5-1-2013

FORAGE QUALITY OF ANDROPOGON GERARDII ACROSS A PRECIPITATION GRADIENT

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by

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B.A. Whittier College, 2009

A Thesis
Submitted in Partial Fulfillment of the Requirements for the
Master of Science Degree

Department of Plant Biology
in the Graduate School
Southern Illinois University Carbondale
May 2013

THESIS APPROVAL

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November 5th, 2012

AN **ABSTRACT** OF THE THESIS OF
JULIETTE M. DONATELLI, for the Master of Science degree in PLANT BIOLOGY, presented on November 5th, 2012, at Southern Illinois University at Carbondale.

TITLE: Forage Quality of *Andropogon gerardii* across a precipitation gradient

MAJOR PROFESSOR: Dr. David J. Gibson

This study focused on the ecotypic variation in forage quality of *Andropogon gerardii* Vitman, a dominant C_4 grass in North American grasslands and an important forage grass for native and introduced grazers. Ecotypes are genetic variations of a plant species adapted to local environmental conditions. *Andropogon gerardii* is represented by many local ecotypes across its range. Forage quality analyses quantify digestibility and nutrition of a plant sample and allow an assessment of nutritional value for grazers. The variability in forage quality among *A. gerardii* ecotypes is unknown. This study aimed to quantify variation in forage quality of *A. gerardii* collected across a precipitation gradient from eastern Colorado to southern Illinois in the North American grassland. Samples of *A. gerardii* plants in four distinct precipitation regions and three-remnant grassland populations within each region were sampled in July 2010 to assess differences in forage quality. In the field study, forage quality increased along an east to west gradient corresponding with a decrease in annual precipitation levels. A greenhouse study, conducted in April to September 2010, was used to test effect of varied precipitation on three *A. gerardii* ecotypes from distinct precipitation regions grown under controlled conditions. In the greenhouse experiment, plant maturity had significant effects on all

forage measurements except lignin (ADF%). Forage quality was most directly connected to environmental conditions and forage maturity, with smaller differences among population sources. Of those tested here, southern IL ecotypes were the most adaptable to variations in precipitation, and will likely maintain high levels of forage quality under projected changes in precipitation resulting from climate change.

DEDICATION

This thesis is dedicated to my family for teaching me to never give up on a commitment, and Fran Wachter for her endless support, love and humor.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. David J. Gibson, for his constant guidance, comments and support. I would not have found SIUC without his mentorship. I would also like to thank Dr. Sara Baer and Dr. Amer AbuGhazaleh for serving on my committee. My specializations in ecological restoration and animal nutrition could not have been possible without their help and guidance.

Thank you to the National Science Foundation (NSF) HEART GK-12 STEM Fellowship for their funding, seminars, and professional development experiences. Thank you to the Plant Biology Department for their classes, facility and faculty. Thank you to the Animal Sciences and Nutrition Laboratory in the College of Agriculture, especially Dr. Rebecca Atkinson, for providing me with research facilitates and guidance. Thank you to the Southern Illinois University of Carbondale (SIUC) Horticulture Research Center in Carbondale, IL in addition to the SIUC greenhouse for providing me with facilities to conduct my greenhouse experiment. I would also like to thank the collaboration with Kansas State University, in addition to the USDA for funding the "Ecotype Project," and in particular, Dr. Loretta Johnson, for her aid in guiding me to remnant prairies throughout the state of Kansas.

Thank you to my colleagues in the Plant Biology department for their support, encouragement and friendship. I would like to specially thank Meredith Mendola and Rachel Goad for their assistance both in the greenhouse and in the field. Ms. Watcher and her 6^{th}, 7^{th}, and 8^{th} grade classes from 2009-2011, my weekly lessons in their classroom brought me great joy and satisfaction. Lastly, I would like to thank my friends and

family for their endless support, humor and love. I could not have done this without them.

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CHAPTER 1: INTRODUCTION

Global Environmental Change

Global environmental change is the most significant research and policy issue facing humankind (Burton et al. 1993). Evidence of global changes are "certain—certain they are happening, and certain they are human-caused (Vitousek 1994)." Human development has altered biogeochemical cycles around the globe. Dramatic increases in greenhouse gases due to continuing rise in industrial emissions are causing rising temperature, greater tropical storm intensities, shifts in global rainfall patterns, melting of polar ice caps and rising sea levels (IPCC 2007). As a result, climate change has energized a vast array of scientific research to better understand ecosystem responses to these environmental changes. For instance, recent statistical models have aimed to project future climate scenarios (IPCC et al. 2000, Botkin et al. 2007), but due to the complexity of ecosystem interactions—from the molecular level to the system level and from abiotic and biotic factors—it is difficult to accurately predict outcomes of such changes. Nevertheless, more research is needed to bridge the gap between basic and applied science to better-forecast future consequences of global change.

The Grassland Ecosystem and Land Use Change

Grasslands occur on every continent, except Antarctica, encompassing an estimated 31-43% of the Earth's surface (Gibson 2009). Human development has reduced distribution of global grasslands to 16% of the land surface (WorldResources 2000), and references

therein). The grassland ecosystem is a major terrestrial carbon (C) sink containing one third of the world's terrestrial carbon due to its extensive root and soil microbial systems (Scurlock and Hall 2002). Accordingly, preservation and restoration of the grassland ecosystem is important to mitigate the effects of global change (Lal 2004, Harris et al. 2006).

The North American Great Plains was the largest biome in North America stretching 4.1×10^8 ha, from east of the Rocky Mountains to Ohio, and from the southern Canadian border into Texas (Samson et al. 2003). This system formed as upwelling of the Rocky Mountains, about 55 million years ago, created a rain shadow effect east of their range. Grassland distribution and composition are driven primarily by temperature and rainfall (Epstein et al. 1997) and water availability is a principle limiting abiotic factor through their distribution (Knapp et al. 2001).

The expanse of the North American grassland is categorized into three community-types: tallgrass prairies, mixed prairie, and shortgrass prairies (Samson and Knopf 1994). These community-types range along a west to east gradient—from short to tallgrass prairies—as a direct result of an increase in precipitation. Moist air from the Gulf of Mexico aids in the formation of the tallgrass prairie in the southeast reaches of its range. Tallgrass prairie has undergone the greatest habitat destruction as compared to mixed and shortgrass prairies (Table 2) due to their nutrient rich soils and lack of rocky outcrops.

Once covering 162 million hectares of the United States (Samson and Knopf 1994, Christopher 1999), an estimated 70% of the original extent of the Great Plains has been destroyed since European settlement (Samson et al. 2004). The Homestead Act of

1862, which encouraged settlement of the Plains, offered free land to individuals willing to farm. European farming practices could be applied to the mid-western United States because the John Deere steel plow (patented in 1837) made it easy to cultivate once unworkable soil. A total of 1.5 million people colonized on to 800,000 km^2 of land (Samson et al. 2004). This settlement coincided with the eradication of native grazers from the prairie, particularly American Bison (*Bison bison*), which were hunted for their skins. By the late 1800s, the American Bison was nearly extinct.

An estimated 85-90% of the tallgrass prairie in the United States has been eliminated (Samson et al. 2004), largely due to industrial agriculture, specifically the production of corn and soybeans. Today, North American tallgrass prairies are classified as an endangered ecosystem. The state of Illinois, for example, has an estimated <0.01% of intact remnant prairies intact.

Biology of *Andropogon gerardii*

Andropogon gerardii Vitman is a dominant, perennial C_4 grass native to the North American tallgrass prairies, compromising up to 80% of biomass in tallgrass prairie (Weaver and Fitzpatrick 1932, Kakani and Raja Reddy 2007). *Andropogon gerardii*, a warm season grass has a six month growing period and flowers in July through early October. In late July, when the plant is beginning to flower, *A. gerardii* grows at a maximum rate of 2 cm/day, reaching a height of 1.8 to 3 m (Weaver et al. 1935). At maturity, *A. gerardii* grows to 2-3 m tall, with characteristic spikelets resembling a "turkey foot," which is where this plant received its nickname. *A. gerardii*'s roots can descend over 2.4 meters belowground (Weaver et al. 1935), and because of this is widely

used in restoration for erosion control. *Andropogon gerardii*'s leafy forage is highly palatable to all classes of livestock, and makes good quality hay if harvested before seed heads emerge (Schwendiman and Hawk 1973). Cultivars of *A. gerardii* have been bred for improved forage yield and digestibility, i.e., 'Bonanza' and 'Goldmine' (Mitchell et al. 2005).

Ranging throughout the North American Great Plains, *A. gerardii's* extensive geographic coverage has made it a model species in grassland research and restoration. A better understanding of variation throughout population sources in dominant species is crucial to adequately restore and ensure the continuity of restored systems. Dominant species drive ecosystem function and community structure (Smith and Knapp 2003); therefore, understanding their responses to change can be a window into the responses of an entire community.

Ecotypes

An ecotype is a genetic variation of a species adapted to local environmental conditions. Ecotypes can express a wide variety of ecophysiological and functional diversity in order for a species to compete for resources in an area. Some of these variations include traits of growth rate, flowering time, productivity, population dynamics and differences in overall ecosystem function (Ackerly et al. 2000).

Local environmental conditions result in intraspecific variation. Environmental differences, i.e. temperature, altitude, precipitation and soil type, all play a role in local adaption among ecotypes across landscapes. Physiological responses can determine

ecotypic differences because these may be a plants first response to altered environmental conditions (Gibson 2002).

For over half a century, studies have focused on ecotypic variation within a species across latitudinal gradients, to include the effect of temperature and length of growing season on plants (Vaartaja 1959, McMillian 1960, McNaughton 1966, McMillian 1969, Heide 1994, Sawada et al. 1994, Li et al. 1998). A milestone study conducted on grassland ecotypes used a common garden in Lincoln, Nebraska (McMillian 1959) to document ecotypic variation of dominant grass species from 43 sites throughout the North American central grasslands. Among one of the dominant species used in the experiment was *Andropogon gerardii*. McMillian concluded that the southern and eastern ecotypes flowered earlier and grew taller than ecotypes from northern and western populations (McMillian 1959). A more recent study by Gustafson et al. (1999), examined the genetic variation within and among populations of *A. gerardii* from Arkansas and Illinois remnant prairies. This study showed greater differences *within* populations of *A. gerardii* than among populations. Genetic variation between populations showed high levels (83-99%) of variability, while low levels (11%) of population divergence throughout (Gustafson et al. 1999).

Restoration Ecology

Restoration Ecology is a science that uses ecological theory to guide the practice of ecological restoration. In an era faced with rapid global change, ensuring restored populations have ecological integrity and adaptive ability to withstand rapid change is critical to the persistence of communities. Restoration ecology often incorporates

population genetics to ensure healthy adaptive variation within restorations. Accordingly, the importance of the origin of seed source used in restorations is under examination.

Aldo Leopold initiated the first ecosystem restoration effort in 1935, a tallgrass prairie restoration at the University of Wisconsin Arboretum, now known as the Curtis Prairie, laying the foundation for ecological restoration efforts (Kindscher and Tieszen 2004). Today there are thousands of restoration initiatives worldwide fostered by both government agencies and non-profit organizations.

Currently, ecological restoration involves planting local population sources to mirror region specific environmental conditions, therefore, maintaining a local gene pool (Schramm 1970, 1992, Gustafson et al. 2005). A growing body of practice is emerging which has caused restorationists to rethink the optimal origin of seed sources for restoration (Lesica and Allendorf 1999) and whether locally adapted sources are too genetically narrow in light of future environmental change (Lesica and Allendorf 1999, Hufford and Mazer 2003, Rice and Emery 2003, O'Neill et al. 2008). Referencing past ecological models is proving to be limited in the face of rapid climate change (Harris et al. 2006). Shifts in climactic conditions may require reestablishing greater genetic heterogeneity within species to preserve biodiversity (Botkin et al. 2007). Current theory indicates the importance of rebuilding for future scenarios by representing genetically diverse populations in restoration projects (Pfadenhauer and Grootjans 1999, Hobbs and Harris 2001, Hufford and Mazer 2003, Harris et al. 2006, Lawler 2009).

Role of Forage Quality

Forage quality is the physical and chemical nutritive value of a plant, which benefits an animal's diet (Owensby et al. 1996, Balasko and Nealson 2003), and refers to how well animals consume a forage and how efficiently the nutrients in the forage are converted into animal products (Linn and Martin 1999). No one measure adequately accounts for a ruminants response to feeds (Van Soest 1973). Therefore, forage quality cannot be directly categorized on the basis of a single measure due to the complexity of the subject. Separate forage analyses (i.e., 1. cell components: protein, sugar, starch and organic acids; and, 2. fibrous or cell wall components: cellulose, hemicellulose and lignin) must be cohesively examined to comprehensively assess forage grade for a ruminant.

Key factors influencing forage quality are species, stage of maturity and storage techniques. Higher quality forage often comes from legumes, rather than grasses, and cool-season rather than warm-season grasses (Ball et al. 2001). Optimal ruminant health and maximizing profit are some of the significant advantages to the production of high-quality forage. Rangelands provide 95% of food for wild ruminants (Semple 1970), accordingly, selecting high quality forage in grassland restoration is of paramount importance. High quality forage is often also very palatable to grazers. Within the grassland ecosystem an integral component to its function is the relationship between ungulates and grasses.

Ungulates are important for ecosystem structure and function in the grassland ecosystem creating spatial heterogeneity, controlling successional processes and regulating the ecosystem between alternative stable states (Hobbs 1996). Restoration managers can benefit greatly from integrating grazers such as bison or cattle into

restoration efforts. Sophisticated restoration efforts use grazers and a balance of native grasses, forbs and legumes, as well as fire, to rebuild the function of the North American grasslands. For example, the Flint Hills of Kansas is the largest, unplowed tallgrass remnant prairie in North America, encompassing a total of 3500 ha (Knapp et al. 1999). Land is regularly burned, and in 1987, Konza Prairie reintroduced bison. Accordingly, Konza Prairie is a site for extensive ongoing ecological research dating back as early as 1972, with hundreds of concurrent research projects occurring on the land. Konza Prairie stands as a key area for prairie research and is used as a model for grassland restoration efforts around the world.

Bison are known to increase plant species richness and spatial heterogeneity in tallgrass prairie (Towne et al 2005). Yet, using cattle to store degraded systems might be more practical due to their current vast majority on grasslands (Allred et al 2011). Studies comparing bison and cattle grazing have shown overall similarities in grazing trends (Towne et al. 2005). Bison and cattle exhibit seasonal differences in their selection for grasses and forbs (Plumb and Dodd 1993). Bison and cattle effects on landscape heterogeneity can be similar if properly managed (Fuhlendorf et al. 2008). Studies evaluating vegetation trends in tallgrass prairies from bison and cattle have shown that plant communities are 85% similar after ten years of grazing (Towne et al. 2005). Overtime, *Andropogon gerardii* increased in cattle-grazed pastures and did not significantly change in bison-grazed pastures over time (Towne et al. 2005). Both species are known to prefer recently burned areas and avoid grazing on steep slopes (Allred et al. 2011). As time since the last burn increased, bison grazed less (Coppedge and Shaw 1998). This dynamic relationship known as pyric herbivory is crucial to

ecosystem structure and function (Fuhlendorf et al. 2008). Bison choose areas based on forage quality rather than quantity (Coppedge and Shaw 1998). Cattle decrease their distance from water whereas bison seem to increase their distance (Allred et al. 2011). It is known that bison reduce their intake during rut, allocating their time towards reproduction rather than resource intake, whereas cattle generally do not show this trend during mating season (Plumb and Dodd 1993).

An increase in atmospheric CO_2 will favor C_3 over C_4 photosynthesis (Bond 2008). It is essential to understand how changes in CO_2, and the subsequent changes in photosynthetic pathway preferences will affect the grassland ecosystem and grazing systems (Chammaillé-Jammes and Bond 2010). Recent studies show it is likely that forage quality of rangeland plants will decrease under elevated CO_2, leading to reduced growth and reproduction of ruminants (Owensby et al. 1996).

Past studies have shown slight differences in mineral content among cultivars of switchgrass (*Panicum virgatum*) (Lemus et al. 2002). This indicates that differences in mineral content of *A. gerardii* ecotypes are highly likely. Moreover, a study evaluating effects of increased CO_2 on tall fescue (*Schedonorus phoenix*) showed elevated CO_2 affected several variables associated with digestibility. Some changes noted in the study were lower neutral detergent fiber levels, higher nitrogen levels and a 21% reduction in crude protein under elevated CO_2 (Newman et al. 2003). These changes in CO_2 level, and subsequently changes in nutrient levels of plants, have the ability to decrease a ruminant's growth and reproduction.

A number of studies have tested variation in forage quality as a result of changes in temperature. Yet, little research has been done on *Andropogon gerardii* ecotypes

across a precipitation gradient, examining a response to varied precipitation levels. Forage quality studies of *A. gerardii* have shown overall high quality performance, and suggest optimal forage quality occurs between late June and early August (Griffin and Jung 1983). Thus, samples for this study were collected in the month of July, at peak forage quality for *A. gerardii*.

Research Questions

What is the degree of intraspecific variation in *Andropogon gerardii* collected from remnant tallgrass prairies along a precipitation gradient in the North American grasslands? What is the effect of ecotypic variation on forage quality measurements?

Research Objectives

The main objective of this study was to quantify the degree of intraspecific variation within *Andropogon gerardii* Vitman ecotypes collected across a precipitation gradient in the North American grasslands testing the effect of ecotypic variation on forage quality measurements, thus allowing an assessment of suitability for grazers. Experimental inquiry was conducted both in the field and the greenhouse.

The specific objectives were to:

1. Quantify intraspecific morphological variation among ecotypes of *Andropogon gerardii* through a greenhouse experiment.
2. Quantify ecotypic variation on forage quality of *Andropogon gerardii.*

Hypotheses

H_1: Ecotypes of *Andropogon gerardii* will exhibit greater levels of forage quality (i.e., high IVDMD, low ADF, high CP) in eastern sites as a result of greater precipitation, and decrease along a precipitation gradient as sites move further west along the gradient.

H_2: Eastern ecotypes of *Andropogon gerardii* will exhibit higher biomass (i.e., above- and belowground biomass) than western ecotypes when grown in a controlled environment.

CHAPTER 2: METHODS

Greenhouse Experiment

A greenhouse experiment was conducted in the summer of 2010 to test for intraspecific variation in forage quality and net primary productivity within wild sources of *A. gerardii* grown under controlled conditions.

Experimental Design

The experiment consisted of three population treatments (Twelve Mile Prairie, Konza Prairie, and Relic Prairie) and two soil moisture treatments (factorial design = 3 x 2). Each population treatment consisted of three source origins from different precipitation regions (Table 2.1). Each treatment combination (population source by soil moisture treatment) was replicated 4 times (n=4).

All population sources were collected from seed by hand in October and November of 2008 from their native population source. Seeds were stored in dry conditions. The central Kansas population source was collected from Relic prairie (38°51' N, 99°22' W), near Hays, KS (hereafter referred to as central KS, CKS). The eastern Kansas population source was collected from Konza Prairie Biological Research Station (39°05' N, 96°32' W), south of Manhattan, Kansas (herein referred to as eastern KS, EKS). The southern Illinois source was collected from the Twelve Mile Prairie (38°44' N, 88°53' W), a remnant railroad prairie near Farina, Illinois (hereafter referred to as southern IL, SIL).

Plants were grown from seed in a greenhouse at Southern Illinois University Carbondale, Carbondale, IL, USA (37°43'N 89°13'W) over a 6-month period from April 2010 to September 2010. Number of seeds sown was chosen based on a preliminary germination test. Seeds were started in Supercell Cone-tainers (21 cm in height by 3.8 cm in diameter) (Stuewe & Sons, Inc. Tangent, Oregon, USA). A generic soil mixture of peat moss, vermiculite and pine bark was used to standardize plant growth (Fafard 3B Mix, Hummert International, Earth City, MO, USA). Cone-tainers were organized in a completely randomized design. At this stage plants were watered 4-6 times a week depending on humidity and temperature.

Ten weeks after emergence of *A. gerardii*, seedlings were transplanted into TPOT4R pots (39.6 cm in height by 20.3 cm in diameter) (Stuewe & Sons, Inc. Tangent, Oregon, USA) and moved to the SIUC Agricultural Research Center Greenhouse. Pots were organized in a completely randomized design, and re-randomized once a week during application of the watering treatment. At this stage seedlings were watered 4-6 times a week depending on humidity and temperature.

Six weeks following transplant of seedlings into the SIUC Agricultural Research Center Greenhouse, *A. gerardii* plants were clipped 5 cm from soil surface (i.e., all plant tissue above 5 cm, including leaves and stems) to assess baseline forage quality of each individual plant. Samples were dried (at 50°C for 7 days), and ground using a Thomas-Wiley Laboratory Mill, Model 4 (Arthur H. Thomas Company, Philadelphia, PA, USA) through a 1 mm screen.

Immediately following baseline forage quality sampling, the experimental watering treatment was initiated. Watering levels were chosen based on field capacity of

soil (Table 2.2). Plants were watered Monday, Wednesday, and Friday and once on Saturday or Sunday for six weeks, totaling 24 watering applications (n=24). Pots were weighed and watering application was calculated for desired watering treatment levels to be achieved. Pots were organized as a completely randomized block and re-randomized prior to each watering treatment to minimize the effects of potential light and temperature gradients. Average day temperatures were 29° C during the day and 23° C at night.

Forage Quality Measurements

Forage quality was measured two times throughout the growing season (18 August 2010 prior to establishment of the watering treatment, and 15 September 2010 at final harvest). Each time individual plants were harvested at >5 cm above the soil surface. Samples were dried (at 50° C for 7 days) and ground, through a 1 mm screen, using a Thomas-Wiley Laboratory Mill, Model 4 (Arthur H. Thomas Company, Philadelphia, PA, USA). Analyses were run dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), In-vitro dry matter digestion (IVDMD), crude fat (CF), crude protein, (CP), ash content and nitrogen (N).

Plant Height and Number of Tillers

Plant height and number of tillers was measured prior to baseline forage quality sampling, and once each week during watering treatment (18 August 2010, 25 August 2010, 1 September 2010, 7 September 2010, 14 September 2010). Plant height was determined from the soil surface to the tip of the longest leaf.

Biomass

At the end of the growing season (15 September 2010), aboveground biomass (AB) and belowground biomass (BB) was determined for each individual plant. To quantify AB, plants were clipped, dried (at 50° C for 7 days), and weighed. To quantify BB, pots were disassembled, roots were separated from soil, washed with water, dried (at 55°C for 7 days), and weighed. AB and BB were combined to determine total biomass (TB).

Forage Analyses

Dry matter and ash content

One gram of dried sample, with two replicates, was placed in a crucible and oven dried 55°C for 24 hours. Prior to oven drying, samples were placed in desiccator for 20 minutes to cool, before being reweighed. The loss of weight due to oven drying is the measure of dry matter (DM).

Ash content measurements were conducted in a Tempco Model No. 293C (Barber-Colman, Ashburn, VA, USA). One gram of sample, with two replicates, was placed in crucibles and ash burn was conducted at 500°C for 24 hours. Crucibles were placed in desiccator for two hours to cool before being weighed. The loss of weight due to burn is the measure of ash content.

Neutral detergent fiber

Samples were weighed to a known quantity of 0.5 g (±0.05 g), with three replicates, into F57 Filter Bags (ANKOM Technology, Macedon NY, USA). Bags were sealed using a

1915/1920 Heat Sealer (ANKOM Technology, Macedon NY, USA). Neutral detergent fiber (NDF) analysis measurements were conducted using an Ankom^{200} Fiber Analyzer (ANKOM Technology, Macedon NY, USA) which ran for 60 minutes at 100°C. After extraction, samples were rinsed with hot water (90-100°C) and alpha-amylase enzyme (ANKOM Technology, Macedon NY, USA), and once more using only hot water. Samples were then soaked in acetone for three minutes, air-dried, and finally oven dried at 55°C for 24 hours. After oven drying, samples were removed from oven, placed in desiccator for 20 minutes to cool and reweighed. The loss of weight due to extraction is the measure of NDF. Neutral detergent fiber represents the total cell wall components (lignin, cellulose and hemicellulose, plus some damaged proteins).

Acid detergent fiber

Samples were weighed to 0.5 g (±0.05 g), with three replicates, placed in F57 Filter Bags (ANKOM Technology, Macedon NY, USA). Bags were sealed using a 1915/1920 Heat Sealer (ANKOM Technology, Macedon NY, USA). Acid detergent fiber (ADF) analysis measurements were conducted using an Ankom^{200} Fiber Analyzer (ANKOM Technology, Macedon NY, USA) which ran for 75 minutes at 100°C. After extraction, samples were rinsed with hot water (90-100°C) three times, soaked in acetone for three minutes, air-dried, and finally oven dried at 55°C for 24 hours. After final oven drying, samples were removed from oven, placed in a desiccator for twenty minutes to cool and reweighed. The loss of weight due to extraction is the measure of ADF.

Acid detergent fiber tests lignin and cellulose content. Lignin is not digestible in the rumen. Therefore, ADF content represents low digestibility; the higher the ADF content, the less the forage is consumed by the ruminant.

In-vitro dry matter digestibility

Two grams (±0.5 g) of sample, with three replicates of each was measured into Screen Bags (Foss North America, Eden Prairie, MN, USA). Ruminal fluid inoculum were collected 2-4 hours after morning feeding from a ruminally fistulated Holstein heifer fed a total mixed ration composed of 35% concentrate mix, 20% corn silage, and 45% alfalfa hay (DM basis). The concentrate mix consisted of ground corn, soybean meal, dried corn distillers plus a vitamins-minerals mix. The rumen contents were brought to the laboratory in a plastic bag under anaerobic conditions, strained through 2 layers of cheesecloth, and used within fifteen minutes. Ruminal contents were mixed with pre-warmed (39°C) buffer solution and *in-vitro* dry matter digestibility (IVDMD) measurements were conducted using a Daisy^{II} Incubator (ANKOM Technology, Macedon, NY, USA). Four samples were run at a time in each container and underwent a 24 hour digestion. After 24 hours, samples were rinsed in deionized (D.I.) water, oven dried for 24 hours at 55°C and reweighed. The loss of weight due to the extraction is a primary measure of sample digestibility.

Crude protein

Samples were weighed to 0.05 grams (±0.005 g) with three replicates in Tin Foil Cups (Leco Corporation, St. Joseph, MI, USA). Crude protein (CP) measurements were taken

using a Leco FP-528 Protein/Nitrogen Determinator (Leco Corporation, St. Joseph, MI, USA).

Crude fat

One gram of each sample, with two replicates, were weighed into XT4 Fat Extraction Filer Bags (ANKOM Technology, Macedon NY, USA) and oven dried at 100°C for three hours to ensure samples were completely dry and free from any water. Samples were removed from oven and placed in a desiccator to cool, then were reweighed and placed back in the desiccator until all bags were weighed and ready for extraction. Crude fat (CF) measurements were conducted using an Ankom^{XT10} (ANKOM Technology, Macedon NY, USA). After extraction, samples were dried at 100°C for one hour and weighed. The loss of weight due to the extraction was the measure of CF content.

Post digestion

Prior to IVDMD, samples were composited and rerun for NDF, CP, Ash and DM.

Statistical Analysis

Data were analyzed with SAS Ver. 9.2 to test for variation in forage quality measurements (DM, Ash, NDF, ADF, IVDMD, CP, CF, N). Repeated measures mixed models were run testing the effects of population sources (n=3, Table 2.1), watering treatment (low, high) and time harvested, and their effect on each forage quality

measurement. *Post-hoc* Tukey tests were run on significant effects to test for differences among treatment levels. Significance was set to $\alpha=0.05$.

A principal component analysis (PCA) was run in Primer-E 6 (Clarke 1993b) on forage quality variables (DM, Ash, NDF, ADF, IVDMD, CP, CF, N) means before watering treatment, and after low and high watering treatments, respectively, in *Andropogon gerardii* population sources for correlation. Principal component axis one and two were graphed to visualize the distribution of sites in ordination space.

Table 2.1. Seed sources used in greenhouse experiment.

Region Source	Name	Seed Source	Latitude (N)	Longitude (W)
Central KS	Hays	Hays, Kansas	38° 52’	99° 19’
Eastern KS	Konza	Manhattan, Kansas	39° 05’	96° 36’
Southern IL	12 Mile	Farina, Illinois	38° 46’	88°50

Table 2.2. Container weight required to establish desired water levels during experimental watering treatments in the greenhouse based upon field capacity of soil.

Watering Treatment	Field Capacity (%)	Container weight (kg)	Total amount of water held (L)
Moist	85	5.32	2.77
Dry	35	3.68	1.14

Field Experiment

An observational field study was used to quantify forage quality of *Andropogon gerardii* throughout four precipitation regions at the time of optimal forage quality for C_4 grasses (Griffin and Jung 1983) during the growing season (2-9 July 2010).

The independent variables were precipitation, region and population source (nested within region). Dependent variables were DM, ash, NDF, ADF, IVDMD, CP, and CF.

Experimental Design

Twelve field sites were located across the North American Great Plains precipitation gradient (Table 2.3). Three remnant prairies were chosen from each of four precipitation regions (Table 2.4). All samples were collected from July 2-9 2010 (Table 2.5). Study sites were high quality prairies, which had never been tilled. Konza Prairie was the only site that was grazed. The grazing herbivore was cattle (May-October) and the burn regime was patch burn.

Field Methods

Within each site, ten $1m^2$ quadrats randomly located in upland areas were sampled. All *A. gerardii* within the quadrat was assessed visually for percent dominance and clipped >5cm from the soil surface and composited for each site. Samples were dried (at 50°C for 7 days), weighed, and finely ground using a Thomas-Wiley Laboratory Mill, Model 4 (Arthur H. Thomas Company, Philadelphia, PA, USA) through a 1 mm screen.

Forage Analyses

See methodologies for greenhouse experiment.

Statistical Analyses

Data were analyzed using SAS Ver. 9.2 to test for variation in forage quality measurements (DM, Ash, NDF, ADF, IVDMD, CP, CF, N) among population sources. Two-way ANOVAs were run on the effects of precipitation region and population source on each forage quality measurement. *Post-hoc* Tukey tests were run on significant effects to test for differences among treatment levels. Significance was set to $\alpha=0.05$.

Principal Component Analysis (PCA) was run using Primer 6 (Clarke 1993a) where each site is represented as a point in multi-dimensional space, calculating correlation among sites (Clarke 1993b). A principal component analysis was run on the correlation matrix of forage variable (DM, Ash, NDF, ADF, IVDMD, CP, CF, N) means of population source samples grown in the greenhouse before and after (low and high) watering treatments. Principal component axis one and two were graphed to see visualize the distribution of sites in ordination space.

Table 2.3. Precipitation regions used in field experiment. Annual average precipitation from www.NOAA.gov (accessed February 15, 2011).

Region Source	**Annual Average Precipitation (mm)**
Boulder, Colorado	405
Hays, Kansas	580
Manhattan, Kansas	830
Carbondale, Illinois	1124

Table 2.4. Location and environmental data for field sites. Data retrieved from http://websoilsurvey.nrcs.usda.gov/ accessed 2/14/2011. (SoilSurveyStaff)

Region	Site Name	Site Abbreviation	County	Latitude (N)	Longitude (W)	Soil Type	Slope (%)	Elevation (m)	Mean annual rainfall (mm)	Mean annual temperature	Visual Assessment of Percent Dominance of *A. gerardii* Within Quadrant Sampled	Growing Degree Days (2010)
Illinois	DeSoto	DES	Jackson	37°51' 14.22"	89°13' 50.49"	Orthents silt-loam	0 to 5	128	1165	54.6°F	25	4474
	Fult's Hill	FTL	Monroe	38°09'26.26"	90°10'53.08"	Menfro silt loam; Stookey silt loam	5 to 10; 35 to 70	218	736 to 1168	54 to 57°F	20	
	12 Mile	12M	Marion	38°44'24.92"	88°53'07.86"	Hoyleton silt loam	0 to 2	192	889 to 1067	52 to 57 °F	20	
Eastern Kansas	Konza	KNZ	Riley	39°05'47.38""	96°32'35.43"	Dwight silt loam, Irwin silty clay loam	1 to 3	366	787 to 1194	51 to 59 °F	25	4105
	Carnahan	CAR	Pottawatomie	39°20'08.20"	96°37'27.19"	Benfield silty clay loam, Florence gravely silt loam	5 to 30	389	787 to 1194	43 to 66 °F	25	
	Top of the World	TOW	Riley	39°13'27.45"	96°37'10.59"	Benfield silty clay loam, Florence gravely silt loam	5 to 30	379	787 to 1194	50 to 57 °F	30	
Central Kansas	Relic	REL	Ellis	38°51'26.69"	99°22'41.35"	Brownell gravelly loam	2 to 10	659	508 to 618	39 to 66 °F	20	4193
	Webster	WEB	Rooks	39°24'19.18"	99°30'19.71"	Wakeen-Harney silt loams	1 to 3	606	508 to 618	41 to 66 °F	30	
	Cedar Bluff	CDB	Trego	38°49'00.10"	99°32'39.21"	Heizer Brownell gravelly silt loam	5 to 30	688	508 to 618	37 to 66 °F	20	
Colorado	Paramount Point	PPT	Boulder	40°00'17.13"	105°17'36.14"	Rock outcrop	20 to 95	1 811	381 to 508	48 to 52 °F	10	3827
	Crown Rock	CRN	Boulder	40°00'04.39"	105°17'39.01"	Rock outcrop	20 to 95	1 879	381 to 508	48 to 52 °F	10	
	Greenbelt	GBT	Boulder	39°55'42.52"	105°14'09.15"	Nederland very cobbly sandy loam	1 to 12	1 802	381 to 508	48 to 52 °F	5	

Table 2.5. Approximate length of growing season in different regions at time of field sampling. Last frost date data from www.NCDC.NOAA.gov (2005) based on 50% probability of mean last frost data from 1971-2000 (accessed September 30, 2012).

Population Region	**Weeks Since Last Frost Date**	**Date Collected (2010)**	**Last Frost Date**
CO	4	July 4-6	May 4
CKS	5	July 6-8	April 26
EKS	6	July 2-3	April 20
SIL	8	July 9	April 15

Synthesis of Greenhouse and Field Experiment

One-way ANOVAs were run by population sources used in the field and greenhouse study to test the treatment effect of field conditions and controlled greenhouse conditions on each forage variable.

A PCA was run on forage variable (DM, Ash, NDF, ADF, IVDMD, CP, CF) means of population sources which were used in greenhouse experiment and field experiment, i.e., Relic Prairie, Konza Prairie and Twelve Mile Prairie. Greenhouse samples were plotted as before watering treatment, and samples after low and high watering treatments. Principal component axis one and two were graphed to map the distribution of sites in ordination space.

CHAPTER 3: RESULTS

Greenhouse Experiment

Growth Measurements

Total biomass (TB)

There was a significant effect of source population on total biomass ($F_{2,18} = 21.76$, $p<0.0001$) (Table 3.1). Overall, means of total biomass showed that the southern IL source was the largest (103.7 ± 14.7 g) and eastern KS source was the smallest (14.9 ± 3.9 g) (Table 3.2) (Figure 3.1) (Appendices Table A1). Total biomass of eastern KS source plants was not statistically different to total biomass of central KS source plants.

Aboveground biomass (AB)

There was a significant effect of source population on aboveground biomass ($F_{2,18}=10.57$, $p=0.0009$) (Table 3.1) (Figure 3.2). Overall, aboveground biomass showed southern IL sources were highest (20.1 ± 2.5 g) and eastern KS was lowest (5.4 ± 1.4 g). Aboveground biomass of central KS plants was not statistically different to aboveground biomass of eastern KS and southern IL plants.

Belowground biomass (BB)

There was a significant effect of source population on belowground biomass ($F_{2,18}=18.31$, $p<0.0001$) (Table 3.1) (Figure 3.3). Overall, means of belowground biomass showed the southern IL source to be the greatest (83.6 ± 13.4 g) and eastern KS source were the

smallest (9.6 ± 2.5 g). Belowground biomass of eastern KS plants was not statistically different to belowground biomass of central KS plants.

Plant height

There were no significant differences among sources in initial height of plants (Table 3.3). There was a two-way interaction between population source and days since cutting on plant height ($F_{8,90}$=2.56, p=0.0145) (Figure 3.4) (Table 3.4). Overall, central KS plants grew to be the tallest (Day 34 = 91.1 ± 8.5 cm) (Table 3.4). For the initial twenty days of plant growth after cutting all sources grew at relatively the same rate. On the twenty-seventh day since cutting central KS plants surpassed eastern KS and southern IL plants in height. Central KS plants grew at a faster rate, surpassing the height of other sources, while eastern KS and southern IL plants leveled out their growth by day thirty-four (Figure 3.4).

There was a marginally significant interaction on height by population source and watering treatment ($F_{2,90}$=3.08, p=0.051) (Table 3.4). Central KS and southern IL source plants were not statistically different to each other (Figure 3.5). Southern IL plants grew marginally taller with higher water treatment than with the low water treatment (Table 3.6, Figure 3.5). Eastern KS plants were statistically similar not different to themselves, and grew shorter with increased watering levels. By contrast, southern IL and central KS sources grew taller when subjected to increased watering levels.

Inflorescence development

Inflorescence development was different among and between ecotypes after watering treatments (Table 3.7). Central KS had five flowering plants, one plant with an emerging inflorescence and two plants with no sign of an inflorescence. Eastern KS had one flowering plant, one plant in the boot stage, one plant with an emerging inflorescence and four plants with no sign of an inflorescence. Southern IL had one plant with an emerging inflorescence and eight plants with no sign of an inflorescence.

Dry matter and ash content

There was a two-way interaction between population source and time harvested on ash content ($F_{2,16}$=3.79, p=0.0449) (Figure 3.6) (Table 3.8). Overall, eastern KS plants had the highest ash content. Central KS plants before cutting were not statistically different to eastern KS plants. Ash content was lowest in plants from southern IL compared with the other sources, regardless of time. Southern IL plants, before and after imposing the watering treatment were not statistically different to the central KS plants after watering treatment. Although ash content in central KS and eastern KS plants decreased after implementing the watering treatment; statistical differences in ash content within a source were restricted to central KS plants.

Neutral detergent fiber

There were no significant treatment, time, or source effects, or interactions on NDF (Table 3.8).

Acid detergent fiber

There was a significant two-way interaction between population source and watering treatment on ADF content, ($F_{2, 16}$=3.77, p=0.0456) (Figure 3.7). ADF content was, on average, greatest in plants from central KS compared to other sources, whereas southern IL plants had the lowest average ADF by source. Eastern KS plants showed the greatest change in ADF value between the low and high watering treatment. ADF of eastern KS low watering treatment plants were marginally less than eastern KS high watering treatment plants (p = 0.09). ADF also decreased significantly with time independent of watering treatment or source (time 1 ADF = 39.7% ± 0.4, time 2 = 37.1% ± 0.4) ($F_{1,16}$ = 12.65, p=0.0026).

In-vitro dry matter digestibility

There was a significant effect of population source and harvest time on IVDMD ($F_{2, 16}$=5.63, p=0.014) (Figure 3.8). Overall, population sources before the watering treatment had a higher IVDMD, and were statistically different (higher) from population sources after watering treatment (lower). Although IVDMD decreased in all sources after the watering treatment, eastern KS plants exhibited the lowest value (38% ± 0.9) and were statistically different from all other sources. IVDMD of central KS and southern IL plants were not statistically different after imposing the watering treatment.

Crude protein

There was a significant effect of time harvested on CP ($F_{1, 16}$=41.73, p<0.0001) (Figure 3.9). Crude protein decreased 3.4% from the first to the second harvest.

Nitrogen

There was a significant effect of time harvested on nitrogen content ($F_{1, 16}$=35.01, p<0.0001) (Figure 3.10). Nitrogen decreased 0.6% following implementation of watering treatment.

Crude fat

There was a significant effect of population source on CF ($F_{2,16}$=4.24, p=0.0334) (Figure 3.11). Overall, southern IL plants had the highest CF content, and were higher than eastern KS plants. Although CF content was lowest in plants from eastern KS, statistical differences in CF were not found between central KS and eastern KS plants.

PCA forage quality

A Principal Component Analysis (PCA) of forage quality measurements, DM, NDF, ADF, IVDMD, CP, and ash, respectively, on *Andropogon gerardii* populations grown in the greenhouse before and after (low and high) watering treatments was run on the correlation matrix. Two PCA axes were retained for interpretation accounting for 97.6% of the total cumulative variation (Table 3.9). A plot of the samples with respect to PCA axes 1 and 2 showed low, negative axis 1 scores for samples before the water treatment was imposed and high, positive axis 1 scores for samples after the water treatment, with the scores increasing for central KS, to southern IL to eastern KS plants (Figure 3.12). The contrast in samples along PCA axis 1 reflected a large negative loading for IVDMD and small but positive loadings for DM and NDF (Table 3.10). PCA axis 2 provided

little separation of plants but was reflective of a contrast in loadings for CF and IVDMD versus NDF and CP.

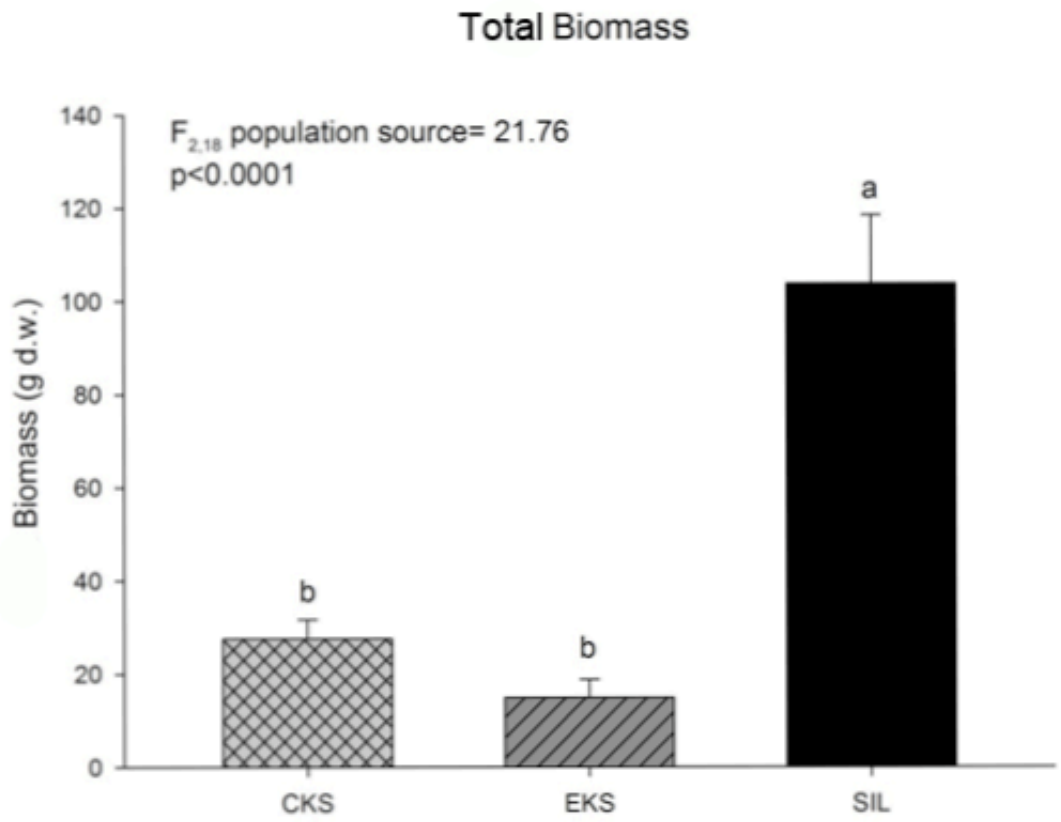

Figure 3.1. Mean (±1 SE) biomass in greenhouse plants of *Andropogon gerardii* at end of growing season (15 September 2010) by region collected. CKS = central Kansas, Relic Prairie; EKS = eastern Kansas, Konza Prairie; SIL = southern Illinois, Twelve Mile Prairie indicate seed sources. Means accompanied by the same letter were not significantly different ($\alpha = 0.05$).

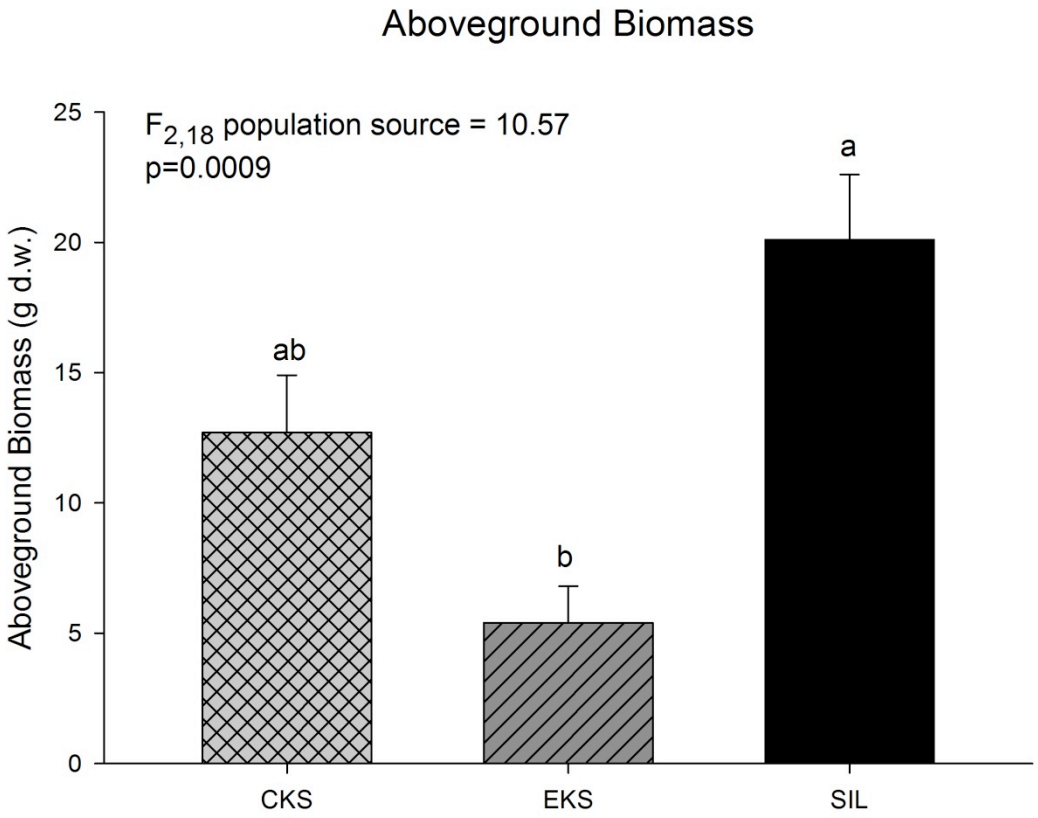

Figure 3.2. Mean (±1 SE) aboveground biomass of greenhouse plants at end of growing season (15 September 2010) by region collected. CKS = central Kansas, Relic Prairie; EKS = eastern Kansas, Konza Prairie; SIL = southern Illinois, Twelve Mile Prairie indicate seed sources. Means accompanied by the same letter were not significantly different ($\alpha = 0.05$).

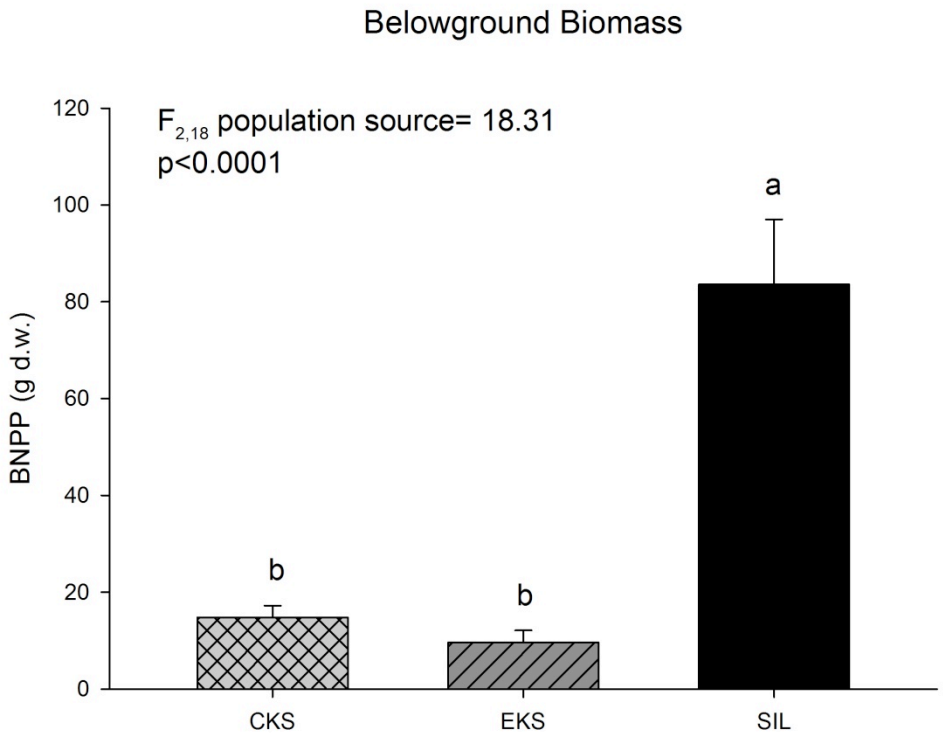

Figure 3.3. Mean (±1 SE) belowground biomass means of greenhouse plants at end of growing season (15 September 2010) by region collected. CKS = central Kansas, Relic Prairie; EKS = eastern Kansas, Konza Prairie; SIL = southern Illinois, Twelve Mile Prairie indicate seed sources. Means accompanied by the same letter were not significantly different ($\alpha = 0.05$).

Table 3.1. Mixed model analysis of the effects of source population and soil moisture treatment on dependent biomass variables in greenhouse samples. Abbreviations correspond to: TB = Total biomass, AB = Aboveground biomass, BB = Belowground biomass. Note that analysis of BB was on log BB to improve normality.

TB	numDF	denDF	F	P value
Source	2	18	21.76	<0.0001
Treatment	2	18	0.18	0.6803
Source*Treatment	2	18	0.40	0.6762

AB	numDF	denDF	F	P value
Source	2	18	10.57	0.0009
Treatment	2	18	0.63	0.4386
Source*Treatment	2	18	0.35	0.7108

BB	numDF	denDF	F	P value
Source	2	18	18.31	<0.0001
Treatment	2	18	1.07	0.3148
Source*Treatment	2	18	0.88	0.4318

Table 3.2. Mean (±1 standard error) Total Biomass (TB), Aboveground Biomass (AB) and Belowground Biomass (BB) by population source.

Source Region	**Population Source**	**TB (g d.w.)**	**AB (g d.w.)**	**BB (g d.w.)**
Central KS	Hays	27.5 ± 4.2	12.7 ± 2.2	14.8 ± 2.4
Eastern KS	Konza	14.9 ± 3.9	5.4 ± 1.4	9.6 ± 2.5
Southern IL	Twelve Mile	103.7 ± 14.7	20.1 ± 2.5	83.6 ± 13.4

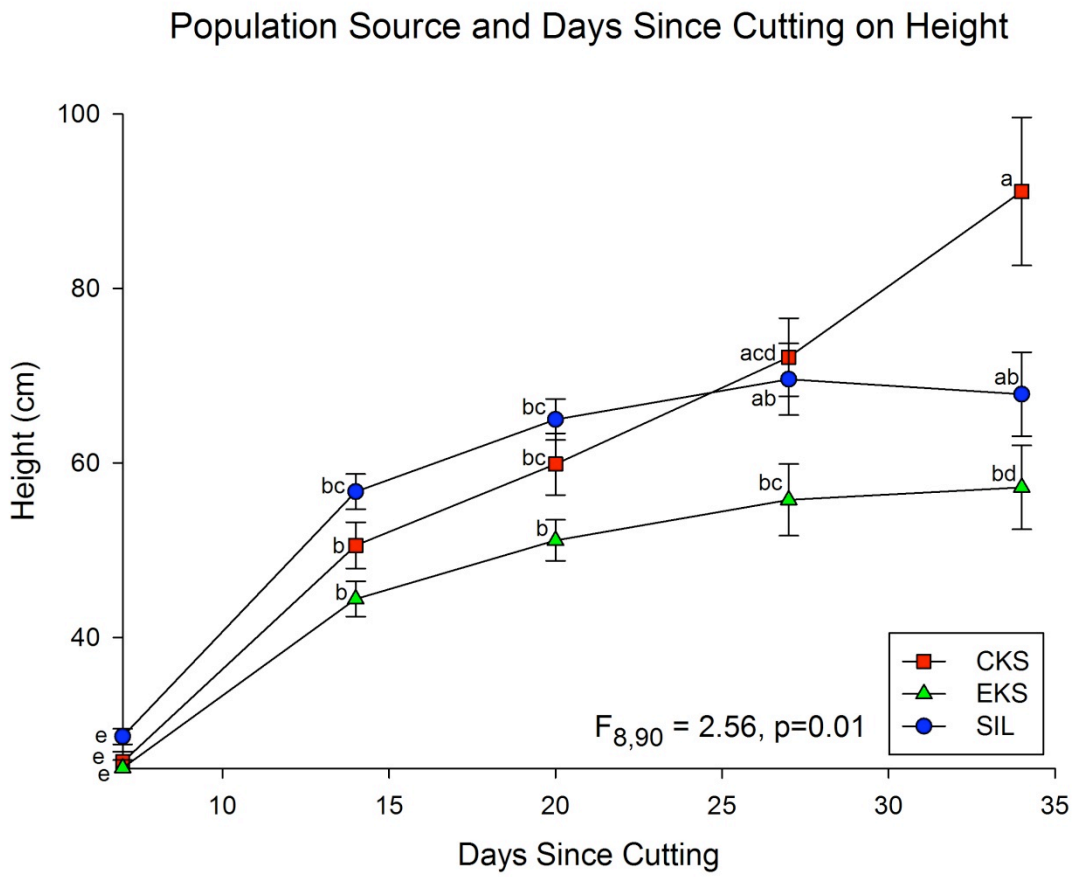

Figure 3.4. Mean (±1 SE) height (cm) of *Andropogon gerardii* ecotypes grown in greenhouse from August 18 2010 to September 15 2010 by population source (CKS = central Kansas, Relic Prairie; EKS = eastern Kansas, Konza Prairie; SIL = southern Illinois, Twelve Mile Prairie) and days since cutting all plants to 5 cm. Means accompanied by the same letter were not significantly different ($\alpha = 0.05$).

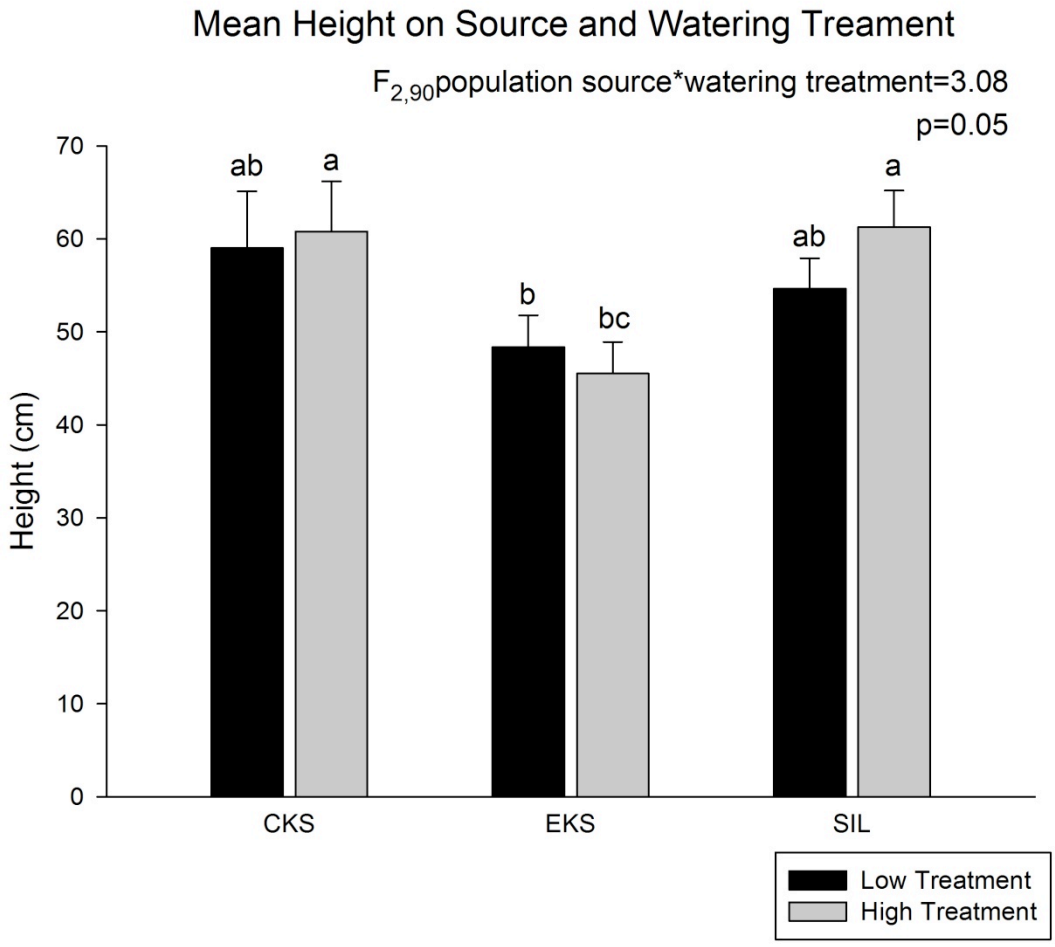

Figure 3.5. Mean (±1 SE) height values of *Andropogon gerardii* ecotypes by source (CKS = central Kansas, Relic Prairie; EKS = eastern Kansas, Konza Prairie; SIL = southern Illinois, Twelve Mile Prairie) and watering treatment (Low treatment = 35% field capacity, High treatment = 85% field capacity). Means accompanied by the same letter were not significantly different ($\alpha = 0.05$; without the Tukey's adjustment to the p-values for the paired comparisons, the 12 Mile plants were significantly different between low and high watering treatment, $\alpha = 0.02$).

Table 3.3. Pre-treatment analysis of height before cutting. Note that analysis of height before cutting was on log height to improve normality.

Height (before)	numDF	denDF	F value	p value
Source	2	18	0.82	0.4570
Treatment	1	18	0.15	0.6999
Source*Treatment	2	18	0.76	0.4837

Table 3.4. Repeated measures analysis of height following regrowth. Note that analysis of height following regrowth was on log height to improve normality.

Height (after)	numDF	denDF	F value	p value
Source	2	90	17.76	<.0001
Day Since Cutting	4	90	125.98	<.0001
Source*Day	**8**	**90**	**2.56**	**0.0145**
Treatment	1	90	1.07	0.3028
Source*Treatment	**2**	**90**	**3.08**	**0.0509**
Day*Treatment	4	90	0.06	0.9930
Source*Day*Treatment	8	90	0.15	0.9965

Table 3.5. Mean (±1 standard error) height (cm) by source (CKS = Relic prairie, central Kansas; EKS = Konza prairie, eastern Kansas; SIL = Twelve mile prairie, southern Illinois) and days after cutting.

Days since cutting					
Source	**7 days**	**14 days**	**20 days**	**27 days**	**34 days**
CKS	25.8 ± 1.2	50.6 ± 2.6	59.9 ± 3.5	72.1 ± 4.5	91.1 ± 8.5
EKS	25.1 ± 0.9	44.4 ± 2.0	51.1 ± 2.3	55.8 ± 4.1	57.2 ± 4.8
SIL	28.7 ± 1.0	56.7 ± 2.5	65 ± 3.0	67.8 ± 3.1	69.6 ± 3.0

Table 3.6. Mean (±1 standard error) height (cm) by source (CKS = Relic prairie, central Kansas; EKS = Konza prairie, eastern Kansas; SIL = Twelve mile prairie, southern Illinois) and watering treatment. (Low treatment = 35% field capacity, high treatment = 85% field capacity).

Source	**Low Treatment (cm)**	**High Treatment (cm)**
CKS	59 ± 6.1	60.8 ± 5.4
EKS	48.4 ± 3.4	45.5 ± 3.4
SIL	54.6 ± 3.3	61.3 ± 4.0

Table 3.7. Flowering stage after watering treatments in greenhouse plants. Source, CKS = Relic prairie, central Kansas; EKS = Konza prairie, eastern Kansas; SIL = Twelve mile prairie, southern Illinois. Treatment, 1 = 35% field capacity watering treatment, 2 = 85% field capacity watering treatment.

Source	Plant	Treatment	Flowering?
SIL	1	1	Emerging
SIL	3	1	No
SIL	7	1	No
SIL	8	1	No
SIL	9	1	No
SIL	2	2	No
SIL	4	2	No
SIL	5	2	No
SIL	6	2	No
EKS	1	1	No
EKS	2	1	No
EKS	3	1	Boot
EKS	4	2	Yes
EKS	5	2	No
EKS	6	2	Emerging
EKS	7	2	No
CKS	2	1	No
CKS	5	1	Yes
CKS	6	1	Yes
CKS	8	1	Yes
CKS	1	2	Yes
CKS	3	2	No
CKS	4	2	Yes
CKS	7	2	Emerging

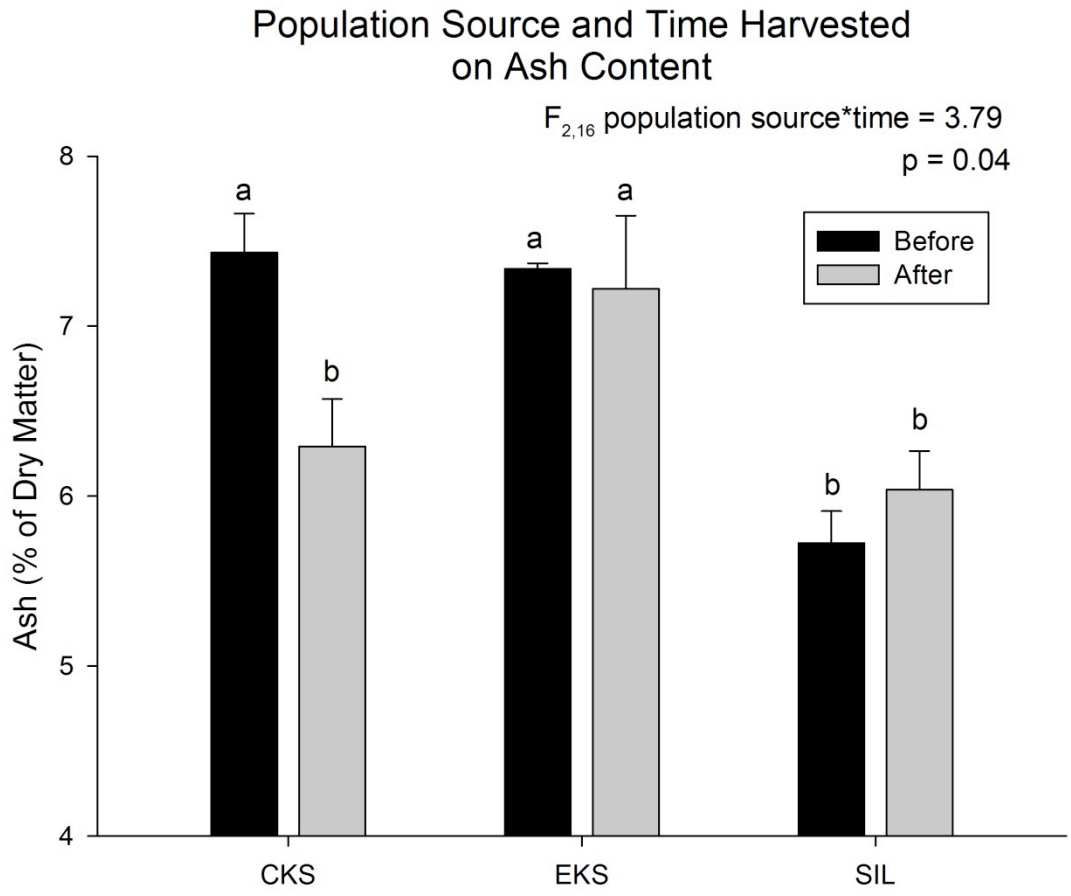

Figure 3.6. Mean (±1 SE) ash content in greenhouse samples by population source (CKS = Relic prairie, central Kansas; EKS = Konza prairie, eastern Kansas; SIL = Twelve mile prairie, southern Illinois) and time harvested (Before = before watering treatment, After = after watering treatment). Means accompanied by the same letter were not significantly different ($\alpha = 0.05$).

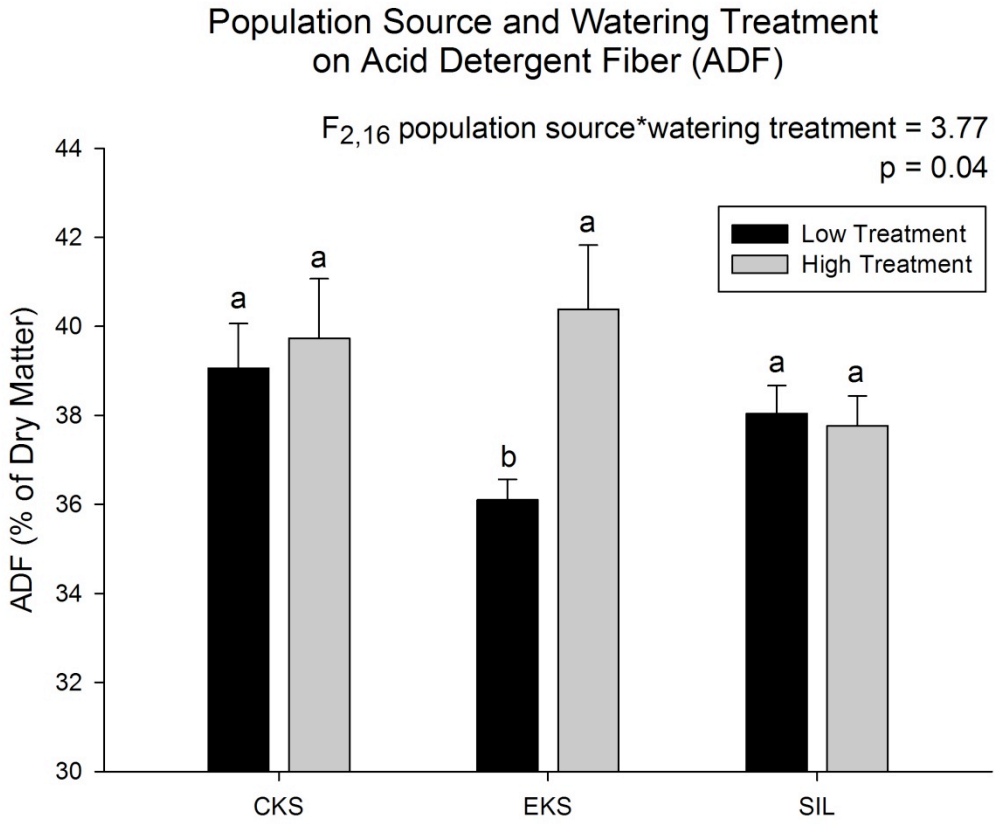

Figure 3.7. Mean (±1 SE) acid detergent fiber content in greenhouse samples by population source (CKS = Relic prairie, central Kansas; EKS = Konza prairie, eastern Kansas; SIL = Twelve mile prairie, southern Illinois) and watering treatment level. Means accompanied by the same letter were not significantly different ($\alpha = 0.09$). Low watering treatment is 35% field capactiy and high watering treatment is 85% field capacity.

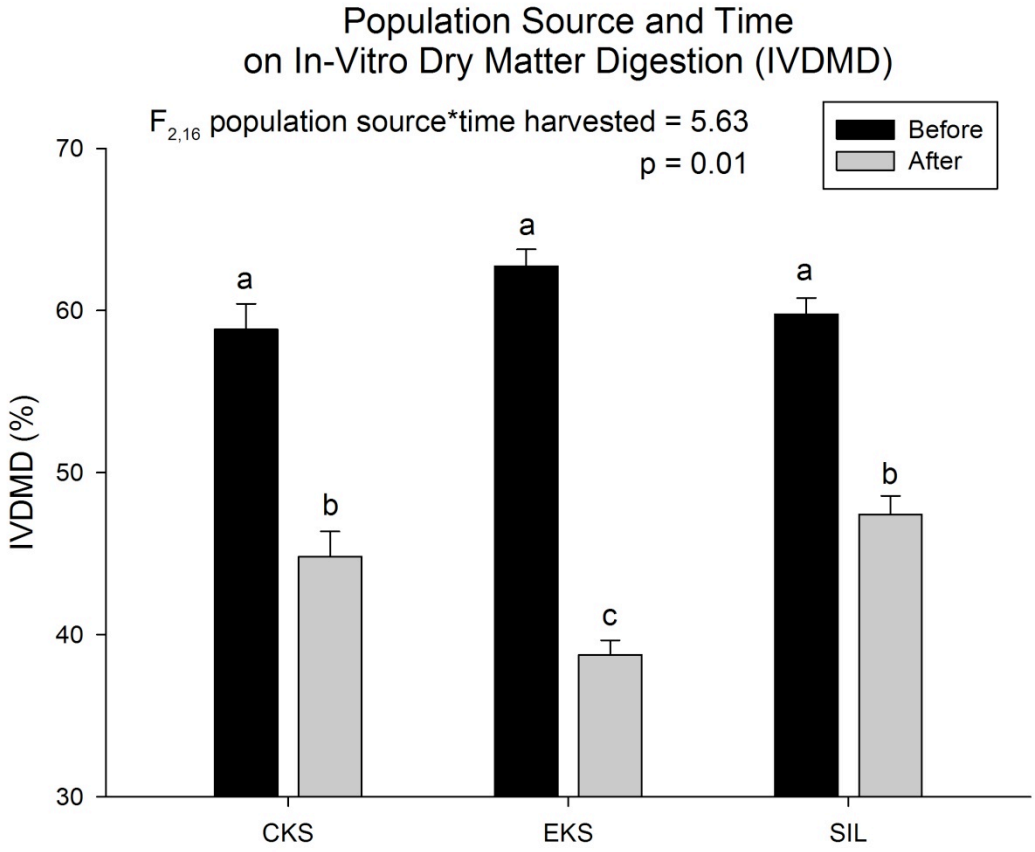

Figure 3.8. Mean (±1 SE) in-vitro dry matter digestion content in greenhouse samples by population source (CKS = Relic prairie, central Kansas; EKS = Konza prairie, eastern Kansas; SIL = Twelve mile prairie, southern Illinois) and time harvested (Before = before watering treatment, After = after watering treatment). Means accompanied by the same letter were not significantly different ($\alpha = 0.05$).

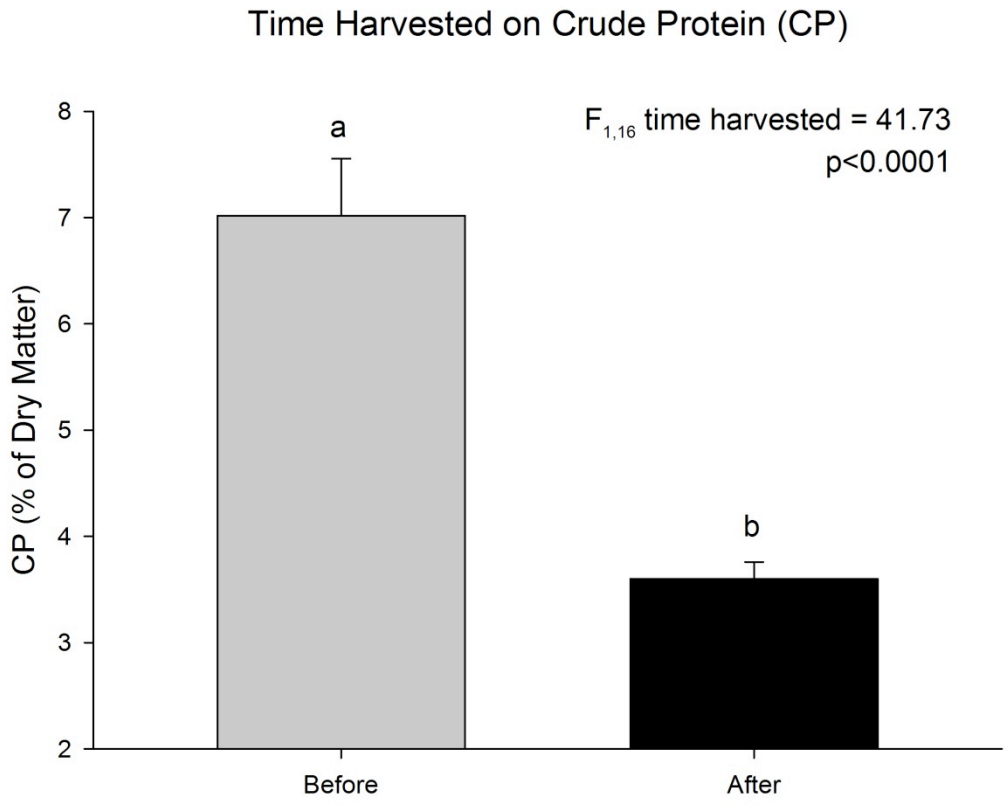

Figure 3.9. Mean (±1 SE) crude protein content in greenhouse plants of *Andropogon gerardii* by time harvested (Before = before watering treatment, After = after watering treatment). Means accompanied by the same letter were not significantly different (α = 0.05).

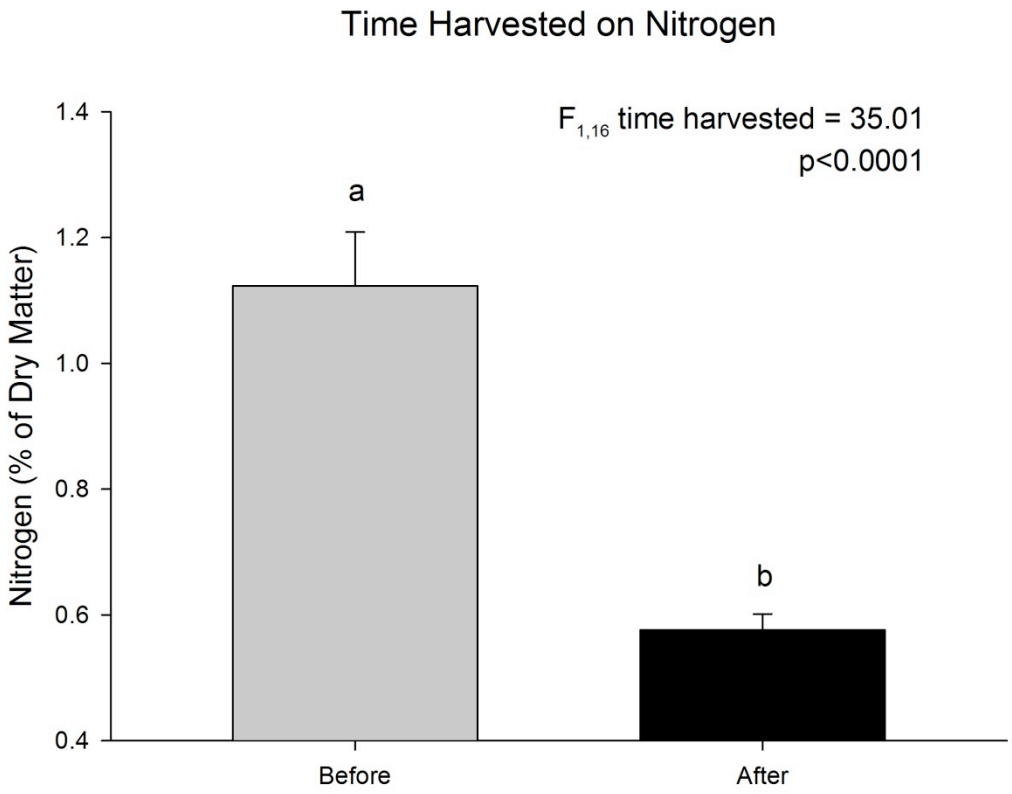

Figure 3.10. Mean (±1 SE) nitrogen content in greenhouse plants of *Andropogon gerardii* by time harvested (Before = before watering treatment, After = after watering treatment). Means accompanied by the same letter were not significantly different ($\alpha = 0.05$).

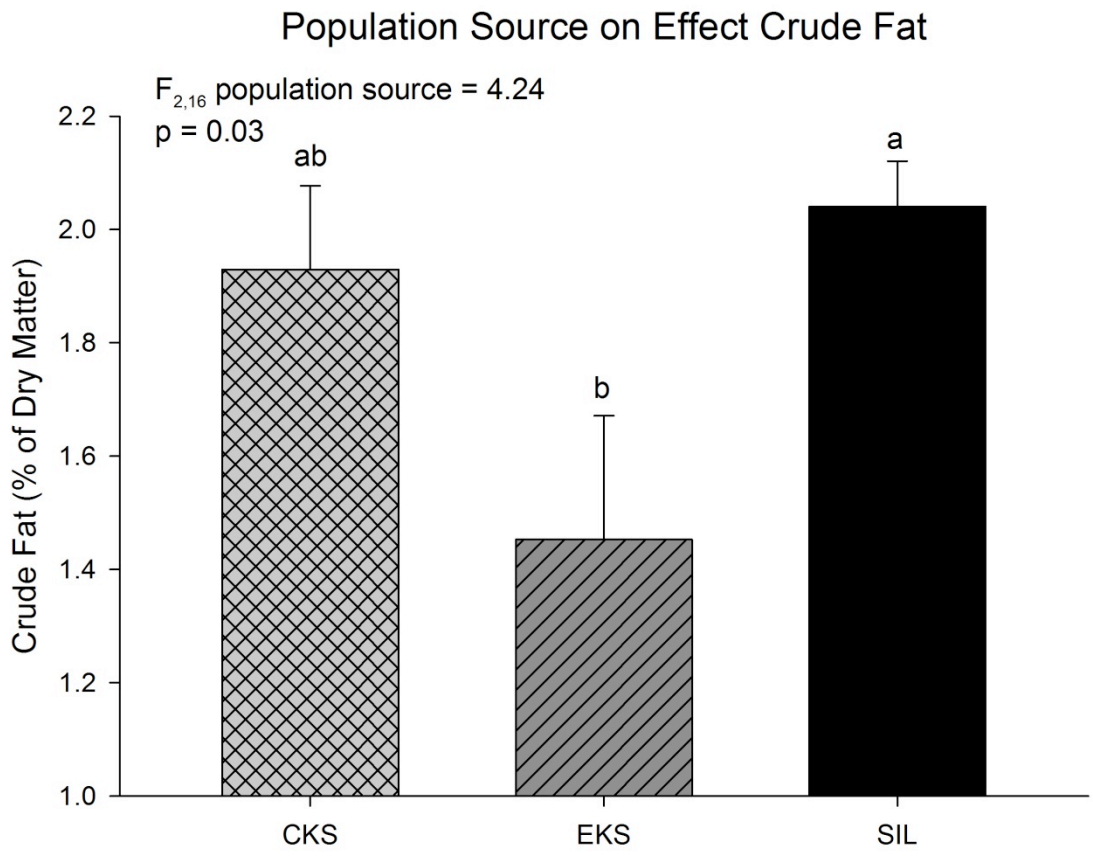

Figure 3.11. Mean (±1 SE) crude fat content in greenhouse plant of *Andropogon gerardii* by population source (CKS = Relic prairie, central Kansas; EKS = Konza prairie, eastern Kansas; SIL = Twelve mile prairie, southern Illinois). Means accompanied by the same letter were not significantly different ($\alpha = 0.05$).

Table 3.8. Mixed model analysis on dependent variables in greenhouse samples.

Ash content	numDF	denDF	F value	p value
Source	2	16	12.54	0.0005
Time	1	16	1.28	0.2737
Source*Time	**2**	**16**	**3.79**	**0.0449**
Treatment	1	16	0.33	0.5749
Source*Treatment	2	16	1.03	0.3781
Time*Treatment	1	16	0.01	0.9163
Source*Time*Treatment	2	16	0.43	0.6593

NDF content	numDF	denDF	F value	p value
Source	2	16	2.03	0.1632
Time	1	16	0.00	0.9837
Source*Time	2	16	0.06	0.9409
Treatment	1	16	0.61	0.4473
Source*Treatment	2	16	0.92	0.4183
Time*Treatment	1	16	0.55	0.4676
Source*Time*Treatment	2	16	1.01	0.3877

Source*Time*Treatment	2	16	0.06	0.9380

ADF content	numDF	denDF	F value	p value
Source	2	16	2.7	0.0977
Time	**1**	**16**	**12.65**	**0.0026**
Source*Time	2	16	0.33	0.7247
Treatment	**1**	**16**	**5.67**	**0.0300**
Source*Treatment	**2**	**16**	**3.77**	**0.0456**
Time*Treatment	1	16	0.82	0.3778

IVDMD	numDF	denDF	F value	p value
Source	2	16	2.05	0.1612
Time	1	16	137.85	<.0001
Source*Time	**2**	**16**	**5.63**	**0.0141**
Treatment	1	16	0.75	0.3994
Source*Treatment	2	16	0.44	0.6495
Time*Treatment	1	16	0.20	0.6615
Source*Time*Treatment	2	16	0.17	0.8424

Crude protein	numDF	denDF	F value	p value
Source	2	16	1.35	0.2868
Time	**1**	**16**	**41.73**	**<.0001**
Source*Time	2	16	2.09	0.1558
Treatment	1	16	1.63	0.2198
Source*Treatment	2	16	1.38	0.2811
Time*Treatment	1	16	1.00	0.3325
Source*Time*Treatment	2	16	0.18	0.8336

Nitrogen	numDF	denDF	F value	p value
Source	2	16	1.17	0.3351
Time	**1**	**16**	**35.01**	**<.0001**
Source*Time	2	16	1.49	0.2541
Treatment	1	16	2.02	0.1748
Source*Treatment	2	16	0.95	0.4089
Time*Treatment	1	16	1.91	0.1856
Source*Time*Treatment	2	16	0.01	0.9944

Crude fat	numDF	denDF	F value	p value
Source	**2**	**16**	**4.24**	**0.0334**
Time	1	16	2.84	0.1115
Source*Time	2	16	2.28	0.1342
Treatment	1	16	1.46	0.2441
Source*Treatment	2	16	1.05	0.3715
Time*Treatment	1	16	0.27	0.6084
Source*Time*Treatment	2	16	0.34	0.7135

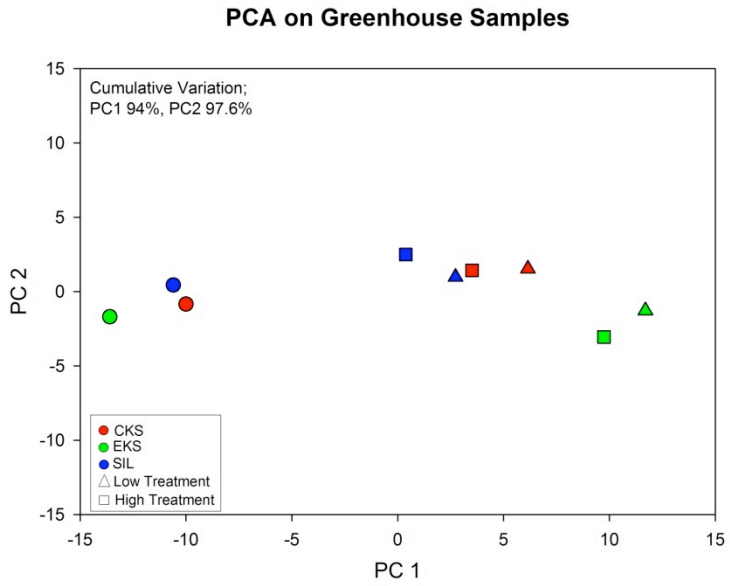

Figure 3.12. Principal component analysis of forage quality (dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), In-vitro dry matter digestion (IVDMD), crude fat (CF), crude protein, (CP), ash content and nitrogen (N)) of *Andropogon gerardii* grown in the greenhouse from three sources under low and high moisture treatments, before watering treatment (circles) and after high (85% field capacity: squares) and low (35% field capacity: triangles) treatments.

Table 3.9. Eigenvalues from PCA on greenhouse sample forage variables (DM, Ash content, NDF, ADF, IVDMD, CP, CF and N).

Eigenvalues			
PC	**Eigenvalues**	**%Variation**	**Cumulative %Variation**
1	86.1	94.0	94.0
2	3.3	3.6	97.6

Table 3.10. Eigenvectors, variable loadings for PCA axes 1 and 2 on forage variables (DM, Ash, NDF, ADF, IVDMD, CP, CF and N) for greenhouse samples before and after low and high watering treatment.

Eigenvectors		
Variable	**PC1**	**PC2**
DM	0.068	0.066
Ash	-0.001	-0.344
CF	-0.026	0.173
IVDMD	-0.974	0.115
NDF	0.031	-0.668
ADF	-0.109	-0.318
CP	-0.182	-0.535
Nitrogen	-0.029	-0.086

Field Experiment

Dry matter and ash content

There were significant effects of region and population source on ash content (Table 3.11). Central Kansas exhibited higher ash content (9.5% ± 2.1) than all other precipitation regions: Colorado (6.2% ± 0.7), eastern Kansas (6.7% ± 0.4), and southern Illinois (6.1% ± 0.7). The Webster ecotype in central Kansas exhibited the highest ash content, 11.9% ± 0.1, and was statistically different from all other ecotype sources (Figure 3.13).

Neutral detergent fiber (NDF)

There were significant effects of region and population source on NDF (Table 3.11). Eastern Kansas (74.1% ± 0.8) and southern Illinois 72.4% ± 1.9) populations exhibited higher NDF value than Colorado (69.3% ± 1.1) and central Kansas (68.5% ± 1.8) precipitation regions (Figure 3.14).

Acid detergent fiber (ADF)

There were significant effects of precipitation region and population source on ADF (Table 3.11). Eastern Kansas exhibited the highest ADF content, 42.3% ± 0.8, followed by southern Illinois, 41.5% ± 0.5, then central Kansas, 40.6% ± 3.3, and lastly, Colorado, 36.3% ± 2.6. Central Kansas ecotypes exhibited significant differences between populations within a region. Webster, a central Kansas source, exhibited the highest

ADF content, 44.7% ± 0.8. Greenbelt, a Colorado source, exhibited the lowest ADF content 35.8% ± 3.6 (Figure 3.15).

In-vitro dry matter digestibility **(IVDMD)**

There were significant effects of precipitation region and source digestibility (Table 3.11). Percentages of IVDMD declined as average annual rainfall increased (Figure 3.16). Colorado ecotypes exhibited the greatest IVDMD, 56% ± 4.3, followed by central Kansas at 50% ± 5.7, eastern Kansas, 46% ± 3.1 and southern Illinois ecotypes exhibited the lowest IVDMD at 43% ± 2.9.

Crude protein **(CP)**

There were significant effects of precipitation region and population source on CP (Table 3.11). Colorado ecotypes exhibited higher CP content than other precipitation regions 7% ± 0.5 (Crown Rock = 7.3% ± 0.2; Greenbelt = 7.2% ± 0.3; Paramount Point = 6.6% ± 0.6) (Figure 3.17). Central Kansas, 4.6% ± 1.1, and southern Illinois, 4.8% ± 0.6, exhibited intermediate crude protein values. Eastern Kansas exhibited lowest values as a region 3.8% ± 0.6, where as the Webster population exhibited the overall lowest value of all samples 3.4% ± 0.7.

Nitrogen

There were significant effects of precipitation region and population source on percent N of samples (Table 3.11). Colorado ecotypes exhibited the highest N content 1.2% ± 0.05

(Crown Rock = 1.17% ± 0.03; Greenbelt = 1.2% ± 0.05; Paramount Point = 1.1% ± 0.09). Colorado ecotypes were not statistically different from each other. Central Kansas, 0.7% ± 0.2, eastern Kansas, 0.6% ± 0.1, and southern Illinois, 0.8% ± 0.1, exhibited similar values. Webster ecotype in central Kansas exhibited the lowest N content of all ecotypes at 0.6% ± 0.05 (Figure 3.18).

***Crude fat* (CF)**

There were no significant treatment effects on CF. Overall, CF means were highest in central Kansas, 4% ± 0.2, followed by Colorado, 3.6% ± 0.3. Eastern Kansas, 3.2 ± 0.8, and southern Illinois values, 3.3 ± 0.7, were similar.

PCA forage quality

Sites sorted on a west to east gradient along PC 1 (Figure 3.19) reflecting a zero loading for DM contrasting with a high positive loading for IVDMD (Table 3.12). The total cumulative variation in PC1 and 2 was 79.4% and 90.9%, respectively (Table 3.13). PC 2 provided little separation of sites but contrasted a positive loading for ADF and a negative loading for ash.

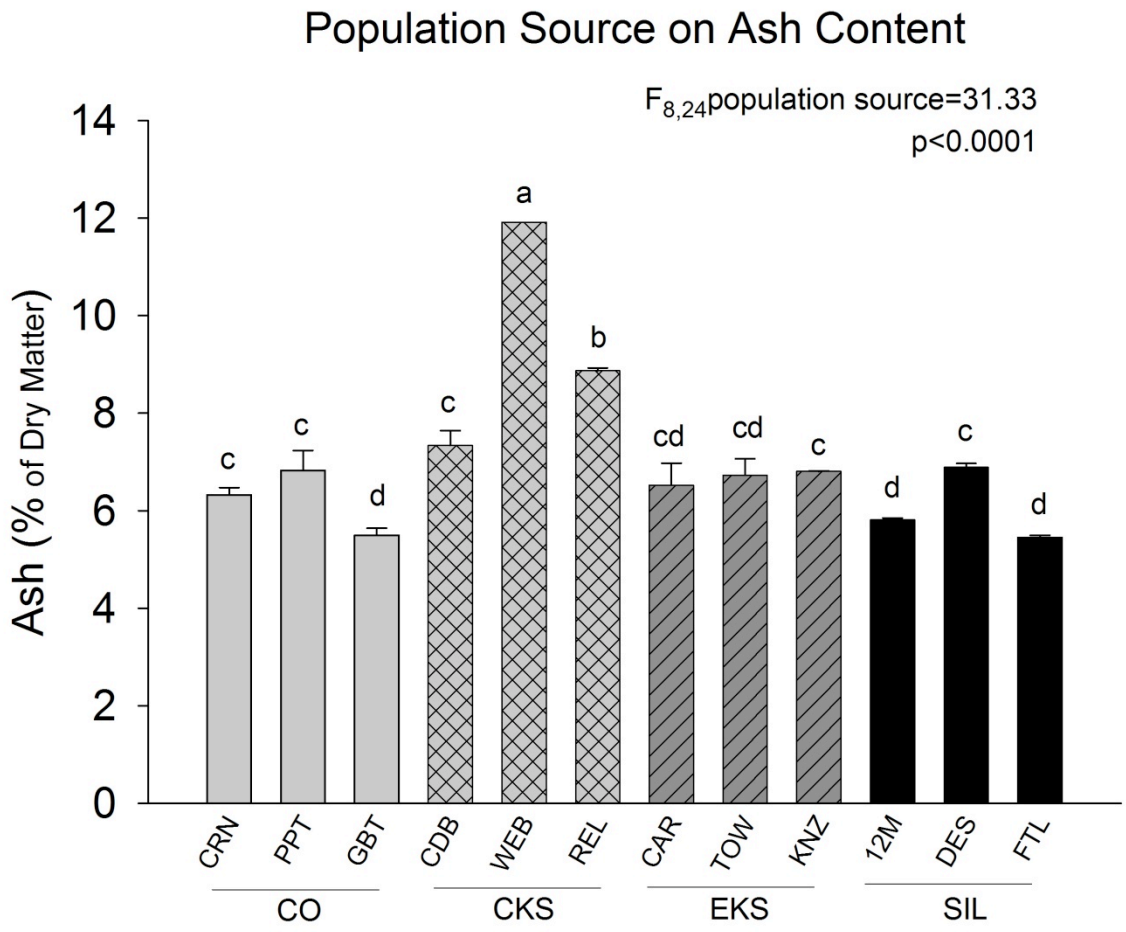

Figure 3.13. Mean (±1 SE) ash content in field samples of *Andropogon gerardii* collected across a precipitation gradient (Abbreviations: CO = Colorado, CKS = central Kansas, EKS = eastern Kansas, SIL = southern Illinois. Population abbreviations see Table 2.4). Means accompanied by the same letter were not significantly different ($\alpha = 0.05$).

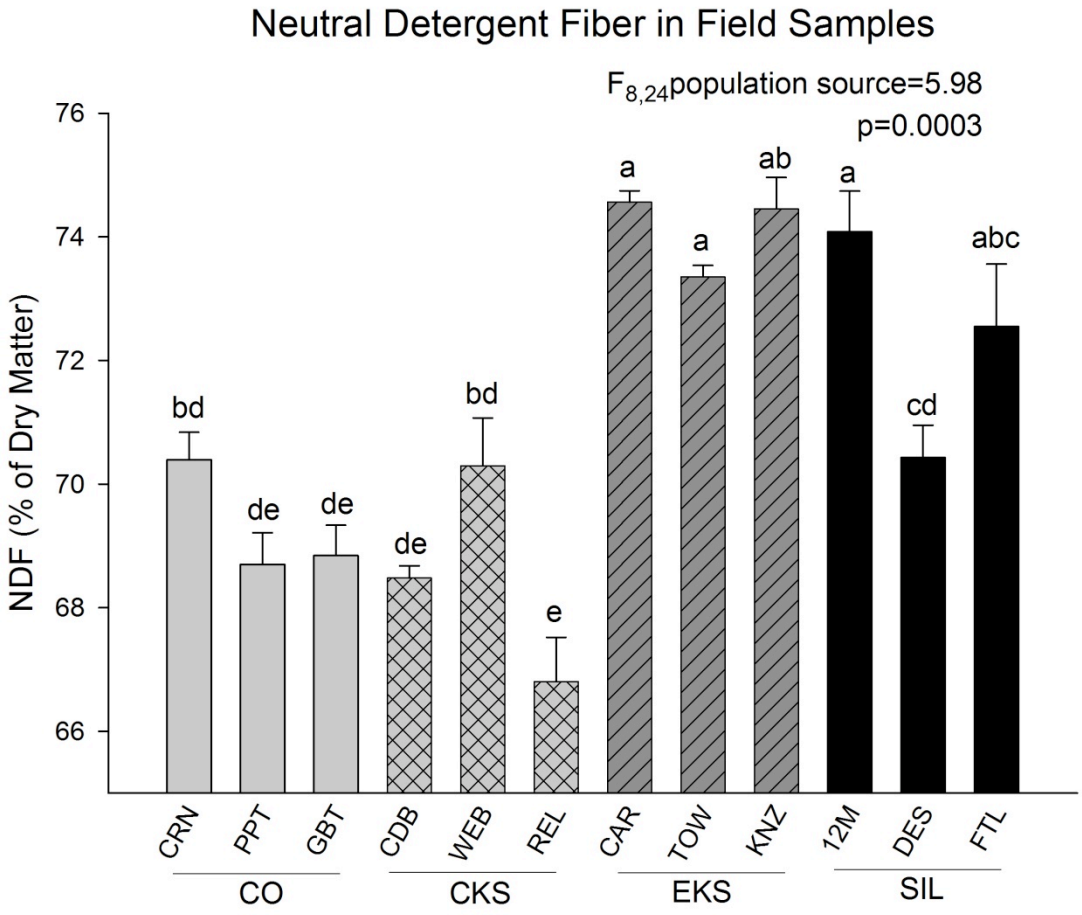

Figure 3.14. Precipitation region and population source on Mean (±1 SE) NDF content in field plants of *Andropogon gerardii* collected across a precipitation gradient (Abbreviations: CO = Colorado, CKS = central Kansas, EKS = eastern Kansas, SIL = southern Illinois. Population abbreviations see Table 2.4). Means accompanied by the same letter were not significantly different ($\alpha = 0.05$).

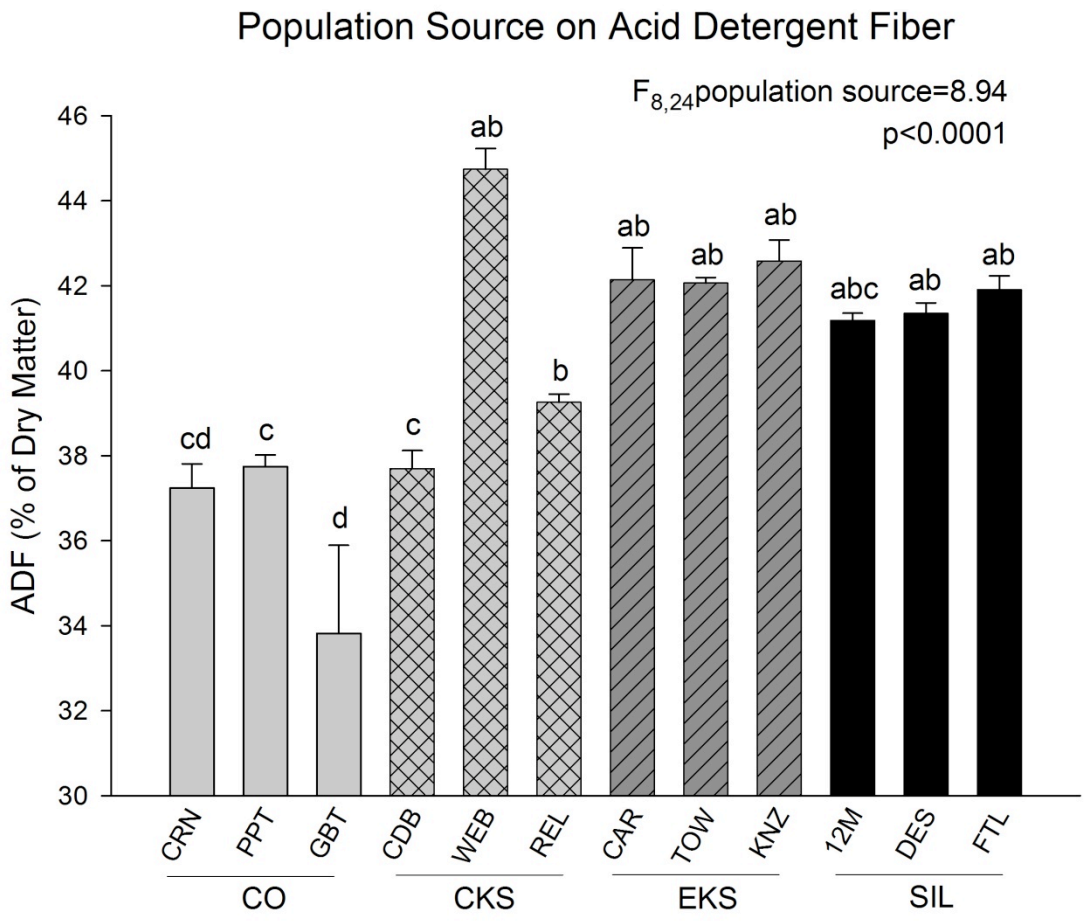

Figure 3.15. Precipitation region and population source on Mean (±1 SE) ADF content in field plants of *Andropogon gerardii* collected across a precipitation gradient (Abbreviations: CO = Colorado, CKS = central Kansas, EKS = eastern Kansas, SIL = southern Illinois. Population abbreviations see Table 2.4). Means accompanied by the same letter were not significantly different ($\alpha = 0.05$).

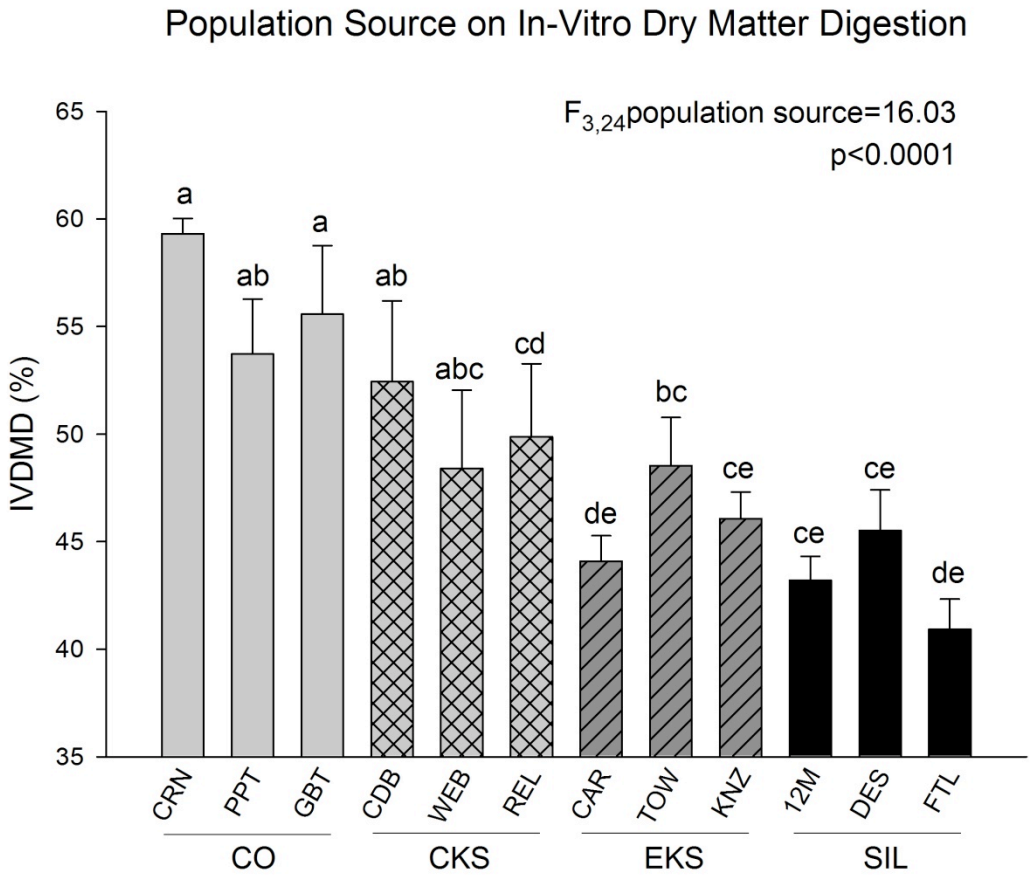

Figure 3.16. Mean (±1 SE) in-vitro dry matter digestion from field plants of *Andropogon gerardii* collected across a precipitation gradient (Abbreviations: CO = Colorado, CKS = central Kansas, EKS = eastern Kansas, SIL = southern Illinois. Population abbreviations see Table 2.4). Means accompanied by the same letter were not significantly different (α= 0.05).

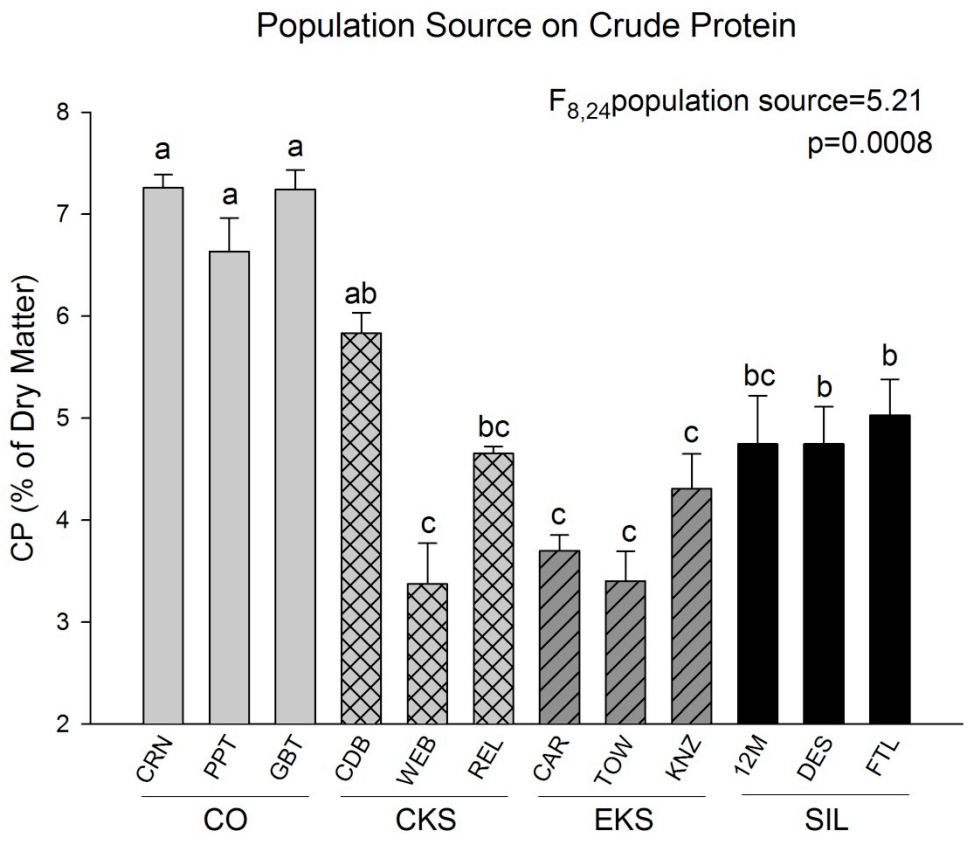

Figure 3.17. Mean (±1 SE) CP content in field plants of *Andropogon gerardii* collected across a precipitation gradient ((Abbreviations: CO = Colorado, CKS = central Kansas, EKS = eastern Kansas, SIL = southern Illinois. Population abbreviations see Table 2.4). Means accompanied by the same letter were not significantly different (α = 0.05).

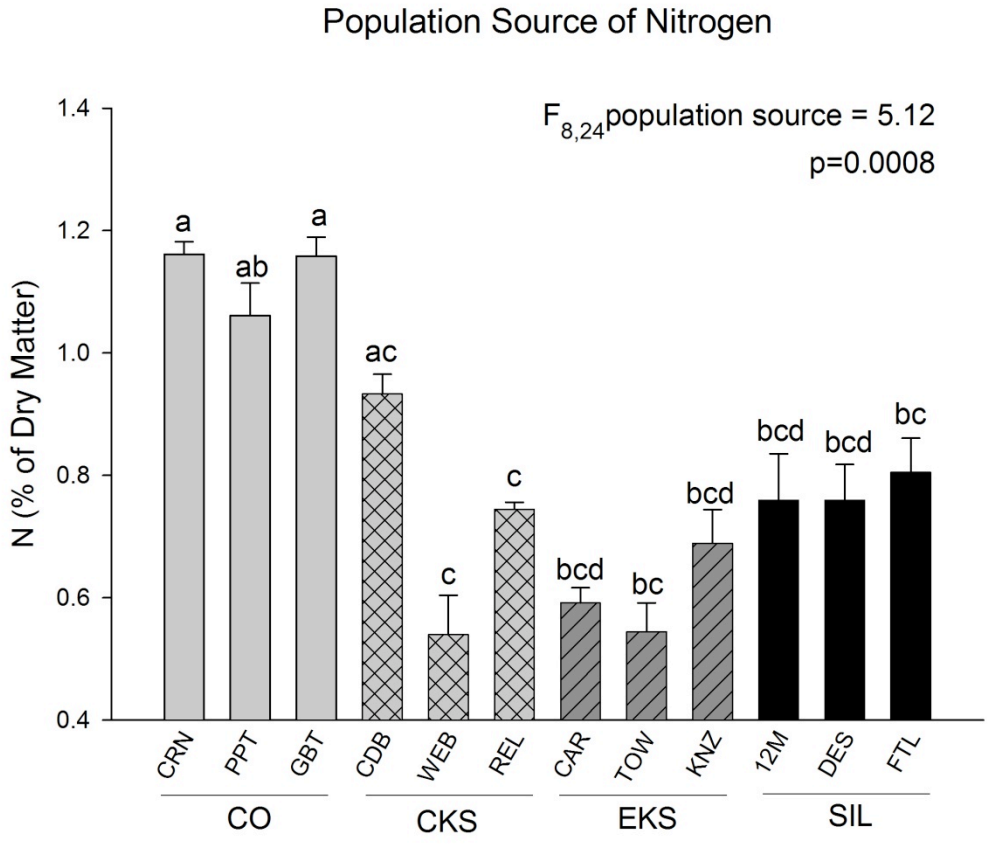

Figure 3.18. Mean (±1 SE) nitrogen content in field plants of *Andropogon gerardii* collected across a precipitation gradient (Abbreviations: CO = Colorado, CKS = central Kansas, EKS = eastern Kansas, SIL = southern Illinois. Population abbreviations see Table 2.4). Means accompanied by the same letter were not significantly different (α = 0.05).

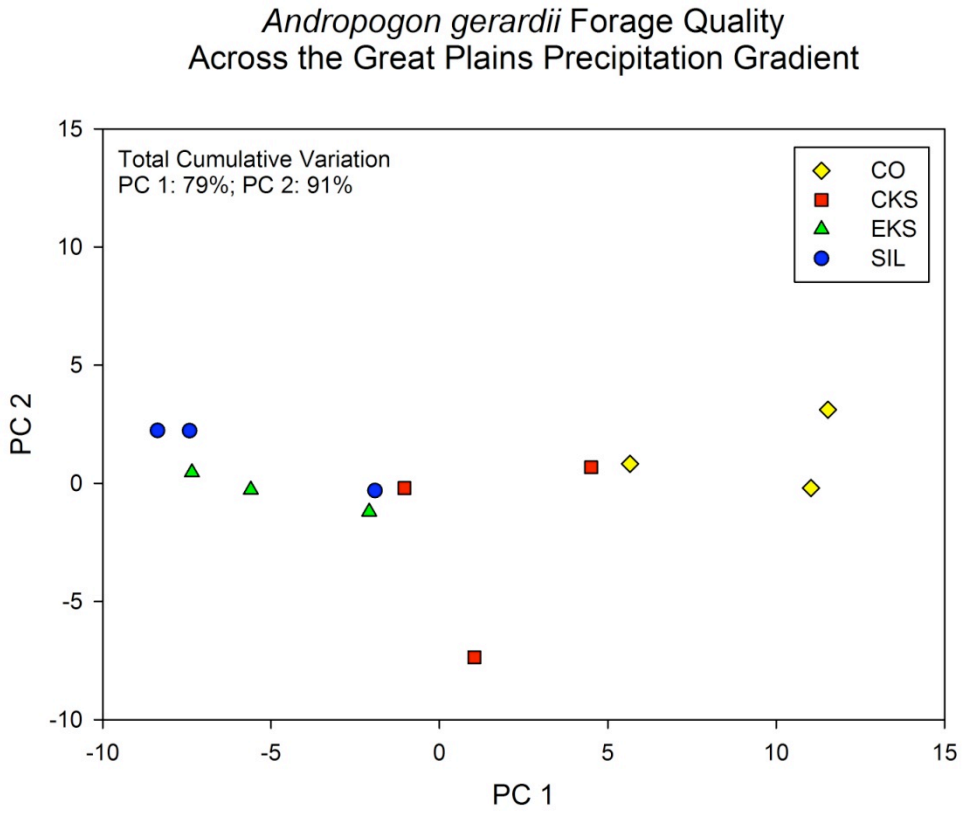

Figure 3.19. Principal component analysis (PCA) of *Andropogon gerardii* ecotypes based on forage quality measurements of dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), in-vitro dry matter digestion (IVDMD), crude fat (CF), crude protein, (CP), ash content and nitrogen (N) in field plants (CO = Colorado, CKS = central Kansas, EKS = eastern Kansas, SIL = southern Illinois).

Table 3.11. Mixed model analysis on dependent variables in forage analysis of field experiment. Sources were nested in region.

Ash	numDF	denDF	F value	p value
Region	3	24	133.67	<0.0001
Source	8	24	31.33	<0.0001

NDF	numDF	denDF	F value	p value
Region	3	24	63.5	<0.0001
Source	8	24	5.98	0.0003

ADF	numDF	denDF	F value	p value
Region	3	24	41.63	<0.0001
Source	8	24	8.94	<0.0001

IVDMD	numDF	denDF	F value	p value
Region	3	24	16.03	<0.0001
Source	8	24	0.95	0.5

Crude protein	numDF	denDF	F value	p value
Region	3	24	64.02	<0.0001
Source	8	24	5.21	0.0008

Nitrogen	numDF	denDF	F value	p value
Region	3	24	64.03	<0.0001
Source	8	24	5.21	0.0008

Crude fat	numDF	denDF	F value	p value
Region	3	24	1.46	0.28
Source	8	24	0.57	0.78

Table 3.12. Variable loadings for PCA axes 1 and 2 on forage variables (DM, Ash, NDF, ADF, IVDMD, CP, CF and N) for field samples.

Eigenvectors		
Variable	**PC1**	**PC2**
CF	0.019	-0.008
CP	0.133	0.277
DM	0.000	0.000
NDF	0.261	-0.055
ADF	0.343	0.684
IVDMD	0.892	-0.278
Ash	0.017	-0.611
Nitrogen	0.021	0.044

Table 3.13. Eigenvalues from PCA on field sample forage variables (DM, Ash, NDF, ADF, IVDMD, CP, CF and N).

Eigenvalues			
PC	**Eigenvalues**	**%Variation**	**Cumulative %Variation**
1	48.0	79.4	79.4
2	7.0	11.6	90.9

Synthesis of Field and Greenhouse Experiment

PCA

All sources of greenhouse plants before watering treatment had similar, high positive scores along PC 1, separate from all other plants (Figure 3.19). Field samples fell into the same area of the PCA as greenhouse samples after they were subjected to watering treatments. There was no distinction between the location of greenhouse samples receiving the low and high watering treatments. The total cumulative variation in PC1 and 2 was 81.6 and 95%, respectively (Table 3.14). PC 1 had negative eigenvector loadings for DM and NDF, and large positive eigenvector loadings for IVDMD. PC 2 provided little separation of sources. Some separation was seen for southern Illinois and eastern Kansas field samples from the post-watering treatment greenhouse samples and the field central Kansas sample.

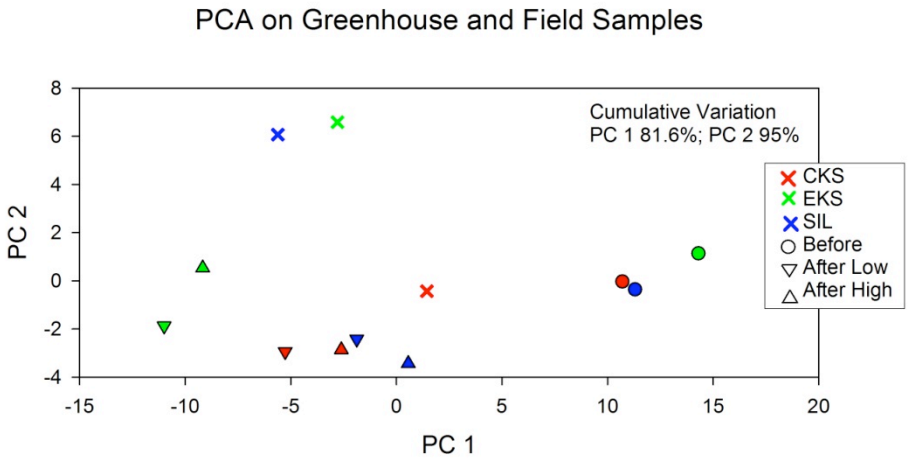

Figure 3.20. PCA on forage quality measurements dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), in-vitro dry matter digestion (IVDMD), crude fat (CF), crude protein, (CP), ash and nitrogen (N) of *Andropogon gerardii* comparing field sample and greenhouse plants before watering treatment and after low (35% field capacity) and high (85% field capacity) watering treatments. (CKS = central Kansas, Relic prairie; EKS = eastern Kansas, Konza prairie; SIL = southern Illinois, Twelve mile prairie).

Table 3.14. Eigenvalues from PCA on field and greenhouse sample forage variables (DM, Ash, NDF, ADF, IVDMD, CP, CF and N).

Eigenvalues			
PC	**Eigenvalues**	**%Variation**	**Cumulative %Variation**
1	66.3	81.6	81.6%
2	10.9	13.4	95%

Table 3.15. Variable loadings for PCA axes 1 and 2 on forage variables (DM, Ash, NDF, ADF, IVDMD, CP, CF and N) for field and greenhouse samples.

Eigenvectors		
Variable	**PC1**	**PC2**
CF	0.006	0.180
CP	0.164	0.218
DM	-0.047	-0.237
NDF	-0.106	0.770
ADF	0.064	0.519
IVDMD	0.977	-0.001
Ash	0.010	0.019
Nitrogen	0.026	0.035

CHAPTER 4: DISCUSSION

The objective of this study was to quantify the effect of ecotypic variation on forage quality in the dominant prairie species *Andropogon gerardii* Vitman. Samples were collected across a precipitation gradient in the North American grasslands from Colorado to southern Illinois and analyzed to determine suitability for ruminant consumption.

Intraspecific variation in growth

Growth differences among populations of A*ndropogon gerardii* were categorized as early as the 1960s, with seminal work by McMillan on ecotypes through common garden experiments in Lincoln, Nebraska (McMillian 1959, McMillan 1965, McMillian 1969). McMillan examined phenology patterns for native prairie grasses collected from sixty-five sites along a north-south gradient ranging over 2,000 km from North Dakota to northern Texas. He noted that ecotypes collected from northern sites flowered earlier when grown in Lincoln, NE, an adaptation due to their shorter photoperiods, than ecotypes from southern sites, where growing seasons are longer.

A more recent study of flowering patterns from a central Illinois tallgrass prairie found number of flowering plants per month was correlated with long-term temperature and precipitation patterns (Kerbart and Anderson 1987). This same general trend was observed in the field study presented here. *A. gerardii* in eastern regions of the field study, where longer growing seasons and greater precipitation occur, were

developmentally more advanced compared to those further west at the time samples were collected for forage analysis. These observations were not supported under the controlled conditions of my greenhouse study. After watering treatments, at the time of final harvest, most plants from central KS were flowering, followed by fewer plants from eastern KS, and, only one plant from southern IL (Table 3.7). Under greenhouse conditions central KS sourced plants were the most advanced, an opposite trend than observed in the field. Yet, this difference could be attributed to the central KS ecotype's adaption to shorter growing seasons, and therefore, they developed faster than the southern IL ecotype, under controlled conditions. Thus, ecotypic variation greatly influences growth patterns of *A. gerardii.*

In the greenhouse study, differences in height among population sources were observed only following regrowth after initial harvest and application of the soil moisture treatments, indicating that baseline growth for all sources were similar. Growth following initial harvest differed among sources, but not as predicted: the central KS source plants grew tallest and the eastern KS source plants grew, shortest. Nevertheless, these differences in height under greenhouse conditions again confirms the occurrence of ecotypic differentiation among the *Andropogon gerardii* population sources modified by a plastic response to precipitation. As in previous studies (Gustafson et al. 2004), height is a useful measure of ecotypic differentiation in *A. gerardii*.

Furthermore, total biomass, including above- and belowground biomass, differed among population sources. Many studies have found a significant relationship between NPP and mean annual precipitation in North American grassland plants (Lauenroth and Sala 1992, Briggs and Knapp 1995) a general trend of production increasing along the

west to east gradient (Sala et al. 1988). In *A. gerardii*, this relationship of greater NPP with greater rainfall has been noted to account for up to 89% of growth variation within the Great Plains (Epstein et al. 1996). However, in the greenhouse study the hypothesis which predicted greater NPP from ecotypes originating from greater rainfall regions was not supported. Plants from the southern IL population source, originating in the area of the greatest annual rainfall across the precipitation gradient, did produce the most biomass of the three sources as predicted. Although, by contrast, plants from the eastern KS source, exhibited the lowest biomass of the sources tested. The central KS source, originating from the driest region and assumed to be the most drought tolerant of the three regions, exhibited intermediate productivity among the three population sources.

Intraspecific variation in forage quality

Forage quality of *A. gerardii* collected from the field varied among the four regions across the precipitation gradient, and, to a lesser extent among populations within each region reflecting both intraspecific variation in forage quality because of the presence of ecotypes of *A. gerardii*, and phenotypic plasticity and phenological variability. Highest forage quality in the field, i.e., high IVDMD, low ADF (lignin) and high crude protein (CP), occurred in CO ecotypes of *A. gerardii* from the driest, western end of the precipitation gradient. However, plants sampled in the western ends of the gradient were observed to be in earlier developmental stages, i.e., smaller leaf to stem ratio, than those sampled from the east.

A reciprocal common garden experiment at locations across the precipitation gradient of the same *A. gerardii* ecotypes studied here found planting location had significant effects on nutritive composition of *A. gerardii*, and was a more valuable

indicator of elemental and chemical composition than ecotype, or the interaction between location and ecotype (Zhang et al. 2012). They also found ecotypic variation had significant effects on digestibility (ADF% and ash content). The results of my study, where the plants were directly sampled from the same remnant prairies, rather than plants grown from seed and reciprocally transplanted into common gardens, is comparable to Zhang et al.,'s (2012) findings where population location in the field was the primary indicator of forage quality, and ecotypic variation and had significant effects on forage quality. Gan at el. (2012), studied plants from the same reciprocal gardens as Zhang et al. (2012) and found southern IL ecotypes, and Carbondale, IL (southern IL) and Manhattan, KS (eastern KS) planting locations produced the highest total cellulose and hemicellulose content (Gan et al. 2012). In my experiment, eastern KS and southern IL plants similarly had the highest NDF content. High cellulose and hemicellulose leads to lower forage quality due to difficulty in digesting these cell wall components. Overall, it is apparent that planting location and population source has significant effects on digestibility, and eastern KS and southern IL ecotypes produce high bio-oil yields, but have lower forage quality than central KS ecotypes in field conditions.

The greenhouse experiment largely supported an interpretation of lower forage quality of as a result of more advanced plant maturity and to a lesser extent lower soil moisture. When *A. gerardii* was raised from seed in the greenhouse, forage quality did not decrease from west to east sources as seen in the field samples. All populations sampled before the watering treatments were imposed exhibited similar high forage quality, with the highest occurring in eastern KS, then southern IL, followed by central KS. Forage quality of all greenhouse plants before the watering treatment were higher

than any population observed in the field. In fact, the high level of forage quality found in the greenhouse grown plants before watering treatments were comparable to strains of Pawnee C3, 'Bonanza', and Kaw C3, 'Goldmine', third generation cultivars of Pawnee and Kaw, respectively, of *A. gerardii* bred for high forage quality (Mitchell et al. 2005, Vogel et al. 2006b, a) (Table 4.1).

Forage quality dropped significantly after regrowth following clipping and imposition of the watering treatments in all sources. Ecotypes were affected differently due to ecotypic variation, i.e., the different climates, particularly, and precipitation levels, to which they are adapted. After the high watering treatment, the southern IL ecotype exhibited the highest forage quality, i.e., high IVDMD, low ADF (lignin) and high crude protein (CP), of the three ecotypes, followed by the southern IL ecotype subject to the low water treatment. These values were comparable to those found in the field. Central KS and eastern KS exhibited low forage quality after implementation of watering treatments, i.e., low IVDMD, high ADF (lignin) and low crude protein (CP), under both watering conditions. Eastern KS values in the greenhouse following watering treatments were lower than any values found in the field. Southern IL ecotypes are adapted to longer growing seasons and greater precipitation compared with central KS ecotypes that evolved under conditions of lower precipitation and shorter growing seasons. Southern IL ecotypes exhibited greater plasticity, maintaining high levels of forage quality in both low and high watering treatments. The difference in adaptation explains the ability of ecotypes from longer growing seasons to better maintain higher rates of forage quality under varied precipitation conditions as seen in the southern IL ecotype and why lower forage quality was exhibited in the central KS ecotype. This adaption confirms ecotypic

variation and phenotypic plasticity in forage quality under controlled conditions with maturity and soil moisture effecting values. Both the field and greenhouse experiments support previous findings indicating that advancing plant maturity is primarily responsible for decreases in forage quality through the growing season (Perry and Baltensperger 1977, Perry and Baltensperger 1979, Griffin and Jung 1983, Mitchell et al. 1994, Cherney and Hall 1998, Jung and Vogel 2006), with environmental factors having a significant albeit lesser role. These results also suggest that southern IL ecotypes of *A. gerardii* exhibit greater phenological plasticity than more westerly derived ecotypes due to their high forage qualities under both watering treatments, and adaption to longer growing seasons.

Implications for Climate Change, Management and Restoration

Shifting global precipitation patterns resulting from climate change (Vitousek 1994, IPCC et al. 2000) requires knowledge of species response to altered conditions if we are to preserve and restore communities that are able to adapt, persist and become self-sustaining over time. Responsible grazing management on prairies can help to restore natural ecosystem functionality and enhance biodiversity on rangelands (Fuhlendorf and Engle 2001) including tallgrass prairies (Collins et al. 1998, Hickman et al. 2004). Nutritious forage is essential for productive animal management, high rates of weight gain and ample milk production (Cherney and Hall 1998, Ball et al. 2001). Grasses are the "backbone" of successful forage management systems (Moser and Nelson 2003).

Andropogon gerardii can be found in high frequency across a wide range of physiographic regions (Tompkins et al. 2010). A recent study examining the gene pool of three natural populations of *A. gerardii* in Wisconsin confirmed three distinct gene pools of the species with overlapping regions and called for preserving its genetic diversity (Price et al. 2012). A study in southwestern Quebec confirmed that the Big Bluestem cultivar 'Niaga' can be grown successfully as far as eastern Canada for forage and biofuel production (Madakadze et al. 1998). Therefore, this species is essential to preserve and use for economic benefits.

If we are to restore grassland communities with grazing using *Andropogon gerardii,* population sources must be considered due to ecotypic differences. Ecotypic differences in forage quality vary with forage maturity, and to a lesser extent soil moisture, and potentially other as yet untested environmental factors. Of those tested here, southern IL ecotypes appear to be the most adaptable to variations in precipitation, and will likely maintain high levels of forage quality under projected changes in precipitation resulting from climate change.

New Questions

In the Midwest and Great Plains regions, models project increased summer aridity, with higher temperatures, longer growing seasons, and greater precipitation in the winter months (Karl et al. 2009). Western ecotypes, which have evolved under shorter growing seasons might not as easily adapt to weather pattern changes, and may experience declines in forage quality as a result. As seen under controlled conditions, the southern IL ecotype exhibited greater phenological plasticity than the more westerly

sourced ecotypes, and can maintain high levels of forage quality under varied precipitation.

From this study new questions arise. Forage quality values in the greenhouse before watering treatments were only slightly lower than Kaw and Pawnee cultivars, but further investigation should be done as to why greenhouse values of forage quality were so high compared with field values, and whether this difference is a result of first generation growth from seed versus re-sprouts of older plants in the field. Age of plant alone was not the reason for differences found in forage quality between the field and greenhouse studies: Greenhouse plants were harvested 16 weeks since planting seed, and field plants were harvested 4-8 weeks since last frost date (Table 2.5). Yet, ecotypes under controlled conditions grown from seed were much higher in initial nutrition value than any field samples.

The effect of other important interacting environmental factors, such as fire, and how they may interact with forage quality and a changing climate should also be considered. Studies show both bison (Knapp et al. 1999, Fuhlendorf et al. 2008) and cattle (Allred et al. 2011) prefer recently burned areas, where forage is highest and choose quality over quantity (Coppedge and Shaw 1998). Do all ecotypes exhibit high nutrition levels after fire? Further investigation on how the forage quality of ecotypes respond to fire should also be explored.

Source	IVDMD (%)	CP (%)	NDF (%)	ADF (%)
Pawnee	59.3	9.5	71.8	.
Pawnee C3 (Bonanza)	60.8	9.4	71.3	.
Kaw	61.2	9.4	71.1	.
Kaw C3 (Goldmine)	61.2	9.7	71.1	.
Central KS (before)	58.8	7.9	67.2	40.5
Eastern KS (before)	62.7	8.0	70.0	39.2
Southern IL (before)	59.3	6.3	68.0	39.4
Central KS (after low)	42.9	2.6	67.1	38.2
Central KS (after high)	46.0	3.6	66.5	38.5
Eastern KS (after low)	37.9	4.4	69.6	35.6
Eastern KS (after high)	39.7	4.0	70.7	39.0
Southern IL (after low)	47.0	4.0	68.0	32.7
Southern IL (after high)	49.0	3.4	67.2	32.0

Table 4.1. Comparison of forage quality in *A. gerardii* cultivars Pawnee, Pawnee C3, Kaw and Kaw C3 data compared with mean values from greenhouse grown Hays, Konza and Twelve Mile ecotypes in this study (cultivar data adapted from Mitchell et al. 2006).

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APPENDICES

APPENDIX A

Greenhouse Experiment

Table A1. Aboveground Net Primary Production (ANPP), Belowground Net Primary Production (BNPP) and Net Primary Production (NPP) in grams (g) on greenhouse samples harvested at end of growing season. Treatment 1=Low moisture treatment of 35% field capacity, and, Treatment 2=High moisture treatment of 85% field capacity

Source	Plant No.	Treatment	ANPP	BGPP	NPP
CKS	1	2	14.9	21.4	36.3
CKS	2	1	4.15	6.11	10.26
CKS	3	2	9.88	15.88	25.76
CKS	4	2	24.54	22.57	47.11
CKS	5	1	16.64	20.57	37.21
CKS	6	1	9.18	15.96	25.14
CKS	7	2	9.97	6.78	16.75
CKS	8	1	12.56	9.14	21.7
EKS	1	1	8.7	17.26	25.96
EKS	2	1	2.65	4.85	7.5
EKS	3	1	6.59	14.18	20.77
EKS	4	2	9.13	11.05	20.18
EKS	5	2	0.4	0.95	1.35
EKS	6	2	8.4	15.99	24.39
EKS	7	2	1.7	2.57	4.27
SIL	1	1	28.8	147.54	176.34
SIL	2	2	26.56	11.63	38.19
SIL	3	1	12.62	51.19	63.81
SIL	4	2	16.78	126.63	143.41
SIL	5	2	12.31	61.5	73.81
SIL	6	2	31.58	88.86	120.44
SIL	7	1	14.81	78.35	93.16
SIL	8	1	14.5	84.97	99.47
SIL	9	1	22.72	101.65	124.37

Table A2. Height data in centimeters (cm), tiller count and base diameter (cm) on greenhouse samples from after first harvest to the end of growing season.

Source	Plant	Treatment	8/18/10	8/25/10	9/1/10	9/7/10	9/14/10	9/21/10	Flowering?	Tiller Count	Base Diameter
CKS	2	1	52	22	45	48	48.5	49	no	13	13
CKS	5	1	104	28	49.5	62	75	105	yes	23	26
CKS	6	1	101	29	58	73	87.5	110	yes	23	27
CKS	8	1	87	21	39.5	50	74	106	yes	24	22
CKS	1	2	85	27.5	62	72	79	94	yes	28	27
CKS	3	2	67	26	52	54	58.5	59	no	21	23
CKS	4	2	95	29.5	54	67	81	112	yes	28	31
CKS	7	2	107	23	44.5	53	73.5	94	emerging	16	18
EKS	1	1	74	26	48	61	64	64	no	11	19
EKS	2	1	77	24.5	49	53	57	57	no	5	15
EKS	3	1	100	25.5	44.5	51	51	50	boot	11	19
EKS	4	2	103	22	38	52	74	82	yes	18	21
EKS	5	2	47	22.5	49	49	50	50	no	1	7
EKS	6	2	96	29	46.5	52	54.5	54.5	emerging	31	26
EKS	7	2	39	26	36	40	40	43	no	5	9
SIL	1	1	97	32	63.5	78	82	76	emerging	71	29
SIL	3	1	73	25	49.5	55	59	60	no	67	24
SIL	7	1	81	25.5	52	60	65.5	67	no	73	27
SIL	8	1	84	27	49.5	60	73	57	no	94	42
SIL	9	1	85	27.5	50	57	57	58	no	106	38
SIL	2	2	84	31.5	61	66	74	71	no	68	30
SIL	4	2	91	29.5	55.5	64	64	64	no	72	28
SIL	5	2	88	27	59	64	69	75	no	57	25
SIL	6	2	93	33	70.5	81	83	83	no	54	29

Table A3. Dry matter (%DM), moisture content (% Moisture) and Ash content (ASH) for each sample before (Time 1) and after (Time 2) watering treatments, 1=Low moisture treatment of 35% field capacity, and, 2=High moisture treatment of 85% field capacity. Source abbreviations: Relic Prairie (CKS), Konza Prairie (ESK), Twelve Mile Prairie (SIL).

Sample	Container No.	Time	Treatment	Replicate	% DM	% Moisture	ASH
CKS	13	1	2	1	94.42	0.06	8.19
CKS	13	1	2	2	94.48	0.06	7.99
CKS	25	1	1	1	94.76	0.05	7.19
CKS	25	1	1	2	94.67	0.05	7.09
CKS	47	1	2	1	94.52	0.05	7.49
CKS	47	1	2	2	95.13	0.05	7.31
CKS	68	1	1	1	94.37	0.06	7.09
CKS	68	1	1	2	94.58	0.05	7.11
CKS	13	2	2	1	95.67	0.04	7.01
CKS	25	2	1	1	96.46	0.04	6.27
CKS	47	2	2	1	95.91	0.04	5.65
CKS	68	2	1	1	96.44	0.04	6.24
EKS	123	1	1	1	95.11	0.05	7.39
EKS	123	1	1	2	94.69	0.05	7.22
EKS	4567	1	2	1	94.18	0.06	7.29
EKS	4567	1	2	2	94.39	0.06	7.45
EKS	123	2	1	1	95.80	0.04	6.79
EKS	4567	2	2	1	95.91	0.04	7.65
SIL	1	1	1	1	94.51	0.05	6.95
SIL	1	1	1	2	94.36	0.06	6.08
SIL	2	1	2	1	94.35	0.06	5.34
SIL	2	1	2	2	94.85	0.05	5.84
SIL	3	1	1	1	94.30	0.06	4.76
SIL	3	1	1	2	94.46	0.06	5.61

SIL	4	1	2	1	94.42	0.06	5.01
SIL	4	1	2	2	94.47	0.06	5.43
SIL	5	1	2	1	94.64	0.05	5.32
SIL	5	1	2	2	94.68	0.05	5.45
SIL	6	1	2	1	94.23	0.06	6.68
SIL	6	1	2	2	94.27	0.06	6.35
SIL	7	1	1	1	94.20	0.06	5.63
SIL	7	1	1	2	94.43	0.06	5.47
SIL	8	1	1	1	94.10	0.06	5.95
SIL	8	1	1	2	94.23	0.06	5.71
SIL	1	2	1	1	95.23	0.05	5.39
SIL	2	2	2	1	95.57	0.04	5.67
SIL	3	2	1	1	95.17	0.05	6.01
SIL	4	2	2	1	95.13	0.05	5.99
SIL	5	2	2	1	95.52	0.04	6.25
SIL	6	2	2	1	95.61	0.04	5.25
SIL	7	2	1	1	95.30	0.05	6.5
SIL	8	2	1	1	95.59	0.04	7.24

Table A4. Neutral detergent fiber (NDF%) for each sample before (Time 1) and after (Time 2) watering treatments, 1=Low moisture treatment of 35% field capacity, and, 2=High moisture treatment of 85% field capacity. Source abbreviations: Relic Prairie (CKS), Konza Prairie (ESK), Twelve Mile Prairie (SIL).

Source	Container No.	Time	Watering Treatment	Bag No.	NDF%
CKS	13	1	2	1	64.97
CKS	13	1	2	2	64.07
CKS	13	1	2	3	64.05
CKS	25	1	1	1	67.12
CKS	25	1	1	2	65.62
CKS	25	1	1	3	65.83
CKS	47	1	2	1	65.10
CKS	47	1	2	2	65.67
CKS	47	1	2	3	65.43
CKS	68	1	1	1	77.09
CKS	68	1	1	2	68.97
CKS	68	1	1	3	72.66
CKS	13	2	2	1	63.58
CKS	13	2	2	2	63.00
CKS	13	2	2	3	63.60
CKS	25	2	1	1	63.05
CKS	25	2	1	2	64.54
CKS	25	2	1	3	61.17
CKS	47	2	2	1	69.73
CKS	47	2	2	2	69.63
CKS	47	2	2	3	69.99
CKS	68	2	1	1	73.79
CKS	68	2	1	2	70.95
CKS	68	2	1	3	69.08
EKS	123	1	1	1	68.87
EKS	123	1	1	2	69.73
EKS	123	1	1	3	71.31
EKS	4567	1	2	1	70.93
EKS	4567	1	2	2	68.83
EKS	4567	1	2	3	67.92
EKS	123	2	1	1	68.81
EKS	123	2	1	2	70.42
EKS	4567	2	2	1	71.93
EKS	4567	2	2	2	69.13
EKS	4567	2	2	3	71.09
SIL	1	1	1	1	68.58
SIL	1	1	1	2	66.90
SIL	1	1	1	3	66.96
SIL	2	1	2	1	68.22
SIL	2	1	2	2	67.49
SIL	2	1	2	3	65.66
SIL	39	1	1	1	69.04

SIL	39	1	1	2	66.82
SIL	39	1	1	3	67.71
SIL	4	1	2	1	68.09
SIL	4	1	2	2	69.17
SIL	4	1	2	3	70.91
SIL	5	1	2	1	68.56
SIL	5	1	2	2	69.61
SIL	5	1	2	3	69.98
SIL	6	1	2	1	66.95
SIL	6	1	2	2	67.94
SIL	6	1	2	3	66.85
SIL	7	1	1	1	67.79
SIL	7	1	1	2	67.22
SIL	7	1	1	3	67.36
SIL	8	1	1	1	66.34
SIL	8	1	1	2	67.01
SIL	8	1	1	3	68.90
SIL	1	2	1	1	66.99
SIL	1	2	1	2	66.54
SIL	1	2	1	3	69.23
SIL	2	2	2	1	66.57
SIL	2	2	2	2	65.72
SIL	2	2	2	3	66.70
SIL	39	2	1	1	68.33
SIL	39	2	1	2	68.58
SIL	39	2	1	3	68.65
SIL	4	2	2	1	67.50
SIL	4	2	2	2	67.47
SIL	5	2	2	1	68.22
SIL	5	2	2	2	68.00
SIL	6	2	2	1	67.04
SIL	6	2	2	2	66.95
SIL	6	2	2	3	67.92
SIL	7	2	1	1	66.07
SIL	7	2	1	2	68.57
SIL	8	2	1	1	69.68
SIL	8	2	1	2	67.56

Table A5. Acid detergent fiber (ADF%) for each sample before (Time 1) and after (Time 2) watering treatments. Source abbreviations: Relic Prairie (CKS), Konza Prairie (ESK), Twelve Mile Prairie (SIL).

Source	Container#	Time	Watering Treatment	Bag No.	ADF %
CKS	13	1	2	1	37.90
CKS	13	1	2	2	38.36
CKS	13	1	2	3	39.64
CKS	25	1	1	1	39.34
CKS	25	1	1	2	38.89
CKS	25	1	1	3	39.23
CKS	47	1	2	1	43.48
CKS	47	1	2	2	43.12
CKS	47	1	2	3	43.52
CKS	68	1	1	1	39.72
CKS	68	1	1	2	40.81
CKS	68	1	1	3	41.52
CKS	13	2	2	1	37.69
CKS	13	2	2	2	36.49
CKS	25	2	1	1	35.53
CKS	25	2	1	2	36.88
CKS	47	2	2	1	39.93
CKS	47	2	2	2	39.73
CKS	68	2	1	1	40.66
CKS	68	2	1	2	39.73
EKS	123	1	1	1	37.01
EKS	123	1	1	2	36.91
EKS	123	1	1	3	35.76
EKS	4567	1	2	1	42.61
EKS	4567	1	2	2	40.83
EKS	4567	1	2	3	42.03
EKS	123	2	1	1	35.68
EKS	123	2	1	2	35.60
EKS	4567	2	2	1	39.01
EKS	4567	2	2	2	38.89
SIL	1	1	1	1	39.25
SIL	1	1	1	2	38.73
SIL	1	1	1	3	37.77
SIL	2	1	2	1	38.78
SIL	2	1	2	2	40.11
SIL	2	1	2	3	38.64
SIL	39	1	1	1	39.42
SIL	39	1	1	2	39.40
SIL	39	1	1	3	40.22
SIL	4	1	2	1	39.28
SIL	4	1	2	2	39.22
SIL	4	1	2	3	39.45
SIL	5	1	2	1	40.46
SIL	5	1	2	2	40.31

SIL	5	1	2	3	40.27
SIL	6	1	2	1	39.05
SIL	6	1	2	2	38.80
SIL	6	1	2	3	38.97
SIL	7	1	1	1	38.35
SIL	7	1	1	2	38.74
SIL	7	1	1	3	38.67
SIL	8	1	1	1	40.94
SIL	8	1	1	2	40.16
SIL	8	1	1	3	40.27
SIL	1	2	1	1	35.87
SIL	1	2	1	2	35.76
SIL	2	2	2	1	35.45
SIL	2	2	2	2	34.99
SIL	39	2	1	1	36.90
SIL	39	2	1	2	36.13
SIL	4	2	2	1	36.46
SIL	4	2	2	2	36.83
SIL	5	2	2	1	36.13
SIL	5	2	2	2	37.03
SIL	6	2	2	1	36.51
SIL	6	2	2	2	35.33
SIL	7	2	1	1	35.69
SIL	7	2	1	2	35.99
SIL	8	2	1	1	39.02
SIL	8	2	1	2	38.72

Table A6. In-Vitro Dry Matter Digestion (Digestibility %) for each sample before (Time 1) and after (Time 2) watering treatments. Source abbreviations: Relic Prairie (CKS), Konza Prairie (ESK), Twelve Mile Prairie (SIL).

Sample	Container No.	Time	Treatment	Digestibility %
CKS	13	1	2	64.35
CKS	13	1	2	62.40
CKS	13	1	2	61.58
CKS	25	1	1	53.13
CKS	25	1	1	52.42
CKS	25	1	1	59.57
CKS	47	1	2	54.49
CKS	47	1	2	65.91
CKS	47	1	2	55.18
CKS	68	1	1	63.94
CKS	68	1	1	58.96
CKS	68	1	1	54.02
CKS	13	2	2	45.15
CKS	13	2	2	46.58
CKS	13	2	2	41.35
CKS	25	2	1	44.03
CKS	25	2	1	49.08
CKS	47	2	2	48.16
CKS	47	2	2	45.98
CKS	47	2	2	48.97
CKS	68	2	1	40.46
CKS	68	2	1	37.08
CKS	68	2	1	44.33
EKS	123	1	1	62.35
EKS	123	1	1	67.48
EKS	123	1	1	61.50
EKS	4567	1	2	64.68
EKS	4567	1	2	66.73
EKS	4567	1	2	53.70
EKS	123	2	1	40.41
EKS	123	2	1	35.33
EKS	4567	2	2	39.94
EKS	4567	2	2	39.37
SIL	1	1	1	61.64
SIL	1	1	1	60.60
SIL	1	1	1	66.33
SIL	2	1	2	61.01
SIL	2	1	2	65.23
SIL	2	1	2	66.13
SIL	39	1	1	58.21
SIL	39	1	1	62.50
SIL	39	1	1	62.69
SIL	4	1	2	63.41

SIL	4	1	2	56.19
SIL	4	1	2	51.58
SIL	5	1	2	60.69
SIL	5	1	2	58.39
SIL	5	1	2	63.30
SIL	6	1	2	61.59
SIL	6	1	2	62.37
SIL	6	1	2	49.26
SIL	7	1	1	48.50
SIL	7	1	1	62.73
SIL	7	1	1	59.24
SIL	8	1	1	55.16
SIL	8	1	1	60.59
SIL	8	1	1	57.30
SIL	1	2	1	48.31
SIL	1	2	1	49.50
SIL	1	2	1	46.79
SIL	2	2	2	50.15
SIL	2	2	2	50.12
SIL	2	2	2	49.03
SIL	3	2	1	48.35
SIL	3	2	1	47.74
SIL	3	2	1	41.85
SIL	4	2	2	48.23
SIL	4	2	2	47.25
SIL	5	2	2	42.86
SIL	6	2	2	51.76
SIL	6	2	2	52.18
SIL	6	2	2	49.40
SIL	7	2	1	43.11
SIL	7	2	1	42.76
SIL	8	2	1	48.86
SIL	8	2	1	52.66

Table A7. Crude protein (%Protein) and nitrogen content (%Nitrogen) for each sample before (Time 1) and after (Time 2) watering treatments. Source abbreviations: Relic Prairie (CKS), Konza Prairie (ESK), Twelve Mile Prairie (SIL).

Source	Container No.	Time	Treatment	Label	%Nitrogen	%Protein
CKS	13	1	2	25	1.15	7.16
CKS	13	1	2	26	1.66	10.35
CKS	13	1	2	27	1.56	9.73
CKS	25	1	1	28	1.01	6.33
CKS	25	1	1	29	1.00	6.22
CKS	25	1	1	30	0.88	5.48
CKS	47	1	2	31	1.32	8.23
CKS	47	1	2	32	1.61	10.06
CKS	47	1	2	33	1.67	10.43
CKS	68	1	1	34	1.08	6.72
CKS	68	1	1	35	1.22	7.63
CKS	68	1	1	36	1.03	6.42
CKS	13	2	2	59	0.45	2.84
CKS	13	2	2	60	0.64	4.00
CKS	25	2	1	61	0.33	2.05
CKS	25	2	1	62	0.34	2.09
CKS	47	2	2	63	0.60	3.74
CKS	47	2	2	64	0.63	3.91
CKS	68	2	1	65	0.45	2.80
CKS	68	2	1	66	0.58	3.61
EKS	123	1	1	37	1.31	8.19
EKS	123	1	1	38	1.10	6.87
EKS	123	1	1	39	1.12	7.00
EKS	4567	1	2	40	1.30	8.12
EKS	4567	1	2	41	1.53	9.54
EKS	4567	1	2	42	1.39	8.67
EKS	123	2	1	67	0.68	4.28
EKS	123	2	1	68	0.73	4.57
EKS	4567	2	2	69	0.70	4.38
EKS	4567	2	2	70	0.61	3.79
SIL	1	1	1	1	1.38	8.60
SIL	1	1	1	2	1.47	9.16
SIL	1	1	1	3	1.38	8.64
SIL	2	1	2	4	1.05	6.58
SIL	2	1	2	5	1.79	11.17
SIL	2	1	2	6	1.45	9.07
SIL	39	1	1	7	0.94	5.88
SIL	39	1	1	8	0.82	5.15
SIL	39	1	1	9	0.69	4.31
SIL	4	1	2	10	0.91	5.69
SIL	4	1	2	11	0.79	4.97
SIL	4	1	2	12	0.78	4.88
SIL	5	1	2	13	0.75	4.72
SIL	5	1	2	14	0.56	3.49

SIL	5	1	2	15	0.77	4.84
SIL	6	1	2	16	1.44	9.03
SIL	6	1	2	17	1.41	8.84
SIL	6	1	2	18	1.40	8.74
SIL	7	1	1	19	0.71	4.43
SIL	7	1	1	20	0.48	3.02
SIL	7	1	1	21	0.62	3.85
SIL	8	1	1	22	0.97	6.08
SIL	8	1	1	23	0.78	4.86
SIL	8	1	1	24	0.90	5.66
SIL	1	2	1	43	0.57	3.57
SIL	1	2	1	44	0.53	3.31
SIL	2	2	2	45	0.56	3.49
SIL	2	2	2	46	0.53	3.28
SIL	39	2	1	47	0.62	3.89
SIL	39	2	1	48	0.67	4.17
SIL	4	2	2	49	0.56	3.48
SIL	4	2	2	50	0.48	2.98
SIL	5	2	2	51	0.58	3.65
SIL	5	2	2	52	0.47	2.96
SIL	6	2	2	53	0.62	3.88
SIL	6	2	2	54	0.53	3.31
SIL	7	2	1	55	0.72	4.52
SIL	7	2	1	56	0.61	3.80
SIL	8	2	1	57	0.70	4.35
SIL	8	2	1	58	0.65	4.06

Table A8. Crude fat content for each sample before (Time 1) and after (Time 2) watering treatments. Source abbreviations: Relic Prairie (CKS), Konza Prairie (ESK), Twelve Mile Prairie (SIL).

Sample	Container No.	Time	Treatment	Bag No.	%CF
CKS	13	1	2	1	1.63
CKS	13	1	2	2	2.42
CKS	25	1	1	1	3.03
CKS	25	1	1	2	1.77
CKS	47	1	2	1	1.66
CKS	47	1	2	2	1.58
CKS	68	1	1	1	2.03
CKS	68	1	1	2	2.29
CKS	13	2	2	1	2.04
CKS	25	2	1	1	2.41
CKS	47	2	2	1	1.34
CKS	68	2	1	1	1.42
EKS	123	1	1	1	2.40
EKS	123	1	1	2	1.40
EKS	4567	1	2	1	1.87
EKS	4567	1	2	2	1.52
EKS	123	2	1	1	1.31
EKS	4567	2	2	1	0.91
SIL	1	1	1	1	1.26
SIL	1	1	1	2	2.87
SIL	2	1	2	1	1.73
SIL	2	1	2	2	1.28
SIL	39	1	1	1	1.74
SIL	39	1	1	2	1.79
SIL	4	1	2	1	3.00
SIL	4	1	2	2	2.00
SIL	5	1	2	1	1.81
SIL	5	1	2	2	1.45
SIL	6	1	2	1	1.84
SIL	6	1	2	2	2.06
SIL	7	1	1	1	1.58
SIL	7	1	1	2	2.15
SIL	8	1	1	1	2.30
SIL	8	1	1	2	2.77
SIL	1	2	1	1	1.68
SIL	2	2	2	1	2.21
SIL	3	2	1	1	2.11
SIL	4	2	2	1	2.41
SIL	5	2	2	1	2.09
SIL	6	2	2	1	2.31
SIL	7	2	1	1	1.75
SIL	8	2	1	1	2.28

Table A9. Post-Digestion DM and ASH in Greenhouse samples before (Time 1) watering treatments. Source abbreviations: Relic Prairie (CKS), Konza Prairie (ESK), Twelve Mile Prairie (SIL).

Source	Container No.	DM%	ASH
CKS	13	97.34	2.31
CKS	25	97.39	3.41
CKS	47	97.69	2.92
CKS	68	97.19	4.45
EKS	123	97.29	3.51
EKS	4567	97.23	3.44
SIL	1	97.18	1.71
SIL	2	97.34	1.64
SIL	39	97.79	1.67
SIL	4	97.47	2.63
SIL	5	97.48	2.32
SIL	6	97.50	2.33
SIL	7	97.72	2.66
SIL	7	97.70	2.67

Table A10. Post-Digestion Crude Protein in Greenhouse samples before (Time 1) watering treatments. Source abbreviations: Relic Prairie (CKS), Konza Prairie (ESK), Twelve Mile Prairie (SIL).

Source	Container No.	Nitrogen %	Protein %
CKS	13	2.52	15.76
CKS	25	1.83	11.45
CKS	25	1.95	12.18
CKS	47	2.36	14.75
CKS	47	2.31	14.43
CKS	68	2.22	13.86
EKS	123	2.84	17.77
EKS	4567	2.42	15.11
SIL	1	1.92	11.97
SIL	1	1.90	11.90
SIL	2	2.05	12.82
SIL	2	2.19	13.72
SIL	39	1.77	11.08
SIL	4	1.86	11.61
SIL	5	3.27	20.44
SIL	5	2.35	14.67
SIL	6	2.44	15.25
SIL	6	2.34	14.64
SIL	7	1.86	11.65
SIL	8	1.88	11.73

Table A11. Post-Digestion Crude Protein in Greenhouse samples after (Time 2) watering treatments. Source abbreviations: Relic Prairie (CKS), Konza Prairie (ESK), Twelve Mile Prairie (SIL).

Source	Container No.	Nitrogen %	Protein %
CKS	13	1.93	12.05
CKS	13	1.93	12.08
CKS	25	1.94	12.16
CKS	47	1.99	12.45
CKS	47	2.02	12.63
CKS	68	1.74	10.86
CKS	68	1.78	11.12
EKS	123	1.70	10.65
EKS	4567	2.13	13.30
SIL	1	2.27	14.22
SIL	1	2.21	13.81
SIL	2	1.97	12.33
SIL	2	1.83	11.45
SIL	3	2.08	12.98
SIL	3	2.03	12.71
SIL	4	1.93	12.08
SIL	4	1.89	11.80
SIL	5	1.86	11.60
SIL	6	2.11	13.20
SIL	6	2.15	13.43
SIL	7	1.82	11.37
SIL	7	2.37	14.80

APPENDIX B

Field Experiment

Table B1. Dry Matter (DM) & ASH content in field samples. Region abbreviations: Colorado (CO), central Kansas (CKS), eastern Kansas (EKS), and southern Illinois (SIL). Source abbreviations: Crown Rock (CRN), Paramount Point (PPT), Greenbelt (GBT), Cedar Bluff (CDB), Webster (WEB), Relic (REL), Carnahan (CAR), Top of the World (TOW), Konza (KNZ), Twelve Mile Prairie (12M), Fult's Hill (FTL), and DeSoto (DES).

Region	Sample	% Moisture	DM %	ASH
CO	CRN	0.07	93.46	6.18
CO	CRN	0.06	93.75	6.47
CO	PPT	0.06	94.10	7.23
CO	PPT	0.06	93.80	6.41
CO	GBT	0.06	94.21	5.64
CO	GBT	0.06	94.33	5.36
CKS	CDB	0.07	92.67	7.64
CKS	CDB	0.07	92.95	7.04
CKS	WEB	0.07	93.30	11.92
CKS	WEB	0.06	93.54	11.92
CKS	REL	0.07	93.15	8.81
CKS	REL	0.07	93.30	8.93
EKS	CAR	0.07	92.95	6.07
EKS	CAR	0.07	92.79	6.98
EKS	TOW	0.07	93.25	6.39
EKS	TOW	0.07	93.35	7.06
EKS	KNZ	0.07	92.86	6.82
EKS	KNZ	0.06	93.73	6.80
SIL	12M	0.06	93.68	5.77
SIL	12M	0.06	93.72	5.85
SIL	FTL	0.07	93.35	5.49
SIL	FTL	0.07	93.05	5.42
SIL	DES	0.06	93.85	6.81
SIL	DES	0.06	94.20	6.97

Table B2. Neutral Detergent Fiber (NDF) in field samples. Region abbreviations: Colorado (CO), central Kansas (CKS), eastern Kansas (EKS), and southern Illinois (SIL). Source abbreviations: Crown Rock (CRN), Paramount Point (PPT), Greenbelt (GBT), Cedar Bluff (CDB), Webster (WEB), Relic (REL), Carnahan (CAR), Top of the World (TOW), Konza (KNZ), Twelve Mile Prairie (12M), Fult's Hill (FTL), and DeSoto (DES).

Region	Sample	Bag No.	NDF %
CO	CRN	1	70.35
CO	CRN	2	69.64
CO	CRN	3	71.18
CO	PPT	1	68.10
CO	PPT	2	68.28
CO	PPT	3	69.72
CO	GBT	1	68.56
CO	GBT	2	68.17
CO	GBT	3	69.80
CKS	CDB	1	68.83
CKS	CDB	2	68.45
CKS	CDB	3	68.15
CKS	WEB	1	71.30
CKS	WEB	2	70.81
CKS	WEB	3	68.78
CKS	REL	1	65.40
CKS	REL	2	67.24
CKS	REL	3	67.77
EKS	CAR	1	74.19
EKS	CAR	2	74.78
EKS	CAR	3	74.71
EKS	TOW	1	73.30
EKS	TOW	2	73.69
EKS	TOW	3	73.06
EKS	KNZ	1	74.43
EKS	KNZ	2	73.59
EKS	KNZ	3	75.35
SIL	12M	1	75.23
SIL	12M	2	74.06
SIL	12M	3	72.96
SIL	FTL	1	70.81
SIL	FTL	2	74.29
SIL	FTL	3	72.56
SIL	DES	1	69.44
SIL	DES	2	71.16
SIL	DES	3	70.71

Table B3. Acid Detergent Fiber (ADF) in field samples. Region abbreviations: Colorado (CO), central Kansas (CKS), eastern Kansas (EKS), and southern Illinois (SIL). Source abbreviations: Crown Rock (CRN), Paramount Point (PPT), Greenbelt (GBT), Cedar Bluff (CDB), Webster (WEB), Relic (REL), Carnahan (CAR), Top of the World (TOW), Konza (KNZ), Twelve Mile Prairie (12M), Fult's Hill (FTL), and DeSoto (DES).

Region	Sample	Bag #	ADF %
CO	CRN	1	38.20
CO	CRN	2	37.30
CO	CRN	3	36.21
CO	PPT	1	37.24
CO	PPT	2	37.80
CO	PPT	3	38.19
CO	GBT	1	35.40
CO	GBT	2	29.70
CO	GBT	3	36.35
CKS	CDB	1	36.90
CKS	CDB	2	38.32
CKS	CDB	3	37.88
CKS	WEB	1	44.91
CKS	WEB	2	45.48
CKS	WEB	3	43.83
CKS	REL	1	38.98
CKS	REL	2	39.18
CKS	REL	3	39.62
EKS	CAR	1	42.03
EKS	CAR	2	43.49
EKS	CAR	3	40.89
EKS	TOW	1	42.19
EKS	TOW	2	41.81
EKS	TOW	3	42.20
EKS	KNZ	1	41.93
EKS	KNZ	2	43.56
EKS	KNZ	3	42.25
SIL	12M	1	41.35
SIL	12M	2	40.85
SIL	12M	3	41.36
SIL	FTL	1	41.30
SIL	FTL	2	42.42
SIL	FTL	3	41.99
SIL	DES	1	41.69
SIL	DES	2	41.49
SIL	DES	3	40.86

Table B4. In-Vitro Dry Matter Digestion (IVDMD) in field samples. Region abbreviations: Colorado (CO), central Kansas (CKS), eastern Kansas (EKS), and southern Illinois (SIL). Source abbreviations: Crown Rock (CRN), Paramount Point (PPT), Greenbelt (GBT), Cedar Bluff (CDB), Webster (WEB), Relic (REL), Carnahan (CAR), Top of the World (TOW), Konza (KNZ), Twelve Mile Prairie (12M), Fult's Hill (FTL), and DeSoto (DES).

Region	Sample	Rep No.	Digestibility %
CO	CRN	1	59.55
CO	CRN	2	60.40
CO	CRN	3	57.99
CO	PPT	1	49.46
CO	PPT	2	58.29
CO	PPT	3	53.41
CO	GBT	1	49.21
CO	GBT	2	58.79
CO	GBT	3	58.72
CKS	CDB	1	46.05
CKS	CDB	2	52.20
CKS	CDB	3	59.05
CKS	WEB	1	51.69
CKS	WEB	2	52.39
CKS	WEB	3	41.14
CKS	REL	1	47.68
CKS	REL	2	45.38
CKS	REL	3	56.55
EKS	CAR	1	43.77
EKS	CAR	2	42.22
EKS	CAR	3	46.28
EKS	TOW	1	44.15
EKS	TOW	2	49.86
EKS	TOW	3	51.56
EKS	KNZ	1	44.11
EKS	KNZ	2	45.75
EKS	KNZ	3	48.34
SIL	12M	1	42.88
SIL	12M	2	41.45
SIL	12M	3	45.28
SIL	FTL	1	43.14
SIL	FTL	2	38.35
SIL	FTL	3	41.31
SIL	DES	1	47.78
SIL	DES	2	47.00
SIL	DES	3	41.79

Table B5. Crude Protein (CP) in field samples. Region abbreviations: Colorado (CO), central Kansas (CKS), eastern Kansas (EKS), and southern Illinois (SIL). Source abbreviations: Crown Rock (CRN), Paramount Point (PPT), Greenbelt (GBT), Cedar Bluff (CDB), Webster (WEB), Relic (REL), Carnahan (CAR), Top of the World (TOW), Konza (KNZ), Twelve Mile Prairie (12M), Fult's Hill (FTL), and DeSoto (DES).

Region	Source	Rep No.	% Nitrogen	% Protein
CO	CRN	1	1.18	7.36
CO	CRN	2	1.12	7.00
CO	CRN	3	1.19	7.41
CO	PPT	1	1.11	6.93
CO	PPT	2	1.12	6.99
CO	PPT	3	0.96	5.97
CO	GBT	1	1.21	7.58
CO	GBT	2	1.16	7.23
CO	GBT	3	1.11	6.91
CKS	CDB	1	0.99	6.21
CKS	CDB	2	0.88	5.52
CKS	CDB	3	0.92	5.77
CKS	WEB	1	0.64	3.98
CKS	WEB	2	0.56	3.53
CKS	WEB	3	0.42	2.62
CKS	REL	1	0.75	4.72
CKS	REL	2	0.76	4.73
CKS	REL	3	0.72	4.51
EKS	CAR	1	0.63	3.96
EKS	CAR	2	0.59	3.71
EKS	CAR	3	0.55	3.43
EKS	TOW	1	0.64	3.99
EKS	TOW	2	0.49	3.05
EKS	TOW	3	0.51	3.17
EKS	KNZ	1	0.80	4.97
EKS	KNZ	2	0.61	3.83
EKS	KNZ	3	0.66	4.11
SIL	12M	1	0.90	5.63
SIL	12M	2	0.64	4.01
SIL	12M	3	0.73	4.59
SIL	FTL	1	0.92	5.72
SIL	FTL	2	0.77	4.79
SIL	FTL	3	0.73	4.57
SIL	DES	1	0.84	5.22
SIL	DES	2	0.80	4.99
SIL	DES	3	0.64	4.03

Table B6. Crude Fat (CF) in field samples. Region abbreviations: Colorado (CO), central Kansas (CKS), eastern Kansas (EKS), and southern Illinois (SIL). Source abbreviations: Crown Rock (CRN), Paramount Point (PPT), Greenbelt (GBT), Cedar Bluff (CDB), Webster (WEB), Relic (REL), Carnahan (CAR), Top of the World (TOW), Konza (KNZ), Twelve Mile Prairie (12M), Fult's Hill (FTL), and DeSoto (DES).

Region	Source	Sample	Rep. No.	%CF
CO	CRN	25	1	3.84
CO	CRN	26	2	3.43
CO	PPT	27	1	3.30
CO	PPT	28	2	3.48
CO	GBT	19	1	4.03
CO	GBT	20	2	3.63
CKS	CDB	5	1	3.79
CKS	CDB	6	2	4.00
CKS	WEB	11	1	4.15
CKS	WEB	12	2	3.51
CKS	REL	21	1	4.04
CKS	REL	22	2	3.94
EKS	CAR	3	1	3.46
EKS	CAR	4	2	3.24
EKS	TOW	13	1	2.97
EKS	TOW	14	2	2.45
EKS	KNZ	23	1	4.69
EKS	KNZ	24	2	2.59
SIL	12M	1	1	3.46
SIL	12M	2	2	4.10
SIL	DES	15	1	3.44
SIL	DES	16	2	2.61
SIL	FTL	17	1	2.32
SIL	FTL	18	2	3.95

APPENDIX C

Post-Digestion Field Experiment

Table C1. Post-Digestion Dry Matter (DM) and ASH content in field samples. Region abbreviations: Colorado (CO), central Kansas (CKS), eastern Kansas (EKS), and southern Illinois (SIL). Source abbreviations: Crown Rock (CRN), Paramount Point (PPT), Greenbelt (GBT), Cedar Bluff (CDB), Webster (WEB), Relic (REL), Carnahan (CAR), Top of the World (TOW), Konza (KNZ), Twelve Mile Prairie (12M), Fult's Hill (FTL), and DeSoto (DES).

Region	Source	%DM	ASH
CO	CRN	95.98	0.07
CO	PPT	97.07	0.06
CO	GBT	95.58	0.03
CKS	REL	96.86	0.09
CKS	CDB	97.42	0.08
CKS	WEB	95.93	0.12
EKS	TOW	95.77	0.06
EKS	CAR	96.72	0.06
EKS	KNZ	95.87	0.07
SIL	12M	97.07	0.06
SIL	DES	97.19	0.08
SIL	FTL	97.16	0.06

Table C2. Post-Digestion Neutral Detergent Fiber (NDF) in field samples. Region abbreviations: Colorado (CO), central Kansas (CKS), eastern Kansas (EKS), and southern Illinois (SIL). Source abbreviations: Crown Rock (CRN), Paramount Point (PPT), Greenbelt (GBT), Cedar Bluff (CDB), Webster (WEB), Relic (REL), Carnahan (CAR), Top of the World (TOW), Konza (KNZ), Twelve Mile Prairie (12M), Fult's Hill (FTL), and DeSoto (DES).

Region	Source	Replication	NDF%
CO	CRN	1	23.42
CO	CRN	2	22.66
CO	CRN	3	22.92
CO	PPT	1	23.88
CO	PPT	2	28.16
CO	PPT	3	25.76
CO	GBT	1	20.72
CO	GBT	2	21.32
CO	GBT	3	22.21
CKS	CDB	1	24.58
CKS	CDB	2	24.01
CKS	CDB	3	23.99
CKS	WEB	1	22.89
CKS	WEB	2	20.16
CKS	WEB	3	21.36
CKS	REL	1	21.02
CKS	REL	2	20.81
CKS	REL	3	22.32
EKS	CAR	1	20.72
EKS	CAR	2	21.18
EKS	CAR	3	20.60
EKS	TOW	1	20.89
EKS	TOW	2	21.80
EKS	TOW	3	21.16
EKS	KNZ	1	22.21
EKS	KNZ	2	21.35
EKS	KNZ	3	21.98
SIL	12M	1	18.02
SIL	12M	2	17.28
SIL	12M	3	18.01
SIL	FTL	1	20.77
SIL	FTL	2	22.59
SIL	FTL	3	20.87
SIL	DES	1	21.56
SIL	DES	2	22.41
SIL	DES	3	21.65

Table C3. Post-Digestion Crude Protein (CP) in field samples. Region abbreviations: Colorado (CO), central Kansas (CKS), eastern Kansas (EKS), and southern Illinois (SIL). Source abbreviations: Crown Rock (CRN), Paramount Point (PPT), Greenbelt (GBT), Cedar Bluff (CDB), Webster (WEB), Relic (REL), Carnahan (CAR), Top of the World (TOW), Konza (KNZ), Twelve Mile Prairie (12M), Fult's Hill (FTL), and DeSoto (DES).

Region	Source	Rep#	Nitrogen %	Protein %
CO	CRN	1	2.16	13.51
CO	CRN	2	1.82	11.38
CO	PPT	1	1.45	9.09
CO	PPT	2	1.95	12.16
CO	GBT	1	1.87	11.68
CO	GBT	2	1.87	11.67
CKS	CDB	1	1.98	12.37
CKS	CDB	2	2.10	13.13
CKS	WEB	1	1.13	7.07
CKS	WEB	2	1.11	6.94
CKS	REL	1	1.86	11.65
CKS	REL	2	1.58	9.89
EKS	CAR	1	1.46	9.11
EKS	CAR	2	1.44	9.01
EKS	TOW	1	1.26	7.85
EKS	TOW	2	1.39	8.68
EKS	KNZ	1	1.55	9.68
EKS	KNZ	2	1.47	9.21
SIL	12M	1	1.48	9.24
SIL	12M	2	1.48	9.25
SIL	FTL	1	1.73	10.78
SIL	FTL	2	1.74	10.90
SIL	DES	1	.	.
SIL	DES	2	1.59	9.96

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Published Abstracts:

Donatelli, J.M., Gibson, D.J., Renzaglia, K., Henson, H., Mumba, F., Sipes, S., & K. Schachel. 2010. Bridging the gap, building the future: STEM fellows teaching global change in primary education. Ecological Society of America, 95th Annual Meeting. August 2nd – 6th, 2010, Pittsburgh, PA. (poster presentation). http://eco.confex.com/eco/2010/techprogram/P24279.HTM

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Thesis Title:

Forage Quality of *Andropogon gerardii* Across a Precipitation Gradient

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