

**An Assessment of Traits Impacting Native Warm-Season Grass Adoption by
Pasture Managers in the Mid-South**

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Dedication

To my family and friends who have shaped my life thus far. Also, dedicated to Ragnar Thorisson, who would have found this a bourgeois extravagance ignorant of its own biases and privilege.

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I would like to thank any or all divine entities for not interacting with my research, direct actions from a conscious deity would have undermined the assumptions of all modern statistical methods.

Abstract

In the Mid-South, Native warm season grasses (NWSG) provide alternative forage to tall fescue (TF) during hot, dry summers. However, NWSG adoption rates are low. This study will evaluate two NWSG pasture types (big bluestem/indiangrass mixture; BBIG; switchgrass; SG) for alternative characteristics that may induce increased adoption of NWSG. The first experiment evaluated the monthly forage characteristics of NWSG (SG and BBIG) and tall fescue (TF) during fall stockpiling (August-December) and winter grazing (January-April) by protein-supplemented yearling beef heifers. Both BBIG and SG nutritive value deteriorated during the fall, but did not continue during winter grazing. Tall fescue provided adequate forage throughout winter for livestock maintenance (89 g kg^{-1} [grams per kilogram] CP; $3,766 \text{ kg ha}^{-1}$ [kilograms per hectare]), while dormant SG had the lowest nutritive value and greatest yields (20 g kg^{-1} CP; $7,489 \text{ kg ha}^{-1}$). The BBIG paddocks had intermediate forage quality compared to TF and SG (31 g kg^{-1} CP; $4,928 \text{ kg ha}^{-1}$). The second experiment evaluated seasonal dynamics of labile nutrients between NWSG and TF pastures. Labile pools such as hot-water extractable carbon (HWE) and nitrogen (HWEN), aromatic content of extracts (Abs_{254}), and potassium permanganate oxidizable carbon (POXC) are potential predictors of “soil health” or future carbon sequestration. Samples were analyzed for 18 mo at two depths. Labile soil pools (HWE, HWEN) had greater seasonal variation relative to more recalcitrant pools (POXC, Abs_{254}). Models indicated greater HWEN (97.4 mg kg^{-1} [milligrams per kilogram]; 77.5 mg kg^{-1}) and Abs_{254} (0.66 cm^{-1} [per centimeter]; 0.58 cm^{-1}) in TF relative to SG. This is consistent with increased microbial activity associated with root traits similar to TF. The third experiment evaluated a gibberellin inhibitor (trinexapac-ethyl) on fall NWSG growth. Fall NWSG growth provides low-quality, high-mass, forage therefore gibberellin inhibitors may provide a beneficial

trade-off. The study applied three concentrations (0, 0.3, 0.6, 1.2 kg a. i. ha⁻¹ [active ingredient per hectare]) to SG and BBIG paddocks. Treatment depressed forage mass and improved CP contents. However, minimal digestibility improvements were observed. Therefore, late season application of gibberellin inhibitors to warm season grasses is unlikely to be useful for pasture managers.

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Introduction

The state of Tennessee lies in the middle of the Fescue Belt. Tall fescue (*Schedonorus phoenix*; TF) is the major component of forage systems in the region bounded by Arkansas and Missouri in the west through the Carolinas and Virginias in the east. Tall fescue is a productive, persistent cool-season (C_3) grass that is adapted to the climate and soil conditions of the mid-south. Over 15 million hectares are currently covered by TF, an area larger than Alabama and equivalent to 80% of the pasture and hay land in the Fescue Belt (Locke and Rogers, 2017; USDA, 2017). Within Tennessee, TF is planted on 70% of pasture and hay land (USDA, 2017).

Although TF is generally persistent and nutritious, the lack of alternative pasture species in the region can cause economic and environmental issues (Washburn et al., 2000; Barnes et al., 2013). Broadly, large scale homogeneity in agricultural systems has been shown to have negative impacts and the regional dominance of a single species tends to lower agroecological resiliency (Benton et al., 2003; Gibon, 2005; Duelli and Obrist, 2003; O'Rourke and Kramm, 2012). Specifically, TF grows slowly during mid-summer and during droughts. Since mid-summer and drought conditions will be amplified by anthropogenic climate change, establishing a more diverse and drought resistant forage base in the Fescue Belt is critical for the economy and the environment (Konrad and Fuhrmann, 2013; IPCC 2014; Hsiang et al., 2017). A large-scale analysis of the 2012 drought determined an overall loss of \$32.4 billion (Smith and Katz, 2013). Beef producers are particularly vulnerable during periods of drought, because the market for feed can increase dramatically. During the 2007 drought in Kentucky, hay producers had a direct loss of \$86 million, a cost that would be passed on to buyers (Craft et al., 2017). In addition to this increased feed cost, beef producers must contend with poor animal performance and herd reductions into flooded markets. The multi-year cost of these droughts is also higher

due to degraded TF stands and the slow recovery of herd sizes. Alternative drought tolerant forages could reduce or eliminate the harm from these drought events.

A lesson can be gleaned from the environmental history of the Southeast. The Fescue Belt lies at the southern limit of C_3 photosynthesis traits (Teeri and Stowe, 1976; Paruelo and Lauenroth, 1996). Prior to European colonization, C_4 grasses dominated many grassland ecosystems of the Southeast (Noss, 2013). Drought adapted C_4 photosynthesis allows greater water and nitrogen use efficiency, as well as increased photosynthetic efficiency at high temperatures (Tjoelker et al., 2005; Taiz, 2015). Interestingly, in the mid-South, C_3 grasses have a competitive advantage over C_4 grasses during years of average precipitation and temperature because they are active during the long and mild spring and fall periods. However, historical C_4 grass dominance in the region is attributed due to their success during rare drought events (Axelrod, 1985; Noss, 2013; Taylor et al., 2014).

Native C_4 grasses, referred to as native warm season grasses (NWSG), offer an alternative or complimentary forage base. These species are heat and drought tolerant due to their deep rooting system and C_4 photosynthesis, and are long-living. In addition, NWSG are environmentally preferable because the species are the foundation to a regionally endangered ecosystem and associated threatened species (Washburn et al., 2000; Monroe, 2014; Noss, 2013).

Adoption of NWSG by pasture managers has been limited due to several economic drawbacks. This includes their lower forage value, slow establishment, and shorter growing season compared to TF. This thesis will assess strategies to improve NWSG utility for livestock producers. This thesis will compare TF and NWSG forage systems regarding 1) forage accumulation and nutrient degradation during fall stockpiling, 2) seasonal labile soil carbon and

nitrogen dynamics, and 3) an attempt to increase fall forage value of NWSG through the application of a novel gibberellin inhibitor (Trinexapac-ethyl).

Literature Review

Tall Fescue

Tall fescue is an uncharacteristically heat and drought tolerant cool season (C_3) perennial grass. Cool season grasses have high nutritive value, but most are not persistent in the region. In the mid-South, cool season grasses have two productive periods: spring and fall (Ball et al., 2007). During the summer, pasture and livestock managers must contend with a period of low growth and potential stand degradation for most cool season species. A fungal endophyte (*Epichloe coenophiala*) helps TF persist during high temperatures, but the wild-type endophyte produces toxic alkaloids (Elbersen and West, 1996; Arachevaleta et al., 1989; Malinowski and Belesky, 2000). These alkaloids reduce forage intake and can cause TF toxicosis (Hemken et al., 1981; Porter and Thompson, 1992; Paterson et al., 1995). Without alternative forage sources, livestock producers in mid-summer must alleviate TF shortage and toxicosis by feeding protein supplements or feeding stored feeds (Read and Camp, 1986).

Native Warm Season Grasses

Native warm season grasses (NWSG) are a group of alternative forage species to TF in the Mid-South. Unlike imported pasture grasses, NWSG grasses have a native range from the continental mountain west to the eastern coast of the United States (Noss, 2013). In the Southeast US, there are multiple commercially available native grasses of importance, this study will focus on three: big bluestem (*Andropogon gerardii*; BB), indiagrass (*Sorghastrum nutans*; IG), and switchgrass (*Panicum virgatum*; SG). Switchgrass is a native C_4 bunchgrass that grows over 2 m and has deep coarse roots. Both BB and IG are native C_4 perennial bunchgrasses that grow from 1-2 m. Although deep-rooted, BB and IG have finer roots, thinner stems, and lower

forage mass accumulation compared to SG (Tjoelker et al., 2005). All three native grasses utilize C_4 photosynthesis, which allows for greater water use efficiency and nitrogen use efficiency at high temperatures. However, C_4 grasses grow slowly at low temperature (Kubien et al., 2003; Bilska and Sowiński, 2010). Native warm season grasses provide lower quality forage (lower protein, greater fiber content) than C_3 alternatives. Of the three NWSG mentioned above, SG has lower forage value than BB and IG (Bonin and Tracy, 2011). Native-warm season grasses become dormant at low temperatures and translocate nitrogen from aboveground biomass, improving nitrogen efficiency but reducing nutritional value (Sarath et al., 2014). In mid-summer, NWSG have shown to support adequate animal gain (Backus, 2014; Monroe, 2014). This is partially attributed to the improved animal intake of warm season grasses (C_4) compared to cool season (C_3) grasses with equivalent NDF (Reid et al., 1988).

The partial adoption of NWSG pastures to supplement TF pastures could result in improved summer and drought production. Therefore, methods to extend the utility of NWSG pastures beyond the warm season could further improve overall economic returns for pasture managers.

Stockpiling

Stockpiling is the practice of allowing forage to accumulate in the field for later use in a different grazing season. Stockpiling can be used to compensate for periods of low productivity and is dependent on a trade-off where forage quality declines (due to plant maturity and weathering) but inputs in labor and equipment are reduced (D'Souza et al., 1990; Poore and Drewnoski, 2010). Therefore, it can be a low-input method for managing variation in productivity. Stockpiling research has been carried out throughout the continental United States

and Canada (Hitz and Russell, 1998; Riesterer et al., 2000; Robinson et al., 2007; Meyer et al., 2009; Baron et al., 2016).

In the Southeast, TF is frequently stockpiled for winter grazing because it resists weathering and produces leafy tillers in the fall instead of less desirable reproductive stems (Fribourg and Bell, 1984; Dierking et al., 2008; Shireman, 2015). In addition, ergovaline, an anti-nutritional compound in TF is low during fall stockpiling (Kallenbach et al., 2003). Recent research has focused on optimizing fall stockpiling by assessing the impacts of initiation dates and nitrogen fertilization (Poore and Drewnoski, 2010; Shireman, 2015; Nave et al., 2016).

Native warm season grass species are considered poor candidates for stockpiling in the mid-South. Although NWSG can accumulate large quantities of forage, the forage has high fiber content and translocates nitrogen below ground in fall (Wayman et al., 2013). The resulting nutritional profile is considered inadequate to support the nutrient requirements of most livestock (Hickman, 2013). However, research indicates that high fiber forage can be economically utilized as winter-feed for livestock with low requirements when provided with proper management and supplementation.

In rangeland settings, prior research has shown protein supplementation may increase intake and digestibility of low-quality forages (Beaty et al., 1994; Köster et al., 1996; Bohnert et al., 2011; Sawyer et al., 2012). However, this strategy has not been attempted in Tennessee. Although the regional forage base in the Southeast does not include extensive regions for rangeland production, NWSG stockpiling provides biomass yields multiple times higher than stockpiled TF, allowing pasture managers to maintain over-wintering livestock on less land and potentially at lower cost.

Growth Regulators

Plant growth regulators offer a potential method to slow stem elongation and improve grass digestibility (Rademacher, 2000). Therefore, growth regulators could provide a useful trade-off in NWSG, since these species produce high biomass but high proportions of low-quality stem materials. Previous forage studies have been carried out on the growth regulator mefluidide to slow stem elongation by suppressing the gibberellin hormone pathway. In addition, suppressing gibberellin expression may weaken apical dominance and result in increased tillering (Ervin and Koski, 1998). In warm season grass forages such as millet and sorghum, applications of mefluidide improved tillering, stem:leaf ratios and stem digestibility (Hernandez, 1984; Bransby et al., 1986; Stair et al., 1991; Redmon et al., 2003). In pasture settings, mefluidide has improved animal intake, digestibility, and rate of gain (Goold et al., 1982; Moyer and Lomas, 1987). However, mefluidide is a cell division inhibitor and slows plant growth. Trinexapac-ethyl inhibits gibberellin synthesis late in the biosynthetic pathway and therefore is potentially less disruptive to growth (Marcum and Jiang, 1997; Ervin and Koski, 1998; Rademacher, 2000). Growth suppression is expected to occur with trinexapac-ethyl, although at rates lower than mefluidide (Luiz et al., 2015). Trinexapac-ethyl has not been evaluated on perennial forage species and could improve NWSG nutrient partitioning.

Soil Organic Matter (SOM) Dynamics in Grassland Systems

Historically, areas dominated by the native tallgrass ecosystem have maintained high levels of soil organic matter (SOM). Improper management has resulted in the loss of large quantities of this organic matter and there is renewed interest in attempting to restore the soil organic matter pool, both to mitigate atmospheric carbon from fossil fuels and to improve

agricultural productivity. Compared to cool-season grass species, NWSG dedicate a greater proportion of photosynthesized carbon to root structures (Hager et al., 2016). Therefore, NWSG may improve SOM accumulation through increased belowground biomass and increased root depth (Rasse et al., 2005; Omonode and Vyn, 2006; Blanco-Canqui et al., 2014, Mazzilli et al., 2015). However, assessments of SOM under NWSG have found mixed results (Corre et al., 1999; Fornara and Tilman, 2008; Mahaney et al., 2008). These contrary results could be due to a difference in root structures, which in turn impacts microbial carbon efficiency.

The species of interest in this study have contrasting plant resource acquisition strategies. These strategies are composed of plant traits favoring either conservative or acquisitive behaviors (Craine et al., 2002; Fort et al., 2013). Acquisitive species have traits that emphasize rapid acquisition and utilization of resources, whereas conservative species rely on outlasting other species through tolerance to stressors. Previous research indicates strong correlations between physical plant traits and plant strategies (Roumet et al., 2016). Tall fescue is an acquisitive species, SG is a conservative species, and both BB and IG are intermediate. Morphologically, acquisitive species have high-quality (low C:N) and short-lived root and leaf material. Conservative species have physically coarse, long-lived, and lower quality root and leaf material. Craine et al. (2002) evaluated the NWSG species involved in this study and SG was a clear outlier in these analyses, with traits more correlated with coarse-stemmed, long-living forb species instead of grass species. Big bluestem and indiangrass were morphologically similar to other C_4 grasses, which were more conservative than C_3 grasses. Within tallgrass prairie species, C_4 photosynthesis is well correlated with conservative traits and C_3 , acquisitive (Tjoelker et al., 2005). Although the overlap of C_4 as conservative and C_3 as acquisitive may not be universal, the trend is very strong within the temperate United States and within the species in this study

(SG, BBIG, TF). Therefore, this review will occasionally use North American C₃/ C₄ comparison studies as proxies for conservative and acquisitive traits.

Although it was not assessed by Craine et al. (2002), multiple Eurasian C₃ pasture species were studied and determined to have traits distinct from NWSG and acquisitive. Based on these traits, C₃ grasses such as TF can be considered acquisitive compared to the NWSG in the study.

Plant resource acquisition strategies have impacts on soil nutrient cycling. Plant strategies alter cycling since acquisitive species create higher supply and demand of labile nutrients through their easily decomposed plant litter and large exudate inputs, which then supports a high activity microbial community (Personeni and Loiseau, 2005; Mahaney et al., 2008). Conservative grasses maintain high C:N, coarse roots (Vivanco and Austin, 2006), which slows microbial degradation (Fornara et al., 2009). A recent study by Kaštovská et al. (2015) found greater overall belowground carbon investment from a conservative grassland species but two-fold greater rate of root exudation in an acquisitive species.

The varying plant strategies impact SOM turnover and potentially SOM sequestration. Personeni and Loiseau (2005) found that conservative species compete for a diffuse pre-existing mineral nitrogen pool, while acquisitive species compete for nitrogen by increasing the microbial cycling of SOM pools. Therefore, acquisitive species must rely on larger and more temporally variable microbial communities that utilize exudates to mineralize nitrogen. Conservative species instead scavenge nutrients from a more diffuse, lower activity microbial community which degrades organic matter at a more temporally and spatially uniform rate (Personeni and Loiseau, 2005; Personeni et al., 2005). There is evidence that more conservative C₄ grass introduction into a competitive C₃ sward down-regulates microbial activity and nitrogen cycling (Fu and Cheng, 2002; Mahaney et al., 2008; Yao et al., 2011). Down-regulation of nitrogen cycling may

limit microbial carbon-use efficiency (see below) and potentially carbon sequestration (Knops and Bradley, 2009; Castellano et al., 2015).

Microbial processing governs the maintenance of a persistent carbon pool. Microbial activity respire a portion of belowground carbon while also degrading plant products into more recalcitrant soil pools (Marschner et al., 2008; Dungait et al., 2012; Bradford and Crowther, 2013; Bradford et al., 2013). Microbial degradation of plant inputs result in a portion of carbon released through respiration and the portion that is absorbed by soil microbes and later deposited as more stable organic matter (Six et al., 2006; Manzoni et al., 2012). The ratio between the carbon respired by microbial activity and retained in soil is carbon-use efficiency (CUE; Bradford and Crowther, 2013; Bradford et al., 2013). A high microbial CUE will maximize potential carbon sequestration rates in an ecosystem (Miltner et al., 2011; Schurig et al., 2012). CUE is dependent on both abiotic (moisture, temperature, soil structure) and biotic (input quality, microbial community) factors. Soil temperature and moisture content of soils can alter the efficiency of processing similar inputs (Grayston et al., 2001; Manzoni et al., 2012; Frey et al., 2013). In addition, historical inputs and microbial community composition can impact CUE (Six et al., 2006; De Deyn et al., 2008; Keiblinger et al., 2010).

Plants deposit carbon into the soil through surface litter, root litter, and root exudates. These deposits can be characterized along a spectrum from labile to recalcitrant (Cotrufo et al., 2013). Labile plant matter has some or all of the following traits: low molecular weight, a low C:N ratio, and simple chemical structure. Recalcitrant plant matter tends to have high C:N, is more chemically complex, and has higher molecular weight. The soil community will degrade these inputs at different rates and the labile carbon pool is preferentially degraded. The labile pool can act as a buffer protecting more persistent organic matter pools or as microbial energy to

enhance breakdown of pre-existing soil carbon (de Graaff et al., 2010; Bradford et al., 2013; Suseela et al., 2013; Mizuta et al., 2015). This is referred to as positive (carbon loss) or negative (carbon protection) soil priming. Generally, C₃ species have been observed to have a stronger positive priming effect on soil carbon compared to C₄ species (Fu and Cheng, 2002). Labile carbon also has higher CUE and therefore contributes proportionally more material to persistent organic pools when decomposed.

Since recalcitrant inputs are processed less efficiently by the soil community (Bradford et al., 2008; Cotrufo et al., 2013) these inputs can result in a lower CUE and therefore result in a lower carbon sequestration rates (Marschner et al., 2008; Bradford et al., 2008; Lee and Schmidt, 2014). More conservative recalcitrant NWSG root litter may have a low carbon sequestration due to low microbial processing efficiency. Multiple studies indirectly support this hypothesis: Fornara and Tilman (2008) reported that the inclusion of legumes into a NWSG paddock improved carbon sequestration rates two-fold and concluded that the combination of legumes and C₄ grasses may be uniquely suited to carbon sequestration. Ampleman et al., (2014) reported an increase in carbon sequestration with the inclusion of forb species, indicating that NWSG monoculture sequestration may be limited by labile organic matter. Monoculture SG stands have shown improved sequestration with moderate N fertilization, which can improve microbial CUE (Jung and Lal, 2011; Gauder et al., 2016). Further study could confirm if labile nutrient pools are lower under NWSG despite high belowground carbon investment.

Seasonal dynamics of labile soil carbon

Research on soil carbon sequestration is often limited due to the slow rate of change in soil carbon pools. However, microbial communities process labile carbon rapidly and therefore,

labile carbon pools should fluctuate over short sampling periods. Labile carbon inputs are primarily through exudation and microbial degradation of organic matter, such as root litter. Root exudates have a half-life from 1-3 days (Kaštovská and Šantrůčková, 2007), and root litter pools also turnover rapidly. Overall root turnover is estimated at 0.9% per day in a cool-season pasture (Reid et al., 2015), and a similar method found root carbon to have a half-life of 2-3 months (Saggar and Hedley, 2001). There is evidence that NWSG will have slower root turnover than cool-season species (Dahlman and Kucera, 1965; Tjoelker et al., 2005). Overall, since exudation and root turnover are governed by plant strategy and seasonal growth habits, the species of interest in this study may have significant intra-annual variation within labile soil pools.

Hot water extractable carbon/nitrogen:

A simple method for monitoring an active carbon pool is with hot-water extraction (Ghani et al., 2003; Sparling et al., 1998). Hot-water extractable carbon is a pool of carbon that includes the microbial population, soluble soil proteins, and microbial-based and microbially-accessible soil carbohydrates (Sparling, et al., 1998; Ghani et al., 2003; Wang et al., 2013; Balaria and Johnson, 2013; Atanassova et al., 2014). In a range of agricultural soils, Chantigny et al., (2014) measured that 30-50% of the extractable carbon was in carbohydrate form, with 10-30% of the extracted carbon was phenolic and up to 20% of extractable N was from the microbial community.

The carbon and nitrogen content of hot-water extract has been found to correlate with many metrics related to soil activity and health (Table 0.1). It should be noted that these studies come from diverse sample environments, some spanning multiple ecosystems and others from

single ecosystem data sets including forest, row-crop, and pasture systems. However, these correlations indicate the potential for hot-water extractable carbon to act as a metric for “soil health”.

Table 0.1: Prior publications indicating correlations between soil health metrics and hot-water extractable carbon (HWE). Threshold for correlation set to $p < 0.05$; $R^2 > 0.75$.

Publication	Variable correlating with HWE
Ćirić et al., 2016	Total organic carbon, aggregate stability
Stevenson et al., 2016	PFLA Biomass, N Mineralization
Thomas et al., 2015	Growing season N supply
Spohn and Giani, 2011	Total Organic Carbon
Ghani et al., 2003	Microbial Biomass C, Mineralizable N, Carbohydrate C
Sparling, et al., 1998	Microbial Biomass C
Ball et al., 1996	Aggregate stability, Bulk Density (inverse)

Relative to other soil metrics, hot-water extractable nutrients are dynamic and change rapidly. In an extraction experiment, Ghani et al., (2012) found respiration of over 50% of hot-water extractable carbon in 21 days across multiple soil types. In forest soils, 12-20% degradation occurred during a 90 day incubation (Bu et al., 2011). In assessing soils in a corn rotation, hot-water extractable carbon degraded 30% during the first day of incubation, and slowly degraded a further 20% during the remaining 40 days (Gregorich et al., 2003). These different outcomes can be explained by an incubation study conducted by Kalbitz et al. (2005), which suggested chemical protection in mineral soil can bind soluble carbon, slowing microbial degradation. These soil protection processes will likely be greater in field conditions (Dungait et al., 2012), particularly when estimating long-term degradation of recalcitrant carbon inputs (Oburger and Jones, 2009). Hot-water extractable carbon correlates with both an available microbial energy source and soil aggregation, a carbon protection method. Therefore, a stable labile carbon pool may act as a buffer limiting microbial decomposition of other SOM pools.

The aromatic content of hot-water extractable carbon impacts microbial processing (Kalbitz et al., 2003; Marschner and Kalbitz, 2003). Soluble aromatic compounds are byproducts of the degradation of complex organic compounds such as lignin and are resistant to microbial degradation (Weishaar et al., 2003). In incubation studies, the proportion of aromatic compounds increases over time, indicating preferential degradation of more labile carbon (Kalbitz et al., 2003; Toosi et al., 2012). In the field, elevated aromatic carbon compounds may indicate a shortage of new labile carbon inputs.

Permanganate Oxidizable Carbon (POXC)

The potassium permanganate oxidizable carbon (POXC) method is frequently used as an estimate of active or microbially accessible carbon (Culman et al., 2012; Hurisso et al., 2016; Wang et al., 2017). This oxidizable portion correlates well with other variables measuring “soil health” (Morrow et al., 2016; Fine et al., 2017). However, the exact content of this carbon pool is undetermined. The method does not react to soil compounds considered labile: carbohydrates, sugars, or amino acids (Tirol-Padre and Ladha, 2004), but a recent analysis indicates that it preferentially reacts with a microbially stabilized carbon pool, rather than fresh plant inputs (Suárez-Abelenda et al., 2014; Culman et al., 2012; Skjemstad et al., 2006).

Degradation of POXC can still be observed when soils are deprived of fresh inputs. Xu et al., (2012) observed a loss of over 75% during a long-term incubation (170 days; variable temperature). A short-term (50-100mg C kg⁻¹year⁻¹ [milligrams carbon per kilogram per year]; 10-20%) and decade long (5-6mg C kg⁻¹ year⁻¹; 36 years) loss of POXC was observed during conversion to a wheat-fallow rotation (Tatzber et al., 2015). Culman et al., (2013) documented POXC fluctuations in a cornfield, indicating elevated POXC during summer and a significant

drop during late summer and fall (up to $100\text{mg C kg}^{-1}\text{month}^{-1}$; 20-25%). Although representative of a more stable carbon pool relative to hot-water extractable carbon, this indicates the potential for POXC to fluctuate during a growing season. Prior evidence of POXC turnover has been focused on row crop systems and due to the robust microbial community and intact soil structure, results are likely to be different under grassland compared to row crops (Skjemstad et al., 2006).

Chapter 1:
Fall Stockpiling of Tall Fescue and
Native Warm-Season Grasses in the Southeastern United States

Abstract

Tall fescue (*Schedonorus phoenix*; TF) is one of the major forage crops in the United States but grows slowly during summer in the Southeast. Native warm-season grasses (NWSG), such as big bluestem (*Andropogon gerardii*; BB), indiangrass (*Sorghastrum nutans*; IG), and switchgrass (*Panicum virgatum*; SG) are potential alternatives, but are dormant during winter. Late summer growth of NWSG results in high forage mass during winter and may provide winter feed if utilized correctly. This experiment evaluated the performance of NWSG (SG and a BB/IG mixture) and tall fescue (TF) during fall stockpiling (August-December) and winter grazing (January-April) by yearling beef heifers supplemented with a protein supplement (0.18 kg, CP heifer¹ day⁻¹). Forage samples were collected monthly to monitor forage mass and assess forage nutritive value. Both BBIG and SG quality deteriorated during the fall stockpiling season, but degradation stabilized during winter grazing. Winter stockpiled TF provided adequate quality feed for animal maintenance, while dormant switchgrass had the lowest nutritive value and greatest yields. A mixture of big bluestem and indiangrass had forage quality intermediate between SG and TF. Leaf sub-samples of NWSG indicated greater forage nutritive quality compared to bulk samples during winter grazing. Under specific conditions, NWSG may provide large quantities of low input stockpiled forage for livestock producers in the Southeast.

Introduction

Cool-season grasses have high forage nutritive value compared to warm-season grasses, but do not thrive in the soils and climate of the mid-South. Tall fescue is unique exception as a cool-season (C₃) grass that is persistent in the region. In addition, it tolerates over grazing, stockpiles efficiently, and has a long growing season (Poore and Drewnoski, 2010). Because of

these advantages, TF now covers over 15 million hectares in the United States, an area larger than Alabama (Locke and Rogers, 2017; USDA, 2017). Tall fescue has several drawbacks, including providing limited habitat for wildlife (Washburn et al., 2000, Barnes et al., 2013) and poor performance under dry or hot conditions ($>30^{\circ}\text{C}$). Although more persistent than other cool-season grasses, TF grows slowly during mid-summer and a fungal endophyte in TF increases production of alkaloid toxins, further lowering the grazing value (Read and Camp, 1986). Without alternative forages or supplements, livestock producers using TF will encounter lower productivity during mid-summer.

Native warm-season grasses (NWSG) have been utilized as a complement forage to TF in the Southeast to fill in during slow summer growth. These species include big bluestem, indiangrass, and switchgrass. Native warm-season grasses utilize the C_4 photosynthetic pathway, which improves drought and heat tolerance by segregating rubisco enzyme activity into bundle sheath cells (Ball et al., 2007). In the current experiment, big bluestem and indiangrass were grown as a mixture (BBIG) and compared to switchgrass (SG). Although NWSG have many similar traits, SG is greater yielding but frequently produces forage of lower nutritive value compared to BBIG. During summer, NWSG can provide economically competitive rates of animal gain (Bonin and Tracy, 2012; Backus 2014; Monroe, 2014; Lowe et al., 2016). The utilization of NWSG pastures to complement TF pastures could result in improved summer and drought outcomes.

For many livestock producers, a major drawback of NWSG is their short growing season. In Tennessee, NWSG begin growth in April and are fully dormant by late September, a major disadvantage, due to the mild winter (Ball et al., 2007). The effective productive period is further narrowed because NWSG are, in many instances, not grazed or mowed during late

summer and fall to maintain stand vigor (Forwood and Magai, 1992; Cuomo et al., 2006). Accumulated fall forage is fibrous and has lower forage nutritive value (Waramit et al., 2012; Wayman et al., 2013; Sarath et al., 2014). Nutritionally, senesced fall NWSG forage has insufficient nutritive value ($>7\%$ CP) for most classes of livestock (Hickman, 2013), but various studies have indicated that stockpiled forage grazing with protein supplementation may provide an economically viable use for low-quality forages (Schoonmaker et al., 2003; Baron et al., 2016).

Stockpiling is the practice of allowing forage to accumulate in the field for later use when other feed options are limited. Stockpiling can be used to compensate for periods of low productivity and is dependent on a trade-off where forage nutritive value is decreased (due to plant maturity and weathering). Labor and equipment costs can be reduced when compared to hay harvesting (D'Souza et al., 1990; Poore and Drewnoski, 2010), therefore it is a potential low-input method for managing variation in forage availability. Stockpiling research has been carried out throughout the continental United States and Canada (Hitz and Russell, 1998; Riesterer et al., 2000; Robinson et al., 2007; Meyer et al., 2009; Baron et al., 2016).

In the Southeast, TF is regularly used for stockpiling because it maintains quality after freezing and produces leaf material in the fall instead of less desirable reproductive stems (Fribourg and Bell, 1984; Dierking et al., 2008; Shireman, 2015). In addition, ergovaline, an anti-nutritional compound in TF is reduced by stockpiling (Kallenbach et al., 2003). Recent research has focused on optimizing fall stockpiling by assessing the impacts of initiation dates and nitrogen fertilization (Poore and Drewnoski, 2010; Shireman, 2015; Nave et al., 2016).

Despite low nutritive value, stockpiling NWSG may be possible with additional supplementation. Specifically, protein supplementation induces increased animal intake and

utilization of low-quality feed (Sanson et al., 1990; DelCurto et al., 1990; Beaty et al., 1994; Köster et al., 1996; Olson et al., 1999; Bohnert et al., 2002). This effect is more apparent in warm-season grasses (Bohnert et al., 2011; Sawyer et al., 2012). By increasing the digestibility and intake rate of fall stockpiled NWSG, protein supplementation can improve utilization of low-quality winter forage, improving the economic return on NWSG pastures. Similar strategies have been successfully implemented in Canada (Jefferson et al., 2004; Legesse et al., 2012), and in the Western United States (Akhtar et al., 1994; Patterson et al., 1999).

Our research objective was to quantify the forage accumulation and nutritive value of switchgrass, mixed big bluestem/indiangrass, and tall fescue stockpiled during fall (August-December) and through the grazing period during winter (January-April). The research hypotheses are that (1) TF will maintain significantly lower forage mass throughout both the stockpiling and grazing period, (2) TF will have a greater rate of dry matter loss during winter grazing due to greater nutritive value, (3) NWSG species will translocate nitrogen belowground during the fall stockpiling period, resulting in a large loss of crude protein and *in vitro* dry matter digestibility (IVTDMD), (4) fully senesced NWSG will lose nutritive value over winter due to leaf loss from grazing and leaf shatter, (5) BBIG mixture will have forage mass and nutritional values intermediate between SG and TF.

Materials and Methods

Site, history, and management

The experiment was conducted at the Middle Tennessee AgResearch and Education Center, in Spring Hill, TN. The soil is Maury silt loam (Typic Paleudalf). The study was conducted during two consecutive seasons (2015-2016, and 2016-2017) with three treatments

(forage types) and five replications. The sampling area consisted of fifteen 1.2-hectare paddocks randomly assigned to one of the three treatments: tall fescue (cv. KY-31), switchgrass (cv. Alamo), and a 1:1 mixture of indiangrass (cv. Rumsey) and big bluestem (cv. OZ-70).

The paddock array was established October 2007 and was used for a NWSG and red clover experiment until 2012 (Keyser et al., 2016). Three cycles of stockpiled winter grazing were carried out prior to the initiation of forage sampling (McFarlane et al., 2017). The management schedule was the following: winter grazing from January-April, regrowth from April-June, management through either haying or grazing during June-July, and mowing in late July to initiate regrowth for winter grazing (beginning the following January).

Winter grazing was carried out on all paddocks by 2 or 3 Angus crossbred yearly heifers per paddock (determined by forage availability) from January until April. Heifers were supplemented with $0.18 \text{ kg CP heifer}^{-1} \text{ day}^{-1}$ through either blood meal/fishmeal or dried distiller's grains (McFarlane 2017).

During summer, paddocks were managed for either hay production or grazing, both followed by mowing in late July-early August to initiate fall stockpiling (20-cm residual height for NWSG, 10-cm for TF). Paddocks that were managed for hay were fertilized with 67 kg ha^{-1} N in June of each year. The remaining paddocks were grazed with a put-and-take system based on forage availability and did not receive supplemental fertilizer. During the 2016 summer grazing period, grazing removed approximately 30% TF, 40% SG, and 45% BBIG forage biomass relative to un-grazed paddocks. In the 2015-16 season, paddocks were mowed on July 1 then allowed to accumulate until grazing began on January 3. In the 2016-2017 season, paddocks were mowed on August 14, 2016 and allowed to accumulate until grazing began on January 4,

2017. No significant impact was observed due to summer management on forage quantity or nutritive value during fall stockpiling.

Routine soil sampling on February 3, 2017 indicated no significant differences between treatments and no micronutrient deficiencies. Soil pH had a mean of 5.96 and 0.19 standard error. Mehlich 1 extractions indicated average phosphorus of 470 kg ha^{-1} (S.E.=361), mean potassium 192 kg ha^{-1} (S.E.=48), mean calcium 3780 kg ha^{-1} (S.E.=1330), and mean magnesium 257 kg ha^{-1} (S.E.=29). The phosphorus variability is due to three outlier paddocks (1157 kg ha^{-1} , 1129 kg ha^{-1} , 930 kg ha^{-1}). This high level of phosphorus did not result in any significant deviations in plant growth.

Forage sampling method

Sampling occurred monthly during one stockpiling (fall 2016) and two winter grazing periods (2016, 2017). Each pasture was sampled for aboveground forage mass (stubble height 8-cm) by collecting from a randomly assigned 0.1 m^2 area. During the 2016 winter grazing period, samples were collected on January 27, 2016, March 3, 2016, and April 8 2016. During the 2016 stockpiling period, forage sampling occurred on August 24, 2016, September 22, 2016, October 26, 2016, and November 30, 2016. During the 2017 winter grazing period, samples were taken on January 4, 2017, February 3, 2017, March 3, 2017, and March 31, 2017. During analysis, the January 4, 2017 data was included within both the stockpiling and grazing season, since grazing began on that date. Forage samples for January 5, 2016 were acquired from a concomitant study by McFarlane et al. (2017). February 3, 2017 sub-samples of leaf material were acquired for BBIG and SG and analyzed for nutritive value.

Forage samples were dried at 60°C for 48 hours up to constant weight and dry weights were recorded. Each sample was then ground through a Wiley Mill Grinder (1-mm screen; Thomas Scientific, Swedesboro, NJ) for near-infrared reflectance spectroscopy (NIRS) analysis of forage nutritive value using a FOSS 6500 NIRS instrument (FOSS NIRS, Laurel, MD) to quantify crude protein (CP), neutral detergent fiber (NDF), *in-vitro* dry matter digestibility at 48 hours (IVDTMD) and neutral fiber digestibility at 48 hours (dNDF). Equations for the forage nutritive analyses were standardized and checked for accuracy with the 2016 mixed hay equation developed by the NIRS Forage and Feed Consortium (NIRSC, Hillsboro, WI). Software used for the NIRS analysis was Win ISI II (Infrasoft International, State College, PA). The global H statistical test compared the samples with the model and other samples within the database for accurate results.

Statistical Analysis

Results were analyzed using JMP statistical software (JMP Pro 12, SAS Institute, Cary, NC). Significance threshold was set at $P < 0.05$. Nutritive value and forage mass data was checked for normal distribution and did not pass the Shapiro-Wilk Test of goodness-of-fit. The data passed a goodness-of-fit test for LogNormal distribution and was transformed for analysis, but will be reported using initial values. Within single sample dates, forage types were compared using a one-way ANOVA with means separation calculated using Tukey's Honestly Significant Difference. A full factorial least-squares regression analysis determined the impact of forage type and date on forage values (forage mass, CP, NDF, ADF, dNDF, IVTDMD) across a season, within, and between years. Significant variation in the regression model due to sampling date for a forage type indicated a rate of change significantly different from zero.

Interaction between date and forage type indicated significantly different rates of change between two forage types for a given variable during the study period.

Results

Environmental Conditions

The fall of 2015 had higher mean temperature and precipitation than the 30 year mean. During the 2016 season, temperatures were higher than average and the highest average recorded in the previous 10 years (Figure 1.1). This was accompanied by a drought conditions from August to late November. This impacted the 2016 fall stockpiling period. The 2017 spring and summer had average precipitation levels and higher than average GDD accumulation (highest in 10 years during 3 out of 7 sampled months; Figure 1.1).

Forage responses to stockpiling

Forage mass was greater for SG during stockpiling compared to BBIG and TF (Table 1.2). Crude protein was greater in TF paddocks compared to SG throughout the stockpiling period, with a negative trend in BBIG and SG indicating nitrogen translocation during fall senescence. Tall fescue NDF was lower than BBIG and SG when pooled across all stockpiling dates. A linear trend upward occurred in NDF in BBIG and SG, with a greater NDF increase in BBIG (Table 1.2). Lignin content was greater in TF and SG compared to BBIG when stockpiling began, but an increase in BBIG and SG eventually resulted in greater lignin content in SG and no difference between BBIG and TF by the end of stockpiling (Table 1.2). The IVTDMD was greater in BBIG than SG at the beginning of stockpiling, but for both NWSG there was a decrease during fall stockpiling, resulting in greater IVTDMD in TF compared to

BBIG and SG (Table 1.2). The dNDF content of all forage types decreased during the stockpiling season, with BBIG maintaining greater dNDF than TF and SG at three of four sampling dates (Table 1.3).

Forage responses to Winter Grazing

During winter grazing, forage mass was greater in SG than BBIG for three sampling dates in 2017 (Table 1.3). Forage mass loss (significant negative slope) occurred during 2016 for BBIG and TF. The CP content was greater in TF than SG during all sampling dates and TF CP was greater than BBIG in all sampling dates except March 3, 2017 (Table 1.3). A greater NDF was found in BBIG and SG compared to TF (Table 1.3). A small upward trend was found in NDF in both BBIG and SG during the 2017 grazing period and a slightly negative trend was found for SG in the 2016 grazing season (Table 1.3). The lignin content of SG was greater than TF and BBIG at sampling dates except March 3, 2017. The IVTDMD content was lower in SG compared to TF at all sampling dates, with BBIG samples containing intermediate and frequently not different from either TF or SG (Table 1.2) during both years. The dNDF content of BBIG was greater than SG during all winter sampling dates in both years, with TF lower than BBIG on March 8, 2016 and all 2017 dates except March 31 (Table 1.2).

Models of forage values that combined the 2016 and 2017 grazing season did not detect forage type effects, except for a relationship between forage mass loss and sampling date during the grazing season in BBIG ($-294\text{kg ha}^{-1}\text{ week}^{-1}$; $P < 0.0001$) and TF ($-26\text{ kg ha}^{-1}\text{ week}^{-1}$; $P < 0.0001$).

Leaf samples from fully dormant warm-season grasses gathered on February 3, 2017 indicated that leaf matter was greater in CP, but mixed or insignificant results were found for

other nutritive value variables (Table 1.4). Contrary to bulk sample results, SG leaf nutritive value measurements were consistently equivalent to BBIG except for CP, where SG was greater than BBIG (Table 1.4).

Discussion

The dissimilar nutritive and forage mass results between the two winter grazing seasons may be due to the 2016 late summer drought resulting in different forage quantity and quality at the beginning of stockpiling (Figure 1.1). Initial winter grazing forage mass during 2017 was approximately half the initial forage mass during 2016 (Table 1.3; January 27, 2016 sample). This impact was more apparent in BBIG and TF, compared to SG, which lost a smaller proportion of forage mass due to drought. The late summer 2016 drought also resulted in uncharacteristic forage loss within both TF and BBIG pastures.

Tall fescue stockpiling nutritive values were consistent with prior results (Fribourg and Bell 1984; Kallenbach et al., 2003; Hickman 2013; Shireman 2015;). In the current study, stockpiling initiation had increased CP and decreased NDF content in TF than those observed by Hickman (2013) at a similar date, but within the range of expectations. The greater than expected 2016 TF stockpiling mass was influenced by heavy warm-season weed pressure during fall stockpiling, which also diluted fall nutritive values. The lower TF CP results during the 2016 grazing season was similar to those found by Fribourg and Bell (1984). The TF NDF content was intermediate of previously observed values Shireman (2015) and Fribourg and Bell (1984). The last month of stockpiling results indicate a high degree of TF resiliency. The 2016 drought ended with a moderate rain event shortly before the November 30, 2016 sampling date. Between that sample and January 4, 2017 TF recovered over 1000kg ha⁻¹ and 40g kg⁻¹ CP. Both 2016 and

2017 winter grazing periods were characterized by only minor forage mass loss for TF (Table 1.2). This was a product of light grazing pressure and warmer than average winters.

The difference in initiation date and fertilization regime explains why the observed stockpiled yields of SG and BBIG are less than half of the only previous NWSG stockpiling observation in the region (Hickman 2013). Surprisingly, no linear relationship was found over time to indicate forage accumulation in BBIG or SG during the stockpiling period (Table 1.2). This was driven by a low rate of growth by BBIG and loss of mass through senescence and leaf shatter during latter stockpiling months. The fall senescence of BBIG and SG, as expected, reduced CP along with an increase in NDF and lignin and a decreased of IVTDMD and dNDF, which are all attributed to increased plant maturity. During the winter grazing period, only BBIG and TF in 2017 had forage loss during the 4 months of grazing pressure (Table 1.3). This was accompanied by a lack of further degradation in any of the nutritive values. The NWSG dormant forage values are comparable to dormant Kansas big bluestem hay tested in an experiment by Del Curto et al., (1990), indicating that further nutritive loss may be unlikely.

Results from nutritive analysis of dormant warm-season leaf sub-samples indicated that livestock that preferentially consume leaf material can obtain marginally improved forage quality, however, these values remained lower than TF (Table 1.4). The nutritive value of leaves, contrary to bulk values, was comparable or superior for SG compared to BBIG.

While not a major focus of this study, dormant NWSG grazing may offer a strategy for reducing cool-season weed pressure, a frequent issue for conservation. Winter grazing may selectively reduce the more nutritious and active cool season species while the native grass species are both dormant and less palatable.

Results supported the conclusion that forage mass will be greater for SG during stockpiling and grazing, but contrary to prior results, BBIG had similar forage mass to TF during winter grazing. Similarly, individual samples indicated BBIG had forage nutritive values intermediate between TF and SG (Table 1.3). One exception was dNDF value, which was greater in BBIG relative to TF for all of 2017 grazing and on January 27, 2016. Further research could assess other high forage mass dormant warm-season species to determine differences in fully dormant nutritional value. Even small improvements in fiber digestibility despite low CP may allow economically viable use of NWSG for winter grazing.

Conclusion

As expected, the study observed a large loss in nutritive value in BBIG and SG due to fall senescence during the stockpiling period (August-January). However, contrary to expectations, further losses were not observed through the winter grazing period (January-April). The lack of nutritive value and forage mass loss indicates that significantly more grazing pressure could be applied to the high biomass NWSG paddocks. While forage nutritive value for BBIG and SG are significantly lower than TF and below thresholds considered necessary to support most classes of livestock, two results indicate avenues for further research: nutritive values of BBIG were occasionally intermediate to SG and TF, indicating variation between dormant NWSG fiber digestibility, despite consistently low CP. Also, the leaf portion of stockpiled NWSG has improved nutritive value relative to bulk samples. Due to the high fall yields of NWSG, available leaf mass in isolation may provide more forage mass for livestock and only marginally lower nutritive value compared to stockpiled tall fescue. By balancing dietary shortages in the

resulting high fiber forage, livestock producers may be able to economically use stockpiled NWSG as winter feed in the Mid-South.

Appendix

Table 1.1: Previous forage mass, crude protein (CP) and neutral detergent fiber (NDF) results from stockpiling or dormant tall fescue, big bluestem, and switchgrass.

Species	Switchgrass	Big Bluestem		Tall Fescue			
Study	Hickman (2013)	Hickman (2013)	Del Curto, et al. [†] (1990)	Hickman (2013)	Kallenbach et al., (2003)	Shireman (2015)	Fribourg and Bell (1984)
Sampling Month	August	August	January	August	December	January	January
Forage Mass (kg ha ⁻¹)	25,000	12,000	N/A	2,800	2,370	1,800	2,000
CP (g kg ⁻¹)	5.5%	5.5%	2.9%	9%	13.3%	9.5%	7%
NDF (g kg ⁻¹)	69%	73%	74%	72%	55%	60%	69%

[†] Del Curto et al., analyzed harvested dormant biomass (IVTDMD48=37.9%; dNDF =35.5%)

Table 1.2: Forage values for tall fescue (TF), a big bluestem/indiangrass mixture (BBIG), and switchgrass (SG) during 2016 fall stockpiling period.

	26-Sep	26-Oct	30-Nov	4-Jan	Slope†
	<u>Forage Mass (kg ha⁻¹)</u>				
TF	4020 ^{ab‡}	3142 ^b	2796 ^b	3790 ^b	
BBIG	3387 ^b	2552 ^b	3158 ^b	4906 ^b	
SG	6909 ^a	9394 ^a	10656 ^a	7200 ^a	
	<u>Crude Protein (g kg⁻¹)</u>				
TF	91.5 ^a	72.3 ^a	76.6 ^a	123.9 ^a	
BBIG	80.6 ^{ab}	38.8 ^{ab}	28.8 ^b	36.0 ^b	-3.1
SG	36.7 ^b	19.7 ^b	17.6 ^b	17.5 ^b	-0.1
	<u>Neutral Detergent Fiber (g kg⁻¹)</u>				
TF	705 ^{ab}	743 ^b	737 ^b	686 ^b	
BBIG	678 ^b	745 ^b	825 ^a	850 ^a	12
SG	788 ^a	844 ^a	866 ^a	885 ^a	7
	<u>Lignin (g kg⁻¹)</u>				
TF	62.1 ^a	56.8 ^b	53.1 ^b	62.6 ^b	
BBIG	43.4 ^b	51.4 ^b	59.1 ^b	67.6 ^b	1.7
SG	73.3 ^a	85.5 ^a	86.4 ^a	98.9 ^a	1.6
	<u>In-vitro Dry Matter Digestibility (48 hours; g kg⁻¹)</u>				
TF	574 ^{ab}	545 ^a	590 ^a	565 ^a	
BBIG	619 ^a	523 ^a	483 ^b	412 ^b	-14
SG	487 ^b	430 ^a	423 ^b	348 ^b	-9
	<u>Neutral Detergent Fiber Digestibility (48 hours; g kg⁻¹)</u>				
TF	350 ^b	354 ^b	357 ^a	296 ^b	-3
BBIG	460 ^a	438 ^a	423 ^a	384 ^a	-5
SG	334 ^b	316 ^b	312 ^a	263 ^b	-5

† Slopes (units week⁻¹) significantly different from zero are reported ($P < 0.05$)

‡ Means within a column without a common letter differ ($P < 0.05$).

Table 1.3: Forage values for tall fescue (TF), a big bluestem/indiangrass mixture (BBIG), and switchgrass (SG) during two winter grazing periods.

2016					2017					
	5-Jan	27-Jan	9-Mar	8-Apr	Slope [†]	4-Jan	3-Feb	3-Mar	31-Mar	Slope [†]
<u>Forage Mass (kg ha⁻¹)</u>										
TF	-	6972 ^{a‡}	4735 ^b	-		3790 ^b	2676 ^c	2864 ^b	1564 ^b	-158
BBIG	-	8110 ^a	5885 ^{ab}	-		4906 ^{ab}	4828 ^b	2920 ^b	2920 ^b	-206
SG	-	9790 ^a	7950 ^a	-		7200 ^a	8124 ^a	4628 ^a	7242 ^a	
<u>Crude Protein (g kg⁻¹)</u>										
TF	86.2 ^a	75.1 ^a	75.6 ^a	71.3 ^a		123.9 ^a	113.5 ^a	56.1 ^a	120.9 ^a	
BBIG	26.6 ^b	31.6 ^b	33.4 ^b	17.0 ^b		36.0 ^b	42.6 ^b	25.6 ^{ab}	44.2 ^b	
SG	18.2 ^b	26.7 ^b	25.5 ^b	11.5 ^b		17.5 ^b	19.4 ^b	19.8 ^b	29.7 ^b	
<u>Neutral Detergent Fiber (g kg⁻¹)</u>										
TF	709 ^b	791 ^b	790 ^b	761 ^b		686 ^b	691 ^c	786 ^b	673 ^b	
BBIG	845 ^a	850 ^a	859 ^a	850 ^a		850 ^a	824 ^b	834 ^{ab}	794 ^a	-4.0
SG	875 ^a	875 ^a	883 ^a	893 ^a	1.0	885 ^a	880 ^a	885 ^a	845 ^a	-3.0
<u>Lignin (g kg⁻¹)</u>										
TF	63.2 ^b	83.0 ^b	81.2 ^a	79.3 ^b		62.6 ^b	65.7 ^b	78.2 ^b	64.4 ^b	
BBIG	76.8 ^a	73.1 ^b	77.8 ^a	80.0 ^b		67.6 ^b	71.6 ^b	69.4 ^b	65.8 ^{ab}	
SG	91.7 ^a	95.1 ^a	95.7 ^a	100.9 ^a	0.06	98.9 ^a	88.7 ^a	98.0 ^a	82.0 ^a	
<u>In-vitro Dry Matter Digestibility (48 hrs; g kg⁻¹)</u>										
TF	527 ^a	428 ^{ab}	423 ^a	469 ^a		565 ^a	523 ^a	427 ^a	546 ^a	
BBIG	413 ^b	431 ^a	396 ^a	416 ^{ab}		401 ^b	394 ^b	396 ^{ab}	435 ^{ab}	
SG	372 ^b	364 ^b	374 ^a	363 ^b		348 ^b	380 ^b	342 ^b	388 ^b	
<u>Neutral Detergent Fiber Digestibility(48 hrs; g kg⁻¹)</u>										
TF	355 ^{ab}	279 ^b	283 ^{ab}	335 ^{ab}		296 ^b	291 ^b	314 ^b	358 ^{ab}	
BBIG	367 ^a	367 ^a	350 ^a	372 ^a		384 ^a	354 ^a	388 ^a	406 ^a	
SG	291 ^b	278 ^b	280 ^b	283 ^b		263 ^b	296 ^b	265 ^b	315 ^b	

[†] Slopes (units week⁻¹) significantly different from zero are reported ($P < 0.05$)

[‡] Means within a column without a common letter differ ($P < 0.05$).

Table 1.4: Whole canopy nutritive values and leaf sub-samples of dormant switchgrass (SG) and a big bluestem/indiangrass mixture (BBIG) taken on February 3rd 2017.

	<i>Bulk</i>		<i>Leaf</i>	
	BBIG	SG	BBIG	SG
Crude Protein(g kg ⁻¹)	31 ^{c‡}	27 ^c	51 ^b	65 ^a
NDF† (g kg ⁻¹)	824 ^b	860 ^a	818 ^b	824 ^b
Lignin (g kg ⁻¹)	69 ^b	89 ^a	67 ^b	62 ^b
IVTDMD (g kg ⁻¹)	401 ^a	368 ^a	410 ^a	431 ^a
dNDF (g kg ⁻¹)	394 ^a	292 ^c	351 ^b	336 ^b

†NDF, Neutral Detergent Fiber; IVTDMD, in-vitro dry matter digestibility (48 hours); dNDF, neutral fiber digestibility.

‡ Letters indicate significant difference within rows between forage type and/or forage component according to two-way t-test ($p < 0.05$; $n=3$).

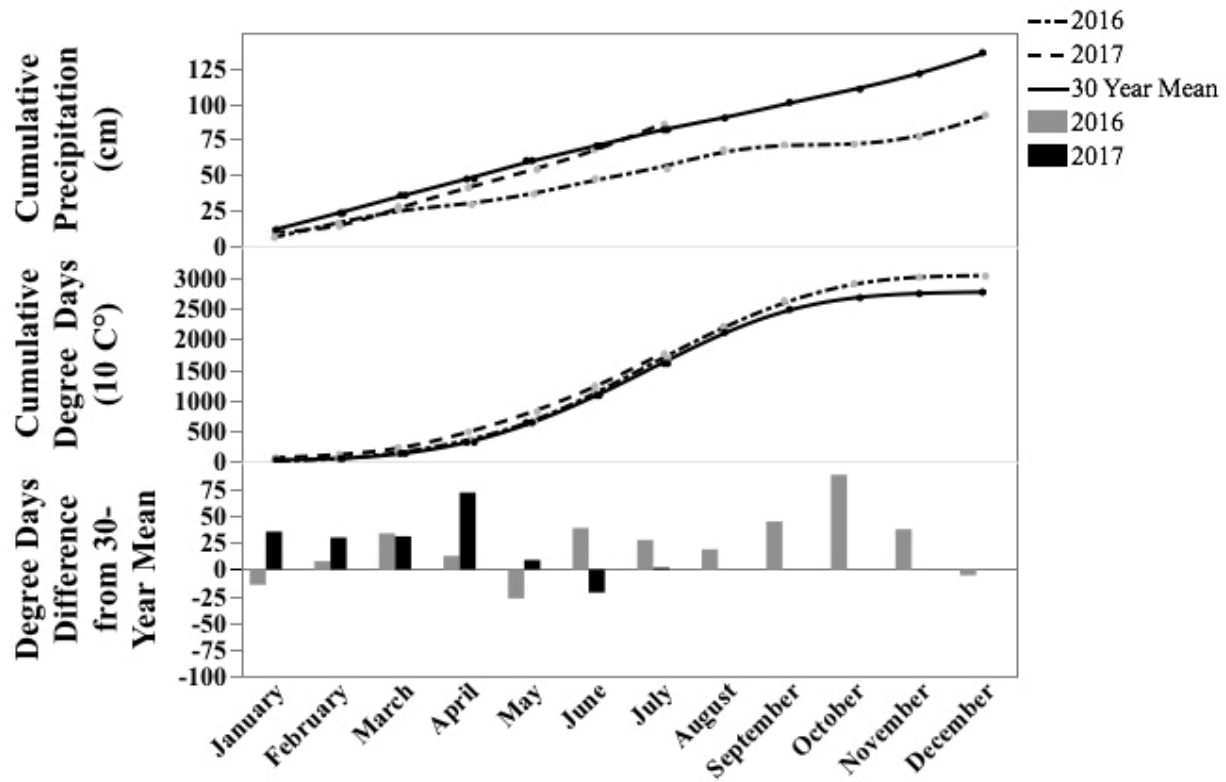


Figure 1.1: Cumulative precipitation (cm), growing degree days (base 10 C°) and monthly difference from 30-year mean growing degree days (base 10 C°).

Chapter 2:
Short-term Variation of Labile Soil Carbon and Nitrogen under
Tall Fescue and Native Grass Forages

Abstract

Labile soil carbon and nitrogen pools can detect short-term changes to carbon and nutrient pools that may correlate with long-term carbon sequestration or soil health improvements. However, the sensitivity of seasonal labile pools may be limited by unexpected seasonal variation. This study provides insight into seasonal cycling within labile carbon and nitrogen pools. To assess fluctuations of labile soil nutrients, this study sampled forage production systems based on forage arrays with contrasting root traits: tall fescue (acquisitive; *Schedonorus phoenix*; TF), switchgrass (conservative; *Panicum virgatum*; SG), and a mixture of big bluestem/indiangrass (intermediate; *Andropogon gerardii*; *Sorghastrum nutans* BBIG). Soil samples across 18 consecutive months were divided into 0-5 cm and 5-15 cm soil depths and analyzed for hot-water extractable carbon (HWE), hot-water extractable nitrogen (HWEN), aromatic content of hot-water extracts (Abs_{254} ; [ultraviolet absorbance at 254nm]), and potassium permanganate oxidizable carbon (POXC). Results were calculated using a repeated measure mixed model to test for forage type effects. A seasonal coefficient of variation was calculated based on monthly means of different labile pools. Labile soil pools (HWE, HWEN) had higher seasonal variation relative to more recalcitrant pools (POXC, Abs_{254}). Models indicated 26% greater HWEN (97.4 mg kg^{-1} ; 77.5 mg kg^{-1}) and 13% greater Abs_{254} (0.66 cm^{-1} ; 0.58 cm^{-1}) in TF and BBIG relative to SG. The HWEN results are consistent with increased soil microbial activity associated with acquisitive species. The unexpectedly low Abs_{254} in SG may indicate increased aromatic degradation. These results document interesting root trait impacts on nutrient cycling and highlight the importance of seasonal variation when attempting to measure sensitive soil indicators.

Introduction

Many agricultural soils of the Southeast United States are heavily weathered Ultisols due to geologic history, warm and humid conditions, and historical mismanagement (Bruce et al., 1995; Triplett and Dick, 2008; Franzluebbers, 2010). These highly developed soils provide sub-optimal conditions for plant growth due to high erodibility and poor soil structure, conditions that can be alleviated through increased soil organic matter (SOM) content (Lal, 2006). Increased SOM also offsets the atmospheric buildup of fossil fuel combustion (IPCC, 2014). Franzluebbers and Follett (2005) compared the sequestration potential of multiple regions and agricultural techniques and found that converting cropland to grassland in the southeast results in the highest potential soil carbon accumulation. By optimizing carbon allocation in forage production systems, researchers and farmers can improve agricultural productivity and social sustainability through SOM sequestration.

The dominant forage species in the mid-South is tall fescue (*Schedonorus phoenix*; TF). TF currently covers over 15 million hectares, an area equivalent to 80% of the pasture and hay land in the Fescue Belt (Locke and Rogers, 2017; USDA 2017). Tall fescue is one of the only C₃ pasture grasses tolerant to the climate and soil conditions of the south (Ball et al., 2007; Fort et al., 2013). Tall fescue grows rapidly within a wide temperature range (7°C-30°C), resulting in high productivity and animal performance for large portions of the year. Despite relatively strong summer performance, TF growth slows substantially above 30°C and it accumulates an endophyte toxin (Read and Camp, 1986). Therefore, during hot or dry periods, such as mid-summer or during drought periods, costly forage shortages can occur for farmers without alternative forage species.

Native warm season grasses (NWSG) are a group of alternative forage species that may provide cost-effective summer forage in the Mid-South. This study will assess three commonly used native grasses: big bluestem (*Andropogon gerardii*; BB), indiangrass (*Sorghastrum nutans*; IG), and switchgrass (*Panicum virgatum*; SG). These native grasses utilize the C₄ photosynthetic pathway, allowing for improved water use efficiency and nitrogen use efficiency at high temperature. However, C₄ photosynthesis improves efficiency through efficient use of RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) concentrated inside bundle sheath cells. Since CO₂ transport into bundle cells decreases at low temperature, C₄ species have a major disadvantage at low temperatures (Kubien et al., 2003; Bilska and Sowiński, 2010). Many native C₄ grasses begin to senesce at overnight temperatures below 15 C°, limiting the window of forage utility (Teeri and Stowe, 1976).

Because of differing plant traits (growth habit, photosynthetic pathway, root systems), TF and the NWSG species can be characterized along a spectrum of plant strategies (Reich 2014; Roumet et al., 2016). Within the NWSG, SG has highly conservative traits, BB and IG have intermediate traits and C₃ grasses such as TF have acquisitive traits (Craine et al., 2002; Fort et al., 2013). Conservative species, generally adapted to harsh or low-resource environments, maintain a larger, low activity, and coarse (high C:N) root systems (Tjoelker et al., 2005; Mahaney et al., 2008). Acquisitive species rely on root systems with smaller, short lived, high activity and high quality (low C:N) roots. These contrasting plant strategies as well as contrasting temperature preferences of NWSG species and TF may result in different rates of soil carbon processing and sequestration.

Research on SOM is limited due to its slow rate of change. Prior research has focused on short-term shifts in labile nutrient pools that may indicate future shifts in SOM. The labile

carbon pool also stimulates microbial activity and therefore nutrient availability to plants, indicating applied agronomic importance (Franzluebbers, 2016). Labile soil carbon has a dual nature, since it stimulates microbial degradation of soil organic matter, which respire a large portion of carbon while also stabilizing carbon byproducts (Schimel and Schaeffer, 2012; Bradford and Crowther, 2013; Bradford et al., 2013). Labile carbon pools represent a methodological challenge, since they are inherently variable and the dominant variables for labile nutrient processing (temperature, moisture, e.g.) are growing season dependent. Therefore, sampling across multiple seasons may be necessary to more thoroughly evaluate contrasting root trait effects on labile carbon.

Hot-water extractable carbon (HWE) and nitrogen (HWE-N) and permanganate oxidizable carbon (POXC) were chosen for methods in this study due to their proposed use as measurements of “soil health”. Hot-water extractable carbon and nitrogen represent highly labile nutrients, primarily microbial and microbially accessible carbohydrates and proteins (Balarin et al., 2009; Balarin and Johnson, 2013). Zhao et al., (2013) and Cepáková et al., (2016) found significant seasonal variation in HWE pools across seasonal sampling in forest systems. Uchida (2012) found similar responses in agricultural soils indicating differences between cropping systems and a fallow period. The aromatic content of hot-water extractable carbon impacts microbial processing (Kalbitz et al., 2003; Marschner and Kalbitz, 2003) and can be compared using the UV absorbance at 254nm (optical units; cm^{-1} ; Weishaar et al., 2003). Soluble aromatic compounds are byproducts of the degradation of complex organic compounds such as lignin and are resistant to microbial degradation. In incubation studies, the proportion of aromatic compounds increases over time, indicating preferential degradation of more labile carbon

(Kalbitz et al., 2003; Toosi et al., 2012). In the field, seasonal increases in aromatic carbon compounds may indicate a shortage of new labile carbon inputs.

The potassium permanganate oxidizable carbon (POXC) method is used as an estimate of active or microbially accessible carbon (Culman et al., 2012; Hurisso et al., 2016; Wang et al., 2017). This oxidizable portion correlates well with other “soil health” variables (Morrow et al., 2016; Fine et al., 2017). However, the exact nature of this carbon pool is debated. The method does not react with soil compounds considered labile: carbohydrates, sugars, or amino acids (Tirol-Padre and Ladha 2004). A recent analysis indicates that POXC preferentially reacts with a microbially stabilized carbon pool, rather than fresh plant inputs (Suárez-Abelenda et al., 2014). However, POXC can still be degraded when soils are deprived of fresh inputs (Xu, et al., 2012).

This study will monitor the variation in labile nutrients due to forage root traits. Based on prior research, several hypotheses can be proposed: Due to its acquisitive plant strategy traits, tall fescue would maintain greater labile soil carbon and nitrogen pools. The NWSG species will support greater labile nutrients during mid-summer, while tall fescue will have greater soil activity during winter due to seasonal differences in plant activity. The deeper, coarser rooting strategy of the NWSG species will also result in higher extract HWE C:N, higher proportion of nutrients at 5-15cm depth, and higher aromatic carbon content.

Materials and Methods

Site, history, and management

The experiment was conducted at the Middle Tennessee AgResearch and Education Center, in Spring Hill, TN. The soil is Maury silt loam (Typic Paleudalf). The sampling array

consisted of 15 randomly assigned 1.2-hectare paddocks to one of three treatments: tall fescue (cv. KY-31), switchgrass (cv. Alamo), and a 1:1 mixture of indiangrass (cv. Rumsey) and big bluestem (cv. OZ-70), with five replications. The paddock array was established October 2007 and was used for a NWSG and red clover experiment until 2012 (Keyser et al., 2016). Three cycles of stockpiled winter grazing were carried out prior to the initiation of sampling, for a total of five years (McFarlane et al., 2017). The management schedule was the following: grazing from January-April, regrowth from April-June, management through either haying or grazing during June-July, and mowing in late July to initiate regrowth for winter grazing (beginning the following January).

Winter grazing was carried out on all paddocks by 2-3 Angus heifers per paddock (determined by forage availability) from January until April. During summer, paddocks were managed during June for either hay or a put-and-take grazing system based on forage availability. Paddocks that were managed for hay were fertilized with $67 \text{ kg ha}^{-1} \text{ N}$ in June of each year and allowed to accumulate. Grazed paddocks were not fertilized due to manure inputs. During the 2016 June grazing period, grazing removed approximately 30% TF, 40% SG, and 45% BBIG forage biomass relative to paddocks grown for hay. All paddocks were mowed (biomass removed) during July or early August (August 14, 2016) to initiate fall stockpiling (20-cm residual height for NWSG, 10-cm for TF).

Sample Collection and Analysis

Soil samples were collected during 2016 on January 27, March 9, May 10, June 6, June 27, July 27, August 24, September 26, October 26, and November 30. In 2017, samples were taken on January 4, February 3, March 3, March 31, May 3, June 14, and July 11. Eight 12-mm

diameter soil cores (0-5cm, 5-15cm depth) were taken from randomly generated points in each paddock and pooled for analysis.

Routine soil sampling on February 3, 2017 indicated no micronutrient deficiencies and no significant differences between treatments. Soil pH had a mean of 5.96 (S.E.=0.19). Mehlich 1 extractions indicated mean phosphorus of 470 kg ha⁻¹ (S.E.=361), mean potassium 192 kg ha⁻¹ (S.E.=48), mean calcium 3780 kg ha⁻¹ (S.E.=1330), and mean magnesium 257 kg ha⁻¹ (S.E.=29). The phosphorus variability is due to three outlier paddocks (1157 kg ha⁻¹, 1129 kg ha⁻¹, 930 kg ha⁻¹) and are attributable to high phosphorus content parent material and shallow soils. This high level of phosphorus did not result in any significant deviations in plant growth or soil variables.

Soil cores were oven dried at 60°C up to constant weight, pulverized, then passed through a 2-mm sieve to remove coarse material. Hot-water extraction procedures were carried out as a single hot-water extraction, omitting an initial cold water extraction. Otherwise, methods followed Ghani et. al. (2003), with a shortened extraction period to focus on nutrient extraction from microbial biomass (Chantigny et al., 2014): 10g of soil (dry wt.) were incubated for 4 hours at 80°C in 0.1 L water. The sample extracts were immediately filtered and refrigerated for analysis. The resulting extract was analyzed for total carbon and total nitrogen content using Shimadzu TOC-5050 analyzer.

Hot-water extract UV-absorbance at 254nm was quantified using a Genesys 6 UV-Vis spectrophotometer (Thermo Scientific, Rochester, NY) with a 1-cm path-length cell to determine aromaticity of extracted carbon. The ratio between the absorbance at 254 nm and total HWEC is an indicator of carbon aromaticity referred to as specific absorbance (SUVA, L mg⁻¹ cm⁻¹; Weishaar et al., 2003; Fernández-Romero et al., 2016). Since HWEC is a labile fraction and its aromatic content is expected to be recalcitrant, short-term SUVA ratios are primarily controlled

by the more variable HWEC value. Therefore, the UV absorbance at 254nm may be a more useful variable since it provides an assessment of the overall extractable aromatic content rather than the aromatic content per carbon unit ($Abs_{254}; cm^{-1}$).

Potassium permanganate oxidizable carbon (POXC) followed methods proposed by Weil et al., (2003). Soil was reacted with $KMnO_4$ for 2 minutes on a shaker, immediately centrifuged (5 min at 3000rpm), and a diluted portion of the supernatant was analyzed for absorbance at 550nm (Powerwave XS, BioTek, Winooski, VT). POXC value was then calculated using the following equation:

$$= [\text{initial concentration } KMnO_4 \text{ (mol } L^{-1}) - (b \times \text{absorbance at 550nm})] \\ \times (9,000 \text{ mg } C \text{ mol}^{-1}) \times (\text{Volume of reactant/weight of soil (kg)})$$

Where b is the slope of a standard curve.

Data Analysis

Results were analyzed using JMP Pro 14 statistical software (SAS Institute, Cary, NC). The results for HWEC, HWEN, and HWE C:N did not pass Shapiro-Wilk test for normal distribution. The HWEC, HWEN, and HWE C:N results matched LogNormal distribution and were converted for analysis but will be reported in original units.

Comparisons were performed using a repeated measures mixed model ANOVA where individual paddocks were treated as subjects with repeated measures across the 18 sampling dates. The model included with a first order auto-regression structure and the fixed effect was forage type. Forage type, season, and the forage type and season interactions were used when evaluating the impact of cool and warm-season samples. Means separation was assessed using Tukey's honestly significant difference test ($P < 0.05$). Warm season months included samples

from late June-September, while cool season months were defined as April-May and October-November. Since shallow (0-5cm) and sub-soil (5-15cm) correlations and trends were expected to differ, the two soil horizons were run in separate models. Ratio of nutrients at depth (nutrients at 0-5cm/nutrients 5-15cm) were created and analyzed using the same repeated measures model.

To assess overall seasonal differences in variation, a coefficient of variation based on monthly averages were created and compared between forage types using a z-test.

Results

Environmental Conditions

During the 2016 season, temperatures were higher than average and the highest average recorded in the previous 10 years (Figure 2.1). A drought occurred from August to late November and prevented 5-15cm depth sampling on September 26, 2016. The 2017 spring and summer had average precipitation levels and higher than average degree day (base 10°C) accumulation (highest in 10 years during 3 out of 7 sampled months).

Soil Variables

Differences were found between forage types in HWEN and Abs₂₅₄ at 0-5cm (Table 2.1). The acquisitive species, tall fescue maintained higher HWEN than the most conservative species, SG (97.4 mg kg⁻¹; 77.5 mg kg⁻¹; Table 2.3). The intermediate, BBIG, was not different from either. Due to strong correlation between HWEN and HWEC, HWEC was similarly related, but did not reach the significance threshold ($P = 0.051$; SG: 764 mg kg⁻¹; TF: 627 mg kg⁻¹). Both TF and BBIG had higher absorbance relative to SG at 0-5 cm depth (Table 2.3). In addition, differences in nutrient distribution (depth ratio) were found between forage types for HWEC and

Abs₂₅₄ (Table 2.3). For both HWEC and Abs₂₅₄, TF had a higher proportion of nutrients at 0-5cm relative to 5-15cm when compared to SG, no differences occurred between BBIG and other species (Table 2.3). No significant forage type effects occurred in models of HWE C:N, SUVA, or POXC at either soil horizon (Table 2.1).

No evidence of differential seasonal responses occurred between forage types in any variables (Table 2.1).

Overall seasonal variation, estimated using the coefficients of variation of monthly means by forage type, indicated differences due to sampling method, depth, and forage type (Table 2.4). Variables related to hot-water extraction (including SUVA) had the greatest seasonal variation, while variables expected to be more recalcitrant, Abs₂₅₄ and POXC, were less variable (HWEC=HWEN>HWE C:N>Abs₂₅₄>POXC; Table 2.4). At the 0-5cm horizon, POXC CV for TF was greater compared to BBIG (p=0.004), with SG not different from either (Table 2.4). Absorption at 254nm coefficient of variation at the 0-5cm horizon was greater for SG compared to TF, with BBIG not different from either (p=0.032; Table 2.4). No significant differences were found among species-level coefficients of variation at the 5-15cm horizon (Table 2.4).

Discussion

The results of this experiment indicate minimal support for the research hypotheses, since variation within the soil pools of interest were driven by environmental conditions, rather than forage type or aboveground management (Figures 2.2-2.5). Seasonal differences between forage types were observed within individual sampling dates, but these were often transitory and not individually meaningful.

Hot-water extractable nutrients results were comparable to other studies on grassland soils, with the caveat that exact extraction methods vary (Ghani et al., 2003; Bu et al., 2011; Fernández-Romero et al., 2016). Extractable nutrients were lower in quantity and lower in C:N ratio relative to extracts higher latitude agricultural soils (Gregorich et al., 2003; Ghani et al., 2003; Fernández-Romero et al., 2016). As expected HWEN was greater at the 0-5cm horizon in the acquisitive species, TF, relative to the species with the most conservative strategy, SG (Table 2.3). This supports the hypothesis and aligns with previous research indicating greater activity related to acquisitive species, associated with increased nitrogen cycling (Personeni and Loiseau, 2005; Mahaney et al., 2008; Fort et al., 2013). There is a strong relationship between HWEC, HWEN, and total SOM accumulation (Sparling et al., 1998; Ghani et al., 2003; Spohn and Giani, 2011; Fan et al., 2013), therefore the current evidence indicates that TF provides more short-term gain in SOM relative to SG at the 0-5cm soil horizon.

A major caveat to these findings is the difference between depth ratios of TF and SG (Table 2.3). For both HWEC and Abs₂₅₄, the acquisitive species, TF, has higher concentrations in the upper horizon (0-5 cm) relative to SG. Xu et al., (2010) reported preferential deep-rooting behavior is a strategy in SG, which may result in low shallow-soil investment even when planted in monoculture. Since microbial activity decreases with soil depth, a greater proportional investment of resources by SG at depth could result in long term SOM accumulation (de Graaff et al., 2014).

Absorption at 254nm was lower than other experiments (Redl, 1990; Bu et al. 2011), however many of these experiments documented more temperate regions with greater organic matter soil than our study (Table 2.3). The range of SUVA and HWEC found in the current study occurred below United Kingdom grassland systems and cereal crops, but greater than

perennial Spanish olive orchards (Fernández-Romero et al., 2016) indicating that climate may be a major control on total extractable carbon and the aromatic content. High temperatures will generally lower the energy threshold for decomposition of recalcitrant carbon (Conant et al., 2011).

Absorption at 254nm indicated higher overall aromatic carbon content in TF and BBIG soil samples relative to SG (Table 2.3). This is counter to the hypothesis that TF would have lower aromatic content due to low C:N roots. Previous studies have reported approximately 6% root lignin content in TF (Creme et al., 2017), while switchgrass has approximately 10% root lignin (Johnson et al., 2007; DeBruyn et al., 2017). White et al., (2011) reported comparable lignin contents between TF and SG, but also indicated a root C:N of 100 in SG compared to a root C:N of 50 in TF. The depressed level of aromatics in SG soil relative to both TF and BBIG despite SG having the coarsest root structure is unexpected. This could be due to two extremes: SG supports a lower activity soil community that degrades recalcitrant SG roots slowly or alternatively, SG supports a community that is nutrient scarce and degrades recalcitrant aromatic byproducts at an increased rate. Since soil respiration rates are generally elevated in SG (Tufekcioglu et al., 2003; Al-Kaisi and Grote, 2007;), the latter explanation is more likely. It is unlikely that TF and BBIG increase aromatic content in soils through high activity, due to the lower SUVA level within this study compared to others (Redl et al., 1990; Bu et al., 2011; Balaria and Johnson, 2013). Therefore, it is likely that SG related microbial communities degrade recalcitrant aromatic carbon at an increased rate due to nutrient scarcity. Reliance on metabolism of recalcitrant carbon reduces microbial carbon use efficiency (Manzoni et al., 2010).

Despite the abundant seasonal variation and contrasting plant activity levels, no species-related seasonal differences were found (Table 2.1). This includes SG and BBIG remaining dormant throughout mild winters while TF was active and the 2016 late summer drought, which severely stressed TF and eventually BBIG while SG was resilient. Post-hoc tests for species variation due to winter or drought did not result in any meaningful trends. An unexpected decrease in POXC under TF occurred on July 27, 2016 (Figure 2.2). This was the beginning of the late summer drought and led to a significantly lower POXC throughout the drought period. However, the largest decrease occurred at the onset of drought and the difference between TF and other species decreased as the drought became more intense. Therefore, it is difficult to connect the depressed POXC to the drought. Within the short-term, variation between plant input timing and quality may be obscured by factors controlled by soil decomposers.

The high POXC CV of TF compared to BBIG at the 0-5 cm horizon provides evidence that TF root traits result in POXC processing during the growing season (Table 2.4), a trend that can be observed in Figure 2.2, where TF has highest POXC values during spring followed by a decrease during the growing season and is significantly below SG for one month during fall before increasing during winter. Despite low CV in POXC samples, no species-level trends were found between species due to the differential species variation. Similarly, the high CV of Abs₂₅₄ in SG relative to TF provides support for the previous observation that the SG soil community is more likely to degrade aromatics (Table 2.4).

The mean POXC at 0-5cm (449 mg kg⁻¹) was lower than a crop-pasture study in the Mid-South (814 mg kg⁻¹), as well as a Pennsylvania dairy (552 mg kg⁻¹), but was greater than a Kansas prairie soil set (378 mg kg⁻¹; Culman et al., 2012). An experiment of a Kansas prairie reported, when controlling for different sampling depths, greater mean POXC compared to the

current study (Xu et al., 2012). The low value of POXC within this study could be a result of high microbial processing due to low latitude and relatively high moisture (Wang et al., 2017; Awale et al., 2017). Overall, the lack of differentiation between forage types after 8 years of establishment indicates that POXC methodology is unlikely to be a reliable predictor of soil nutrient cycling improvements in pasture systems.

The HWE C:N values (8.0, 0-5cm; 8.8, 5-15cm) were similar to other pasture studies (Ghani et al., 2010; Stevenson et al., 2016) and is similar to ratios expected for soil microbial communities (Cleveland and Liptzin, 2007). Interestingly, HWE C:N had a lower seasonal CV relative to its components (HWEC; HWEN) indicating that it may be a more reliable soil variable and that the quantity, rather than composition of hot-water extracts change.

Conclusion

Measurements of labile nutrients are sensitive to seasonality and microbial activity. This experiment indicates a minor relationship between labile pools and plant traits despite major physiological differences between forage species. Evidence of increased nitrogen cycling was found in TF relative to SG, consistent with prior evidence and expectations (Fort et al., 2013). Higher seasonal variation and lower quantities of Abs₂₅₄ was documented in SG for relative to TF, providing evidence for increased aromatic decomposition. Higher seasonal variation occurred in POXC for TF relative to SG, indicating potentially increased use or degradability of the pool by soil communities associated with TF. The dominance of seasonal variation over plant species effects in this forage production system highlights the importance of short term variation due to microbial activity. Environmental variation could limit the application of labile nutrient pools to metrics of soil health. Further research on seasonal interactions between labile

soil pools and environmental impacts could improve the accuracy of assessments using labile nutrients.

Appendix

Table 2.1: ANOVA F-value results for labile soil pools under TF, BBIG, or SG. Hot-water extractable carbon (HWEC), hot water extractable nitrogen (HWEN), the ratio of HWEC to HWEN (HWE C:N), UV absorbance of hot water extract at 254nm (Abs₂₅₄).

	HWEC (mg kg ⁻¹)	HWEN (mg kg ⁻¹)	HWEC: HWEN	POXC (mg kg ⁻¹)	Abs ₂₅₄ (cm ⁻¹)	SUVA (L mg ⁻¹ cm ⁻¹)
0-5cm	2.58	3.79*†	0.05	1.49	13.95***	0.05
5-15cm	0.37	1.10	1.18	2.06	0.83	0.29
Depth Ratio‡	3.73*	2.80	0.97	0.51	3.94*	0.14
Season*Forage Type						
0-5cm	1.00	0.69	0.64	0.56	0.56	1.01
5-15cm	1.53	1.13	0.90	0.55	0.69	1.52

† Symbols *, and *** refer to significant effects at $P < 0.05$, and $P < 0.001$, respectively.

‡Depth ratio is between the 0-5cm and 5-15cm value.

Table 2.2: Least squared means estimates for model variables: hot water extractable carbon (HWEC), hot-water extractable nitrogen (HWEN), the ratio of HWEC and HWEN (HWE C:N), POXC, absorbance at 254nm and SUVA.

	HWEC (mg kg ⁻¹)	HWEN (mg kg ⁻¹)	HWE C:N	POXC (mg kg ⁻¹)	Abs ₂₅₄ (cm ⁻¹)	SUVA (L mg ⁻¹ cm ⁻¹)
0-5cm	702	87.5*†	8.00	449	0.633*	1.01
5-15cm	263	31.6	8.80	229	0.282	1.18

†* indicate significant underlying variation due to forage type ($P < 0.05$; Table 2.4).

Table 2.3: Least squared means estimates for significant model variables: hot water extractable carbon (HWEC), hot-water extractable nitrogen (HWEN) and absorbance at 254nm.

	0-5 cm		Depth Ratio‡	
	HWEN (mg kg ⁻¹)	Abs ₂₅₄ (cm ⁻¹)	HWEC (mg kg ⁻¹)	Abs ₂₅₄ (cm ⁻¹)
Tall Fescue	97.4 ^{a†}	0.66 ^a	3.00 ^a	2.54 ^a
Big Bluestem/ Indiangrass	87.7 ^{ab}	0.66 ^a	2.81 ^{ab}	2.38 ^{ab}
Switchgrass	77.5 ^b	0.58 ^b	2.58 ^b	2.18 ^b

† Letters indicate significant differences according to a Tukey's honestly significant difference test ($P < 0.05$).

‡Depth ratio is between the 0-5cm and 5-15cm value.

Table 2.4: Coefficients of variation between monthly means of sampling methods: hot-water extractable carbon (HWE C), hot water extractable nitrogen (HWEN), the ratio of HWE C to HWEN (HWE C:N), UV absorbance of hot water extract at 254nm (Abs₂₅₄), specific absorbance (SUVA), and permanganate oxidizable carbon (POXC).

	Forage Type	HWE C (mg kg ⁻¹)	HWEN (mg kg ⁻¹)	SUVA (L mg ⁻¹ cm ⁻¹)	HWE C:N	Abs ₂₅₄ (cm ⁻¹)	POXC (mg kg ⁻¹)
0-5 cm	Big Bluestem/ Indiangrass	31.7	27.4	26.4	22.8	9.27 ^{ab†}	5.97 ^b
	Tall Fescue	30.4	29.8	26.6	18.9	7.72 ^b	9.37 ^a
	Switchgrass	32.6	28.9	30	19.2	10.4 ^a	7.21 ^{ab}
5-15 cm	Big Bluestem/ Indiangrass	29.3	36.6	25.5	14	15.5	13.9
	Tall Fescue	27.8	35	32.6	14.8	14.2	14.6
	Switchgrass	29.1	33.8	27	14.8	13.8	12.1
Sampling Method‡		A	A	A	B*	C*	D*

† Lowercase letters indicate significant differences between forage types within soil horizon according to z-test (p<0.05).

‡ Uppercase letters indicate significant differences between CV of sampling methods (p<0.05). Asterisk indicate significantly different CV values between soil horizons.

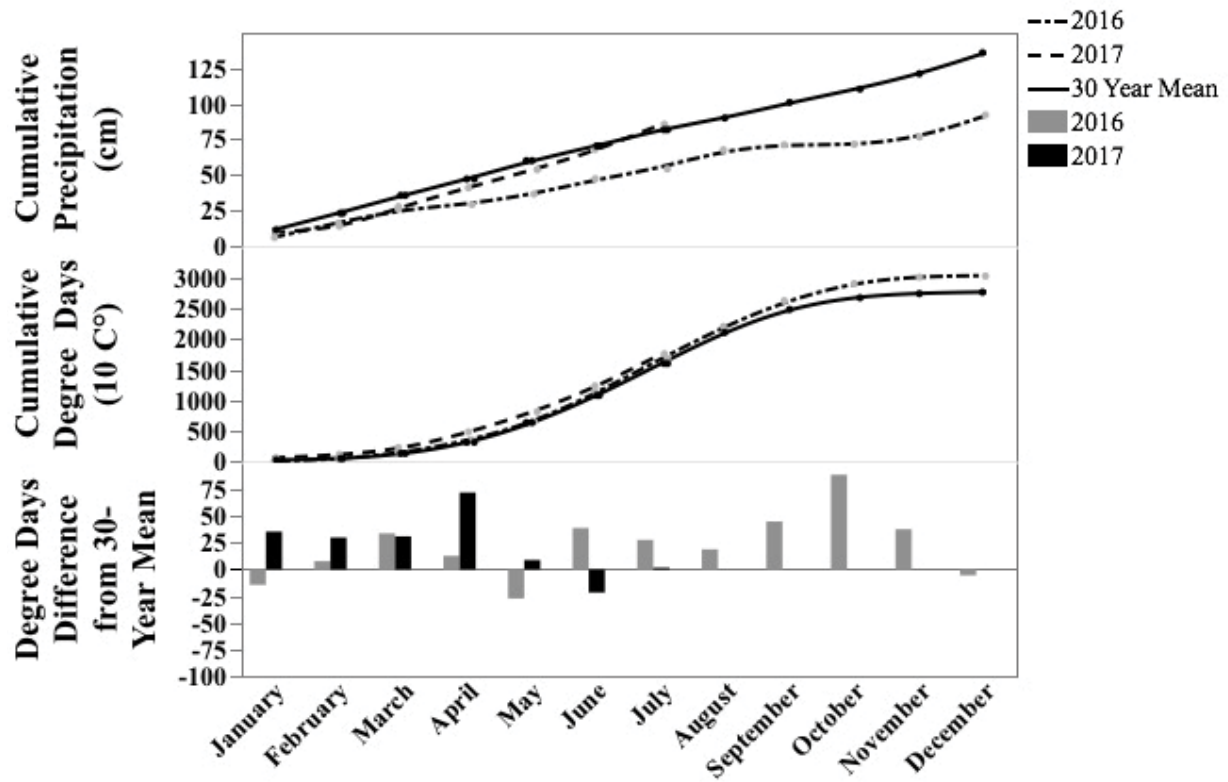


Figure 2.1: Cumulative precipitation (cm), growing degree days (base 10 C°) and monthly difference from 30-year mean growing degree days (base 10 C°).

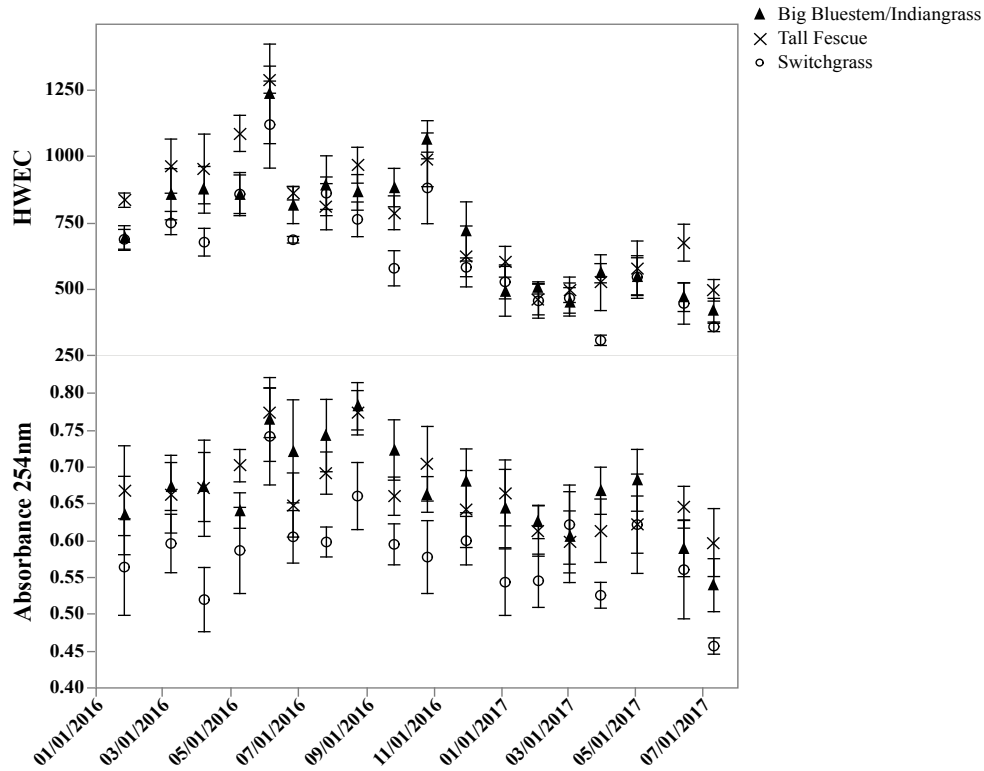


Figure 2.2: Hot-water extractable carbon (mg kg^{-1}) and UV absorbance at 254nm (cm^{-1}) across 18 sampling months and three forage types (big bluestem/indiangrass mixture, tall fescue, and switchgrass) at the 0-5 cm soil horizon.

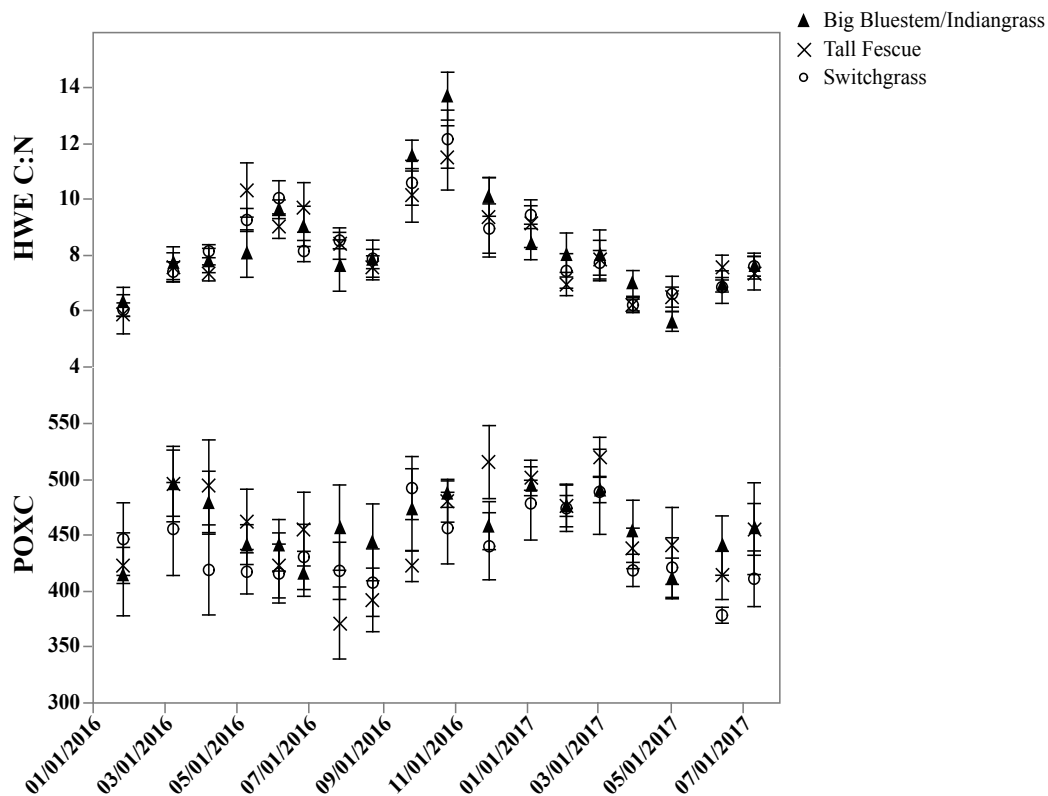


Figure 2.3: The ratio of hot-water extractable carbon:nitrogen (mg kg^{-1}) and permanganate oxidizable carbon (mg kg^{-1}) across 18 sampling months and three forage types (big bluestem/indiangrass mixture, tall fescue, and switchgrass) at the 0-5 cm soil horizon.

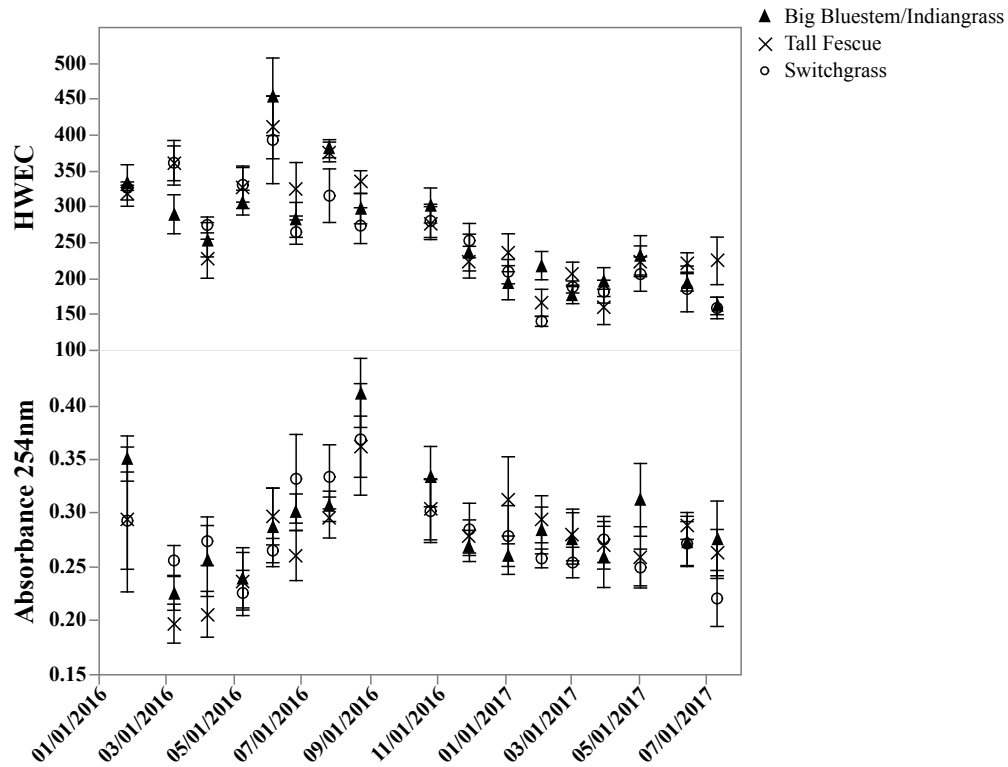


Figure 2.4: Hot-water extractable carbon (mg kg^{-1}) and UV absorbance at 254nm (cm^{-1}) across 18 sampling months and three forage types (big bluestem/indiangrass mixture, tall fescue, and switchgrass) at the 5-15 cm soil horizon.

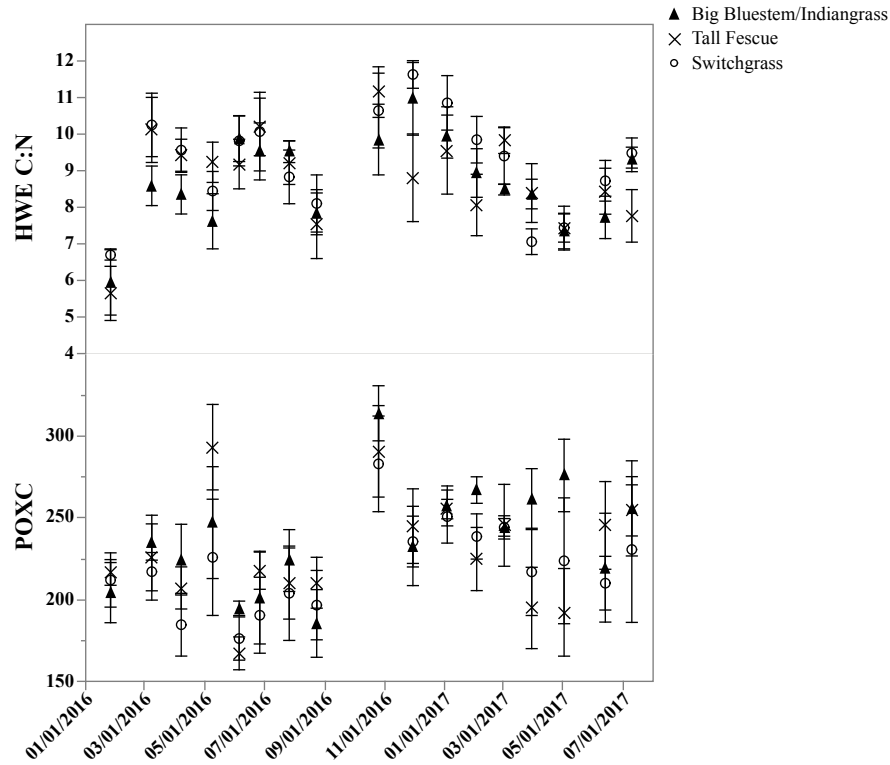


Figure 2.5: The ratio of hot-water extractable carbon:nitrogen (mg kg^{-1}) and permanagnate oxidizable carbon (mg kg^{-1}) across 18 sampling months and three forage types (big bluestem/indiangrass mixture, tall fescue, and switchgrass) at the 5-15 cm soil horizon.

Chapter 3:
Forage Characteristics of Native Grasses Treated
with Plant Growth Regulator Trinexapac-ethyl

Abstract

Native warm season grasses can provide mid-summer forage for livestock producers in the Mid-South, but are rested during the late-summer and fall to build root reserves. Gibberellin inhibitors may provide a unique trade-off during this period by decreasing stem growth and improving forage nutritive value. This study evaluated the effect of late July trinexapac-ethyl treatments at three concentrations (0, 0.3, 0.6, 1.2 kg a. i. ha⁻¹) on switchgrass (*Panicum virgatum*) and a mixture of big bluestem (*Andropogon gerardii*) and indiangrass (*Sorghastrum nutans*) during 2016 and 2017. Forage mass and nutritive values (crude protein, neutral detergent fiber, acid detergent fiber, in-vitro dry matter digestibility) were evaluated for 2-3 months post-treatment. Results indicate depressed forage mass and improvements in crude protein during both years and forage types. During 2017, all forage nutritive values (crude protein, neutral detergent fiber, acid detergent fiber, in-vitro dry matter digestibility) improved due to treatment for big bluestem/indiangrass mixture. However, improvements in nutritive values relative to forage mass loss indicated a higher loss in crude protein relative to forage mass in treated rather than untreated paddocks. Therefore, late season application of gibberellin inhibitors to warm season grasses is unlikely to be useful for pasture managers.

Introduction

Forage producers in the southeastern United States rely heavily on cool season species such as tall fescue (*Schedonorus phoenix*; TF). Despite strong persistence and production, TF presents multiple issues for producers during mid-summer, resulting in poor pasture and animal health (Ball et al., 2007). Native warm-season grasses (NWSG) provide an alternative summer forage for producers, but adoption has been limited due its lower nutritive value, shorter growing

season, and high establishment cost. One drawback is the fall rest period required to allow NWSG to build root reserves (Forwood and Magai, 1992; Cuomo et al., 2006). The resulting forage is has a high proportion stem material and contains low protein and high fiber (Waramit et al., 2012). Plant growth regulators which inhibit gibberellin synthesis offer a potential method to slow stem elongation and improve grass digestibility (Rademacher, 2000). Late-summer application of a gibberellin inhibitor could improve nutritive value of fall stockpiled NWSG biomass.

Previous forage studies have been carried out on the growth regulator mefluidide to slow stem elongation by suppressing the gibberellin hormone pathway. In addition, suppressing gibberellin expression may weaken apical dominance and result in increased tillering (Ervin and Koski 1998). In warm-season grass forages such as millet and sorghum, applications of mefluidide improved tillering, stem:leaf ratios and stem digestibility (Hernandez 1984; Bransby et al., 1986; Stair et al., 1991; Redmon et al., 2003). In pasture settings, mefluidide has improved animal intake, digestibility, and rate of gain (Goold et al., 1982; Moyer and Lomas 1987). More recent research has been carried out on low-dose metsulfuron application in pasture settings to reduce tall fescue seedhead production (Aiken et al. 2012). However, mefluidide and metsulfuron are cell division inhibitors and slow overall plant growth. Trinexapac-ethyl (TE) inhibits gibberellin synthesis later in the biosynthetic pathway relative to mefluidide and therefore is potentially less disruptive to growth (Marcum and Jiang 1997; Ervin and Koski 1998; Rademacher 2000). Growth suppression is expected to occur with TE, potentially at rates lower than mefluidide (Luiz et al., 2015). Trinexapac-ethyl has not been evaluated on perennial forage species and could improve NWSG nutrient partitioning and stand health.

Switchgrass (*Panicum virgatum*), big bluestem (*Andropogon gerardii*), and indiangrass (*Sorghastrum nutans*) are NWSG used for biofuels and forage. They are characterized by high forage mass but low nutritive value, especially when mature. Therefore, a tradeoff of mass for nutritive value could be beneficial in NWSG swards.

The objective in this study is to assess forage characteristics of fall accumulation of NWSG treated with TE. The study will assess if TE either improves short-term forage nutritive value by directly suppressing stem growth or alternatively TE may improve long term forage quality through suppressing apical dominance and increased tiller growth. This could result in improved forage nutritive traits over 2-3 months.

Materials and Methods

Site and study design

The study was carried out on paddocks planted to switchgrass (cv. Alamo) and a 1:1 mixture of big bluestem (cv. OZ-70)/indiangrass (cv. Rumsey). During 2016, the paddocks were located at the University of Tennessee AgResearch and Education Center in Greeneville, TN (Dunmore loam; kaolinitic, mesic Typic Paleudult). During 2017, the paddocks were located at the Highland Rim AgResearch and Education center near Springfield, TN (Dickson silt loam; superactive, mesic Typic Hapludoll). In both locations, established adjacent unfertilized paddocks were divided into four replications of four treatments (PrimoMaxx, Syngenta Crop Science, Raleigh, North Carolina; control, 0.3, 0.6, 1.2 kg a. i. ha⁻¹) in a randomized block design. Treatment units were 10m by 10m with 2-m buffer zone between units. Plots were clipped to 20-cm on the 5th of July in 2016 and the 12th of July in 2017. Clipped biomass was

raked off the experimental area. On July 25th (2016 and 2017), foliar applications of TE occurred.

Sampling methods

Samples were collected on August 22, 2016, September 21, 2016, October 20, 2016, August 25, 2017 and September 26, 2017. Forage mass above 8-cm was collected and dried from two 0.1 m² areas in each experimental unit. Forage samples were dried at 60°C for 48 hours up to constant weight and dry weights were recorded. Sub-samples of September samples (control and 1.2 kg a.i. ha⁻¹) were divided into stem and leaf portions to be analyzed separately.

Each sample was ground through a Wiley Mill Grinder (1-mm screen; Thomas Scientific, Swedesboro, NJ) for near-infrared reflectance spectroscopy (NIRS) analysis of forage nutritive value using a FOSS 6500 NIRS instrument (FOSS NIRS, Laurel, MD) to quantify crude protein (CP), neutral detergent fiber (NDF), *in-vitro* dry matter digestibility at 48 hours (IVDTMD) and acid detergent fiber (ADF). Equations for the forage nutritive analyses were standardized and checked for accuracy with the 2016 mixed hay equation developed by the NIRS Forage and Feed Consortium (NIRSC, Hillsboro, WI). Software used for the NIRS analysis was Win ISI II (Infrasoft International, State College, PA). The global H statistical test compared the samples with the model and other samples within the database for accurate results.

Data Analysis

Results were analyzed using JMP statistical software (JMP Pro 12, SAS Institute, Cary, NC). Significance threshold was set at $P < 0.05$. A mixed model was created to determine significant determinants of forage values (forage mass, CP, NDF, ADF, IVTDMD). Fixed

effects were forage type, treatment, and forage type-treatment interaction. Block alone was used as a random effect since there was significant year interaction.

Since forage mass is impacted by treatment and forage mass is a major covariate of forage nutritive value, a separate model was run that included forage mass and treatment-forage mass interactions as a fixed variable to test if forage quality measurements are altered beyond that predicted by plant growth rate.

Leaf and stem nutritive values were compared through a full factorial mixed model including year, forage type, and treatment.

Results

Environmental Conditions

During the 2016 season, temperatures were greater than average and precipitation was lower than average (Figure 3.1). The 2017 season at Springfield, TN had average temperature and greater than average precipitation.

Forage Response

Due to major variation between years and locations, bulk forage models were also run independently for each year. In both years, TE treatment reduced forage regrowth rate across both forage types (Table 3.1; Figure 3.2; Figure 3.3). Crude protein content increased due to treatment across both years and both forage types (Table 3.1). Other forage nutritive values (NDF, ADF, IVTDMD) did not respond to treatment during 2016. Only IVTDMD indicated a difference due to forage type in 2016. During 2017, NDF and ADF decreased and IVTDMD increased due to treatment (Table 3.1). An interaction between forage type and treatment was found for CP, IVTDMD, NDF and ADF (Table 3.1). When models were run separately by

species for nutritive values in 2017, BBIG responded to treatment in all models (CP: $p < 0.001$; IVTDMD: $p = 0.005$; NDF: $p < 0.001$; ADF: $p = 0.003$) while SG had no treatment response (CP: $p = 0.52$; IVTDMD: $p = 0.54$; NDF: $p = 0.59$; ADF: $p = 0.72$).

Within models including forage mass as a covariate for predicting nutritive values response to treatment, no treatment effect was detected (Table 3.1). Crude protein indicated an interaction between forage mass and treatment. Visualization of the relationship between CP and forage mass for different treatment levels (Figure 3.4) indicated that CP decreased more rapidly with forage mass accumulation in treated paddocks relative to untreated paddocks.

Leaf CP, NDF, ADF and IVTDMD and stem CP improved for BBIG (Table 3.2). No improvements were found in SG leaf or stem nutritive values.

Discussion

The two years in the study had highly contrasting precipitation rates. The high temperature and low rainfall in 2016 reduced biomass accumulation for all treatments and BBIG had lower than expected nutritive values (Figure 3.2; Figure 3.3). Abundant rainfall in 2017 resulted in a larger expression of TE effects, specifically in BBIG. Despite variation between years, treatment decreased forage regrowth mass and increased CP during both years (Table 3.1). The increased CP content concurs with prior observations of increased chlorophyll related to TE applications (Luiz et al., 2015). Leaf nutritive values for BBIG were also improved due to treatment, in agreement with prior literature (Redmon et al., 2003; Macedo et al., 2017; Table 3.2). No improvement was found in stem digestibility, but CP of BBIG stem material increased (Table 3.2). This improvement was smaller than other observations of improved stem

digestibility of annual warm-season grasses (Stair et al., 1991; Hernandez, 1984; Macedo et al., 2017).

The interaction between forage mass and CP relative to treatment level implies a more rapid decrease in treated paddock CP with increasing forage mass (Figure 3.4). This indicates a poor trade-off between nutritive value for forage mass through late-season TE treatment of NWSG. However, due to the lack of high forage mass samples from treated pastures and likely non-linear relationship between forage mass and crude protein, this conclusion is tentative. Additionally, NWSG translocate nitrogen belowground during this period. Therefore, increased CP will be rapidly lost during fall and potentially be counter-productive.

Although bulk nutritive improvements were significant, particularly in BBIG during 2017, they were more attributable to decreased forage mass (Table 3.1). The low rate of stem suppression can be attributed to the late-season application. Resource allocation to stem production is high during this period and may be difficult to depress. Goold et al. (1982) found diminishing returns with late season stem growth suppression and Moyer and Lomas (1987) found the highest difference between treated and untreated during June in a tall fescue pasture, a period coinciding with early stem production.

Conclusion

These results indicate an improvement in CP, but only minor digestibility improvements in NWSG during fall with trinexepac-ethyl application. A slight improvement was observed in leaf and stem nutritive values, primarily in BBIG. The overall response to treatment was greater for BBIG, indicating variation in response between NWSG. Chemical suppression of stem growth and overall fiber accumulation is challenging biologically and may be unfeasible.

Further research could quantify the impact of growth regulators on sward health, such as altered belowground growth or tiller production.

Appendix

Table 3.1: Mixed model *P*-value results for predictors of forage mass and nutritive value across two years and two locations in Tennessee.

	2016					2017				
	Forage Mass	Crude Protein	NDF†	ADF	IVTDMD	Forage Mass	Crude Protein	NDF	ADF	IVTDMD
Forage Types	0.004	0.523	0.246	0.132	0.035	0.018	<0.001	0.003	<0.001	<0.001
Treatment	0.016	0.060	0.159	0.140	0.268	<0.001	0.001	0.0013	0.007	0.036
Interaction	0.633	0.837	0.955	0.883	0.928	0.810	0.003	0.005	0.0043	0.008
Forage Mass	-	<0.001	0.003	0.012	0.004	-	<0.001	<0.001	0.999	0.010
Treatment	-	0.389	0.303	0.222	0.444	-	0.058	0.299	0.475	0.663
Interaction	-	0.567	0.399	0.414	0.427	-	0.010	0.108	0.101	0.238

Random variables included block and month.

Forage type, treatment, and forage type-treatment interactions were reported (first three rows).

Forage mass and treatment models included forage type and forage type-forage mass interactions as fixed variables (last three rows).

†NDF, neutral detergent fiber; ADF, acid detergent fiber IVTDMD, in-vitro dry matter digestibility (48 hours).

Table 3.2: Mixed model least-square means estimates of nutritive values of September leaf and stem components two forage types (switchgrass and big bluestem/indiangrass mixture).

Leaf		Crude Protein (g kg ⁻¹)	NDF † (g kg ⁻¹)	ADF (g kg ⁻¹)	IVTDMD (g kg ⁻¹)
Switchgrass	Treated	100.6	588.1	348.8	697.1
	Untreated	91.1	610.1	366.8	684.3
Big bluestem/ Indiangrass	Treated	87.6*‡	635.5*	380.7*	672.3*
	Untreated	79.1*	653.3*	401.0*	644.7*
Stem					
Switchgrass	Treated	23.7	781.2	465.6	537.4
	Untreated	18.7	800.4	484.9	527.9
Big bluestem/ Indiangrass	Treated	20.6*	828.5	512.1	478.9
	Untreated	12.6*	832.8	522.4	483.7

Treated samples were collected from 1.2 kg a.i. trinexapac-ethyl ha⁻¹ paddocks and untreated were from control paddocks.

† NDF, neutral detergent fiber; ADF, acid detergent fiber IVTDMD, in-vitro dry matter digestibility (48 hours).

‡ * indicate significant differences due to treatment (p<0.05).

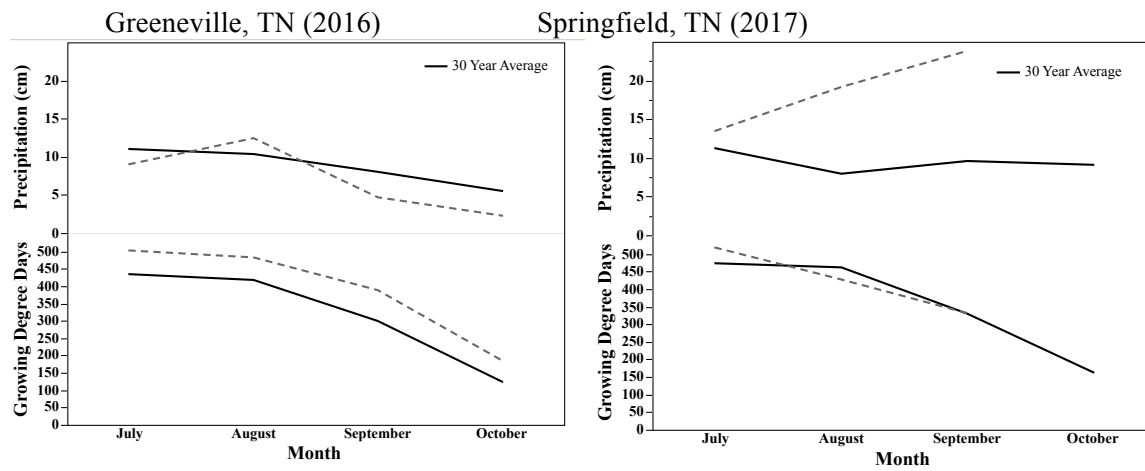


Figure 3.1: Monthly precipitation (cm) and growing degree days (base 10 C°) for Greeneville, TN and Springfield, TN.

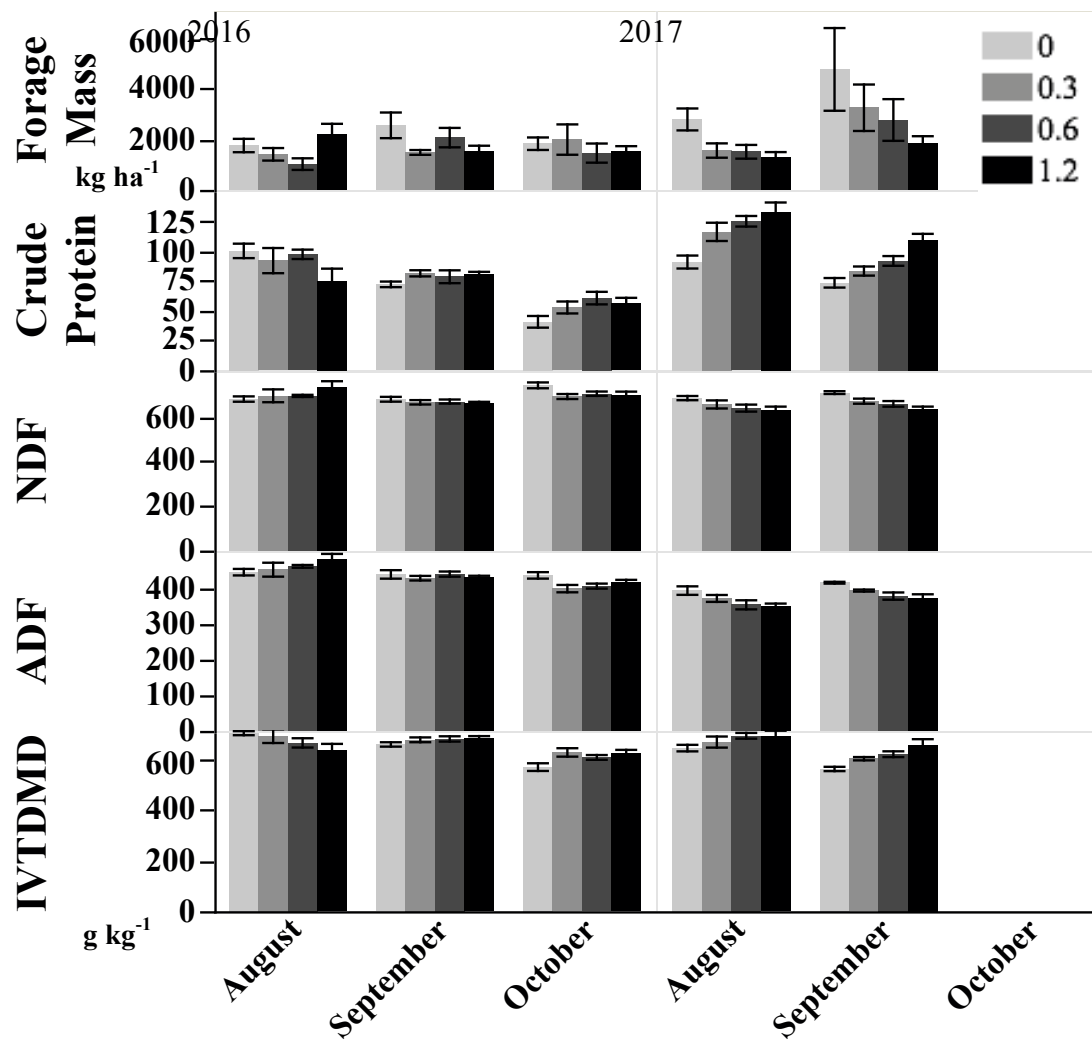


Figure 3.2: Forage mass and nutritive value of mixed big bluestem/indiangrass paddocks across two years treated with 0, 0.3, 0.6 or 1.2 kg a.i. trinexapac-ethyl ha⁻¹. NDF, neutral detergent fiber; ADF, acid detergent fiber IVTDMD, in-vitro dry matter digestibility (48 hours).

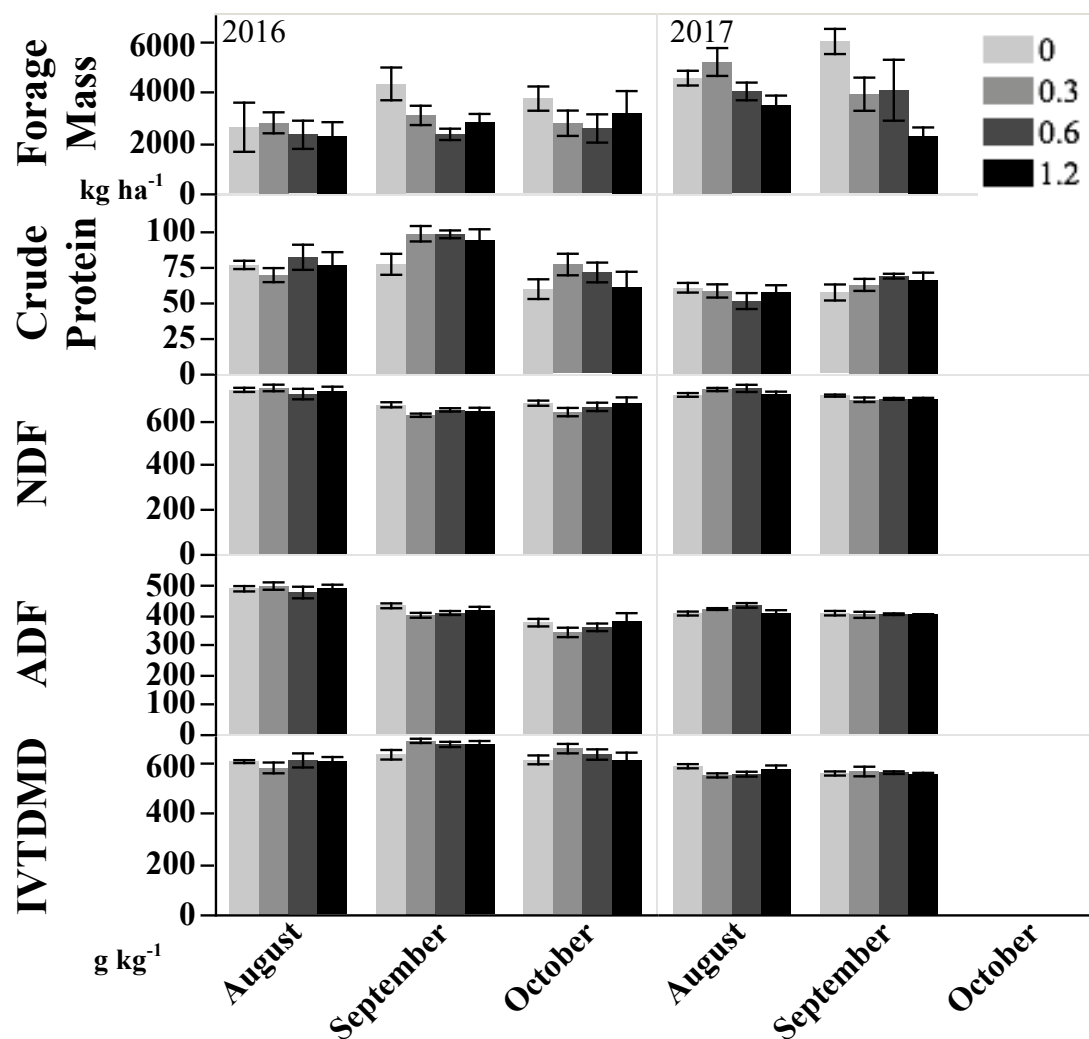


Figure 3.3: Forage mass and nutritive value of switchgrass paddocks across two years treated with 0, 0.3, 0.6 or 1.2 kg a.i. trinexapac-ethyl ha⁻¹. Forage mass is measured in kg ha⁻¹. NDF, neutral detergent fiber; ADF, acid detergent fiber IVTDMD, in-vitro dry matter digestibility (48 hours).

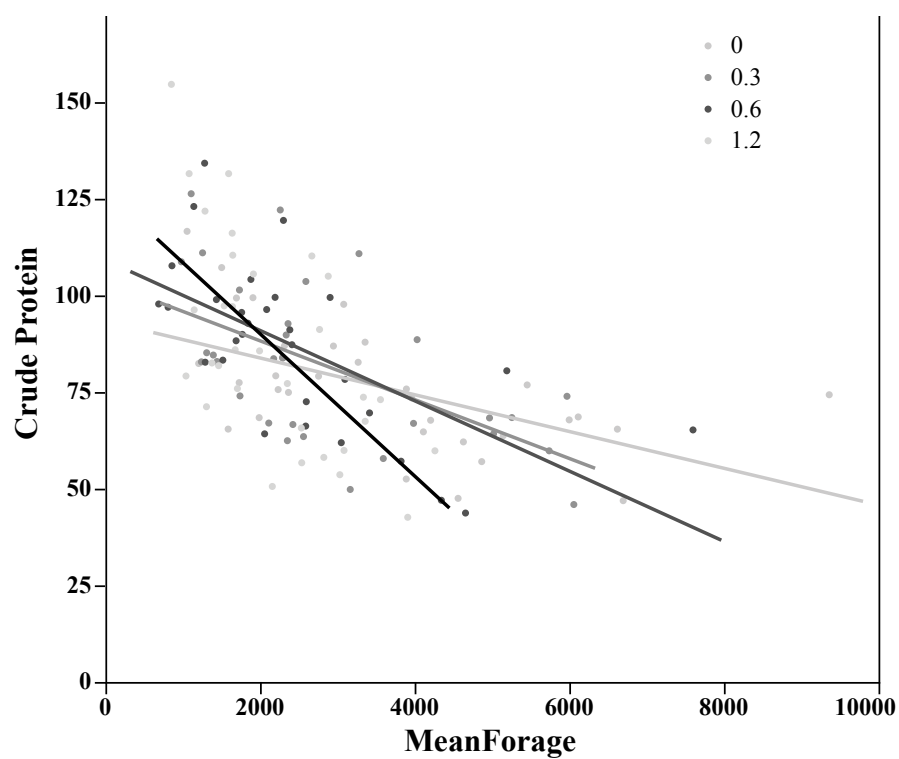


Figure 3.4: A display of the interaction between trinexapac-ethyl treatment, forage mass (kg ha^{-1}) and crude protein content of forage (g kg^{-1}) of switchgrass and big bluestem/indiangrass paddocks across two years treated with 0, 0.3, 0.6 or 1.2 kg a.i. ha^{-1} .

Conclusion

Native warm-season grass adoption offers an opportunity to support an endangered ecosystem while improving economic outcomes for pasture managers. This thesis provides evidence to assist in decision making when evaluating this alternative forage system. Bulk forage samples of winter stockpiled NWSG support the conclusion that they are below the threshold for livestock maintenance diets. However, the variation between species digestibility and increased leaf nutritive value indicate that there are potential methods for improving the utility of this low-input forage source. A separate assessment of the gibberellin inhibitor trinexapac-ethyl evaluated the potential to chemically improve the forage value of late summer NWSG growth. Treatment resulted in a significant decrease in forage quantity, but only limited improvements in digestibility.

Year-round soil sampling found differences in soil nitrogen and aromatic carbon cycling between NWSG and tall fescue. These samples indicated lower soil cycling within NWSG, specifically switchgrass. This conflicts with claims of improved “soil health” with NWSG establishment, but the misunderstanding could equally be attributed to generalizations about soil health metrics. Further research is necessary to improve methods to measure soil nutrient dynamics in perennial forage systems.

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