower yield. Further research should be conducted to explore this possibility.

Table 3.1 Effect of harvest frequency and nitrogen fertility on mean cumulative dry matter yield (kg ha^{-1}) for environments Year 1 Starkville 2013 and Year 1 Brooksville 2014.

		Number of Harvests per Season							
		1		2		3		4	
Species	**Nitrogen**	**2013†**	**2014†**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**
	kg ha^{-1} yr^{-1}	kg ha^{-1}							
Orchardgrass	0	2198.4	1188.3	3138.6	1131.6§	2226.8	1399.7	2742.0	1730.4
	134	3576.8	1719.5	4889.3	1963.3	4385.2	1928.3	3991.4	2211.8
	202	3846.7	2115.3	4614.2	2189.6	4384.4	2241.4	4245.6	2224.5
	269	4457.7	3181.8	6302.3‡	2576.9	3969.4	2807.7	5012.7	2468.3
Southeastern wildrye	0	3476.7	3194.7	3969.1	2724.4	3104.4	2528.1	2599.3	2649.2
	134	4458.9	4079.2	5215.6	4131.5	3822.8	3972.4	3238.5	4008.9
	202	4521.3	4320.5	5173.6	4551.8	4226.3	4099.7	3530.5	3960.8
	269	4289.8	3667.0	5721.8	4449.0	4448.9	4218.1	3315.5	4309.8
Tall fescue	0	2699.6	1449.2	2395.3	2102.6	2455.4	2042.4	2630.0	2182.2
	134	3881.2	2865.2	3875.9	2520.0	4211.6	2866.9	4013.9	3037.2
	202	3545.7	2777.9	4464.3	2339.9	4544.7	2989.4	4801.2	2770.7
	269	3988.5	3051.7	4833.9	3435.3	5430.7	3807.8	4910.3	3674.4

*Mean cumulative dry matter yield was not significant for environment x species x nitrogen x harvests per season at $\alpha = 0.05$, $P = 0.1469$.

†Environment was significant at $\alpha = 0.05$, $P = 0.0029$, so environments were not pooled. Years correspond with locations: 2013-Starkville Year 1; 2014-Brooksville Year 1.

‡Highest mean cumulative dry matter yield

§Lowest mean cumulative dry matter yield

Table 3.2 Effect of harvest frequency and nitrogen fertility on mean height (cm) using rising platemeter for environments Year 1 Starkville 2013 and Year 1 Brooksville 2014.

		Number of Harvests per Season							
		1		2		3		4	
Species	Nitrogen	2013[†]	2014[†]	2013	2014	2013	2014	2013	2014
	kg ha^{-1} yr^{-1}	cm							
Orchardgrass	0	14.7 H-N[‡‖]	12.4 j-s[§]	12.0 M-Q	9.4 s-y	8.5 S-V	7.5 y	8.6 R; UV	8.0 xy
	134	18.2 A-G	11.5 m-w	16.5 E-J	11.2 n-x	11.9 M-R	8.0 xy	10.6 O-U	8.3 w-y
	202	17.1 C-I	13.8 h-o	15.7 G-L	12.0 k-s; uv	12.1 M-Q	8.1 xy	11.5 N-T	8.6 t; w-z
	269	18.2 A-G	17.6 e-g	18.7 A-F	12.7 j-r	12.4 K-P	8.2 w-y	11.8 M-Q; ST	9.0 s-y
Southeastern wildrye	0	19.6 A-E	23.7 bc	13.3 J-O	13.8 h-n	10.5 O-U	11.0 n-y	7.9 U	10.3 o-y
	134	17.2 D-I	26.2 b	19.7 A-E	19.0 de	11.9 M-S	15.9 e-j	9.2 P-U	12.1 m-u
	202	20.5 A-C	32.3 a	20.0 A-E	18.0 e-g	11.7 M-T	15.6 f-l	10.9 O-T	11.5 m-x
	269	15.1 G-M	31.7 a	18.0 B-H	21.3 cd	12.7 K-O	16.3 e-i	11.5 N-T	12.4 k-s
Tall fescue	0	15.7 F-L	13.1 i-q	10.7 O-U	10.2 q-y	8.3 T-V	8.5 v-z	8.5 Q-U	8.6 u-z
	134	20.5 A-D	18.6 d-f	14.4 I-N	13.4 h-p	11.8 M-S	10.0 q-y	10.9 O-U	9.6 r-y
	202	21.1 AB	17.6 e-g	15.8 F-K	12.1 l; n-t	13.2 J-O	9.7 q-y	10.6 O-U	9.3 r-y
	269	21.6 A	16.4 e-h	17.5 C-H	14.8 g-k; m	12.9 K-O	11.1 n-x	12.3 L-P	10.1 p-y

*Mean height using rising platemeter was significant for environment x species x N application rate x harvests per season at $\alpha = 0.05$, $P = 0.0097$.
[†]Environments were not pooled. Years correspond with locations: 2013-Starkville Year 1; 2014-Brooksville Year 1.
[‡]UPPERCASE letters indicate significant differences among Year 1 Starkville 2013.
[§]lowercase letters indicate significant differences among Year 1 Brooksville 2014.
[‖]Letter groups consisting of three or more sequential letters are written with the first and last letter with a dash in between.

Table 3.3 Effect of harvest frequency and nitrogen fertility on mean crude protein (%) for environments Year 1 Starkville 2013 and Year 1 Brooksville 2014.

		Number of Harvests per Season							
		1		2		3		4	
Species	Nitrogen	2013[†]	2014[†]	2013	2014	2013	2014	2013	2014
	kg ha^{-1} yr^{-1}	%							
Orchardgrass	0	11.3 N; P[‡]	11.9 l-n; p; rs[§]	13.6 E-N	13.8 d; g-n	14.1 D-J	16.5 a-c	14.5 B-I	12.6 k-n; pq
	134	14.3 B-J[‖]	14.7 c-k	15.1 A-H	15.9 a-c; ef	15.7 A-G	16.2 a-d	14.5 B-I	11.4 n; p; r-u
	202	15.7 A-G	15.3 b-f; hi	13.6 E-N	15.6 b-g	14.3 B-J	15.6 b-e; g	15.3 A-H	13.0 i-p
	269	17.4 A	13.4 g; j-o	15.0 B-H	15.3 b-i	15.7 A-G	13.5 f; h-n	15.0 B-H	13.7 e-m
Southeastern wildrye	0	9.6 P	7.9 w	14.2 C-I	15.3 b-i	13.4 G-O	17.2 ab	14.9 B-H	12.5 k-n; p-r
	134	11.6 MN	10.0 rs; uv	13.9 E-M	15.5 b-h	14.1 D-M	16.2 a-c	14.1 D-J; L	13.1 h-p
	202	11.7 L-N;Q	10.1 rs; uv	13.0 H-N	16.7 a-c	13.9 E-M	15.6 b-g	14.9 B-H	10.1 s; u-w
	269	12.3 I-N	11.7 l-n; p; rs	12.0 J-N; P	16.8 a-c	14.5 B-J	15.1 c-j	15.7 A-G	9.2 u-w
Tall fescue	0	11.7 K;MN;PQ	11.8 m; p; r-t	15.0 B-H	16.3 a-c	13.3 F; H-O	16.4 a-c	15.7 A-F	10.8 p; rs; u
	134	13.6 E-N	12.8 j; l-n; pq	14.9 B-H	18.1 a	13.4 E-N	15.5 b-g	16.3 A-D	9.1 u-w
	202	14.8 B-H	13.7 e-l; n	15.4 A-G	16.3 a-c	14.5 B-I	15.9 a-e	16.5 A-C	8.7 vw
	269	16.6 AB	14.8 c-i; k	15.0 B-H	15.9 b-e	14.2 B-K	14.8 c-k	16.3 A-D	9.1 u-w

*Mean crude protein value was significant for environment x species x N application rate x harvests per season at $\alpha = 0.05$, $P = 0.0059$.
[†]Environments were not pooled. Years correspond with locations: 2013-Starkville Year 1; 2014- Brooksville Year 1.
[‡]UPPERCASE letters indicate significant differences among Year 1 Starkville 2013.
[§]lowercase letters indicate significant differences among Year 1 Brooksville 2014.
[‖]Letter groups consisting of three or more sequential letters are written with the first and last letter with a dash in between.

Table 3.4 Effect of harvest frequency and nitrogen fertility on mean cumulative dry matter yield (kg ha^{-1}) for Year 2 Starkville 2013.

		Number of Harvests per Season			
Species	**Nitrogen**	1	2	3	4
	kg ha^{-1} yr^{-1}	kg ha^{-1}			
Orchardgrass	0	1541.6 j*	2359.7 gij	1435.5 j	2018.9 ij
	134	2741.5 e-i†	3706.5 b-f	2771.2 cghi	2677.0 f-i
	202	3203.2 b-g	4149.9 b	2865.8 cghi	2812.2 dfgh
	269	3761.3 bcd	5221.3 a	3643.6 bdef	3694.9 bce
Southeastern wildrye	0	3486.9 de*	1442.2 hij	1218.0. ij	940.4 j§
	134	5100.8 b	3403.0 de	2243.4 fgh	1763.6 ghi
	202	4897.4 b	3788.4 d	2588.7 efg	1847.5 ghi
	269	6461.2 a	4501.9 bc	3750.5 cd	2656.1 ef
Tall fescue	0	3695.7 f*	3325.5 f	2945.6 f	1500.5 g
	134	4518.5 de	4912.7 cd	5054.5 d	3814.0 ef
	202	4979.2 cd	4753.9 cde	5758.0 bc	3851.9 ef
	269	5053.3 cd	6448.2 ab	6572.0 a‡	4849.5 cd

*Letters indicate significant differences within species x N application rate x harvests per season with respect to species at $\alpha = 0.05$, P = 0.0037.

†Letter groups consisting of four or more sequential letters are written with the first and last letter with a dash in between.

‡Highest numerical mean cumulative dry matter yield across species x N application rate x harvests per season

§Lowest numerical mean cumulative dry matter yield across species x N application rate x harvests per season

Table 3.5 Effect of harvest frequency and nitrogen fertility on mean height (cm) using rising platemeter for Year 2 Starkville 2013.

		Number of Harvests per Season			
Species	**Nitrogen**	**1**	**2**	**3**	**4**
	kg ha^{-1} yr^{-1}	**cm**			
Orchardgrass	0	12.7 d; f-h*†	11.4 gh	8.8 h	9.1 gh
	134	17.2 bc; e	13.4 e-h	11.5 f-h	9.7 gh
	202	19.3 ab	15.1 b-f	12.1 fg	10.2 gh
	269	20.4 a	17.4 a-d	13.1 c-g	11.7 f-h
Southeastern wildrye	0	21.5 cd	12.4 fg	8.2 gh	5.5 h
	134	27.4 b	18.5 c-e	11.0 g	8.1 gh
	202	27.0 b	17.2 de	10.9 g	8.6 gh
	269	31.2 a	21.1 c	14.2 ef	10.0 fg
Tall fescue	0	29.0 a	13.8 f-h	12.9 hi	9.0 i
	134	26.3 a	18.5 b-e	15.1 e-h	12.6 f-h
	202	22.7 b	16.7 d-h	17.4 c-f	12.5 gh
	269	20.8 b-d	21.2 bc	16.7 c-g	14.3 e-h

*Letters indicate significant differences for species x N application rate x harvests per season interaction within each species at $\alpha = 0.05$, $P < 0.0001$.

†Letter groups consisting of three or more sequential letters are written with the first and last letter with a dash in between.

Table 3.6 Effect of harvest frequency and nitrogen fertility on mean relative forage quality (RFQ) for Year 2 Starkville 2013.

Species	Nitrogen	Number of Harvests per Season			
		1	2	3	4
	kg ha^{-1} yr^{-1}				
Orchardgrass	0	75.5 o-r*†	82.3 l-n	97.2 b-h	96.3 c-i
	134	78.0 n-p	85.4 kl	102.1 a	100.7 a-d
	202	73.5 p-s	84.7 l	98.7 a-f	101.9 ab
	269	72.7 q-s	83.7 lm	102.6 a‡	99.6 a-d
Southeastern wildrye	0	66.0 tu	83.6 l-n	92.6 h-j	91.0 i; k
	134	66.3 tu	83.8 l	99.1 a-e	99.8 a-d
	202	66.6 tu	80.8 l-o	93.1 f; h-j	98.2 a-e; g
	269	63.1 u	83.6 l-n	96.0 d-i	102.0 abc
Tall fescue	0	61.6 u§	78.0 m; o-q	93.6 e-i	92.9 g; ij
	134	71.2 r-t	82.2 l-n	96.2 d-i	97.9 a-f; h
	202	69.4 st	83.3 l; n	95.9 d-i	98.2 a-f; h
	269	73.2 p-s	81.7 l-n	97.6 a-h	97.8 a-f; h

*Letters indicate significant differences for species x N application rate x harvests per season interaction at $\alpha = 0.05$, $P = 0.0165$.

†Letter groups consisting of three or more sequential letters are written with the first and last letter with a dash in between.

‡Highest numerical mean RFQ for species x N application rate x harvests per season

§Lowest numerical mean RFQ for species x N application rate x harvests per season

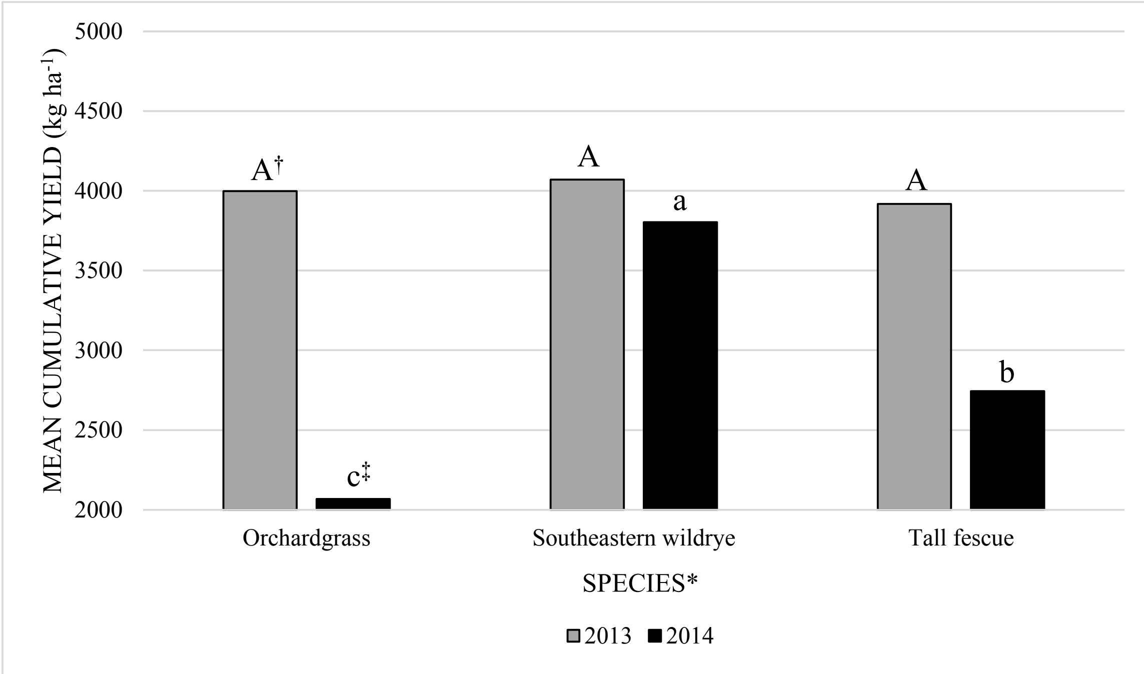

Figure 3.1 Mean cumulative dry matter yield for cool-season grass species by environment for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

*Year 1 environments x species indicate significant differences at $\alpha = 0.05$, $P = 0.0005$, thus data were not pooled among environments.
†UPPERCASE letters indicate significant differences among Year 1 Starkville 2013.
‡lowercase letters indicate significant differences among Year 1 Brooksville 2014.

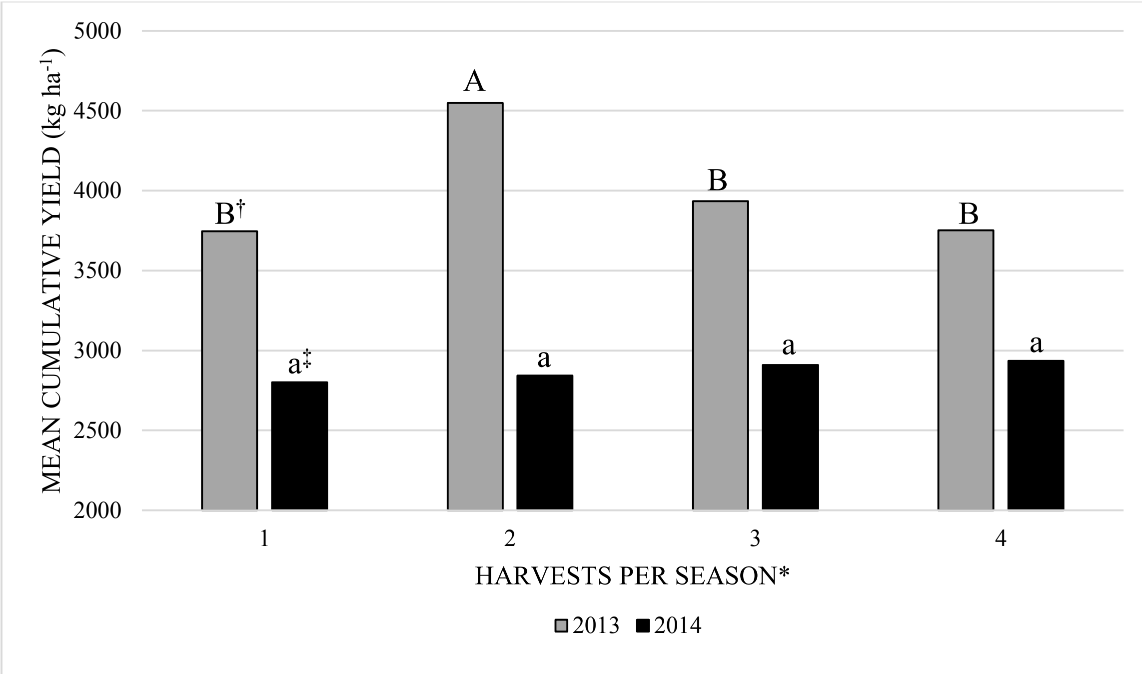

Figure 3.2 Mean cumulative dry matter yield for harvests per season by environment for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

*Year 1 environments x harvests per season indicate significant differences at $\alpha = 0.05$, $P = 0.0260$, thus data were not pooled among environments.
†UPPERCASE letters indicate significant differences among Year 1 Starkville 2013.
‡lowercase letters indicate significant differences among Year 1 Brooksville 2014.

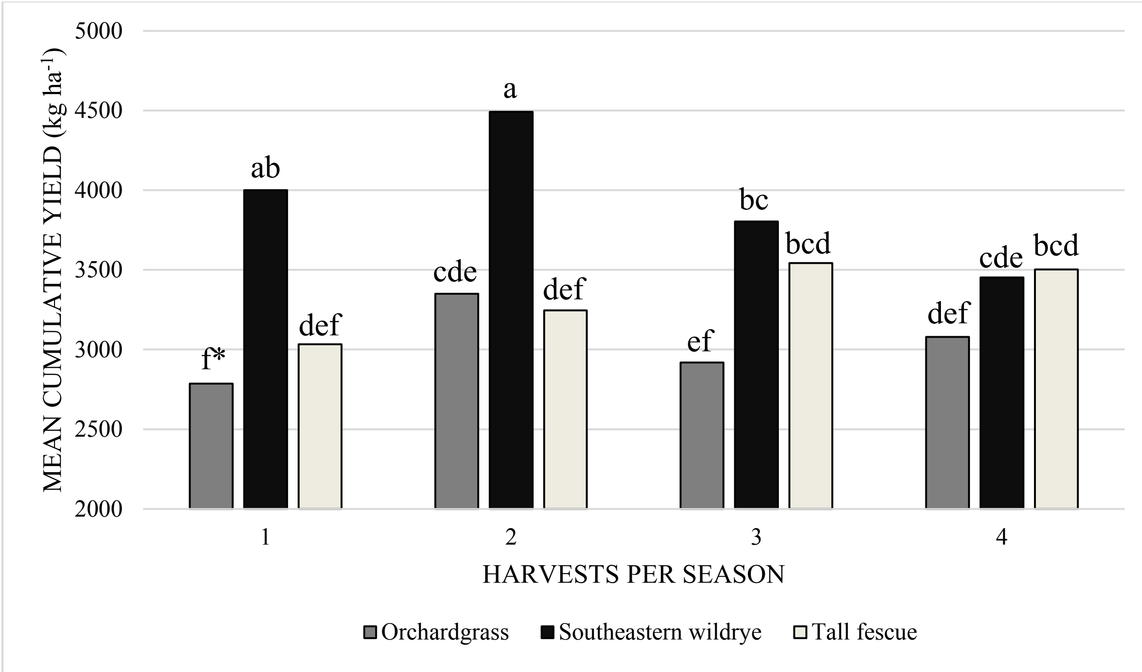

Figure 3.3 Mean cumulative dry matter yield for harvests per season by cool-season grass species pooled across environment for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

*Letters indicate significant differences at $\alpha = 0.05$.

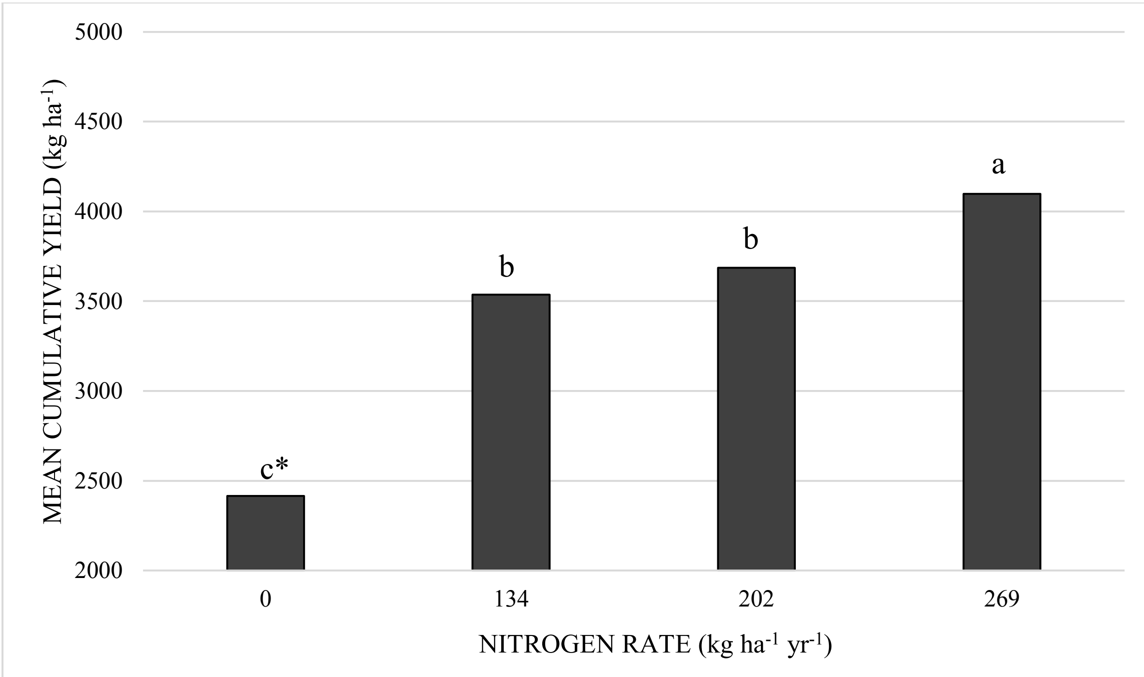

Figure 3.4 Mean cumulative dry matter yield by nitrogen rate pooled across environments for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

*Letters indicate significant differences at $\alpha = 0.05$.

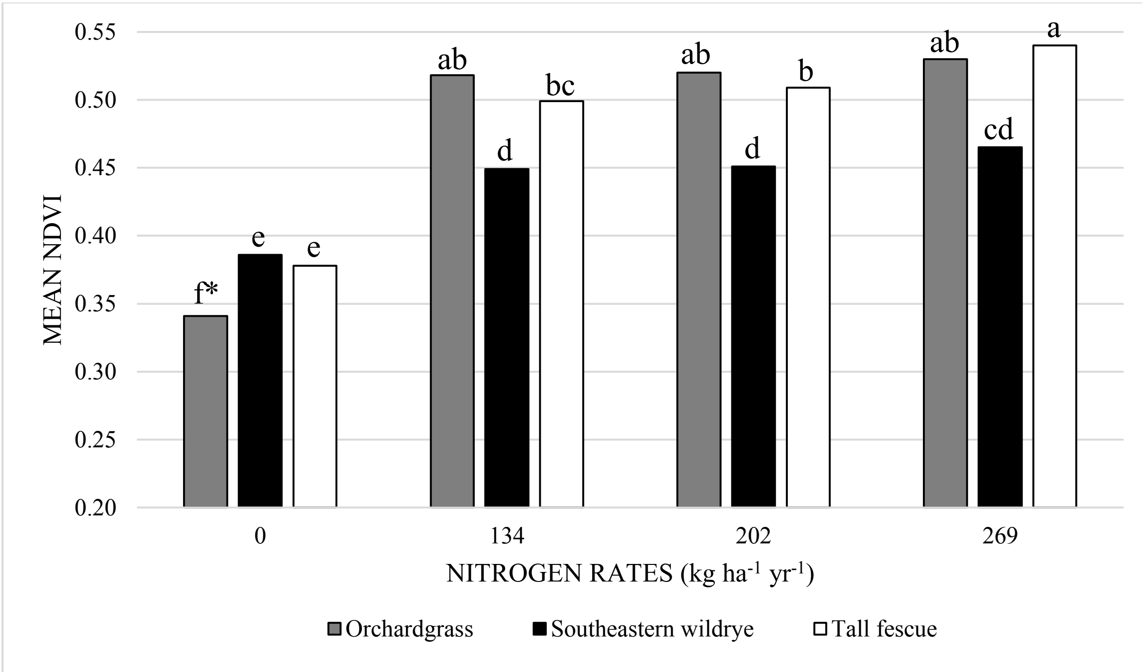

Figure 3.5 Mean normalized difference vegetation index (NDVI) for nitrogen rates by cool-season grass species for Year 1 Starkville 2013 since data were not pooled across environments.

*Letters indicate significant difference at $\alpha = 0.05$.

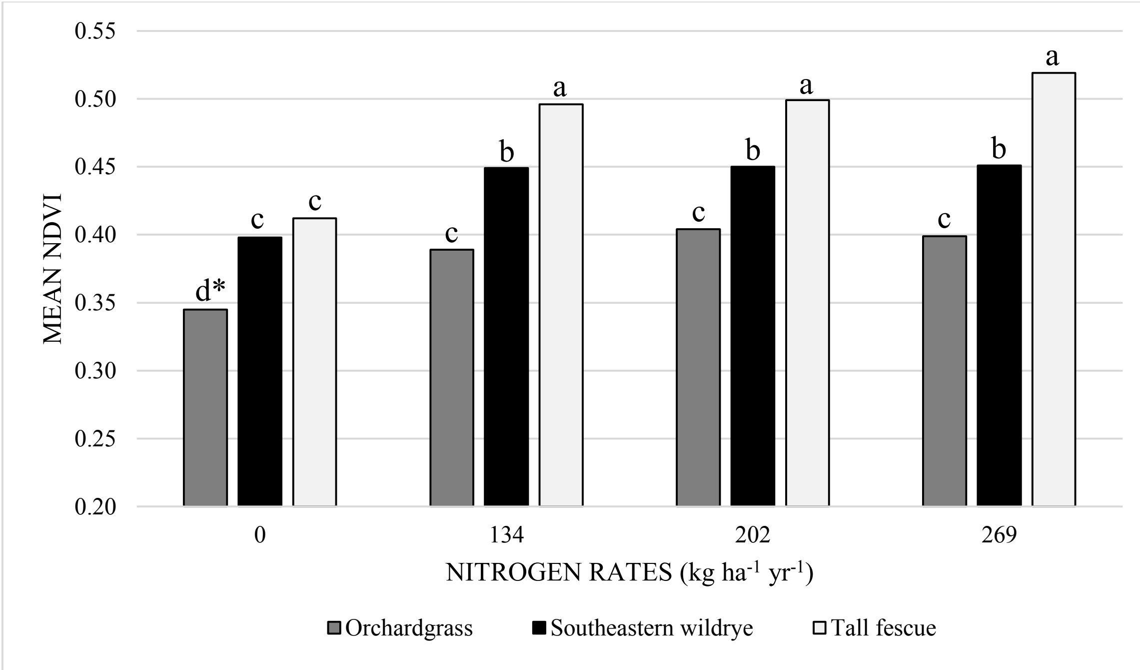

Figure 3.6 Mean normalized difference vegetation index (NDVI) for nitrogen rates by cool-season grass species for Year 1 Brooksville 2014 since data were not pooled across environments.

*Letters indicate significant difference at $\alpha = 0.05$.

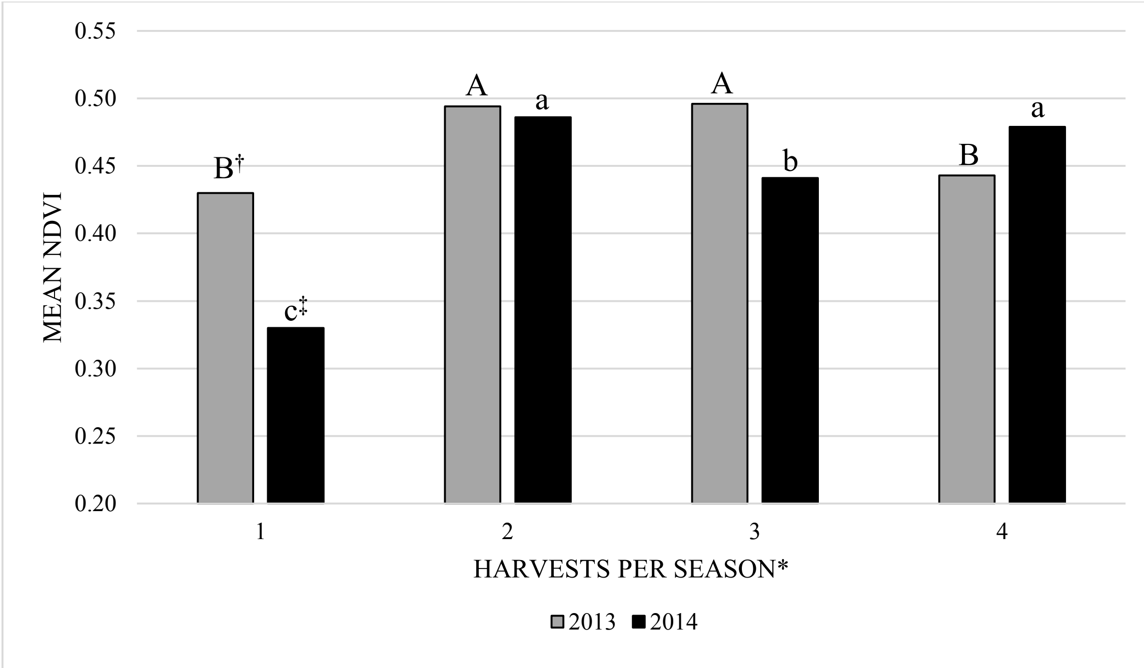

Figure 3.7 Mean normalized difference vegetation index (NDVI) for harvests per season by environment for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

*Year 1 environments x harvests per season indicate significant differences at $\alpha = 0.05$, $P < 0.0001$, thus data were not pooled among environments.
†UPPERCASE letters indicate significant differences among Year 1 Starkville 2013.
‡lowercase letters indicate significant differences among Year 1 Brooksville 2014.

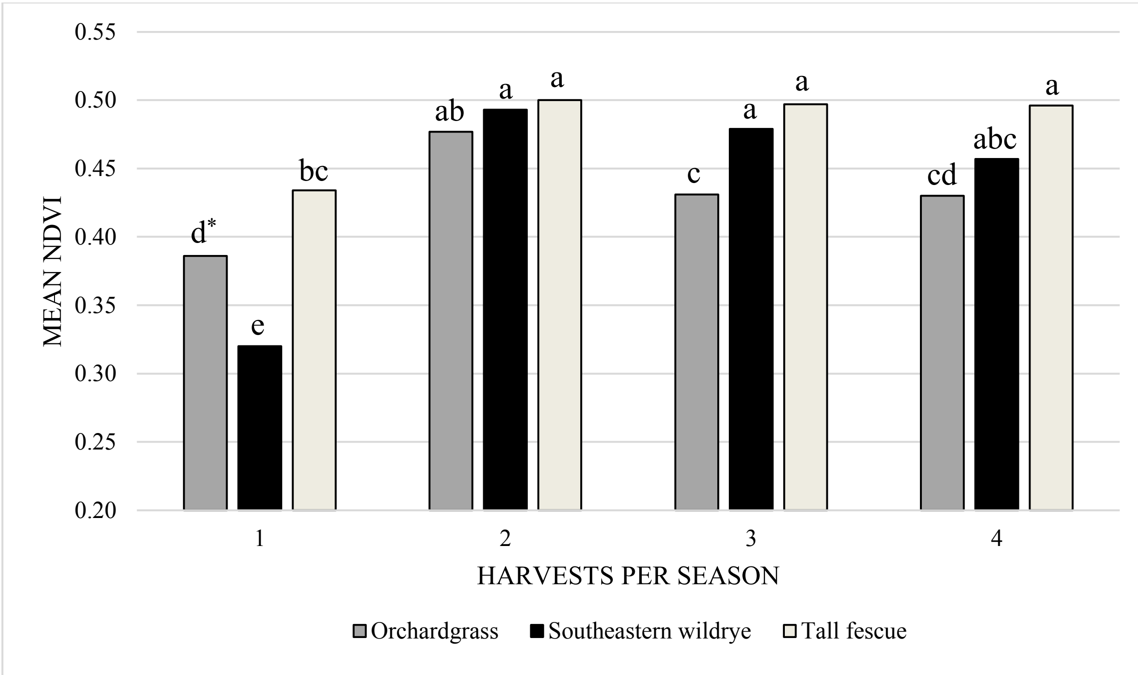

Figure 3.8 Mean normalized difference vegetation index (NDVI) for harvests per season by cool-season grass species pooled across Year 1 Starkville 2013 and Year 1 Brooksville 2014.

*Letters indicate significant difference at $\alpha = 0.05$.

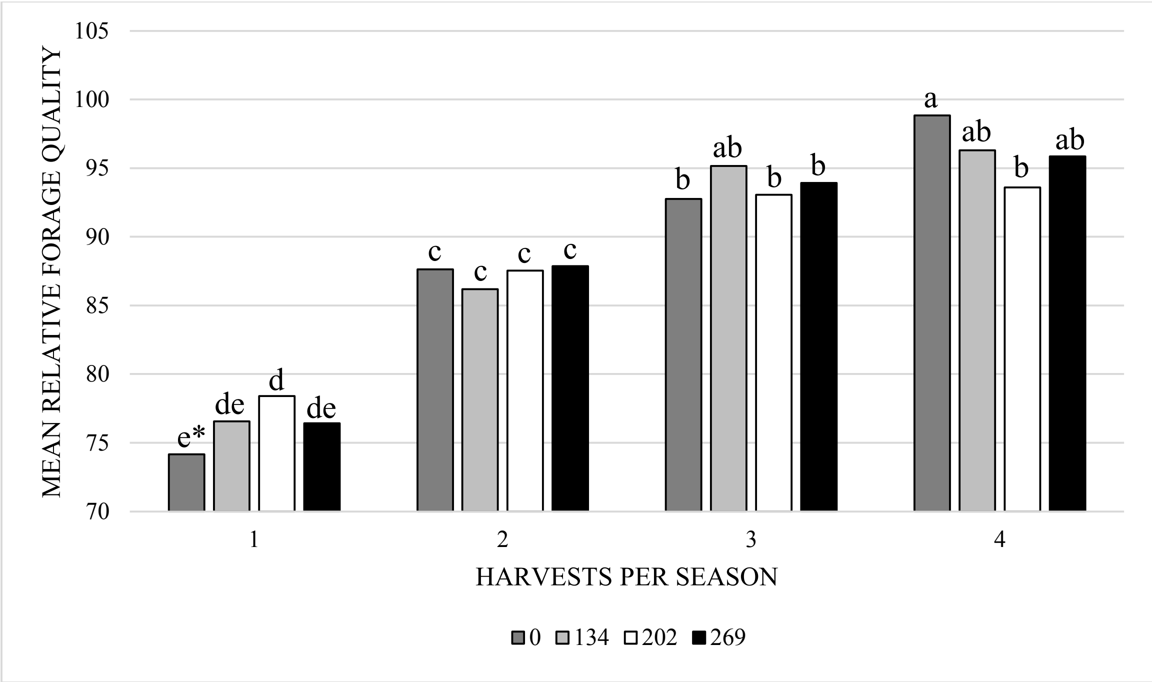

Figure 3.9 Mean relative forage quality (RFQ) for harvests per season by nitrogen rates (0, 134, 202, and 269 kg ha^{-1} yr^{-1}) for Year 1 Starkville 2013.

*Letters indicate significant difference at $\alpha = 0.05$.

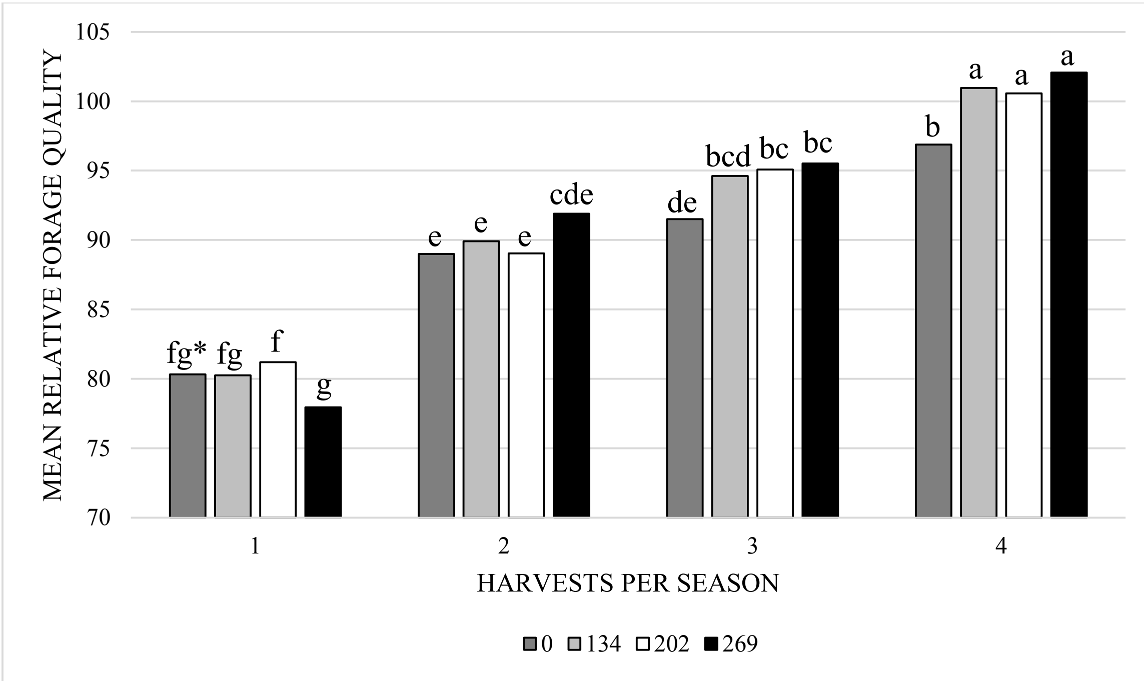

Figure 3.10 Mean relative forage quality (RFQ) for harvests per season by nitrogen rates (0, 134, 202, and 269 kg ha^{-1} yr^{-1}) for Year 1 Brooksville 2014.

*Letters indicate significant difference at α = 0.05.

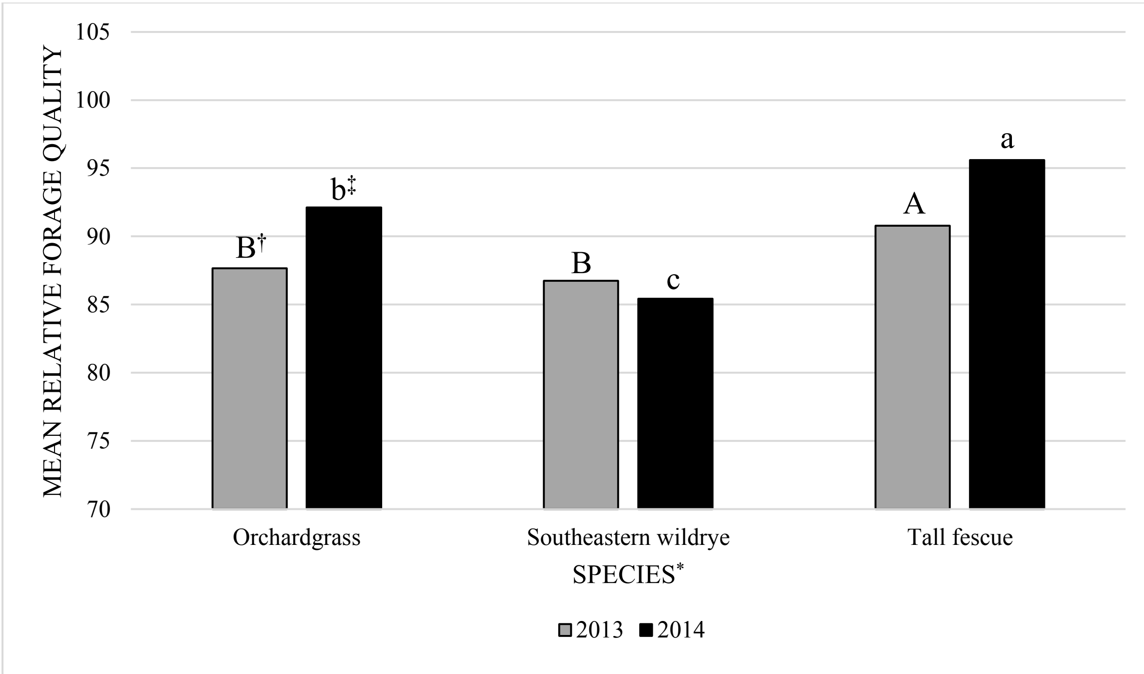

Figure 3.11 Mean relative forage quality (RFQ) for cool-season grass species by environment for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

*Year 1 environments x species indicate significant differences at $\alpha = 0.05$, $P = 0.0150$, thus data were not pooled among environments.
†UPPERCASE letters indicate significant differences among Year 1 Starkville 2013.
‡lowercase letters indicate significant differences among Year 1 Brooksville 2014.

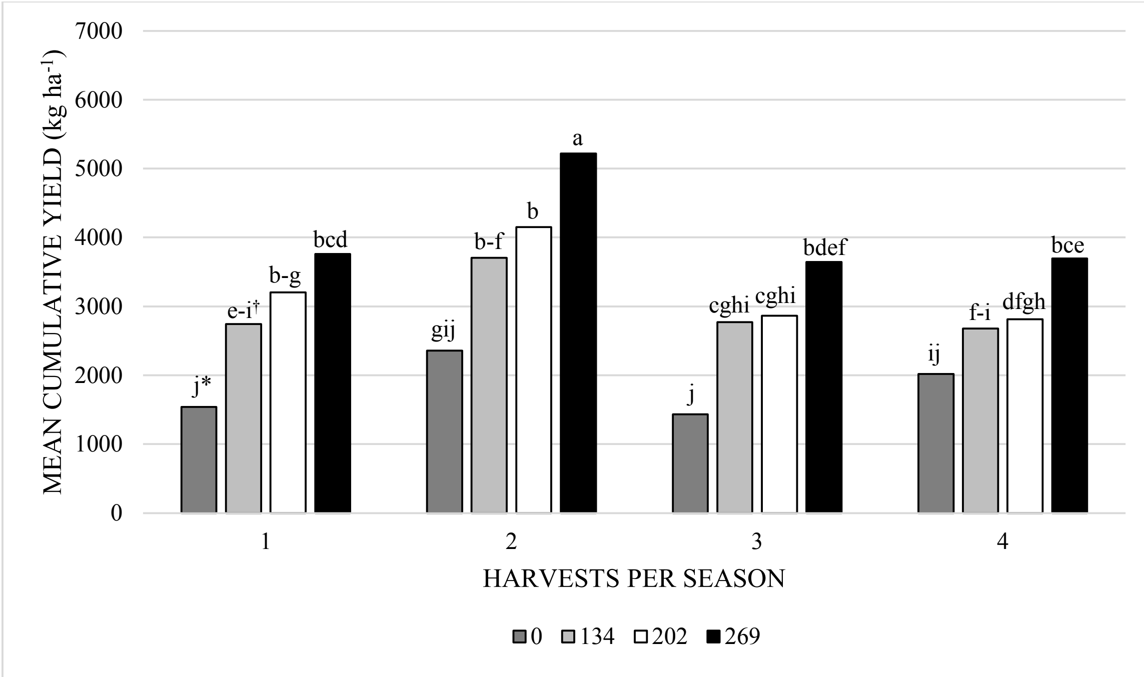

Figure 3.12 Mean cumulative dry matter yield for orchardrgrass for harvests per season by nitrogen rates (0, 134, 202, and 269 kg ha^{-1} yr^{-1}) for Year 2 Starkville 2013.

*Letters indicate significant difference at α = 0.05.

†Letter groups consisting of four or more sequential letters are written with the first and last letter with a dash in between.

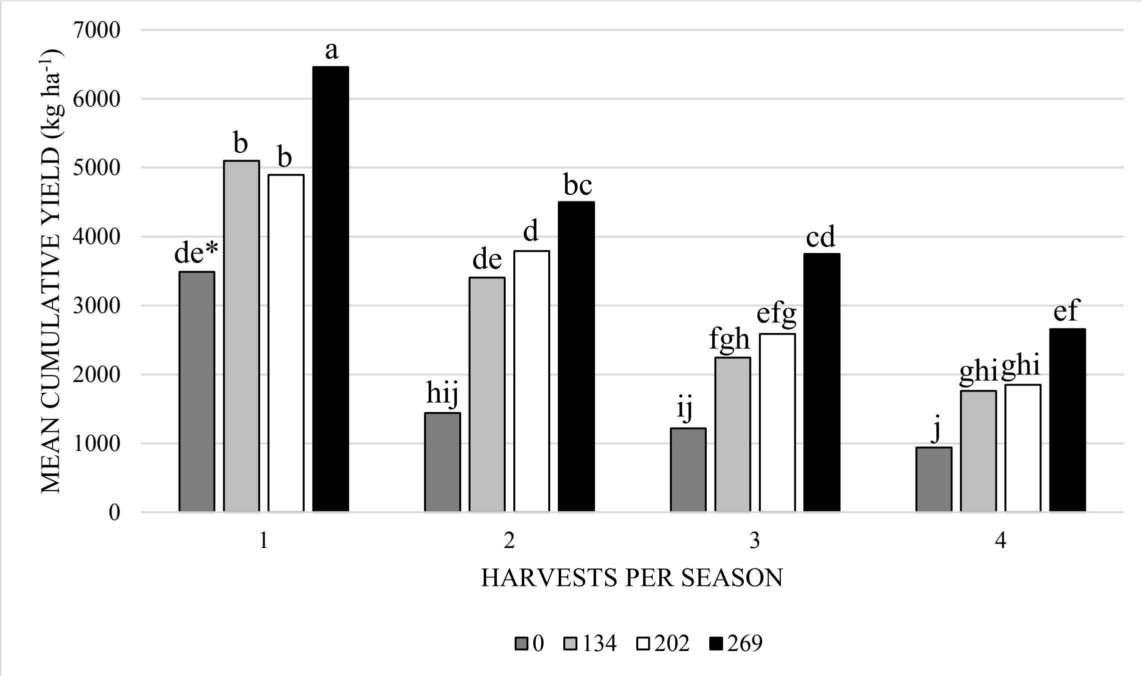

Figure 3.13 Mean cumulative dry matter yield for southeastern wildrye for harvests per season by nitrogen rates (0, 134, 202, and 269 kg ha^{-1} yr^{-1}) for Year 2 Starkville 2013.

*Letters indicate significant difference at α = 0.05.

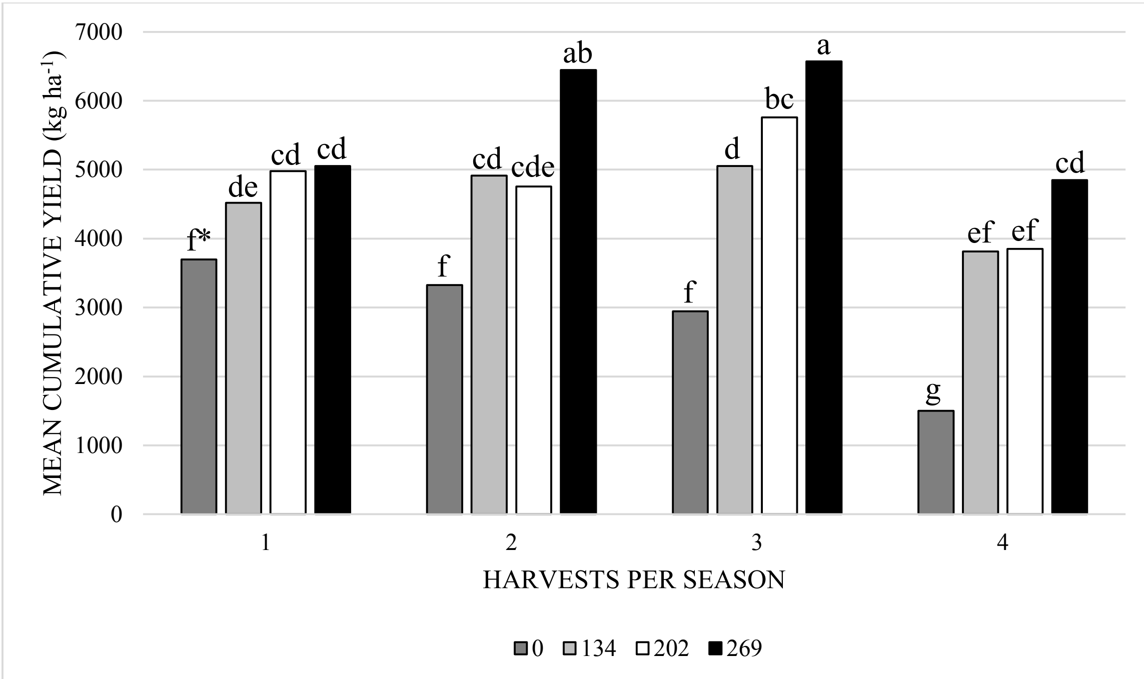

Figure 3.14 Mean cumulative dry matter yield for tall fescue for harvests per season by nitrogen rates (0, 134, 202, and 269 kg ha^{-1} yr^{-1}) for Year 2 Starkville 2013.

*Letters indicate significant difference at α = 0.05.

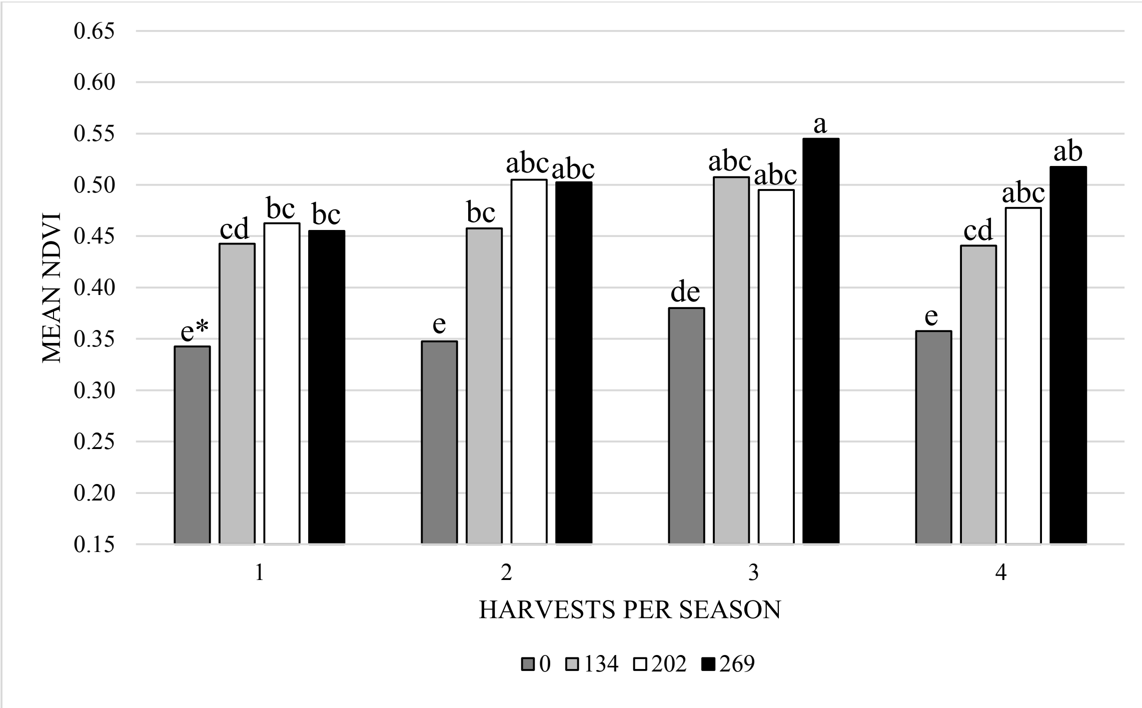

Figure 3.15 Mean normalized difference vegetation index (NDVI) for orchardgrass for harvests per season by nitrogen rates (0, 134, 202, and 269 kg ha^{-1} yr^{-1}) for Year 2 Starkville 2013.

*Letters indicate significant difference at $\alpha = 0.05$.

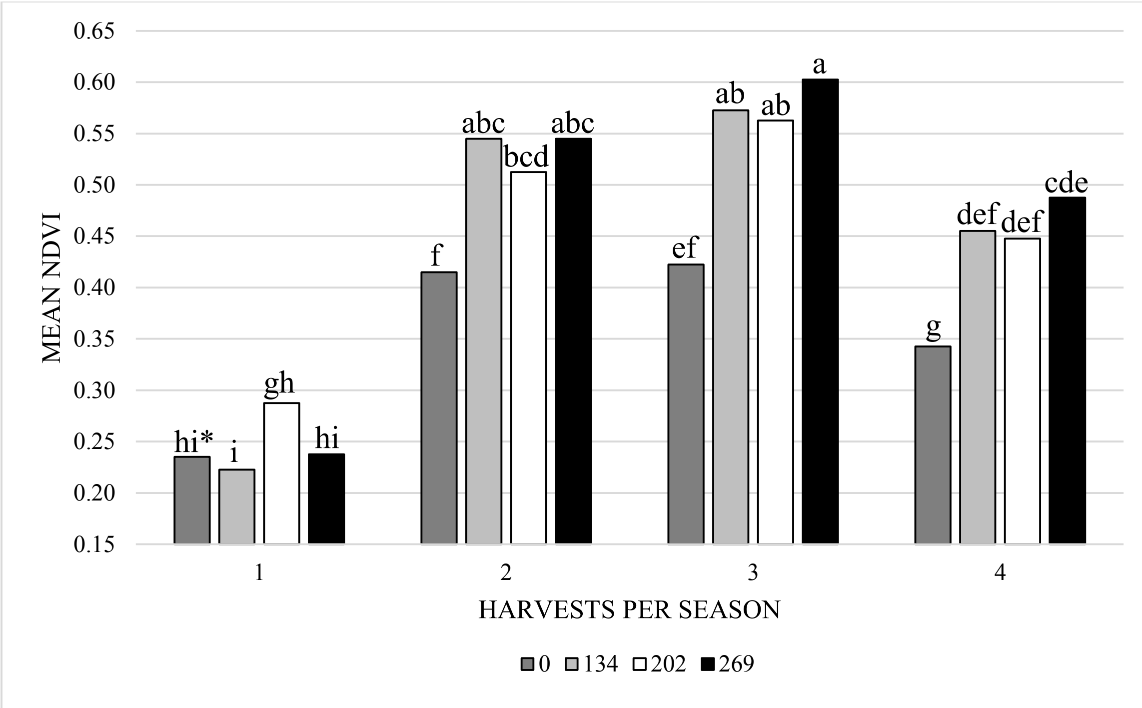

Figure 3.16 Mean normalized difference vegetation index (NDVI) for southeastern wildrye for harvests per season by nitrogen rates (0, 134, 202, and 269 kg ha^{-1} yr^{-1}) for Year 2 Starkville 2013.

*Letters indicate significant difference at α = 0.05.

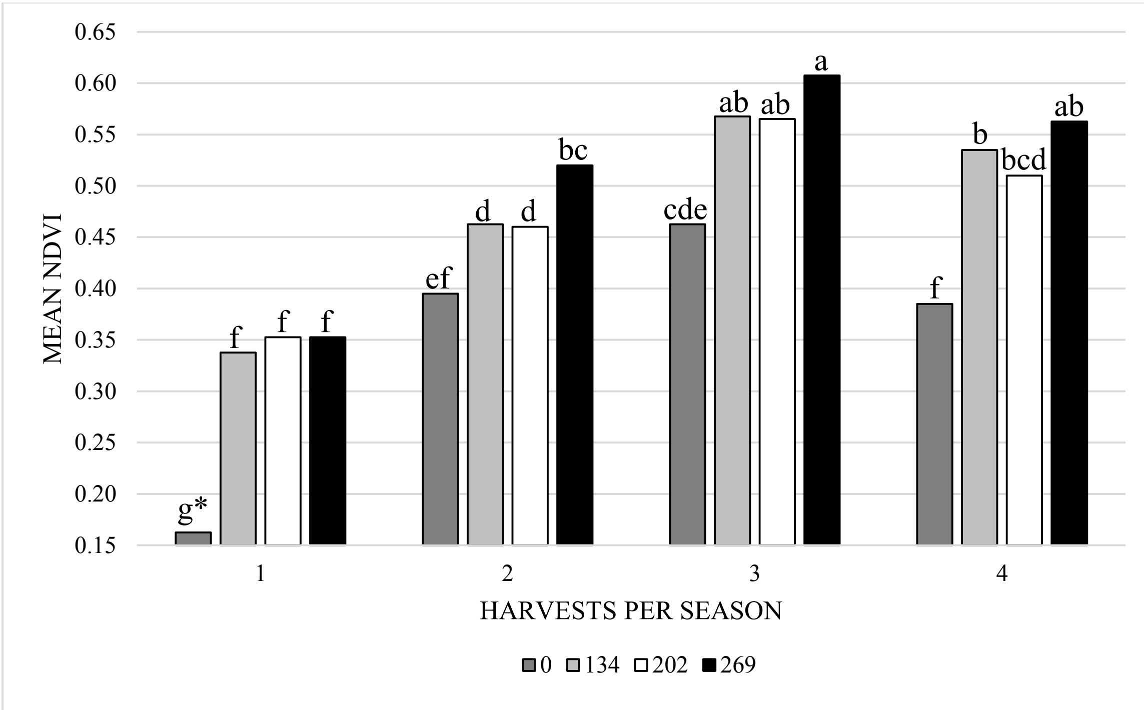

Figure 3.17 Mean normalized difference vegetation index (NDVI) for tall fescue for harvests per season by nitrogen rates (0, 134, 202, and 269 kg ha^{-1} yr^{-1}) for Year 2 Starkville 2013.

*Letters indicate significant difference at $\alpha = 0.05$.

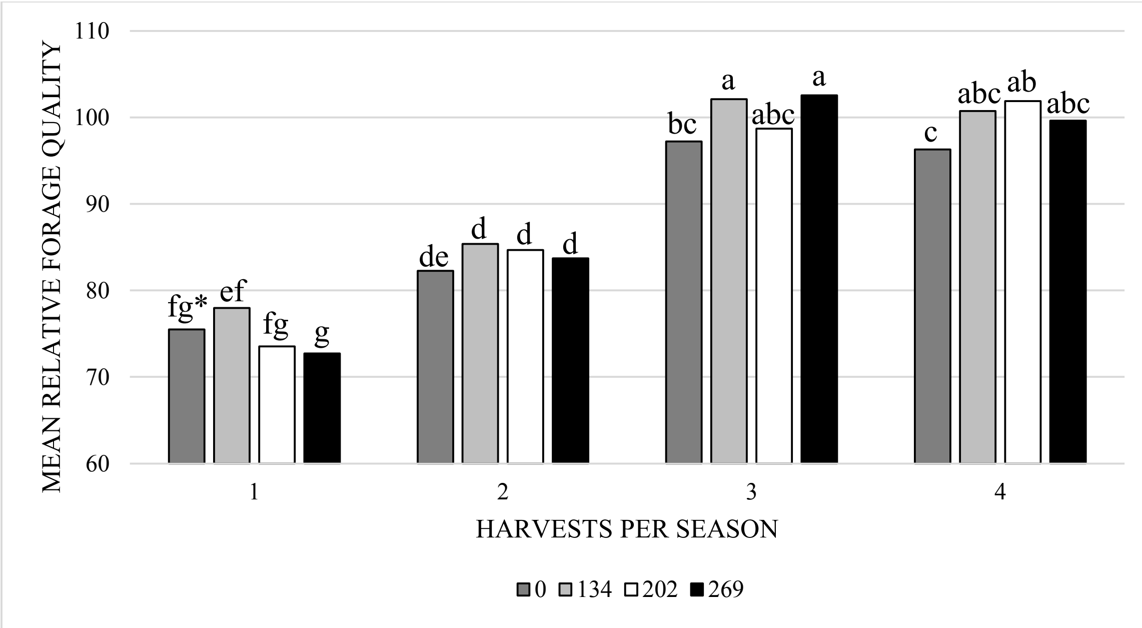

Figure 3.18 Mean relative forage quality (RFQ) for orchardgrass for harvests per season by nitrogen rates (0, 134, 202, and 269 kg ha^{-1} yr^{-1}) for Year 2 Starkville 2013.

*Letters indicate significant difference at α = 0.05.

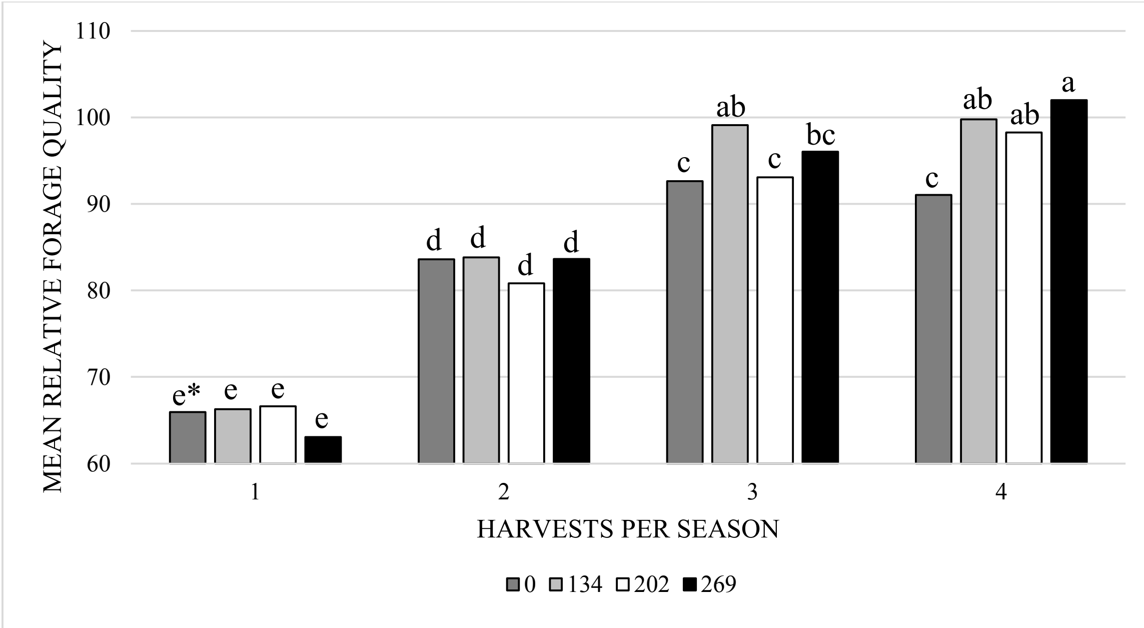

Figure 3.19 Mean relative forage quality (RFQ) for southeastern wildrye for harvests per season by nitrogen rates (0, 134, 202, and 269 kg ha^{-1} yr^{-1}) for Year 2 Starkville 2013.

*Letters indicate significant difference at α = 0.05.

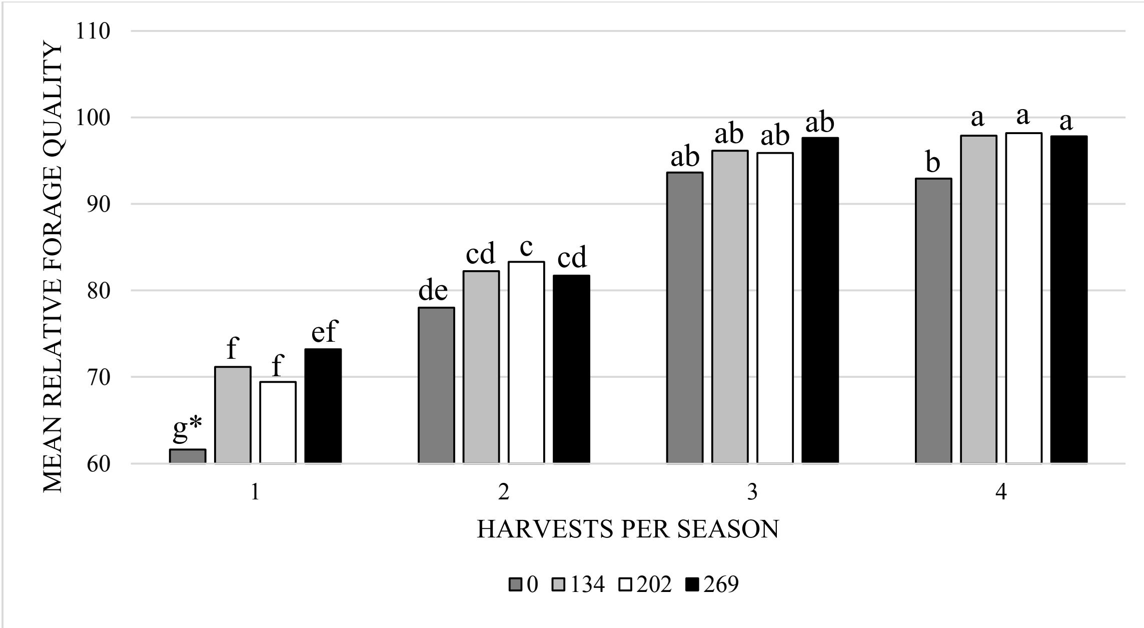

Figure 3.20 Mean relative forage quality (RFQ) for tall fescue for harvests per season by nitrogen rates (0, 134, 202, and 269 kg ha^{-1} yr^{-1}) for Year 2 Starkville 2013.

*Letters indicate significant difference at α = 0.05.

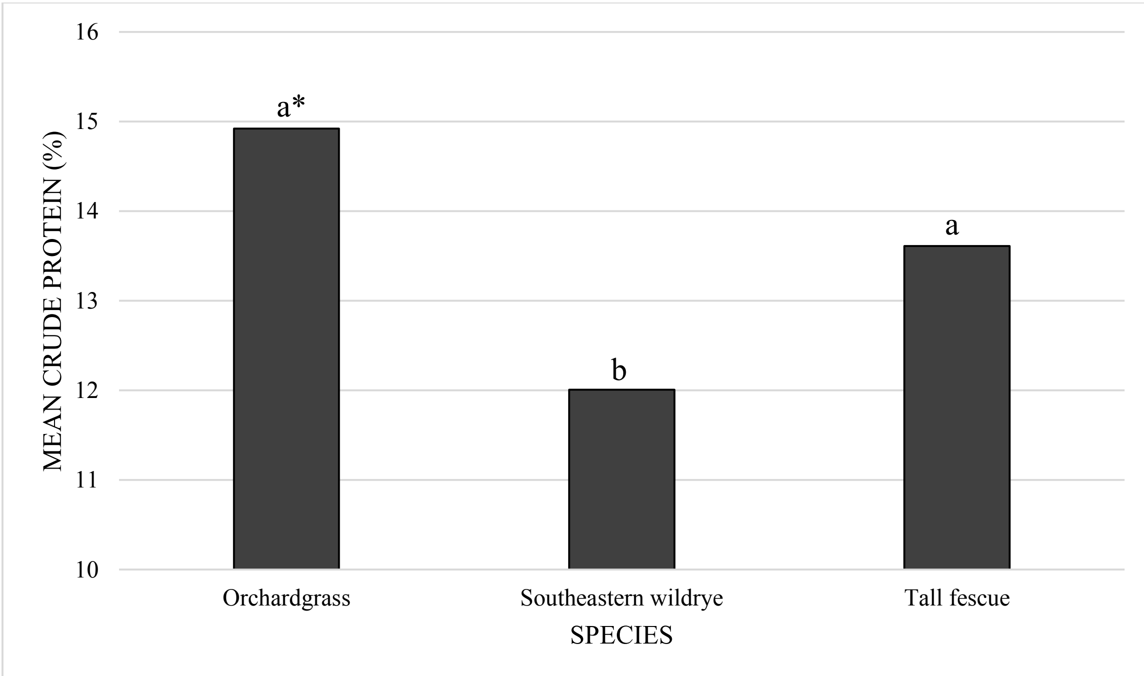

Figure 3.21 Mean crude protein (%) for cool-season grass species for Year 2 Starkville 2013.

*Letters indicate significant difference at α = 0.05.

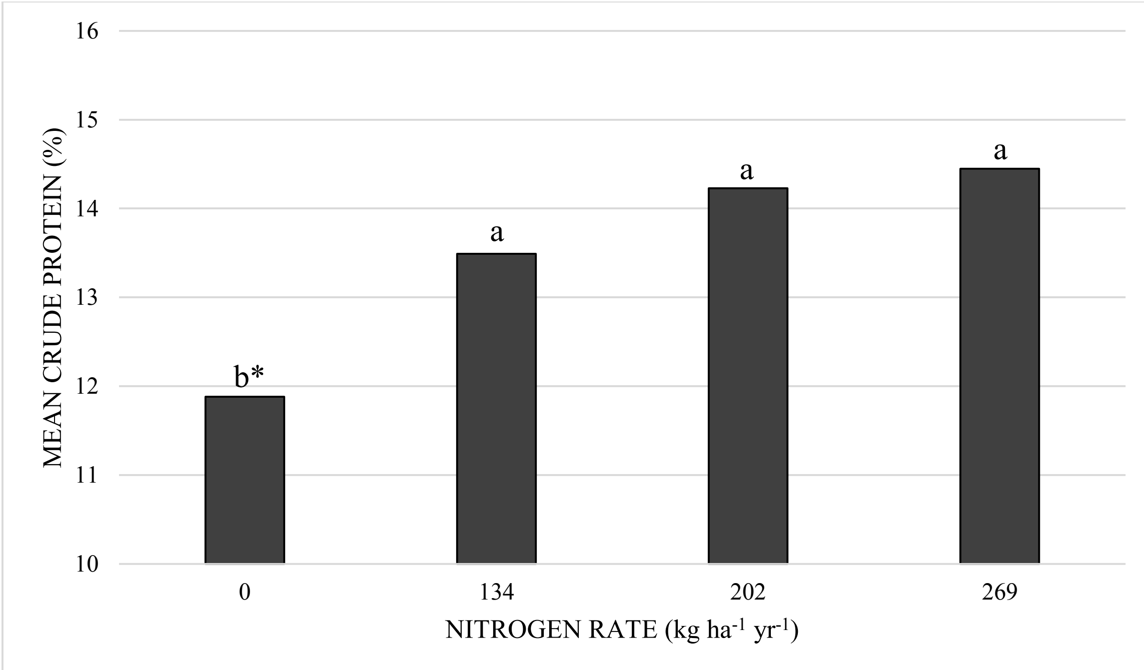

Figure 3.22 Mean crude protein (%) for nitrogen application rates for Year 2 Starkville 2013.

*Letters indicate significant difference at α = 0.05.

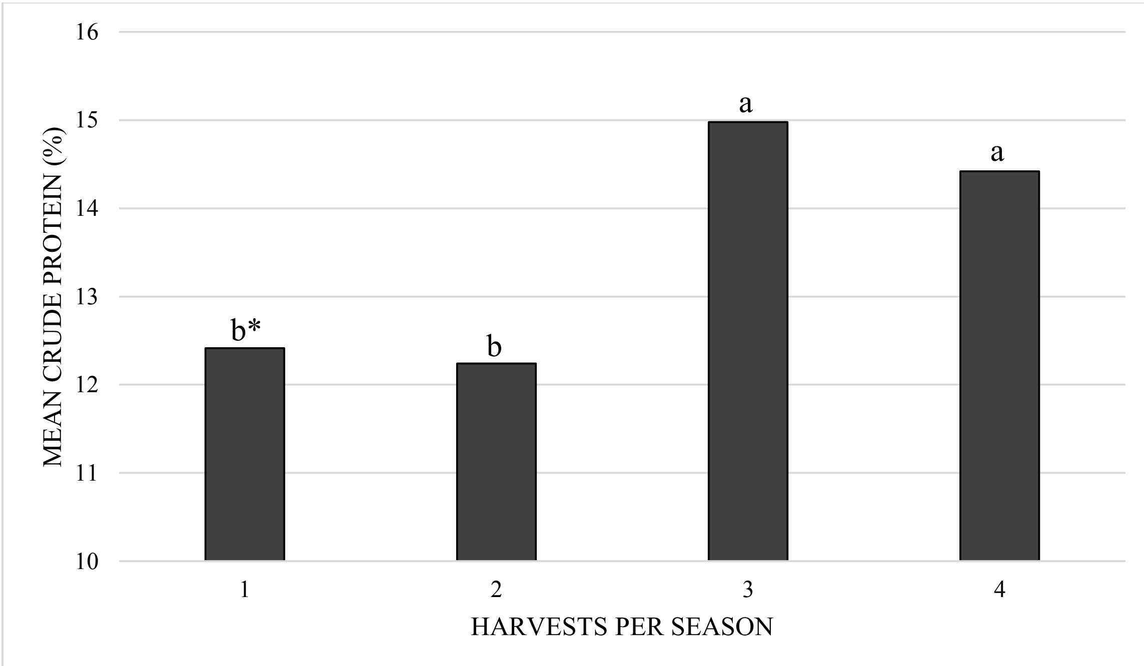

Figure 3.23 Mean crude protein (%) for harvests per season for Year 2 Starkville 2013.

*Letters indicate significant difference at α = 0.05.

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CHAPTER IV

NITROGEN USE EFFICIENCY OF THREE COOL-SEASON GRASSES SPECIES IN NORTH CENTRAL MISSISSIPPI

Abstract

Nitrogen fertilizer is the largest cost to a forage producer. While grasses respond well to nitrogen application, they (as do other crops) do not capture all the nitrogen applied to the field. The objective of this study was to evaluate the nitrogen use efficiency (NUE) of three cool-season grasses when combined with three N application rates and four harvest regimes. Two field studies were established in Starkville and Brooksville, MS, in October 2013 and 2014, respectively. Treatments were: cool-season grass species (orchardgrass, southeastern wildrye, and tall fescue); three N application rates (134, 202, and 269 kg ha^{-1} yr^{-1}, as well as an unfertilized control); and four harvest regimes (one, two, three, or four times during a 112-day season). All plots were fertilized in split applications every 28 days. Plots were harvested to a 10 cm stubble height. Subsamples were analyzed using near infrared reflectance spectroscopy for crude protein percentage (nitrogen content). Crop nitrogen use efficiency (NUE) was mathematically calculated using four equations: partial factor productivity (PFP), agronomic nitrogen use efficiency (ANUE), apparent nitrogen recovery (ANR), and physiological nitrogen use efficiency (PNUE). For Year 1 at both environments, as N application rates increased from 134 to 269 kg N ha^{-1} yr^{-1}, NUE as measured by PFP, ANUE, and ANR

decreased. Nitrogen use efficiency as measured by ANUE did not follow the same trend in Year 2 as Year 1. For Year 2 Starkville 2013 species was significant in combination with either harvest frequency or N application rate for NUE as measured by PFP, ANUE, and ANR. No matter which equation was used, environment and species, either in treatment combinations, or independently, were influenced by NUE to the greatest degree.

Introduction

Forage fertilization recommendations vary depending on geographic location, soil type, species composition, and management goals. The main goal of fertilization in any forage production system is to produce high-quality herbage that will meet the nutritional requirements of livestock while yielding an amount adequate to minimize the need for supplemental feed (Snyder and Leep, 2007). Proper utilization of fertilizer, appropriate application time, and in the optimal amount, on cool-season grass (CSG) forage production can be highly profitable.

The quantity and availability of nutrients within the soil profile impact the yield and quality of forage (Fales and Fritz, 2007). Nitrogen (N) is an essential element in chlorophyll construction and thus aids in photosynthesis (Ball et al., 2007). According to Ball et al. (2007) N is the macro-nutrient that has the greatest effect on overall plant development. Of the three primary plant nutrients (N, P, and K), N is needed in the largest quantity to maximize growth and is considered least readily available (Snyder and Leep, 2007).

Nitrogen fertilizer application, whether in solid or liquid form, is the prominent source of N for managed grasses. Forage plants require large amounts of N for growth, and considering the potential for loss of N, through leaching, ammonia volatilization, denitrification, etc., more than one application should be applied during the growing season (Ball et al., 2007). To help avoid N loss, fertilizers should be applied immediately prior to, or during, active forage growth (Snyder and Leep, 2007).

Cool-season grasses do not have the potential to produce as much vegetation as WSG and, as such, usually do not require as much nitrogen (Snyder and Leep, 2007). Tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.] is tolerant of low amounts of nutrients within the soil but reacts exceptionally well to fertilization (Ball et al., 2007). Balasko (1977) reported tall fescue yields in West Virginia to be two to three times greater when fertilized with N as compared to no N supplementation. Belesky et al. (1982) observed greater fresh and dry matter yields when using 200 kg N ha^{-1} on tall fescue in Georgia than when not applying N.

According to van Santen and Sleper (1996) orchardgrass [*Dactylis glomerata* L.] has been found to be one of the most responsive CSG to N fertilization due to the considerably higher dry matter yields produced when fertilized with N. Reynolds et al. (1969) studied orchardgrass in Tennessee and found that applying 224 kg N ha^{-1} produced greater dry matter yield than using 112 kg N ha^{-1} at three, four, and six week harvest intervals. Studies by Wedin (1974) on CSG fertility showed yields three to six times higher than normal with N as the primary nutrient supplied.

Studies have not only been conducted on maximizing yield with N but also on the efficiency plants to utilize applied N (Power, 1985; Guillard et al., 1995; Zemenchik and Albrecht, 2002; Lemus et al., 2008). Nitrogen use efficiency (NUE) for grasses is how much dry matter forage is produced per each unit of N applied (Zemenchik and Albrecht, 2002). Grass NUE can be influenced by multiple factors such as temperature, soil type, geographic location, species, and the amount of N applied (Wright and Davison, 1964).

Our objective was to evaluate orchardgrass, southeastern wildrye [*Elymus glabriflorus* (Vasey ex L.H. Dewey) Scribn. & C.R. Ball], aspeciesnd tall fescue to evaluate the impacts of N application and defoliation events on the NUE of each species.

Materials and Methods

A field trial was established at two locations: Henry H. Leveck Animal Research Center (South Farm) at Mississippi State University near Starkville, MS (33°26'15.63" N, 88°47'50.51" W) and at the Black Belt Branch Experiment Station of Mississippi State University near Brooksville, MS (33°15'38.72" N, 88°32'26.64" W). The soil type at the Starkville location was a Catalpa silty clay loam (fine, smectic, thermic, Fluvaquentic Hapludolls), moderately well drained with a pH of 5.6. The soil type at the Brooksville location was a Brooksville silty clay (fine, smectic, thermic, Aquic Hapludert), somewhat poorly drained with a pH of 7.2. Weather data for both locations was recorded (Tables D.1 and D.2). An initial soil test was taken prior to planting for each location. Fertilization, with the exception of N, was administered based on a soil test with recommendations for perennial cool-season forage grasses (Mississippi State University Soil Testing Lab). Pelletized lime ($CaCO_3$) was applied at a rate of 2.24 Mg ha^{-1} in Starkville 2013 prior to planting bringing soil pH to 6.2. Fertilization recommendations and soil pH did not indicate Brooksville 2014 required any additional lime.

Pre-plant burndown for both locations was achieved by applying Eraser™ A/P® glyphosate (N-[phosphonomethyl] glycine, isopropyl-amine salt; 41%) 2.76 kg ae ha^{-1}

once prior to tillage and again following tillage. Post emergence application of Banvel® [dimethylamine salt of dicamba (3,6-dichloro-o-anisic acid; 40%) 48.2%] was applied at 0.56 kg ae ha^{-1} for control of broadleaf weeds.

Three CSG species were established at Starkville (Year 1) and Brooksville (Year 1) on October 7 and 17, 2013 and 2014, respectively, in a prepared seed bed. The Starkville 2013 site was harvested in the spring of 2014 (Year 1/Establishment Year) and again in the spring of 2015 (Year 2). Brooksville 2014 was harvested in the spring of 2015 (Year 1/Establishment Year).

The three CSG species used were: southeastern wildrye (Foundation Seed, Mississippi State, MS), 'Potomac' orchardgrass (Ernst Conservation Seeds Inc., Meadville, PA), and 'Kentucky-31' tall fescue purchased from Oktibbeha Co. Farmers' Cooperative (Starkville, MS). Seed were drilled to a prepared seed bed using an Almaco® (Almaco, Nevada, IA) 8-row light duty grain drill at a depth of 0.6 cm. Planting rate was based on a pure live seed (PLS) rate of 16.8 kg ha^{-1} corresponding to a bulk seed rate of 56.3, 17.2, and 20.0 kg ha^{-1} for southeastern wildrye, orchardgrass, and tall fescue, respectively. Seeding rates correspond with those used by Rushing and Baldwin (2013) for southeastern wildrye, by Bates (1999) for orchardgrass, and by the Mississippi Cool-Season Forage Variety Trial Testing Program for tall fescue (White et al., 2013).

The study design consisted of a split plot in strips, with three treatments: CSG species, nitrogen application, and harvest regime. Each block was first split by species. Each species plot was superimposed by N application and harvest regime. Each block was randomized and replicated four times across the field. Individual plots were 1.8 m x

3.0 m with eight drilled rows per plot with 25.4 cm spacing. Nitrogen was applied using a Gandy® (Gandy Co., Owatonna, MN) 1.8 m drop spreader. Plots received 134, 202, and 269 kg $ha^{-1}yr^{-1}$ N of 33-0-0 S (ammonium sulfate & urea) in four split applications per season per specified plot every 28 days. An unfertilized control was also included. Plots were harvested one, two, three, or four times throughout the 112-day growing season. A Ferris® (Ferris, Munnsville, NY) zero-turn mower equipped with a bagging system and a 132.1 cm cutting width was used to harvest the center of each plot at a 10 cm stubble height (Brink and Casler, 2009, 2012; White et al., 2013). First harvest was conducted when 75% of the plots were ≥ 38 cm in height for both years, spring 2014 and spring 2015. In fall 2014, prior to second-year harvest for Starkville 2013, above ground biomass of deceased summer annuals were removed by hand to allow for cool-season grass growth.

Following each harvest a biomass subsample was collected from each plot and dried at 50°C until no further weight change was observed. For dry matter determination subsamples were ground to pass a 1 mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) for forage analysis. Nutritive value measurements of percent crude protein (CP) were obtained from near infrared reflectance spectroscopy using a Foss 6500C® (Foss North America, Eden Prairie, MN) using the grass hay equation from the NIR Forage and Feed Testing Consortium (Hillsboro, WI). Percentage N per plot was calculated using percentage crude protein values and the following equation:

Calculation: CP (dry matter basis) = % N (dry matter basis) x F

Where: F = 6.25 for all forages and feeds except wheat grain (4.1)

The following four equations were utilized for determining N use efficiency (NUE):

Partial Factor Productivity (PFP) (Cassman et al., 1998)

Calculation: PFP (kg kg^{-1}) = ($Y_0 + \Delta Y$) / N_r (4.2)

Where: Y_0 = yield at 0 N

ΔY = increment in yield that results from N application

N_r = rate of N applied

Agronomic Nitrogen Use Efficiency (ANUE) (Novoa and Loomis, 1981)

Calculation: ANUE (kg kg^{-1}) = (Biomass N_x – Biomass N_0) / NA (4.3)

Where: N_x = applied N rate

N_0 = control

NA = N applied

Apparent Nitrogen Recovery (ANR) (Crasswell and Godwin, 1984)

Calculation: ANR (%) = ((N Uptake* N_x – N Uptake N_0) / NA) x 100 (4.4)

Where: N_x = applied N rate

N_0 = control

NA = N applied

*To determine N uptake, multiply biomass x N concentration.

Physiological Nitrogen Use Efficiency (PNUE) (Cassman et al., 1998)

Calculation: PNUE (kg kg-1) = (Biomass Nx – Biomass N0) / (N Uptake* Nx – N Uptake N_0) (4.5)

Where: Biomass N_x = biomass of applied N rate

Biomass N_0 = biomass of control

N Uptake N_x = N content of biomass at applied N rate

N Uptake N_0 = N content of control

*To determine N uptake, multiply biomass x N concentration.

Statistical analysis for mean separation of each NUE equation value was conducted using PROC MIXED using SAS® software, Version 9.4 (SAS Institute, Cary, NC, 2013). Mean separations were based on Tukey's protected least significant difference (LSD) and considered significant at $\alpha = 0.05$. Since there is a difference in year x location, year x location will be referred to in this document as environment.

Results and Discussion

The NUE results varied significantly among environments. Each NUE calculation for Year 1, environments were significantly different from one another. Year 1 data for both environments were analyzed together. Year 2 Starkville 2013 data were analyzed separately from Year 1 environments.

Year 1 Results for Starkville 2013 and Brooksville 2014 Environments

Partial Factor Productivity (PFP)

Partial factor productivity was calculated using mean cumulative dry matter yields across harvests. Analysis of data indicated significant interactions of: environment x species ($P = 0.0004$), environment x N application ($P = 0.0072$), environment x harvests ($P = 0.0186$), species x N application ($P = 0.0060$), and species x harvests ($P = 0.0142$) (Table B.2). Since there was a significant interaction between environment x species, environment x N application, and environment x harvests, environment data sets were not pooled. For Year 1 Starkville 2013, NUE as measured by PFP for orchardgrass, southeastern wildrye, and tall fescue were 23.6, 23.1, and 23.0 kg kg^{-1}, respectively, and were not different from one another (Figure 4.1). For Year 1 Brooksville 2014, southeastern wildrye (22.2 kg kg^{-1}) was greater than tall fescue (15.8 kg kg^{-1}). Both were greater than orchardgrass (11.9 kg kg^{-1}). Nitrogen application rate also affected NUE as measured by PFP. For Year 1 Starkville 2013, N applied at 134 kg N ha^{-1} yr^{-1} had a PFP of 30.8 kg kg^{-1} and was significantly greater than the 202 kg N ha^{-1} yr^{-1} treatment with a PFP of 21.4 kg kg^{-1}; which was significantly greater than the 269 kg N ha^{-1} yr^{-1} treatment with a PFP of 17.6 kg kg^{-1} (Figure 4.2). The same results, but slightly lower values, were observed with Year 1 Brooksville 2014. This trend for both Year 1 environments indicate that NUE as measured by PFP decreases as N application rate increases. Greater waste occurs with more N applied. Harvest regime also affected PFP as a measure of NUE. For Year 1 Starkville 2013, two harvests per season (26.4 kg kg^{-1}) had greater efficiency than all other treatments: one, three, or four harvests (21.7, 23.3, 21.7 kg kg^{-1}, respectively) (Figure 4.3); with one, three, and four harvests not different from one another. For Year 1 Brooksville 2014 data, no differences were noted for NUE as measured by PFP for harvest regime due to lack of rain or timing of rain following fertilization. Fertilizer prills were observed in tact prior to the first harvest of the 38-day interval. For all of the aforementioned environment x species, environment x N application, and environment x harvests interactions, only N application had the same trend for both Year 1 environments. These results were consistent with those found by Lemus et al. (2008). They found that increasing N applied from 90 kg N ha^{-1} to 180 kg N ha^{-1} decreased NUE of switchgrass in Blacksburg, VA. Brink and Casler (2009) also found that applying more than 134 kg N ha^{-1} yr^{-1} to meadow fescue [*Schedonorus pratensis* (Huds.) P. Beauv.], tall fescue, and orchardgrass caused a decrease in NUE as measured by PFP.

Due to a lack of significant difference by environment, interactions of species x N application and species x harvests were pooled across environments. For species x N application southeastern wildrye at the 134 kg N ha^{-1} yr^{-1} treatment, with a PFP of 30.7 kg kg^{-1}, was greater than all other combinations with respect to NUE as measured by PFP (Figure 4.4). Orchardgrass at the 269 kg N ha^{-1} yr^{-1} treatment with a PFP of 14.3 kg kg^{-1} was the lowest, but not different from southeastern wildrye and tall fescue at the same N application rate and orchardgrass at the 202 kg N ha^{-1} yr^{-1} (16.0, 15.4, and 16.0 kg kg^{-1}, respectively). When each species was separated from one another, the N application rate of 134 kg N ha^{-1} yr^{-1} resulted in significantly greater PFP than those of 202 and 269 kg N ha^{-1} yr^{-1} for all species (Figure 4.4). When each N application is examined individually, southeastern wildrye was significantly more efficient as measured by PFP than orchardgrass and tall fescue at the 134 kg N ha^{-1} yr^{-1} treatment (30.7, 23.0, and 25.4 kg

kg^{-1}, respectively) and the 202 kg N ha^{-1} yr^{-1} treatment (21.3, 16.0, and 17.5, respectively). Estimates of NUE by PFP are also affected by a species x harvests interaction. Southeastern wildrye harvested two times during the 112-day season (25.9 kg kg^{-1}) was greater than all other treatment combinations (Figure 4.5). Orchardgrass harvested once (16.2 kg kg^{-1}) was the lowest but not different from orchardgrass harvested three (17.5 kg kg^{-1}) or four times (17.7 kg kg^{-1}) or tall fescue harvested one (18.0 kg kg^{-1}) or two times (18.7 kg kg^{-1}).

Evaluating each harvest to distinguish which species had the greatest NUE as measured by PFP showed that when harvested one or two times southeastern wildrye (22.8 and 25.9 kg kg^{-1}, respectively) was greater than orchardgrass (16.2 and 19.6 kg kg^{-1}, respectively) and tall fescue (18.0 and 18.7 kg kg^{-1}, respectively) (Figure 4.5). When harvested three times, southeastern wildrye (21.9 kg kg^{-1}) and tall fescue (20.7 kg kg^{-1}) had greater efficiency than orchardgrass (17.5 kg kg^{-1}) with neither of the former being significantly different from each other. However when harvested four times, no significant differences were observed for PFP value among any of the species.

Agronomic Nitrogen Use Efficiency (ANUE)

Agronomic NUE was calculated using mean cumulative dry matter yields across harvests. No significant interactions were observed. Environment ($P = 0.0115$) and N application ($P = 0.0019$) were independently significant with respect to NUE as measured by ANUE (Table B.3). Year 1 Starkville 2013 as a whole had significantly greater ANUE (8.2 kg kg^{-1}) than Year 1 Brooksville 2014 (5.8 kg kg^{-1}) (Figure 4.6). Since N application was independently significant, environments were pooled. The lowest N application rate, 134 kg N ha^{-1} yr^{-1} (8.4 kg kg^{-1}), had greater ANUE than 202 and 269 kg

N ha^{-1} yr^{-1} (6.3 and 6.3 kg kg^{-1}, respectively) (Figure 4.7). Power (1985) reported greater NUE when fertilizing multiple CSG in North Dakota with the lowest treatment (45 kg N ha^{-1}) as compared to the greatest N rate (225 kg N ha^{-1}). He reported NUE values two to three times greater at the lowest treatment, which is not consistent with our data. Marino and other (2004) reported NUE decreasing in annual ryegrass [*Lolium multiflorum* Lam.] as N rates increased from 50 to 250 kg N ha^{-1}. Nitrogen use efficiency as measured by ANUE followed the same basic trend as for PFP. Plots fertilized with 134 kg N ha^{-1} yr^{-1} had greater NUE as measured by ANUE and PFP than 202 and 269 kg N ha^{-1} yr^{-1}. These results were consistent with Lemus et al. (2008).

Apparent Nitrogen Recovery (ANR)

Apparent nitrogen recovery was calculated using mean cumulative dry matter yields across harvests. There was a significant interaction between environment x species (P = 0.0195) (Table B.4). Since environments were different, data were not pooled. For Year 1 Starkville 2013 both orchardgrass and tall fescue were greater for NUE as measured by ANR (26.2% and 24.6%, respectively) than southeastern wildrye (12.1%) (Figure 4.8); orchardgrass and tall fescue were not different from one another. For Year 1 Brooksville 2014 there was no significant species effect due to lack of rain or timing of rain following fertilization.

The independent treatments of N application (P = 0.0012) and harvests (P = 0.0442) were significant with respect to NUE as measured by ANR. The lowest N application rate of 134 kg N ha^{-1} yr^{-1} had an ANR of 20.7% and was greater than the ANR of the 202 and 269 kg N ha^{-1} yr^{-1} treatments (15.3 and 15.5%, respectively) (Figure 4.9). This recovery percentage pattern was the same as the PFP and ANUE pattern that lower N application rates resulted in greater N recovery (use efficiency) and consistent with Lemus et al. (2008). Hallock et al. (1973) found the percentage of average annual N uptake by tall fescue in Virginia decreased as N treatments increased from 263 kg N ha^{-1} yr^{-1} to 1,120 hg N ha^{-1} yr^{-1}. Wagner (1954) found that 90 and 179 kg N ha^{-1} applied to orchardgrass in Maryland was greater for NUE as measured by ANR than using 269 kg N ha^{-1}. For harvest treatments, plots harvested one (19.2%) or two (18.8%) times had significantly greater ANR than plots harvested four (13.0%) times, but harvesting three (17.7%) times was the same as the one or two harvest treatments (Figure 4.10). This indicates that as number of harvests per season increase beyond two harvests, the NUE of the grass species decreases.

Physiological Nitrogen Use Efficiency (PNUE)

Physiological nitrogen use efficiency was calculated using mean cumulative dry matter yields across harvests. After calculating PNUE from raw data (n = 288), all negative PNUE values and those beyond three standard deviations from the mean (outliers) were removed (n = 42) from the data set. There was a significant interaction between environment x harvests (P = 0.0207) (Table B.6). Since environments were different, data were not pooled. For Year 1 Starkville 2013 two (46.7 kg kg^{-1}), three (44.1 kg kg^{-1}), and four (41.7 kg kg^{-1}) harvests per season had greater NUE as measured by PNUE than one (31.6 kg kg^{-1}) harvest (Figure 4.11). Plots harvested two, three, and four times were not different from one another. For Year 1 Brooksville 2014 the highest frequency of harvests, four (67.7 kg kg^{-1}), was greater for NUE as measured by PNUE than the other treatments: one, two, or three harvests (39.3, 40.0, or 48.6 kg kg^{-1}, respectively).

Year 2 Results for Starkville 2013 Environment

Nitrogen use efficiency results were calculated using cumulative mean dry matter yields across harvests. For each NUE equation for Year 2 environments, species were significantly different from one another, either in combination with another treatment or independently.

Partial Factor Productivity (PFP)

The interactions of species x harvests ($P < 0.0001$) and species x N application ($P = 0.0033$) were significant with respect to PFP (Table B.8).

Interactions indicate species behaved differently under the various harvest regimes. For the species x harvests interaction (Figure 4.12), southeastern wildrye at one (28.8 kg kg^{-1}) harvest and tall fescue at two (28.1 kg kg^{-1}) and three (30.2 kg kg^{-1}) harvests had greater NUE as measured by PFP (25.8 kg kg^{-1}) than the other treatments, except for tall fescue at one harvest. These results were not the same as Year 1 Starkville 2013 (Figure 4.5). In Year 1 southeastern wildrye declined in NUE as measured by PFP as harvest frequency increased beyond two harvests. In Year 2 southeastern wildrye declined in NUE as measured by PFP as harvest regimes increased. However, tall fescue increased in NUE as measured by PFP with the higher harvest frequencies, with a maximum of three harvests, in Year 2. Southeastern wildrye also decreased in NUE as measured by PFP at each harvests, except for the single harvest, from Year 1 to Year 2. Tall fescue increased in NUE as measured by PFP at each harvest from Year 1 to Year 2.

For the species x N application interaction (Figure 4.13), all species had the greatest NUE as measured by PFP at the lowest N application rate (134 kg N ha^{-1} yr^{-1}). Tall fescue under the 134 kg N ha^{-1} yr^{-1} treatment (34.1 kg kg^{-1}) showed the greatest NUE as measured by PFP compared to all other treatment combinations. Orchardgrass and southeastern wildrye were no different from one another at any N application rate. Again PFP indicated greater NUE at lower N application rates which was the same trend observed in Year 1. However, southeastern wildrye and tall fescue behaved differently in Year 2 than Year 1. Southeastern wildrye declined in NUE as measured by PFP at each N application rate from Year 1 to Year 2, whereas tall fescue increased in NUE as measured by PFP at each N application rate from Year 1 to Year 2.

Agronomic Nitrogen Use Efficiency (ANUE)

For ANUE there was a significant interaction for species x harvests (P = 0.0079) and independently for N application rate (P = 0.0005) (Table B.9). Addressing the species x harvests interaction first, tall fescue harvested three (14.4 kg kg-1) or four (13.8 kg kg-1) times had greater NUE as measured by ANUE than orchardgrass harvested one, three, or four times (8.5, 8.4, and 5.0 kg kg-1, respectively), southeastern wildrye harvested three or four times (7.9 and 5.7 kg kg-1, respectively), and tall fescue harvested one time (5.8 kg kg-1) (Figure 4.14). While each species performs differently for NUE as measured by ANUE, it appears tall fescue is able to maintain relatively high NUE under the most rigorous harvest regimes. Nitrogen application rate during Year 2 in Starkville was independently significant for NUE as measured by ANUE. The ANUE for the N treatment of 202 kg N ha-1 yr-1 (8.1 kg kg-1) was lower than either 134 or 269 kg N ha-1 yr-1 (10.4 or 9.5 kg kg-1, respectively) (Figure 4.15). This trend does not mimic prior NUE measurements of PFP, ANR, or ANUE (Year 1), and we are at a loss as to the reason for these results.

Apparent Nitrogen Recovery (ANR)

For ANR there was a significant interaction for species x harvests (P = 0.0242). (Table B.10). Tall fescue harvested three times had greater NUE (40.7%) as measured by ANR than all other treatment combinations, except for southeastern wildrye harvested one time (29.9%) and tall fescue harvested four times (35.2%) (Figure 4.16). Analyzing each species for harvests, no species was significantly different from one another when harvesting one or two times. Apparent nitrogen recovery followed the same trend as PFP and ANUE at the three harvest regime. Tall fescue was greater than orchardgrass and southeastern wildrye with neither being different from one another.

Physiological Nitrogen Use Efficiency (PNUE)

For NUE as measured by PNUE, there was a significant difference among species (P = 0.0120). (Table B.11). Southeastern wildrye recovered 45.9 kg kg^{-1} of N applied as measured by PNUE (Figure 4.17). This was greater than both orchardgrass and tall fescue (32.0 and 31.5 kg kg^{-1}, respectively). Southeastern wildrye was not more efficient than the other species in any other NUE calculation for Year 2 Starkville 2013. It should also be noted that NUE of southeastern wildrye could have been inflated by the infestation of summer annuals observed in plots harvested more than once (Table A.15).

Summary

When calculating NUE, the specific equation used defines the significance among all treatments. No matter which equation was used, environment and species, either in treatment combinations or independently, influenced the measurement of NUE to the greatest degree. For Year 1 across both environments, each equation was significant by environment, either in a treatment combination or independently. Environmental factors, such as average rainfall (water availability) and soil texture, could have played a role in the amount of N taken up by the plant, volatilized, or leached (Tables D.1 and D.2). In Year 1 when harvesting the first of three harvests in Brooksville, fertilizer applied ten days earlier following the 28 day fertilizing increment was observed to be intact on top of the soil. The first fertilizer application was not followed by a rain event or not a substantial one since fertilizer prills were observed on top of the ground ten days later. The fertilizer was unable to penetrate the soil surface for plant uptake and could have reduced NUE for the subsequent harvest. Hargrove et al. (1977) reported 40-41% N loss due to ammonia volatilization when applying ammonium sulfate as pelleted fertilizer to calcareous soils. Gasser (1964) reported ammonium sulfate has greater losses on calcareous soils than urea, but Raun and Johnson (1999) noted that when urea is not properly incorporated into the soil following application, 40% can be lost as ammonia. The fertilizer used in our study was 50% urea 50% ammonium sulfate so ammonia volatilization was possible.

For Year 2 of Starkville 2013 all NUE calculations were significant by species, either in a treatment combination or independently. Species behave differently with respect to NUE. Tall fescue increased efficiency with respect to NUE as measured by PFP, ANUE, and ANR as harvest regimes increased compared to orchardgrass and southeastern wildrye. Southeastern wildrye had the greatest NUE measured by PNUE. However, as previously stated, NUE could have been inflated by the infestation of summer annuals observed in the southeastern wildrye plots harvested more than once.

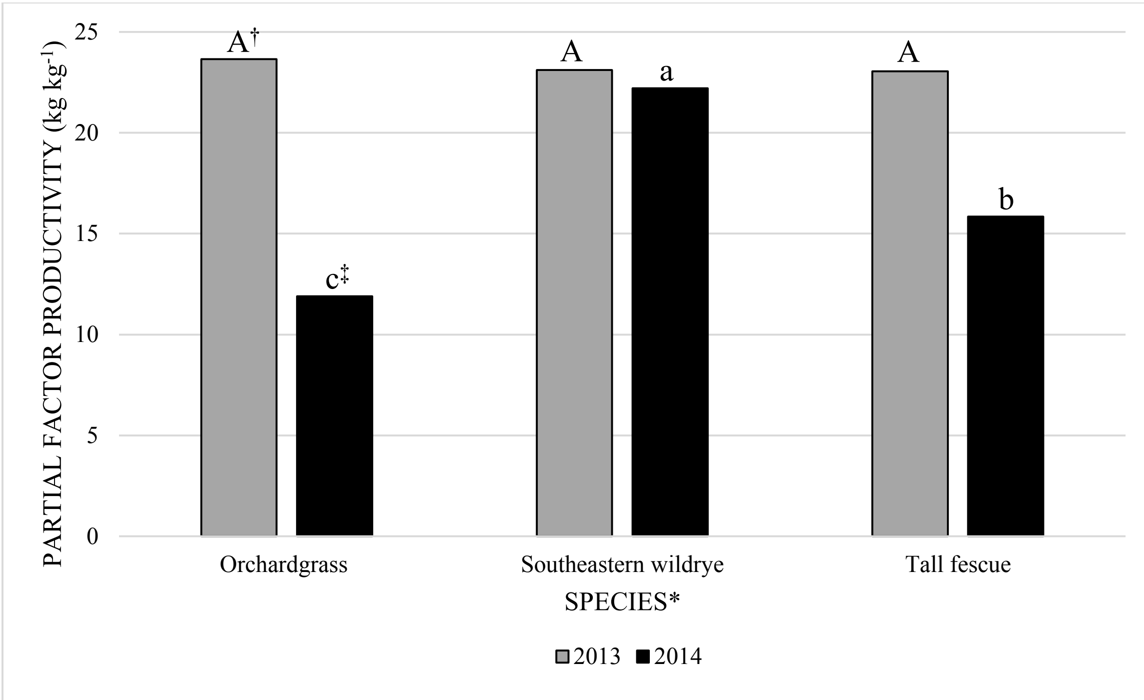

Figure 4.1 Mean partial factor productivity (PFP) for cool-season grass species by environment for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

*Cool-season grass species x Year 1 environments indicate significant differences at $\alpha = 0.05$, $P = 0.0004$, thus data were not pooled among environments.
†UPPERCASE letters indicate significant differences among Year 1 Starkville 2013.
‡lowercase letters indicate significant differences among Year 1 Brooksville 2014.

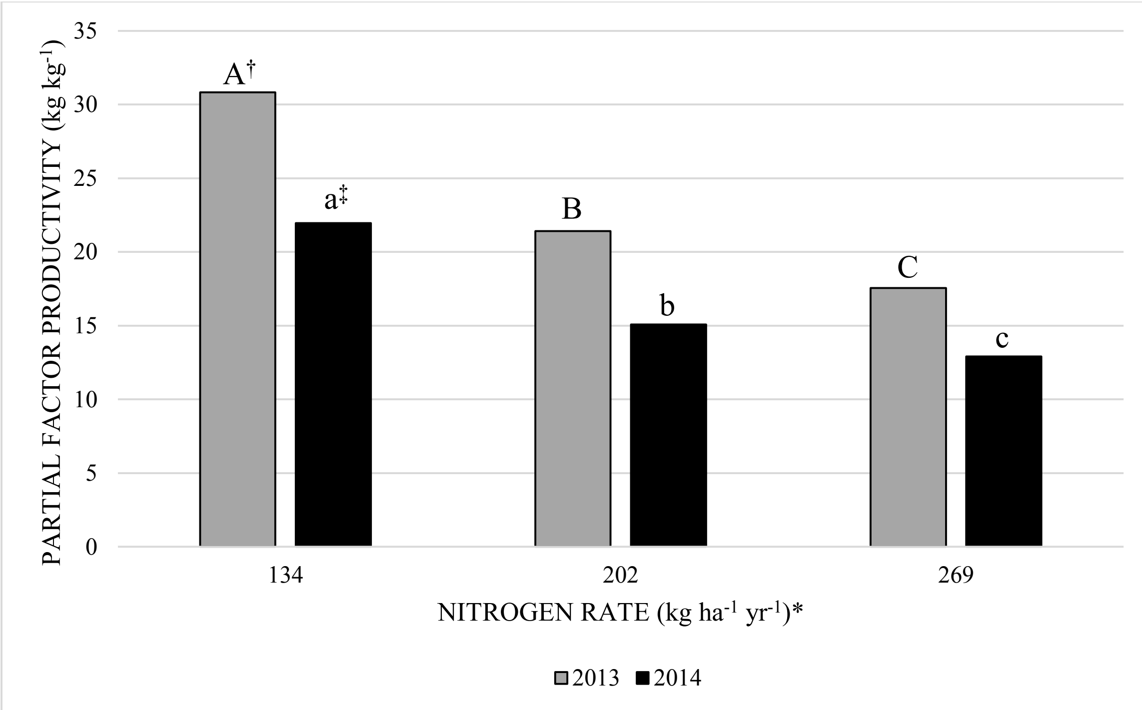

Figure 4.2 Mean partial factor productivity (PFP) for nitrogen rate by environment for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

*Nitrogen rate x Year 1 environments indicate significant differences at $\alpha = 0.05$, $P = 0.0195$, thus data were not pooled among environments.
†UPPERCASE letters indicate significant differences among Year 1 Starkville 2013.
‡lowercase letters indicate significant differences among Year 1 Brooksville 2014.

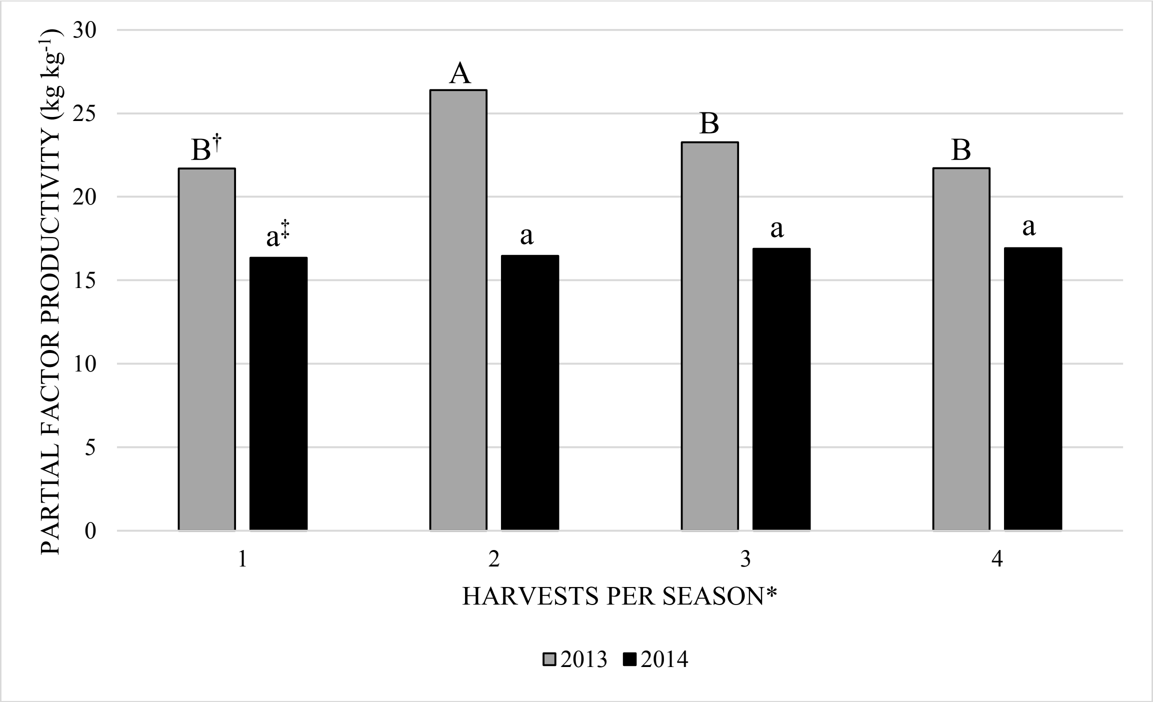

Figure 4.3 Mean partial factor productivity (PFP) for harvests per season by environment for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

*Harvests per season x Year 1 environments indicate significant differences at $\alpha = 0.05$, $P = 0.0186$, thus data were not pooled among environments.
†UPPERCASE letters indicate significant differences among Year 1 Starkville 2013.
‡lowercase letters indicate significant differences among Year 1 Brooksville 2014.

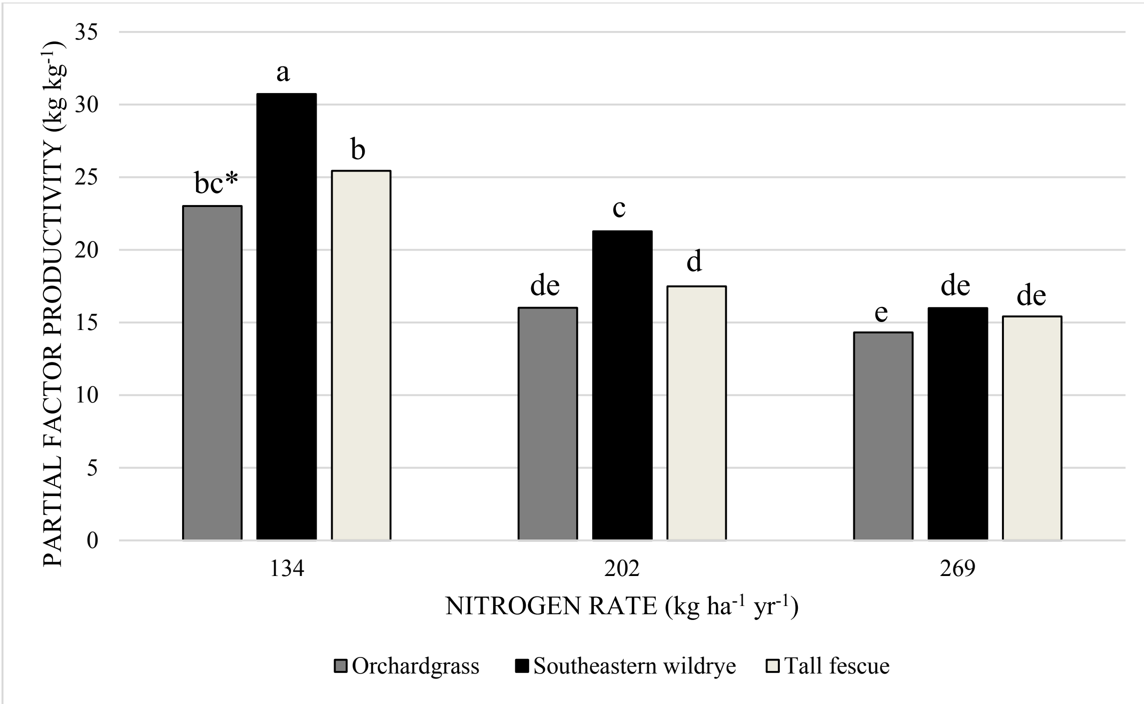

Figure 4.4 Mean partial factor productivity (PFP) for nitrogen rates by cool-season grass species pooled across Year 1 Starkville 2013 and Year 1 Brooksville 2014.

*Letters indicate significant difference at $\alpha = 0.05$.

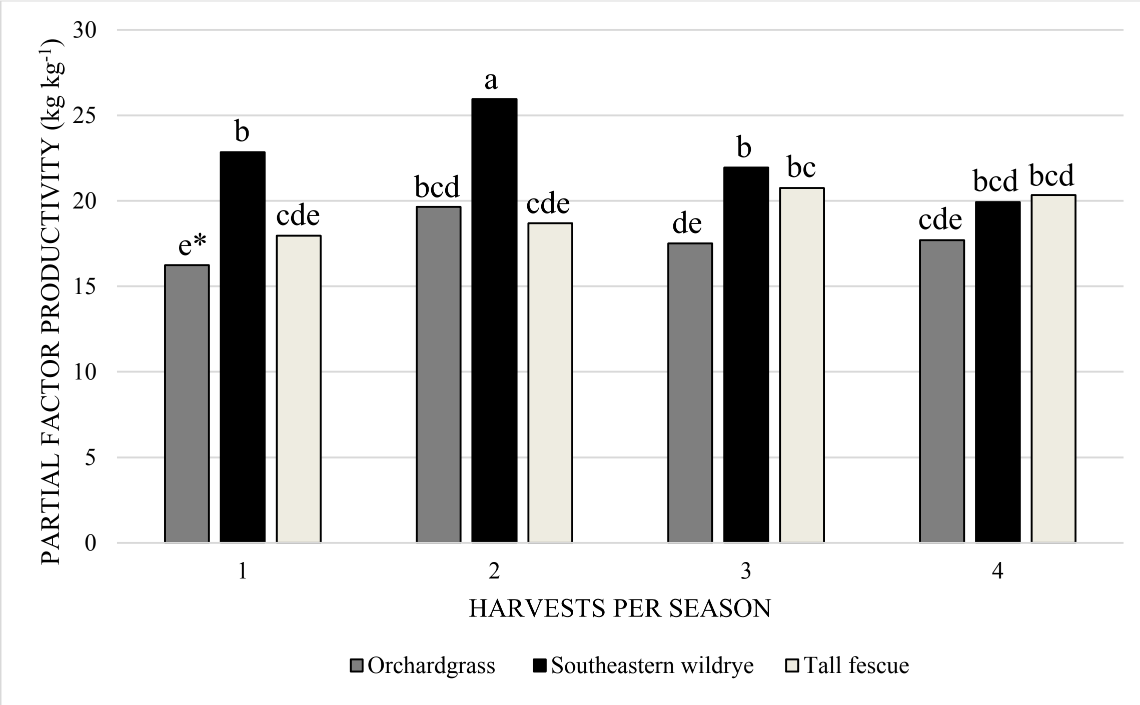

Figure 4.5 Mean partial factor productivity (PFP) for harvests per season by cool-season grass species pooled across Year 1 Starkville 2013 and Year 1 Brooksville 2014.

*Letters indicate significant difference at $\alpha = 0.05$.

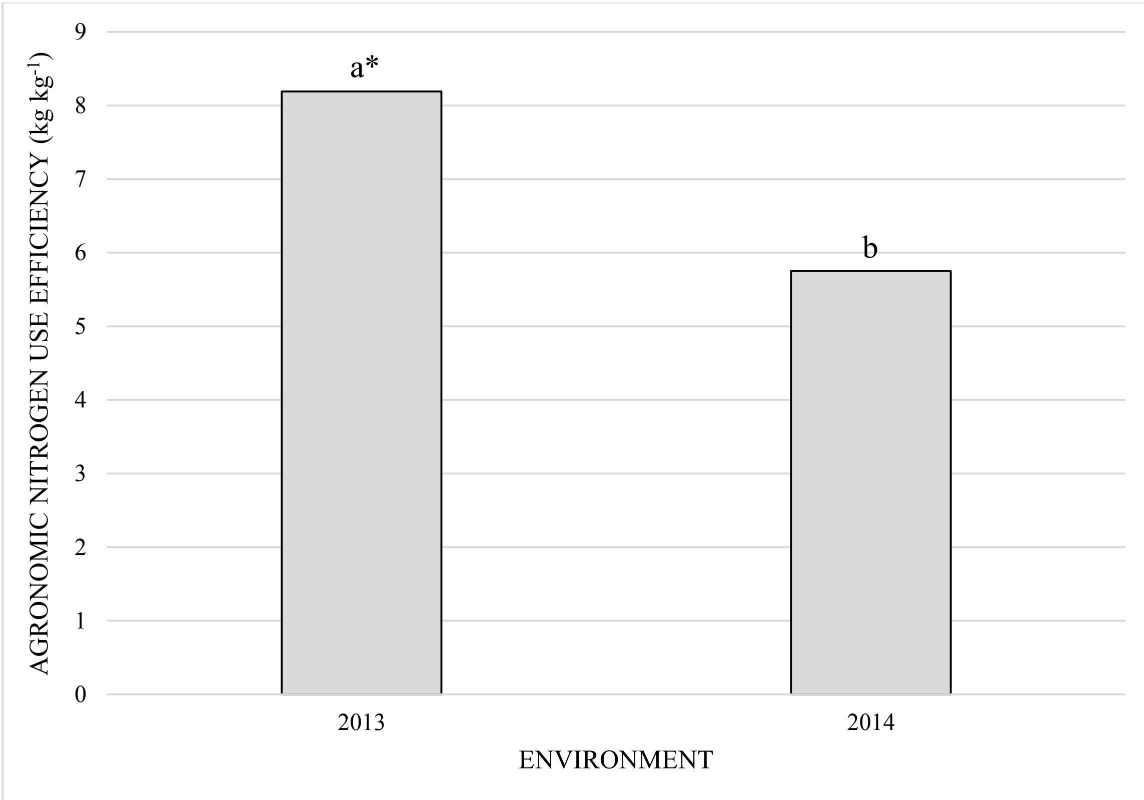

Figure 4.6 Mean agronomic nitrogen use efficiency (ANUE) for environment for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

*Letters indicate significant difference at $\alpha = 0.05$.

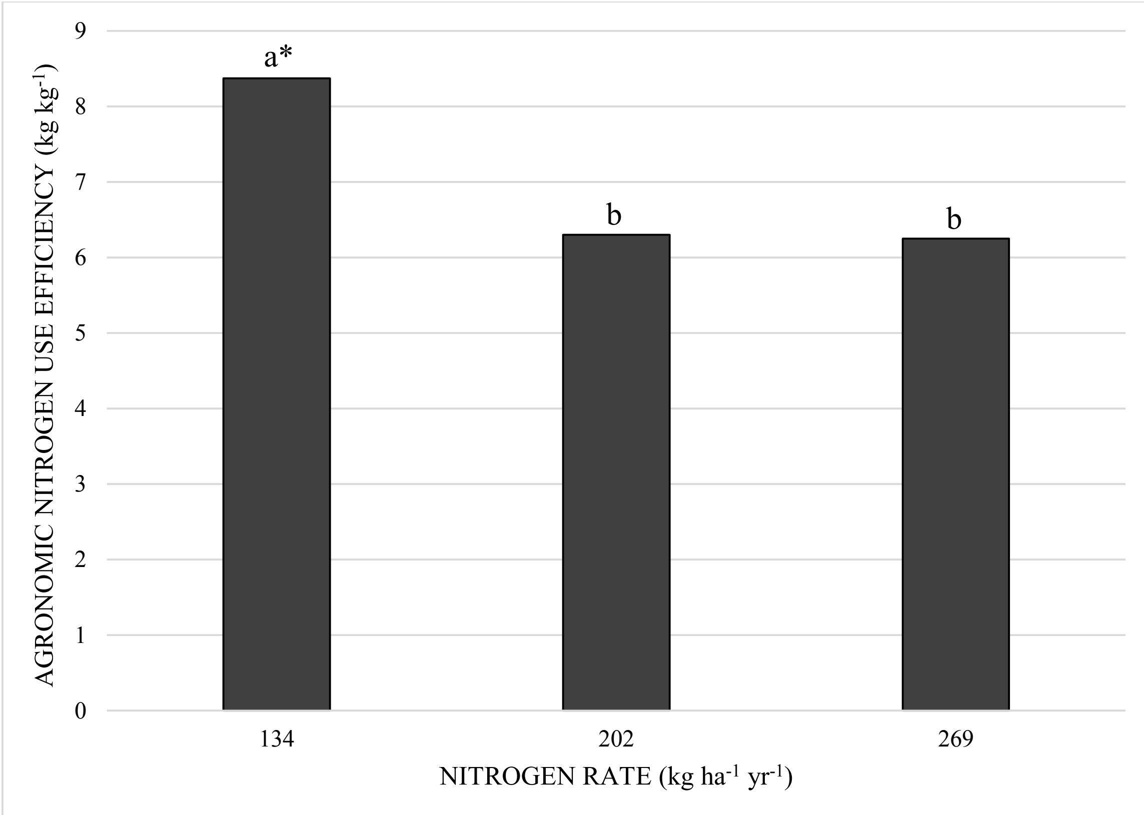

Figure 4.7 Mean agronomic nitrogen use efficiency (ANUE) for nitrogen rates pooled across environments for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

*Letters indicate significant difference at $\alpha = 0.05$.

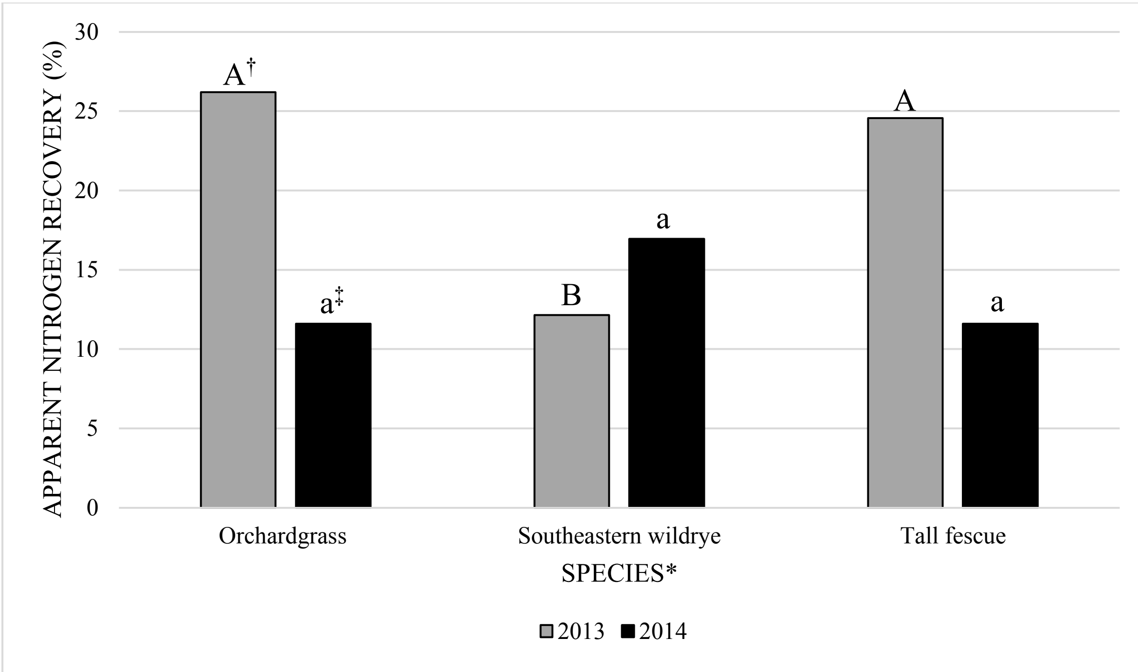

Figure 4.8 Mean apparent nitrogen recovery (ANR) for cool-season grass species by environment for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

*Cool-season grass species x Year 1 environments indicate significant differences at $\alpha = 0.05$, $P = 0.0195$, thus data were not pooled among environments.
†UPPERCASE letters indicate significant differences among Year 1 Starkville 2013.
‡lowercase letters indicate significant differences among Year 1 Brooksville 2014.

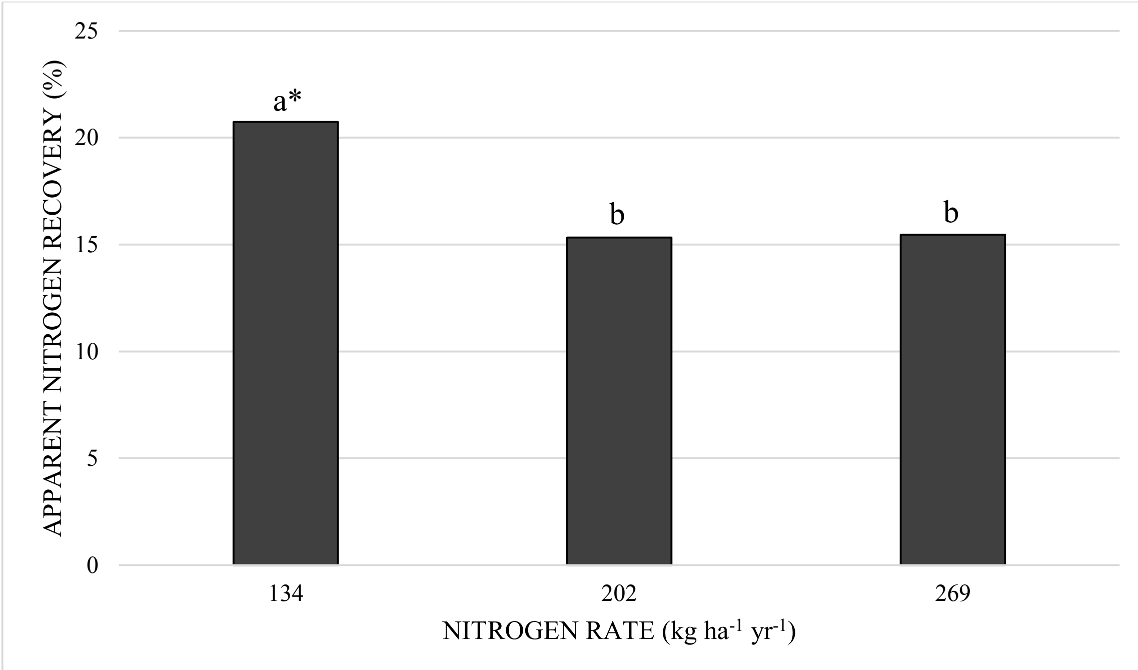

Figure 4.9 Mean apparent nitrogen recovery (ANR) for nitrogen rates pooled across environments for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

*Letters indicate significant difference at α = 0.05.

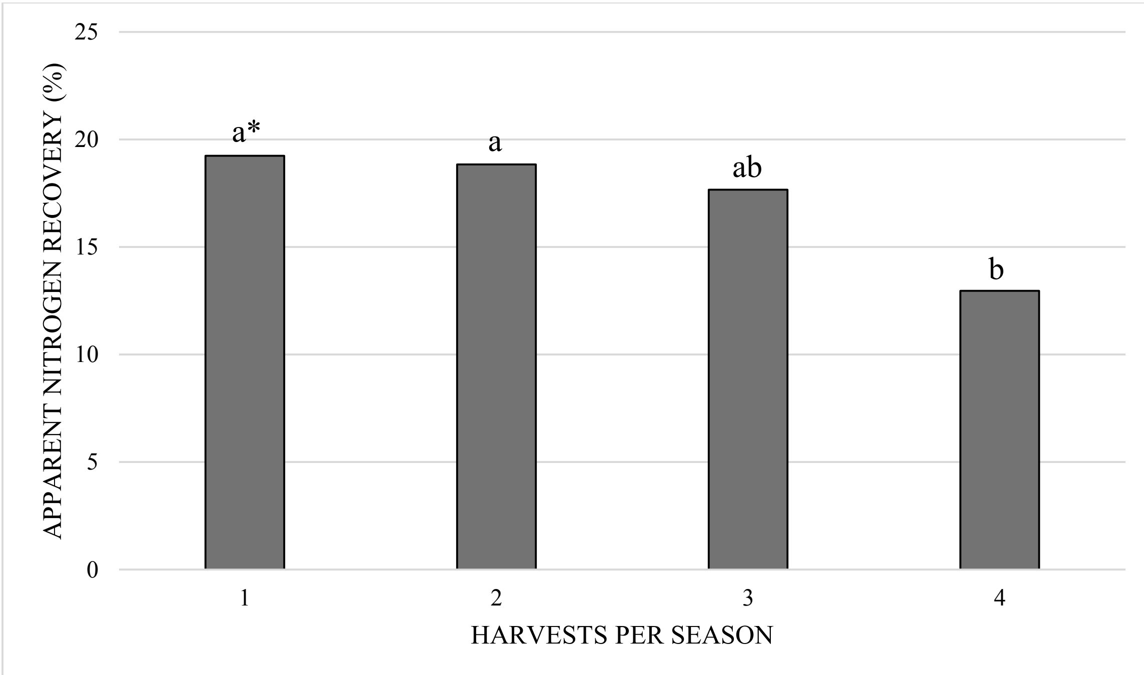

Figure 4.10 Mean apparent nitrogen recovery (ANR) for harvests per season pooled across environments for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

*Letters indicate significant difference at α = 0.05.

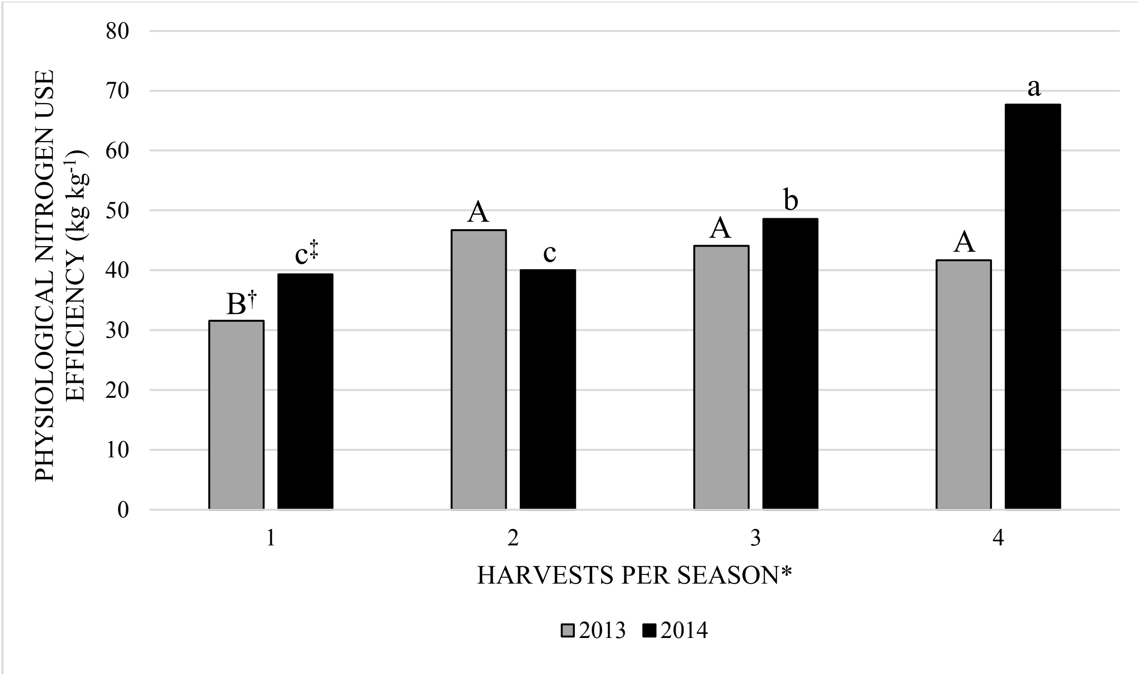

Figure 4.11 Mean physiological nitrogen use efficiency (PNUE) for harvests per season by environment for Starkville 2013 Year 1 and Brooksville 2014 Year 1.

*Harvests per season x Year 1 environments indicate significant differences at $\alpha = 0.05$, $P = 0.0207$, thus data were not pooled among environments.
†UPPERCASE letters indicate significant differences among Year 1 Starkville 2013.
‡lowercase letters indicate significant differences among Year 1 Brooksville 2014.

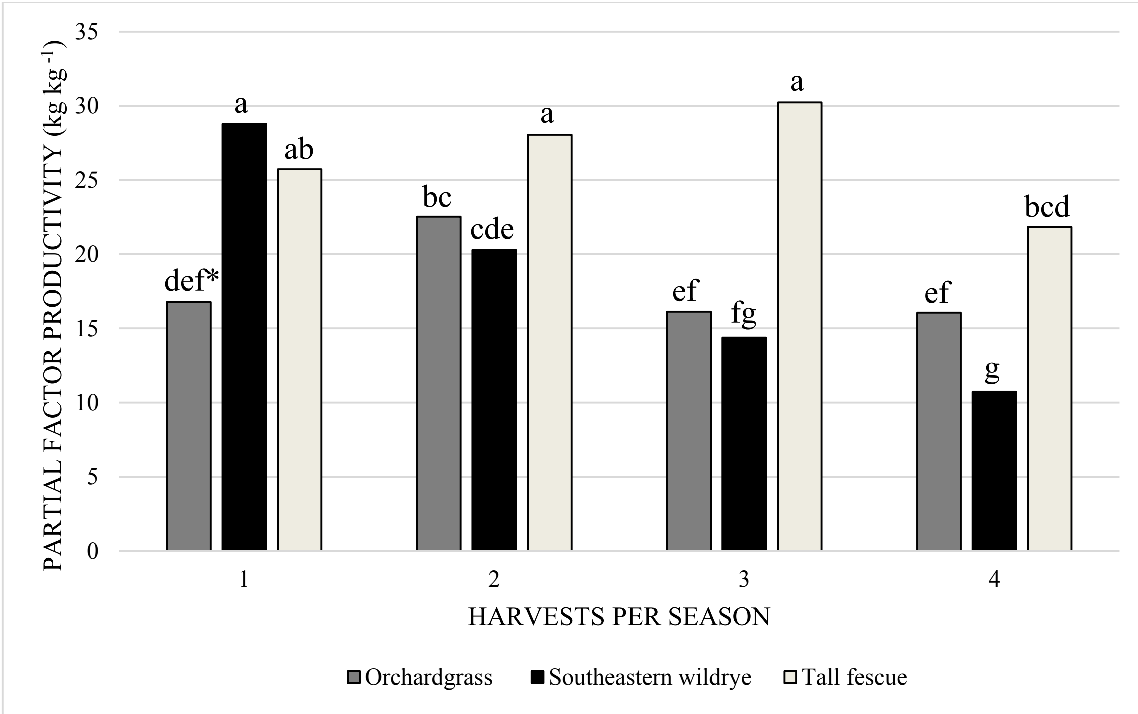

Figure 4.12 Mean partial factor productivity (PFP) for harvests per season by cool-season grass species for Year 2 Starkville 2013.

*Letters indicate significant difference at α = 0.05.

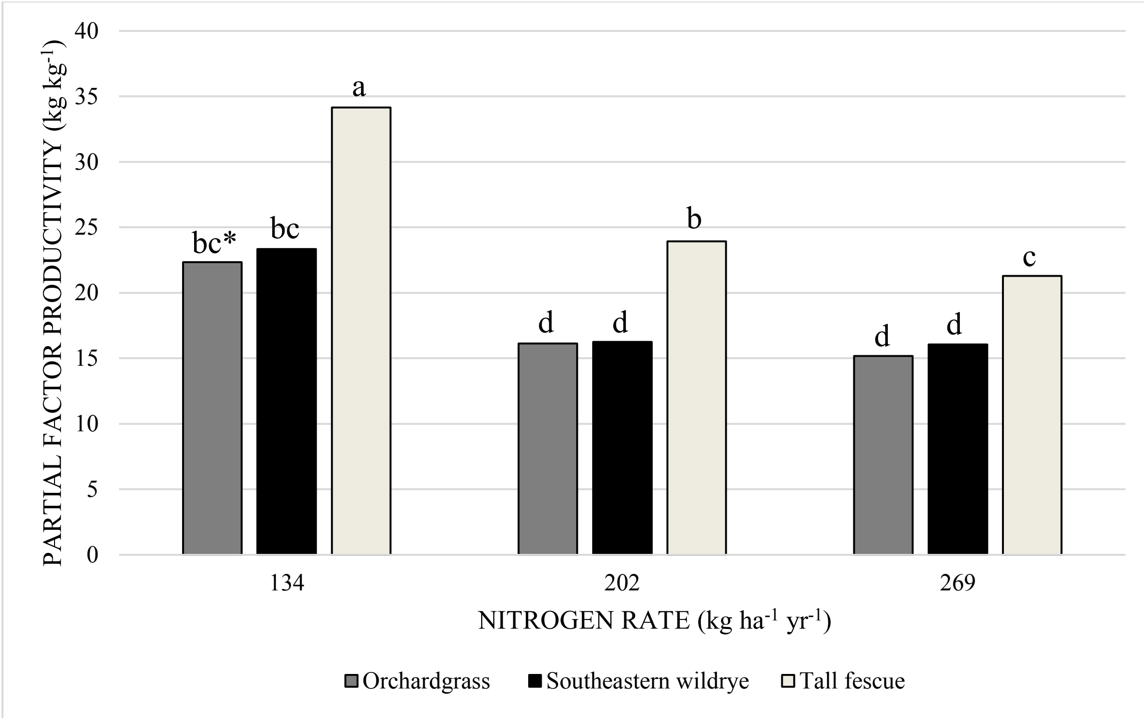

Figure 4.13 Mean partial factor productivity (PFP) for nitrogen rates by cool-season grass species for Year 2 Starkville 2013.

*Letters indicate significant difference at $\alpha = 0.05$.

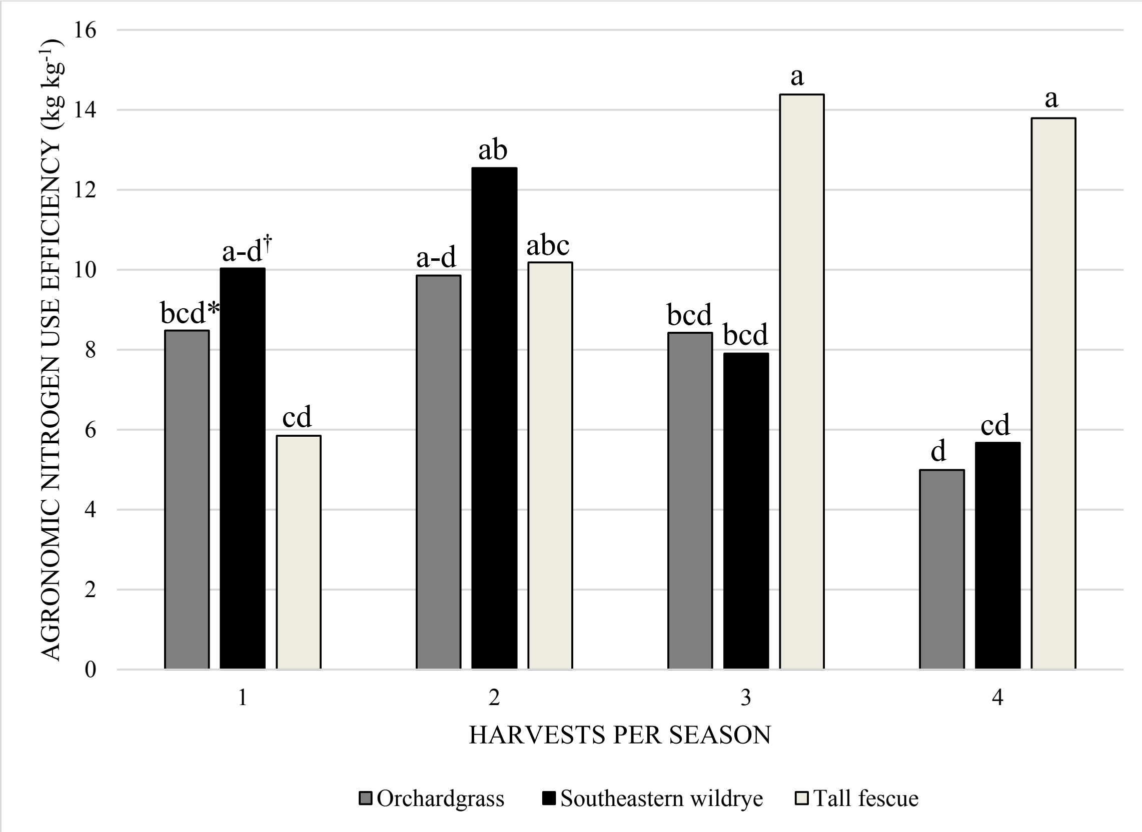

Figure 4.14 Mean agronomic nitrogen use efficiency (ANUE) for harvests per season by cool-season grass species for Year 2 Starkville 2013.

*Letters indicate significant difference at $\alpha = 0.05$.
†Letter groups consisting of four or more sequential letters are written with the first and last letter with a dash in between.

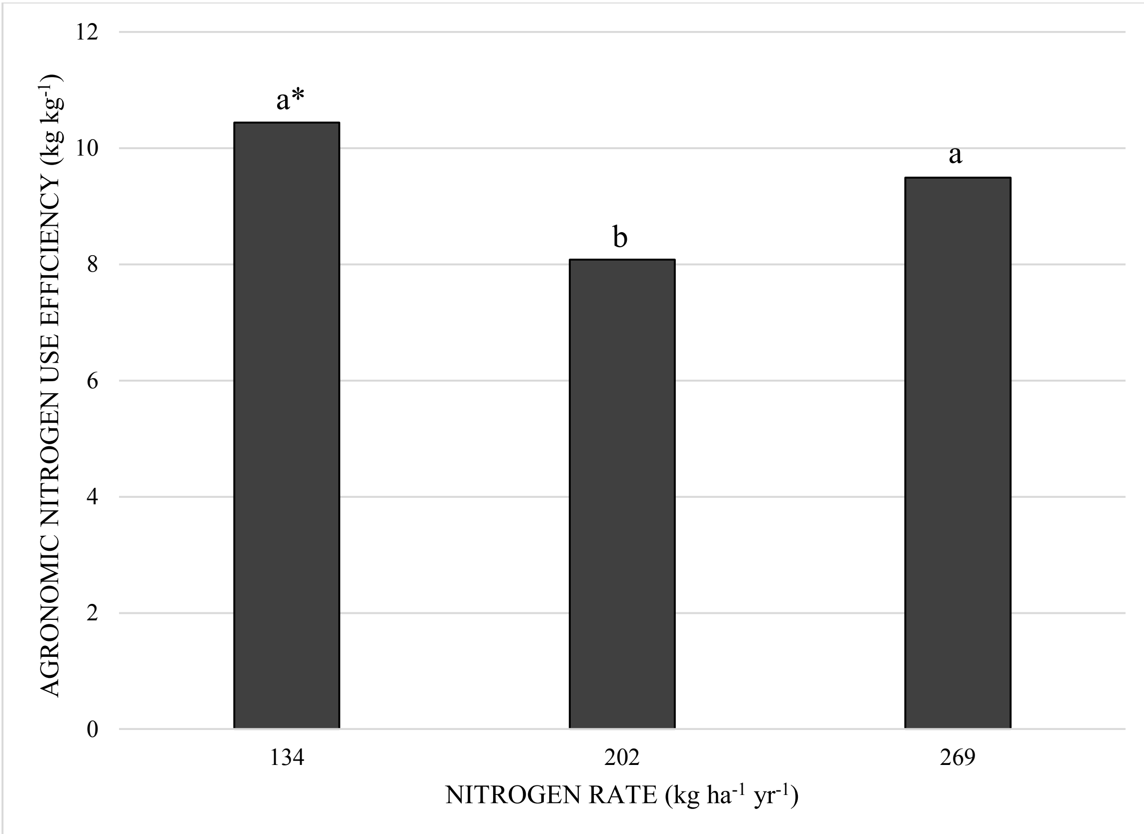

Figure 4.15 Mean agronomic nitrogen use efficiency (ANUE) for nitrogen rates Year 2 Starkville 2013.

*Letters indicate significant difference at α = 0.05.

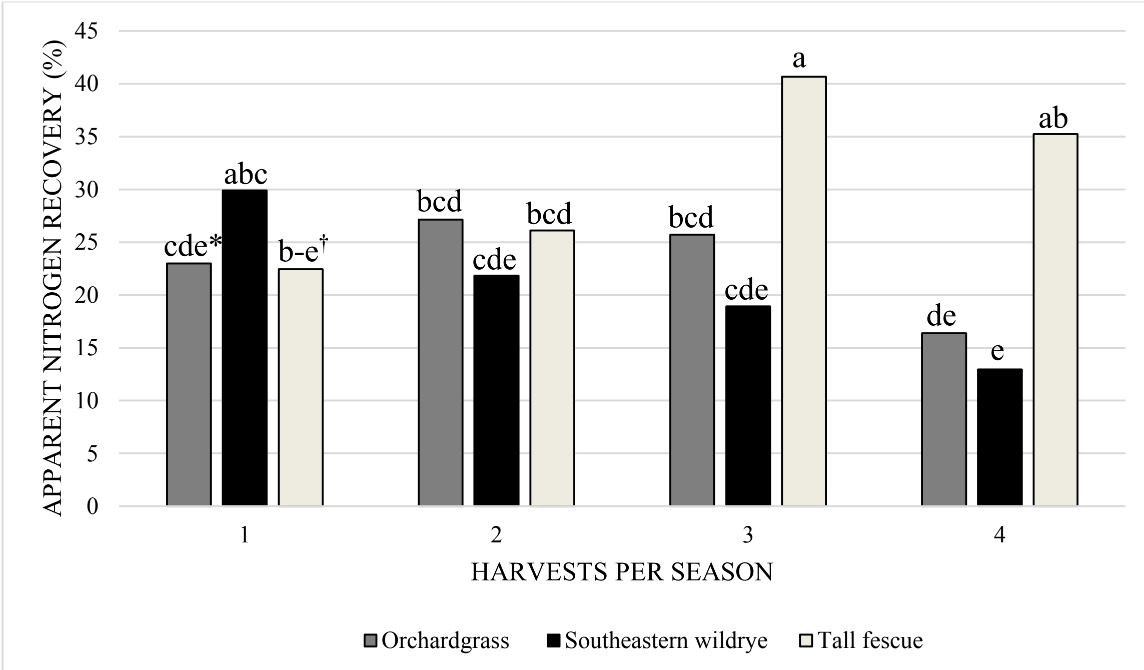

Figure 4.16 Mean apparent nitrogen recovery (ANR) for harvests per season by cool-season grass species for Year 2 Starkville 2013.

*Letters indicate significant difference at $\alpha = 0.05$.
†Letter groups consisting of four or more sequential letters are written with the first and last letter with a dash in between.

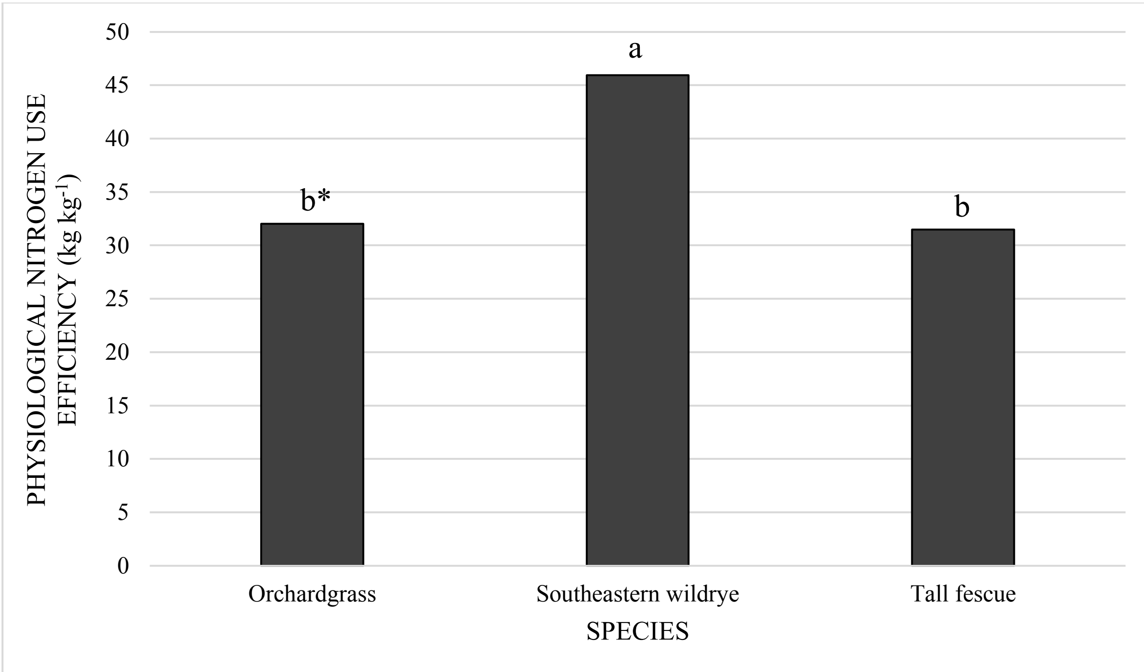

Figure 4.17 Mean physiological nitrogen use efficiency (PNUE) for cool-season grass species for Year 2 Starkville 2013.

*Letters indicate significant difference at $\alpha = 0.05$.

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CHAPTER V

PERSISTENCE OF SOUTHEASTERN WILDRYE AS AFFECTED BY NITROGE APPLICATION AND HARVEST INTERVAL

Abstract

Abiotic stress from frequent defoliation or harvesting aboveground biomass at inappropriate heights often plays a role in decreased plant longevity and therefore stand persistence. A field study was established in Starkville, MS, in 2013 using southeastern wildrye, a North American native cool-season grass. The study was harvested for two years, spring 2014 and 2015. Plots were fertilized with 134, 202, and 269 kg ha^{-1} N and harvested to a 10 cm stubble height either once (at the end of the 112 day season), twice (one every 56 days), three times (one every 37 days), or four times (one every 28 days) during the 112 day season. Following the final harvest in 2015, plant counts were taken within a random 1 m^2 within each plot. There was a significant difference for plant survival among harvests only. Southeastern wildrye plants under the single harvest regime had significantly higher mean number of plants m^{-2} than any other treatment.

Introduction

Incorporating a perennial cool-season grass (CSG) into a grass production system is beneficial to the livestock's feeding regime. Cool-season grasses help alleviate production expenses by filling the forage gaps and reduce feeding costs (Riesterer et al., 2000). Cool-season grasses are not widely utilized across the southern United States due to drought potential and high summer temperatures associated with the region. Both abiotic, such as those mentioned, and biotic stresses hinder the persistence of CSG. Southeastern wildrye has proven to be highly drought tolerant and can proliferate in the humid climate of this region. Determination of optimum agronomic management practices for this grass in order to lessen abiotic stress of harvest is essential if southeastern wildrye is to gain acceptance in production systems.

Livestock producers in the southern United States are open to the possibility of incorporating persistent and productive CSG into forage production systems (Hopkins and Alison, 2006). Appropriate removal of above ground biomass, removing an adequate amount without decreasing plant vigor, is crucial to perennial forage persistence (Owensby et al., 1974). Agronomic practices such as nutrient management and harvest regime are integral to the longevity of forage grasses. In order for the plant to regain nutrients, photosynthetic capacity removed during harvesting, and to preserve vigor, proper rest periods following harvest must be implemented. Photosynthetic vegetation produces carbohydrates needed for plant growth, development, maintenance, and persistence (Owensby et al., 1974; McKendrick et al., 1975).

Harvesting frequently, as well as seasonal timing, can negatively impact regrowth potential and increase mortality of forage crops. Sustainable forage production hinges on the plant's capacity to tolerate moderate to heavy utilization (Kinsinger and Hopkins, 1961). Stress from frequent harvesting or inappropriate harvest height often leads to decreased stand persistence (Ethredge et al., 1973; Anderson and Matches, 1983). Brink and Casler (2009; 2012) used a 10 cm stubble height to harvest plots consisting of both endophyte-free and -infected tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.], orchardgrass [*Dactylis glomerata* L.], Kentucky bluegrass [*Poa pratensis* L.], common quackgrass [*Elymus repens* (L.) Gould], meadow fescue [*Schedonorus pratensis* (Huds.) P. Beauv.], reed canarygrass [*Phalaris arundinacea* L.], smooth brome [*Bromus inermis* Leyss.], and timothy [*Phleum pratense* L.]. Griffith and Teel (1965) researched the difference in persistency of orchardgrass when using a 5 and 10 cm cutting height in Indiana. The greater percent stand reduction occurred in plots cut at 5 cm at a 4, 5½, and 7 week cutting interval. Wagner (1952) found that orchardgrass grown in Beltsville, MD, will tolerate various harvest regimes, from traditional three-cut system to harvesting more frequently.

Replenishing nutrients lost during harvest is important to stand longevity. Nitrogen plays an integral part in the development of forage grasses. It serves a fundamental role in the formation of amino acids and proteins and is essential as a primary component of chlorophyll. Ample plant nitrogen levels produce dark green leaf coloration while nitrogen deficiency contributes to stunted growth and yellowing of leaf tissue (Snyder and Leep, 2007).

Our objective was to evaluate the persistence of southeastern wildrye, a native, perennial CSG, when harvested and fertilized following similar agronomic management schemes commonly used with exotic, perennial CSG.

Materials and Methods

A two-year field trial was established on October 7, 2013, at the Henry H. Leveck Animal Research Center (South Farm) at Mississippi State University near Starkville, MS (33°26'15.63" N, 88°47'50.51" W) on a Catalpa silty clay loam (fine, smectic, thermic, Fluvaquentic Hapludolls), moderately well drained with a pH of 5.6. Weather data for

Starkville was recorded (Table D.1). An initial soil test was taken prior to planting. Fertilization, with the exception of nitrogen (N), was administered based on soil test results with recommendations for perennial cool-season forage grasses (Mississippi State University Soil Testing Lab). Pelletized lime ($CaCO_3$) was applied at a rate of 2.24 Mg ha^{-1} prior to planting bringing soil pH to 6.2.

Herbicides were used to control weed pressure. Pre-plant burndown was achieved by applying glyphosate (N-[phosphonomethyl] glycine, isopropyl-amine salt; 41%) 2.76 kg ae ha^{-1} once prior to tillage and again following tillage. Post emergence broadcast application of dicamba [dimethylamine salt of dicamba (3,6-dichloro-o-anisic acid; 40%) 48.2%] was applied at 0.56 kg ae ha^{-1} for control of winter broadleaf weeds.

Southeastern wildrye seed was obtained from the Foundation field (Mississippi State, MS). Seed was drilled on a prepared seed bed using an Almaco® (Almaco, Nevada, IA) 8-row light duty grain drill at a depth of 0.6 cm. Planting was at a bulk seed rate of 56.3 kg ha^{-1} corresponding to a pure live seed (PLS) rate of 16.8 kg ha^{-1}. Seeding rates correspond with those used by Rushing and Baldwin (2013) for southeastern wildrye.

The study design consisted of a split plot in strips, with two treatments: nitrogen application and harvest regime. Each plot had nitrogen application and harvest regime superimposed on it. Each block was rerandomized and replicated four times across the field. Individual plots were 1.8 m x 3.0 m with eight drilled rows per plot with 25.4 cm spacing. For two consecutive years, plots were fertilized with nitrogen using a Gandy® (Gandy Co., Owatonna, MN) 1.8 m drop spreader. Plots received 0, 134, 202, and 269 kg N $ha^{-1}yr^{-1}$ as 33-0-0 S (50% urea and 50% ammonium sulfate commercial available mix) in four split applications per season per specified plot every 28 days. Plots were harvested one, two, three, or four times throughout the 112 day growing season. A Ferris® (Ferris, Munnsville, NY) zero-turn mower equipped with a bagging system and a 132.1 cm cutting width was used to harvest the center of each 1.8 m wide plot (to decrease the potential impact of differing nitrogen rates on adjacent plots) at a 10 cm stubble height (Brink and Casler, 2009, 2012; White et al., 2013). First harvest was conducted when 75% of the plots were ≥ 38 cm in height for both years, spring 2014 and spring 2015. In fall 2014, prior to second-year harvest, above ground biomass of deceased summer annual weeds were removed by hand to maximize cool-season grass growth.

Following the final harvest in 2015 (two years after inception), plant counts were taken within a random $1m^2$ within each plot. Statistical analysis for each trial was conducted using PROC MIXED using SAS® software, Version 9.4 (SAS Institute, Cary, NC, 2013). Mean separations were based on Tukey's protected least significant difference (LSD) and considered significant at $\alpha = 0.05$.

Results and Discussion

Analysis of data indicated persistence of southeastern wildrye was not significantly impacted by nitrogen x harvest ($P = 0.2770$) with respect to mean number of plants m^{-2}. Since the combination of treatments was not significant, nitrogen and harvest were analyzed independently.

Persistence of southeastern wildrye was not significantly affected by nitrogen ($P = 0.1156$) with respect to mean number of plants m^{-2} (Table C.2). This indicates that nitrogen fertilization did not have a significant effect on persistence of southeastern wildrye when fertilized with either 0, 134, 202, or 269 kg N ha^{-1} yr^{-1}.

Number of harvests ($P < 0.0001$) during the 112 day growing season was significant with respect to mean number of southeastern wildrye plants m^{-2} remaining in the plots (Table C.2). Harvest treatments showed significant differences in mean number of plants m^{-2} with one harvest per season (18.3 plants m^{-2}) having significantly more mean number of plants m^{-2} than plots harvested two (5.9 plants m^{-2}), three (5.6 plants m^{-2}), and four (3.9 plants m^{-2}) times per season (Figure 5.1). These data suggest a single harvest at the end of the 112-day growing season for two consecutive years regardless of N application results in the least plant mortality as compared to the other treatments used in this study.

These results were consistent with Harper and others (2004). He noted that generally, harvesting native grasses for haying or grazing should not occur until they are at least 76 or 30 cm tall, respectively, with at least a 10 cm stubble height remaining (Harper et al., 2004). Leaving a 15 cm stubble height allows more photosynthetic vegetation for regrowth, and therefore these researchers recommend leaving a stubble height of 20 cm (Harper et al. 2004, 2007). Harvesting native grasses continually lower than a 10-12.7 cm height results in reduced persistence of the stand (Harper et al., 2004). Our study was harvested at 10 cm each time to determine if southeastern wildrye can persist through management practices recommended for tall fescue. The southeastern wildrye used in this study is two generations out of the wild with limited selection pressure on the population, no selection for grazing or harvest potential has been made.

Summary

In this study, harvest frequency impacts stand persistence more than N application in southeastern wildrye. Fertilizing southeastern wildrye with N will increase dry matter yield but will not significantly impact mortality. Harvesting should be kept to a minimum for stand longevity. Further research should be conducted by combining harvest frequency with harvest intensity for southeastern wildrye. This would aid in understanding the height at which southeastern wildrye can be harvested without impacting persistence. The maturity stage at which the grass is harvested should also be examined. Perry, Jr. and Chapman (1975) found that when basin wildrye [*Elymus cinerus* Scribn. and Merr.] was harvested at boot stage, the grass contained a low percentage of total nonstructural carbohydrates (TNC) indicating that plants were under stress (Perry, Jr. and Chapman, 1974). In order to combine both frequency of harvest and height of harvest, the growth stage of the grass should be considered to determine the appropriate harvest time and not be limited to a specified number of days as was the case in our study.

In this trial, the southeastern wildrye germplasm evaluated demonstrated poor vigor and response to frequent defoliation events based on management recommendation for tall fescue. This study clearly expresses the need for germplasm development within southeastern wildrye for the generation of lines with enhanced forage quality characteristics, particularly tolerance to frequent cuttings.

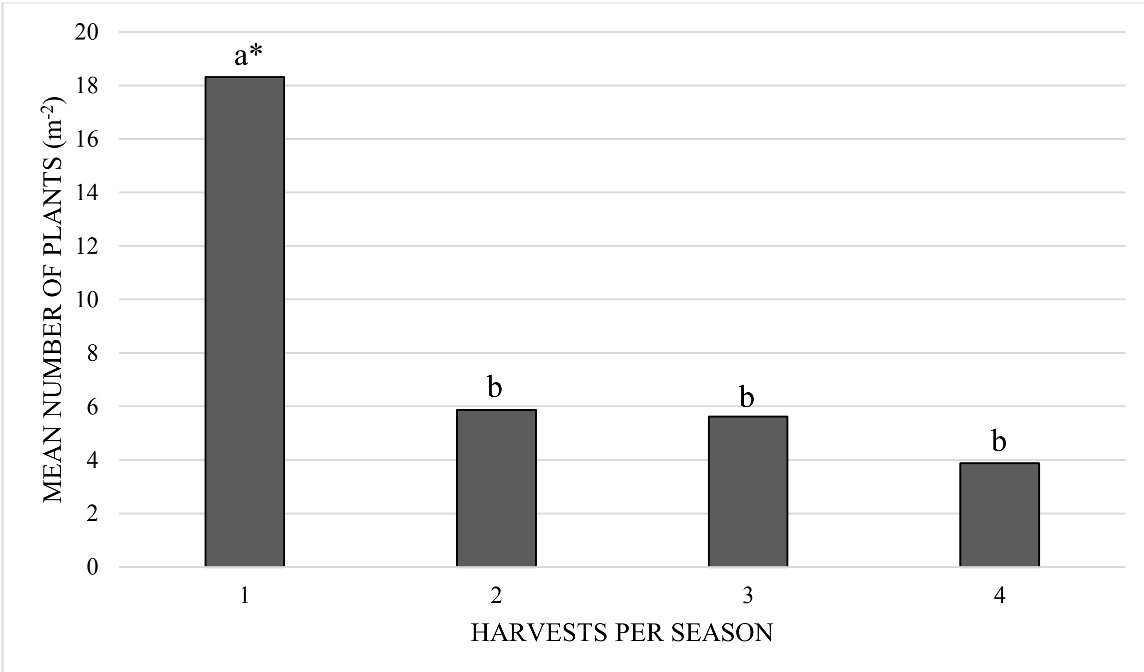

Figure 5.1 Mean number of plants (m^{-2}) for harvests per season following Year 2 Starkville 2013.

*Letters indicate significant difference at $\alpha = 0.05$.

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APPENDIX A

EFFECT OF NITROGEN APPLICATION AND HARVEST INTERVAL ON YIELD AND NUTRITIVE VALUE OF THREE COOL-SEASON GRASS SPECIES

Table A.1 Expected mean square for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

Source	Expected Mean Square
ENV*	Var(Residual) + 4 Var(REP(ENVxSPECIESxH))+ 4 Var(REP(ENVxSPECIESxN))+ 16 Var(REP(ENVxSPECIES))+ 48 Var(REP(ENV))+ Q(ENV, ENVxSPECIES, ENVxN, ENVxSPECIESxN, ENVxH, ENVxSPECIESxH, ENVxNxH, ENVxSPECIESxNxH)
SPECIES†	Var(Residual) + 4 Var(REP(ENVxSPECIESxH))+ 4 Var(REP(ENVxSPECIESxN))+ 16 Var(REP(ENVxSPECIES))+ Q(SPECIES, ENVxSPECIES, SPECIESxN, ENVxSPECIESxN, SPECIESxH, ENVxSPECIESxH, SPECIESxNxH, ENVxSPECIESxNxH)
ENVxSPECIES	Var(Residual) + 4 Var(REP(ENVxSPECIESxH))+ 4 Var(REP(ENVxSPECIESxN))+ 16 Var(REP(ENVxSPECIES))+ Q(ENVxSPECIES, ENVxSPECIESxN, ENVxSPECIESxH, ENVxSPECIESxNxH)
N‡	Var(Residual) + 4 Var(REP(ENVxSPECIESxN))+ Q(N, ENVxN, SPECIESxN, ENVxSPECIESxN, NxH, ENVxNxH, SPECIESxNxH, ENVxSPECIESxNxH)
ENVxN	Var(Residual) + 4 Var(REP(ENVxSPECIESxN))+ Q(ENVxN, ENVxSPECIESxN, ENVxNxH, ENVxSPECIESxNxH)
SPECIESxN	Var(Residual) + 4 Var(REP(ENVxSPECIESxN))+ Q(SPECIESxN, ENVxSPECIESxN, SPECIESxNxH, ENVxSPECIESxNxH)
ENVxSPECIESxN	Var(Residual) + 4 Var(REP(ENVxSPECIESxN))+ Q(ENVxSPECIESxN, ENVxSPECIESxNxH)
H§	Var(Residual) + 4 Var(REP(ENVxSPECIESxH))+ Q(H, ENVxH, SPECIESxH, ENVxSPECIESxH, NxH, ENVxNxH, SPECIESxNxH, ENVxSPECIESxNxH)
ENVxH	Var(Residual) + 4 Var(REP(ENVxSPECIESxH))+ Q(ENVxH, ENVxSPECIESxH, ENVxNxH, ENVxSPECIESxNxH)
SPECIESxH	Var(Residual) + 4 Var(REP(ENVxSPECIESxH))+ Q(SPECIESxH, ENVxSPECIESxH, SPECIESxNxH, ENVxSPECIESxNxH)
ENVxSPECIESxH	Var(Residual) + 4 Var(REP(ENVxSPECIESxH))+ Q(ENVxSPECIESxH, ENVxSPECIESxNxH)
NxH	Var(Residual) + Q(NxH, ENVxNxH, SPECIESxNxH, ENVxSPECIESxNxH)
ENVxNxH	Var(Residual) + Q(ENVxNxH, ENVxSPECIESxNxH)
SPECIESxNxH	Var(Residual) + Q(SPECIESxNxH, ENVxSPECIESxNxH)
ENVxSPECIESxNxH	Var(Residual) + Q(ENVxSPECIESxNxH)
REP‖(ENV)	Var(Residual) + 4 Var(REP(ENVxSPECIESxH))+ 4 Var(REP(ENVxSPECIESxN))+ 16 Var(REP(ENVxSPECIES))+ 48 Var(REP(ENV))
REP(ENVxSPECIES)	Var(Residual) + 4 Var(REP(ENVxSPECIESxH))+ 4 Var(REP(ENVxSPECIESxN))+ 16 Var(REP(ENVxSPECIES))
REP(ENVxSPECIESxN)	Var(Residual)+ 4 Var(REP(ENVxSPECIESxN))
REP(ENVxSPECIESxH)	Var(Residual) + 4 Var(REP(ENVxSPECIESxH))
Residual	Var(Residual)

*Environment
†Cool-season grass species
‡Nitrogen application rate
§Harvests per season
‖Replication across field

Table A.2 Analysis of variance for mean cumulative dry matter yield for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

Source	DF	Sum of Squares	Mean Square	Error Term	Error DF	F Value	Pr > F
ENV*	1	121130438.0	121130438.0	MS(REP(ENV))	6	23.36	0.0029
SPECIES[†]	2	54274789.0	27137394.5	MS(REP(ENVxSPECIES))	12	18.47	0.0002
ENVxSPECIES	2	44535110.0	22267555.0	MS(REP(ENVxSPECIES))	12	15.16	0.0005
N[‡]	3	149028006.0	49676002.0	MS(REP(ENVxSPECIESxN))	54	87.06	<0.0001
ENVxN	3	3958815.0	1319605.0	MS(REP(ENVxSPECIESxN))	54	2031.00	0.0863
SPECIESxN	6	5728118.0	954686.3	MS(REP(ENVxSPECIESxN))	54	1.67	0.1454
ENVxSPECIESxN	6	6770737.0	1128456.2	MS(REP(ENVxSPECIESxN))	54	1.98	0.0850
H[§]	3	9875317.0	3291772.3	MS(REP(ENVxSPECIESxH))	54	2.89	0.0439
ENVxH	3	11406442.0	3802147.3	MS(REP(ENVxSPECIESxH))	54	3.33	0.0260
SPECIESxH	6	19374197.0	3229032.8	MS(REP(ENVxSPECIESxH))	54	2.83	0.0181
ENVxSPECIESxH	6	7644438.0	1274073.0	MS(REP(ENVxSPECIESxH))	54	1.12	0.3648
NxH	9	3051539.0	339059.9	MS(Residual)	162	1.13	0.3451
ENVxNxH	9	2520479.0	280053.2	MS(Residual)	162	0.93	0.4985
SPECIESxNxH	18	6651407.0	369522.6	MS(Residual)	162	1.23	0.2426
ENVxSPECIESxNxH	18	7467762.0	414875.7	MS(Residual)	162	1.38	0.1469
REP[‖](ENV)	6	31118865.0	5186477.5	MS(REP(ENVxSPECIES))	12	3.53	0.0300
REP(ENVxSPECIES)	12	17630408.0	1469200.7	MS(REP(ENVxSPECIESxN)) + MS(REP(ENVxSPECIESxH)) - MS(Residual)	64.89	1.04	0.4234
REP(ENVxSPECIESxN)	54	30813075.0	570612.5	MS(Residual)	162	1.90	0.0011
REP(ENVxSPECIESxH)	54	61588647.0	1140530.5	MS(Residual)	162	3.80	<0.0001
Residual	162	48646866.0	300289.3	-	-	-	-

*Environment
[†]Cool-season grass species
[‡]Nitrogen application rate
[§]Harvests per season
[‖]Replication across field

Table A.3 Analysis of variance for normalized difference vegetation index (NDVI) values for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

Source	DF	Sum of Squares	Mean Square	Error Term	Error DF	F Value	Pr > F
ENV*	1	0.095	0.095	MS(REP(ENV))	6	8.17	0.0289
SPECIES[†]	2	0.195	0.098	MS(REP(ENVxSPECIES))	12	15.24	0.0005
ENVxSPECIES	2	0.184	0.092	MS(REP(ENVxSPECIES))	12	14.34	0.0007
N[‡]	3	0.705	0.235	MS(REP(ENVxSPECIESxN))	54	149.10	<0.0001
ENVxN	3	0.076	0.025	MS(REP(ENVxSPECIESxN))	54	15.98	<0.0001
SPECIESxN	6	0.056	0.009	MS(REP(ENVxSPECIESxN))	54	5.97	<0.0001
ENVxSPECIESxN	6	0.043	0.007	MS(REP(ENVxSPECIESxN))	54	4.55	0.0008
H[§]	3	0.670	0.223	MS(REP(ENVxSPECIESxH))	54	28.05	<0.0001
ENVxH	3	0.251	0.084	MS(REP(ENVxSPECIESxH))	54	10.52	<0.0001
SPECIESxH	6	0.168	0.028	MS(REP(ENVxSPECIESxH))	54	3.51	0.0053
ENVxSPECIESxH	6	0.020	0.003	MS(REP(ENVxSPECIESxH))	54	0.43	0.8573
NxH	9	0.011	0.001	MS(Residual)	162	1.08	0.3767
ENVxNxH	9	0.014	0.002	MS(Residual)	162	1.37	0.2075
SPECIESxNxH	18	0.018	0.001	MS(Residual)	162	0.87	0.6133
ENVxSPECIESxNxH	18	0.013	0.001	MS(Residual)	162	0.64	0.8611
REP[‖](ENV)	6	0.070	0.012	MS(REP(ENVxSPECIES))	12	1.81	0.1802
REP(ENVxSPECIES)	12	0.077	0.006	MS(REP(ENVxSPECIESxN)) + MS(REP(ENVxSPECIESxH)) - MS(Residual)	57.809	0.76	0.6872
REP(ENVxSPECIESxN)	54	0.085	0.002	MS(Residual)	162	1.42	0.0507
REP(ENVxSPECIESxH)	54	0.430	0.008	MS(Residual)	162	7.15	<0.0001
Residual	162	0.180	0.001	-	-	-	-

*Environment
[†]Cool-season grass species
[‡]Nitrogen application rate
[§]Harvests per season
[‖]Replication across field

Table A.4 Analysis of variance for plant height using rising platemeter for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

Source	DF	Sum of Squares	Mean Square	Error Term	Error DF	F Value	Pr > F
ENV*	1	22.67	22.67	MS(REP(ENV))	6	3.36	0.1165
SPECIES†	2	1247.95	623.98	MS(REP(ENVxSPECIES))	12	30.46	<0.0001
ENVxSPECIES	2	924.21	462.11	MS(REP(ENVxSPECIES))	12	22.56	<0.0001
N‡	3	768.75	256.25	MS(REP(ENVxSPECIESxN))	54	46.38	<0.0001
ENVxN	3	13.43	4.48	MS(REP(ENVxSPECIESxN))	54	0.81	0.4938
SPECIESxN	6	35.03	5.84	MS(REP(ENVxSPECIESxN))	54	1.06	0.3999
ENVxSPECIESxN	6	125.88	20.98	MS(REP(ENVxSPECIESxN))	54	3.80	0.0032
H§	3	4674.10	1558.03	MS(REP(ENVxSPECIESxH))	54	129.82	<0.0001
ENVxH	3	132.98	44.33	MS(REP(ENVxSPECIESxH))	54	3.69	0.0172
SPECIESxH	6	408.79	68.13	MS(REP(ENVxSPECIESxH))	54	5.68	0.0001
ENVxSPECIESxH	6	391.14	65.19	MS(REP(ENVxSPECIESxH))	54	5.43	0.0002
NxH	9	100.74	11.19	MS(Residual)	162	3.38	0.0008
ENVxNxH	9	78.37	8.71	MS(Residual)	162	2.63	0.0073
SPECIESxNxH	18	117.92	6.55	MS(Residual)	162	1.98	0.0137
ENVxSPECIESxNxH	18	121.41	6.75	MS(Residual)	162	2.04	0.0106
REP‖(ENV)	6	40.49	6.75	MS(REP(ENVxSPECIES))	12	0.33	0.9087
REP(ENVxSPECIES)	12	245.83	20.49	MS(REP(ENVxSPECIESxN)) + MS(REP(ENVxSPECIESxH)) - MS(Residual)	61.208	1.44	0.1723
REP(ENVxSPECIESxN)	54	298.36	5.53	MS(Residual)	162	1.67	0.0077
REP(ENVxSPECIESxH)	54	648.09	12.00	MS(Residual)	162	3.62	<0.0001
Residual	162	536.81	3.31	-	-	-	-

*Environment
†Cool-season grass species
‡Nitrogen application rate
§Harvests per season
‖Replication across field

Table A.5 Analysis of variance for relative forage quality (RFQ) for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

Source	DF	Sum of Squares	Mean Square	Error Term	Error DF	F Value	Pr > F
ENV*	1	678.44	678.45	MS(REP(ENV))	6	3.32	0.1182
SPECIES†	2	3243.82	1621.91	MS(REP(ENVxSPECIES))	12	26.11	<0.0001
ENVxSPECIES	2	755.44	377.72	MS(REP(ENVxSPECIES))	12	6.08	0.0150
N‡	3	96.42	32.14	MS(REP(ENVxSPECIESxN))	54	2.38	0.0795
ENVxN	3	82.62	27.54	MS(REP(ENVxSPECIESxN))	54	2.04	0.1189
SPECIESxN	6	189.15	31.53	MS(REP(ENVxSPECIESxN))	54	2.34	0.0445
ENVxSPECIESxN	6	107.74	17.96	MS(REP(ENVxSPECIESxN))	54	1.33	0.2596
H§	3	21449.00	7149.66	MS(REP(ENVxSPECIESxH))	54	97.52	<0.0001
ENVxH	3	175.95	58.65	MS(REP(ENVxSPECIESxH))	54	0.80	0.4993
SPECIESxH	6	965.80	160.97	MS(REP(ENVxSPECIESxH))	54	2.20	0.0574
ENVxSPECIESxH	6	558.41	93.07	MS(REP(ENVxSPECIESxH))	54	1.27	0.2869
NxH	9	229.50	25.50	MS(Residual)	162	1.50	0.1503
ENVxNxH	9	365.66	40.63	MS(Residual)	162	2.40	0.0141
SPECIESxNxH	18	182.33	10.13	MS(Residual)	162	0.60	0.8976
ENVxSPECIESxNxH	18	297.06	16.50	MS(Residual)	162	0.97	0.4925
REP‖(ENV)	6	1225.82	204.30	MS(REP(ENVxSPECIES))	12	3.29	0.0376
REP(ENVxSPECIES)	12	745.51	62.13	MS(REP(ENVxSPECIESxN)) + MS(REP(ENVxSPECIESxH)) - MS(Residual)	46.617	0.89	0.5635
REP(ENVxSPECIESxN)	54	728.56	13.49	MS(Residual)	162	0.80	0.8332
REP(ENVxSPECIESxH)	54	3958.93	73.31	MS(Residual)	162	4.33	<0.0001
Residual	162	2745.79	16.95	-	-	-	-

*Environment
†Cool-season grass species
‡Nitrogen application rate
§Harvests per season
‖Replication across field

Table A.6 Analysis of variance for crude protein as analyzed by near-infrared spectroscopy for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

Source	DF	Sum of Squares	Mean Square	Error Term	Error DF	F Value	Pr > F
ENV*	1	24.54	24.54	MS(REP(ENV))	6	29.49	0.0016
SPECIES†	2	93.53	46.77	MS(REP(ENVxSPECIES))	12	18.77	0.0002
ENVxSPECIES	2	17.21	8.61	MS(REP(ENVxSPECIES))	12	3.45	0.0653
N‡	3	36.39	12.13	MS(REP(ENVxSPECIESxN))	54	6.73	0.0006
ENVxN	3	29.41	9.80	MS(REP(ENVxSPECIESxN))	54	5.44	0.0024
SPECIESxN	6	15.63	2.61	MS(REP(ENVxSPECIESxN))	54	1.44	0.2149
ENVxSPECIESxN	6	12.68	2.11	MS(REP(ENVxSPECIESxN))	54	1.17	0.3349
H§	3	387.84	129.28	MS(REP(ENVxSPECIESxH))	54	19.17	<0.0001
ENVxH	3	540.67	180.22	MS(REP(ENVxSPECIESxH))	54	26.72	<0.0001
SPECIESxH	6	195.50	32.58	MS(REP(ENVxSPECIESxH))	54	4.83	0.0005
ENVxSPECIESxH	6	99.05	16.51	MS(REP(ENVxSPECIESxH))	54	2.45	0.0363
NxH	9	158.12	17.57	MS(Residual)	162	9.52	<0.0001
ENVxNxH	9	38.01	4.22	MS(Residual)	162	2.29	0.0192
SPECIESxNxH	18	36.35	2.02	MS(Residual)	162	1.09	0.3626
ENVxSPECIESxNxH	18	71.92	4.00	MS(Residual)	162	2.16	0.0059
REP‖(ENV)	6	4.99	0.83	MS(REP(ENVxSPECIES))	12	0.33	0.9060
REP(ENVxSPECIES)	12	29.90	2.49	MS(REP(ENVxSPECIESxN)) + MS(REP(ENVxSPECIESxH)) - MS(Residual)	47.785	0.37	0.9674
REP(ENVxSPECIESxN)	54	97.34	1.80	MS(Residual)	162	0.98	0.5274
REP(ENVxSPECIESxH)	54	364.23	6.75	MS(Residual)	162	3.65	<0.0001
Residual	162	299.01	1.85	-	-	-	-

*Environment
†Cool-season grass species
‡Nitrogen application rate
§Harvests per season
‖Replication across field

Table A.7 Effect of harvest frequency and nitrogen fertility on mean visual coverage of desired species within plots relative to present vegetation prior to final harvest of all plots.

		Number of Harvests per Season							
		1		2		3		4	
Species	Nitrogen	2013†	2014†	2013	2014	2013	2014	2013	2014
	kg ha^{-1} yr^{-1}								
Orchardgrass	0	4.75*	4.25	5.00	3.75	4.75	3.75	5.00	3.75
	134	4.75	3.75	5.00	3.25	4.75	4.50	5.00	3.75
	202	5.00	4.00	5.00	3.50	5.00	3.75	5.00	3.75
	269	5.00	3.25	5.00	3.00	4.75	4.00	5.00	4.00
Southeastern wildrye	0	3.75	3.00	1.25	1.50	1.75	1.00	1.50	1.00
	134	3.25	2.75	1.75	1.00	1.25	1.00	1.00	1.00
	202	3.50	2.25	1.25	1.00	1.25	1.00	1.00	1.00
	269	3.00	2.75	1.25	1.00	1.00	1.00	1.00	1.00
Tall fescue	0	5.00	4.75	4.50	5.00	4.25	4.75	5.00	4.75
	134	4.00	4.50	4.25	4.50	4.00	4.50	4.75	4.75
	202	4.75	4.75	4.00	4.75	4.75	4.75	5.00	4.50
	269	5.00	4.50	4.25	4.50	4.75	5.00	4.50	5.00

*Scale of 1 to 5; 5 being greater than 80% coverage of desired species relative to present vegetation
†Years correspond with locations: 2013 – Starkville Year 1; 2014 – Brooksville Year 1

Table A.8 Expected mean square for mean cumulative dry matter yield for Year 2 Starkville 2013.

Source	Expected Mean Square
SPECIES*	Var(Residual) + 3.9042 Var(REP(SPECIESxH))+ 3.9042 Var(REP(SPECIESxN))+ 15.617 Var(REPxSPECIES)+ Q(SPECIES, SPECIESxN, SPECIESxH, SPECIESxNxH)
N†	Var(Residual) + 3.9051 Var(REP(SPECIESxN))+ Q(N, SPECIESxN, NxH, SPECIESxNxH)
SPECIESxN	Var(Residual) + 3.9082 Var(REP(SPECIESxN))+ Q(SPECIESxN, SPECIESxNxH)
H‡	Var(Residual) + 3.9051 Var(REP(SPECIESxH))+ Q(H, SPECIESxH, NxH, SPECIESxNxH)
SPECIESxH	Var(Residual) + 3.9082 Var(REP(SPECIESxH))+ Q(SPECIESxH, SPECIESxNxH)
NxH	Var(Residual) + Q(NxH, SPECIESxNxH)
SPECIESxNxH	Var(Residual) + Q(SPECIESxNxH)
REP§	Var(Residual) + 3.9051 Var(REP(SPECIESxH))+ 3.9051 Var(REP(SPECIESxN))+ 15.621 Var (REPxSPECIES) + 46.862 Var(REP)
REPxSPECIES	Var(Residual) + 3.9082 Var(REP(SPECIESxH))+ 3.9082 Var(REP(SPECIESxN))+ 15.633 Var (REPxSPECIES)
REP(SPECIESxN)	Var(Residual)+ 3.9259 Var(REP(SPECIESxN))
REP(SPECIESxH)	Var(Residual) + 3.9259 Var(REP(SPECIESxH))
Residual	Var(Residual)

*Cool-season grass species
†Nitrogen application rate
‡Harvests per season
§Replication across field

Table A.9 Analysis of variance for mean cumulative dry matter yield for Year 2 Starkville 2013.

Source	DF	Sum of Squares	Mean Square	Error Term	Error DF	F Value	Pr > F
SPECIES*	2	84904835.0	42452417.5	0.999 MS(REPxSPECIES) + 0.001 MS(Residual)	6.0015	20.02	0.0022
N†	3	157171279.0	52390426.3	0.9947 MS(REP(SPECIESxN)) + 0.0053 MS(Residual)	27.381	268.96	<0.0001
SPECIESxN	6	2228914.0	371485.7	0.9955 MS(REP(SPECIESxN)) + 0.0045 MS(Residual)	27.324	1.91	0.1156
H‡	3	58604481.0	19534827.0	0.9947 MS(REP(SPECIESxH)) + 0.0053 MS(Residual)	27.057	15.03	<0.0001
SPECIESxH	6	67666986.0	11277831.0	0.9955 MS(REP(SPECIESxH)) + 0.0045 MS(Residual)	27.048	8.67	<0.0001
NxH	9	3278591.0	364287.9	MS(Residual)	79	1.42	0.1947
SPECIESxNxH	18	11228306.0	623794.8	MS(Residual)	79	2.43	0.0037
REP§	3	4219306.0	1406435.3	0.9992 MS(REPxSPECIES) + 0.0008 MS(Residual)	6.0011	0.66	0.6044
REPxSPECIES	6	12735275.0	2122545.8	0.9955 MS(REP(SPECIESxN)) + 0.9955 MS(REP(SPECIESxH)) - 0.991 MS(Residual)	23.688	1.71	0.1616
REP(SPECIESxN)	27	5250273.0	194454.6	MS(Residual)	79	0.76	0.7900
REP(SPECIESxH)	27	35251062.0	1305594.9	MS(Residual)	79	5.08	<0.0001
Residual	79	20295712.0	256907.7	-	-	-	-

*Cool-season grass species
†Nitrogen application rate
‡Harvests per season
§Replication across field

Table A.10 Expected mean square for normalized difference vegetation index (NDVI), plant height using rising platemeter, relative forage quality (RFQ), and crude protein (CP) as analyzed by near-infrared spectroscopy for Year 2 Starkville 2013.

Source	**Expected Mean Square**
SPECIES*	Var(Residual) + 3.9518 Var(REP(SPECIESxH))+ 3.9518 Var(REP(SPECIESxN))+ 15.807 Var(REPxSPECIES)+ Q(SPECIES, SPECIESxN, SPECIESxH, SPECIESxNxH)
N†	Var(Residual) + 3.9524 Var(REP(SPECIESxN))+ Q(N, SPECIESxN, NxH, SPECIESxNxH)
SPECIESxN	Var(Residual) + 3.954 Var(REP(SPECIESxN))+ Q(SPECIESxN, SPECIESxNxH)
H‡	Var(Residual) + 3.9524 Var(REP(SPECIESxH))+ Q(H, SPECIESxH, NxH, SPECIESxNxH)
SPECIESxH	Var(Residual) + 3.954 Var(REP(SPECIESxH))+ Q(SPECIESxH, SPECIESxNxH)
NxH	Var(Residual) + Q(NxH, SPECIESxNxH)
SPECIESxNxH	Var(Residual) + Q(SPECIESxNxH)
REP§	Var(Residual) + 3.9524 Var(REP(SPECIESxH))+ 3.9524 Var(REP(SPECIESxN))+ 15.81 Var (REPxSPECIES) + 47.429 Var(REP)
REPxSPECIES	Var(Residual) + 3.954 Var(REP(SPECIESxH))+ 3.954 Var(REP(SPECIESxN))+ 15.816 Var (REPxSPECIES)
REP(SPECIESxN)	Var(Residual) + 3.963 Var(REP(SPECIESxN))
REP(SPECIESxH)	Var(Residual) + 3.963 Var(REP(SPECIESxH))
Residual	Var(Residual)

*Cool-season grass species
†Nitrogen application rate
‡Harvests per season
§Replication across field

Table A.11 Analysis of variance for normalized difference vegetation index (NDVI) values for Year 2 Starkville 2013.

Source	DF	Sum of Squares	Mean Square	Error Term	Error DF	F Value	Pr > F
SPECIES*	2	0.020	0.010	0.9994 MS(REPxSPECIES) + 0.0006 MS(Residual)	6.0013	1.17	0.3714
N†	3	0.559	0.186	0.9973 MS(REP(SPECIESxN)) + 0.0027 MS(Residual)	27.186	147.34	<0.0001
SPECIESxN	6	0.012	0.002	0.9977 MS(REP(SPECIESxN)) + 0.0023 MS(Residual)	27.157	1.54	0.2015
H‡	3	1.048	0.349	0.9973 MS(REP(SPECIESxH)) + 0.0027 MS(Residual)	27.045	66.83	<0.0001
SPECIESxH	6	0.357	0.059	0.9977 MS(REP(SPECIESxH)) + 0.0023 MS(Residual)	27.038	11.38	<0.0001
NxH	9	0.023	0.003	MS(Residual)	80	1.57	0.1383
SPECIESxNxH	18	0.059	0.003	MS(Residual)	80	2.00	0.0190
REP§	3	0.012	0.004	0.9996 MS(REPxSPECIES) + 0.0004 MS(Residual)	6.001	0.48	0.7104
REPxSPECIES	6	0.050	0.008	0.9977 MS(REP(SPECIESxN)) + 0.9977 MS(REP(SPECIESxH)) - 0.9955 MS(Residual)	21.487	1.72	0.1634
REP(SPECIESxN)	27	0.034	0.001	MS(Residual)	80	0.78	0.7661
REP(SPECIESxH)	27	0.141	0.005	MS(Residual)	80	3.22	<0.0001
Residual	80	0.130	0.002	-	-	-	-

*Cool-season grass species
†Nitrogen application rate
‡Harvests per season
§Replication across field

Table A.12 Analysis of variance for plant height using rising platemeter values for Year 2 Starkville 2013.

Source	DF	Sum of Squares	Mean Square	Error Term	Error DF	F Value	Pr > F
SPECIES*	2	547.37	273.68	0.9994 MS(REPxSPECIES) + 0.0006 MS(Residual)	6.001	10.50	0.0110
N†	3	566.19	188.73	0.9973 MS(REP(SPECIESxN)) + 0.0027 MS(Residual)	27.073	23.81	<0.0001
SPECIESxN	6	122.27	20.38	0.9977 MS(REP(SPECIESxN)) + 0.0023 MS(Residual)	27.062	2.57	0.0422
H‡	3	4410.89	1470.30	0.9973 MS(REP(SPECIESxH)) + 0.0027 MS(Residual)	27.018	46.25	<0.0001
SPECIESxH	6	641.61	106.94	0.9977 MS(REP(SPECIESxH)) + 0.0023 MS(Residual)	27.015	3.36	0.0132
NxH	9	69.70	7.74	MS(Residual)	80	1.92	0.0600
SPECIESxNxH	18	355.42	19.75	MS(Residual)	80	4.91	<0.0001
REP§	3	116.27	38.76	0.9996 MS(REPxSPECIES) + 0.0004 MS(Residual)	6.0008	1.49	0.3103
REPxSPECIES	6	156.50	26.08	0.9977 MS(REP(SPECIESxN)) + 0.9977 MS(REP(SPECIESxH)) - 0.9955 MS(Residual)	31.904	0.73	0.6286
REP(SPECIESxN)	27	214.27	7.94	MS(Residual)	80	1.97	0.0105
REP(SPECIESxH)	27	860.42	31.87	MS(Residual)	80	7.92	<0.0001
Residual	80	321.91	4.02	-	-	-	-

*Cool-season grass species
†Nitrogen application rate
‡Harvests per season
§Replication across field

Table A.13 Analysis of variance for relative forage quality (RFQ) for Year 2 Starkville 2013.

Source	DF	Sum of Squares	Mean Square	Error Term	Error DF	F Value	Pr > F
SPECIES*	2	708.60	354.30	0.9994 MS(REPxSPECIES) + 0.0006 MS(Residual)	6.0012	6.25	0.0341
N†	3	498.42	166.14	0.9973 MS(REP(SPECIESxN)) + 0.0027 MS(Residual)	27.093	10.31	0.0001
SPECIESxN	6	108.36	18.06	0.9977 MS(REP(SPECIESxN)) + 0.0023 MS(Residual)	27.079	1.12	0.3765
H‡	3	25484.00	8494.52	0.9973 MS(REP(SPECIESxH)) + 0.0027 MS(Residual)	27.072	406.75	<0.0001
SPECIESxH	6	355.58	59.26	0.9977 MS(REP(SPECIESxH)) + 0.0023 MS(Residual)	27.061	2.84	0.0283
NxH	9	151.12	16.79	MS(Residual)	80	1.61	0.1260
SPECIESxNxH	18	381.83	21.21	MS(Residual)	80	2.04	0.0165
REP§	3	97.13	32.38	0.9996 MS(REPxSPECIES) + 0.0004 MS(Residual)	6.009	0.57	0.6546
REPxSPECIES	6	340.45	56.74	0.9977 MS(REP(SPECIESxN)) + 0.9977 MS(REP(SPECIESxH)) - 0.9955 MS(Residual)	26.113	2.13	0.0831
REP(SPECIESxN)	27	435.37	16.12	MS(Residual)	80	1.55	0.0696
REP(SPECIESxH)	27	564.62	20.91	MS(Residual)	80	2.01	0.0090
Residual	80	833.62	10.42	-	-	-	-

*Cool-season grass species
†Nitrogen application rate
‡Harvests per season
§Replication across field

Table A.14 Analysis of variance for crude protein as analyzed by near-infrared spectroscopy for Year 2 Starkville 2013.

Source	DF	Sum of Squares	Mean Square	Error Term	Error DF	F Value	Pr > F
SPECIES*	2	264.73	132.36	0.9994 MS(REPxSPECIES) + 0.0006 MS(Residual)	6.0027	10.47	0.0110
N†	3	194.24	64.75	0.9973 MS(REP(SPECIESxN)) + 0.0027 MS(Residual)	27.122	10.97	<0.0001
SPECIESxN	6	20.49	3.41	0.9977 MS(REP(SPECIESxN)) + 0.0023 MS(Residual)	27.103	0.58	0.7439
H‡	3	274.16	91.39	0.9973 MS(REP(SPECIESxH)) + 0.0027 MS(Residual)	27.099	12.56	<0.0001
SPECIESxH	6	7.54	1.26	0.9977 MS(REP(SPECIESxH)) + 0.0023 MS(Residual)	27.084	0.17	0.9819
NxH	9	56.77	6.31	MS(Residual)	80	1.27	0.2686
SPECIESxNxH	18	119.20	6.62	MS(Residual)	80	1.33	0.1933
REP§	3	40.96	13.65	0.9996 MS(REPxSPECIES) + 0.0004 MS(Residual)	6.002	1.08	0.4259
REPxSPECIES	6	75.86	12.64	0.9977 MS(REP(SPECIESxN)) + 0.9977 MS(REP(SPECIESxH)) - 0.9955 MS(Residual)	18.921	1.54	0.2181
REP(SPECIESxN)	27	159.37	5.90	MS(Residual)	80	1.18	0.2764
REP(SPECIESxH)	27	196.61	7.28	MS(Residual)	80	1.46	0.0993
Residual	80	398.76	4.98	-	-	-	-

*Cool-season grass species
†Nitrogen application rate
‡Harvests per season
§Replication across field

Table A.15 Effect of harvest frequency and nitrogen fertility on mean visual coverage of desired species within plots relative to present vegetation prior to final harvest of all plots.

		Number of Harvests per Season			
Species	**Nitrogen**	**----------- 1 -----------**	**------------ 2 ------------**	**------------ 3 ------------**	**------------ 4 ------------**
	kg ha^{-1} yr^{-1}				
Orchardgrass	0	4.75*	5.00	4.00	4.75
	134	5.00	5.00	4.25	4.25
	202	4.75	5.00	5.00	4.50
	269	5.00	5.00	4.00	4.50
Southeastern wildrye	0	4.75	2.25	1.75	1.00
	134	4.75	1.75	1.00	1.00
	202	4.50	1.25	1.00	1.00
	269	4.50	1.25	1.00	1.00
Tall fescue	0	5.00	4.25	4.25	3.50
	134	5.00	4.00	3.50	3.00
	202	5.00	4.25	4.50	4.00
	269	5.00	4.00	4.50	3.50

*Scale of 1 to 5; 5 being greater than 80% coverage of desired species relative to present vegetation

APPENDIX B

NITROGEN USE EFFICIENCY OF THREE COOL-SEASON GRASS SPECIES IN NORTH CENTRAL MISSISSIPPI

Table B.1 Expected mean square for partial factor productivity (PFP), agronomic nitrogen use efficiency (ANUE), and apparent nitrogen recovery (ANR) for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

Source	**Expected Mean Square**
ENV*	Var(Residual) + 3 Var(REP(ENVxSPECIESxH))+ 4 Var(REP(ENVxSPECIESxN))+ 12 Var(REP(ENVxSPECIES))+ 36 Var(REP(ENV))+ Q(ENV, ENVxSPECIES, ENVxN, ENVxSPECIESxN, ENVxH, ENVxSPECIESxH, ENVxNxH, ENVxSPECIESxNxH)
SPECIES†	Var(Residual) + 3 Var(REP(ENVxSPECIESxH))+ 4 Var(REP(ENVxSPECIESxN))+ 12 Var(REP(ENVxSPECIES))+ Q(SPECIES, ENVxSPECIES, SPECIESxN, ENVxSPECIESxN, SPECIESxH, ENVxSPECIESxH, SPECIESxNxH, ENVxSPECIESxNxH)
ENVxSPECIES	Var(Residual) + 3 Var(REP(ENVxSPECIESxH))+ 4 Var(REP(ENVxSPECIESxN))+ 12 Var(REP(ENVxSPECIES))+ Q(ENVxSPECIES, ENVxSPECIESxN, ENVxSPECIESxH, ENVxSPECIESxNxH)
N‡	Var(Residual) + 4 Var(REP(ENVxSPECIESxN))+ Q(N, ENVxN, SPECIESxN, ENVxSPECIESxN, NxH, ENVxNxH, SPECIESxNxH, ENVxSPECIESxNxH)
ENVxN	Var(Residual) + 4 Var(REP(ENVxSPECIESxN))+ Q(ENVxN, ENVxSPECIESxN, ENVxNxH, ENVxSPECIESxNxH)
SPECIESxN	Var(Residual) + 4 Var(REP(ENVxSPECIESxN))+ Q(SPECIESxN, ENVxSPECIESxN, SPECIESxNxH, ENVxSPECIESxNxH)
ENVxSPECIESxN	Var(Residual) + 4 Var(REP(ENVxSPECIESxN))+ Q(ENVxSPECIESxN, ENVxSPECIESxNxH)
H§	Var(Residual) + 3 Var(REP(ENVxSPECIESxH))+ Q(H, ENVxH, SPECIESxH, ENVxSPECIESxH, NxH, ENVxNxH, SPECIESxNxH, ENVxSPECIESxNxH)
ENVxH	Var(Residual) + 3 Var(REP(ENVxSPECIESxH))+ Q(ENVxH, ENVxSPECIESxH, ENVxNxH, ENVxSPECIESxNxH)
SPECIESxH	Var(Residual) + 3 Var(REP(ENVxSPECIESxH))+ Q(SPECIESxH, ENVxSPECIESxH, SPECIESxNxH, ENVxSPECIESxNxH)
ENVxSPECIESxH	Var(Residual) + 3 Var(REP(ENVxSPECIESxH))+ Q(ENVxSPECIESxH, ENVxSPECIESxNxH)
NxH	Var(Residual) + Q(NxH, ENVxNxH, SPECIESxNxH, ENVxSPECIESxNxH)
ENVxNxH	Var(Residual) + Q(ENVxNxH, ENVxSPECIESxNxH)
SPECIESxNxH	Var(Residual) + Q(SPECIESxNxH, ENVxSPECIESxNxH)
ENVxSPECIESxNxH	Var(Residual) + Q(ENVxSPECIESxNxH)

Table B.1 (continued)

REP[‖](ENV)	Var(Residual) + 3 Var(REP(ENVxSPECIESxH))+ 4 Var(REP(ENVxSPECIESxN))+ 12 Var(REP(ENVxSPECIES))+ 36 Var(REP(ENV))
REP(ENVxSPECIES)	Var(Residual) + 3 Var(REP(ENVxSPECIESxH))+ 4 Var(REP(ENVxSPECIESxN))+ 12 Var(REP(ENVxSPECIES))
REP(ENVxSPECIESxN)	Var(Residual)+ 4 Var(REP(ENVxSPECIESxN))
REP(ENVxSPECIESxH)	Var(Residual) + 3 Var(REP(ENVxSPECIESxH))
Residual	Var(Residual)

*Environment
[†]Cool-season grass species
[‡]Nitrogen application rate
[§]Harvests per season
[‖]Replication across field

Table B.2 Analysis of variance for partial factor productivity (PFP) for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

Source	DF	Sum of Squares	Mean Square	Error Term	Error DF	F Value	Pr > F
ENV*	1	3150.1	3150.1	MS(REP(ENV))	6	23.82	0.0028
SPECIES[†]	2	1186.9	593.5	MS(REP(ENVxSPECIES))	12	13.37	0.0009
ENVxSPECIES	2	1422.1	711.0	MS(REP(ENVxSPECIES))	12	16.02	0.0004
N[‡]	2	6393.5	3196.8	MS(REP(ENVxSPECIESxN))	36	168.19	<0.0001
ENVxN	2	215.6	107.8	MS(REP(ENVxSPECIESxN))	36	5.67	0.0072
SPECIESxN	4	327.1	81.8	MS(REP(ENVxSPECIESxN))	36	4.30	0.0060
ENVxSPECIESxN	4	185.3	46.3	MS(REP(ENVxSPECIESxN))	36	2.44	0.0648
H[§]	3	249.4	83.1	MS(REP(ENVxSPECIESxH))	54	3.14	0.0326
ENVxH	3	287.6	95.9	MS(REP(ENVxSPECIESxH))	54	3.62	0.0186
SPECIESxH	6	470.7	78.5	MS(REP(ENVxSPECIESxH))	54	2.96	0.0142
ENVxSPECIESxH	6	174.2	29.0	MS(REP(ENVxSPECIESxH))	54	1.10	0.3763
NxH	6	14.2	2.4	MS(Residual)	108	0.26	0.9539
ENVxNxH	6	65.3	10.9	MS(Residual)	108	1.20	0.3109
SPECIESxNxH	12	158.3	13.2	MS(Residual)	108	1.46	0.1525
ENVxSPECIESxNxH	12	90.2	7.5	MS(Residual)	108	0.83	0.6194
REP[‖](ENV)	6	793.6	132.3	MS(REP(ENVxSPECIES))	12	2.98	0.0580
REP(ENVxSPECIES)	12	532.6	44.4	MS(REP(ENVxSPECIESxN)) + MS(REP(ENVxSPECIESxH)) - MS(Residual)	55.788	1.22	0.2938
REP(ENVxSPECIESxN)	36	684.3	19.0	MS(Residual)	108	2.10	0.0018
REP(ENVxSPECIESxH)	54	1429.2	26.5	MS(Residual)	108	2.92	<0.0001
Residual	108	978.5	9.1	-	-	-	-

*Environment
[†]Cool-season grass species
[‡]Nitrogen application rate
[§]Harvests per season
[‖]Replication across field

Table B.3 Analysis of variance for agronomic nitrogen use efficiency (ANUE) for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

Source	DF	Sum of Squares	Mean Square	Error Term	Error DF	F Value	Pr > F
ENV*	1	428.8	428.8	MS(REP(ENV))	6	12.90	0.0115
SPECIES[†]	2	54.5	27.2	MS(REP(ENVxSPECIES))	12	0.30	0.7452
ENVxSPECIES	2	683.9	342.0	MS(REP(ENVxSPECIES))	12	3.79	0.0531
N[‡]	2	280.1	140.0	MS(REP(ENVxSPECIESxN))	36	7.47	0.0019
ENVxN	2	20.4	10.2	MS(REP(ENVxSPECIESxN))	36	0.54	0.5856
SPECIESxN	4	63.3	15.8	MS(REP(ENVxSPECIESxN))	36	0.84	0.5061
ENVxSPECIESxN	4	100.7	25.2	MS(REP(ENVxSPECIESxN))	36	1.34	0.2728
H[§]	3	135.2	45.1	MS(REP(ENVxSPECIESxH))	54	1.34	0.2701
ENVxH	3	81.0	27.0	MS(REP(ENVxSPECIESxH))	54	0.80	0.4968
SPECIESxH	6	135.4	22.6	MS(REP(ENVxSPECIESxH))	54	0.67	0.6720
ENVxSPECIESxH	6	285.1	47.5	MS(REP(ENVxSPECIESxH))	54	1.42	0.2255
NxH	6	19.3	3.2	MS(Residual)	108	0.31	0.9315
ENVxNxH	6	44.7	7.5	MS(Residual)	108	0.72	0.6377
SPECIESxNxH	12	109.7	9.1	MS(Residual)	108	0.88	0.5713
ENVxSPECIESxNxH	12	116.0	9.7	MS(Residual)	108	0.93	0.5216
REP[‖](ENV)	6	199.4	33.2	MS(REP(ENVxSPECIES))	12	0.37	0.8855
REP(ENVxSPECIES)	12	1083.9	90.3	MS(REP(ENVxSPECIESxN)) + MS(REP(ENVxSPECIESxH)) - MS(Residual)	55.483	2.16	0.0272
REP(ENVxSPECIESxN)	36	674.5	18.7	MS(Residual)	108	1.80	0.0110
REP(ENVxSPECIESxH)	54	1811.8	33.6	MS(Residual)	108	3.22	<0.0001
Residual	108	1124.8	10.4	-	-	-	-

*Environment
[†]Cool-season grass species
[‡]Nitrogen application rate
[§]Harvests per season
[‖]Replication across field

Table B.4 Analysis of variance for apparent nitrogen recovery (ANR) for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

Source	DF	Sum of Squares	Mean Square	Error Term	Error DF	F Value	Pr > F
ENV*	1	4139.8	4139.8	MS(REP(ENV))	6	36.62	0.0009
SPECIES†	2	1027.8	513.9	MS(REP(ENVxSPECIES))	12	1.03	0.3868
ENVxSPECIES	2	5560.7	2780.3	MS(REP(ENVxSPECIES))	12	5.57	0.0195
N‡	2	1828.7	914.3	MS(REP(ENVxSPECIESxN))	36	8.13	0.0012
ENVxN	2	11.0	5.5	MS(REP(ENVxSPECIESxN))	36	0.15	0.9523
SPECIESxN	4	387.4	96.9	MS(REP(ENVxSPECIESxN))	36	0.86	0.4963
ENVxSPECIESxN	4	819.9	205.0	MS(REP(ENVxSPECIESxN))	36	1.82	0.1457
H§	3	1803.7	601.2	MS(REP(ENVxSPECIESxH))	54	2.88	0.0442
ENVxH	3	731.9	244.0	MS(REP(ENVxSPECIESxH))	54	1.17	0.3302
SPECIESxH	6	334.2	55.7	MS(REP(ENVxSPECIESxH))	54	0.27	0.9500
ENVxSPECIESxH	6	1675.2	279.2	MS(REP(ENVxSPECIESxH))	54	1.34	0.2568
NxH	6	162.2	27.0	MS(Residual)	108	0.40	0.8783
ENVxNxH	6	332.2	55.4	MS(Residual)	108	0.82	0.5591
SPECIESxNxH	12	717.0	59.7	MS(Residual)	108	0.88	0.5678
ENVxSPECIESxNxH	12	1096.0	91.3	MS(Residual)	108	1.35	0.2029
REP‖(ENV)	6	678.3	113.0	MS(REP(ENVxSPECIES))	12	0.23	0.9602
REP(ENVxSPECIES)	12	5992.1	499.3	MS(REP(ENVxSPECIESxN)) + MS(REP(ENVxSPECIESxH)) - MS(Residual)	53.48	1.97	0.0459
REP(ENVxSPECIESxN)	36	4047.0	112.4	MS(Residual)	108	1.66	0.0244
REP(ENVxSPECIESxH)	54	11273.0	208.8	MS(Residual)	108	3.08	<0.0001
Residual	108	7320.1	67.8	-	-	-	-

*Environment
†Cool-season grass species
‡Nitrogen application rate
§Harvests per season
‖Replication across field

Table B.5 Expected mean square for physiological nitrogen use efficiency (PNUE) for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

Source	Expected Mean Square
ENV*	Var(Residual) + 2.0155 Var(REP(ENVxSPECIESxH))+ 2.5302 Var(REP(ENVxSPECIESxN))+ 7.5906 Var(REP(ENVxSPECIES))+ 22.764 Var(REP(ENV))+ Q(ENV, ENVxSPECIES, ENVxN, ENVxSPECIESxN, ENVxH, ENVxSPECIESxH, ENVxNxH, ENVxSPECIESxNxH)
SPECIES[†]	Var(Residual) + 2.0468 Var(REP(ENVxSPECIESxH))+ 2.5698 Var(REP(ENVxSPECIESxN))+ 7.7095 Var(REP(ENVxSPECIES))+ Q(SPECIES, ENVxSPECIES, SPECIESxN, ENVxSPECIESxN, SPECIESxH, ENVxSPECIESxH, SPECIESxNxH, ENVxSPECIESxNxH)
ENVxSPECIES	Var(Residual) + 2.0364 Var(REP(ENVxSPECIESxH))+ 2.5514 Var(REP(ENVxSPECIESxN))+ 7.6541 Var(REP(ENVxSPECIES))+ Q(ENVxSPECIES, ENVxSPECIESxN, ENVxSPECIESxH, ENVxSPECIESxNxH)
N[‡]	Var(Residual) + 2.4307 Var(REP(ENVxSPECIESxN))+ Q(N, ENVxN, SPECIESxN, ENVxSPECIESxN, NxH, ENVxNxH, SPECIESxNxH, ENVxSPECIESxNxH)
ENVxN	Var(Residual) + 2.4307 Var(REP(ENVxSPECIESxN))+ Q(ENVxN, ENVxSPECIESxN, ENVxNxH, ENVxSPECIESxNxH)
SPECIESxN	Var(Residual) + 2.5021 Var(REP(ENVxSPECIESxN))+ Q(SPECIESxN, ENVxSPECIESxN, SPECIESxNxH, ENVxSPECIESxNxH)
ENVxSPECIESxN	Var(Residual) + 2.5021 Var(REP(ENVxSPECIESxN))+ Q(ENVxSPECIESxN, ENVxSPECIESxNxH)
H[§]	Var(Residual) + 1.7979 Var(REP(ENVxSPECIESxH))+ Q(H, ENVxH, SPECIESxH, ENVxSPECIESxH, NxH, ENVxNxH, SPECIESxNxH, ENVxSPECIESxNxH)
ENVxH	Var(Residual) + 1.7915 Var(REP(ENVxSPECIESxH))+ Q(ENVxH, ENVxSPECIESxH, ENVxNxH, ENVxSPECIESxNxH)
SPECIESxH	Var(Residual) + 1.8498 Var(REP(ENVxSPECIESxH))+ Q(SPECIESxH, ENVxSPECIESxH, SPECIESxNxH, ENVxSPECIESxNxH)
ENVxSPECIESxH	Var(Residual) + 1.8428 Var(REP(ENVxSPECIESxH))+ Q(ENVxSPECIESxH, ENVxSPECIESxNxH)
NxH	Var(Residual) + Q(NxH, ENVxNxH, SPECIESxNxH, ENVxSPECIESxNxH)
ENVxNxH	Var(Residual) + Q(ENVxNxH, ENVxSPECIESxNxH)

Table B.5 (continued)

SPECIESxNxH	Var(Residual) + Q(SPECIESxNxH, ENVxSPECIESxNxH)
ENVxSPECIESxNxH	Var(Residual) + Q(ENVxSPECIESxNxH)
REP[‖](ENV)	Var(Residual) + 2.03 Var(REP(ENVxSPECIESxH))+ 2.5699 Var(REP(ENVxSPECIESxN))+ 7.7097 Var(REP(ENVxSPECIES))+ 23.122 Var(REP(ENV))
REP(ENVxSPECIES)	Var(Residual) + 2.03 Var(REP(ENVxSPECIESxH))+ 2.5699 Var(REP(ENVxSPECIESxN))+ 7.7097 Var(REP(ENVxSPECIES))+ 23.122 Var(REP(ENV)) Var(Residual) + 2.1173 Var(REP(ENVxSPECIESxH))+ 2.6805 Var(REP(ENVxSPECIESxN))+ 8.0414 Var(REP(ENVxSPECIES))
REP(ENVxSPECIESxN)	Var(Residual)+ 2.9167 Var(REP(ENVxSPECIESxN))
REP(ENVxSPECIESxH)	Var(Residual) + 2.3529 Var(REP(ENVxSPECIESxH))
Residual	Var(Residual)

*Environment
[†]Cool-season grass species
[‡]Nitrogen application rate
[§]Harvests per season
[‖]Replication across field

Table B.6 Analysis of variance for physiological nitrogen use efficiency (PNUE) for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

Source	DF	Sum of Squares	Mean Square	Error DF	F Value	Pr > F
ENV*	1	2881.1	2881.1	6.1042	9.04	0.0233
SPECIES†	2	1331.8	665.9	12.558	2.35	0.1357
ENVxSPECIES	2	933.2	466.6	12.661	1.65	0.2302
N‡	2	31.6	15.8	47.701	0.09	0.9125
ENVxN	2	455.4	227.7	47.701	1.32	0.2763
SPECIESxN	4	716.9	179.2	45.676	1.03	0.3996
ENVxSPECIESxN	4	207.1	51.8	45.676	0.30	0.8772
H§	3	6339.1	2113.0	71.624	10.68	<0.0001
ENVxH	3	2053.2	684.4	71.924	3.46	0.0207
SPECIESxH	6	1796.6	299.4	69.235	1.50	0.1910
ENVxSPECIESxH	6	1462.1	243.7	69.554	1.22	0.3054
NxH	6	857.5	142.9	69	1.01	0.4264
ENVxNxH	6	908.3	151.4	69	1.07	0.3894
SPECIESxNxH	12	2307.0	192.3	69	1.36	0.2076
ENVxSPECIESxNxH	12	1912.2	159.4	69	1.13	0.3546
REP‖(ENV)	6	1924.7	320.8	12.511	1.14	0.3974
REP(ENVxSPECIES)	12	3464.2	288.7	34.848	1.19	0.3254
REP(ENVxSPECIESxN)	36	6423.1	178.4	69	1.26	0.2030
REP(ENVxSPECIESxH)	51	10982.0	215.3	69	1.52	0.0523
Residual	69	9769.0	141.6	-	-	-

*Environment
†Cool-season grass species
‡Nitrogen application rate
§Harvests per season
‖Replication across field

Table B.7 Error terms for analysis of variance for physiological nitrogen use efficiency (PNUE) for Year 1 Starkville 2013 and Year 1 Brooksville 2014.

Source	Error Term
ENV*	0.9845 MS(REP(ENV)) + 474E-7 MS(REP(ENV*SPECIES)) + 0.0072 MS(REP(ENV*SPECIES*H)) + 0.0083 MS(Residual)
SPECIES†	0.9587 MS(REP(ENV*SPECIES)) + 0.0072 MS(REP(ENV*SPECIES*H)) + 0.0341 MS(Residual)
ENVxSPECIES	0.9518 MS(REP(ENV*SPECIES)) + 0.009 MS(REP(ENV*SPECIES*H)) + 0.0392 MS(Residual)
N‡	0.8334 MS(REP(ENV*SPECIES*N)) + 0.1666 MS(Residual)
ENVxN	0.8334 MS(REP(ENV*SPECIES*N)) + 0.1666 MS(Residual)
SPECIESxN	0.8579 MS(REP(ENV*SPECIES*N)) + 0.1421 MS(Residual)
ENVxSPECIESxN	0.8579 MS(REP(ENV*SPECIES*N)) + 0.1421 MS(Residual)
H§	0.7641 MS(REP(ENV*SPECIES*H)) + 0.2359 MS(Residual)
ENVxH	0.7614 MS(REP(ENV*SPECIES*H)) + 0.2386 MS(Residual)
SPECIESxH	0.7862 MS(REP(ENV*SPECIES*H)) + 0.2138 MS(Residual)
ENVxSPECIESxH	0.7832 MS(REP(ENV*SPECIES*H)) + 0.2168 MS(Residual)
NxH	MS(Residual)
ENVxNxH	MS(Residual)
SPECIESxNxH	MS(Residual)
ENVxSPECIESxNxH	MS(Residual)
REP‖(ENV)	0.9587 MS(REP(ENV*SPECIES)) + 274E-7 MS(REP(ENV*SPECIES*H)) + 0.0412 MS(Residual)
REP(ENVxSPECIES)	0.919 MS(REP(ENVxSPECIESxN)) + 0.8999 MS(REP(ENVxSPECIESxH)) - 0.8189 MS(Residual)
REP(ENVxSPECIESxN)	MS(Residual)
REP(ENVxSPECIESxH)	MS(Residual)
Residual	-

*Environment
†Cool-season grass species
‡Nitrogen application rate
§Harvests per season
‖Replication across field

Table B.8 Expected mean square for partial factor productivity (PFP), agronomic nitrogen use efficiency (ANUE), and apparent nitrogen recovery (ANR) for Year 2 Starkville 2013.

Source	**Expected Mean Square**
SPECIES*	Var(Residual) + 2.8938 Var(REP(SPECIESxH))+ 3.8584 Var(REP(SPECIESxN))+ 11.575 Var(REPxSPECIES)+ Q(SPECIES, SPECIESxN, SPECIESxH, SPECIESxNxH)
N†	Var(Residual) + 3.8584 Var(REP(SPECIESxN))+ Q(N, SPECIESxN, NxH, SPECIESxNxH)
SPECIESxN	Var(Residual) + 3.8626 Var(REP(SPECIESxN))+ Q(SPECIESxN, SPECIESxNxH)
H‡	Var(Residual) + 2.8953 Var(REP(SPECIESxH))+ Q(H, SPECIESxH, NxH, SPECIESxNxH)
SPECIESxH	Var(Residual) + 2.9003 Var(REP(SPECIESxH))+ Q(SPECIESxH, SPECIESxNxH)
NxH	Var(Residual) + Q(NxH, SPECIESxNxH)
SPECIESxNxH	Var(Residual) + Q(SPECIESxNxH)
REP§	Var(Residual) + 2.8953 Var(REP(SPECIESxH))+ 3.8604 Var(REP(SPECIESxN))+ 11.581 Var (REPxSPECIES) + 34.744 Var(REP)
REPxSPECIES	Var(Residual) + 2.9003 Var(REP(SPECIESxH))+ 3.867 Var(REP(SPECIESxN))+ 11.601 Var (REPxSPECIES)
REP(SPECIESxN)	Var(Residual)+ 3.8889 Var(REP(SPECIESxN))
REP(SPECIESxH)	Var(Residual) + 2.9259 Var(REP(SPECIESxH))
Residual	Var(Residual)

*Cool-season grass species
†Nitrogen application rate
‡Harvests per season
§Replication across field

Table B.9 Analysis of variance for partial factor productivity (PFP) for Year 2 Starkville 2013.

Source	DF	Sum of Squares	Mean Square	Error Term	Error DF	F Value	Pr > F
SPECIES*	2	2140.5	1070.3	0.9978 MS(REPxSPECIES) + 0.0022 MS(Residual)	6.0042	20.13	0.0022
N[†]	2	2243.2	1121.6	0.9922 MS(REP(SPECIESxN)) + 0.0078 MS(Residual)	18.341	158.73	<0.0001
SPECIESxN	4	165.0	41.2	0.9932 MS(REP(SPECIESxN)) + 0.0068 MS(Residual)	18.293	5.84	0.0033
H[‡]	3	1292.3	430.8	0.9895 MS(REP(SPECIESxH)) + 0.0105 MS(Residual)	27.148	13.36	<0.0001
SPECIESxH	6	1642.8	273.8	0.9912 MS(REP(SPECIESxH)) + 0.0088 MS(Residual)	27.124	8.48	<0.0001
NxH	6	88.5	14.8	MS(Residual)	52	1.75	0.1279
SPECIESxNxH	12	172.3	14.4	MS(Residual)	52	1.70	0.0929
REP[§]	3	164.6	54.9	0.9983 MS(REPxSPECIES) + 0.0017 MS(Residual)	6.0032	1.03	0.4431
REPxSPECIES	6	319.7	53.3	0.9944 MS(REP(SPECIESxN)) + 0.9912 MS(REP(SPECIESxH)) - 0.9856 MS(Residual)	22.504	1.72	0.1618
REP(SPECIESxN)	18	127.0	7.1	MS(Residual)	52	0.84	0.6503
REP(SPECIESxH)	27	877.6	32.5	MS(Residual)	52	3.86	<0.0001
Residual	52	438.2	8.4	-	-	-	-

*Cool-season grass species
[†]Nitrogen application rate
[‡]Harvests per season
[§]Replication across field

Table B.10 Analysis of variance for agronomic nitrogen use efficiency (ANUE) for Year 2 Starkville 2013.

Source	DF	Sum of Squares	Mean Square	Error Term	Error DF	F Value	Pr > F
SPECIES*	2	225.6	112.8	0.9978 MS(REPxSPECIES) + 0.0022 MS(Residual)	6.007	3.10	0.1187
N†	2	139.5	69.7	0.9922 MS(REP(SPECIESxN)) + 0.0078 MS(Residual)	18.467	11.88	0.0005
SPECIESxN	4	16.1	4.0	0.9932 MS(REP(SPECIESxN)) + 0.0068 MS(Residual)	18.401	0.69	0.6109
H‡	3	199.5	66.5	0.9895 MS(REP(SPECIESxH)) + 0.0105 MS(Residual)	27.154	1.89	0.1551
SPECIESxH	6	786.2	131.0	0.9912 MS(REP(SPECIESxH)) + 0.0088 MS(Residual)	27.129	3.72	0.0079
NxH	6	9.4	1.6	MS(Residual)	52	0.16	0.9852
SPECIESxNxH	12	112.3	9.4	MS(Residual)	52	0.98	0.4789
REP§	3	173.4	57.8	0.9983 MS(REPxSPECIES) + 0.0017 MS(Residual)	6.0054	1.59	0.2875
REPxSPECIES	6	218.5	36.4	0.9944 MS(REP(SPECIESxN)) + 0.9912 MS(REP(SPECIESxH)) - 0.9856 MS(Residual)	20.184	1.15	0.3687
REP(SPECIESxN)	18	105.2	5.8	MS(Residual)	52	0.61	0.8735
REP(SPECIESxH)	27	957.7	35.5	MS(Residual)	52	3.72	<0.0001
Residual	52	496.2	9.5	-	-	-	-

*Cool-season grass species
†Nitrogen application rate
‡Harvests per season
§Replication across field

Table B.11 Analysis of variance for apparent nitrogen recovery (ANR) for Year 2 Starkville 2013.

Source	DF	Sum of Squares	Mean Square	Error Term	Error DF	F Value	Pr > F
SPECIES*	2	2837.8	1418.9	0.9978 MS(REPxSPECIES) + 0.0022 MS(Residual)	6.0274	17.71	0.0030
N†	2	433.1	216.6	0.9922 MS(REP(SPECIESxN)) + 0.0078 MS(Residual)	18.256	2.38	0.1210
SPECIESxN	4	219.1	54.8	0.9932 MS(REP(SPECIESxN)) + 0.0068 MS(Residual)	18.22	0.60	0.6667
H‡	3	707.2	235.7	0.9895 MS(REP(SPECIESxH)) + 0.0105 MS(Residual)	27.191	0.97	0.4212
SPECIESxH	6	4293.7	715.6	0.9912 MS(REP(SPECIESxH)) + 0.0088 MS(Residual)	27.16	2.94	0.0242
NxH	6	363.7	60.6	MS(Residual)	52	0.74	0.6181
SPECIESxNxH	12	488.5	40.7	MS(Residual)	52	0.50	0.9062
REP§	3	1235.7	411.9	0.9983 MS(REPxSPECIES) + 0.0017 MS(Residual)	6.0208	5.14	0.0425
REPxSPECIES	6	480.6	80.1	0.9944 MS(REP(SPECIESxN)) + 0.9912 MS(REP(SPECIESxH)) - 0.9856 MS(Residual)	23.141	0.32	0.9215
REP(SPECIESxN)	18	1641.8	91.2	MS(Residual)	52	1.12	0.3637
REP(SPECIESxH)	27	6607.4	244.7	MS(Residual)	52	3.00	0.0003
Residual	52	4247.2	81.7	-	-	-	-

*Cool-season grass species
†Nitrogen application rate
‡Harvests per season
§Replication across field

Table B.12 Analysis of variance for physiological nitrogen use efficiency (PNUE) for Year 2 Starkville 2013.

Source	DF	Sum of Squares	Mean Square	Error Term	Error DF	F Value	Pr > F
SPECIES*	2	5991.0	2995.5	0.9978 MS(REPxSPECIES) + 0.0022 MS(Residual)	6.017	10.08	0.0120
N†	2	234.5	117.2	0.9922 MS(REP(SPECIESxN)) + 0.0078 MS(Residual)	18.368	0.80	0.4642
SPECIESxN	4	263.1	65.8	0.9932 MS(REP(SPECIESxN)) + 0.0068 MS(Residual)	18.316	0.45	0.7717
H‡	3	2011.3	670.4	0.9895 MS(REP(SPECIESxH)) + 0.0105 MS(Residual)	27.252	1.58	0.2172
SPECIESxH	6	3932.6	655.4	0.9912 MS(REP(SPECIESxH)) + 0.0088 MS(Residual)	27.211	1.54	0.2022
NxH	6	998.2	166.4	MS(Residual)	52	0.88	0.5135
SPECIESxNxH	12	2423.0	201.9	MS(Residual)	52	1.07	0.4016
REP§	3	1009.7	336.6	0.9983 MS(REPxSPECIES) + 0.0017 MS(Residual)	6.0129	1.13	0.4084
REPxSPECIES	6	1785.3	297.6	0.9944 MS(REP(SPECIESxN)) + 0.9912 MS(REP(SPECIESxH)) - 0.9856 MS(Residual)	17.324	0.78	0.5990
REP(SPECIESxN)	18	2630.8	146.2	MS(Residual)	52	0.78	0.7165
REP(SPECIESxH)	27	11531.0	427.1	MS(Residual)	52	2.27	0.0056
Residual	52	9789.0	188.2	-	-	-	-

*Cool-season grass species
†Nitrogen application rate
‡Harvests per season
§Replication across field

APPENDIX C

PERSISTENCE OF SOUTHEASTERN WILDRYE AS AFFECTED BY NITROGEN APPLICATION AND HARVEST INTERVAL

Table C.1 Expected mean square for persistence of southeastern wildrye plants for Starkville 2013 following Year 2.

Source	**Expected Mean Square**
N*	Var(Residual) + 4 Var(REP(N))+ Q(N, NxH)
H†	Var(Residual) + 4 Var(REP(H))+ Q(H, NxH)
NxH	Var(Residual) + Q(NxH)
REP‡	Var(Residual) + 4 Var(REP(H))+ 4 Var(REP(N))+ 16 Var (REP)
REP(N)	Var(Residual) + 4 Var(REP(N))
REP(H)	Var(Residual)+ 4 Var(REP(H))
Residual	Var(Residual)

*Nitrogen application rate
†Harvests per season
‡Replication across field

Table C.2 Analysis of variance for persistence of southeastern wildrye plants for Starkville 2013 following Year 2.

Source	**DF**	**Sum of Squares**	**Mean Square**	**Error Term**	**Error DF**	**F Value**	**Pr > F**
N*	3	57.80	19.27	MS(REP(N))	9	2.61	0.1156
H†	3	2124.92	708.31	MS(REP(H))	9	25.63	<0.0001
NxH	9	94.14	10.46	MS(Residual)	27	1.31	0.2770
REP‡	3	192.17	64.06	MS(REP(N)) + MS(REP(H)) - MS(Residual)	7.8365	2.37	0.1480
REP(N)	9	66.39	7.38	MS(Residual)	27	0.92	0.5197
REP(H)	9	248.77	27.64	MS(Residual)	27	3.46	0.0058
Residual	27	215.42	7.98	-	-	-	-

*Nitrogen application rate
†Harvests per season
‡Replication across field

APPENDIX D

WEATHER DATA, HARVEST DATES, AND FERTILIZATION DATES FOR ALL ENVIRONMENTS

Table D.1 Mean monthly temperatures (°C) and total monthly precipitation (mm) for Starkville, MS, from planting in 2013 to final harvest in 2015.

Year	Month	Temperature (°C)	Precipitation (mm)
2013	October	17.4	104.6
	November	8.9	78.9
	December	5.7	134.8
2014	January	2.2	77.3
	February	4.4	92.6
	March	8.6	125.2
	April	15.3	27.0
	May	22.2	162.6
	June	26.1	260.8
	July	24.4	45.0
	August	25.9	33.3
	September	22.3	9.4
	October	19.0	13.5
	November	9.0	0.0
	December	9.0	0.0
2015	January	5.7	0.0
	February	4.3	122.9
	March	13.4	146.8
	April	18.3	149.8
	May	22.0	119.3
	June	26.2	63.1
	July	28.3	89.0

Table D.2 Mean monthly temperatures (°C) and total monthly precipitation (mm) for Brooksville, MS, from planting in 2014 to final harvest in 2015.

Year	Month	Temperature (°C)	Precipitation (mm)
2014	October	19.1	11.7
	November	8.7	23.2
	December	9.3	59.7
2015	January	5.3	53.1
	February	4.0	47.3
	March	12.9	43.4
	April	18.6	45.8
	May	22.0	23.4
	June	25.7	25.3
	July	27.6	17.8

Table D.3 Harvest and fertilization dates at all environments for three cool-season grass species.

		Date for Each Harvest			
Environment	Harvests per Season	1	2	3	4
Starkville	1	7-17			
2013	2	5-22	7-17		
Year 1*	3	5-6	6-12	7-17	
	4[‡]	4-25	5-22	6-20	7-17
Brooksville	1	7-27			
2014	2	6-1	7-27		
Year 1[†]	3	5-14	6-19	7-27	
	4[‡]	5-4	6-1	6-30	7-27
Starkville	1	7-2			
2013	2	5-7	7-2		
Year 2[†]	3	4-19	5-26	7-2	
	4[‡]	4-9	5-7	6-4	7-2

*Harvests conducted in Spring 2014
[†]Harvests conducted in Spring 2015
[‡]Fertilization for all plots with respective N kg-1 ha-1 yr-1 requirements occurred following each harvest