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A PLANT TRAIT-BASED APPROACH TO EVALUATE THE ABILITY OF NATIVE C_3 AND C_4 GRASSES TO RESTORE FUNCTIONALITY TO A REMNANT BLUEGRASS SAVANNA-WOODLAND IN KENTUCKY, USA.

Jann E. Fry
University of Kentucky, jann.fry@gmail.com

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Jann E. Fry, Student

Dr. Scott K. Gleeson, Major Professor

Dr. David Westneat, Director of Graduate Studies

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DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Arts and Sciences
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By
Jann E. Fry

Director: Dr. David Westneat, Professor of Biology

Lexington, Kentucky

ABSTRACT OF THESIS

A PLANT TRAIT-BASED APPROACH TO EVALUATE THE ABILITY OF NATIVE C_3 AND C_4 GRASSES TO RESTORE FUNCTIONALITY TO A REMNANT BLUEGRASS SAVANNA-WOODLAND IN KENTUCKY, USA.

Temperate Midwestern oak savannas are considered imperiled ecosystems with < 1 % remaining since European settlement and are identified as critical areas for preservation. Restoration of Midwestern oak savannas is challenging due to several factors including lack of accurate historical data, few intact remnants remaining to study, and lack of restoration ecology studies. A plant trait-based approach was used to evaluate the ability of six C_3 and three C_4 native bunchgrasses to restore functionality to a remnant savanna–woodland of the Bluegrass Region of Kentucky. The response and effect framework was used to assess the response of the nine native grasses according to the habitat filters of interannual precipitation, inter- vs. intra-specific competition, and simulated grazing. The effect traits associated with plant-soil nitrogen and carbon cycling were also assessed. The response traits of interannual competition and inter- vs. intra-specific competition along with the effect traits plant-soil nitrogen and carbon cycling were measured in a monoculture experiment conducted at Griffith Woods WMA. The simulated grazing or clipping experiment was conducted over three months in a heated greenhouse experiment.

Four of the C_3 species were of the genus *Elymus* which had significant differences in life history traits compared to the other species made them particularly well adapted to the Bluegrass Savanna-Woodland. The *Elymus* species were not well adapted to the most intense clipping treatment (clip to 7 cm). For the other two C_3 species, *C. latifolium* would be a better competitor than *D. clandestinum* under normal conditions. *D. clandestinum* had the most number of plastic traits and was the only species to exhibit all three grazing strategies. Comparing the C_4 species, *T. flavus* and *P. anceps* grew well in the monoculture but *A. virginicus* did not. The life history traits of *A. virginicus* does not make this species a good candidate for restoration at this site. The three C_4 species were well adapted to clipping. The results of this study suggest that the C_3 species, particularly the *Elymus*, are well adapted to the eutrophic mesic conditions of the Bluegrass Savanna-Woodland, and that the C_4 species are better adapted to disturbance.

KEYWORDS: savanna, restoration ecology, C_3 and C_4 grasses, response traits, effect traits.

Jami E Day
5-1-2014

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By

Jann Elise Fry

Dr. Scott K. Gleeson
Director of Dissertation

Dr. David Westnest
Director of Graduate Studies

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Chapter 1: Introduction

Savannas are grassland ecosystems characterized by the trees being either small or widely spaced so that the tree canopy is not closed (McPherson 1997), and are influenced by fire, climate, topography and soil type (Nuzzo 1986). Savannas cover 20 % of the Earth's land area and can be divided into tropical and temperate groups. Tropical savannas cover 15 % of the Earth's land area, are generally better represented in the scientific literature, and are extensive in Africa, Australia, and S. America (McPherson 1997). While temperate savannas of North America were historically common at the time of European settlement, most of these landscapes have been reduced to < 1 % of their original area, are considered to be endangered (Anderson, Fralish et al. 1999), and are identified as critical areas for preservation (Klopatek et. al 1979). Furthermore, temperate savannas are not as well studied or represented in the scientific literature as tropical savannas (McPherson 1997, Anderson, Fralish et al. 1999).

At the time of European settlement of Midwestern North America, oak savannas occurred in the northern half of the central United States in a region that includes Minnesota, Iowa, Missouri, Wisconsin, Illinois, Michigan, Indiana, and Ohio (Nuzzo 1986). The oak savanna was mainly a transitional community located between the western prairie and eastern deciduous forest (Nuzzo 1986, McPherson 1997). The dominant trees were primarily *Quercus* sp., giving rise to names for the savanna such as oak savanna and oak savanna-woodland. Bray (1960) and Nuzzo (1986) characterized open savannas as usually dominated by burr oak (*Quercus macrocarpa*) and primarily found on flatter more mesic areas than scrub savannas. Scrub savannas were dominated by white oak (*Quercus alba*) and black oak (*Quercus velutina*) and were generally found on dry to dry-mesic areas of steeper topography.

Within 20 to 40 years, after the Midwest was settled by Europeans in the eighteenth century, oak savannas all but disappeared due to fire cessation and conversion of land to agricultural or urban development (Nuzzo 1986, Anderson, Fralish et al. 1999). The fact that only 2% of Midwestern Oak Savannas remained by 1986 (Nuzzo 1986) has caused this habitat to be listed as a "globally imperiled" ecosystem (Heikens and Robertson 1994). Conservation and restoration efforts of Midwestern Oak Savannas are difficult due to: 1) the limited amount of historical data, which were recorded mainly by European pioneers and land surveyors, and the unknown validity and motivation for these records (Nuzzo 1986); and 2) the lack of restoration ecology studies that could guide ecological restoration practices and management of these systems (McPherson 1997).

One potential reason for the lack of research activity on restoration of Midwestern oak savannas and temperate savannas in general, is the absence of a professional discipline associated with savannas that promotes an understanding of the role and importance of savannas in temperate regions (McPherson

1997). Since Midwestern oak savannas are generally transitional zones between grasslands and oak forests (McPherson 1997), boundaries between the three vegetation types are subjective. Midwestern oak savannas can be utilized as grasslands for grazing animal production and managed accordingly or utilized as woodlands with forest management. Another potential reason for the lack of research activity for Midwestern oak savannas could be inconsistent definitions and/or interpretations of the term savanna (McPherson 1997). Midwestern oak savannas can be referred to as oak savanna, oak opening, oak barrens, scrub prairie, brush prairie, and brush savannas (Nuzzo 1986) Thus, definitions of a Midwestern oak savanna are variable and can be site specific (McPherson 1997, Anderson, Fralish et al. 1999). Bray (1960) and Nuzzo (1986) classified Midwestern oak savannas as open savanna or scrub savanna and these two savanna types can vary over time and disturbance levels. The amount of canopy cover in Midwestern oak savannas is also highly variable. According to McPherson (1997), the woody plant cover of Midwestern oak savannas can range from < 1% to about 30%, while Nuzzo (1986) reported canopy cover of Midwestern oak savannas ranging from 10% to 100%. The species composition of the understory also determines the difference between a forest, grassland, and a savanna. Nuzzo (1986) categorized the savanna understory as having less grass and more forbs and shrubs than a prairie, but more grass and fewer forbs, vines, and shrubs than oak forests. Thus, while the definition of savannas include a grassland and tree component, savannas can broadly differ in the way they look and, most likely, the way they function.

The general definition of a savanna found in most textbooks also can be misleading when identifying Midwestern oak savannas. For example, while frequent low intensity fires, a distinct annual dry season, extended droughts, and grazing by large herbivores are characteristics often associated with savannas (Enger and Smith 2004), these characteristic may be more common to African Tropical Savannas than Midwestern oak savannas (McPherson 1997). The climate of most Midwestern Oak savannas does not promote frequent low intensity natural fires or extended droughts, and the dry season is generally more variable than in tropical savannas. While natural fires may not be common in Midwestern Oak savannas, fire is considered to be an important disturbance in the maintenance of these savannas, with fires started by Native Americans playing an important role historically (Mann 2011).

With the lack of research conducted on Midwestern oak savannas and few intact oak savannas remnants remaining, restoration of a functional savanna community requires an alternative approach. The plant trait-based approach views a species as a set of inter-connected traits that are both the result of its evolutionary history and determine the ability of the species to respond to or affect biotic and abiotic environmental filters (Adler, Milchunas et al. 2004). The response-and-effect framework uses this plant trait-based approach that views a species as a set of interconnected traits which can both respond to

abiotic and biotic habitat filters and can affect ecosystem properties (Garnier and Navas 2012). The response-and-effect framework includes a performance trait (e.g., annual net primary production - ANPP), which is an overall indicator of plant fitness that can be explained by morphological or physiological response traits (Garnier and Navas 2012). In this study, morphological traits are referred to as macroscopic traits since they are easily observed and measured, and physiological traits are referred to as microscopic traits since they are not easily observed or measured. Macroscopic and microscopic response trait values can vary with differing abiotic and biotic habitat filters, which can then affect traits that influence ecosystem functioning (Lavorel and Garnier 2002) through the direct effects of habitat filters as well as feedback loops that affect ecosystem function (Garnier and Navas 2012). These response and effect traits can be interrelated and may or may not be correlated (Couso and Fernandez 2012).

By growing prospective species in monocultures, performance, response and effect traits can be measured to determine each species characteristics and niche which then can be used to predict how they might function in a mixed species community setting. The purpose of this restoration ecology study was to study the plant traits of six C_3 and three C_4 perennial grasses to help evaluate possible components of a restored functional grassland community for the historic Oak Savanna-Woodland located in the Inner Bluegrass Region of Kentucky, USA. The Bluegrass Savanna-Woodland was considered by Braun (1943) to be anomalous or unexpected in the middle of the mixed mesophytic forest biome. Wharton and Barbour (1991) characterized this area as a savanna-woodland with an open forest whereby the trees are dominant but with a well-developed grassy undergrowth. This savanna-woodland was described at the time of European settlement in the mid to late 1700's as having a rolling mildly karst topography, fertile, deep, and well drained silt loam soil produced over highly phosphatic Ordovician Limestone, vast cane breaks (*Arundinaria gigantea*), large mature trees including oak (*Quercus sp.*) and ash (*Fraxinus sp.*), and a graminoid dominated herbaceous layer (McInteer 1952, Wharton and Barbour 1991, Campbell 2004). With European settlement, native grasses were rapidly replaced by non-native C_3 forage grasses (*Poa pratensis* and *Festuca arundinacea*) so that no intact savanna grassland remains in this region today (Bryant, Wharton et al. 1980). The native C_3 grasses were thought to be dominant in both abundance and number of species in woodlands (Wharton and Barbour 1991) with mesic eutrophic soils as well as in the more open woods (Campbell 2004). The native C_4 grasses were thought to be fewer in the number of species and found in local openings on poorer soils or openings created by disturbance such as fire or bison trails (Campbell 2004). Common prairie grasses of more western prairie regions were not common in this region (Campbell 2004).

The two experiments included in this study were a field monoculture experiment and a greenhouse clipping experiment. The monoculture experiment was conducted in a relatively flat, tall fescue (*Festuca arundinacea*) dominated abandoned paddock located at Griffith Woods Wildlife Management Area (WMA). Griffith Woods WMA is considered to be the best Bluegrass Savanna-Woodland remnant in the Inner Bluegrass Region of Kentucky. It includes 302 hectares in southern Harrison County, Kentucky, and lies on the northern edge of the Inner Bluegrass Region of Kentucky. While the vegetation of Griffith Woods WMA is known for its remnant Blue Ash-Oak savanna-woodland with 150 – 350 year old trees of *Fraxinus quadrangulata* (Blue Ash), *Quercus macrocarpa* (Burr Oak), *Quercus muhlenbergii* (Chinquapin Oak), and *Quercus shumardii* (Shumard Oak), the herbaceous layer is dominated by non-native C_3 forage grasses (e.g., *Festuca arundinacea* and *Poa pratensis*).

In the field monoculture experiment, characteristics for each of the nine native grass species, the performance trait of annual net primary production (ANPP), macroscopic traits and microscopic traits were measured in 2010 and 2011. Since these two years had significant differences in interannual precipitation, plant traits for each species were analyzed between the relatively dry year (2010) and the wet year (2011). A species mixture treatment was added to the monoculture experiment to compare how the species performed in the monoculture (with only intra-specific competition) and the species mixture treatment (with inter-specific competition). This comparison was analyzed for both the dry year and the wet year.

Chapter 1 includes how each species performed in the monoculture in general, the response trait comparisons between the dry vs. wet year (drought effects), and the response trait comparisons between inter- vs. intra-specific competition that were measured in both the dry and wet year (competition x drought effects). This information can then be used to predict how they might function in a community setting. My hypotheses included: 1) The C_3 and C_4 grasses will differ in the macroscopic and microscopic plant traits that explain the performance trait (ANPP); 2) ANPP and macroscopic and microscopic response traits will be differentially affected by the habitat filters of drought and drought x competition; 3) In response to the habitat filter of drought and competition, the C_3 species would show trait differences in the performance trait and macroscopic traits, and that the C_4 species will be more stress tolerant and show trait differences only in microscopic traits; 4) The macroscopic and microscopic traits of the four *Elymus* species will not be plastic in response to drought as their plant traits were measured before the summer drought of 2010. The *Elymus* species should have experienced the least amount of precipitation variability as both years had a wet spring. The macroscopic and microscopic traits of the other two C_3 species that were actively grow during the summer will show plasticity in traits as they did experience summer interannual precipitation variability, and the C_4 species will be stress tolerant and only

plastic in the microscopic traits; and 5.) Drought and competition will have differing effects on C_4 and C_3 species whereby C_3 species should be at a competitive advantage over the C_4 species in the wet year (2011), and the C_4 species should be at a competitive advantage over the C_3 species in the dry year (2010). Results of this experiment can be used to better understand the dynamics of this Bluegrass Savanna-Woodland and how these nine species might perform in a mixed species community. A variety of effect traits associated with plant-soil nitrogen and carbon cycling were also assessed in the monoculture experiment and are presented in Chapter 2. The goal of this study was to determine if the species were fast N cycling or slow N cycling species and how these characteristics affected N and C soil pools and soil nutrient concentrations. Chapter 2 included species characteristics, an inorganic N resin pools, litter decomposition, and soil nutrient analyses. I hypothesized that: 1) The C_3 grasses will have plant traits that promote fast N cycling, and C_4 grasses will have plant traits that promote more conservative or slow cycling N plant traits; and 2) If N is limiting at the ecosystem level, slow N cycling species should store N in more slowly cycling, recalcitrant pools more than fast N cycling species according to the resource-competition theory.

The greenhouse clipping experiment is presented in Chapter 3. If grazing was an important disturbance in the Bluegrass Savanna-Woodland, these native grasses should have evolved grazing strategies to tolerate, deter, or avoid grazing. Since savannas are maintained by disturbance, the goal of this experiment was to better understand the ability of the nine grass species to respond to grazing, and to recommend effective mowing regimes that would maintain a functional grassland community within the Bluegrass Savanna-Woodland. The clipping experiment had a factorial design with two clipping heights (intensities) and two clipping frequencies designed to mimic frequent intense grazing to less intense rotational grazing, with a non-clipped control included for comparison. I hypothesized that: 1) Frequency will have a bigger impact on plant traits than intensity as predicted by Augustine and McNaughton (1998); 2) The C_4 species will be better adapted to grazing than the C_3 grasses because they generally have higher nitrogen use efficiency, a higher C:N ratio, and a higher water use efficiency that should make them less affected by biomass loss; and 3) The grasses may employ different grazing tolerance? strategies at different frequency and intensity treatment levels. Results of this experiment can be used to recommend mowing regimes for ecological restoration that will maintain these grasses in a community setting, and provide insights for future restoration efforts.

Considering the response-and-effect framework, this study measured response traits across the abiotic habitat filter of drought and the biotic habitat filters of competition and grazing, and effect traits that impacted the cycling of N and C and soil nutrient concentrations. This information was used to help inform how these nine species would perform in a community and the biogeochemical effects they might

have on the plant-soil system. The nine native species used in this study were identified as potentially good candidates for the ecological restoration of the Bluegrass Savanna-Woodland (Table 1.1). The six C_3 grasses included in this study are associated with wooded habitats, and the three C_4 species are associated with more open habitats (Wharton and Barbour 1991, Campbell 2004). Of the six C_3 species, four are from the genus *Elymus* or wildryes which are well documented in historical records and are thought to have been abundant at the time of European settlement in the mid to late 1700's (Wharton and Barbour 1991). The *Elymus* species have a different life history pattern with significant niche differentiation from the other species. They flower in the spring or early summer, set seed, and then go dormant during the hottest months of the summer. They regrow tillers in the fall which overwinter and produce flowering culms the next spring. The *Elymus* species flower before the other five species (Figure 1.1). *Dichantheilium clandestinum* and *Chasmanthium latifolium* were the last two C_3 species to flower (Figure 1.1). *D. clandestinum* may have been referred to as buffalo grass in historical records where it is frequent in open woods, thickets, and fencerows, especially on low ground (Wharton and Barbour 1991). *D. clandestinum* also has life history traits that differ from the other species in this study. *D. clandestinum* first produces cleistogamous flowering culms, and then later in the season they produce self-fertilizing chasmogamous flowers on small inflorescences that are usually hidden within the sheathes. Both types of flowers produce viable seeds. While this species does not produce a lot of tillers, it had the greatest ability for tiller branching, so one tiller could be quite large and heavy. *C. latifolium* is frequent on wooded stream banks, on floodplains, and in other moist habitats (Wharton and Barbour 1991). *C. latifolium* is also used in horticultural plantings and can be quite invasive. The three C_4 species are generally found in more open sites and flowered after the C_3 species (Figure 1.1). *P. anceps* is found less commonly and on moist ground, and *T. flavus* is common in old fields, woodland borders, open woods, pastures, and roadsides (Wharton and Barbour 1991). *Andropogon virginicus* is common in old fields and overgrazed pastures and is the last of the C_4 species to bolt and produce flowering culms (Wharton and Barbour 1991).

Thus, the plant trait method was used evaluate the ability of native grasses to restore functionality of the grassland component of the oak savanna-woodland in central KY. Specific hypothesis were tested in a greenhouse and field experiment, and the results provide us with new insights into how to select native grasses for use in restoration projects.

Tables

Scientific Name	Common Name	Photosynthetic Pathway
1. *Elymus macgregorii* R. Brooks & J.J.N. Campb.	Early wildrye	C_3
2. *Elymus villosus* Muhl. ex Willd.	Nodding wildrye	
3. *Elymus virginicus* L.	Virginia wildrye	
4. *Elymus hystrix* L.	Bottlebrush	
5. *Dichanthelium clandestinum* (L.) Gould	Deer tongue	
6. *Chasmanthium latifolium* (Michx.) Yates	River Oats	
7. *Panicum anceps* Michx.	Beaked panicgrass	C_4
8. *Tridens flavus* (L.) Hitchc.	Purple top/grease grass	
9. *Andropogon virginicus* L,	Broomsedge	

Table 1.1: The nine native perennial bunchgrass species used in this experiment listed in order of flowering time.

Figures

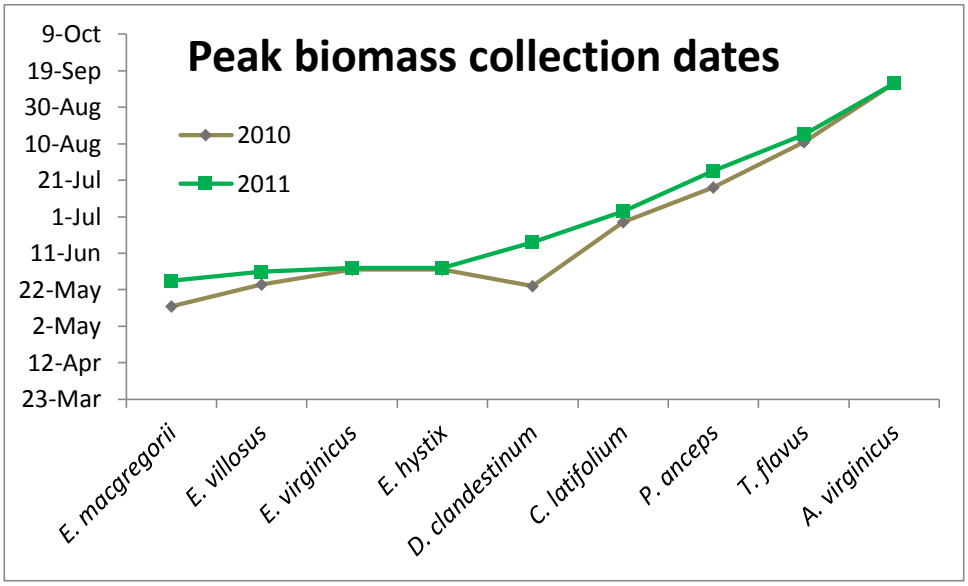
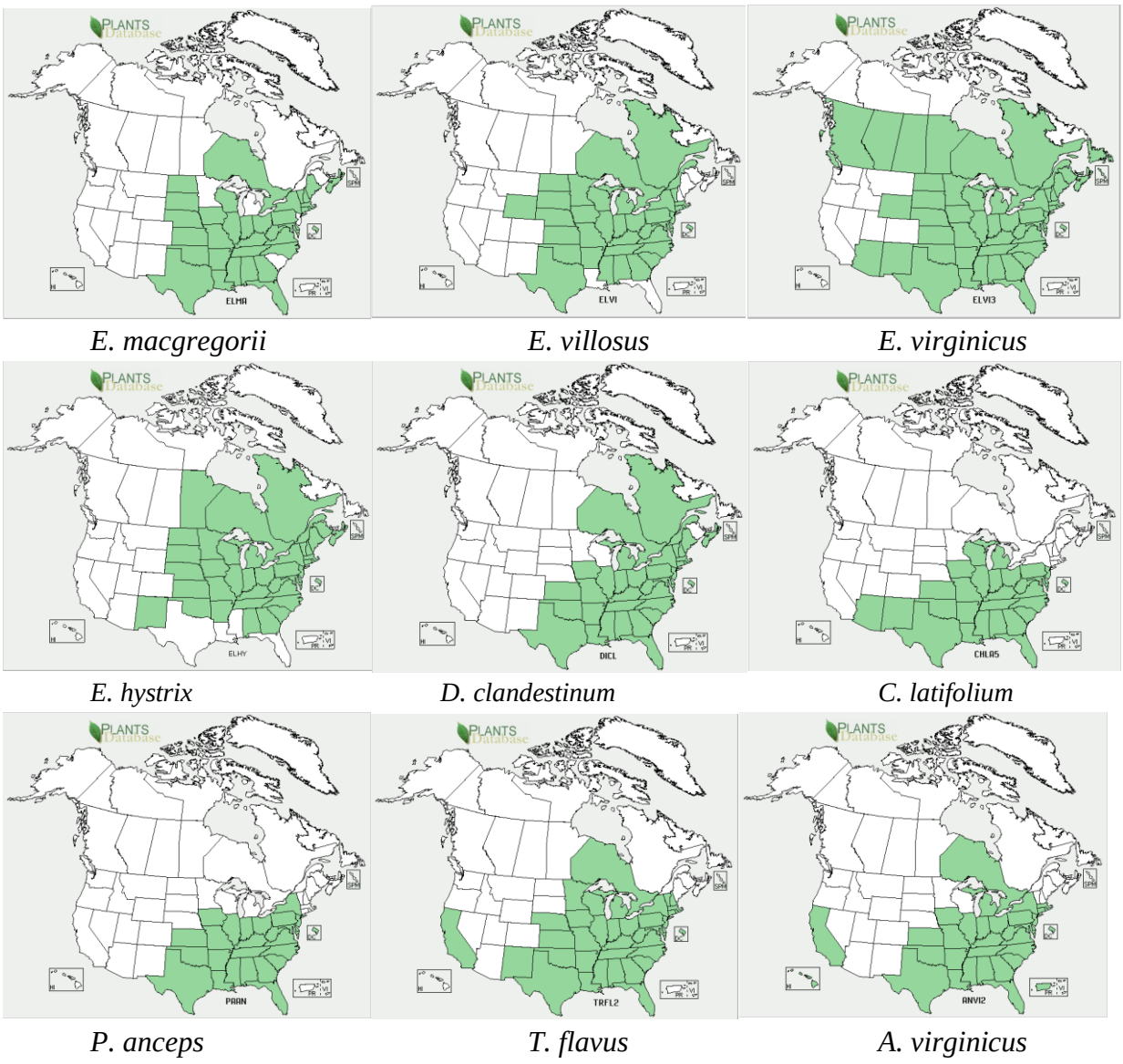


Figure 1.1: The dates of data collection according to each species time of flowering or peak biomass.



E. macgregorii *E. villosus* *E. virginicus*

E. hystrix *D. clandestinum* *C. latifolium*

P. anceps *T. flavus* *A. virginicus*

Present absent/not reported

Figure 1.2: Distribution maps for the nine species taken from the NRCS plants database.

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Chapter 2: Do C_3 and C_4 bunchgrasses differ in phenotypic plasticity and stress tolerance in response to drought and competition?

Abstract

Since oak savannas of North America have been reduced to < 1 % of their historic ranges, restoration of these habitats is important to maintain the biodiversity and ecosystem properties of these landscapes. Efforts to restore oak savannas are hindered by the lack of dependable historic data describing these savannas before they were converted to other uses, and by lack of guidelines for ecological restoration. Since no intact remnant oak savanna remains to be studied and replicated, restoration of a functional savanna community requires an alternative approach. The goal of this study was assess potential vegetation dynamics of the historic Bluegrass Savanna-Woodland grassland community in central Kentucky (USA) by studying the plant trait responses of six C_3 and three C_4 native bunchgrass species to the habitat filters of interannual variability in rainfall and inter- vs. intra-specific competition.

Using the plant trait framework, a monoculture experiment was conducted that included a species mixture treatment to assess the performance trait of annual net primary production (ANPP), macroscopic traits (morphological), and microscopic traits (physiological) for each species, which then can be used to predict how they might function in a community setting. The C_3 species were expected to be more phenotypically plastic in the performance and macroscopic traits (morphological), and the C_4 species were expected to be more stress tolerant and show plasticity in only microscopic (physiological) traits. In response to interannual variability in rainfall, the macroscopic trait of plant height was most affected by drought, and generally the microscopic traits were more affected than the performance trait and macroscopic traits. In response to competition the performance and macroscopic traits were more affected than the microscopic traits. In response to drought and competition, the C_3 species were plastic in the performance and macroscopic traits as predicted but were plastic in microscopic traits as well. The C_4 species were stress tolerant in response to drought as predicted but in response to competition, the C_4 species were plastic only in the performance and macroscopic traits which was opposite of what was predicted. *E. virginicus* was the best inter-specific competitor in both the wet and dry year most likely due to life history traits which may have a bigger impact on competitive outcomes than plasticity in trait values. The results of this experiment suggests that the C_3 species are more plastic and thus, better adapted to the heterogeneous environment of the Bluegrass Savanna-Woodland. The C_3 grasses, particularly the *Elymus* species, are recommended for use in ecological restoration and maintenance of a functional savanna grassland community not only in the Bluegrass Savanna-Woodland of Kentucky but in

other temperate regions with oak savannas. The plant trait methodology also can be used in other savanna systems to better understand savanna grassland community dynamics.

Introduction

Savannas are grassland ecosystems characterized by the trees being sufficiently small or widely spaced so that the tree canopy is not closed McPherson (1997) and are influenced by fire, climate, topography and soil (Nuzzo 1986). Savannas cover 20 % of the Earth's land area and can be divided into tropical and temperate groups. Tropical savannas cover 15 % of the Earth's land area, generally are well represented in the scientific literature, and are extensive in Africa, Australia, and S. America (McPherson 1997). While temperate savannas of North America were historically common at the time of European settlement, most of these landscapes have been reduced to less than 1 % of their original area, are considered to be endangered landscapes (Anderson, Fralish et al. 1999), and are identified as critical areas for preservation (Klopatek, Olson et al. 1979). Furthermore, temperate savannas are not as well studied or represented in the scientific literature as tropical savannas (McPherson 1997, Anderson, Fralish et al. 1999). Some potential reasons for the difference in level of research activity are the absence of a professional discipline associated with savannas, limited understanding of the role and importance of savannas in temperate regions, and inconsistent definitions and/or interpretations of the term savanna (McPherson 1997). Thus, there is a lack of knowledge of the ecological relationships and ecological management practices for temperate savannas compared to adjacent forest, desert, or grassland landscapes (McPherson 1997).

With European settlement in the eighteenth century, Midwestern Oak savannas in the USA all but disappeared within 20 to 40 years due to fire cessation and conversion of land to agricultural or urban development (Nuzzo 1986, Anderson, Fralish et al. 1999). The fact that only 2 % of Midwest Oak Savannas remained by 1986 (Nuzzo 1986) has caused this habitat to be listed as a "globally imperiled" ecosystem (Heikens and Robertson 1994). Conservation and restoration efforts of oak savannas are difficult due to: 1) the limited amount of historical data which was recorded mainly by European pioneers and land surveyors, and the unknown validity and motivation for these records (Nuzzo 1986), and 2) lack of restoration ecology studies to guide ecological restoration practices in the field (McPherson 1997).

If no intact remnant oak savanna remains as a reference system, restoration of a functional savanna community becomes challenging and requires an alternative approach. The plant trait-based approach views a species as a set of inter-connected traits that are both the result of its' evolutionary history and determine the ability of the species to respond to or affect biotic and abiotic habitat filters (Adler, Milchunas et al. 2004). The plant trait framework (Violle, Navas et al. 2007) includes a performance trait (annual net primary productivity ANPP) which is an overall indicator of plant fitness

that can be explained by morphological or physiological response traits. Response traits can vary with differing abiotic and biotic habitat filters and can also be interrelated (Couso and Fernandez 2012). By growing prospective species in monocultures, performance and microscopic and macroscopic response traits can be measured to determine the characteristics and niche of each species, and the information can be used to predict how they might function in a community setting. The ability of a species to respond to abiotic and biotic habitat filters are important in determining that species niche in the community. While fire and grazing are major disturbances in savanna, other factors including competition for light, water, and nutrients, and drought tolerance can also play a role in community dynamics. In addition, a plant must respond to multiple abiotic or biotic factors at the same time.

The phenotypic plasticity vs. stress tolerant tradeoff or the fixed–plastic continuum (Couso and Fernandez 2012) predicts that species that grow in eutrophic heterogeneous environments will have more traits that are plastic, and species that grow in resource limited or disturbed environments will be stress tolerant and less plastic. While phenotypic plasticity is usually measured on individual plants and varies within a species across differing environmental conditions, this study focuses on plasticity at the species level, or population phenotypic plasticity (Valladares, Sanchez-Gomez et al. 2006). Phenotypic plasticity is the ability of a species to change a trait value in response to an environmental factor, and is an adaptive characteristic that is influenced by, the genotype, the environment, and the plant trait of interest (Bradshaw 1965). Species with phenotypically plastic traits are expected to be adapted to a heterogeneous environment where optimizing phenotype to the current environment can increase fitness (Avolio and Smith 2013). Phenotypically plastic traits are usually macroscopic or morphological in nature (Violle, Navas et al. 2007, Couso and Fernandez 2012) and are easily observed and measured. For grasses, examples of macroscopic traits are changes in plant height and number of tillers in response to drought and grazing (Gilgen and Buchmann 2009, Barbosa, do Nascimento et al. 2011, N'Guessan and Hartnett 2011, Ge, Sui et al. 2012).

Stress tolerance is the ability of a plant species to survive different forms of severe stress (Grime 1977), resulting in little or no effect on plant growth (Couso and Fernandez 2012). Stress tolerant species are expected to be found in less heterogeneous, more stable environments where selective pressures are relatively constant (e.g., aridity in deserts) and fixed trait values promote one optimal phenotype (Couso and Fernandez 2012). This one optimal phenotype may be maintained by plasticity in microscopic or physiological trait values in response to environmental variability (Valladares, Sanchez-Gomez et al. 2006) that are not as easily observed or measured. For example , microscopic traits of grasses that may help them endure stress associated with drought include reduced specific leaf area (SLA) (Gilgen and

Buchmann 2009), and increased rhizosheath thickness and fine root development (Hartnett, Wilson et al. 2013).

The purpose of this study was to use the plant trait framework to assess the performance and response traits of six C_3 and three C_4 native grasses to help predict a functional grassland community assembly as part of the ecological restoration of the historic Oak Savanna-Woodland located in the Inner Bluegrass Region of Kentucky, U.S.A. The Bluegrass Savanna-Woodland was considered by Braun (1943) to be anomalous or unexpected in the middle of the mixed mesophytic forest biome. Wharton and Barbour (1991) characterized this area as a savanna-woodland with an open forest whereby the trees are dominant but with a well-developed grassy undergrowth. This savanna-woodland was described at the time of European settlement in the mid to late 1700's as having a rolling mildly karst topography, fertile, deep, and well drained silt loam soil produced over highly phosphatic Ordovician Limestone, vast cane breaks (*Arundinaria gigantea*), large mature trees including Oak (*Quercus sp.*) and Ash (*Fraxinus sp.*), and a graminoid dominated herbaceous layer (McInteer 1952, Wharton and Barbour 1991, Campbell 2004). With European settlement, native grasses were rapidly replaced by non-native C_3 forage grasses (*Poa pretensis* and *Festuca arundinacea*) so that no intact savanna grassland remains in this region (Bryant, Wharton et al. 1980). It is thought that C_3 grasses were dominant in both abundance and number in the original savannas (Wharton and Barbour 1991, Campbell 2004), and that C_4 grasses fewer in the number of species and occurred in local openings on poorer soils or openings created by disturbance such as fire or bison trails (Campbell 2004).

The goal of this experiment was 1) to compare and explain the performance of these nine grass species in general, and 2) assess the traits of these nine grass species in response to the abiotic habitat filter of interannual variability in rainfall, the biotic habitat filter of inter vs. intra-specific competition, and the interaction between the two habitat filters. This information can then be used to predict how they might function in a community setting. I hypothesize that

1) The C_3 and C_4 grasses will differ in the macroscopic and microscopic plant traits that can explain the performance trait (ANPP).

2) ANPP and macroscopic and microscopic response traits will be differentially affected by the habitat filters of drought and drought x competition.

3) In response to the habitat filter of drought and competition, the C_3 species would show trait differences in the performance trait and macroscopic traits, and that the C_4 species will be more stress tolerant and show trait differences only in microscopic traits.

4) The macroscopic and microscopic traits of four *Elymus* species will not be plastic in response to drought as their plant traits were measured before the summer drought of 2010. The *Elymus* species should have experienced the least amount of precipitation variability as both years had a wet spring. The macroscopic and microscopic traits of the other two C_3 species that were actively grow during the summer will show plasticity in traits as they did experience summer interannual precipitation variability, and the C_4 species will be stress tolerant and only plastic in microscopic traits.

5.) Drought and competition will have differing effects on C_4 and C_3 species whereby C_3 species should be at a competitive advantage over the C_4 species in the wet year (2011), and the C_4 species should be at a competitive advantage over the C_3 species in the dry year (2010).

Results of this experiment can be used to better understand the dynamics of this Bluegrass Savanna-Woodland and how these nine species would assemble in the community. This study also uses methodology that could be used in other savanna landscapes that could guide ecological restoration efforts of endangered oak savanna landscapes.

Materials and Methods

Study Site

The experiment was conducted in a relatively flat, tall fescue (*Festuca arundinacea*) dominated abandoned paddock located at Griffith Woods Wildlife Management Area (WMA). Griffith Woods WMA is considered to be the best Bluegrass Savanna-Woodland remnant in the Inner Bluegrass Region of Kentucky. It includes 746 acres in southern Harrison County, Kentucky (Latitude N 38.33457, Longitude W -84.354) and lies on the northern edge of the Inner Bluegrass Region of Kentucky. While the vegetation of Griffith Woods WMA is known for its remnant Blue Ash-Oak savanna-woodland with 150 – 350 year old trees of *Fraxinus quadrangulata* (Blue Ash), *Quercus macrocarpa* (Burr Oak), *Quercus muhlenbergii* (Chinquapin Oak), and *Quercus shumardii* (Shumard Oak), the herbaceous layer is dominated by non-native C_3 forage grasses (*Festuca arundinacea, and Poa pretensis*). While there is a long history of human occupation and agricultural use (Wharton and Barbour 1991), one management goal is to restore a portion of the property back to pre-European settlement savanna–woodland vegetation. Ecological restoration efforts this far have included native tree planting, native cane planting (*Arundinaria gigantea*), and invasive species removal. However, for a complete restoration native grasses need to be introduced.

The Inner Bluegrass Region of Kentucky encompasses about 2,400 square miles and is underlain by Ordovician Limestone which was pushed up over the millennia by the Jessamine Dome of the Cincinnati Arch, and produces a mildly karst topography (Wharton and Barbour 1991). This highly

phosphatic limestone generally produces a silt loam soil that is fertile, deep, and well drained (Wharton and Barbour 1991). The warm, temperate, and humid climate is continental and highly variable (Wharton and Barbour 1991). Average yearly precipitation for the Bluegrass Region is 112 cm/year with typical wet springs and dry autumns (Wharton and Barbour 1991). The mean length of the growing season is 181 days, and mean annual temperature of 13° Celsius with generally mild winters and hot summers (Wharton and Barbour 1991).

Species

The nine native bunchgrasses (Wharton and Barbour 1991, Campbell 2004) included in this study are listed in Table 2.1 in the order of their flowering times. The nine species are categorized in two functional groups C_3 (or cool season) grasses and C_4 (or warm season) grasses. The six C_3 grasses included in this study are associated with wooded habitats, and the three C_4 species are associated with more open habitats (Wharton and Barbour 1991, Campbell 2004). Four of the C_3 grasses are *Elymus* species or wildryes. The *Elymus* species are well documented in historical records and are thought to have been abundant at the time of European settlement in the mid to late 1700's (Wharton and Barbour 1991). *Elymus virginicus* is common in open woods, thickets and old fields, and *Elymus villosus* is frequent in dry and moist open woods (Wharton and Barbour 1991). *Elymus macgregorii* can be confused with *E. virginicus* but flowers a month earlier and is also found in woods and thickets (Committee 2002), and *Elymus hystrix* is frequent in the woods (Wharton and Barbour 1991). The *Elymus* species have a different life history pattern with significant niche differentiation from the other five species. They flower in the spring or early summer, set seed, and then go dormant during the hottest months of the summer. They regrow tillers in the fall which overwinter and produce flowering culms the next spring.

Dichantheilium clandestinum, which may have been referred to as "buffalos grass" in historical records, is frequent in open woods, thickets, and fencerows, especially on low ground (Wharton and Barbour 1991). *D. clandestinum* also has life history traits that differ from the other species in this study. *D. clandestinum* first produces cleistogamous flowering culms, and then later in the season they produce self-fertilizing chasmogamous flowers on small inflorescences that are usually hidden within the sheathes. Both types of flowers produce viable seeds. While this species did not produce a lot of tillers, it had the greatest ability for tiller branching, so one tiller could be quite large and heavy. *Chasmanthium latifolium* is frequent on wooded stream banks, on floodplains, and in other moist habitats (Wharton and Barbour 1991). *C. latifolium* also is used for in horticultural plantings and can be quite invasive. *C. latifolium* also has the ability for tiller branching.

The three C_4 species generally are found in open sites. *P. anceps* is not common and is found on moist ground, and *T. flavus* is common in old fields, woodland borders, open woods, pastures, and roadsides (Wharton and Barbour 1991). *Andropogon virginicus* is common in old fields and overgrazed pastures (Wharton and Barbour 1991). *A. virginicus* grew really well the first year it was planted but did successively worse each year. Since the percent cover was very low in the monoculture and particularly low in the competition plots, *A. virginicus* had low replication in the monoculture plots and was completely dropped from the competition analysis.

Experimental procedures

Seeds for each species were collected in the Bluegrass Region of Kentucky and cold (wet) stratification requirements were determined through the seed testing laboratory of the Regulatory Services at the University of Kentucky. The stratified seeds were germinated in a heated greenhouse on a flooding table in 72 hole plant trays filled with Pro-Mix potting soil. These plugs were planted in the field plots at 169 plugs/2 meter^2 plot with a hand trowel to minimize disturbance.

In a completely randomized design, the nine bunchgrass species monocultures plus one species mixture treatment were each replicated 10 times to produce 100-2 meter^2 plots. The species mixture treatment was a completely randomized planting with six species: *E. virginicus*, *D. clandestimum*, *C. latifolium*, *P. anceps*, *T. flavus*, and *A. virginicus*. Only one species of *Elymus* was added to the mixture treatment so that the genus *Elymus* would not be over represented.

Initial preparation of the field site included mowing after which the grass clippings were raked into piles and burned. The field was then sprayed with Roundup herbicide at recommended concentrations to kill all the vegetation. A second application of Roundup was applied to areas that did not die back after the initial Roundup treatment. The plots were watered as needed with a garden hose after initial planting, and rainfall was recorded at the site. The C_3 species were planted in March through May, and the C_4 species were planted in June and July. The first field season (2008) *Elymus virginicus*, *Elymus villosus*, *Elymus mcgregorii*, *Panicum anceps*, *Tridens flavus*, and *Andropogon virginicus* were planted with the remaining species planted the second growing season (2009). An 18 inch path was maintained around each of the plots by mowing. The experiment and the surrounding area were maintained by hand weeding, spot spraying with Roundup, and mowing.

Environmental Factors

There was little variation in monthly average temperatures between 2010 and 2011 (Kentucky Mesonet), and both years were similar to the long term average (1895 to 2013) of the Bluegrass Region (NOAA/ESRL http://www.esrl.noaa.gov/psd/data/timeseries) (Figure 2.1).

There was significant precipitation variation between 2010 and 2011. The year 2010 was generally a dry year but with a wet spring, and the year 2011 received near record annual rainfall (Kentucky Mesonet) compared to the long term monthly precipitation average for the Bluegrass Region (Figure 2.1). From January to April, 2010 received 43 % less precipitation, and 2011 received 39 % more precipitation compared to the long-term average of the Bluegrass Region. From July to October, 2010 received 41 % less precipitation, and 2011 received 4 % more precipitation compared to the long-term average of the Bluegrass Region. Compared to 2011, 2010 received 59 % less precipitation from January through April, and 43 % less precipitation from July to October (Figure 2.1).

Plant Trait measurements

Due to the large seasonal variation of flowering times of the nine grass species, plant trait values were taken for each species at peak biomass (or time of flowering) and ranged from May to September (Table 2.1). A fifteen cm^2 area was randomly chosen for each plot where maximum plant height was measured, the number of tillers and flowering culms was counted, and aboveground biomass 5 cm above soil level was clipped, dried at 55º C for several days, and weighed. Average tiller size was calculated as aboveground biomass/tiller number. The microscopic traits of total organic carbon and nitrogen concentrations in plant aboveground biomass material were measured using the Elementar vario MAX CNS Analyzer through the soil testing laboratory of the Regulatory Services at the University of Kentucky.

To assess the mobile versus structural carbon component for each species, a palatability study (or forage quality analysis) was done for the 2010 and 2011 peak biomass samples. Procedures for the Ankom^{200} Fiber Analyzer are found at http://www.ANKOM.com under the procedures tab. The Ankor Fiber Analyzer measures neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL). NDF digests the cell solubles which leaves the total % plant fiber or cell wall including hemicellulose, cellulose, and lignin. ADF measures the % cellulose and % lignin. Cellulose can only be digested by animals with the right bacteria in their rumen. ADL is a measurement of % lignin which is indigestible by animal enzymes. The different carbon components were calculated as: % cell solutes = 100% - % ADL, % hemicellulose = % NDF - % ADF, % cellulose = % ADF - % ADL, and % lignin = % ADL. Cell solutes are considered mobile and hemicellulose, cellulose, and lignin are considered structural.

Statistics

The statistical program PAST (Hammer 2001) was used to normalize the data and ANOVA's were performed in SAS (9.3: SAS Institute, Cary, NorthCarolina, USA) using PROC MIXED. The

ANOVA that looked at drought effects for each plant trait included all nine species, the 2 years (2010 and 2011) and the interaction between species x year (or drought). Another ANOVA for each plant trait was performed for each species to look at drought effects (or differences between the 2 years). An overall ANOVA for each plant trait was done for the competition x drought which included all five species, two levels of competition (monoculture vs. species mixture treatment) and the 2 years of drought (2010 and 2011). This included species effects, competition effects, drought effects, and all interactions. Another ANOVA for each species was performed for each plant trait to assess competition effects, drought effects, and competition x drought interaction effects. For each species, to assess differences in competitive ability between the wet year and the dry year, for each plant trait, the trait value for the monoculture was subtracted from the average trait value of the mixture which created a competition value for each year. An ANOVA was then performed for each species to compare this competition value between the two years.

Multivariate analysis was performed in the program PC-ORD (6.08: MjM Software, Gleneden Beach, Oregon, U.S.A.) using Principle Components Analysis (PCA)using the Euclidean distance measurement (McCune and Mefford 2011). The data was not standardized and all response variable were included in the analysis. The Euclidean distance measurement was also used with Multi-Response Permutation Procedures (MRPP) within PC-ORD to discern significant differences between the nine species, and the four competition x drought treatments. MRPP was also used for pairwise comparisons using the Euclidean distance measurement. For the MRPP analysis, acceptable p values were determined by dividing 0.05 by the number of species or treatments.

Results

Species performance

An ANOVA analysis that included all species and both years was performed to assess species differences in ANPP (Figure 2.3). *C. latifolium* and *T. flavus* were the top performers followed by *E. virginicus* (Figure 2.3). *E. macgregorii*, *E. villosus*, and *D. clandestinum* had the lowest ANPP and thus had the lowest performance. *C. latifolium* grew tall plants with lots of tillers that then produced flowering culms (Figure 2.3). *C. latifolium* also had a high percentage tissue C mainly in the form of lignin and cellulose (Figure 2.4). *T. flavus* grew the tallest plants with fewer but larger tillers (Figure 2.3). *E. virginicus*, was the most prolific in producing tillers that then became flowering culms (Figure 2.3). *E. virginicus* had a high percentage of tissue C that was allocated mainly to cell solutes. *E. hystrix*, *P. anceps*, and *A. virginicus* were the mid performers. The lowest performers, *E. macgregorii*, *E. villosus*, and *D. clandestinum* generally produced plants with more but smaller tillers (Figure 2.3). *E. macgregorii* had a lower percentage of tissue C that was allocated mainly to cell solutes (Figure 2.4). *E. villosus* and

D. clandestinum had a high percentage of tissue C that was allocated to cell solutes and lignin (Figure 2.4). *D. clandestinum* also had a high percentage of tissue N which resulted in a low lignin/N. The trade-off to produce ANPP by growing more small tillers or fewer big tillers was observed between the C_3 and C_4 species (Figure 2.2A). In general, the C_4 species compared to the C_3 species grew fewer but bigger tillers with fewer flowering culms (Figures 2.2 and 2.3), produced smaller seeds which require stratification with more seeds per spikelet, and had a higher C:N that allocated more C to structural C than cell solutes. The C_3 species generally grew more but smaller tillers that then produced flowering culms (Figure 2.2A and Figure 2.3). The *Elymus* species and *C. latifolium* produce bigger seeds with fewer seeds per spikelet and allocated more C to cell solutes and lignin. The seeds of the *Elymus* species requires little or no stratification. The C_3 species generally allocate

In the multivariate PCA analysis, differences in species means were detected using MRPP and pairwise comparisons. The three top performers in ANPP (dry wt.) and the lowest performer *D. clandestinum* were significantly different from each other and from all other species in the multivariate analysis using all traits (Figure 2.2B). Plant height, tiller size, and the microscopic traits explained the variance for axis 1 and the mcroscopic traits explained the variance for axis 2 (Figure 2.2 B). The species means of *E. macgregorii*, *E. villosus*, and *E. hystrix* in multivariate analysis including all traits were statistically the same, and the species means of *P. anceps* and *A. virginicus* (Broom) were statistically the same for the analysis using all traits (Figure 2.2 B). Species groupings were similar between the analysis using all traits and the macroscopic traits analysis (Figure 2.2 B and C). ANPP (drywt), flowering culms, and tiller number explained the variance for axis 1, and plant height and tiller size explained the variance for axis 2 (Figure 2.2 C). In the microscopic trait analysis, only *P. anceps* and *E. virginicus* were not significantly different, and *C. latifolium* and *E. virginicus* were not significantly different (Figure 2.2 D). Total % N and C:N explained the most variance for axis 1, and lignin explained the most variance for axis 2 (Figure 2.2 D).

Drought effects

In the ANOVA analysis that included all species and both years, the performance trait (ANPP) had a significant species effects ($p<.0001$), year effect ($p=.01$) and species x year interaction effect ($p=.007$). All macroscopic and microscopic traits had a significant species effect (all $p<.0001$). Significant year effects were found for all macroscopic traits (all $p<.0009$) except for flowering culms ($p=.60$), and significant species x year interactions were found for all macroscopic traits (all $p<.004$). The microscopic traits of C:N % C and % N had significant year effects (all $p<.0001$) and species x year interaction effects (all $p<.003$). For the carbon components, hemicellulose and cellulose had a significant year effect (both $p=.0004$) and hemicellulose and lignin:N had significant species x year interaction

effects (both p=.02). Six separate multivariate PCA analyses were performed for 2010 and 2011 for all traits, macroscopic traits only, and microscopic traits only. While there were no clear species groupings, MRPP results revealed significant species effects (all p<.0001) and C_3 species vs. C_4 species effects (all p<.003) for all six PCA multivariate analyses.

Plant height was the trait that was most affected by drought whereby all species except for *T. flavus* grew significantly shorter plants in the dry than wet year (2010) (21.2 Figure 2.3). Percent tissue C was the next most significantly affected trait by drought whereby five species increased % C in the dry year. Four species decreased C:N and increased % N in the dry year (Table 2.2 and Figure 2.4). Hemicellulose significantly increased in the dry year for four species (Table 2.2 and Figure 2.4). All other traits had less species significantly affected by drought (Table 2.2)

From May to September in 2010 and 2011, plant traits were measured for each species at the time of peak biomass for both the monocultures and the species mixture treatment. Plant traits for the *Elymus* species were measured from mid-May to the beginning of June so these species should have been more affected by the winter drought as they overwinter their tillers and flowered before the 2010 summer drought (Figure 2.1). Plant traits for the other two C_3 species were measured from the end of May to the beginning of July so they may have been more affected by the 2010 summer drought. Plant traits of the C_4 species were measured from mid-July to mid-September so these species were actively growing during the 2010 summer drought.

While only *E. macgregorii*, *D. clandestinum*, and *C. latifolium* significantly decreased in the performance trait of ANPP in the dry year (2010), *E. virginicus* showed the same trend. This loss in ANPP may have been caused by the winter drought for *E. macgregorii* and *E. virginicus.* For the other two C_3 species, ANPP of *D. clandestinum*, and *C. latifolium* may have been more negatively affected by the summer drought. *E. macgregorii*, *D. clandestinum*, and *C. latifolium* were also the only species to reduce tiller size or tiller number in response to drought, and *A. virginicus* was the only species to increase tiller number in response to drought (Table 2.2 Figure 2.3). The number of flowering culms decreased for *C. latifolium* and increased for *A. virginicus* in the dry year (Figure 2.3). Considering the microscopic traits, *D. clandestinum* was the most affected by drought. *D. clandestinum* lowered C:N and lignin:N in the dry year by increasing % N and % hemicellulose, and lowering % cellulose. *E. macgregorii* decreased C:N in response to drought by increasing % N and increasing % C in the form of hemicellulose (Table 2.2 and Figure 2.3). The other three *Elymus* species decreased % C in response to drought. The microscopic traits of *C. latifolium* were not affected by drought.

Of the three C_4 species, *A. virginicus* was the most affected by drought in the macroscopic traits, and *P. anceps* was the most affected by drought in the microscopic traits. *T. flavus* was not affected by drought in the macroscopic traits, and *A. virginicus* was not affected by drought in the microscopic traits (Table 2.2). *A. virginicus* was the only species to increase ANPP, tiller number and number of flowering culms in the dry year.

Plasticity in plant traits was measured as the change in trait value between the 2 years (dry year – wet year) (Figures 1.3 and 1.4, Table 2.3). An ANOVA s was performed to see if plasticity of traits differed between the species. Plasticity in the performance trait of ANPP was not found to significantly differ between the species. However plasticity in tiller number, tiller size and plant height did significantly differ between species (Figure 2.3). Plasticity in the microscopic traits of % N, % C, C:N, % cell solutes, and ash/silica also were found to differ significantly between species. In the multivariate PCA analysis that included all plant traits, all species except *D. clandestinum* and *P. anceps* were similar in most traits, with the microscopic traits of % tissue N, % lignin, and C:N explaining the most variation between species (Figure 2.5). For the macroscopic traits analysis, plant height and tiller size explained the most variation in plasticity between the species. For the microscopic trait analysis, C:N, and % N explained the most variation in plasticity between the species (Figure 2.5).

To assess the plasticity of traits for each species, if the error bar for the plasticity of a trait mean did not cross the x-axis, it was considered plastic (Table 2.3, Figures 2.3 and 2.4). All species except for *E. villosus* and *A. virginicus* were plastic in more microscopic traits than macroscopic traits (Table 2.3). The four C_3 species that were plastic in the performance trait of ANPP also were plastic in the most number of traits. *E. macgregorii* (nine traits), *E. virginicus* (ten traits), *D. clandestinum* (ten traits), and *C. latifolium* (seven traits) were plastic in both macroscopic and microscopic traits (Figures 2.2 and 2.3). *E. villosus* and *E. hystrix* were plastic in a fewer number of traits (both six traits) but were plastic in both macroscopic and microscopic traits. Of the C_4 species, *T. flavus* was only plastic in the microscopic traits (6 traits). *P. anceps* also was plastic in the microscopic traits (six traits) and the macroscopic trait plant height *A. virginicus* was plastic in two macroscopic traits and two microscopic traits (Table 2.3, Figures 2.2 and 2.3).

Competition x drought

For the competition x drought analysis, only six species were used in the species mixture treatment so the three *Elymus* species (*E. macgregorii*, *E. villosus* and *E. hystrix*) were not included in this analysis. Also, *A. virginicus* was excluded from this analysis because it did poorly in the monoculture experiment in general. In the ANOVA analysis that included the five species, inter vs. intraspecific

competition, and both years (or drought effects), significant species effects were found for the performance trait of ANPP, all macroscopic traits, and all microscopic traits (all $p<.0001$). Significant drought effects were found for the performance trait ($p<.0001$), the macroscopic traits of tiller number ($p<.0001$), tiller size ($p=.03$), and plant height ($p<.0001$), and for all microscopic traits (all $p<.0001$). Significant competition effects were found for the performance trait ($p<.0001$) and the macroscopic traits ($p<.001$) but no microscopic traits. Significant species x competition interactions were found for the performance trait ($p<.0001$), all macroscopic traits (all $p< .0017$) and all microscopic traits (all $p<.02$). For the species x drought interaction, significant effects were found for the macroscopic traits of plant height ($p<.0001$) and number of flowering culms ($p=.0008$) along with all three microscopic traits (all $p<.0001$). For the competition x drought interaction, significant effects were found for only the macroscopic traits of tiller number ($p=.02$), plant height ($p=.003$) and culms ($p=.0006$) but no microscopic traits. For the three way interaction, significant effects were found for the performance trait ($p=.006$), the macroscopic traits of tiller size ($p=.01$) and plant height ($p<.0001$), and the microscopic trait of % C ($p=.02$). A multivariate PCA analysis was performed that included all species, inter vs. intraspecific competition, and both years. The MRPP results revealed significant species effects ($p<.0001$), C_3 species vs. C_4 species effects ($p<.0001$), inter vs. intraspecific competitive effects ($p=.0003$), and year effects ($p<.0001$). All five species were significantly different from each other in multivariate space ($p<.025$) (Figure 2.6).

The performance trait of ANPP was significantly different between inter vs. intra-specific competition treatments for all five species (Table 2.4 and supplemental). *E. virginicus* significantly increased ANPP in inter-specific competition, while the other four species significantly decreased ANPP in inter-specific competition (Figure 2.7). The number of flowering culms was significantly different between inter vs. intra-specific competition treatments for all five species, and tiller number, tiller size, and plant height was significantly different for four of the five species (Table 2.4 and supplemental). For *P. anceps*, tiller size and plant height was not significantly different, and *T. flavus* was not significantly different in tiller number between inter vs. intra-specific competition treatments (Table 2.4 and supplemental). *E. virginicus* was the only species to perform better inter-specifically for all macroscopic traits (Figure 2.7). The other four species performed better in intra-specific competition. The microscopic traits were much less affected by competition than the macroscopic traits (Figure 2.8). *D. clandestinum* and *C. latifolium* significantly lowered % N in interspecific competition, *E. virginicus* significantly lowered % C in interspecific competition, and *C. latifolium* increased C:N in interspecific competition (Figure 2.8). The three C_3 species were significantly affected by competition for the performance trait and all macroscopic traits. For the C_4 species, *P. anceps* was not significantly affected

by competition for the macroscopic traits of tiller size and plant height, and *T. flavus* was not significantly affected by competition for the macroscopic trait of tiller number. Only C_3 species showed significant differences in microscopic traits (Figure 2.8).

To assess how drought affected competitive ability, a measure of competitive plasticity was calculated for each trait (average species mixture treatment – monoculture treatment) and then compared between the drought year (2010) and the wet year (2011) (Figures 2.7 and 2.8). A trait was considered plastic if the error bar for the plasticity of a trait mean did not cross the x-axis (Figure 2.5, Figures 2.7 and 2.8). *E. virginicus* had higher ANPP in the species mixture treatment in both years and all higher macroscopic trait values except that tiller size showed no difference in competitive ability in the wet year. The other two C_3 species competed better in the monocultures in both years for ANPP and all macroscopic traits. The two C_4 species had higher ANPP in the monoculture in both years, and higher macroscopic trait values in the dry year. *P. anceps* showed no difference in trait values in the wet year for tiller size and flowering culms (Table 2.5). *T. flavus* showed no difference in trait values in the wet year for any macroscopic traits. *D. clandestinum* and *C. latifolium* showed differences in trait values in the wet year for all microscopic traits with a higher C:N in the mixture treatment. *E. virginicus*, *T. flavus*, and *P. anceps* showed more differences in trait values for microscopic traits in the dry year compared to the wet year.

A multivariate PCA analysis was performed for each species to determine if there were significant differences between the four treatments: the 2010 monoculture treatment, 2011 monoculture treatment, 2010 species mixture treatment, and 2011 species mixture treatment (Figure 2.9). All five species had significant competitive effects, and treatment effects (supplemental). Only *E. virginicus* did not have a significant year effect (supplemental). All four treatments were significantly different for *E. virginicus* and *D. clandestinum*. For *E. virginicus*, plant height and % C explained differences in drought effects, and macroscopic traits explained differences in inter vs. intraspecific competition (Figure 2.9). For *D. clandestinum*, plant height., tiller size, C:N and %N were the main traits that explained differences in drought effects, and tiller number and flowering culms explained differences in inter vs. intraspecific competition (Figure 2.9). For *C. latifolium*, the mixture 2011 treatment and the mono 2010 treatment were not significantly different (Figure 2.9) Plant traits that explained variation in competition and drought were not clear. The two C_4 species responded similarly to drought and competition as the PCA graphs look similar in both plant traits and species means. For *P. anceps*, the monoculture treatment and the species mixture treatment were not significantly different in 2011. The species mixture treatment in 2010 had lower trait values for all traits except for % N compared to the other three treatments. For *T.*

flavus, only the mixture treatment was significantly different from the other three treatments (Figure 2.9). The mixture 2010 treatment was negatively correlated to all traits except for % N.

Discussion

I hypothesized that the C_3 and C_4 species will differ in the macroscopic and microscopic plant traits that can be used to explain the performance trait (ANPP). The three top performing species used different strategies to produce ANPP. *T. flavus* grew the tallest plants with fewer but larger tillers that were supported by high amounts of recalcitrant C. *C. latifolium* grew more but smaller tillers than *T. flavus* that were tall with high amounts of recalcitrant C. *E. virginicus* was the most prolific producer of tillers, which were shorter and smaller and had high amounts of lignin and cell solutes compared to those of *C. latifolium* and *T. flavus*. The other three *Elymus* species were similar to *E. virginicus* but produced less tillers. In general, the C_3 species produced more smaller tillers with a lower C:N ratio that allocated more C to cell solutes than the C_4 species. In general, the C_4 species produced bigger but fewer tillers with a high C:N and allocated more C to lignin and cellulose than C_3 species. Because the *Elymus* species flower by late spring and are dormant during the hot summer months, allocating C to cell solutes may allow more plasticity in C allocation. The other two C_3 species (*D. clandestinum* and *C. latifolium*) were actively growing during the summer months so allocation to recalcitrant C may be beneficial for tiller structure during the dry months of summer.

My second hypothesis was that plant traits will be differentially affected in response to the habitat filters of drought and drought x competition. Drought affected four of the nine species for the performance trait, and plant height was the most affected macroscopic trait whereby eight of the nine species grew shorter plants in the dry year. The microscopic traits of % tissue N, % tissue C, C:N, % hemicellulose, and lignin/N were the most affected by drought. Thus, the microscopic traits were generally more effected by drought than the performance or macroscopic traits. Competition had a significant effect on the performance trait and all macroscopic traits, but a small effect on microscopic traits. Significant drought effects were found when interspecific competition was added for the three macroscopic traits of tiller number, plant height, and the number of flowering culms.

My third hypothesis was that in response to the habitat filters of drought and competition, C_3 species would show trait differences in the performance trait and macroscopic traits, and the C_4 species will be more stress tolerant and show trait differences in only microscopic traits. Only three C_3 species had significant performance trait differences in response to drought. Also, in response to drought, C_3 macroscopic trait values had more differences than the C_4 species macroscopic trait values. While the C_4 species microscopic trait values differed as expected, the C_3 species microscopic trait values differed as well. Thus, as predicted, in response to drought, C_3 species trait values differed in the performance trait

and macroscopic traits, and C_4 species trait values differed in the microscopic traits. However, the C_3 species microscopic trait values also differed which was not predicted.

In response to competition, differences in ANPP were found for all five species. The three C_3 species had significant traits differences for all macroscopic traits with fewer microscopic trait differences. For the C_3 species, the microscopic trait values of *E. virginicus* and *D. clandestinum* differed in one trait, and the microscopic trait values of *C. latifolium* different in two traits. For the C_4 species, only macroscopic trait values differed. Drought had a bigger effect when competing inter-specifically for two C_3 species (*E. virginicus* reduced tiller number and *D. clandestinum* reduced the number of flowering culms) and the two C_4 species (*P. anceps* reduced tiller number and *T. flavus* reduced tiller number and plant ht). Thus, as predicted, in response to competition, the trait values of the C_3 species were different in the performance trait and the macroscopic traits but were also different in the microscopic traits. However, the C_4 did not respond to competition as predicted as their trait values only differed in the performance trait and the macroscopic traits. All species except *C. latifolium* had macroscopic traits that were more sensitive to drought when competing inter-specifically compared to competing intra-specifically.

E. virginicus was a top performer in monoculture where it was the most prolific species at producing small tillers that became flowering culms. In response to drought, *E. virginicus* was plastic in ANPP, macroscopic traits and the most microscopic traits. In response to competition, *E. virginicus* was the only species that competed better in the species mixture treatment than the monoculture in both the wet and dry year. This same effect was seen for the macroscopic traits as well. In the species mixture treatment, *E. virginicus* had a competitive advantage of both light and space as this species began actively growing and flowered before the other species. At the time the other species were actively growing, the plants of *E. virginicus* were dying back which then lodged and further shaded out neighboring plants. For this reason, I think that the life history traits of *E. virginicus* had a bigger effect on competitive ability than plasticity in traits. The other three *Elymus* species were not top performers in monoculture as they produced less tillers than *E. virginicus* but have similar life history traits as *E. virginicus*. *E. macgregorii* was the earliest flowering species thus, ANPP was most likely negatively affected by the winter drought. The traits of *E. macgregorii* were plastic in both macroscopic and microscopic traits. The ANPP of *E. villosus* and *E. hystrix* was not affected by the winter drought.

Reduced ANPP of other two C_3 species were expected to be caused by the onset of the summer drought. *C. latifolium* was a top performer in the monoculture treatment where it grew a lot of relatively big and tall tillers. *D. clandestinum* was one of the lowest performers in monoculture where it produced

fewer, smaller, and shorter tillers than *C. latifolium*. *D. clandestinum* was more plastic than *C. latifolilum* in both macroscopic and microscopic traits. *D. clandestinum* was the only species that was plastic in all four macroscopic traits. In the dry year, *D. clandestinum* produced short plants with a low number of tillers and flowering culms, and in the wet year it produces tall plants with big tillers, which were probably due to tiller branching. *D. clandestinum* and *C. latifolium* had higher trait values in the monoculture than the species mixture treatment in both the dry year and the wet year for ANPP and all macroscopic traits. While both of these species were plastic in all three microscopic traits in the wet year, *C. latifolium* had no plastic microscopic traits, and *D. clandestinum* only was plastic in the microscopic trait of C:N in the dry year.

All species grew well in monoculture except for *A. virginicus*. The C_4 species plots were generally weedier in the spring than those of the *Elymus* species because the overwintered tillers of the *Elymus* began growing early in the spring before the weedy species became established. The C_4 species began growing later in the spring after the weedy species were well established. *A. virginicus* was the last species to begin in the growing season and generally remained in a rosette until it bolted in late summer to produce flowering culms. *A. virginicus* did not have the ability to bolt through the weedy layer of plants like the other two C_4 species. For this reason, *A. virginicus* was not a good competitor for light, which may explain in part why it is found on poor disturbed sites where competition for nutrients may be stronger than competition for light. This may also explain why *A. virginicus* was the only species to increase ANPP, tiller number, and the number of flowering culms in the dry year when the plots were less weedy and light competition may have been reduced compared to the wet year.

The ANPP was not affected by drought for any of the C_4 species even though they were actively growing during the summer. *T. flavus* was a top performer in the monoculture where it produced a low number of tillers that were big and taller tillers than the other species. *T. flavus* was the only species that was not plastic in plant height in response to drought. *P. anceps* and *A. virginicus* produced the same number of tillers but smaller and shorter tillers than *T. flavus*. In response to drought, *T. flavus* was not plastic in any macroscopic traits and *P. anceps* was only plastic in the macroscopic trait of plant height Both *T. flavus* and *P. anceps* were plastic in microscopic traits in response to drought. *T. flavus* and *P. anceps* had a higher ANPP in the monoculture compared to the species mixture treatment for both years. In the dry year (2010), *T. flavus* and *P. anceps* had higher macroscopic trait values in the monoculture. In the wet year (2011), *T. flavus* and *P. anceps* competed better in the species mixture treatment in both macroscopic and microscopic traits because the macroscopic traits for *T. flavus* were not different between the monoculture and species mixture treatment, and the macroscopic traits of tiller size and the number of flowering culms for *P. anceps* were not different between the monoculture and species mixture

treatment. Also, in the wet year, *T. flavus* and *P. anceps* increased %tissue C in the species mixture treatment.

My prediction that the four *Elymus* species will be the least affected by drought as their plant traits were measured before the summer drought was not supported. *E. macgregorii* and *E. virginicus* were plastic in ANPP which may have been caused by the winter drought. All four *Elymus* species were plastic in three macroscopic traits and at least three microscopic traits. *E. macgregorii* and *E. virginicus* had more plastic microscopic traits than *E. villosus* and *E. hystrix*. My prediction that the two C_3 species that were actively growing during the drought would be highly plastic in response to drought was partially supported. Both species were plastic in ANPP, and *D. clandestinum* was the only species that was plastic in all four macroscopic traits. My prediction that the C_4 species would be the least plastic and stress tolerant in response to drought was supported. Excluding *A. virginicus*, *T. flavus* was only plastic in six microscopic traits and *P. anceps* was plastic in seven microscopic traits and one macroscopic trait.

My last prediction that C_3 species will be more competitive in wet year and the C_4 species will be more competitive in the dry year was not supported. Because of the life history traits of *E. virginicus*, *E. virginicus* was a better competitor for light and space in the species mixture treatment in both years. Opposite of my prediction, the two C_4 species competed better in the species mixture treatment in the wet year.

In conclusion, the C_3 and C_4 grasses did differ in how they performed in the monoculture treatment which was generally explained by the trade-off of allocating biomass to fewer but bigger tillers, or more but smaller tillers. In response to interannual rainfall, plant height was most affected macroscopic trait in response to drought, and generally the microscopic traits were more affected than the performance trait and microscopic traits. In response to competition the performance and macroscopic traits were more affected than the microscopic traits. In response to interannual rainfall and competition, the C_3 species were plastic in the performance and macroscopic traits as predicted but were plastic in microscopic traits as well. The C_4 were stress tolerant in response to interannual rainfall as predicted but in response to competition, the C_4 species were plastic only in the performance and macroscopic traits which was opposite of what was predicted. Plasticity in trait values for the *Elymus* species may have been a result of the winter drought, and plasticity in trait values for the other two C_3 species may have been a result of the summer drought. *E. virginicus* was the best inter-specific competitor in both the wet and dry year which was most likely due to life history traits that give it a head start over the other species. All this evidence supports the idea that the C_3 species may be better adapted to the Bluegrass Savanna-Woodland's mesic heterogeneous environment. The *Elymus* species may be at a particular advantage

because they overwinter their tillers which then begins growing early in the spring. This early growth may give them a competitive advantage in both light and space over the later growing species. Also, the *Elymus* species are actively growing before the canopy closes on the Bluegrass Savanna-Woodland. All these factors would make them good candidate species in the restoration of this savanna-woodland.

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Tables

Table 2.1: The nine native perennial bunchgrass species used in this experiment listed in order of flowering time. The abbreviations are used in the tables and graphs.

Scientific Name	Abbreviation	Common Name	Photosynthetic Pathway
1. *Elymus macgregorii* R. Brooks & J.J.N. Campb.	Emg	Early wildrye	C_3
2. *Elymus villosus* Muhl. ex Willd.	Evl	Nodding wildrye	
3. *Elymus virginicus* L.	Evg	Virginia wildrye	
4. *Elymus hystrix* L.	Ehy	Bottlebrush	
5. *Dichanthelium clandestinum* (L.) Gould	Dclan	Deer tongue	
6. *Chasmanthium latifolium* (Michx.) Yates	Clat	River Oats	
7. *Panicum anceps* Michx.	Panc	Beaked panicgrass	C_4
8. *Tridens flavus* (L.) Hitchc.	Tflav	Purple top/grease grass	
9. *Andropogon virginicus* L,	Broom	Broomsedge	

Table 2.2: Plant traits that were significantly affected by interannual differences in rainfall for each species. (* p= .05, ** p<.001, *** p<.0001). For the macroscopic traits, the green cell indicates that the species did significantly better in the wet year, and the brown cell indicates that the species did significantly better in the dry year. For the microscopic traits, the green cell indicates that the trait value was significantly higher in the wet year, and the brown cell indicates that the trait value was significantly higher in the dry year.

Species x Drought Effects									
	C_3 species						C_4 species		
	Emg	Evl	Evg	Ehy	Dclan	Clat	Panc	Tflav	Broom
Performance trait									
ANPP	***				*	**			
Macroscopic traits									
tiller number	***					***			*
tiller size					***				
Plant ht.	**	***	***	***	***	*	***		***
Flowering culms						*			***
Microscopic traits									
%tissue N	***				***		*	*	
%tissue C	**	***	***	***			*		
C/N	**				***		*	*	
Mobile vs. structural Carbon components									
Lignin/N	*			*	*				
%cell solutes			*				*		
%hemicellulose	*		*		*		*		
%cellulose		*			*				
%lignin				*					
%ash/silica		***							

Table 2.3: Trait plasticity was measured as the change in trait value between the two years (dry year – wet year). To assess the plasticity of each trait, if the error bar for the plasticity of a trait mean did not cross the x-axis, it was considered plastic. If the cell is green, the trait value was higher in the wet year. If the cell is brown, the trait value was higher in the dry year.

Plasticity in response to drought									
	C_3 species						C_4 species		
	Emg	Evl	Evg	Ehy	Dclan	Clat	Panc	Tflav	Broom
Performance trait									
ANPP									
Macroscopic traits									
tiller number									
tiller size									
Plant ht.									
Flowering culms									
Microscopic traits									
%tissue N									
%tissue C									
C/N									
Mobile vs. structural Carbon components									
Lignin/N									
%cell solutes									
%hemicellulose									
%cellulose									
%lignin									
%ash/silica									

Table 2.4: Plant traits that were significantly affected by differences in competitive ability (Average mixture – monoculture). Mix indicates that the species had significantly higher trait values in the species mixture treatment, and mono indicates that the species had significantly higher trait values in the monoculture experiment.

Competitive effects for each species					
	C_3 species			C_4 species	
	Evg	Dclan	Clat	Panc	Tflav
Performance trait					
ANPP	mix	mono	mono	mono	mono
Macroscopic traits					
tiller number	mix	mono	mono	mono	
tiller size	mix	mono	mono		mono
Plant ht.	mix	mono	mono		mono
flowering culms	mix	mono	mono	mono	mono
Microscopic traits					
% tissue N		mono	mono		
% tissue C	mono				
C:N			mix		

Table 2.5: Trait plasticity was measured as the change in trait value between inter-specific competition (mix) and intra-specific competition (mono). To assess the plasticity of each trait, if the error bar for the plasticity of a trait mean did not cross the x-axis, it was considered plastic. Mono indicates a higher trait value in the monoculture treatment, and mix indicates a higher trait value in the species mixture treatment. Plasticity between inter and intra-specific competition was assessed for 2010 (dry year) and 2011 (wet year).

Plasticity in response to competition x drought										
	Evg		Dclan		Clat		Panc		Tflav	
	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011
Performance trait										
ANPP	mix	mix	mono	mono	mono	mono	mono	mono	mono	mono
Macroscopic traits										
Tiller #	mix	mix	mono	mono	mono	mono	mono	mono	mono	
Tiller size	mix		mono	mono	mono	mono	mono		mono	
Plant ht.	mix	mix	mono	mono	mono	mono	mono	mono	mono	
Culms	mix	mix	mono	mono	mono	mono	mono		mono	
Microscopic traits										
%tissue N	mono	mix		mono		mono	mix		mix	
%tissue C	mono			mono		mono	mono	mix		mix
C:N	mix	mono	mix	mix		mix	mono		mono	

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Supplemental

Table 2.6:ANOVA results for drought effects for all species. Significant p values are in bold.

Drought Effects									
Trait	Species effects			Y effects			Drought x species		
	Df	F	p	Df	F	p	Df	F	p
Performance trait									
biomass	8,151	12.6	**<.0001**	1,151	6.28	**0.0133**	8,151	2.78	**.0067**
Macroscopic traits									
tiller number	8,151	31.68	**<.0001**	1,151	11.52	**0.0009**	8,151	5.82	**<.0001**
tiller size	8,151	6.23	**<.0001**	1,151	431.4	**<.0001**	8,151	63.62	**<.0001**
plant height	8,151	213.9	**<.0001**	1,151	857.8	**<.0001**	8,151	43.48	**<.0001**
flowering culms	8,151	22.97	**<.0001**	1,151	0.28	0.5987	8,151	3.01	**0.0036**
Microscopic traits									
%tissue N	8,147	12.44	**<.0001**	1,147	37.08	**<.0001**	7,147	3.65	**0.0012**
%tissue C	8,147	9.2	**<.0001**	1,147	40.32	**<.0001**	7,147	4.61	**0.0001**
C/N	8,147	10.93	**<.0001**	1,147	24.73	**<.0001**	7,147	3.25	**0.0031**
Mobile vs. structural Carbon componentss									
%cell solutes	8,145	35.87	**<.0001**	1,145	1.81	0.1811	8,145	1.03	0.4138
%hemicellulose	8,145	8.78	**<.0001**	1,145	13.05	**0.0004**	8,145	2.41	**0.0182**
%cellulose	8,145	11.90	**<.0001**	1,145	12.94	**0.0004**	8,145	0.87	0.5411
%lignin	8,145	7.01	**<.0001**	1,145	0.54	0.4631	8,145	1.22	0.2917
%ash/silica	8,145	5.07	**<.0001**	1,145	0.03	0.8555	8,145	1.71	0.1017
Lignin/N	8,143	5.42	**<.0001**	1,143	2.90	0.0908	7,143	2.39	**0.0244**

Table 2.7: ANOVA results drought effects for each species. Significant values are in bold.

Drought effects for each species												
	E. macgregorri			***E. villosus***			***E. virginicus***			***E. hystrix***		
Trait	df	F	p	df	F	p	df	F	p	df	F	p
Performance trait												
Biomass	1,18	16.63	**0.0007**	1,18	0	0.9517	1,18	3.81	0.0667	1,17	0.05	0.8323
Macroscopic traits												
Tiller number	1,18	30.22	**<.0001**	1,18	3.5	0.4185	1,18	2.81	0.1107	1,17	1.77	0.2013
Tiller size	1,18	1.03	0.3237	1,18	766.14	0.0778	1,18	3.15	0.093	1,17	2.64	0.1288
Plant height	1,18	688.89	**<.0001**	1,18	1.7	**<.0001**	1,18	286.86	**<.0001**	1,17	498.26	**<.0001**
Culms	1,18	1.14	0.3	1,18	1.7	0.2083	1,18	0.07	0.7889	1,17	0.66	0.2013
Microscopic traits												
%tissue N	1,18	17.23	**0.0006**	1,18	17.59	0.6774	1,18	4.24	**0.0542**	1,17	0.03	0.8596
%tissue C	1,18	9.64	**0.0061**	1,18	3.57	**0.0005**	1,18	83.8	**<.0001**	1,17	21.19	**0.0003**
C/N	1,18	14.91	**0.0011**	1,18	3.57	0.0749	1,18	1.68	0.2119	1,17	0.02	0.8816
Mobile vs. structural Carbon components												
%cell solutes	1,18	0	0.9669	1,18	0.24	0.7687	1,17	5.99	**0.0256**	1,17	1.71	0.2079
%hemicellulose	1,19	7.34	**0.0144**	1,18	5.32	0.6294	1,17	6.75	**0.0188**	1,17	0.1	0.7532
%cellulose	1,20	3.85	0.0653	1,18	0.19	**0.0333**	1,17	3.96	0.0631	1,17	0.63	0.4383
%lignin	1,21	0.76	0.3961	1,18	28.14	0.6715	1,17	2.71	0.1178	1,17	7.37	**0.0147**
%ash/silica	1,22	0.42	0.5254	1,18	0.41	**<00001**	1,17	1.98	0.1776	1,17	0.09	0.7688
Lignin/N	1,23	5.74	**0.0277**	1,18		0.5305	1,17	0.12	0.7373	1,17	7.13	**0.0162**
%cell solutes	1,17	0.62	0.4429	1,16	5.07	**0.0468**	1,16	0.51	0.4843	1,6	0.56	0.481
%hemicellulose	1,17	0	0.9776	1,16	0.01	**0.0371**	1,16	3.35	0.0858	1,6	4	0.0924
%cellulose	1,17	1.84	0.1924	1,16	1.59	0.924	1,16	1.72	0.2087	1,6	0	0.9675
%lignin	1,17	0	0.9852	1,16	3.18	0.2233	1,16	1.3	0.2709	1,6	2.36	0.1754
%ash/silica	1,17	0.2	0.6584	1,16	1.56	0.0916	1,16	0.87	0.3657	1,6	0.83	0.3972
Lignin/N	1,17	0.44	0.517	1,16	1.56	0.2283	1,16	0.49	0.4952			

Drought effects for each species												
	D. clandestinum			***C. latifolium***			***P. anceps***			***T. flavus***		
Trait	df	F	p	df	F	p	df	F	p	df	F	p
Performance trait												
Biomass	1,18	4.67	**0.0444**	1,18	12.83	**0.0021**	1,18	0.01	0.9219	1,16	0.34	0.567
Macroscopic traits												
Tiller number	1,18	3.47	0.0788	1,18	30.18	**<.0001**	1,18	1.01	0.3271	1,16	1.01	0.3294
Tiller size	1,18	15.93	**0.0009**	1,18	0.03	0.8611	1,18	1.21	0.2854	1,16	0.81	0.3809
Plant height	1,18	128.4	**<.0001**	1,18	5.13	**0.0362**	1,18	28.32	**<.0001**	1,16	1.78	0.201
Culms	1,18	2.8	0.1117	1,18	4.36	**0.0513**	1,18	0.37	0.553	1,16	0	0.9735
Microscopic traits												
%tissue N	1,18	17.92	**0.0005**	1,18	1.21	0.2862	1,18	7.92	**0.0115**	1,16	6.45	**0.0218**
%tissue C	1,18	2.62	0.1227	1,18	0.01	0.932	1,18	6.3	**0.0218**	1,16	3.66	0.0737
C/N	1,18	19.28	**0.0004**	1,18	0.67	0.4254	1,18	7.27	**0.0148**	1,16	7.27	**0.0148**
Mobile vs. structural Carbon components												
%cell solutes	1,18	1.43	0.2474	1,17	0.62	0.4429	1,16	4.56	**0.0468**	1,16	0.51	0.4843
%hemicellulose	1,18	11.17	**0.0036**	1,17	0	0.9776	1,16	5.07	**0.0371**	1,16	3.35	0.0858
%cellulose	1,18	5.78	**0.0272**	1,17	1.84	0.1924	1,16	0.01	0.924	1,16	1.72	0.2087
%lignin	1,18	0.02	0.888	1,17	0	0.9852	1,16	1.59	0.2233	1,16	1.3	0.2709
%ash/silica	1,18	0.14	0.7087	1,17	0.2	0.6584	1,16	3.18	0.0916	1,16	0.87	0.3657
Lignin/N	1,18	5.37	**0.0324**	1,17	0.44	0.517	1,16	1.56	0.2283	1,16	0.49	0.4952

	A. virginicus		
Trait	df	F	p
Performance trait			
Biomass	1,18	2.48	0.146
Macroscopic traits			
Tiller number	1,18	7.04	**0.0242**
Tiller size	1,18	0.48	0.506
Plant height	1,18	28	**0.0004**
Culms	1,18	0.03	0.8771
Microscopic traits			
%tissue N			
%tissue C			
C/N			
Mobile vs. structural Carbon components			
%cell solutes	1,6	0.56	0.481
%hemicellulose	1,6	4	0.0924
%cellulose	1,6	0	0.9675
%lignin	1,6	2.36	0.1754
%ash/silica	1,6	0.83	0.3972
Lignin/N			

Table2.8: ANOVA results for competition x drought effects for all species including all interactions. Significant p values are in bold.

Competition x Drought effects															
Trait	**Species**			**competition**			**drought**			**Spec*comp**			**Spec*drought**		
	df	F	p	df	F	p	df	F	p	df	F	p	df	F	p
Performance trait															
ANPP	4,170	49.52	**<.0001**	1,170	32.89	**<.0001**	1,170	35.44	**<.0001**	4,170	18.89	**<.0001**	4,170	1.41	0.2318
Macroscopic trait															
tiller #	4,170	96.52	**<.0001**	1,170	34.61	**<.0001**	1,170	28.55	**<.0001**	4,170	12.57	**<.0001**	4,170	2.34	0.0569
tiller size	4,170	90.33	**<.0001**	1,170	11.21	**.001**	1,170	4.85	**0.0291**	4,170	4.52	**0.0017**	4,170	2.36	0.055
plant ht.	4,170	263.9	**<.0001**	1,170	35.69	**<.0001**	1,170	381.44	**<.0001**	4,170	7.08	**<.0001**	4,170	30	**<.0001**
culms	4,170	152.6	**<.0001**	1,170	29.82	**<.0001**	1,170	5.69	**0.051**	4,170	8.48	**<.0001**	4,170	5.01	**0.0008**
Microscopic traits															
% N	4,169	15.1	**<.0001**	1,169	0.25	.6172	1,169	101.22	**<.0001**	4,169	3.7	**0.0065**	4,169	8.54	**<.0001**
% C	4,169	8.16	**<.0001**	1,169	0.98	.3229	1,169	35.92	**<.0001**	4,169	3.24	**0.0135**	4,169	13.65	**<.0001**
C/N	4,169	13.51	**<.0001**	1,169	0.22	0.643	1,169	88.26	**<.0001**	4,169	3.01	**0.0199**	4,169	9.23	**<.0001**

Competition x Drought effects						
Trait	**Comp*drought**			**Spec*comp*drought**		
	df	F	p	df	F	p
Performance trait						
ANPP	1,170	3.78	0.0535	4,170	3.76	**0.0059**
Macroscopic trait						
tiller #	1,170	5.25	**0.0232**	4,170	1.77	0.138
tiller size	1,170	2.45	0.1196	4,170	3.28	**0.0127**
plant ht.	1,170	9.04	**0.003**	4,170	7.7	**<.0001**
culms	1,170	12.27	**0.0006**	4,170	1.71	0.1503
Microscopic traits						
% N	1,169	2.8	0.0963	4,169	2.13	0.079
% C	1,169	0.38	0.5367	4,169	2.9	**0.0234**
C/N	1,169	3.39	0.0672	4,169	2.26	0.0643

Table 2.9: ANOVA for each species for competition x drought

	E. virginicus			*D. clandestinum*			*C. latifolium*			*P. anceps*			*T. flavus*		
trait	df	F	p	df	F	p	df	F	p	df	F	p	df	F	p
Performance trait															
biomass															
Competition	1,36	25.22	**<.0001**	1,36	52.81	**<.0001**	1,36	23.13	**<.0001**	1,36	21.30	**<.0001**	1,31	7.89	**0.0081**
Drought	1,36	0.77	0.3873	1,36	5.96	**0.0195**	1,36	12.92	**0.001**	1,36	2.12	0.1545	1,31	0.10	0.7579
CxD	1,36	1.77	0.1919	1,36	0.39	0.5356	1,36	0.2	0.6541	1,36	2.57	0.1175	1,31	0.71	0.4051
Macroscopic traits															
tiller number															
Competition	1,36	19.05	**0.0001**	1,36	61.22	**<.0001**	1,36	13.84	**0.0007**	1,36	17.46	**0.0002**	1,31	2.29	0.1406
Drought	1,36	6.09	**0.0185**	1,36	2.01	0.1645	1,36	14.71	**0.0005**	1,36	5.30	**0.0272**	1,31	7.94	**0.0083**
CxD	1,36	0.04	0.8350	1,36	1.38	0.2474	1,36	0.82	0.3672	1,36	1.33	0.2572	1,31	1.97	0.1701
tiller size															
Competition	1,36	17.32	**0.0002**	1,36	5.93	**0.0200**	1,36	7.59	**0.0091**	1,36	3.71	0.0620	1,31	6.59	**0.0153**
Drought	1,36	2.75	0.1057	1,36	8.87	**0.0052**	1,36	1.16	0.2893	1,36	0.43	0.5170	1,31	0.76	0.3893
CxD	1,36	16.54	**0.0002**	1,36	2.26	0.1416	1,36	1.68	0.2038	1,36	0.29	0.5907	1,31	3.81	0.0599
plant height															
Competition	1,36	19.25	**<.0001**	1,36	5.97	**0.0199**	1,36	63.31	**<.0001**	1,33	1.37	0.2503	1,31	7.97	**0.0082**
Drought	1,36	6.09	**0.0185**	1,36	200.59	**<.0001**	1,36	27.46	**<.0001**	1,33	37.91	**<.0001**	1,31	5.80	**0.0221**
CxD	1,36	0.04	0.8350	1,36	2.04	0.1623	1,36	8.29	**0.0067**	1,33	0.02	0.8976	1,31	15.85	**0.0004**
culms															
Competition	1,36	15.42	**0.0004**	1,36	5.70	**0.0223**	1,36	23.32	**<.0001**	1,36	13.42	**0.0008**	1,31	6.63	**0.0150**
Drought	1,36	0.01	0.9437	1,36	5.17	**0.0290**	1,36	10.82	**0.0023**	1,36	0.28	0.5990	1,31	3.44	0.0731
CxD	1,36	0.23	0.6334	1,36	0.29	0.5939	1,36	0.17	0.6787	1,36	2.07	0.1585	1,31	6.13	**0.0189**

	E. virginicus			*D. clandestinum*			*C. latifolium*			*P. anceps*			*T. flavus*		
trait	df	F	p	df	F	p	df	F	p	df	F	p	df	F	p
Microscopic traits															
%tissue N															
Competition	1,36	0.08	0.7733	1,36	5.67	**0.0277**	1,36	11.09	**0.002**	1,36	0.00	0.9729	1,30	2.67	0.1130
Drought	1,36	0.19	0.6663	1,36	45.69	**<.0001**	1,36	10.86	**0.0022**	1,36	15.12	**0.0004**	1,30	35.72	**<.0001**
CxD	1,36	5.78	**0.0215**	1,36	1.77	0.1923	1,36	1.81	0.1872	1,36	0.98	0.3290	1,30	2.56	0.1202
%tissue C															
Competition	1,36	9.90	**0.0033**	1,34	1.95	0.1716	1,36	1.72	0.1985	1,36	2.87	0.0989	1,30	1.72	0.1997
Drought	1,36	124.81	**<.0001**	1,34	3.52	0.0694	1,36	2.57	0.1176	1,36	0.65	0.4313	1,30	3.45	0.0733
CxD	1,36	0.0	0.9523	1,34	0.34	0.5641	1,36	6.45	**0.0156**	1,36	0.21	0.6501	1,30	2.98	0.0946
C/N															
Competition	1,36	0.03	0.8700	1,34	3.40	0.0741	1,36	7.34	**0.0103**	1,33	1.65	0.2079	1,30	1.27	0.2691
Drought	1,36	0.35	0.5570	1,34	38.82	**0.0001**	1,36	14.08	**0.0006**	1,33	22.63	**<.0001**	1,30	22.77	**<.0001**
CxD	1,36	4.96	**0.0322**	1,34	1.72	0.1982	1,36	6.49	**0.0153**	1,33	0.94	0.3386	1,30	2.80	0.1044

Table 2.10: ANOVA results for plant trait differences (Avg mixture – monoculture) between the dry year (2010) and the wet year (2011) for each species.

ANOVA results for differences in plant traits between inter vs. intra specific competition between the dry and wet year															
	E. virginicus			*D. clandestinum*			*C. latifolium*			*P. anceps*			*T. flavus*		
trait	df	F	p	df	F	p	df	F	p	df	F	p	df	F	p
Performance trait															
biomass	1,18	5.54	**0.0301**	1,18	0.01	0.9111	1,18	1.51	0.2354	1,18	8.64	**0.0088**	1,17	4.71	**0.0444**
Macroscopic traits															
tiller number	1,18	0.12	0.7291	1,18	7.65	**0.0127**	1,18	4.46	**0.0488**	1,18	10.73	**0.0333**	1,17	1.89	0.1871
tiller size	1,18	35.72	**<0.0001**	1,18	0.71	0.409	1,18	1.38	0.2559	1,18	2.78	0.113	1,17	2.78	0.1139
plant height	1,18	0.07	0.7896	1,18	5.54	**0.0302**	1,18	20.78	**0.0002**	1,18	0	0.968	1,17	58.51	**<.0001**
culms	1,18	3.48	0.0785	1,18	2.75	0.1143	1,18	0.37	0.5512	1,18	6.35	**0.0214**	1,17	7.33	**0.0156**
Microscopic traits															
%tissue N	1,18	10.69	**0.0043**	1,18	1.95	0.1796	1,18	7.99	**0.0112**	1,18	2.15	0.1596	1,16	6.85	**0.0187**
%tissue C	1,18	5.79	**0.027**	1,18	1.57	0.2256	1,18	4.2	**0.0554**	1,18	5.31	**0.0333**	1,16	3.89	0.0662
C/N	1,18	11.68	**0.0031**	1,18	4.89	**0.0402**	1,18	11.94	**0.0028**	1,18	1.07	0.315	1,16	4.3	**0.0546**

Table 2.11: ANOVA results for competition (monoculture vs. mixture), drought (dry year vs. wet year), and drought x competition interaction . (* p= .05, ** p<.001, *** p<.0001). The species shaded in blue are the C_3 species and the species shaded in orange are the C_4 species. For competition, the pink indicates that the species did significantly better in monoculture, and the blue indicates the species did significantly better in mixture. For drought, the green arrow indicates that the species did significantly better in the wet year, and the brown arrow indicates it the species did significantly better in the dry year.

Competition, Drought, and Competition x Drought interactions					
	C_3 species			C_4 species	
	E. vrg	*D. clan*	*C. lat*	*P.anc*	*T. flav*
Performance trait					
biomass					
Comp	***	***	***	***	**
Drought		↑ *	↑ **		
CxD					
Macroscopic traits					
tiller number					
Comp	***	***	***	***	
Drought	↑ *		↑ ***	↑ *	↑ **
CxD					
tiller size					
Comp	***	*	**		*
Drought		↑ **			
CxD	***				
Plant ht.					
Comp	***	*	***		**
Drought	↑ *	↑ ***	↑ ***	↑ ***	↑ *
CxD			**		***
Flowering culms					
Comp	***	*	***	***	*
Drought		↑ *	↑ **		
CxD					*
Microscopic traits					
%tissue N					
Comp		*	**		
Drought		↑ ***	↑ **	↑ ***	↑ ***
CxD	*				
%tissue C					
Comp	**				
Drought	↑ ***				
CxD			*		
C/N					
Comp			*		
Drought		↑ ***	↑ ***	↑ ***	↑ ***
CxD	*		*		

Table 2.12: Multivariate competitive results performed for each species. MRPP pairwise comparisons determined by differences between intra vs. interspecific competition (monoculture treatment vs. species mixture treatment), between the 2010 and 2011, differences between the four treatments.

Multivariate analysis for each species using all traits					
	Evg	Dclan	Clat	Panc	Tflav
p values determined by MRPP					
Intra vs. interspecific comp	<.0001	.002	<.0001	.01	.005
Year	0.79	<.0001	.0012	<.0001	.013
treatment	<.0001	<.0001	<.0001	<.0001	<.0001

Figures

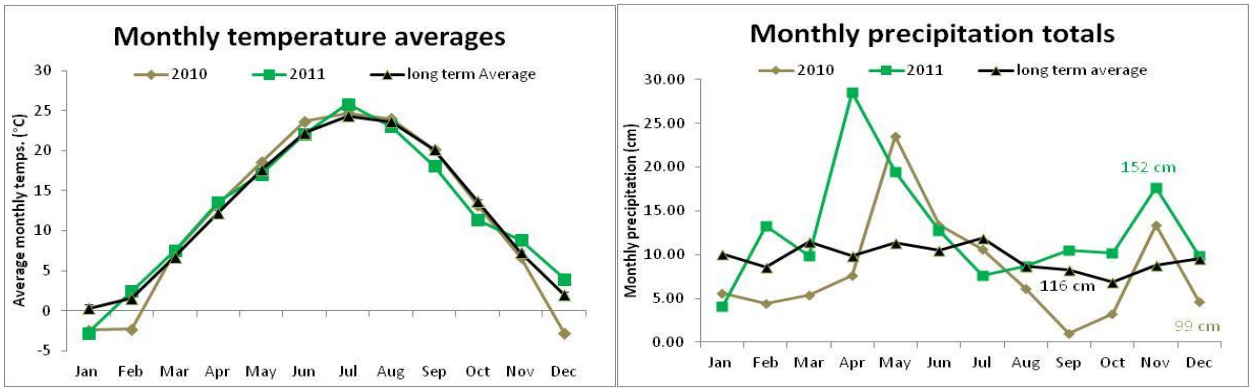


Figure 2.1: Monthly temperature averages for the drought year (2010) and the wet year (2011) compared to the long term average in the Bluegrass Region of Kentucky (± 1std error). Monthly precipitation totals for the drought year (2010) and the wet year (2011) compared to the long term average in the Bluegrass Region of Kentucky (± 1std error). The color coded numbers are yearly precipitation totals.

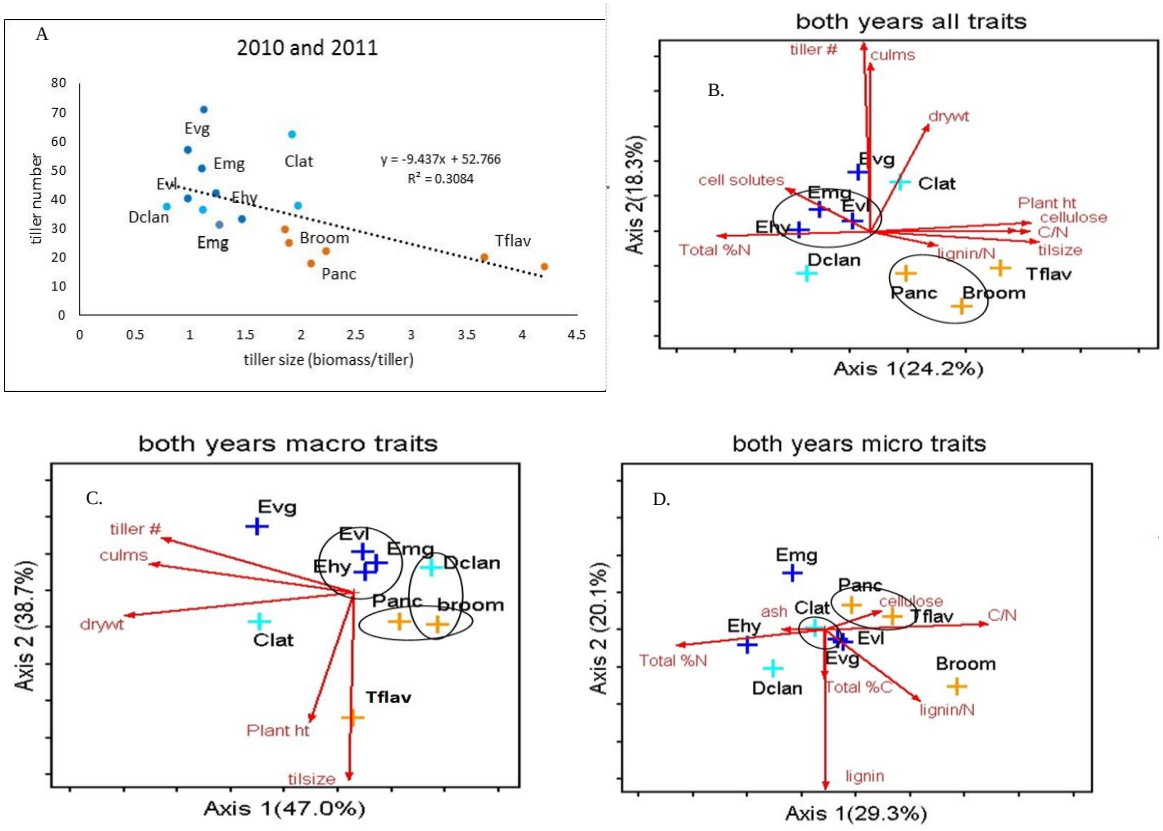
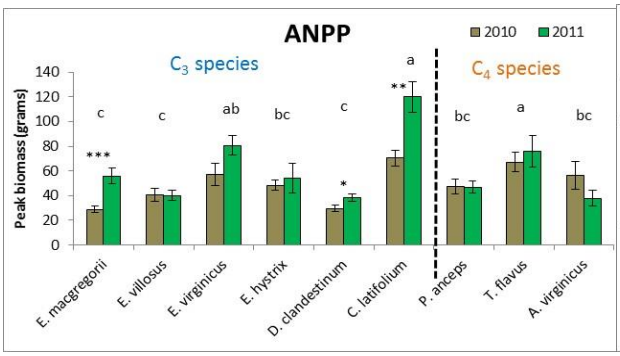
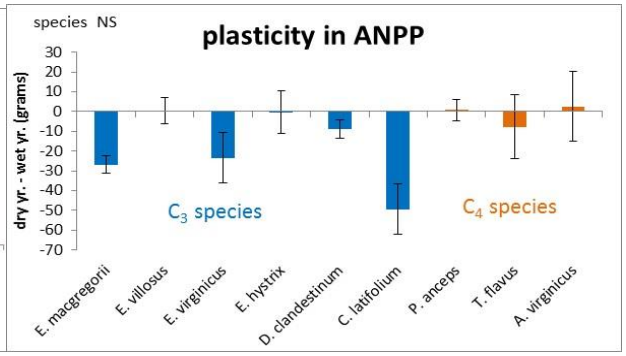
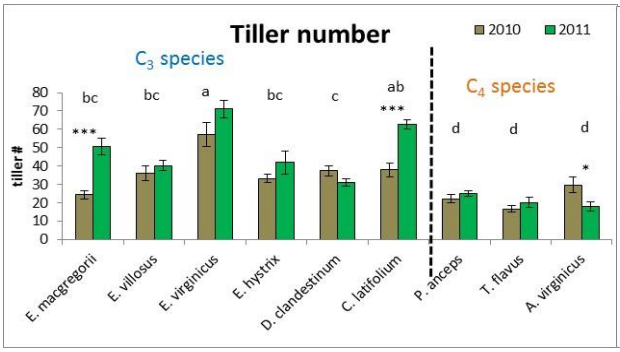
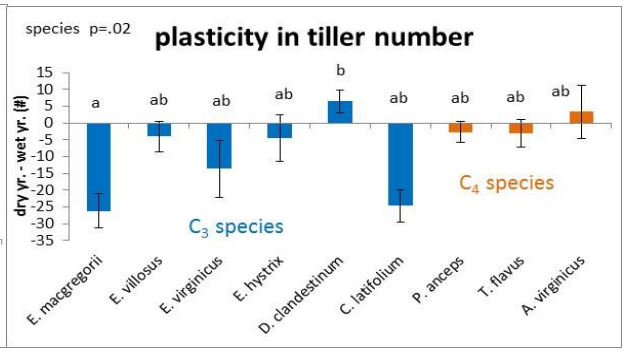


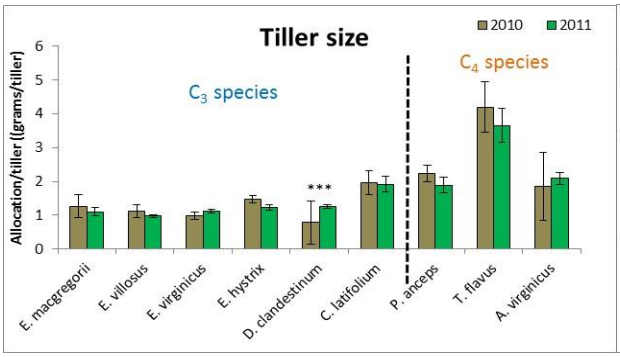
Figure 2.2: A. regression of tiller size and tiller number using data from 2010 and and 2011. B. C. and D. PCA results including all traits and both years, macroscopic traits for both years, and microscopic traits for both years. The circles indicate the species means that are not significantly different in pairwise comparisons using MRPP (p<.025). The percent of variance explained for each axis is in parenthesis.

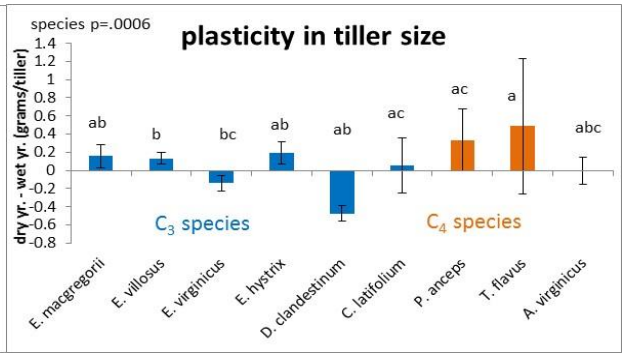

ANPP
2010
2011
C3 species
C4 species
Peak biomass (grams)
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus

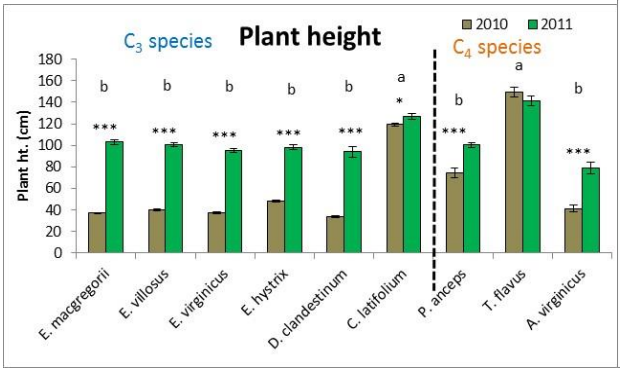

species NS
plasticity in ANPP
dry yr. - wet yr. (grams)
C3 species
C4 species

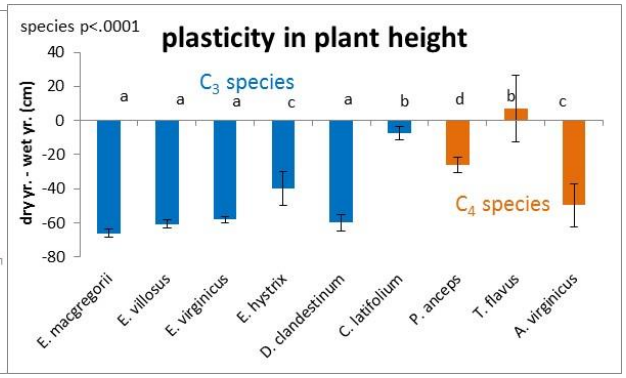

Tiller number
2010
2011
C3 species
C4 species
tiller #


species p=.02
plasticity in tiller number
dry yr. - wet yr. (#)
C3 species
C4 species


Tiller size
2010
2011
C3 species
C4 species
Allocation/tiller (grams/tiller)


species p=.0006
plasticity in tiller size
dry yr. - wet yr. (grams/tiller)
C3 species
C4 species


Plant height
2010
2011
C3 species
C4 species
Plant ht. (cm)


species p<.0001
plasticity in plant height
dry yr. - wet yr. (cm)
C3 species
C4 species

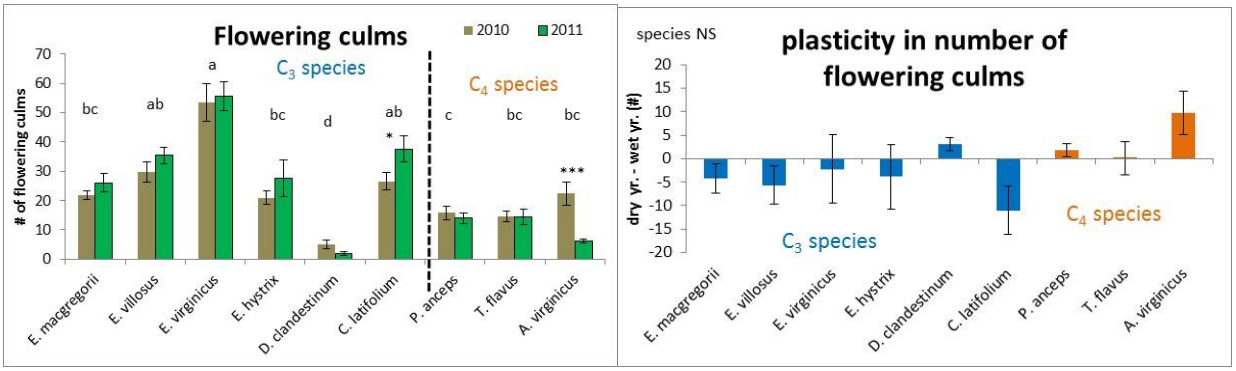
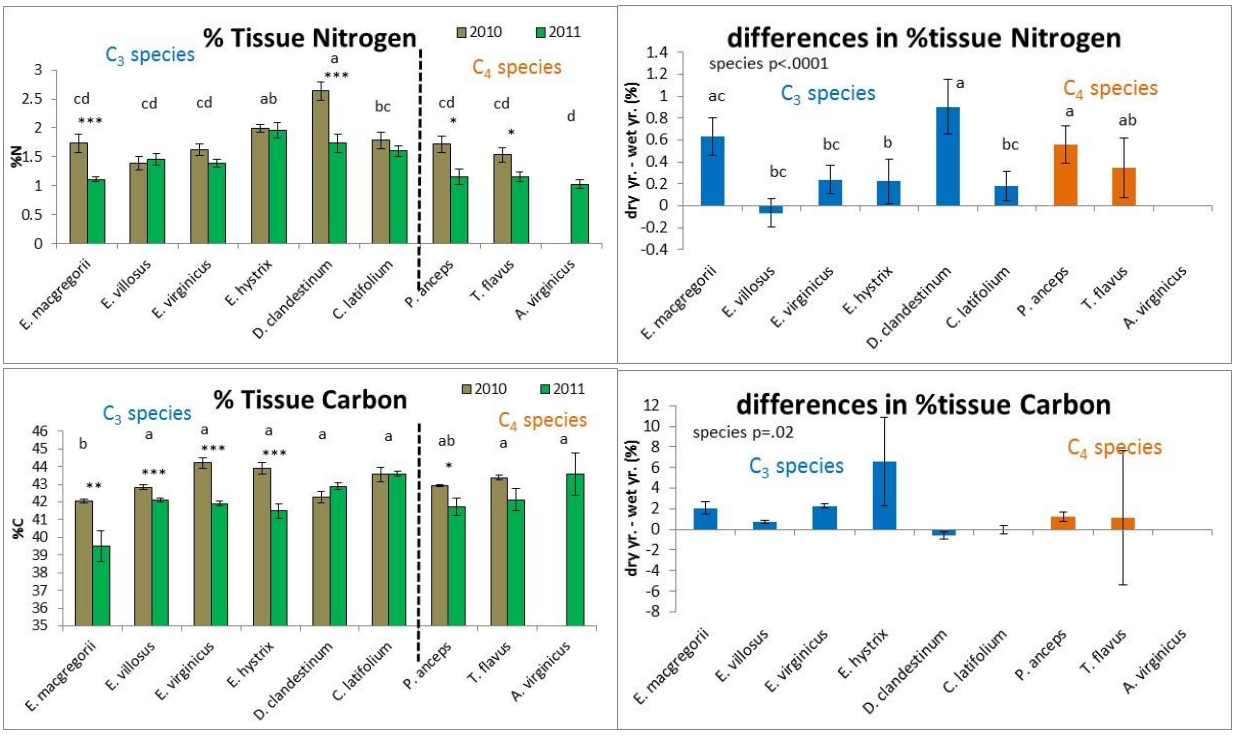
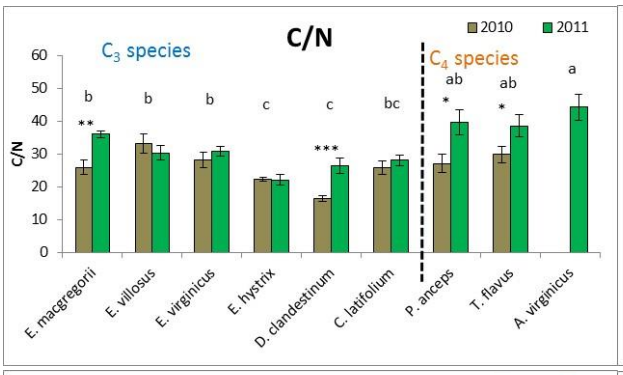
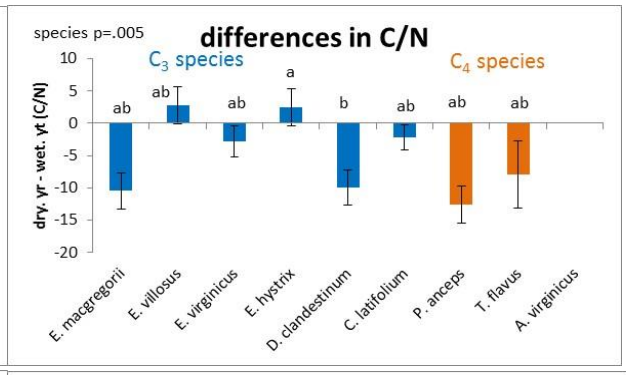
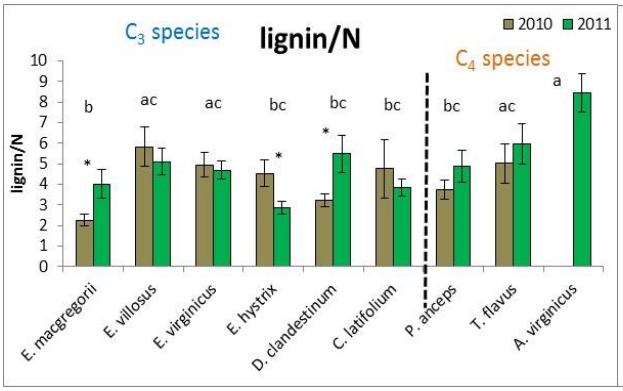


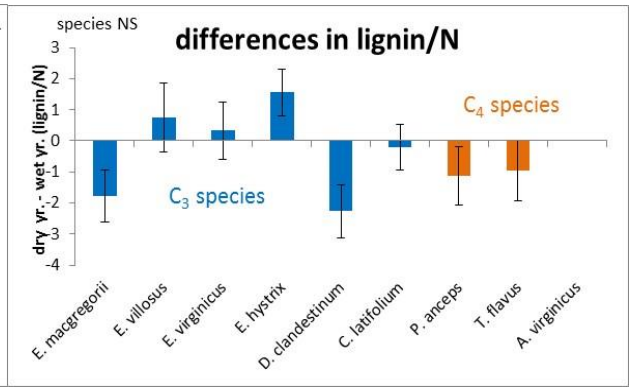
Figure 2.3: Drought effects for the Performance trait (ANPP) and macroscopic traits measured at the time of peak biomass. The species are listed on the x-axis in order of their flowering time with mean (± 1 SE). The left panel of graphs are species meansg (± SE) for each year (2010= dry year and 2011= wet year). The letter indicate significant differences between species with both years combined ($p<.05$). The asterisks above the bars indicate a significant difference between the years for a species (* $p<.05$, ** $p<.001$. *** $p<.0001$). The right panel of graphs shows the species means (± 1 SE) for plasticity or change in trait values between the two years (dry yr – wet yr.) If the bar is above the line it had a higher value in the dry year. If the bar is below the line it had a higher value in the wet year. Different letters represent significant differences between species means (P value ≤ 0.05) determined by adhoc Tukeys.

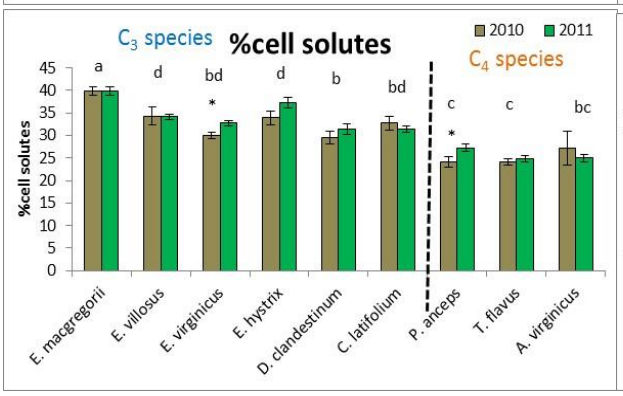


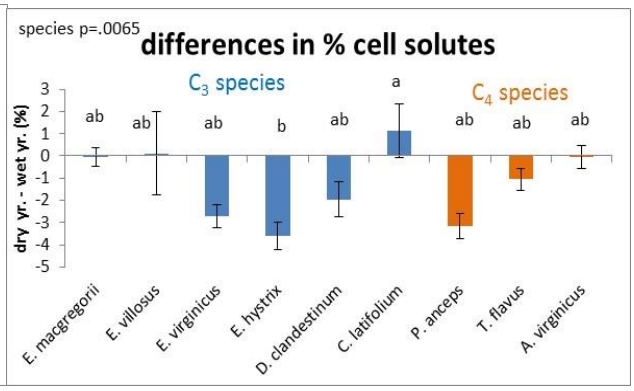

C/N
2010
2011
C3 species
C4 species
C/N
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus

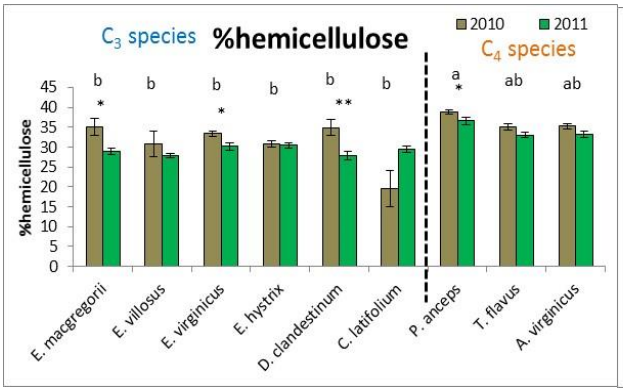

species p=.005
differences in C/N
C3 species
C4 species
dry. yr - wet. yt (C/N)
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus

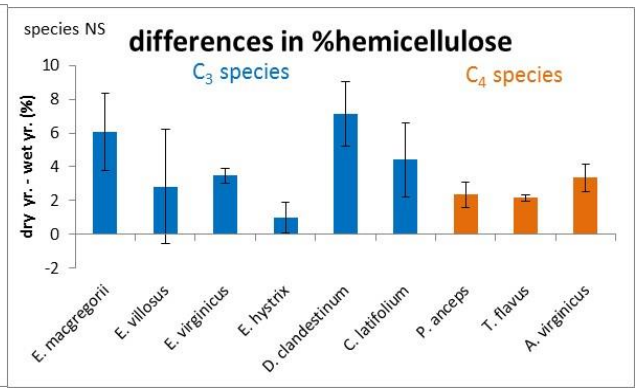

C3 species
lignin/N
2010
2011
C4 species
lignin/N
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus


species NS
differences in lignin/N
C4 species
C3 species
dry yr. - wet yr. (lignin/N)
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus


C3 species
%cell solutes
2010
2011
C4 species
%cell solutes
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus


species p=.0065
differences in % cell solutes
C3 species
C4 species
dry yr. - wet yr. (%)
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus


C3 species
%hemicellulose
2010
2011
C4 species
%hemicellulose
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus


species NS
differences in %hemicellulose
C3 species
C4 species
dry yr. - wet yr. (%)
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus

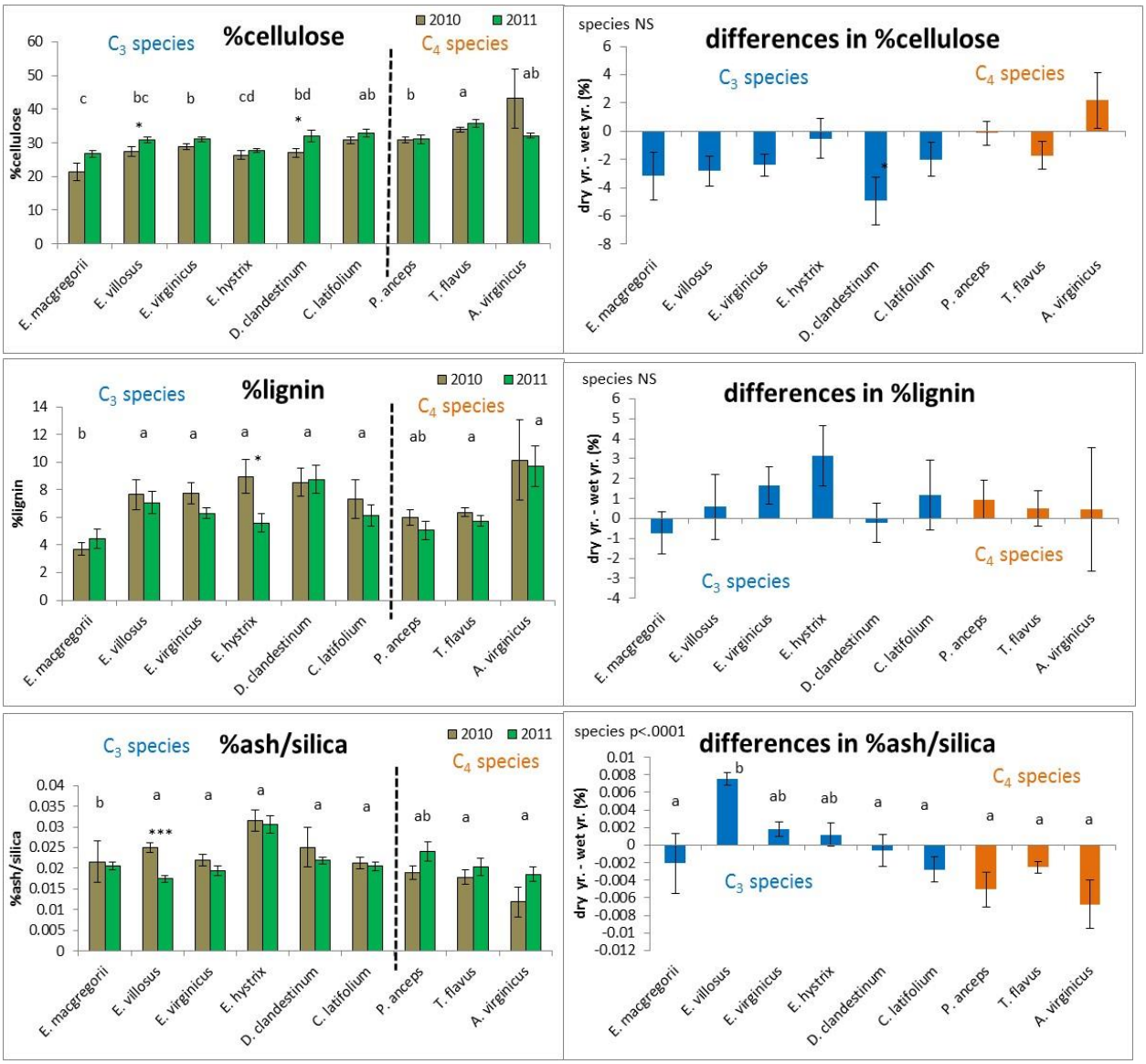


Figure 2.4: Drought effects for microscopic trait graphs for drought effects measured at the time of peak biomass. The species are listed on the x-axis in order of their flowering time with mean (± 1 SE). The left panel of graphs show species averages (± SE) for each year (2010= dry year and 2011= wet year). The letter indicate significant differences ($p<.05$) between species with both years combined. The asterisks above the bars indicate a significant difference between the years for a species (* $p<.05$, ** $p<.001$. *** $p<.0001$). The right panel of graphs shows the species means (± 1 SE) for plasticity or change in trait values between the two years (dry yr – wet yr.) If the bar is above the line it did better in the dry year. If the bar is below the line it did better in the wet year. Different letters represent significant differences between species means ($p<0.05$) determined by adhoc Tukeys.

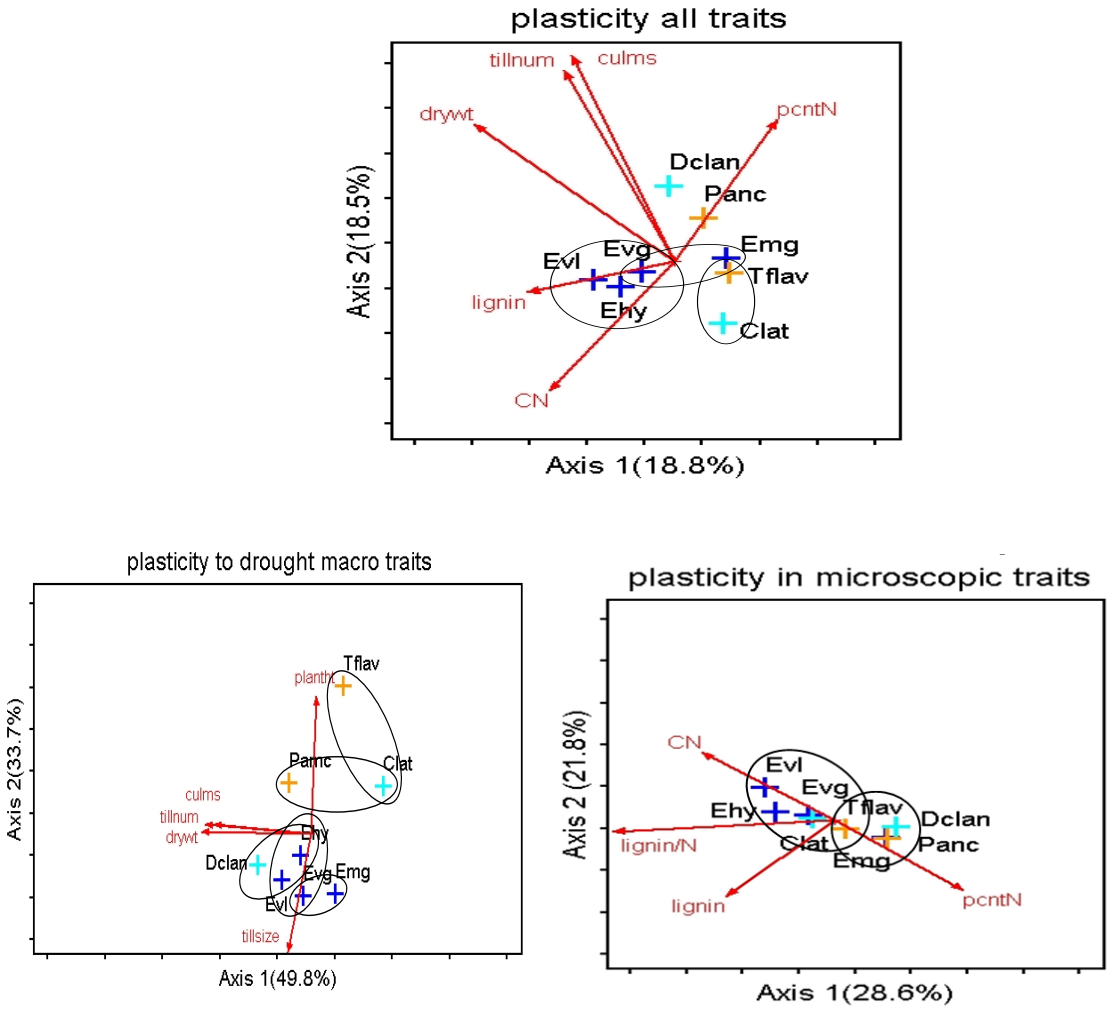


Figure 2.5: PCA drought results including all plasticity traits, macroscopic plasticity traits, and microscopic plasticity traits. Plasticity trait values are dry year- wet year. The circles indicate the species means that are not significantly different in pairwise comparisons using MRPP ($p<.025$). The percent of variance explained for each axis is in parenthesis beside the axis.

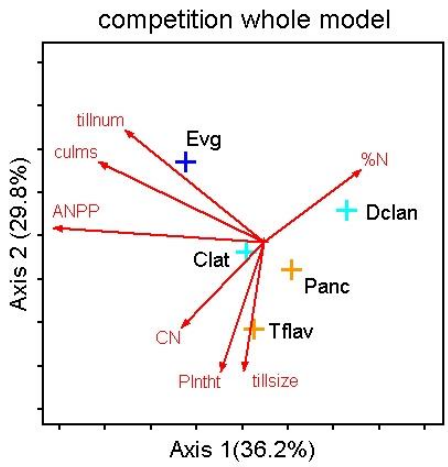


Figure 2.6: PCA results for competitive effects including all species, all traits, mono vs mixture treatments, and both years. All species means were significantly different determined by pairwise comparisons using MRPP ($p<.025$). The percent of variance explained for each axis is in parenthesis beside the axis.

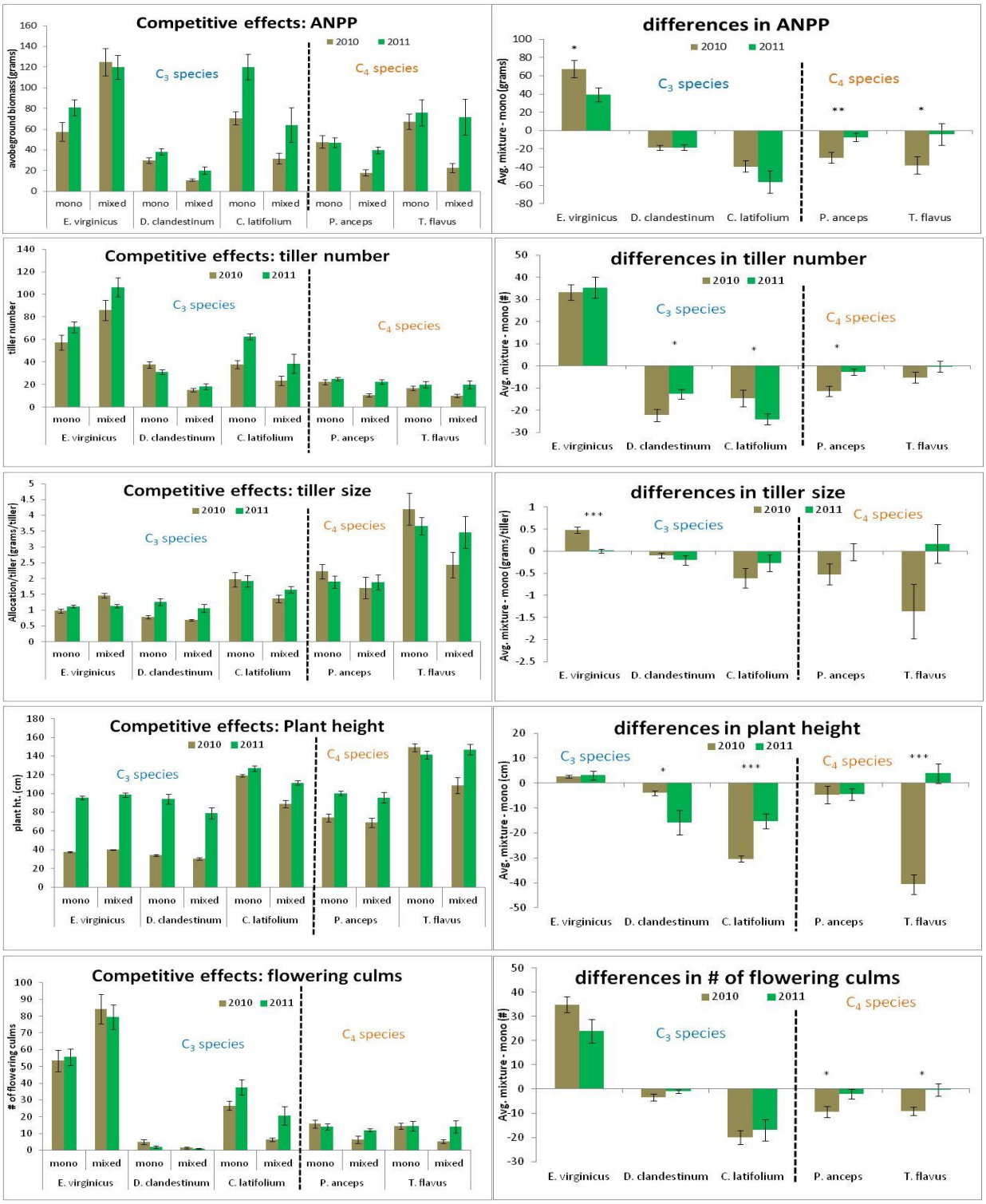


Figure 2.7: Performance and Macroscopic trait graphs for differences in competitive ability between the two years (2010= dry year and 2011= wet year). The left side is the species means raw data and the right side is difference in competitive ability (Avg. mixture – monoculture) for the two years. If the bar is positive, it did better in the mixture treatment than the monoculture. If the bar is negative, it did better in the monoculture. The bigger the bar, the bigger difference there was between Average mixture and the monoculture. Error bars are ±1std error. (* p<.05, ** p<.001. *** p<.0001

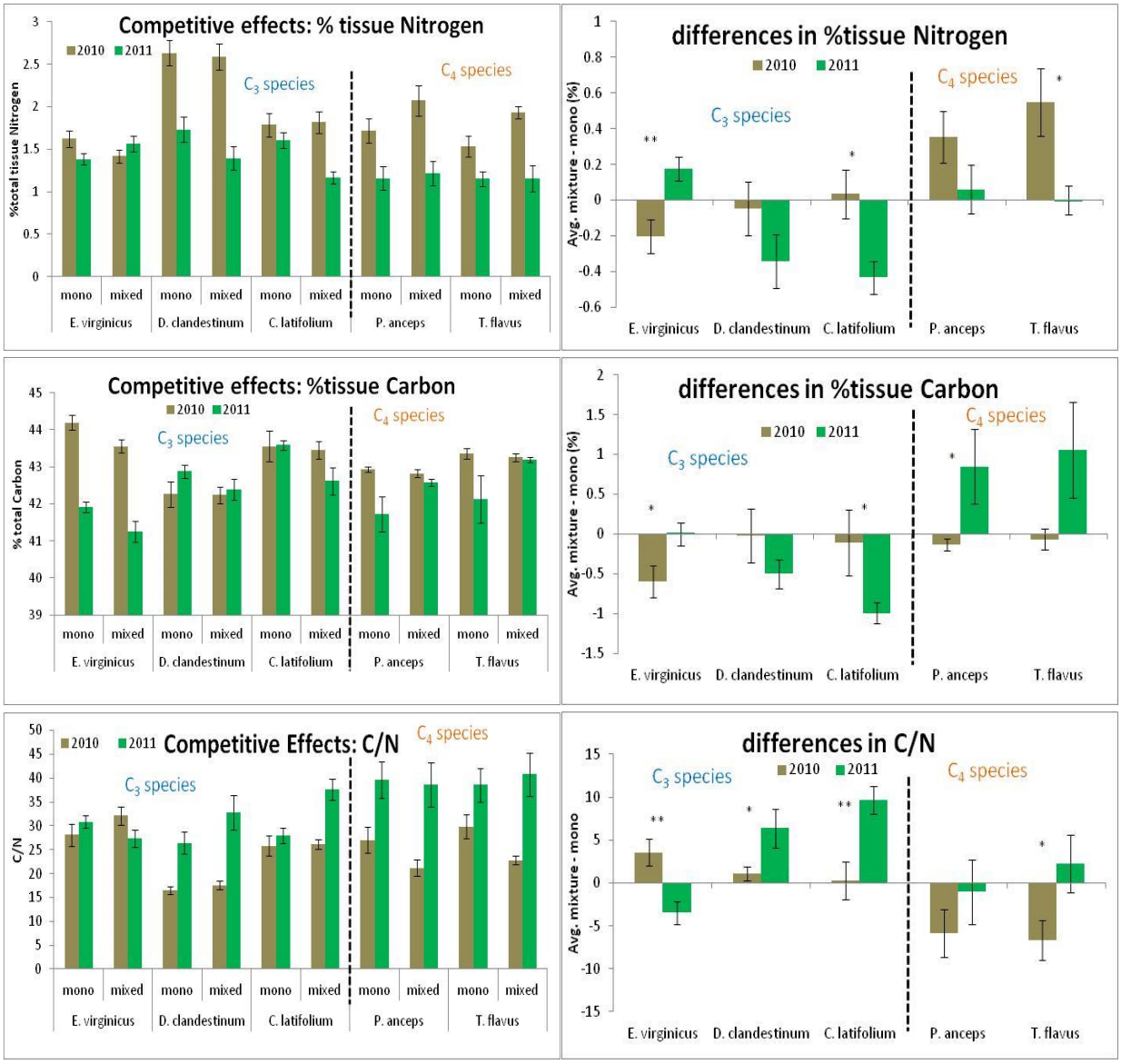


Figure 2.8: Microscopic trait graphs for differences in competitive ability between the two years (2010= dry year and 2011= wet year). The left side is the raw data and the right side is difference in competitive ability (Avg. mixture – monoculture) for the two years. If the bar is positive, it did better in the mixture treatment than the monoculture. If the bar is negative, it did better in the monoculture. The bigger the bar, the bigger difference there was between Average mixture and the monoculture. Error bars are ±1std error. (* p<.05, ** p<.001. *** p<.0001

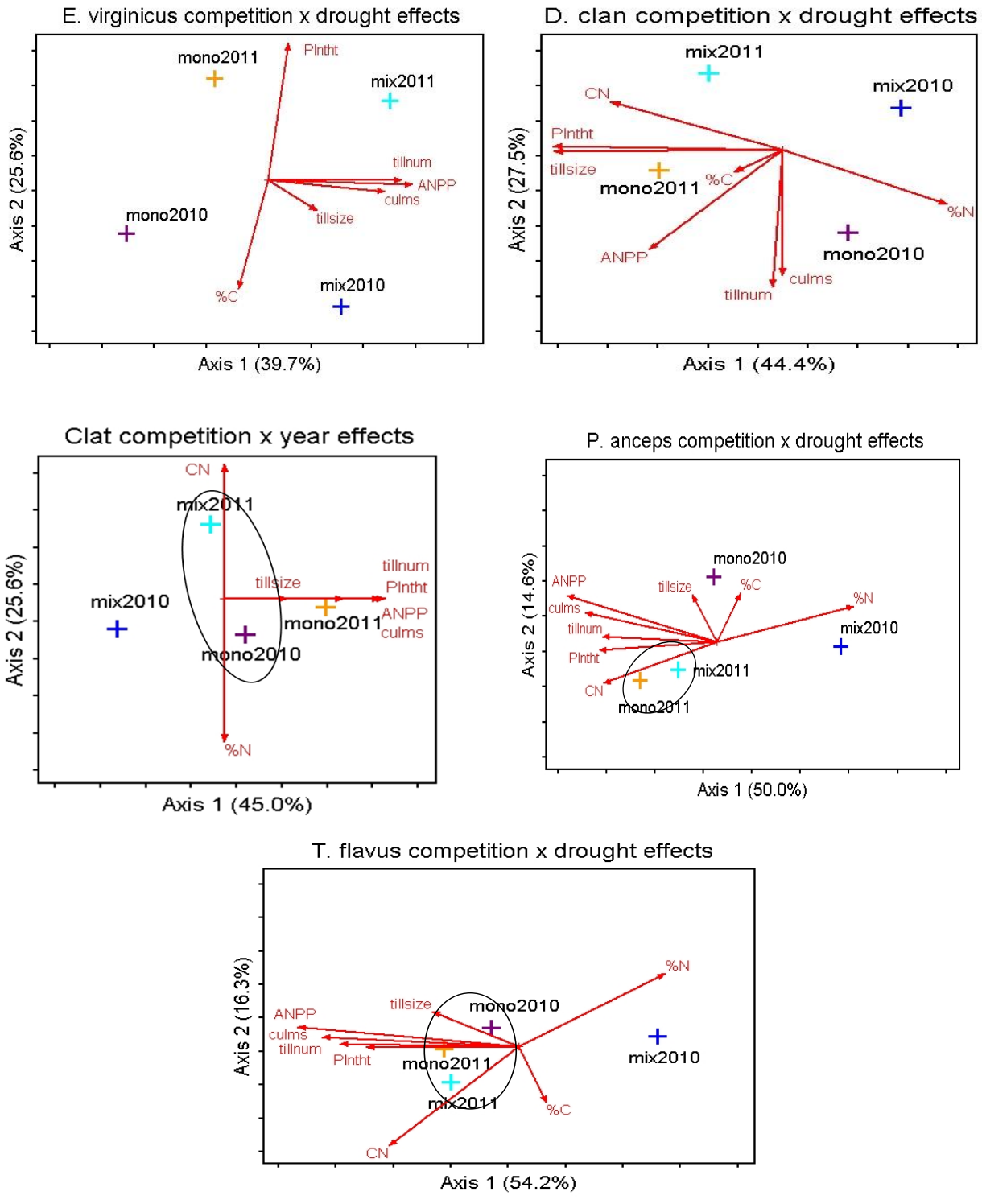


Figure 2.9: Competition and drought PCA results for each species comparing treatment means for all traits. The circles indicate the species means that are not significantly different in pairwise comparisons using MRPP ($p<.025$). The percent of variance explained is denoted in parenthesis for each axis.

Chapter 3: Differences in ecosystem properties between C_3 and C_4 grasses native to a historic North American Oak Savanna

Abstract

Since oak savannas of North America have been reduced to < 1 % of their historic ranges, restoration of these habitats are important to maintain the biodiversity and ecosystem functioning of these landscapes. Efforts to restore these oak savannas are hindered by the lack of dependable historic data describing these savannas before they were largely converted to other uses and by lack of restoration ecology guidelines for ecological restoration. To better understand the ecosystem dynamics of the herbaceous layer of a temperate oak savanna, a field monoculture experiment was performed to assess the ecosystem characteristics of six C_3 and three C_4 native bunchgrasses. This information can then be used to help predict how these species might function in a community setting, and to recommend ecological restoration guidelines to restore and maintain a functional grassland community assembly of the historic Bluegrass Oak Savanna-Woodland of Kentucky. This methodology can also be used in other savanna systems to better understand savanna grassland ecosystem functioning.

The monoculture experiment included nine native bunchgrass species that were replicated 10 times to produce 90-2 meter^2 plots in a completely randomized design. The three experiments included in this study are: 1) plant characteristics were measured at peak biomass for each species, 2) a litter decomposition experiment was performed over 15 months, 3) a resin nitrate (NO_3^-) and ammonium (NH_4^+) bag experiment was performed during the growing season of 2010, and 4) a soil nutrient study was performed in 2008 and again in 2012. The goal of this study was to measure the plant traits of these species and determine if the species were fast N cycling or slow N cycling species. The C_3 species were predicted to be fast N cycling species as they were thought to be found in more mesic eutrophic soils where N is less likely to be limiting. The C_4 species were predicted to be slow N cycling species as they were thought to be found in more nutrient poor and disturbed sites where N is more likely to be limiting. My results found that only C_3 species had trait values that promoted fast N cycling, but both C_3 and C_4 species had traits values that promoted slow N cycling. I concluded that N is not limiting in this experiment because: 1) decomposition of species with low quality litter was not hindered, 2) retention of N was found in the litter bags for all species, and 3) resin NO_3-N and NH_4-N levels were similar for species that were predicted to promote both mineralization and immobilization. Soil nutrient studies supported these observations as the nine species were not found to differentially deplete soil N levels. These findings do not support the resource-competition theory which is dependent upon N limitation. The results of this study suggests that other factors such as fire or grazing may have bigger impacts on the community setting of these grasses than competition for limiting N.

Introduction

Savannas are grassland ecosystems characterized by the trees being sufficiently small or widely spaced so that the tree canopy is not closed (McPherson 1997) and are influenced by fire, climate, topography and soil (Nuzzo 1986). Savannas constitute 20 % of the Earth's land area and can be divided into tropical and temperate groups. Tropical savannas cover 15 % of the Earth's land area, are generally well represented in the scientific literature, and are extensive in Africa, Australia, and S. America (McPherson 1997). While temperate savannas of North America were historically common at the time of European settlement, most of these landscapes have been reduced to less than 1 % of their original area, are considered to be endangered landscapes (Anderson, Fralish et al. 1999), and are identified as critical areas for preservation (Klopatek, Olson et al. 1979). Furthermore, temperate savannas are not as well studied or represented in the scientific literature as tropical savannas(McPherson 1997, Anderson, Fralish et al. 1999). Some potential reasons for this difference in the level of research activity are the absence of a professional discipline associated with savannas, limited understanding of the role and importance of savannas in temperate regions, and inconsistent definitions and/or interpretations of the term savanna (McPherson 1997). Thus, there is a lack of knowledge of the ecological relationships and ecological management practices for temperate savannas compared to adjacent forest, desert, or grassland landscapes (McPherson 1997).

With European settlement in the eighteenth century, Midwestern Oak savannas in the USA all but disappeared within 20 to 40 years due to fire cessation and conversion of land to agricultural or urban development (Nuzzo 1986, Anderson, Fralish et al. 1999). The fact that only 2 % of Midwest Oak Savannas remained by 1986 (Nuzzo 1986) has caused this habitat to be listed as a "globally imperiled" ecosystem (Heikens and Robertson 1994). Conservation and restoration efforts of oak savannas are difficult due to: 1) the limited amount of historical data which was recorded mainly by European pioneers and land surveyors, and the unknown validity and motivation for these records (Nuzzo 1986), and 2) lack of restoration ecology studies to guide ecological restoration practices in the field (McPherson 1997).

Benefits to restoring these historic landscapes include an increase in biodiversity and restoration of ecosystem functioning (McPherson 1997). Ecosystem functioning is influenced by climate, topography, nature of parent material, living organisms, and time (Brady 1990). Ecosystem functioning of oak savannas is further influenced by the more interactive factors such as fire, grazing, biogeochemical processes, and biotic interactions (Chapin, Matson et al. 2002). Savanna grasses can impact ecosystem functioning through positive or negative feedback loops that can influence fire or grazing frequencies, nutrient cycling, and soil nutrient availability (Bardgett, Mawdsley et al. 1999, Knops, Bradley et al. 2002).

Wei et al. (2013) characterized soil C and N stoichiometry is a main driver of ecosystem functioning of temperate grasslands in part because N is found to be a primary limiting nutrient in many grasslands (Polley and Detling 1988, Schlesinger 1991, Vitousek and Howarth 1991). Nitrogen is cycled through the plant, soil, and microbial communities and is intricately linked to the C cycle (Knops, Bradley et al. 2002, Wei, Yu et al. 2013). Plant species take up available N from the soil, produce biomass which eventually decomposes and returns the C and N back to the soil. Soil microbes regulate mineralization and mobilization rates of N in part by the amount of N in soil organic matter, and the quantity and quality of C and N of decomposing plant organic matter. Mineralization and immobilization of N by the soil microbes in turn determines the amount N that is available for plant uptake.

Plant species differ in the amount and quality of biomass and litter they produce, N uptake rates, and the efficiency with which they use N (Wedin and Tilman 1990, Knops, Bradley et al. 2002, Jiang, Han et al. 2011). These species differences can result in positive or negative feedback loops (Vitousek 1982). A positive feedback loop is the result of a plant species that produces a low C:N biomass with high percent N and a low percent C particularly low in the recalcitrant forms of lignin and cellulose. This low C:N biomass produces a high quality litter that is then easily decomposed by the soil microbes. When soil microbes are not limited by N, mineralization occurs which coverts organic N to plant available inorganic N that is optimized under warm, moist soil conditions (McClellan, Deenik et al. 2007). Thus, fast N cycling species cycle nitrogen more rapidly through the plant, litter, and soil, and promotes plant available N. Similarly, a plant species that produces a high C:N biomass with low percent N and a high percentage of C particularly recalcitrant C. This high C:N biomass produces a low quality litter that is N limited that promotes immobilization. Immobilization is the process whereby soil microbes use inorganic N to break down the N limited litter that can ultimately result in soil N deficiencies and limit plant N availability (McClellan, Deenik et al. 2007). Thus, slow N cycling species cycle less nitrogen more slowly through the plant litter and soil which limits plant available N. Also since lignin in the litter turns into humic substances in soil organic matter, litter with high amounts of lignin leads to higher soil organic C and N pools (De Deyn, Cornelissen et al. 2008). Moreover, slow N cycling plants should be selected for when water or nutrients are limiting plant growth. Thus, N limitation creates a positive feedback that results in slower C cycling and an increase of soil organic C and N (De Deyn, Cornelissen et al. 2008).

N cycling is also influenced by a plants ability to retranslocate N from dying aboveground biomass to belowground parts, and synchronizing the seasonality of plant activity with precipitation. Grass species are known to vary in their ability of retranslocate N from dying aboveground biomass to crowns and roots (Norris and Reich 2009). Retranslocation of N is thought to be favored in N limited

environments where the conservation of N is important, and less important in more mesic environments where soil N may not limiting (Norris and Reich 2009). The retranslocation of N increases the C:N of the litter and promotes immobilization which in turn further limits plant available N. Moreover, plant uptake of N is also dependent on water availability as uptake of nitrate (NO_3^-) and ammonium (NH_4^+) happens in the root hairs and is dependent on water. Plants that temporally synchronize N uptake at the time they are most actively growing with seasonal precipitation can show an increase of % tissue N. This would lower C:N of litter and promote mineralization and increase plant available N. Plants that do not synchronize plant N uptake with precipitation could experience decreased plant N availability and uptake, and possibly the accumulation of nitrates in the soil that then becomes susceptible to leaching (McCulley, Burke et al. 2009).

While these positive and negative feedback loops can have effects at the ecosystem scale, plant available N also differs at more local scales that can select for fast or slow N cycling species. This local scale can have major impacts on the spatial distribution of species in a community. At the ecosystem level, the resource-competition theory predicts that in N limited environments, the species that conserves N and most efficiently reduces soil N availability will have a competitive advantage over neighboring species that have higher N demands (Fargione and Tilman 2006). In N limiting conditions, slow N cycling species are predicted be better competitors than fast N cycling species which would result in increased production and abundance of slow N cycling species in the community (Fargione and Tilman 2006). However if N is not limiting, faster cycling N species should be at a competitive advantage which would result in their increased production and abundance in the community. At more local scales, fast N cycling species would persist on more fertile sites, and slow N species would persist on less fertile and more disturbed sites.

The purpose of this study is to test these theories using six C_3 grasses and three C_4 perennial bunchgrasses to help predict a functional grassland community setting as part of the ecological restoration of the historic Oak Savanna-Woodland located in the Inner Bluegrass Region of Kentucky. U.S.A. The Bluegrass Savanna-Woodland was considered by Braun (1943) to be anomalous or unexpected in the middle of the mixed mesophytic forest biome. Wharton and Barbour (1991) characterized this area as a savanna-woodland with an open forest dominated by trees but retaining a well-developed grassy undergrowth. This savanna-woodland was best described at the time of European settlement in the mid to late 1700's as having rolling topography that is mildly karst, a highly phosphatic Ordovician Limestone parent material that produces a silt loam soil that is fertile, deep, and well drained, vast cane breaks (*Arundinaria gigantea*), large mature trees including Oak (*Quercus*) and Ash (*Fraxinus*), and a C_3 graminoid dominated herbaceous layer (McInteer 1952, Wharton and Barbour 1991, Campbell 2004).

With European settlement, native grasses were rapidly replaced by non-native C_3 forage grasses (*Festuca arundinacea, and Poa pretensis*) so that no intact savanna grassland remains in this region (Bryant, Wharton et al. 1980). The C_3 grasses were thought to be dominant in both abundance and number of species where they frequented the woodlands (Wharton and Barbour 1991) with mesic eutrophic soils as well as the more open woods (Campbell 2004). The C_4 grasses were predicted to be fewer in the number of species and found in local openings on poorer soils or openings created by disturbance such as fire or bison trails (Campbell 2004).

These nine grasses were grown in a monoculture experiment to assess the plant traits associated with N and C cycling of each species individually. I hypothesized that:

1) The C_3 grasses will promote fast N cycling, and C_4 grasses will promote more conservative or slow N cycling.

2) If N is limiting at the ecosystem level, fast N cycling species should deplete soil N less than slow N cycling species according to the resource-competition theory.

Results of this experiment can be used to better understand the dynamics of this savanna-woodland and how these nine species would function in a community. This study also uses methodology that could be used in other savanna landscapes that could guide ecological restoration efforts of these endangered oak savanna landscapes.

Materials and Methods

Study Site

The experiment was conducted in a relatively flat, tall fescue (*Festuca arundinacea*) dominated abandoned paddock located at Griffith Woods Wildlife Management Area (WMA). Griffith Woods WMA is considered to be the best Bluegrass Savanna-Woodland remnant in the Inner Bluegrass Region of Kentucky. It includes 746 acres in southern Harrison County, Kentucky (Latitude N 38.33457, Longitude W -84.354) and lies on the northern edge of the Inner Bluegrass Region of Kentucky. While the vegetation of Griffith Woods WMA is known for its remnant Blue Ash-Oak savanna-woodland with 150 – 350 year old trees of *Fraxinus quadrangulata* (Blue Ash), *Quercus macrocarpa* (Burr Oak), *Quercus muhlenbergii* (Chinquapin Oak), and *Quercus shumardii* (Shumard Oak), the herbaceous layer is dominated by non-native C_3 forage grasses (*Festuca arundinacea, and Poa pretensis*). While there is a long history of human occupation and agricultural use (Wharton and Barbour 1991), one management goal was to restore a portion of the property back to pre-European settlement savanna–woodland vegetation. Ecological restoration efforts include native tree planting, native cane planting (*Arundinaria gigantea*), and invasive species removal.

The Inner Bluegrass Region of Kentucky encompasses about 2,400 square miles and is underlain by Ordovician Limestone which was pushed up over the millennia by the Jessamine Dome of the Cincinnati Arch that has produces a mildly karst rolling topography (Wharton and Barbour 1991). This highly phosphatic limestone produces a silt loam soil that is fertile, deep, and well drained (Wharton and Barbour 1991). The warm, temperate, and humid climate is continental and highly variable (Wharton and Barbour 1991). Average yearly precipitation for the Bluegrass Region is 112 cm/year with typical wet springs and dry autumns (Wharton and Barbour 1991). On Average the growing season is 181 days with average annual temperature of 13° Celsius with generally mild winters and hot summers (Wharton and Barbour 1991).

Species

The nine native bunchgrasses (Wharton and Barbour 1991, Campbell 2004) included in this study are listed in Table 3.2.1in the order of their flowering times. The nine species are categorized in two functional groups C_3 (or cool season) grasses and C_4 (or warm season) grasses. The six C_3 grasses included in this study are associated with wooded habitats, and the three C_4 species are associated with more open habitats (Wharton and Barbour 1991, Campbell 2004). Four of the C_3 grasses are *Elymus* species or wildryes. The *Elymus* species are well documented in historical records and are thought to have been abundant at the time of European settlement in the mid to late 1700's (Wharton and Barbour 1991). *Elymus virginicus* is common in open woods, thickets and old fields, and *Elymus villosus* is frequent in dry and moist open woods (Wharton and Barbour 1991). *Elymus macgregorii* can be confused with *E. virginicus* but flowers a month earlier and is also found in woods and thickets (Committee 2002), and *Elymus hystrix* is frequent in the woods (Wharton and Barbour 1991). The *Elymus* species have a different life history pattern with significant niche differentiation from the other species. They flower in the spring or early summer, set seed, and then go dormant during the hottest months of the summer. They regrow tillers in the fall which overwinter and produce flowering culms the next spring.

Dichantheilium clandestinum may have been referred to as buffalos grass in historical records where it is frequent in open woods, thickets, and fencerows, especially on low ground (Wharton and Barbour 1991). *D. clandestinum* also has life history traits that differ from the other species in this study. *D. clandestinum* first produces cleistogamous flowering culms, and then later in the season they produce self-fertilizing chasmogamous flowers on small inflorescences that are usually hidden within the sheathes. Both types of flowers produce viable seeds. While this species did not produce a lot of tillers, it had the greatest ability for tiller branching, so one tiller could be quite large and heavy. *Clandestinum latifolium* is frequent on wooded stream banks, on floodplains, and in other moist habitats (Wharton and Barbour 1991). *C. latifolium* is also used for in horticultural plantings and can be known to be quite invasive.

The three C_4 species are generally found in more open sites. *P. anceps* is found less commonly and on moist ground, and *T. flavus* is common in old fields, woodland borders, open woods, pastures, and roadsides (Wharton and Barbour 1991). *Andropogon virginicus* is common in old fields and overgrazed pastures (Wharton and Barbour 1991). *A. virginicus* grew really well the first year it was planted, but did successively worse each year.

Experimental procedures

Seeds for each species were collected in the Bluegrass Region of Kentucky and cold (wet) stratification requirements were determined through the seed testing laboratory at the Regulatory Services at the University of Kentucky. The stratified seeds were germinated in a heated greenhouse on a flooding table in 72 hole plant trays filled with Pro-Mix potting soil. These plugs were planted in the field plots at 169 plugs/2 meter^2 plot with a hand trowel to minimize disturbance.

In a completely randomized design, nine native bunchgrass species monocultures plus one species mixture treatment were each replicated 10 times to produce 100-2 meter^2 plots. The species mixture treatment was not included in this analysis.

Initial preparation of the field site included mowing after which the grass clippings were raked into piles and burned. The field was then sprayed with Roundup herbicide at recommended concentrations to kill all the vegetation. A second application of Roundup was applied to areas that did not die back after the initial Roundup treatment. The plots were watered as needed with a garden hose after initial planting, and rainfall was recorded at the site. The C_3 species were planted in March through May, and the C_4 species were planted in June and July. The first field season (2008) *Elymus virginicus*, *Elymus villosus*, *Elymus mcgregorii*, *Panicum anceps*, *Tridens flavus*, and *Andropogon virginicus* were planted with the remaining species planted the second growing season (2009). An 18 inch path was maintained around each of the plots by mowing. The experiment and the surrounding area were maintained by hand weeding, spot spraying with Roundup, and mowing.

In 2010 the net primary production N content data was collected and the inorganic nitrogen plant availability study was performed. There was little variation in monthly average temperatures between 2010 (Kentucky Mesonet), and the (1895 to 2013) long term average of the Bluegrass Region (NOAA/ESRL http://www.esrl.noaa.gov/psd/data/timeseries) (Figure 3.3b). There was significant precipitation variation between 2010 which was a relatively dry year (Kentucky Mesonet), compared to the (1895 to 2013) long term monthly precipitation average for the Bluegrass Region (Figure 3.3a). The year 2010 witnessed a 15% decrease in annual precipitation primarily in January to March and August to October compared to the Bluegrass Region long term average (Figure 3.6a).

Soil analysis

The soils at the site are well drained uplands of Faywood silty clay loam according to Web Soil Survey maintained by the Natural Resources Conservation Service (NRCS). Available water capacity is low (about 12cm) and it is considered not prime farmland. Soil horizons are: 0 to 15 cm: silty clay loam, 15 to 76 cm: clay, 76 to 86 cm: and unweathered bedrock. The soil for this site was a silt loam with an average of 17% sand (range 16.42 – 18.29), 68% silt (range 66.48 – 70.43) and 14% clay (range 12.82-16.5). The other soil parameters for this site that were assessed at the initiation of the study are shown in Table 3.2.

Site level data were collected in October 2008, soil cores were taken in the top 10 cm of each plot. Those samples were pooled and mixed thoroughly by species and the species mixture treatment to create one sample for each species and the species mixture treatment. Initial assessments of cation exchange capacity (CEC), base saturation, soil texture class, and water holding capacity were performed on these (Table 3.2). Bulk density samples were collected in October 2012 with a replication of 5 for each species plus the species mixture treatment. Since bulk density was not measured at the beginning of the experiment (2008), "control" soil cores were collected in 2012 in relatively undisturbed spots around the perimeter of the experiment as a proxy for initial conditions. Soil nutrient concentrations were converted to pool sizes using the bulk density.

Additional soil cores for each plot were collected in October 2008 and October 2012. For individual plot samples, the Mehlich 3 test was performed by Regulatory Services at the University of Kentucky to determine soil levels of phosphorous, potassium, calcium, magnesium, zinc, pH and buffer pH. Total % soil carbon and % soil nitrogen also were determined by Regulatory Services at the University of Kentucky using the Elementar vario MAX CNS Analyzer.

Net primary production N content

Due to the large seasonal variation of flowering times of the nine grass species, aboveground net primary production (ANPP) values were taken for each species at peak biomass (or time of flowering) and ranged from May to September (Table 3.1). A 15 cm^2 area was randomly chosen for each plot where aboveground biomass 5 cm above soil level was clipped, dried, and weighed. Total organic carbon and total organic nitrogen concentrations in plant biomass were measured using the Elementar vario MAX CNS Analyzer by the soil testing laboratory of the Regulatory Services at the University of Kentucky. The acid-fiber digest procedure was used to determine cellulose and lignin concentrations of the 2010 peak biomass samples using the Ankom^{200} Fiber Analyzer according to the procedures found at http://www.ANKOM.com under the procedures tab. ANPP-N was calculated by multiplying ANPP times the %total tissue Nitrogen and Nitrogen Use Efficiency (NUE) was calculated as 1/%tissue Nitrogen (Table 3.6).

Inorganic Nitrogen Resin Study

Plant available inorganic nitrogen was measured for 7 months over the growing season (March through October) in 2010 using resin bags that absorb both ammonium (NH_4^+) and nitrate (NO_3^-) ions. Resin bags were constructed using a nylon mesh bag filled with a mixture of 7.4 milliliter of a cation and 7.4 milliliter of an anion exchange resin. The bags were knotted on each end and a neon orange fishing line was attached to the bag for easy retrieval from the plots. For each plot, a resin bag was flatly placed 5 centimeters below the soil surface in April 2010. The bags were collected and replaced each month throughout the growing season until October 2010. Seven monthly pickups of 100 plots per pickup resulted in 700 resin bags analyzed. Using the KCl extraction technique, one resin bag was placed in 50 mls of 2N KCl, and the extractants were analyzed colorimetrically for NO3-N and NH4-N using a Bran-Luebbe auto-analyzer. Nitrates were analyzed using the hydrazine sulfate - copper sulfate reduction method and ammonium was analyzed using the sodium nitroprusside – phenol method.

Litter decomposition

To assess litter decomposition rates for each species, 5 grams of standing dead dried leaves and stems were collected in the Autumn of 2009, clipped to ~2 cm long pieces, and sealed into 10 x 10 cm^2 bags made of fiberglass-nylon mesh with 1.4 mm^2 openings. The bags were sealed with a TISH-200 impulse sealer and secured to the soil surface with metal ground staples. The bags were set out in replicates with one replicate consisting of one litter bag for each species or nine bags. At each pickup time four replicates (or 36 bags) were picked up. There were six pickup times and four replicates picked up, resulting in 216 litterbags in total. When the samples were picked up, they were oven dried at 55º C and weighed. They then were ground using a coffee grinder and sent to the soils laboratory at Regulatory Services to be analyzed for total % nitrogen and total % carbon using the Elementar vario MAX CNS Analyzer. Each sample was adjusted for ash free weight by burning ~0.5 grams of a sample in a muffle oven for 5 hours at 525°C and then in a drying oven for 2 hours at 105°C. That weight was recorded and divided by the sample weight to get the % ash of the sample. The % ash was deducted from the total litter weight to get the ash free weight of the whole sample. The bags were set out in January 25, 2010 in a flat area adjacent to the monoculture experiment. The first five pick-up dates were at 2 month intervals from March to November 2010. The last set of replicates was picked up in June 20, 2011 with a 7 month interval.

Statistics

The statistical program PAST (Hammer 2001) was used to normalize the data and each ANOVA was performed in SAS (9.3: SAS Institute, Cary, NorthCarolina, USA) using PROC MIXED (SAS 2010). Multivariate analysis was performed in the program PC-ORD (6.08: MjM Software, Gleneden Beach, Oregon, U.S.A.) using Principle Components Analysis (PCA) using the Euclidean distance measurement

(McCune and Mefford 2011). The data was not standardized and all response variable were included in the analysis. The Euclidean distance measurement was also used with Multi-Response Permutation Procedures (MRPP) within PC-ORD to discern significant species effects.

Repeated measures analysis was performed in SAS (9.3: SAS Institute, Cary, NorthCarolina, USA) using PROC MIXED for the resin data and the litter decomposition data. For the resin data, a repeated measures model was run for NH_4-N and NO_3-N including species and time effects, and also a repeated measures model was run for each species which looked at time effects.

Results

Species characteristics

While significant species differences were found for every species characteristic (Figure 3.2), there was no clear pattern between C_3 and C_4 species. Plant traits that promote fast N cycling include high amounts of N, low NUE, low amounts of C and recalcitrant C, and plants that do not retranslocate significant amount of N to crowns and roots. Plant traits that promote slow N cycling include low amounts of N, high NUE, high amounts of C and recalcitrant C, and plants that are effective at retranslocating N to crowns and roots. To characterize the nine species as promoting either fast N cycling or slow N cycling, I compared significant differences between species for each plant trait. *E. macgregorii* had the most traits that promoted fast N cycling (Figure 3.2). While *E. macgregorii* did not have high amounts of N, it did have low amounts of recalcitrant C and was not effective at retranslocating N. *E. hystrix* and *D. clandestinum* had plant trait values that promoted both fast and slow N cycling. *E. hystrix* had high ANPP, low C:N and low recalcitrant C which promotes fast cycling, but also had high lignin:N, low levels of tissue N, high levels of lignin that promotes slow N cycling. Also, *E. hystrix* was effective at retranslocating N. The fast N cycling traits for *D. clandestinum* were low C:N, high % tissue N, and low NUE, and the slow N cycling traits were a high percent of cellulose and lignin. *D. clandestinum* was significantly better than all the other species at retranslocating N. The other six species had more plant trait values that promoted slow N cycling compared to fast N cycling. *E. villosus* and *E. virginicus* generally had the same species characteristics with high lignin:N, low % tissue N, high % lignin, and high NUE that promoted slow N cycling. *E. villosus* and *E. virginicus* were not effective at retranslocating N. For *C. latifolium* and *T. flavus*, high ANPP-N was the only trait that promoted fast N cycling. Slow N cycling plant traits for *C. latifolium* and *T. flavus* were low % tissue N, high NUE, and high % cellulose. *T. flavus* also had a high lignin:N and high amounts of recalcitrant C. *P. anceps* was the only species to have just slow N cycling traits with low % tissue N, high NUE, high % cellulose, and was effective at retranslocating N. In conclusion, I found that three C_3 species, (*E. macgregorii*, *E. hystrix*, and *D.*

clandestinum) had the most number of plant traits that promoted fast N cycling, and the other six species had more plant traits that promoted slow N cycling.

Litter Decomposition

Significant species differences were found for initial litter quality, litter decomposition rate (k value) and C:N at the last pickup date (Figure 3.3). Litter decomposition characteristics that promote fast N cycling are low C:N, high N content, low C content, and a fast litter decomposition rate. Litter decomposition characteristics that promote slow N cycling are high C:N, low N content, high C content, and a slow litter decomposition rate. To characterize the nine species as having either fast N cycling or slow N cycling litter, I compared significant differences between species for each litter characteristic. In general I found that the litter characteristics of the C_3 species promoted fast N cycling, and the litter characteristics of the C_4 species promoted slow N cycling. *E. villosus* and *E. virginicus* had the most definite litter characteristics that promoted fast N cycling. *E. villosus* and *E. virginicus* had the highest initial quality litter compared to the other seven species with significantly higher % litter N, and significantly lower litter C:N (Figure 3.3). Also, the litter of *E. villosus* decomposed the fastest, and *E. virginicus* had the lowest litter C:N at the last litter bag pickup (Figure 3.3B and C). The litter of *E. macgregorri* and *D. clandetinum* promoted fast N cycling with a relatively higher % litter N (Figure 3.3). The only C_3 species that promoted slow N cycling was *E. hystrix* that had a high litter C:N. Significant litter characteristics of the three C_4 species promoted only slow N cycling. *A. virginicus* had the most litter characteristics that promoted slow N cycling with low % litter N, and a high litter C:N. *A. virginicus* also had the slowest decomposition rate and the highest litter C:N at the last litter bag pickup date (Figure 3.3). Litter characteristics of *P. anceps* were similar to *A. virginicus* except that *P. anceps* had a faster litter decomposition rate (Figure 3.3). The high litter C:N of *T. flavus* promoted slow N cycling (Figure 3.3).

The ANOVA repeated measures analysis for the litter decomposition experiment had significant species effects, time (days incubated) effects, and species x time effects for all response variables listed in Table 3.4 except that % C did not have a significant species x time interaction. Therefore, % C tended to decline through time for all species. Since a significant interaction term was detected for all variables except for %C, a one way ANOVA was performed for each species. All species had a significant time effect for all variables listed in Table 3.4 except that *C. latifolium*, *P. anceps*, and *T. flavus* did not have a significant time effect for litter N. Therefore, the amount of N in the litter for *C. latifolium*, *P. anceps*, and *T. flavus* did not significantly change over the course of this experiment.

Litter C concentration, % of initial amount remaining, and total amount tended to decline the over the course of the experiment for all species (Figure 3.5). However, litter N was variable over the course of the experiment and between species in both concentration and % of initial amount remaining (Figure 3.5). The variation in % N resulted in a relatively constant total amount of N over the course of the experiment and for all species (Figure 3.5). Thus, the litter was losing mass and C but retaining N. *E. villosus* and *E. virginicus* had a high amount of N in initial litter, but that N was quickly lost within the first 60 days of incubation (Figure 3.5). Also, *E. villosus* and *E. virginicus* were the only species to lower % N below initial % N levels (Figure 3.5). All other species maintained or increased % N during the course of the experiment (Figure 3.5). The C_4 species, *C. latifolium* and *E. hystrix* increased % N by 50 % or more by the end of the experiment (Figure 3.5).

Patterns of total litter N (% N x litter wt.) and C (% C x litter wt.) from January 2010 to June 2011 for each species are shown in Figure 3.6. Since total amounts of litter N and C were graphed, litter C generally declines and litter N remains relatively constant over the course of the experiment. *E. villosus*, *E. virginicus*, and *E. hystrix*, had significantly lower amounts of litter N after the first 60 days, and then relatively constant litter N amounts for the rest of the experiment. *D. clandestinum* also had a drop in litter N in the first 60 days, but then increased litter N over the course of the growing season. All species except for *P. anceps* generally lose carbon until July which is the beginning of the drought and temperatures are at the maximum (Figure 3.6). *P. anceps* continues to reduce C into September which is at the peak of the drought. *D. clandestinum* and *P. anceps* reduced litter C the slowest with *D. clandestinum* retaining the most litter C over the first five intervals (until October).

Nitrate and ammonium resin data

Significant species differences were found for NO_3-N, NH_4-N, total resin N, N soil pools, C soil pools, and bulk density (Figure 3.7). I predicted that fast cycling N species with high litter N and low litter C:N should decompose more rapidly and promote mineralization. Mineralization occurs when litter C:N is less than 20:1 (Namuth 2014). Initial litter C:N of *E. villosus* and *E. virginicus* had the lowest litter C:N (avg. 23.22 and 20.40 respectively) followed by *D. clandestinum* (avg. 39.98), *E. macgregorii* (avg. 42.58) (Table 3.3). A fast cycling N species should then be adapted to quickly take up plant available N. I predicted that slow cycling N species with low litter N and high litter C:N should decompose more slowly and promote immobilization. Immobilization occurs when litter C:N is greater than 20:1 (Namuth 2014). Initial litter C:N of *P. anceps* was the highest (avg. 70.00) followed by *A. virginicus* (avg. 66.03), *T. flavus*, *E. hystrix*, and *C. latifolium* (Table 3.3). Immobilization limits plant available N and can lower soil N. Since well decomposed litter has a C:N of around 10:1 (Namuth 2014), all nine species were relatively well decomposed at the end of 528 days of incubation with *P. anceps*

(avg. 18.61) and *A. virginicus* (avg. 17.01) the least decomposed (Table 3.3). However, by the end of the experiment *E. villosus* and *E. virginicus* lowered litter C:N by 35% and 30% respectively, while *A. virginicus, P. anceps, T. flavus* and *E. hystrix* lowered C:N by at least 70 % (Table 3.3). This suggests that N was not limiting litter decomposition for the species with high initial litter C:N.

Also, species that had higher % lignin were predicted to have high C and N soil pool levels which should then lower bulk density. I did not find a noteworthy positive correlation between % lignin (Figure 3.2) and N and C soil pools, and bulk density (Figure 3.2) but *E. virginicus, E. hystrix, D. clandestinum, C. latifolium*, and *T.flavus* did show the general trend.

Uptake of plant available N is optimal if it is synced with seasonal precipitation. Since only the amount of resin NO_3-N and NH_4-N was measured for each species, plant available N and the amount of N that was taken up by the plant could not be teased apart. For this instance, I will assume that there was more plant available N for *E. macgregorii, E. villosus, E. virginicus*, and *D. clandestinum* which were the species with high quality litter. Of these four species, *E. virginicus* and *D. clandestinum* were the only two species that efficiently depleted both NO_3-N and NH_4-N throughout the growing season (Figure 8). Although *E. villosus* had the highest litter quality and decomposed the fastest (Figure 3.3), it was able to efficiently deplete NH_4-N but not NO_3-N (Figure 3.7 and 2.8). The peak in NO_3-N coincides with the time *E. villosus* goes dormant, and also the time the nitrifying bacteria may be most active (McClellan, Deenik et al. 2007). Resin bag levels for *E. macgregorii* were similar to *E. villosus, E. virginicus* but *E. macgregorii* did not deplete NH_4-N as efficiently as *E. villosus.*

The assumption that there was less plant available N for the species that had low litter quality does not hold true for my data. *E. hystrix, P. anceps, T. flavus*, and *A. virginicus* had low quality litter but did not have low levels resin NO_3-N and NH_4-N (Figures 2.7 and 2.8). In fact *E. hystrix, P. anceps*, and *A. virginicus* had the highest levels of total resin N (Figure 3.7). Since these four species had higher levels of resin N, I have to assume that plant available N is similar between the species and that my resin data may be a better measurement of plant uptake. If this is true, the four species with low quality litter (*E. hystrix, P. anceps, T. flavus*, and *A. virginicus*) were less efficient at N uptake than the four species with high quality litter (*E. macgregorii, E. villosus, E, virginicus*, and *D. clandestinum*).

Significant species, time, and species x time effects were found in the repeated measures analysis for resin NO_3-N and NH_4-N (Table 3.5). Since the species x time interaction was significant, a separate repeated measures was performed for each species. For NO_3-N, all species had a significant time effect, and all species except for *E. villosus* had a significant time effect for NH_4-N (Table 3.5). In general, NO_3-N levels varied more over the growing season than NH_4-N levels (Figures 2.7 and 2.8). All species

except for *E. virginicus*, *D. clandestimnum*, and *T. flavus* showed a significant increase in resin NO_3-N in July (Figure 3.8) when the monthly temperature average was the highest for the year (~25º C) and precipitation fell below the long term average for the Bluegrass Region of Kentucky (Figure 12.). This July spike also coincides with the time the nitrifying bacteria may have been most active which may account for the drop in resin NH_4-N for *E. macgregorii* and *C. latifolium* (Figure 3.8). The late summer drought that lasted from July to October and was most severe in September (Figure 3.1) which coincides with the increased NO_3-N levels for *E. villosus* and *E. hystrix* even though these two species were dormant during the beginning of this drought. Even though the four *Elymus* species were dormant during the month of July, resin NH_4-N levels remained relatively constant.

Soil data

At the beginning of the experiment in October 2008, significant species differences were found for phosphorous, potassium, magnesium, and pH (Figure 3.9). The only significant adhoc tukeys species pairwise comparisons were found for magnesium and pH (Figure 3.9). At the end of the experiment in October 2012, no significant species effects were found for any of the soil nutrients or pH (Figure 3.9). Comparing all species and both years (2012 and 2008), significant species effects and year effects were found for pH (spec $p = .012$, year $p<.0001$), phosphorous (spec $p = .002$, year $p=.05$), potassium (spec $p<.0001$, year $p<.0001$), calcium (spec $p = .0002$, year $p<.0001$), and magnesium (spec $p = .012$, year $p<.0001$). No significant species x year interactions were detected. Soil pH, and levels of potassium, calcium, and magnesium were lower in 2012, and soil levels for phosphorous were higher in 2012.

To determine if the species had differing effects on soil nutrients, an ANOVA analysis was performed that analyzed the amount of change between the 2 years (2012 - 2008). Only pH was found to have significant species effects where *E. hystrix* lowered pH significantly more than *T. flavus* (Figure 3.9). To test if the species significantly varied in multivariate space, the differences between the two years for all soil nutrients were plotted using principle components analysis (PCA). The PCA graph was sorted by species and a multi-response Permutation Procedures (MRPP) was performed using Euclidean distances to test for significant pairwise differences between species. No significant species differences were detected ($p=.604$) using differences (2012-2008) for all soil nutrients, pH and buffer pH. I also performed the same PCA analysis excluding pH and buffer pH with similar results.

Discussion

My first hypothesis was partially confirmed in that C_3 species had plant traits that promoted fast N cycling. My first hypothesis was also partially falsified because both C_3 and C_4 species had plant traits that promoted slow N cycling. Considering all the C_3 species, my hypothesis was supported by three *Elymus* species (*E. macgregorii, E. villosus*, and *E. virginicus*), partially supported for *D. clandestinum*,

and generally not supported for *E. hystrix*, and *C. latifolium*. *E. virginicus* had the most plant traits that supported the fast N cycling strategy with high quality litter that rapidly decomposed, and was efficient at taking up both NO_3-N and NH_4-N. After *E. virginicus*, *E. villosus* and *E. macgregorii* had the most plant traits that promoted fast N cycling except that *E. villosus* was less efficient at NO_3-N uptake, and *E. macgregorii* had lower quality litter and was less efficient at taking up both NO_3-N and NH_4-N. *D. clandestinum* had traits that promoted both fast N cycling and slow N cycling. Traits that promoted fast N cycling were high litter N and efficiency at taking up both NO_3-N and NH_4-N. The slow N cycling traits of *D. clandestinum* were retranslocation of the most N to crowns and roots, and losing litter C the slowest compared to all the other species. My first hypothesis was supported by the C_4 species which had traits that promoted only slow N cycling. While all three C_4 species had low litter quality and were inefficient at taking up both NO_3-N and NH_4-N, the litter of *A. virginicus* decomposed the slowest, and *P. anceps* was better at retranslocating N, lost litter C the slowest, and was the least decomposed at the end of the experiment. *C. latifolium* and *E. hystrix* were the two C_3 species that tended to have slow N cycling traits with lower litter quality than the other C_3 species, and inefficient at taking up NO_3-N.

My data does not support the second prediction that slow N cycling species will have a positive feedback loop where poor litter quality will promote immobilization, and limit plant available N. For fast N cycling species as well as slow N cycling species, similar levels of resin NO_3-N and NH_4-N were observed. Also decomposition of litter was not limited by N as all species except for *E. villosus* and *E. virginicus* increased percent litter N over the course of the experiment. Thus, similar to other litter decomposition studies (Melillo, Aber et al. 1982, Hobbie 1996) the litter was losing mass and C but retaining N. Also species with initially high litter C:N reduced litter C:N by over 70 % over the course of the experiment which again suggests no N limitation. Knops et al. (2002) suggests that the negative slow N cycling feedback loop does not limit plant available N because species differences in litter quality have a limited impact on plant available N compared to the N in the soil organic pool which accounts for 90 % of total ecosystem N. Most N gained from the decomposing litter is retained and incorporated into the soil organic matter, which prevents immediate feedbacks to the plants. The soil organic matter has a bigger impact on mineralization and immobilization and ultimately plant available N compared to plant and litter characteristics (Knops, Bradley et al. 2002).

At the ecosystem level, the soil data does not suggest that the species significantly affected soil parameters over the four years this experiment was conducted. Percent C and % N did not significantly differ for any species over the course of this experiment. Thus, these nine species did not differentially deplete soil N as was predicted by the resource-competition theory. All this data is evidence that N may not be the primary limiting nutrient for this savanna-woodland which is opposite of what has been found

to be true for many temperate grasslands (Polley and Detling 1988, Schlesinger 1991, Vitousek and Howarth 1991). Knops et al (2002) suggested that species differences in quantity and quality of litter did not have large impacts on N cycling but by plant species impacts on nitrogen inputs and losses. Furthermore other studies suggest that other factors besides plant and litter characteristics have a bigger impact on ecosystem N cycling such as the diversity and abundance of soil microbial communities, and disturbances such a fire and grazing (Reich, Grigal et al. 1997, Knops, Bradley et al. 2002).

These results are consistent with the reported species distribution in the field. The fast N cycling species will have traits that make them better adapted for habitats that are not limited by N and water. The four fast N cycling C_3 species, *E. macgregorii*, *E. villosus*, *E. virginicus* and *D. clandestinum* do frequent the Bluegrass savanna-woodlands with mesic eutrophic soils as well as the more open woods (Wharton and Barbour 1991, Campbell 2004). The *Elymus* species may also be best adapted at taking up plant available N because the time they are actively growing and plant N demands are high, coincides with the Bluegrass Region's wet spring. Also, the *Elymus* species produce high quality litter during the summer months when soil microbes are most active. My data also supports the prediction that the slow N cycling species will be best adapted for N limited habitats. The C_4 grasses had more conservative N traits that promote slow N cycling which would explain why they are found in local openings on poorer soils in the Bluegrass savanna-woodland or openings created by disturbance such as fire or bison trails (Campbell 2004). The C_4 species actively grow during the summer months which was during the summer drought. The lack of soil water during the summer drought may have limited the uptake of inorganic N. Also, increased nitrifying bacteria activity during the summer months may partially explain the NO_3-N peaks during the summer months which can be seen for most of the species (Figure 3.8).

In conclusion, most C_3 species were found to have fast N cycling traits, and C_4 species were found to have slow N cycling traits that could explain their local distribution for the Bluegrass savanna-woodland. Unlike many other temperate grassland systems, N limitation was not found to be a main determinant in sorting species in a community assembly. Other factors besides plant mediated competition for N may have bigger impacts on ecosystem N cycling in the savanna-woodland. Since savannas are dependent on disturbance, fire and grazing could have major impacts of N inputs and losses at the ecosystem level. Future restoration ecology studies to investigate the effect of fire and grazing on these savannas can provide more effective ecological restoration guidelines.

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Tables

Table 3.1: The nine native perennial bunchgrass species used in this experiment listed in order of flowering time. The abbreviations are used in the multivariate graphs.

Scientific Name	Abbreviation	Common Name	Photosynthetic Pathway
1. *Elymus macgregorii* R. Brooks & J.J.N. Campb.	Emg	Early wildrye	C_3
2. *Elymus villosus* Muhl. ex Willd.	Evl	Nodding wildrye	
3. *Elymus virginicus* L.	Evg	Virginia wildrye	
4. *Elymus hystrix* L.	Ehy	Bottlebrush	
5. *Dichanthelium clandestinum* (L.) Gould	Dclan	Deer tongue	
6. *Chasmanthium latifolium* (Michx.) Yates	Clat	River Oats	
7. *Panicum anceps* Michx.	Panc	Beaked panicgrass	C_4
8. *Tridens flavus* (L.) Hitchc.	Tflav	Purple top/grease grass	
9. *Andropogon virginicus* L,	Broom	Broomsedge	

Table 3.2: Site level soil parameters that were pooled by species (10 replicates mixed into one sample/species measured in 2008).

Soil parameters for data that was pooled by species (1 sample/species)									
	C_3 species						C_4 species		
	E. macg	*E. vill*	*E. virg*	*E. hyst*	*D. clan*	*C. lat*	*P. anc*	*T. flav*	*A. virg*
TC	Silt loam								
%sand	17.26	17.7	17.23	18.04	16.57	16.94	16.65	16.42	17.15
%silt	68.29	68.56	68.62	69.14	70.43	68.95	66.85	69.68	66.56
%clay	14.44	13.73	14.15	12.82	13	14.11	16.5	13.9	16.29
CEC	24.92	24.92	26	26	24.92	24.92	26	24.92	26
%Base S	91.59	97.89	92.29	87.18	94.19	95.23	90.4	91.18	93.87
Meq K	1.31	1.84	1.52	1.33	1.62	1.66	1.44	1.65	1.32
Meq C	18.97	19.88	19.86	18.79	19.24	19.4	19.23	18.49	20.47
Meq Mg	2.47	2.63	2.56	2.48	2.56	2.63	2.76	2.53	2.57
Meq Na	0.08	0.05	0.06	0.07	0.05	0.04	0.07	0.05	0.06
%PAW	21.39	19.69	19.86	18.12	18.72	21.57	21.26	20.49	19.44
%Field C	44.26	43.21	43.67	42.19	43.12	43.87	44.75	43.41	44.12
%Wilting	22.87	23.52	23.81	24.07	24.4	22.3	23.49	22.92	24.68

Table 3.3: Litter decomposition characteristics showing the one way ANOVA species effect (significant p-vlaues in bold). Species means (±SE) for the litter decomposition rate (k value), initial litter quality, and plant reabsorption of N. [a]initial litter quality of N, C and C:N content is in the initial litter at time 0 for each species. [b]plant reabsorption of litter is an estimate of how much N each species reabsorbed from the dying aboveground biomass (ANPP-N – litter N)

	Litter Decomp rate K value	Initial litter quality[a]			Plant Reabsortion of N[b]	% litter C:N at last pickup date	% change in litter C:N
		litter N (%N x biomass)	litterC (%C x biomass)	litterC:N			
Species effect	**.0438**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**.0206**	
E. macgregorii	.64(.094)	4.89(.22)	207.0(.56)	42.58(1.83)	.69(.16)	15.23(.98)	64
E. villosus	.72(.015)	8.72(.47)	200.1(.39)	23.22(1.20)(	.51(.12)	15.15(.29)	35
E. virginicus	.64(.092)	10.12(.23)	207.0(.91)	20.40(.54)	.41(.10)	14.20(.27)	30
E. hystrix	.64(.094)	3.96(.28)	211.4(.43)	54.24(4.13)	1.32(.07)	16.24(.51)	70
D. clandestinum	.45(.043)	4.84(.32)	190.9(1.7)	39.98(2.94)	1.92(.15)	16.43(.25)	59
C. latifolium	.60(.044)	4.00(.15)	207.6(2.4)	52.10(2.33)	.89(.14)	15.87(.43)	58
P. anceps	.60(.060)	3.15(.29)	214.7(2.3)	70.00(7.14)	1.26(.15)	18.61(2.09)	70
T. flavus	.63(.062	4.00(.21)	223.0(.40)	56.17(2.69)	.81(.12)	15.37(.34)	73
A. virginicus	.38(.068)	3.48(.24)	226.1(.55)	66.03(4.92)		17.01(.19)	74

Table 3.4: Repeated measures ANOVA results for the litter decomposition experiment. Overall fixed effects for species, pickup times (or days incubated) and species x time interaction for litter wt., %C/N, litter C:N, %N, litter N, %C, and litter C. One way ANOVA results for differences in pickup times (or days incubated) for each species

Repeated measures litter decomposition analysis									
	Litter wt. grams			%C:N			Litter C:N (biomass x %C)/(biomass x %N)		
	df	F	p	df	F	p	df	F	p
All Species	8,144	16.96	**<.0001**	8,144	45.19	**<.0001**	8,144	45.19	**<.0001**
Days incubated	6,18	191.11	**<.0001**	6,18	185.49	**<.0001**	6,18	185.49	**<.0001**
Species x time	48,144	2.00	**.0009**	48,144	7.48	**<.0001**	48,144	7.48	**<.0001**
Time (days incubated) effects for each species									
E. macgregorri	6,18	39.78	**<.0001**	6,18	17.60	**<.0001**	6	23.09	**<.0001**
E. villosus	6,18	29.76	**<.0001**	6,18	15.60	**<.0001**	6	18.09	**<.0001**
E. virginicus	6,18	35.44	**<.0001**	6,18	28.88	**<.0001**	6	33.22	**<.0001**
E. hystrix	6,18	28.59	**<.0001**	6,18	23.09	**<.0001**	6	42.74	**<.0001**
D. clandestinum	6,18	28.23	**<.0001**	6,18	44.74	**<.0001**	6	44.74	**<.0001**
C. latifolium	6,18	66.17	**<.0001**	6,18	111.56	**<.0001**	6	46.72	**<.0001**
P. anceps	6,18	9.73	**<.0001**	6,18	39.01	**<.0001**	6	39.01	**<.0001**
T. flavus	6,18	24.79	**<.0001**	6,18	17.44	**<.0001**	6	22.51	**<.0001**
A virginicus	6,18	22.48	**<.0001**	6,18	77.68	**<.0001**	6	77.68	**<.0001**

Repeated measures litter decomposition analysis												
	%Nitrogen			Litter N (biomass x %N)			%Carbon			Litter C (biomass x %C)		
	df	F	p	df	F	p	df	F	p	df	F	p
All Species	8,144	48.41	**<.0001**	8,144	25.31	**<.0001**	8,144	3.12	**.0028**	8,144	16.83	**<.0001**
Days incubated	6,18	21.76	**<.0001**	6,18	12.03	**<.0001**	6,18	36.69	**<.0001**	6,18	169.33	**<.0001**
Species x time	48,144	5.70	**<.0001**	48,144	10.84	**<.0001**	48,144	1.06	0.3817	48,144	2.07	**.0005**
Time (days incubated) effects for each species												
E. macgregorri	6	4.94	**.0038**	6	14.21	**<.0001**	6	11.63	**<.0001**	6	79.72	**<.0001**
E. villosus	6	7.31	**.0004**	6	30.65	**<.0001**	6	14.77	**<.0001**	6	136.30	**.0004**
E. virginicus	6	16.89	**<.0001**	6	54.64	**<.0001**	6	15.16	**<.0001**	6	84.56	**<.0001**
E. hystrix	6	16.98	**<.0001**	6	5.34	**.0018**	6	14.63	**<.0001**	6	80.70	**<.0001**
D. clandestinum	6	18.67	**<.0001**	6	9.17	**<.0001**	6	10.99	**<.0001**	6	45.01	**<.0001**
C. latifolium	6	10.44	**<.0001**	6	2.18	.0868	6	8.86	**.0041**	6	51.83	**<.0001**
P. anceps	6	14.17	**<.0001**	6	2.17	.0869	6	11.34	**<.0001**	6	29.93	**<.0001**
T. flavus	6	7.09	**.0005**	6	1.89	.1302	6	10.44	**.0005**	6	58.96	**<.0001**
A virginicus	6	12.84	**<.0001**	6	3.75	**.0202**	6	24.36	**<.0001**	6	61.47	**<.0001**

Table 3.5: Repeated measures ANOVA results for monthly NO_3-N and NH_4-N over for the growing season (March thru October) of 2010. One way ANOVA results for differences in season totals between NO_3-N and NH_4-N for each species.

Repeated measures Resin data analysis							Significant difference for season totals between NO_3-N and NH_4-N		
	NO_3-N			NH_4-N					
	df	F	p	df	F	p			
All Species	9,90	4.79	**<.0001**	9,90	3.79	**.0004**			
Time (monthly)	6,531	62.88	**<.0001**	6,532	19.75	**<.0001**			
Species x time	54,531	3.31	**<.0001**	54,532	2.19	**<.0001**			
time (monthly) effects for each species							df	F	p
E. macgregorri	6,53.5	6.02	**<.0001**	6,53.4	2.67	**.0244**	1	0.43	0.5196
E. villosus	6,53	7.34	**<.0001**	6,53.2	0.69	.6591	1	15.11	**0.0011**
E. virginicus	6,54	7.39	**<.0001**	6,54	2.50	**.0329**	1	9.68	**0.006**
E. hystrix	6,54	10.66	**<.0001**	6,54	3.06	**.0120**	1	19.73	**0.0003**
D. clandestinum	6,54	3.42	**0.0062**	6,54	8.51	**<.0001**	1	0.27	0.6098
C. latifolium	6,53.4	6.44	**<.0001**	6,53.3	8.04	**<.0001**	1	0.51	0.4849
P. anceps	6,53.5	14.20	**<.0001**	6,53.5	4.92	**.0004**	1	2.47	0.1338
T. flavus	6,53.3	6.72	**<.0001**	6,53.8	8.58	**<.0001**	1	4.33	0.0519
A virginicus	6,54	41.33	**<.0001**	6,54	4.61	**.0008**	1	33.42	**<.0001**

Supplemental

Table 3.6: Overall ANOVA for all species comparing soil nutrients measured in 2008 and 2012. Significant values are in bold.

	2008 species effects			2012 species effects			2008 and 2012 Soil Nutrients Effects (Species and year)								
							Species effects			Year effects			Year x species		
	Df	F	p	df	F	p	Df	F	p	Df	F	p	Df	F	p
Soil Nutrients															
%Nitrogen	9,90	0.88	0.5421	10,43	0.61	.7937	10,84	0.68	.74	1,86	0.73	.3943	9,84	0.5	.763
%Carbon	9,90	0.56	0.8272	10,45	1.16	.3397	10,86	1.1	.3706	1,86	0.04	0.8481	9,86	0.93	.501
pH	9,90	2.14	**0.0341**	10,45	1.83	.0832	10,86	2.48	**.0117**	1,86	207.1	**<.0001**	9,86	1.24	.2799
Buffer pH	9,90	1.33	0.2352	10,45	1.19	.3249	10,86	1.53	.143	1,86	0.32	0.5703	9,86	0.36	.9522
Soil Nutrients (mg/kg)															
Phosphorous	9,90	2.04	**0.0436**	10,45	1.20	.3188	10,86	3.13	**.0019**	1,86	4.05	**.0473**	9,86	0.67	.7338
Potassium	9,90	3.07	**0.0030**	10,45	2.44	**.0202**	10,86	4.71	**<.0001**	1,86	40.66	**<.0001**	9,82	1.22	.2963
Calcium	9,90	1.04	0.4160	10,45	1.83	.0832	10,86	4.02	**.0002**	1,86	58.1	**<.0001**	9,86	0.38	.9403
Magnesium	9,90	3.64	**0.0007**	10,45	1.17	.3382	10,86	2.47	**.012**	1,86	91.02	**<.0001**	9,86	0.3	.9728
Zinc	9,90	1.36	0.2164	10,45	1.61	.1355	10,86	2.08	.35	1,84	0.09	.7625	9,84	0.19	.9949

Table 3.7: ANOVA results for species effects

ANOVA results for species effects			
	Df	F	p value
Soil nutrient differences between years (2012 – 2008)			
%Carbon	9,41	1.77	.1040
%Nitrogen	9,39	1.15	0.3504
Phosphorous mg/kg	9,41	1.92	.0762
Potassium mg/kg	9,41	1.74	.1115
Calcium mg/kg	9,41	0.68	.7204
Magnesium mg/kg	9,41	1.45	.2000
Zinc mg/kg	9,39	0.67	.7302
pH	9,41	2.60	**.0179**
Buffer pH	9,41	1.24	.2985
Species characteristics			
%tissue Nitrogen	7,71	8.80	**<.0001**
%cellulose	8,72	6.79	**<.0001**
%lignin	8,72	4.02	**.0005**
C:N	7,71	5.51	**<.0001**
Lignin:N	7,70	4.76	**.0002**
ANPP-N	7,71	5.65	**<.0001**
Recalcitrant C	8,75	7.99	**<.0001**
Plant and soil N cycling parameters			
NUE	7,71	6.94	**<.0001**
NO_3-N	9,90	7.57	**<.0001**
NH_4-N	9,89	3.88	**.0003**
Inorganic N total	9,89	6.63	**<.0001**
N soil pool	10,45	3.81	**.0009**
C soil pool	10,44	4.13	**.0005**
Bulk density	10,44	4.90	**<.0001**
Litter decomposition			
Kvalues	8,27	2.38	**.0438**
Reabsorption of litter N	7	15.41	**<.0001**
%C:N litter at last pickup date	8,27	2.82	**.0206**
Initial litter quality			
Litter N	8	45.52	**<.0001**
Litter C	8	67.27	**<.0001**
Litter C:N	8	22.69	**<.0001**

Table 3.8: Species characteristics determined from peak biomass samples collected in 2010. One way ANOVA p-vlaue for the overall species effect, and mean (±1SE) for each species. ANPP-N - amount of nitrogen in aboveground net primary production. NUE - nitrogen use efficiency.

	%tissue N	%Cellulose	%Lignin	C:N	Lignin: N	ANPP-N g N	NUE[a] 1/%N	Recalcitrant C %cell + %lignin
Species effect p vlaue	**<.0001**	**<.0001**	**.0005**	**<.0001**	**.0002**	**<.0001**	**<.0001**	**<.0001**
E. macgregorii	1.7(0.2)	21.5(2.6)	3.7(0.43)	26.0(2.3)	2.2(0.3)	50.3(7.0)	0.62(.056)	25.18(2.45)
E. villosus	1.4(0.1)	27.3(1.4)	7.6(1.1)	33.1(3.0)	5.8(0.9)	57.7(9.2)	0.77(.069)	34.99(1.39)
E. virginicus	1.6(0.1)	28.8(0.76)	7.7(0.8)	28.1(2.3)	4.9(0.6)	96.7(21.3)	0.64(.038)	36.58(0.65)
E. hystrix	2.0(0.06)	26.3(1.3)	8.9(1.2)	22.3(0.7)	4.5(0.6)	96.1(8.5)	0.51(.016)	35.26(0.79)
D. clandestinum	2.6(0.2)	27.1(1.3)	8.5(1.0)	16.5(0.8)	3.2(0.3)	78.5(9.1)	0.39(.023)	35.62(1.19)
C. latifolium	1.8(0.1)	30.8(1.1)	7.3(1.4)	25.8(2.2)	4.8(1.4(	123.4(12.4)	0.59(.052)	42.95(4.60)
P. anceps	1.7(0.1)	30.9(0.92)	6.0(0.5)	27.0(2.8)	3.7(0.5)	79.2(11.5)	0.63(.065)	36.93(1.11)
T. flavus	1.5(0.1)	33.95(0.64)	6.4(0.3)	29.8(2.5)	5.0(1.0)	104.4(14.2)	0.69(.051)	40.86(1.00)
A. virginicus		43.17(8.90)	10.2(2.9)		.	.	.	55.33(11.51)

Table 3.9: Soil N cycling parameters. One way ANOVA p-vlaue for the overall species effect, and mean (±1SE) for each species.

[a]NO_3-N, NH4-N and Total resin represents the sum of inorganic nitrogen captured in the resin bags that were placed 5 cm below the soil surface and collected and replaced throughout the growing season of 2010.

[b]Soil cores were collected in in 2008 and 2012. Since no significant year effect was detected, 2012 N and C soil pool means (±SE) are presented here.

[C]Bulk density was measured in 2012.

	NO_3-N[a] mg	NH4-N[a] mg	Total resin N[a] mg	N soil pool[b] g m^{-2}	C soil pool[b] g m^{-2}	Bulk density[c] g cm^{-3}
Species effect p vlaue	**<.0001**	**.0003**	**<.0001**	**.0009**	**.0005**	**<.0001**
E. macgregorii	1.15(.17)	1.14(.180)	2.14(.21)	320.8(28.2)	3226.4(292.5)	.857(.055)
E. villosus	1.51(.17)	0.78(.083)	2.29(.19)	277.5(37.1)	2768.8(331.9)	.663(.046)
E. virginicus	0.88(.10)	0.54(.041)	1.42(.096)	408.6(28.3)	4057.4(258.8)	.967(.028)
E. hystrix	1.78(.20)	0.80(.095)	2.57(.24)	403.8(26.5)	3917.6(240.3)	.932(.018)
D. clandestinum	0.81(.14)	0.73(.076)	1.530.15)	365.7(12.0)	3519.1(167.3)	.913(.032)
C. latifolium	0.96(.20)	0.81(.082)	1.76(.24)	381.0(77.3)	3574.2(695.9)	.783(.027
P. anceps	1.37(.11)	1.13(.112)	2.47(.18)	396.7(35.6)	3821.6(212.8)	.877(.027)
T. flavus	1.24(.17)	0.874(.055)	2.11(.18)	329.2(6.2)	3172.6(127.0)	.825(.027)
A. virginicus	1.64(.10)	0.884(.086)	2.53(.15)	362.8(20.1)	3395.8(206.0)	.898(.045)

Figures

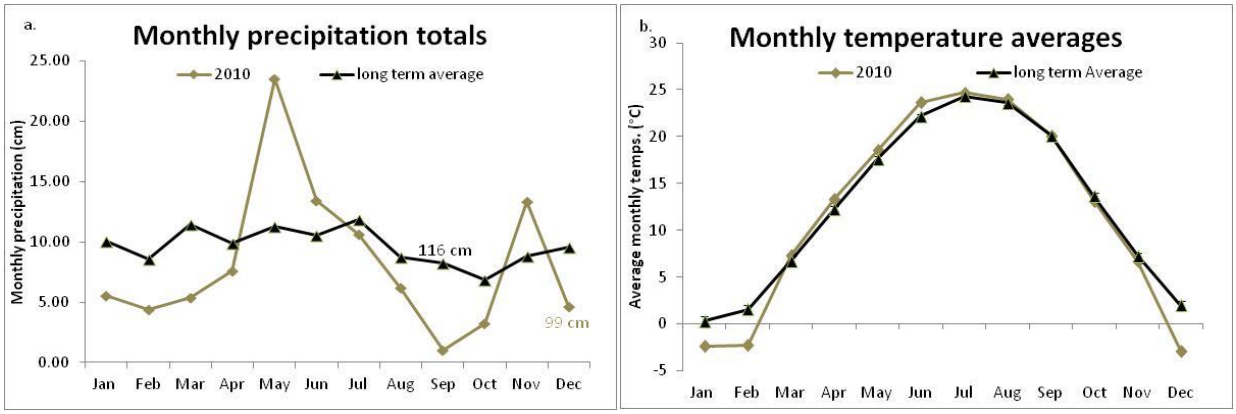


Figure 3.1: a. Monthly temperature averages for the year of study (2010) compared to the long term average in the Bluegrass Region of Kentucky (± 1 SE). b. Monthly precipitation totals for the year of study (2010) compared to the long term average in the Bluegrass Region of Kentucky (± 1 SE). The color coded numbers are yearly precipitation totals.

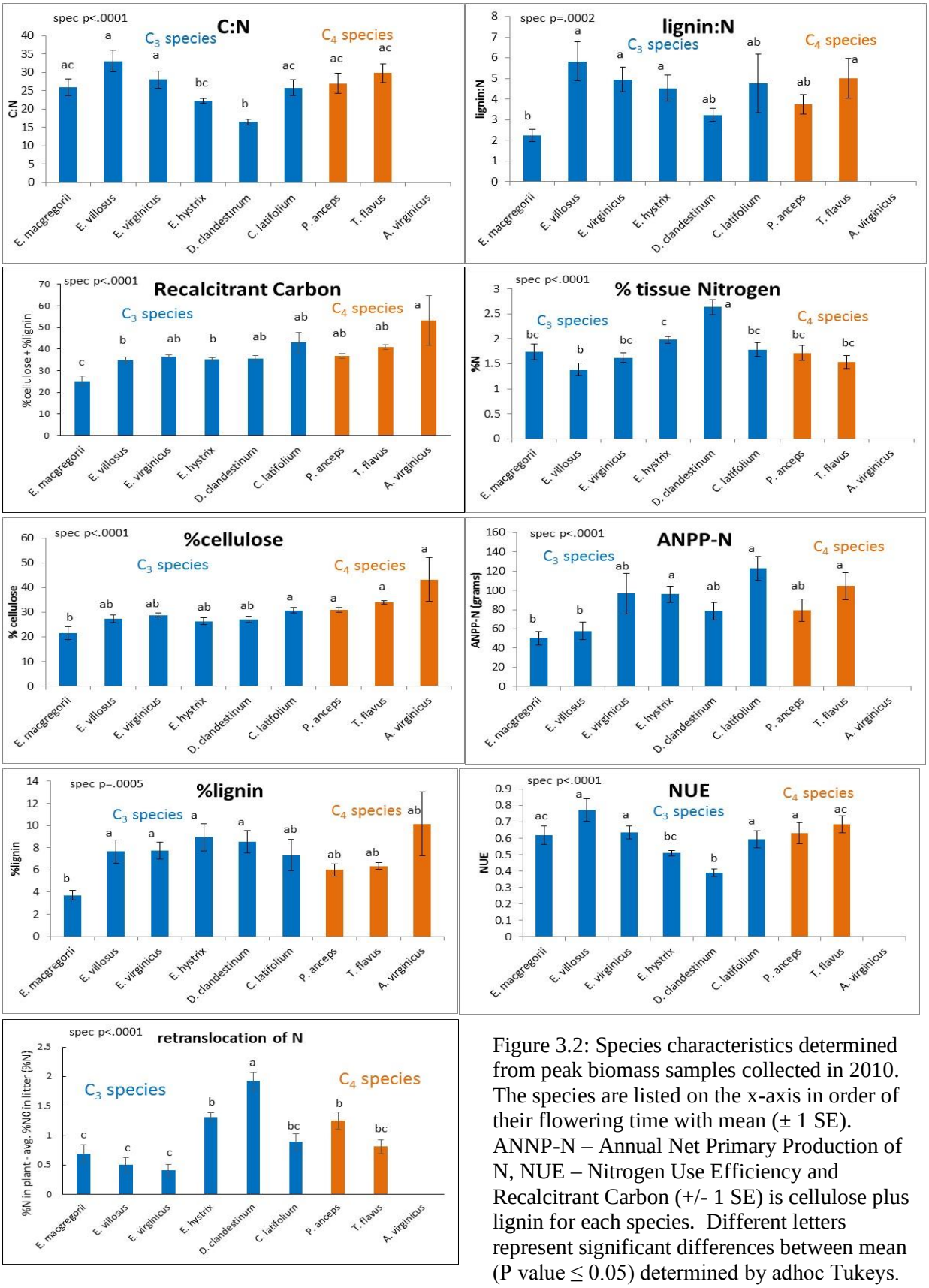


Figure 3.2: Species characteristics determined from peak biomass samples collected in 2010. The species are listed on the x-axis in order of their flowering time with mean (± 1 SE). ANNP-N – Annual Net Primary Production of N, NUE – Nitrogen Use Efficiency and Recalcitrant Carbon (+/- 1 SE) is cellulose plus lignin for each species. Different letters represent significant differences between mean (P value ≤ 0.05) determined by adhoc Tukeys.

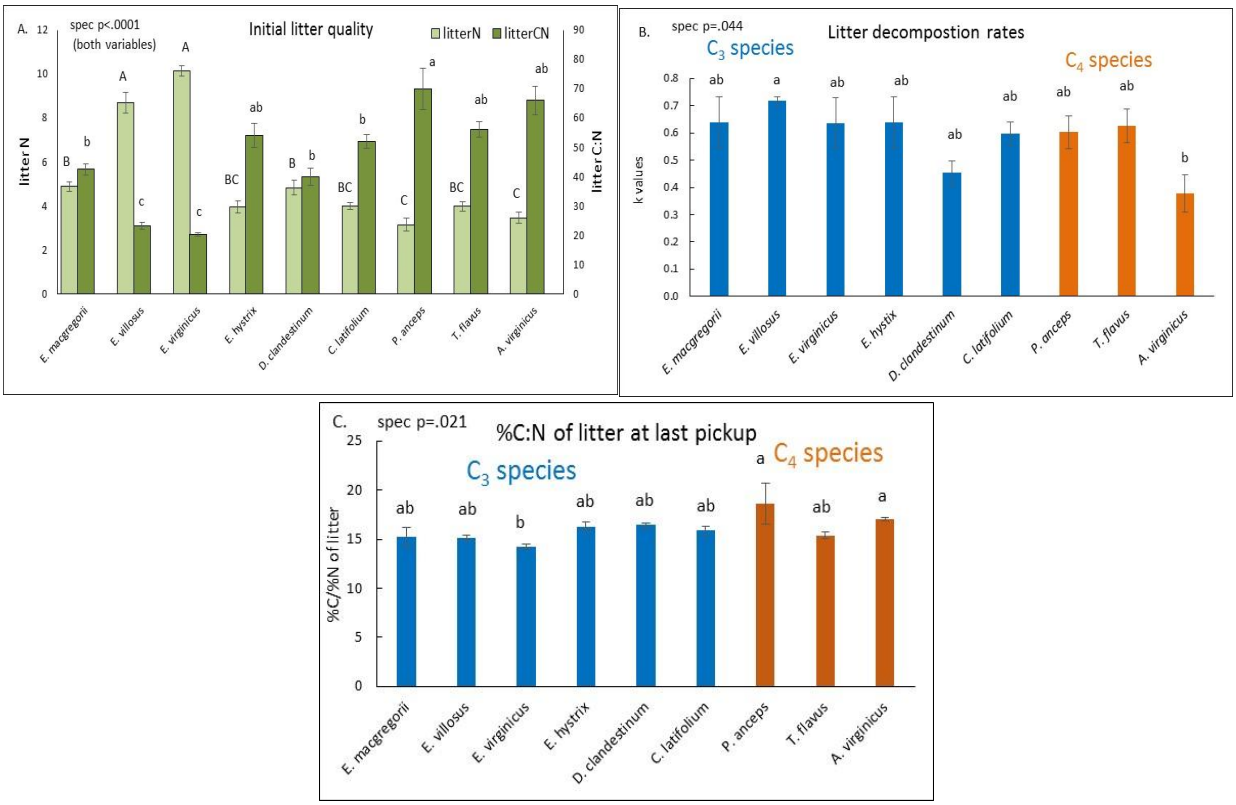


Figure 3.3: Species averages (±SE) for: A. initial litter quality for litter N (%N x biomass) and litter C:N (%C x biomass/%N x biomass), B. litter decomposition rates with calculated k value, and C. %C:N of litter at the last pickup date. Different letters represent significant differences between mean (P value ≤ 0.05). For graph A. litter N letters are capitalized.

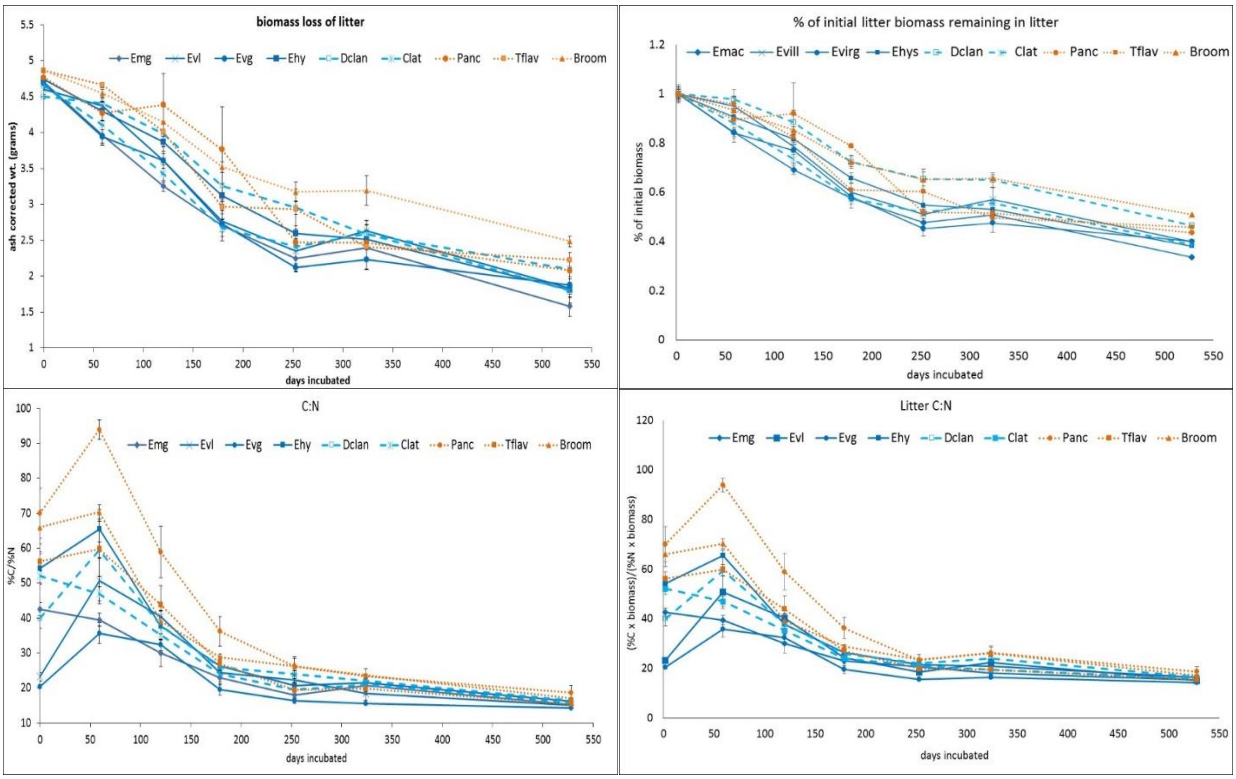


Figure 3.4: Litter bag averages (±SE) for the number of days incubated for biomass loss of litter, % of initial litter remaining in the litter, and C:N that were collected from January 2010 to June 2011. Litter bag averages (Mean ±SE) for each pickup date for litter C:N (%C x biomass/%N x biomass). Dark blue legend lines represent the *Elymus* species, light blue legend lines represent the other two C_3 species, and the orange legend lines represent the C_4 species.

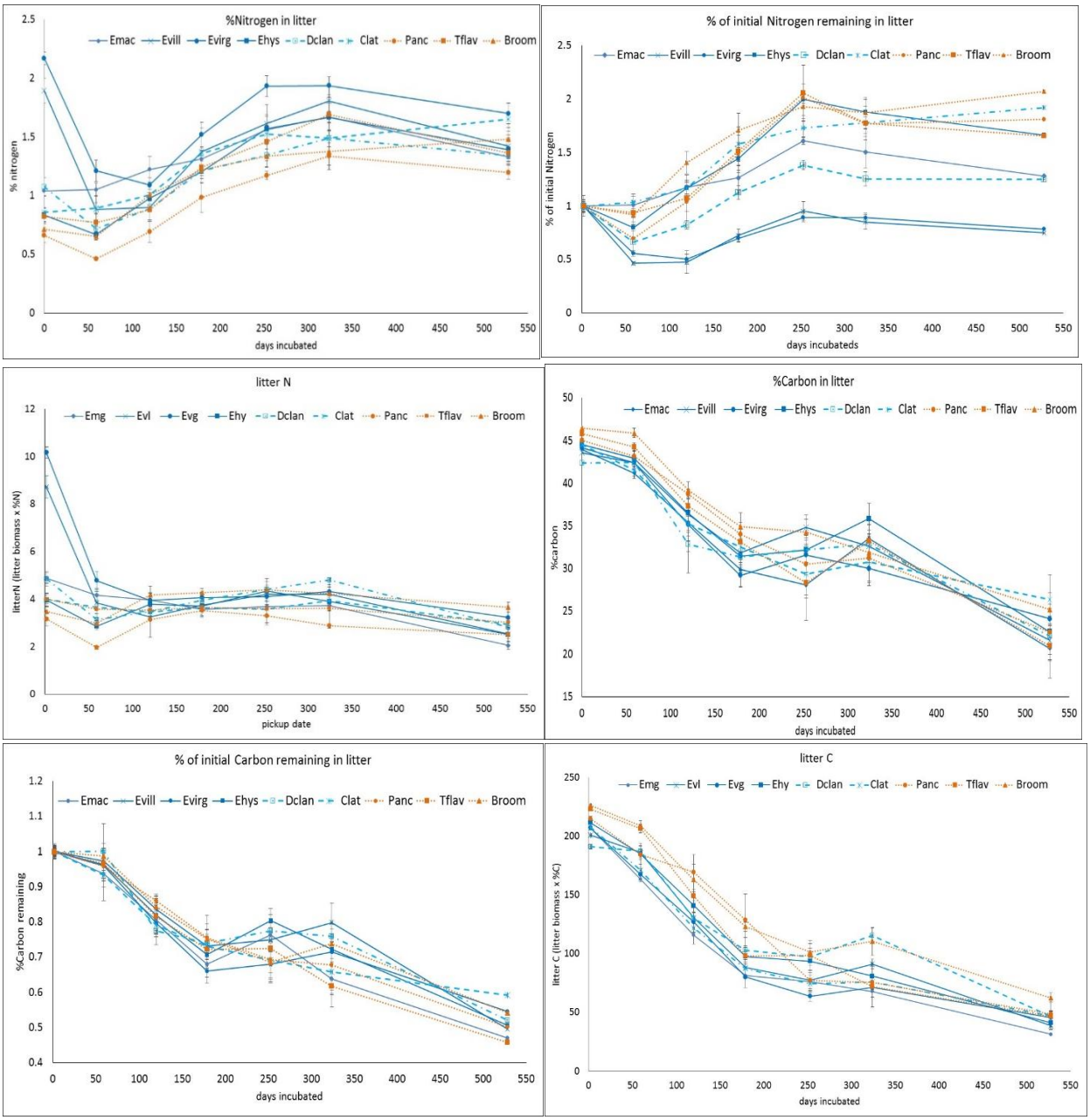


Figure 3.5: Litter bag averages (±SE) for the number of days incubated for %Nitrogen and %Carbon in the litter, and % of initial Carbon and Nitrogen remaining in the litter that were collected from January 2010 to June 2011. Litter bag averages (±SE) for each pickup date for litter C (%C x biomass) and litter N (%N x biomass). Dark blue legend lines represent the *Elymus* species, light blue legend lines represent the other two C_3 species, and the orange legend lines represent the C_4 species.

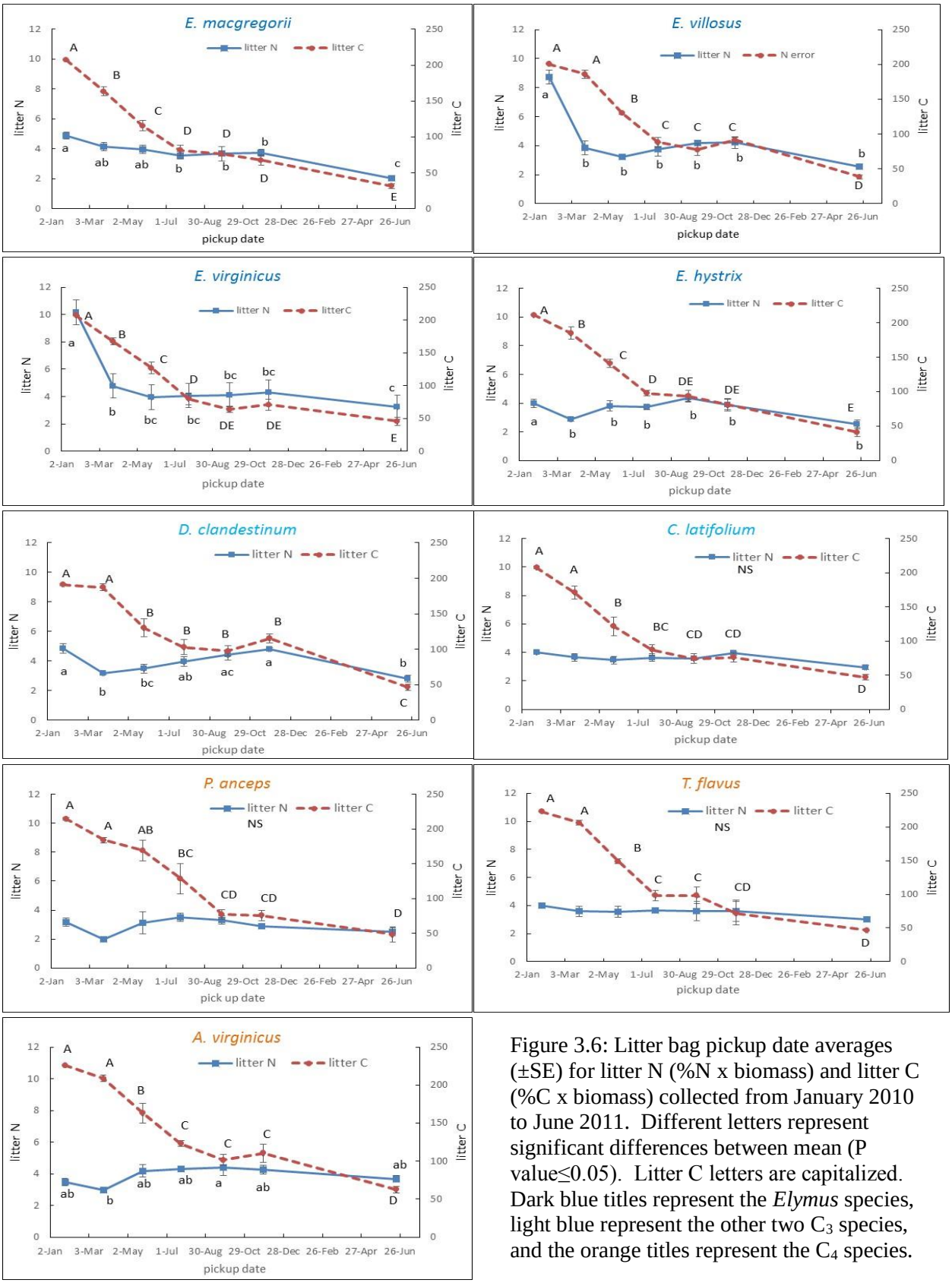


Figure 3.6: Litter bag pickup date averages (±SE) for litter N (%N x biomass) and litter C (%C x biomass) collected from January 2010 to June 2011. Different letters represent significant differences between mean (P value≤0.05). Litter C letters are capitalized. Dark blue titles represent the *Elymus* species, light blue represent the other two C_3 species, and the orange titles represent the C_4 species.

Figure 3.7: Soil N cycling parameters. Season totals for NO_3-N, NH_4-N and inorganic N were determined with resin bags. Differences between NH_4-N and $N0_3$-N season totals show significant differences between N types (* p<.05, ** p<.001. *** p<.0001) for each species. Nitrogen and carbon soil pools and bulk density were determined using 2012 soil samples. (Mean ± 1 SE) Different letters represent significant differences between mean (P value ≤ 0.05).

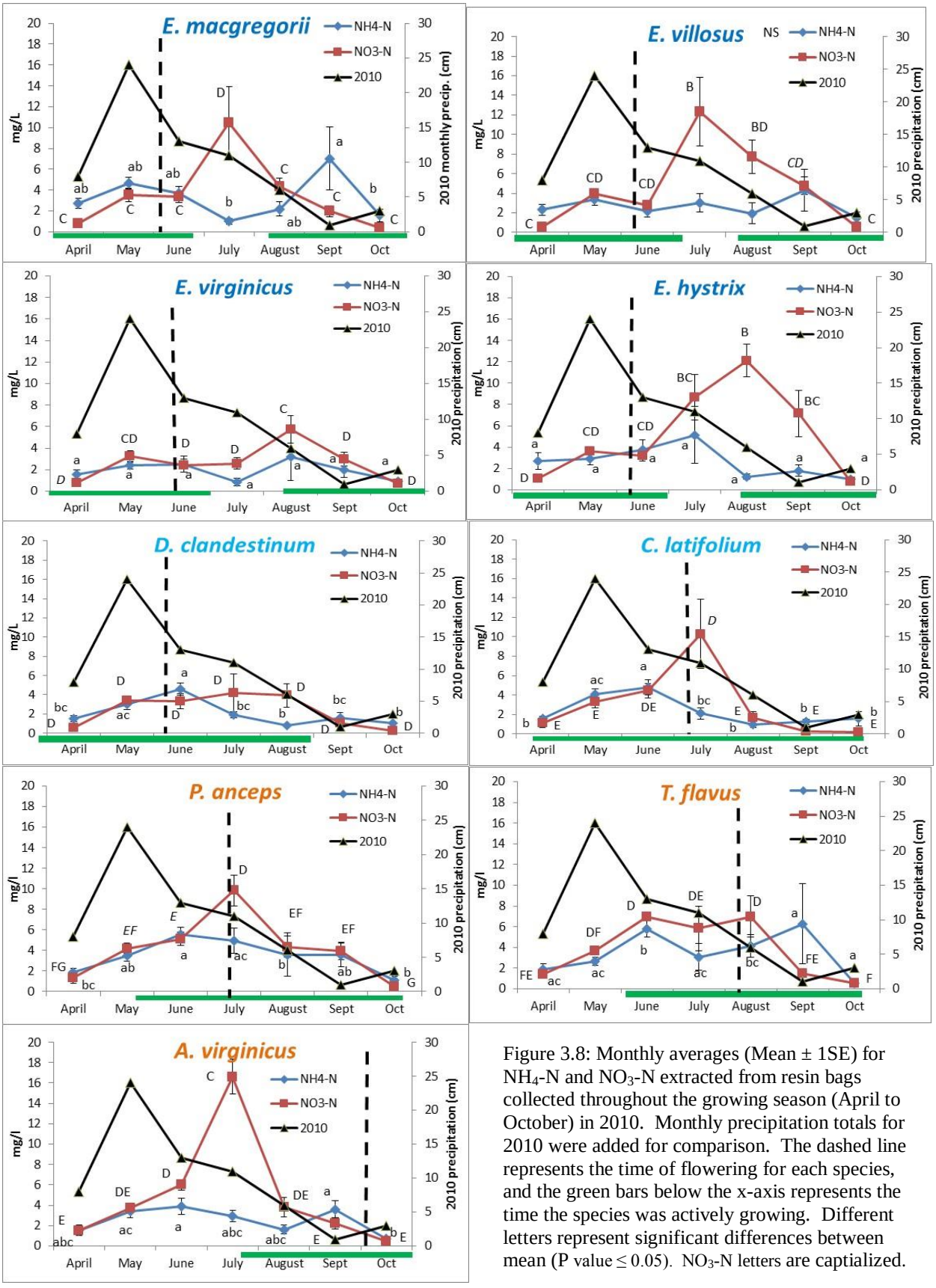
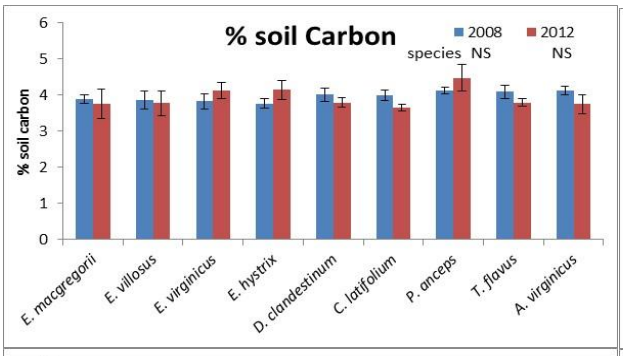
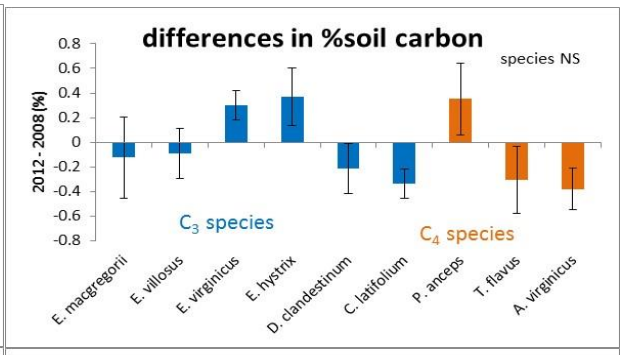
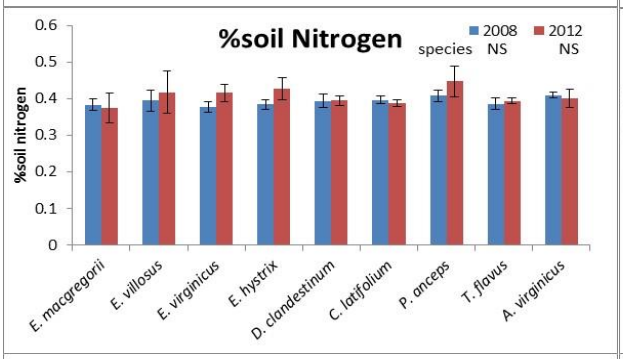
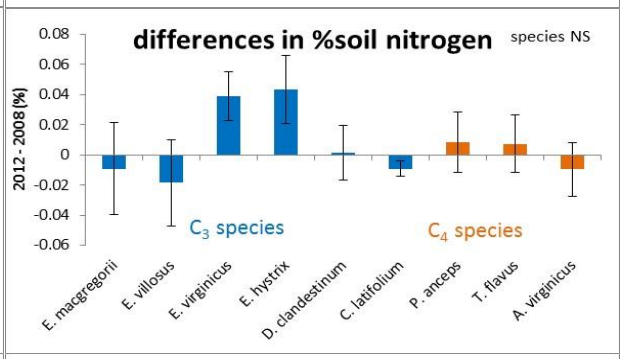


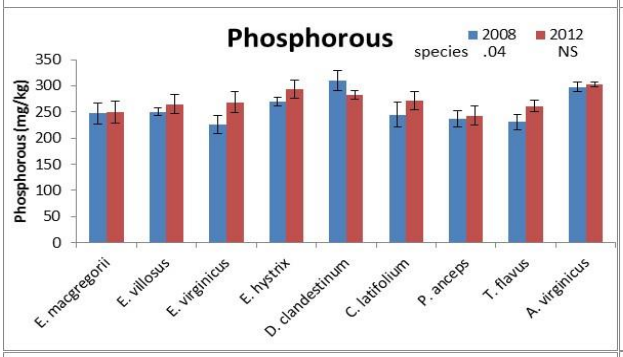
Figure 3.8: Monthly averages (Mean ± 1SE) for NH_4-N and NO_3-N extracted from resin bags collected throughout the growing season (April to October) in 2010. Monthly precipitation totals for 2010 were added for comparison. The dashed line represents the time of flowering for each species, and the green bars below the x-axis represents the time the species was actively growing. Different letters represent significant differences between mean (P value ≤ 0.05). NO_3-N letters are captialized.

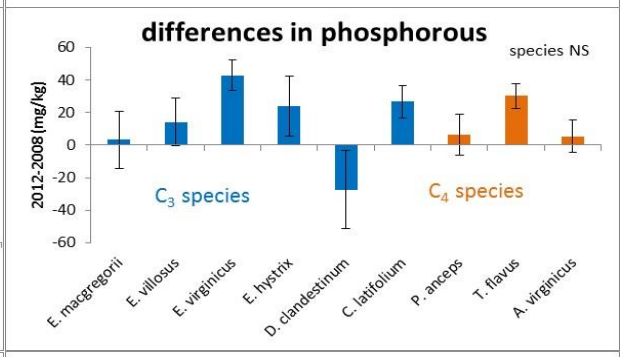

% soil Carbon
2008
2012
species NS
NS
% soil carbon
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus

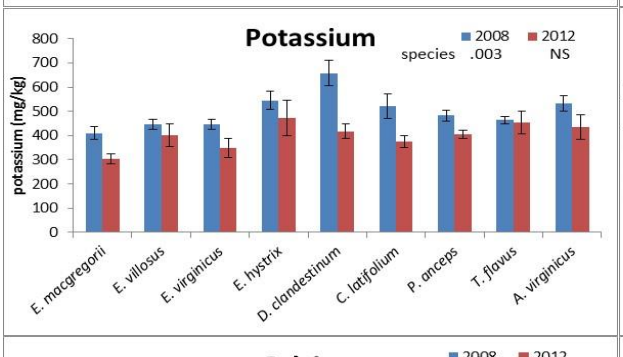

differences in %soil carbon
species NS
2012 - 2008 (%)
C3 species
C4 species

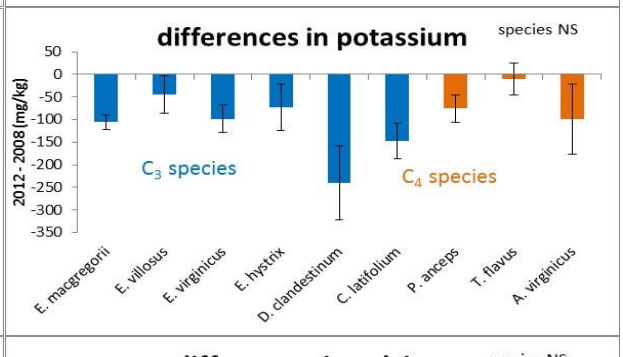

%soil Nitrogen
2008
2012
species NS
NS
%soil nitrogen

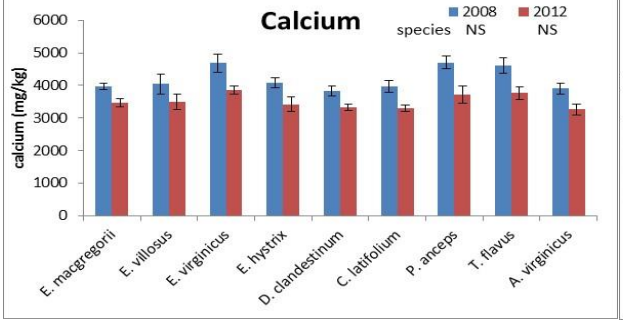

differences in %soil nitrogen
species NS
2012 - 2008 (%)
C3 species
C4 species

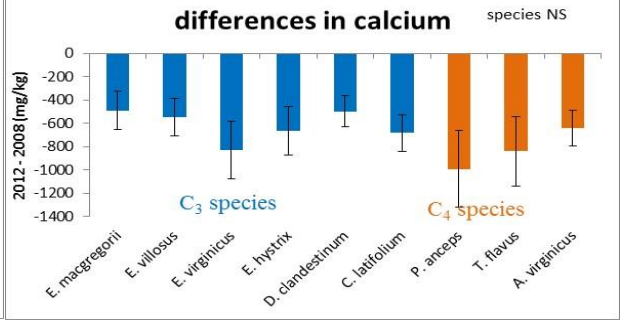

Phosphorous
2008
2012
species .04
NS
Phosphorous (mg/kg)


differences in phosphorous
species NS
2012-2008 (mg/kg)
C3 species
C4 species


Potassium
2008
2012
species .003
NS
potassium (mg/kg)


differences in potassium
species NS
2012 - 2008 (mg/kg)
C3 species
C4 species


Calcium
2008
2012
species NS
NS
calcium (mg/kg)


differences in calcium
species NS
2012 - 2008 (mg/kg)
C3 species
C4 species

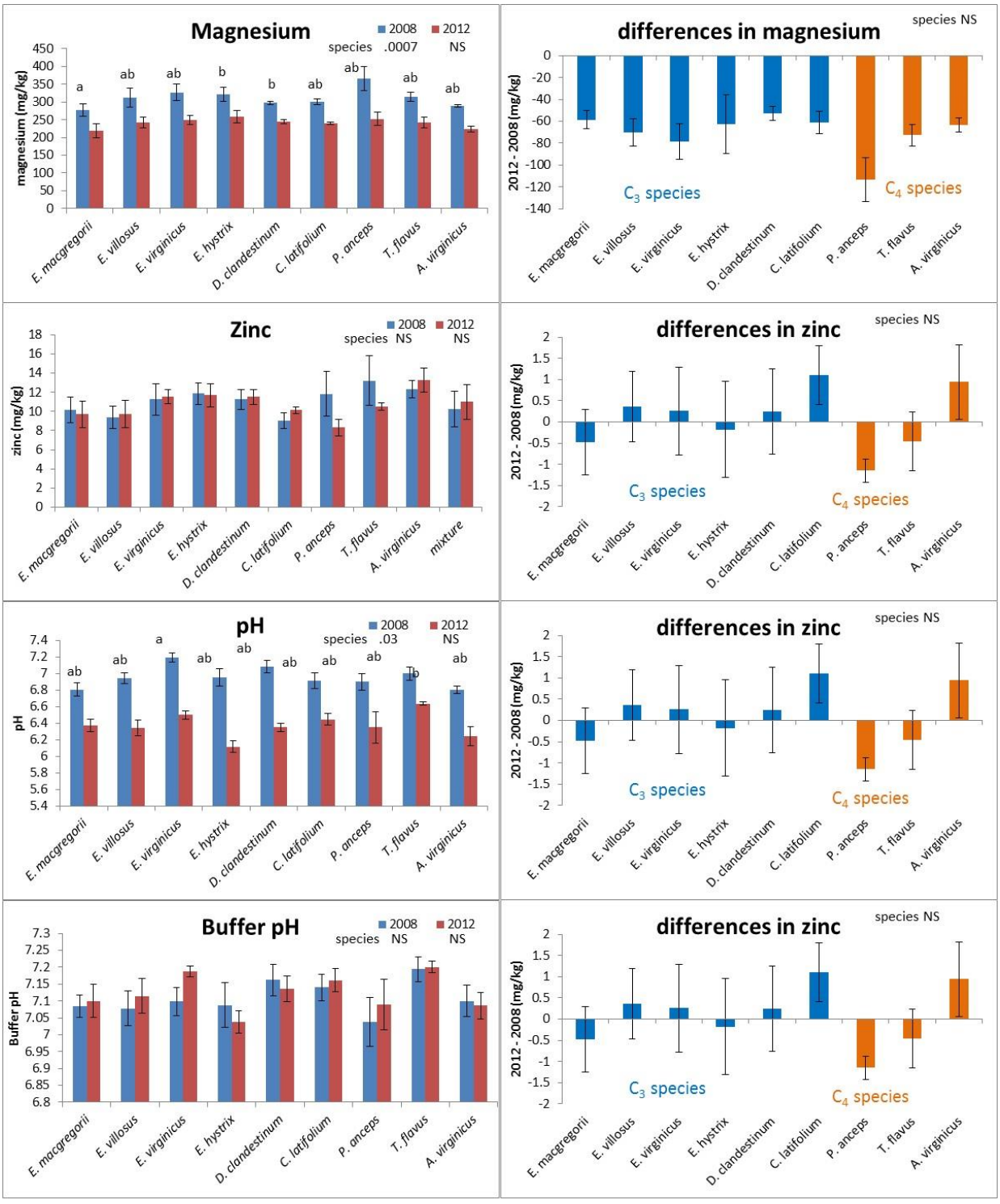


Figure 3.9: Left side panel shows averages (+/- 1 stderr) for soil nutrients (mg/kg), pH and buffer pH for 2008 and 2012. P values for species differences for 2008 and 2012 are denoted underneath the legend. Different letters represent significant differences between mean (P value ≤ 0.05). The right side panel shows averages (+/- 1 stderr) for the differences between 2012 and 2008 for soil nutrients, pH and buffer pH. Only soil pH had a significant species effect.

Chapter 4: Grazing strategies of C_3 and C_4 bunchgrasses native to a historic Oak Savanna-Woodland

Abstract

Since oak savannas of North America have been reduced to < 1 % of their historic ranges, restoration of these habitats is important to maintain the biodiversity and ecosystem properties of these landscapes. Restoration efforts of oak savannas are hindered by the lack of dependable historic data describing these savannas before they were converted to other uses and by lack of guidelines for ecological restoration. To better understand the dynamics associated with grazing effects, nine native bunchgrasses were studied in a clipping experiment that was designed to assess the effects of grazing on a savanna-woodland where no remnants remain to be studied, to compare the species for evidence of differences in grazing strategy (tolerance, deterrence and avoidance) and to recommend effective mowing regimes that would maintain a functional grassland community setting of the historic Bluegrass Oak Savanna-Woodland of Kentucky.

This clipping experiment included a factorial design with two clipping frequency treatments and two clipping intensity treatments to mimic a range of grazing regimes from frequent intense grazing to less intense rotational grazing. A non-clipped control treatment was added for comparison. In a heated greenhouse, the clipping treatments lasted from June to September of 2010 with 14 weekly clippings and 4 monthly clippings at both a 7 and 15 cm height (intensity). Plant height, tiller number, and clipped wt. were recorded at each clipping. Root wt. and shoot wt. were harvested at the end of the experiment. Percent tissue C and % tissue N were assessed for the root wt. and shoot wt. This experiment included three C_4 and six C_3 native perennial bunchgrasses. Four of the C_3 grasses were from the genus *Elymus* which were well recorded in historical documents and have significant life history trait differences compared to the other six species.

I found a significant effect of clipping on grass productivity overall, and a significant intensity effect but no significant frequency effect overall, and for the microscopic traits (tissue C/N) considered separately. For the macroscopic traits (productivity, biomass) considered separately, a significant frequency effect was detected but only at the most intense clipping treatment. In general, the three C_4 species and *Dichanthelium clandestinum* outperformed the *Elymus* species and *Chasmanthium latifolium*. All species except for *Elymus macgregorii* and *Panicum anceps* displayed evidence of more than one grazing strategy. While the most obvious strategies were seen at the 1/week 15 cm intensity clipping treatment, all nine species also were productive at the 1/month frequency treatments. Although grazing tolerance and deterrence strategies were mostly determined by clipping treatments, the avoidance strategy was more species specific with only *D. clandestinum* and *Andropogon virginicus* demonstrating an

avoidance strategy. *D. clandestinum* was the most plastic species, and the only one to demonstrate all three grazing strategies.

The results of this experiment suggest that the Bluegrass Savanna-Woodland grassland was historically not frequently and intensively grazed. Mowing regime recommendations to sustain a community setting of these grasses would include less intense more frequent grazing, or more intense less frequent mowing treatments. The diversity of forbs and the control of woody vegetation also should be taken into account when determining a mowing regime, particularly when managing the Bluegrass Savanna-Woodland without the use of fire.

Introduction

Savannas are grassland ecosystems characterized by the trees being sufficiently small or widely spaced so that the tree canopy is not closed (McPherson 1997) and are influenced by fire, climate, topography and soil (Nuzzo 1986). Savannas cover 20 % of the Earth's land area and can be divided into tropical and temperate groups. Tropical savannas cover 15 % of the Earth's land area, are generally well represented in the scientific literature, and are extensive in Africa, Australia, and S. America (McPherson 1997). While temperate savannas of North America were historically common at the time of European settlement, most of these landscapes have been reduced to < 1 % of their original area, are considered to be endangered landscapes (Anderson, Fralish et al. 1999), and are identified as critical areas for preservation (Klopatek, Olson et al. 1979). Furthermore, temperate savannas are not as well studied or represented in the scientific literature (McPherson 1997, Anderson, Fralish et al. 1999). Some potential reasons for this difference in level of research activity are the absence of a professional discipline associated with savannas, limited understanding of the role and importance of savannas in temperate regions, and inconsistent definitions and/or interpretations of the term savanna (McPherson 1997). Thus, there is a lack of knowledge of the ecological relationships and ecological management practices for temperate savannas compared to adjacent forest, desert, or grassland landscapes (McPherson 1997).

With European settlement in the eighteenth century, Midwestern Oak savannas in the U. S. A. all but disappeared within 20 to 40 years due to fire cessation and conversion of land to agricultural or urban development (Nuzzo 1986, Anderson, Fralish et al. 1999). The fact that only 2 % of Midwest Oak Savanna remained by 1986 (Nuzzo 1986) has caused this habitat to be listed as a "globally imperiled" ecosystem (Heikens and Robertson 1994). Conservation and restoration efforts of Oak Savannas are difficult due to: 1) the limited amount of historical data which were recorded, mainly by European pioneers and land surveyors, and the unknown validity and motivation for these records (Nuzzo 1986), and 2) lack of restoration ecology studies to guide ecological restoration practices in the field (McPherson 1997).

This restoration ecology study is designed to provide ecological guidelines to create and maintain a functional grassland community in the region where oak savanna once occurred. However no remnants remain to be studied and replicated, and the factors that created and maintained the savannas are unknown. A plant trait approach was used which views a species as a set of inter-connected traits that are both the result of its' evolutionary history and the ability of the species to respond to or affect community biotic and abiotic factors (Adler, Milchunas et al. 2004). This plant trait approach can reveal what ecological pressures a species may have evolved under and also help predict how the species will adapt to future selective pressures. With this approach, past ecological pressures and historical disturbance regimes can be inferred by studying plant traits in response to different environmental factors.

Disturbance is important and necessary for the maintenance of savannas. Frequent low intensity fires, a distinct annual dry season, extended droughts, and grazing by large herbivores are disturbances that often are associated with savannas (Enger and Smith 2004). However, these disturbances may be more characteristic of African Tropical Savannas than Midwestern Oak savannas (McPherson 1997). For example, the climate of most Midwestern Oak savannas does not promote frequent natural fires or extended droughts, and the dry season is generally more variable. While natural fires may not be common in Midwestern Oak savannas, fire is considered to be an important disturbance in the maintenance of Oak Savannas with Native Americans playing an important role (Mann 2011).

The Bluegrass Savanna-Woodland located in the Inner Bluegrass Region of Kentucky was considered by Braun (1943) to be anomalous or unexpected in the middle of the mixed mesophytic forest biome. Wharton and Barbour (1991) characterized this area as a savanna-woodland with an open forest whereby the trees are dominant but with a well-developed grassy undergrowth. This savanna-woodland was best described at the time of European settlement in the mid to late 1700's as having a mildly karst rolling topography, fertile, deep, and well drained silt loam soil produced over highly phosphatic Ordovician Limestone, vast cane breaks (*Arundinaria gigantea*), large mature trees including Oak (*Quercus sp.*) and Ash (*Fraxinus*), and a graminoid dominated herbaceous layer (McInteer 1952, Wharton and Barbour 1991, Campbell 2004). With European settlement, native grasses were rapidly replaced by non-native C_3 forage grasses (*Poa pretensis* and *Festuca arundinacea*) so that no intact savanna grassland remains in this region (Bryant, Wharton et al. 1980). It is thought that C_3 grasses were dominant in both abundance and number in the original savannas (Wharton and Barbour 1991, Campbell 2004), and that C_4 grasses fewer in the number of species and occurred in local openings on poorer soils or openings created by disturbance such as fire or bison trails (Campbell 2004).

Using Nuzzo's classification (1986), the Bluegrass savanna-woodland would have been classified as an open savanna that was maintained by frequent low intensity fires set by Native Americans (Mann 2011). Good evidence that the barrens in the Mississippian Plateau Region just west of the Bluegrass Region of Kentucky were created and maintained by anthropogenic fire (Anderson, Fralish et al. 1999) is further evidence that fire was used in this area. The use of fire by Native Americans may have been used in part to manage the large grazers or browsers of this system which were bison (*Bison bison*), elk (*Cervus canadensis*), and white tailed deer (*Odocoileus virginianus*) at the time of European settlement (Wharton and Barbour 1991).

If these savanna grasslands evolved under heavy grazing, native grasses would be expected to have been selected for strategies to tolerate, avoid, or deter grazing. Furthermore, the grazing history of this Bluegrass Savanna-Woodland can be inferred by studying the response of native grasses to differing grazing intensities and frequencies. Augustine and McNaughton's (1988) review of clipping experiments found that the frequency of clipping had a bigger impact than the intensity of clipping. After grasses are grazed, the timing between grazing events was important because competition for the newly available light is time sensitive (Augustine and McNaughton 1998).

The three grazing strategies optimize different suites of traits. Tolerance grazing strategies include rapid regrowth of tillers using the newly available light that was created by the grazing event, thus the grazing tolerant plant can outcompete its neighbors for light (Augustine and McNaughton 1998). Plant traits associated with grazing tolerance are increased photosynthetic rate, regrowth of photosynthetic biomass, lower investment of reproductive shoots, increased relative growth rates, increased root/shoot ratio, decreased C:N ratios, plasticity in carbon and nitrogen allocation, and reduced transpiration costs (Caldwell, Richards et al. 1981, Vandermeijden, Wijn et al. 1988, Augustine and McNaughton 1998, Pontes, Soussana et al. 2007). Grazing tolerant strategies are optimal under high grazing intensity and frequency environments where shading by neighboring plants is not an important factor. Strategies to deter grazing include the production of toxic secondary compounds or with mutualistic relationships with endophytes, and the accumulation of silica and/or recalcitrant carbon (Augustine and McNaughton 1998, Melo 2010). Grazing deterrence strategies promote the unpalatability of grasses that lower digestibility and nutritional value for herbivores. The avoidance grazing strategy is to be less conspicuous to herbivores and includes a low growth stature with low apical meristems, increased allocation to crown and roots, increased root/shoot ratio, and accumulation of standing senesced leaves and stems (Milchunas and Noy-Meir 2002, Adler, Milchunas et al. 2004, Quiroga, Golluscio et al. 2010). Structural carbon also would be important for the accumulation of aboveground dead biomass. Both avoidance and deterrence strategies are dependent on herbivore selection and preference, so these

strategies would be most beneficial in environments of intermediate grazing intensity and frequency where a variety of plants would be available for the herbivores (Vesk and Westoby 2001).

A greenhouse clipping experiment was designed using six C_3 and three C_4 native bunchgrasses (Wharton and Barbour 1991, Campbell 2004) to assess tolerance and look for evidence of other grazing strategies of these nine grasses and infer the historic grazing pattern for each of them. A factorial design included two clipping heights (intensities) and two clipping frequencies that were designed to mimic a range of grazing regimes from intensive grazing to rotational grazing. A control treatment was added for comparison. I hypothesis that: 1) frequency will have a bigger impact on plant traits than intensity as predicted by Augustine and McNaughton (1998), 2) the C_4 species will be better adapted to grazing than the C_3 grasses because they generally have higher nitrogen use efficiency, a higher C:N ratio, and a higher water use efficiency that should make them less affected by biomass loss, 3) that the grasses may have different grazing strategies at different frequency and intensity treatment levels. Results of this experiment can be used to recommend mowing regimes for ecological restoration that will maintain these grasses in a community setting, and provide insights for future restoration efforts.

Methods

Experimental Design

This clipping experiment was conducted using nine perennial bunchgrasses (six C_3 and three C_4) (Table 4.1) in a heated greenhouse at the University of Kentucky. A factorial design was used that included two clipping frequencies (1/week and 1/month) and two clipping heights (hereafter intensities; clip down to 7 cm and 15 cm above the soil surface) with a non-clipped control added for comparison. With a replication of 5, this completely randomized experiment produced 225 experimental units. An experimental unit consisted of one plant grown in a 16.6 cm depth, 16 cm width pot with drainage holes filled with 50% maury silt loam treated with methyl bromide, 50% coarse silica sand, and 15 milliliters of osmocote fertilizer.

Seeds of each species were collected in the Bluegrass Region of Kentucky and cold (moist) stratification requirements were determined by the seed testing laboratory at the Regulatory Services at the University of Kentucky. Plants were stratified as needed, and placed to germinate on a flooding table in a heated greenhouse on January 28, 2010. They were grown in the 72-well plant trays filled with Pro-Mix potting mix soil before being transferred into the experimental pots on April 6, 2010 when they were completely randomized on greenhouse tables. The plants were hand watered with a hose as needed, and maximum and minimum temperatures of the greenhouse were recorded weekly (Figure 4.1). The grasses were grown in the experimental pots for 10 weeks before the clipping treatments began.

Clipping treatments began on June 15th and lasted for 14 weeks until September 14th. Before each clipping, maximum plant height, number of tillers, and number flowering culms were recorded. To clip down to 7 cm or 15 cm, a metal collar that was either 7 cm or 15 cm tall was put around the plant and rested on the soil surface. The plant biomass above the collar was then clipped, dried at 55º C for 3 days, and weighed. Fourteen weekly and 4 monthly plant measurements were recorded. The 1week 7 cm treatment was designed to mimic intense grazing. The 1 week 15 cm treatment represented less intense grazing, and the 1 month 7 cm treatment simulated intense rotational grazing and the 1 month 15 cm treatment simulated less intense rotational grazing. The control treatment mimicked no grazing.
At the end of the experiment, shoots and roots were harvested, dried, and weighed. The roots were thoroughly rinsed over a mesh screen to remove as much sand and soil as possible. After the roots and shoots were dried and weighed, they were ground in a coffee grinder and analyzed for total %carbon and total %nitrogen with the Elementar vario MAX CNS Analyzer at the soils laboratory at Regulatory Services at the University of Kentucky.

Species

The nine native bunchgrasses (Wharton and Barbour 1991, Campbell 2004) included in this study are listed in Table 4.1 in the order of their flowering times. The nine species are categorized in two functional groups C_3 (or cool season) grasses and C_4 (or warm season) grasses. According to Wharton and Barbour (1991), the six C_3 grasses included in this study are associated with wooded habitats, and the three C_4 species are associated with more open habitats. Of the C_3 grasses, four species are from the genus *Elymus* or wildryes. *Elymus* species are well documented in historical records and are thought to have been abundant at the time of European settlement in the mid to late 1700's (Wharton and Barbour 1991). *E. virginicus* is common in open woods, thickets and old fields, and *E. villosus* is frequent in dry and moist open woods (Wharton and Barbour 1991). *E. macgregorii* can be confused with *E. virginicus* but flowers a month earlier and is also found in woods and thickets (Committee 2002), and *E. hystrix* is frequent in woods (Wharton and Barbour 1991). The *Elymus* species have a different life history pattern with significant niche differentiation from the other five species used in this study. They flower in the spring or early summer, set seed, and then go dormant during the hottest months of the summer. Plants regrow tillers in the autumn that will overwinter and produce flowering culms the next spring.

Dichantheilium clandestinum, which may have been referred to as "buffalos grass" in historical records, is frequent in open woods, thickets, and fencerows, especially on low ground (Wharton and Barbour 1991). *D. clandestinum* also has life history traits that differ from the other species in this study. *D. clandestinum* first produces cleistogamous flowering culms in the spring, and then later in the season plants produce self-fertilizing chasmogamous flowers on small inflorescences that are usually hidden

within the sheathes. Both types of flowers produce viable seeds. While this species did not produce a lot of tillers, it had the greatest ability for tiller branching, so one tiller could be quite large and heavy. *C. latifolium* is frequent on wooded stream banks, on floodplains, and in other moist habitats (Wharton and Barbour 1991), and it is used for in horticultural plantings and can be quite invasive.

The three C_4 species used in this study generally are found in more open sites. *P. anceps* is found less commonly and on moist ground, and *T. flavus* is common in old fields, woodland borders, open woods, pastures, and roadsides (Wharton and Barbour 1991). *A. virginicus* is common in old fields and overgrazed pastures (Wharton and Barbour 1991).

Plant traits

Macroscopic and microscopic traits were recorded for each species. The macroscopic traits are morphological and were counted by observation (tiller number and number of flowering culms), measured (plant height), or weighed (shoot wt. root. wt., and clipped wt.). Tiller size was calculated by dividing the shoot wt. by the number of tillers (grams/tiller). Aboveground Net Primary Production (ANPP) represents the shoot wt. plus the clipped wt. For the control treatment, the shoot wt. was also used for ANPP as no clipping occurred. Total plant biomass is the sum of ANPP and the root wt. The microscopic traits are physiological in nature and include measurements of % total N or % total C and were not directly measured or observed. Percent C and % N in both roots and shoots were analyzed with the Elementar vario MAX CNS Analyzer. Shoot C and Shoot N represent the total amount of C and N in the shoot and were calculated by multiplying the biomass of the shoot by the % C or % N of the shoot. Root C and root N were calculated in the same way. % C:N shoots, % C:N roots, shoot C:N and root C:N are the ratios of the respective numbers.

Considering the plant traits that were measured in this experiment, the three grazing strategies would optimize different suites of traits. Grazing tolerant plants would optimize regrowth (% N shoot, clipping wt.), which would lower shoot C:N and minimize sexual reproduction (number of flowering culms). The traits that would be optimized are clipped wt., percent shoot N, and root:shoot ratio, and minimize the number of flowering culms. Grazing deterrence strategies would optimize root:shoot ratio, % C shoot, shoot C, and C:N ratio, and minimize plant height, and the amount of N in the shoot. A tolerant plant would try to regrow tillers with the new light availability that was created by the grazing event, which would increase % N shoot, clipping wt. and plant height (Table 5).

Statistics

The statistical program PAST (Hammer 2001) was used to normalize the data and each ANOVA was performed in SAS (9.3: SAS Institute, Cary, NorthCarolina, USA) using PROC GLM (SAS 2010). Adhoc Tukeys tests were used for pairwise comparisons. To incorporate the control treatment into the

factorial 2x2 design, a partially hierarchical design was used. This design included the following factors: species (9 levels), group (2 levels: control vs. all others), frequency (nested within group; 2 levels: monthly vs. weekly), intensity (nested within group; 2 levels: 7cm vs. 15cm), as well as all identifiable two-way and three-way interactions. The total number of treatment combinations was 45. This analysis approach allowed us to test for main and interaction effects for each trait. Type I Sums of Squares were used to test these effects.

Multivariate analysis was performed in the program PC-ORD (6.08: MjM Software, Gleneden Beach, Oregon, U.S.A.) using Principle Components Analysis (PCA) using the Euclidean distance measurement (McCune and Mefford 2011). The data were not standardized and all response variables were included in the analysis. The Euclidean distance measurement also was used with Multi-Response Permutation Procedures (MRPP) within PC-ORD to discern significant differences between the nine species, five treatments (1wk7cm, 1wk15cm, 1mnth7cm, 1mnth15cm, and control), three intensities (7cm, 15cm, and control) and three frequencies (1week, 1month, and control). MRPP also was used for pairwise comparisons using the Euclidean distance measurement. For the MRPP analysis, acceptable p values were determined by dividing 0.05 by the number of treatments. For intensity and frequency effects, the acceptable $p<0.017$, for treatment effects $p<0.01$, and for species $p<0.006$. All pairwise comparisons used $p<.025$.

Results

Maximum weekly temperatures taken in the greenhouse were consistently and significantly higher than ambient maximum monthly temperature averages for Fayette County, Kentucky (Figure 4.1). Thus, the plants experienced higher than average maximum temperatures in the greenhouse than they would have experienced in the field. The high heat in the greenhouse during this experiment was probably most detrimental to the *Elymus* species because under field conditions, these species would have been dormant at that time. The number of plants that died during the experiment was higher in the 1/week frequency than the 1/month treatments with only *Elymus* species dying (Table 4.2). Also, one plant of *E. villosus* and *A. virginicus* died in the controls (Table 4.2).

Group, Species and Treatment Effects:ANOVA

Overall, there were significant effects of group (C_3 vs. C_4), species, intensity and frequency on macroscopic traits (Table 4.4) as well as numerous interactions. In multiple comparison, with the exception of total clipped weight (Figure 4.5), the three C_4 species and *D. clandestinum* produced significantly more total plant biomass, ANPP, and number of flowering culms than the four *Elymus* species and *C. latifolium* (Figure 4.5). *T. flavus* produced more shoot wt. than the four *Elymus* species and *C. latifolium* (Figure 4.5). *P. anceps*, *T. flavus*, and *D.clandestinum* produced more root wt. and grew

fewer but bigger tillers than the other species (Figure 4.5). *T. flavus*, and *P. anceps* increased plant height significantly more than the four *Elymus* species and *D. clandestinum*. *P. anceps* and *D. clandestinum* had a significantly higher root:shoot compared to *E. macgregorri* and *E. hystrix* (Figure 4.5).
For the microscopic traits (Table 4.5 and Figure 4.6), a significant species effect was detected for all traits except for % C roots. The same as the whole model species pattern (Figure 4.4 D), the C_4 species and *D. clandestinum* had significantly higher % C:N root and % N root than the four *Elymus* species and *C. latifolium* (Figure 4.6). The C_4 species and *C. latifolium* were significantly higher in % C:N shoots, % N shoots, and shoot C:N than the four *Elymus* species and *D. clandestinum* (Figure 4.6). There were no clear species grouping patterns for %C shoots, shoot C, shoot N, root C:N, root C, and root N (Figure 4.6).

Group, Species and Treatment Effects:PCA

To simplify the analysis and examine these overall results in more depth, PCA was used to organize the trait responses into more inclusive, correlated categories.

In the multivariate PCA analysis using all plant traits, significant intensity, frequency, treatment, and species differences were found (all $p<.0001$) (Figure 4.2A-D). The axes aggregate the traits fairly cleanly into macroscopic (Axis 1) and microscopic (Axis 2) traits. Many of the significant differences were primarily on Axis 1, but Axis 2 played a role as well. While intensity showed significant differences between all three treatments (7cm, 15 cm and control) (all $p<.0001$), the two frequency treatments (1/week and 1/month) were significantly different from the control ($p<.0001$) but not significantly different from each other ($p=.06$) (Figure 4.2A and B). When the PCA analysis was grouped by treatment, all four factorial clipping treatments were significantly different from the control (Figure 4.2C). There was an intensity effect with the two 15 cm treatments being significantly different than the two 7 cm treatments but no frequency effect. When the PCA analysis was grouped by species, two species groupings were apparent with the four *Elymus* species and *C. latifolium* in one group, and the three C_4 species and *D. clandestinum* in the other group (Figure 4.2D). In multivariate space, overall, the three C_4 species and *D. clandestinum* were more productive, and the four *Elymus* species and *C. latifolium* were the least productive (Figure 4.2D). *T. flavus* was the most productive species.

A separate PCA analysis was performed for the macroscopic and the microscopic traits to discern if these two types of traits were affected differently (Figure 4.2E and F). The macroscopic axes (Figure 4.2E) now separate out into productivity (Axis 1) and allocation (Axis 2), and the microscopic axes (Figure 4.2F) are less clear but appear to be root N (Axis 1) and shoot N (Axis 2). Both types of traits were significantly affected by intensity, frequency, treatment and species (all effects $p<.0001$) (Figure 4.2E and F). For the macroscopic traits, all three intensity treatments (7 cm, 15 cm and control) and all

three frequency treatments (1/week, 1/month and control) were significantly different (all effects $p<.0001$).

Species Differences at each Treatment Level

A PCA analysis was performed for each of the five treatments and grouped by species (Figure 4.4) to compare how the species were grouped at each treatment level compared to the overall model pattern using all treatments (Figure 4.2D). The same general species grouping between the four *Elymus* species plus *C. latifolium*, and the three C_4 species plus *D. clandestinum* (Figure 4.2 D) was found for both of the 15 cm intensity treatments (1week15cm and 1month15cm) (Figure 4.4 B, and D). The 1month7cm treatment (Figure 4.4 C) was similar to the overall pattern using all treatments (Figure 4.2 D) except *T. flavus* was significantly more productive than the other species. For the control treatment, all the species are loosely grouped but *T. flavus* was significantly more productive than the other species (Figure 4.4 E). For the 1week 7cm treatment, there is no clear grouping of species except that *E. macgregorri* did significantly worse than all the other species (Figure 4.4 A).

Pairwise comparisons were done using MRPP for all treatment combinations, which are shown in Table 4.3. The shaded cells represent a significant difference between the two treatments at that frequency or intensity level. If a frequency cell is shaded, the interpretation is that there is a significant intensity effect between those two frequency treatments. If an intensity cell is shaded, the interpretation is that there is a significant frequency effect between those two intensity treatments. When pairwise comparisons were done at the treatment level for the macroscopic traits, a significant frequency effect was found between the two 7 cm intensity treatments (Table 4.3). This significant frequency effect between the two 7 cm intensity treatments was not detected in the overall model (Table 4.3). Similar to the overall model, significant intensity effects were found when comparing both frequency treatments at both 1/week and 1/month intensity treatments. When the analysis was grouped by species, the species groupings also were different looking at just the macroscopic traits compared to the overall pattern using all plant traits (Figure 4.2 D and E) with the four *Elymus* species being loosely grouped and all other species being significantly different from each other. For the four *Elymus* species, *E. villosus* was statistically the same as *E. macgregorri* and *E. hystrix*, and *E. virginicus* was statistically the same as *E. hystrix*. (Figure 4.2E).

For the microscopic traits, intensity and frequency had the same effects as the whole model using all plant traits. Intensity was significantly different between all three treatments (7 cm, 15 cm and control), and the two frequency treatments (1/week and 1/month) were significantly different from the control (both $p<.0001$) but not significantly different from each other ($p=.06$). When pairwise comparisons were done at the treatment level, significant intensity effects were found between the two levels at the 1/week frequency and between the two levels at the 1/month frequency (Table 4.3). This was the same pattern as the overall model using all plant traits. Species groupings for the microscopic traits were similar to the

overall pattern using all plant traits (Figure 4.2 D and F). The only difference was that *A.virginicus* (Broom) and *P. anceps* were not significantly different in the microscopic plant trait analysis (Figure 4.2 D and F).

Treatment Differences for each Species

Another PCA analysis was performed for each species and grouped by treatment using all plant traits (Figure 4.7). *E. macgregorri*, *E. villosus*, and *E. hystrix* were the only species to have treatments that were not significantly different from the controls (Figure 4.7).

Looking at the PCA analysis done for each species that included all plant traits (Figure 4.7), all nine species had a significant intensity effect (all species $p<.0007$) but only *E. virginicus* and *D. clandestinum* had a significant frequency effect (both species $p<.0001$). For each species, pairwise comparisons were done using MRPP for all treatment combinations (Table 4.3). The shaded cells represent a significant difference between the two treatments at that frequency or intensity level. If a frequency cell is shaded, the interpretation is that there is a significant intensity effect between those two frequency treatments. If an intensity cell is shaded, the interpretation is that there is a significant frequency effect between those two intensity treatments. No clear patterns were seen between C_3 and C_4 species. Looking at all plant traits, all nine species had a significant intensity effect between the two 1/week frequency treatments, and six species had a significant intensity effect between the two 1/month frequency treatments (Table 4.3). Five species had significant frequency effects at both intensity levels (Table 4.3). Comparing the macroscopic and microscopic traits, the macroscopic traits had more species with significant frequency effects at both levels of intensity, and the microscopic traits had more species with intensity effects at both levels of frequency (Table 4.3). For the microscopic traits, all nine species had significant intensity effects at both frequency levels (Table 4.3) with five species had significant frequency effects at the 7 cm intensity level and three species had frequency effects at the 15 cm intensity level (Table 4.3). For the macroscopic traits, seven species had a significant intensity effect between the two 1/week frequency treatments, and seven species had a significant frequency effect between the 7 cm intensity treatments (Table 4.3). Four species had significant intensity effects at the 1/month frequency and four species had significant frequency effects between the 15 cm intensity treatments. *E. macgregorii* was the only species with the same effects for all plant traits, macroscopic traits, and microscopic traits (Table 3). *P. anceps* was the only other species besides *E. macgregorri* to have the same macroscopic and microscopic effects. Four C_3 species (*E. villosus*, *E. hystrix*, *D. clandestinum*, and *C. latifoium*) had the same effects for all plant traits and microscopic traits (Table 4.3). *T. flavus* and *E. virginicus* had different effects for all plant traits, macroscopic traits, and microscopic traits (Table 4.3).

Grazing strategies

Since a species was predicted to show different grazing strategies at different treatment levels, a PCA analysis was done using all plant traits for each species and grouped by the five treatments (Figure 4.7). To discern how each species responded to the clipping treatments, another PCA analysis was performed for each species with the control excluded (Figure 4.7). With the plant traits measured in this experiment, I categorized the most important plant traits for each grazing strategy and designated if the trait was positively or negatively correlated with that grazing strategy (Table 4.6). For each species, I assessed the grazing strategy at each clipping treatment level by determining what traits were positively or negatively correlated on the PCA graph according to each strategy (Table 4.7). Those predictions were verified using Figures 3.5, 3.6 and Supplemental graphs. If a treatment did not have a discernable pattern, it was left blank (Table 4.7). While these results are somewhat subjective, I found evidence of all three grazing strategies.

A treatment received a tolerance designation if it had an increased level of % N shoot along with increased clipped wt. and/or plant ht. (Figures 3.5, 3.6, and 3.7). Evidence of the tolerance strategy was found only at the 1/week frequency level treatments and for every species except for *A. virginicus* (Broom) and *D. clandestimum* (Table 4.7). In general, the *Elymus* species did not grow well at the 1/week 7cm treatment level and could not sustain the tolerance strategy throughout the experiment (Figure 4.5 and Supplemental). At the 1/week 15cm treatment, *E. macgregorii*, *E. virginicus* and *E. hystrix* were able to maintain the tolerance strategy but they did not maintain as much clipped wt. as *C. latifolium* (Supplemental). At the 1/week 7cm treatment level, *D. clandestinum* had higher clipped wt. than *C. latifolium* and *T. flavus* (Figure 4.5 and Supplemental). *E. villosus, P. anceps,* and *T. flavus* were designated as tolerant at the 1/week 15 cm treatment because %N shoot was positively correlated to plant ht. (Figures 3.5 and 3.6). *P. anceps* at the 1/week 7 cm treatment also was designated at tolerant. At the 1/week 15 cm treatment, *T. flavus* produced more total plant biomass compared to the other three clipping treatments by investing more in shoot wt. and root wt. *T.flavus* also had the highest plant ht., shoot carbon, shoot nitrogen, root carbon and root nitrogen at 1/week 15 cm treatment compared to the other three clipping treatments (Figures 3.5 and 3.6). *P. anceps* responded similarly as *T. flavus* at the 1/week 15 cm treatment but also produced significantly more flowering culms at this treatment level (Figure 4.5).

To assess the deterrence strategy, I predicted % C:N shoot, shoot C:N (high % C and low % N in shoots), and bigger tillers would be increased, while tiller number would be minimized (Table 4.6). The three C_4 species and *C. latifolium* had significantly higher % C:N shoots and shoot C:N and significantly lower %N shoots which implies they are better able to deter grazing than the other species (Figure 4.6). At the 1/month 7 cm clipping treatment, *E. virginicus, E. hystrix, D. clandestinum, C. latifolium, T. flavus,* and *A. virginicus* had the highest % C:N shoots compared to all five treatments (Figure 4.6).

These same six species plus *E. villosus* also had the lowest % N shoots at the1/month 7 cm clipping treatment (Figure 4.6).

To assess the avoidance grazing strategy, I predicted that shoot wt., root wt. root:shoot, tiller number, root C , and root N would increase, while clipped wt., plant height, and tiller size would decrease (Table 4.6). The clearest evidence of the avoidance grazing strategy was found for *D. clandestinum* at the 1/week 15 cm treatment (Table 4.7). *D. clandestinum* allocated biomass to leaves and stems below the 15 cm clipping height, produce little clipped wt. while still producing as much biomass as the control (Figure 4.5). At the 1/week 15 cm treatment, *D. clandestinum* grew more tillers than the other three treatments (Supplemental) and invested more biomass in root wt. which gave it a high root:shoot ratio (Figure 4.5). *D. clandestinum* also had significantly more root C and N at the 1/week 15cm treatment than the other 4 treatments (Figure 4.6). The only other species that displayed avoidance traits was *A. virginicus* (Broom) which allocated more biomass and nitrogen to the roots, produced more tillers, and grew shorter plants at the 15 cm intensity treatments compared to the control treatment (Figures 3.5 and 3.6 and Supplemental). *A. virginicus* (Broom) was also characterized as avoidance at the 1/week 7 cm treatment because it responded similarly as at the 15 cm frequency treatment except for producing less root wt, and reducing clipped wt. instead of plant ht. (Figure 4.6).

Discussion

The intensity of clipping had a bigger effect than the frequency of clipping on the macroscopic and microscopic traits for all species except for *E. virginicus* and *D. clandestinum* (Table 4.3). For the overall multivariate analysis including all nine species and all plant traits, a significant intensity effect was detected but not a significant frequency effect with both levels of frequency with both levels of intensity being significantly different from the control (Figure 4.2 A and B). These results are opposite of what Augustine and McNaughton (1988) found in their review of clipping experiments. My results may not support the conclusions of Augustine and McNaughton (1998) because 1) the short length of time the experiment was conducted, and 2) temperate bunchgrasses may not be as grazing tolerant as the tropical species they generally used in their review. When a separate PCA analysis was performed on the macroscopic and microscopic traits, a significant frequency effect was detected for the macroscopic traits between the two 7 cm intensity treatments (Table 4.3). Thus, frequency became important when the grasses were more intensively clipped. The species were more significantly different between the macroscopic than the microscopic traits (Figure 4.3 E and F). A frequency effect at lowest intensity treatment was seen for *E. macgregorii*, *E. virginicus*, *P. anceps* and *T. flavus* for the macroscopic traits and *E. macgregorii*, *P. anceps* and *T. flavus* for the microscopic traits (Table 4.3). When frequency and intensity effects were analyzed at the species level, all species had a significant intensity effect but only *E. virginicus*, and *D. clandestinum* had a significant frequency effect. At the treatment level analysis (Table

4.3), *E. macgregorii*, *E. hystrix*, *P. anceps*, and *T. flavus* had significant frequency effects for both macroscopic and microscopic traits, *C. latifoium* and *A. virginicus* had significant frequency effects for only the macroscopic traits, and *E. villosus* had no frequency effects (Table 3). It could be that frequency has a bigger effect on tropical species because they are better adapted to more intense grazing. The bunchgrass species used in this experiment seem to be better adapted to more frequent but less intense grazing events, therefore, they are more sensitive to intensity.

Considering how the nine species performed in this greenhouse environment with no imposed clipping (control), the C_4 species produced the most total biomass, followed by *C. latifolium* and *D. clandestinum*, with the four *Elymus* producing the least amount of biomass (Figure 4.4 E). While the C_4 species were positively correlated to all the macroscopic traits, the C_3 species were positively correlated to only a few microscopic traits (Figure 4.4 E). The high heat in the greenhouse (Figure 4.1) and the life history traits of the *Elymus* species may have been partly to blame for the poor performance of the *Elymus*. When clipping treatments were added to the analysis, the C_4 species and *D. clandestinum* performed better than the other five C_3 species (Figure 4.2 D). The C_4 species generally outperformed the *Elymus* species in all macroscopic traits except for root:shoot and tiller number (Figure 4.3B and Figure 4.5). Three of the *Elymus* species (*E. macgregorri*, *E. villosus*, and *E. hystrix*) had 15 cm intensity treatments that were not significantly different than the controls (Figure 4.7). This may indicate that these three *Elymus* species were not significantly affected at those treatment levels. *C. latifolium* performed similarly to the *Elymus* species in all macroscopic trait except that *C. latifolium* grew less tillers. *D. clandestinum* performed similarly to the C_4 species except *D. clandestinum* grew shorter plants with less clipped wt. (Figure 4.5). For the microscopic traits, the C_4 species and *C. latifolium* had lower tissue nitrogen concentrations in the shoots which gave them a higher shoot C:N compared to the other five C_3 species (Figure 4.3C and Figure 4.6). The C_4 species and *D. clandestinum* had lower tissue N in the roots that resulted in a higher C:N in the root compared to the other five species. Thus, the C_4 species had a higher a C:N ratio in both the root and shoot compared to the *Elymus* species, and the *Elymus* species had higher percent tissue N in shoots and roots.

Grazing strategies

This experiment was primarily designed to assess grazing tolerance rather than grazing deterrence and avoidance. Grazing deterrence and avoidance are dependent on herbivore selection which was not included in this design. However, the results can be interpreted from this broader perspective. In response to clipping, *T. flavus* was generally the top performer in all clipping treatments with *P. anceps* performing well at the 15 cm treatments, and *D. clandestinum* performing well at the 1 week 7 cm treatment (Figure 4.4). While the *Elymus* species and *C. latifolium* were the poorest performers at all treatment levels, *E.*

macgregorii performed the worst at the 7 cm intensity treatments (Figure 4.4). With higher percent shoot tissue N concentrations, the *Elymus* species and *D. clandestimum* would be expected to be able to replace eaten biomass and therefore be more grazing tolerant. With higher shoot C:N, the C_4 species and *C. latifolium* would generally be expected to be less nutritious and therefore deter grazing. While evidence for both of these strategies were found, these two grazing strategies were determined more by frequency and not by the C_3 and C_4 species grouping (Table 4.7). Only the avoidance strategy seemed to be species specific (Table 4.7).

Evidence for tolerance strategies was found at the 1/week frequencies. The most clear example of tolerance was for *T. flavus* at the 1/week 15 cm treatment where increased concentrations of N was found in the root and shoot which was allocated to growing taller plants. Higher amount of N in the roots and shoots imply increased N uptake at the 1/week 15 cm treatment. An explanation for why the *Elymus* did so poorly at the 1/week 7cm treatments may be that plants could not sustain the tolerance strategy throughout the experiment. With the high % N demand of the shoots, N uptake from the soil may have become limited as the clipping continued until the plants died or looked necrotic. Since the *Elymus* species are generally found in more mesic low lands and shaded wooded areas, they may not be adapted for frequent high intensity clipping under high heat and light conditions.

Evidence for the deterrence grazing strategy was found at the 1/month 7 cm treatment for all species except for *E. macgregorii* and *P. anceps*. Since the C_4 species and *C. latifolium* had higher C:N ratios than the C_3 species, they are expected to be better at deterring grazing. While this pattern was true for all of those species except for *P. anceps*, three of the *Elymus* species and *D. clandestinum* also had increased shoot C:N at the 1/month 7 cm treatment. Since species that are found in more open areas (C_4 species) and wooded areas (C_3 species) displayed deterrence strategies at the 1/month 7 cm treatment, there must be enough time between clippings at this lowest clipping intensity for plants to reallocate C to the shoots.

D. clandestinum and *A. virginicus* (Broom) were the only species that exhibited traits that promoted the avoidance strategy (Table 6). *D. clandestinum* exhibited clear avoidance traits at the 1/week 15 cm treatment where it concentrated biomass below the clipping height and in the roots with reduced clipping wt. (Figure 4.5). *D. clandestinum* also was the most plastic species, since it responded significantly different between all treatments (Figure 4.7). *A. virginicus* (Broom) displayed avoidance traits at the 15 cm intensity treatments and the 1/week 7 cm treatment by allocating more biomass to roots, increasing % N root, increasing tiller number, and growing shorter plants (Figures 3.5 and 3.6 and Supplemental). *A. virginicus* (Broom) was also considered avoidance at the1/month 15cm treatments and

at the 1/week 7cm where it allocated more biomass and nitrogen to the roots, produced more tillers, and grew shorter plants (Figures 5 and 6). The avoidance strategy for *A. virginicus* may be optimal in habitats without intense light competition from other plants which may be why this species is found commonly in old fields and overgrazed pastures (Wharton and Barbour 1991).

Conclusion

In conclusion, my hypothesis that clipping frequency would have a bigger effect on these nine species than clipping intensity was not supported. I found clipping intensity to have a bigger effect for both macroscopic and microscopic traits than clipping frequency, and a clipping frequency effect was found for only the macroscopic traits at the most intense (7 cm) clipping treatment. I predict that these bunchgrasses should be more sensitive to intensity if these savanna-woodland grasses historically experienced frequent but less intense grazing. My second hypothesis was partially supported as the C_4 species were more productive than all the C_3 species except for *D. clandestinum*. *D. clandestinum* had the most plastic response to grazing, and it was the only species to display traits for all three grazing strategies. *T. flavus* was the most productive of the C_4 grasses, and the *Elymus* species, particularly *E. macgregorri*, were the least productive. The *Elymus* species were probably the least adapted to the high light and heat environment of the greenhouse which was at the same time they would have been dormant under field conditions. Dormancy of the *Elymus* species at the same time the other species are active also may be a good grazing avoidance strategy that was not assessed in this experiment. I also predicted that the *Elymus* species would be eliminated from this savanna-woodland grassland community under high frequency and intensity grazing regimes. My third hypothesis that these grasses would have different grazing strategies at different frequency and intensity treatment levels was supported for all the species except for *E. macgregorii* and *P. anceps*. The most obvious grazing strategies were at the 1/week 15 cm treatment where *D. clandestinum* displayed clear avoidance traits, *T. flavus* displayed clear tolerance traits, and *P. anceps* optimized sexual reproduction through the production of flowering culms. The results of this experiment suggest the Bluegrass Savanna-Woodland grassland was not historically intensively grazed at high frequencies but that these grasses may be able to sustain this level of grazing for a short time. These results also suggest that these grasses are more adapted to less intense more frequent grazing, or more intense less frequent grazing. Mowing regimes at these intensity and frequency levels would most likely maintain a community settimg of these grasses. However, since fire is thought to be an important tool to keep woody species at bay, when fire is not used as a management tool, woody species management also would have to be taken into account when prescribing mowing regimes to maintain the Bluegrass Woodland-Savanna landscape. In some savannas, light grazing has apparently helped to preserve the savanna by inhibiting woody invasion without eliminating the ground layer (Nuzzo

1986). To be able to manage higher diversity of the savanna-woodland, future clipping experiments should include other functional groups such as woody species and forbs.

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Tables

Table3.1: The nine native perennial bunchgrass species used in this experiment listed in order of flowering time. The abbreviations are used in the multivariate graphs.

Scientific Name	Abbreviation	Common Name	Photosynthetic Pathway
1. *Elymus macgregorii* R. Brooks & J.J.N. Campb.	Emg	Early wildrye	C_3
2. *Elymus villosus* Muhl. ex Willd.	Evl	Nodding wildrye	
3. *Elymus virginicus* L.	Evg	Virginia wildrye	
4. *Elymus hystrix* L.	Ehy	Bottlebrush	
5. *Dichanthelium clandestinum* (L.) Gould	Dclan	Deer tongue	
6. *Chasmanthium latifolium* (Michx.) Yates	Clat	River Oats	
7. *Panicum anceps* Michx.	Panc	Beaked panicgrass	C_4
8. *Tridens flavus* (L.) Hitchc.	Tflav	Purple top/grease grass	
9. *Andropogon virginicus* L,	Broom	Broomsedge	

Table 4.2. Number of plant deaths for each species by treatment.

Species	Number of dead plants by treatment				
	1week7cm	1week15cm	1month7cm	1month15cm	control
Emg	1				
Evl	2				1
Evg		1			
Ehy	2				
Dclan					
Clat					
Panc					
Tflav					
Broom					1

Table 4.3: PCA results for treatment effects for all plant traits combined, macroscopic traits, and microscopic traits. The P value is for the overall model for all species and each species. The shaded cells represent a significant difference (p<.025) between the two treatments at that frequency or intensity level. If an intensity cell is shaded, the interpretation is that there is a significant intensity effect between those two frequency treatments. If a frequency cell is shaded, the interpretation is that there is a significant frequency effect between those two intensity treatments.

PCA results for significant treatment effects										
	All species	Emg	Evl	Evg	Ehy	Dclan	Clat	Panc	Tflav	Broom
All plant traits										
P value	<.0001	.0004	.005	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Intensity (p< .025)										
1/week										
1/month										
Frequency (p< .025)										
7cm										
15cm										
	Macroscopic traits									
Pvalue	<.0001	<.0001	.15	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	Intensity (p< .025)									
1/week										
1/month										
	Frequency (p< .025)									
7cm										
15cm										
	Microscopic traits									
Pvlaue	<.0001	.0004	.005	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	Intensity (p< .025)									
1/week										
1/month										
	Frequency (p< .025)									
7cm										
15cm										

Table 4.4 Whole model macroscopic traits ANOVA results including nine species, two clipping frequencies (weekly and monthly), 2 clipping intensities (7 cm and 15 cm), plus all interactions. Type I Sums of Squares were used to test these effects.

	Total biomass (grams)			ANPP (grams)		Shoot wt grams		Root wt. (grams)		Root:shoot	
	Df	F	p	F	p	F	p	F	p	F	p
Group	1	63.18	**<.0001**	60.86	**<.0001**	300.72	**<.0001**	38.48	**<.0001**	26.04	**<.0001**
Species	8	120.72	**<.0001**	145.5	**<.0001**	52.43	**<.0001**	50.01	**<.0001**	13.67	**<.0001**
Frequency	1	48.13	**<.0001**	82.68	**<.0001**	10.92	**.0012**	7.91	**.0055**	1.36	.246
Intensity	1	120.67	**<.0001**	101.33	**<.0001**	305.09	**<.0001**	84.55	**<.0001**	53.36	**<.0001**
Freq*inten	1	9.91	**.0019**	5.86	**.0165**	1.54	.2165	11.1	**.0011**	12.05	**.0007**
Spec*freq	16	3.04	**.0002**	3.76	**<.0001**	3.36	**<.0001**	3.13	**<.0001**	6.24	**<.0001**
Spec*inten	8	2.84	**.0055**	3.51	**.0009**	2.50	**.0137**	2.4	**.0176**	2.72	**.0075**
Sp*fre*int	8	3.00	**.0035**	3.2	**.002**	2.76	**.0068**	1.53	.1487	0.47	.8752

	Plant ht (cm)			Tiller number		Tiller size (tiller#/grams)	
	Df	F	p	F	p	F	p
Group	1	394.03	**<.0001**	11.24	**.001**	204.77	**<.0001**
Species	8	46.18	**<.0001**	31.32	**<.0001**	87.17	**<.0001**
Frequency	1	237.09	**<.0001**	3.22	.0744	.06	.7992
Intensity	1	158.15	**<.0001**	76.79	**<.0001**	43.88	**<.0001**
Freq*inten	1	58.73	**<.0001**	5.98	**.0155**	6.20	**.0137**
Spec*freq	16	3.5	**<.0001**	1.76	**.0408**	10.53	**<.0001**
Spec*inten	8	5.18	**<.0001**	6.83	**<.0001**	9.24	**<.0001**
Sp*fre*int	8	1.97	.0528	0.46	.8839	10.24	**<.0001**

Table 4.5 Whole model microscopic traits ANOVA results including nine species, two clipping frequencies (weekly and monthly), 2 clipping intensities (7 cm and 15 cm), plus all interactions. Type I Sums of Squares were used to test these effects.

	%C/%N shoot			%C shoot		%N shoot		C/N shoot (grams/grams)		C shoot (grams)	
	Df	F	p	F	p	F	p	F	p	F	p
Group	1	10.02	**.0019**	164.18	**<.0001**	14.88	**.0002**	.57	.4495	285.16	**<.0001**
Species	8	124.22	**<.0001**	7.05	**<.0001**	121.88	**<.0001**	138.35	**<.0001**	37.67	**<.0001**
Frequency	1	98.85	**<.0001**	1.88	.1720	91.87	**<.0001**	135.03	**<.0001**	24.33	**<.0001**
Intensity	1	6.93	**.0093**	46.03	**<.0001**	5.67	**.0185**	.06	.8104	249.89	**<.0001**
Freq*inten	1	24.62	**<.0001**	.02	.8998	22.58	**<.0001**	22.30	**<.0001**	5.67	**.0184**
Spec*freq	16	2.82	**.0005**	.89	.5853	3.31	**<.0001**	2.86	**.0004**	4.94	**<.0001**
Spec*inten	8	1.58	.1344	1.74	.0925	1.50	.1624	3.21	**.0021**	.85	.5622
Sp*fre*int	7	1.08	.3777	1.58	.1444	1.38	.2186	1.79	.0922	1.17	.3257

	N shoot (grams)			%C:%N root		%C root		%N root		C/N root (grams/grams))	
	Df	F	**p**	F	p	F	p	F	p	F	p
Group	1	361.37	**<.0001**	5.03	**.0262**	121.41	**<.0001**	65.98	**<.0001**	.27	.6014
Species	8	23.32	**<.0001**	42.62	**<.0001**	1.90	.0628	43.35	**<.0001**	5.20	**<.0001**
Frequency	1	.24	.6273	5.12	**.0250**	14.25	**.0002**	20.22	**<.0001**	2.66	.1050
Intensity	1	199.62	**<.0001**	.78	.3773	.86	.3354	0.61	.4345	.94	.3332
Freq*inten	1	.39	.5327	2.86	.0930	51.02	**<.0001**	3.98	**.0477**	1.98	.1615
Spec*freq	16	3.55	**<.0001**	4.16	**<.0001**	2.14	**.0089**	1.33	.1838	.70	.7949
Spec*inten	8	6.51	.8475	.78	.6251	.35	.9463	.93	.4932	.38	.9306
Sp*fre*int	8	1.35	.2306	3.63	**.0006**	.73	.6650	3.25	**.0019**	.77	.6262

	C root (grams)				N root (grams)	
	Df	F	p		F	p
Group	1	1.54	4.78		2.97	.0868
Species	8	37.00	**<.0001**		14.34	**<.0001**
Frequency	1	14.29	**.0002**		2.11	.1487
Intensity	1	92.06	**<.0001**		71.20	**<.0001**
Freq*inten	1	5.34	**.0221**		6.74	**.0103**
Spec*freq	16	4.78	**<.0001**		3.53	**<.0001**
Spec*inten	8	1.69	.1054		1.33	.2302
Sp*fre*int	8	.41	.9107		1.08	.3799

Table 4.6: Plant traits that are predicted to signify the three different grazing strategies that are denoted with a (↑) if positively correlated and (↓) if negatively correlated.

	Grazing strategies		
	Tolerance	Deterrence	Avoidance
Macroscopic traits			
Total plant biomass			
ANPP			
Shoot wt.			↑
Total clipped wt.	↑		↓
Root wt.			↑
Root:shoot			↑
Cumulative plant height	↑		↓
Tiller number	↑	↓	↑
Tiller size	↓	↑	↓
Flowering culms	↓		
Microscopic traits			
%C/N shoots	↓	↑	
%C shoots	↓	↑	
%N shoots	↑	↓	
Shoot C/N		↑	
Shoot C		↑	
Shoot N		↓	
%C/N roots			
%C roots			
%N roots			
Root C/N			
Root C			↑
Root N			↑

Table 4.7: Assessment of observed grazing strategy patterns for each species at each clipping treatment. The grazing strategy designations were determined by comparing the expected trait correlations for each strategy in Table 4.6 and comparing these predictions to Figures 3.5, 3.6, 3.7 and Supplemental.

Grazing strategy predictions				
Species	1/week 7 cm	1/week 15cm	1/month 7cm	1/month 15cm
Emg	tolerance	tolerance		
Evl	tolerance	tolerance	deterrence	
Evg	tolerance	tolerance	deterrence	
Ehy	tolerance	tolerance	deterrence	
Dclan	tolerance	avoidance	deterrence	
Clat	tolerance	tolerance	deterrence	
Panc	tolerance	tolerance		
Tflav	tolerance	tolerance	deterrence	
Broom	avoidance	avoidance	deterrence	avoidance

Figures

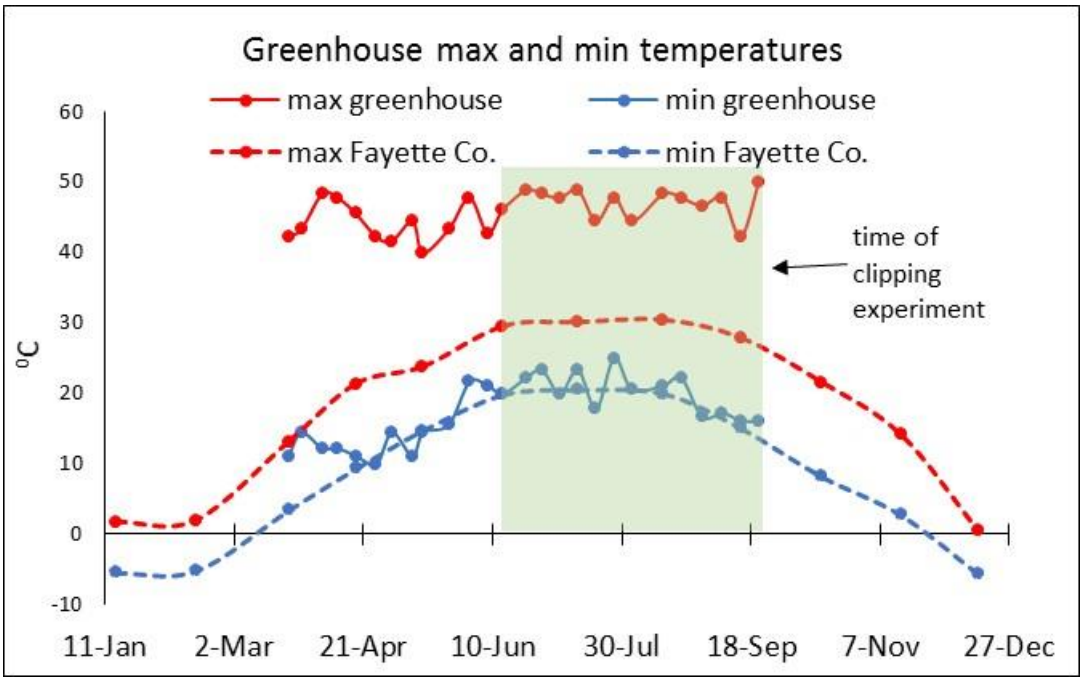


Figure 4.1: Maximum and minimum greenhouse temperatures (°C) recorded from the time the seeds germinated until the end of the 3 month clipping experiment. Max and min monthly averages for Fayette County in 2010 were added for comparison (Ky Mesonet).

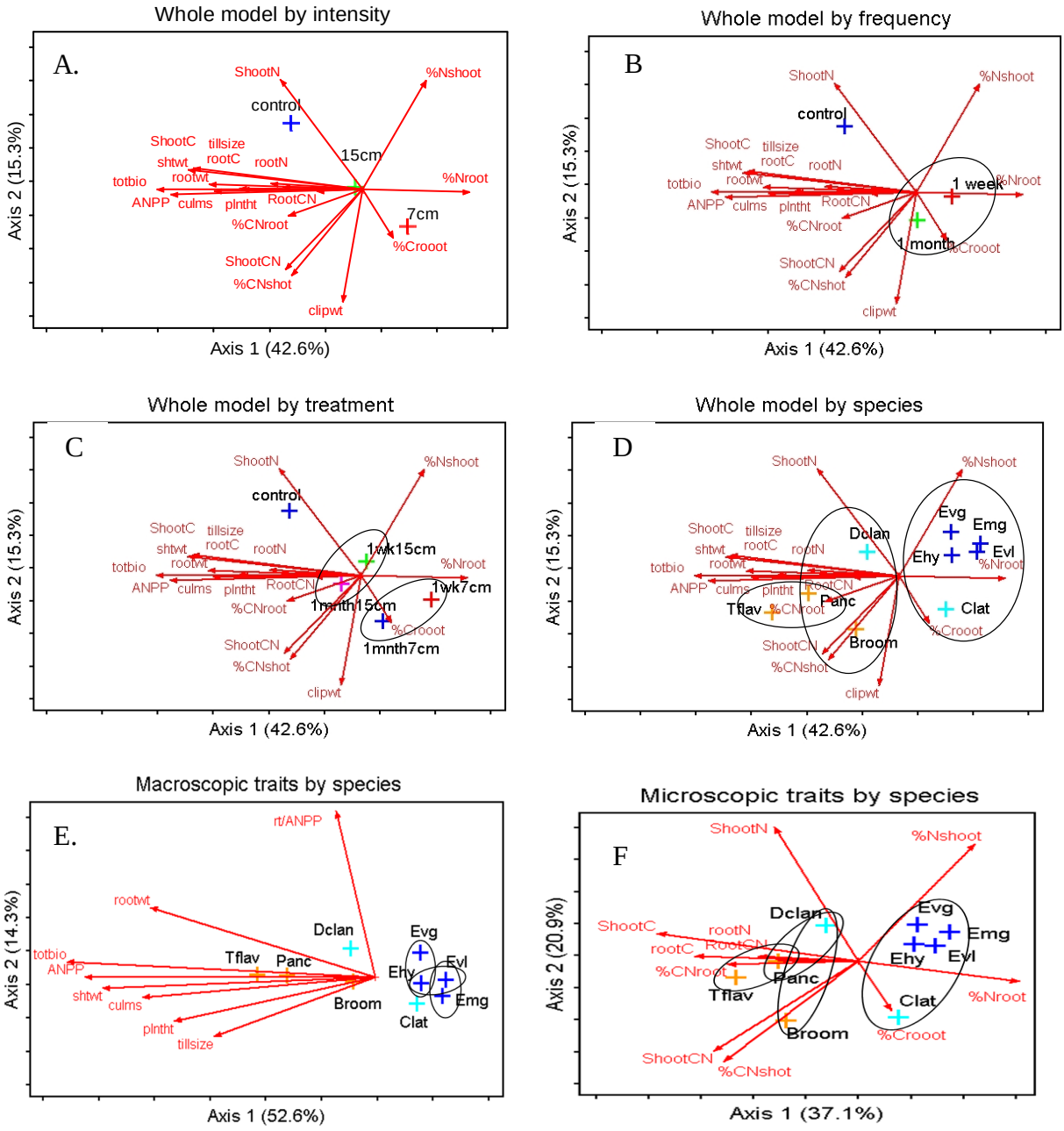


Figure 4.2: PCA results A.) all traits grouped by intensity, B.) all traits grouped by frequency, C.) all traits grouped by the five treatments, D.) all traits grouped by species. E.) macroscopic traits grouped by species, and F.) microscopic traits grouped by species. The circles represent the species means that are not significantly different in pairwise comparisons using MRPP ($p<.025$). The percent of variance explained for each axis is in parenthesis.

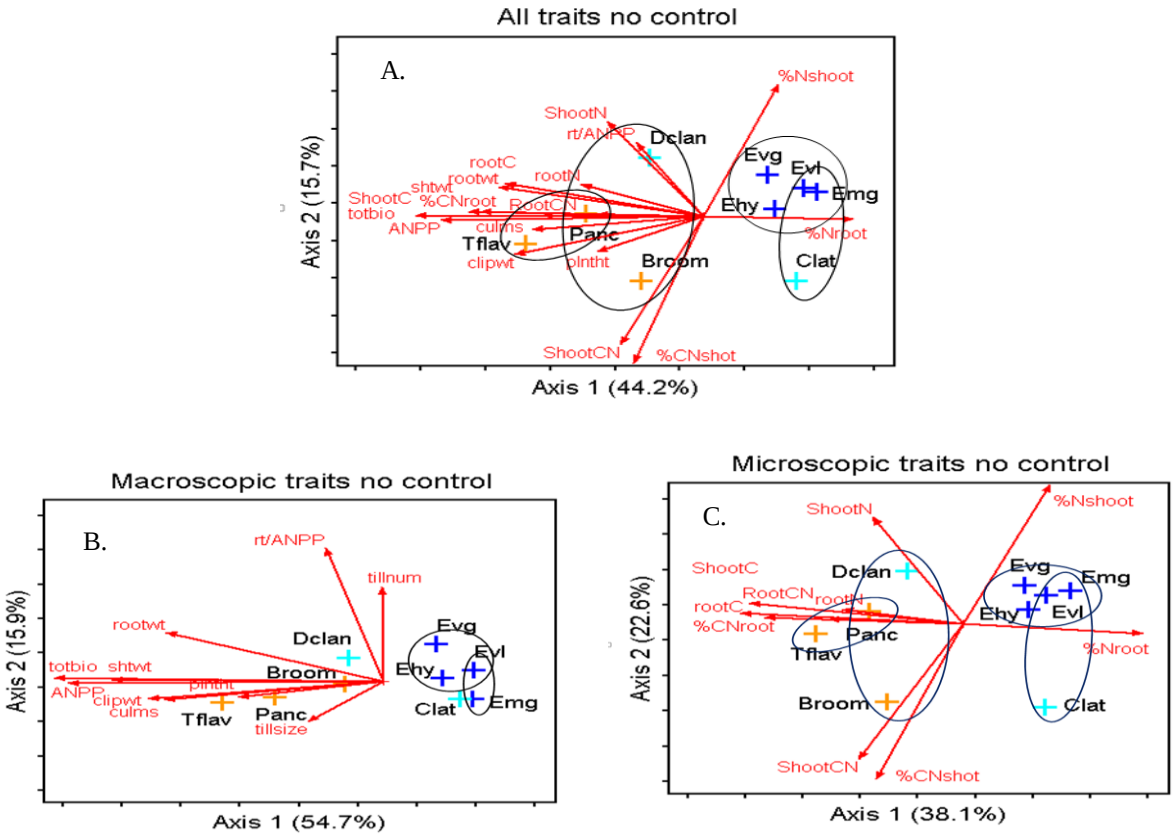


Figure 4.3: PCA results for the clipping treatments only A.) all traits with the control treatment excluded, B.) macroscopic traits with the control treatment excluded, C.) microscopic traits with the control treatment excluded. The circles represent the means that are not significantly different in pairwise comparisons using MRPP ($p<.025$). The percent of variance explained for each axis is in parenthesis.

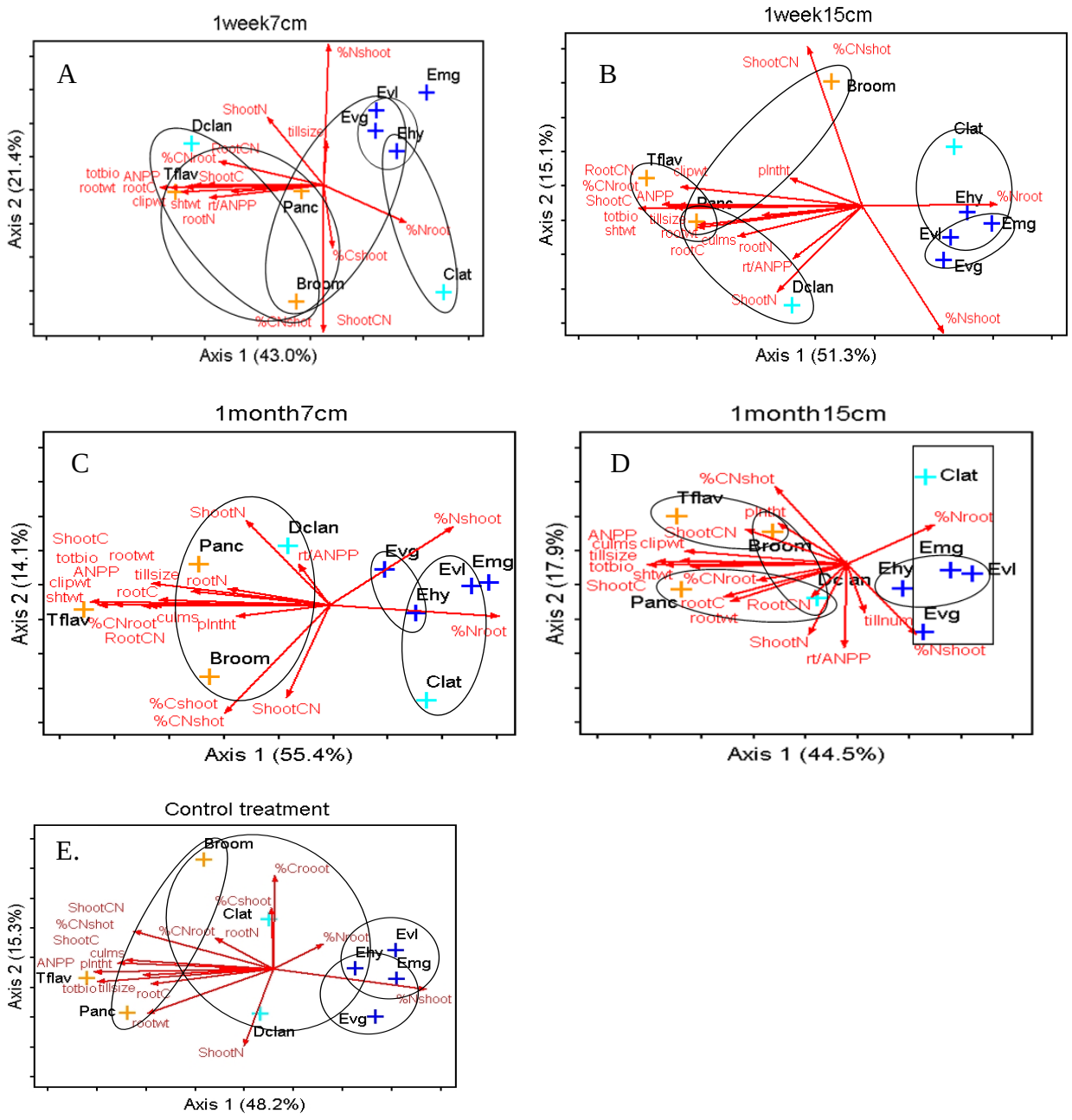
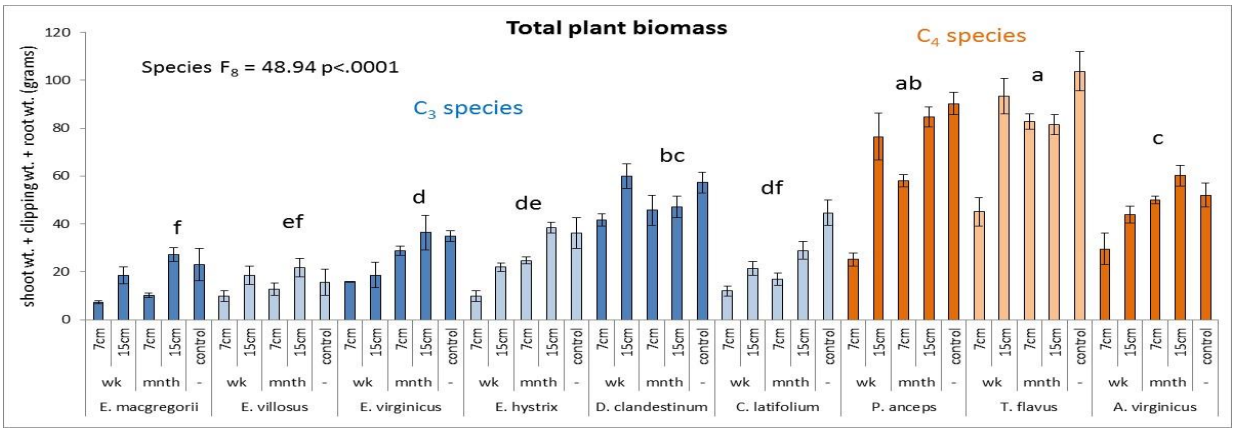
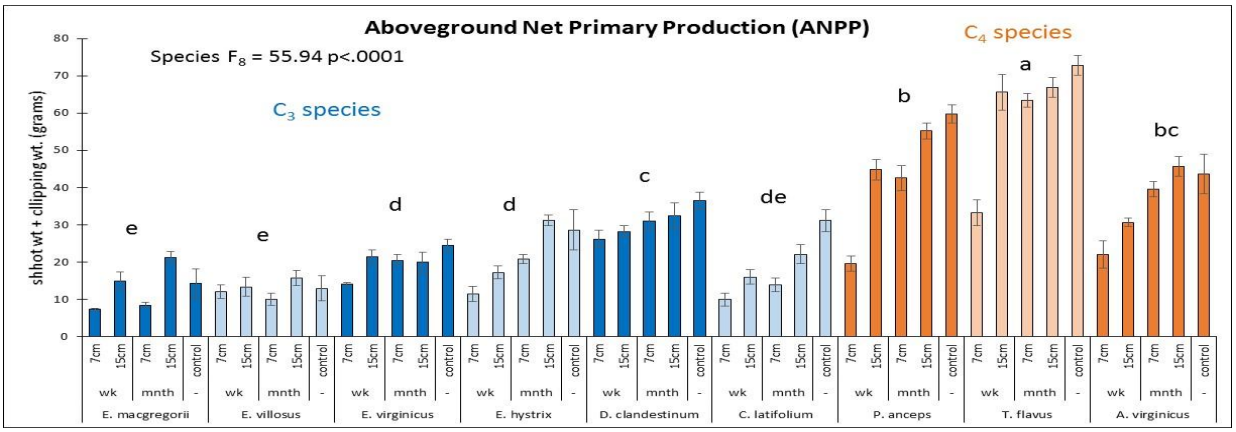
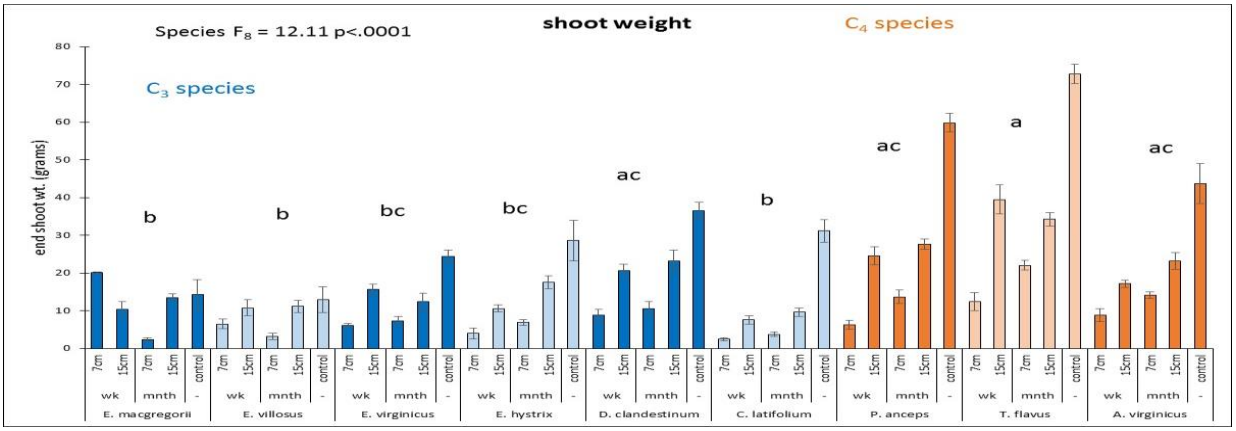
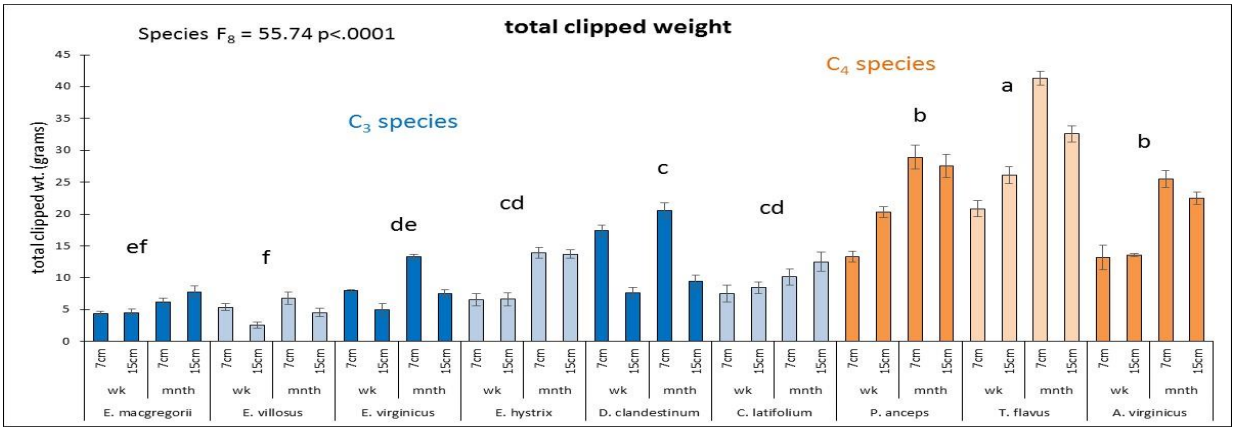


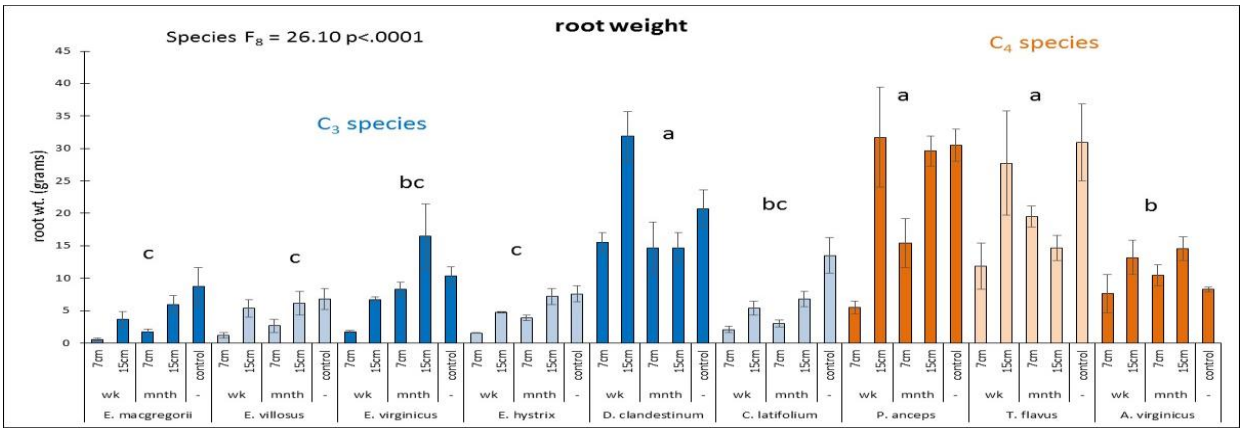
Figure 4.4. PCA results for each treatment using all traits grouped by species. The circles represent the species means that are not significantly different in pairwise comparisons using MRPP ($p<.025$). The percent of variance explained for each axis is reported.

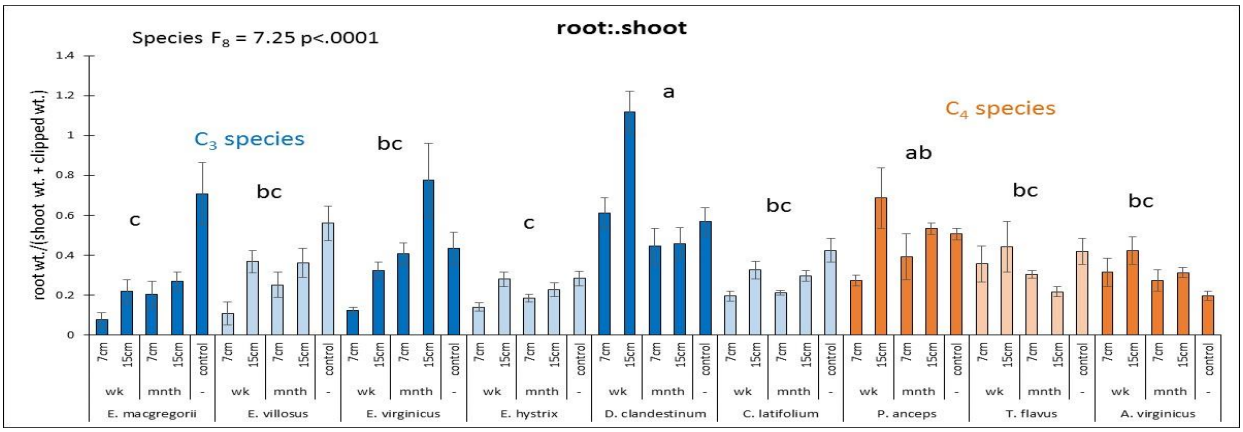

Total plant biomass
Species F_8 = 48.94 p<.0001
C_3 species
C_4 species
shoot wt. + clipping wt. + root wt. (grams)
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus

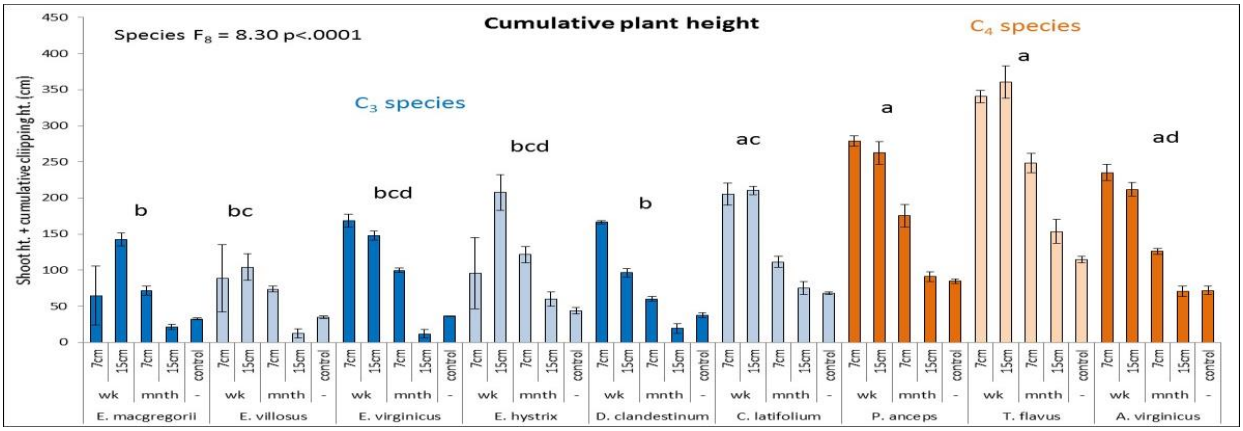

Aboveground Net Primary Production (ANPP)
Species F_8 = 55.94 p<.0001
C_3 species
C_4 species
shhot wt + clipping wt. (grams)
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus

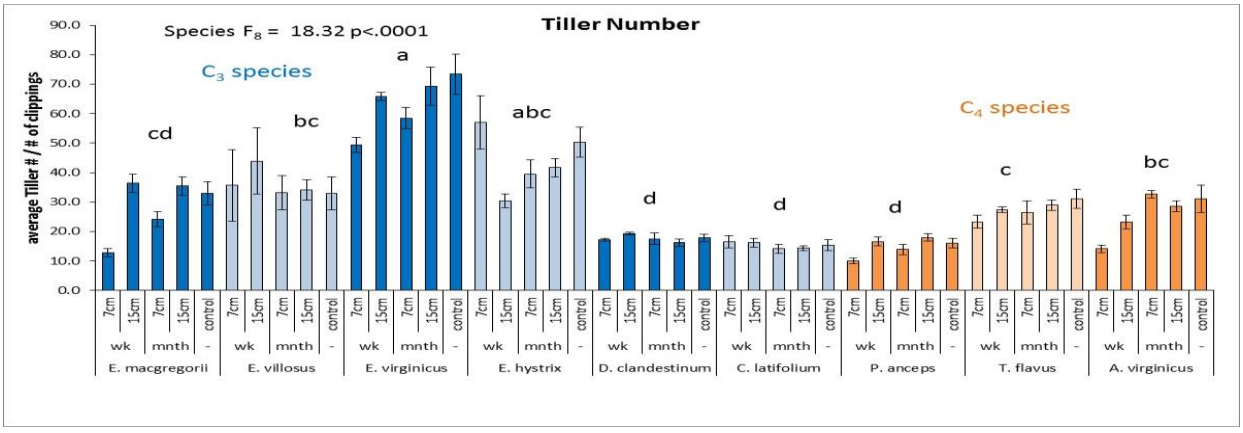

shoot weight
Species F_8 = 12.11 p<.0001
C_3 species
C_4 species
end shoot wt. (grams)
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus


total clipped weight
Species F_8 = 55.74 p<.0001
C_3 species
C_4 species
total clipped wt. (grams)
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus


root weight
Species F_8 = 26.10 p<.0001
C_3 species
C_4 species
root wt. (grams)
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus


root:.shoot
Species F_8 = 7.25 p<.0001
C_3 species
C_4 species
root wt./(shoot wt. + clipped wt.)
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus


Cumulative plant height
Species F_8 = 8.30 p<.0001
C_3 species
C_4 species
Shoot ht. + cumulative clipping ht. (cm)
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus


Tiller Number
Species F_8 = 18.32 p<.0001
C_3 species
C_4 species
average Tiller # / # of clippings
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus

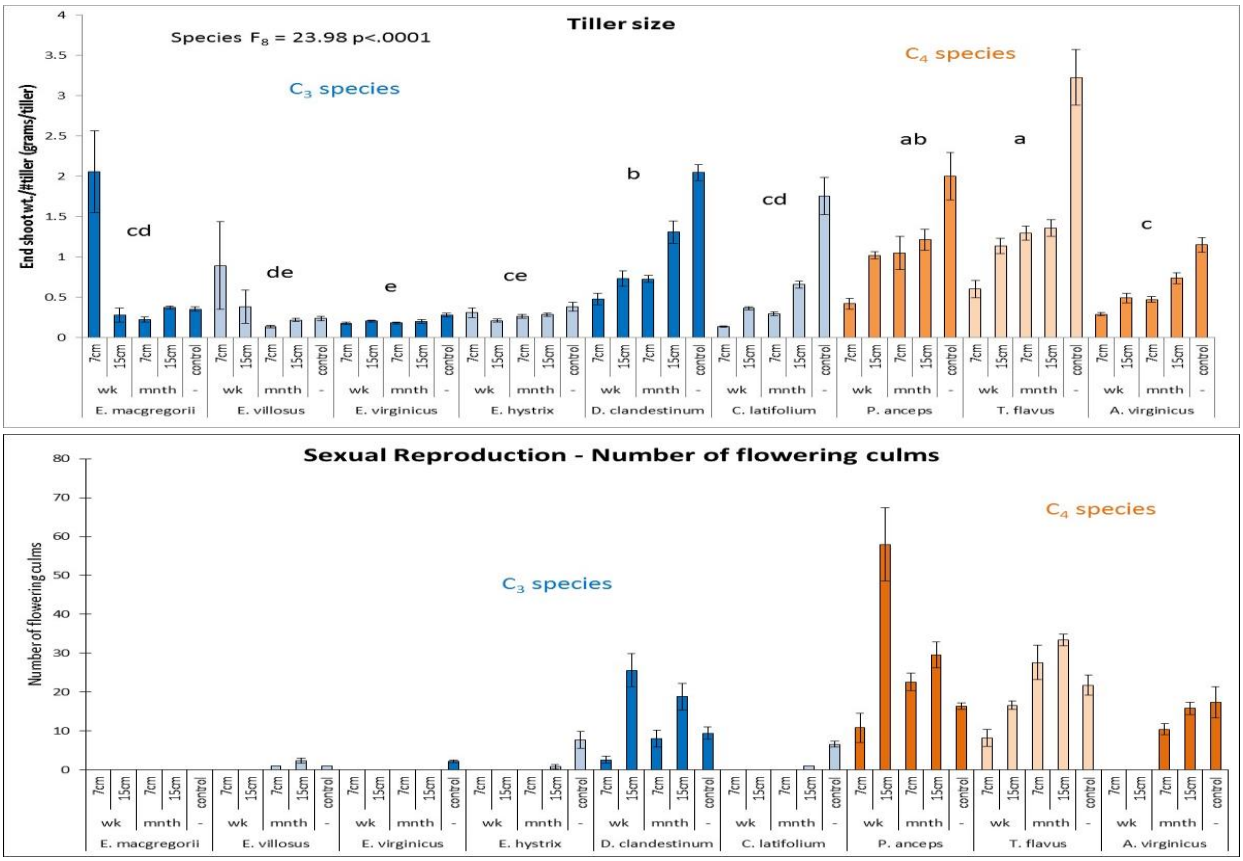
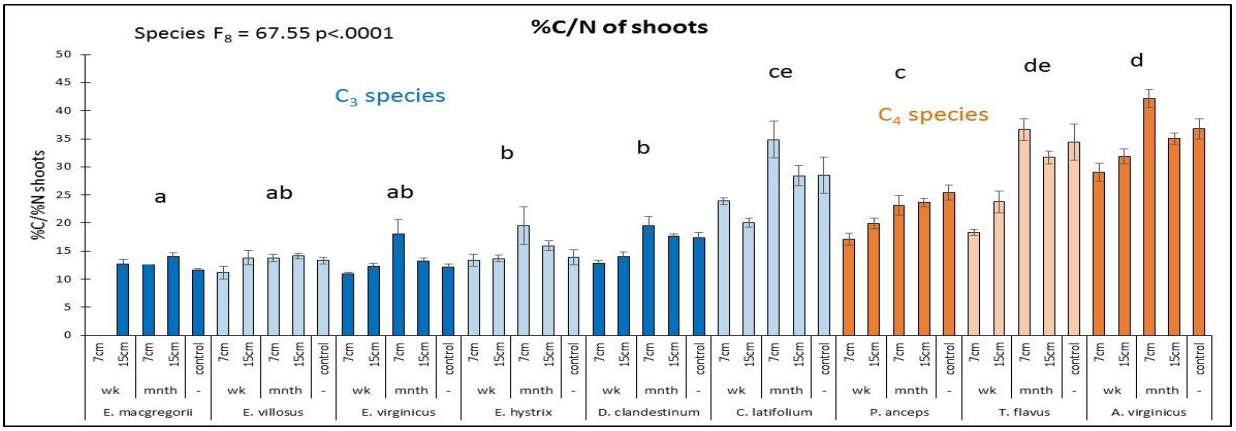
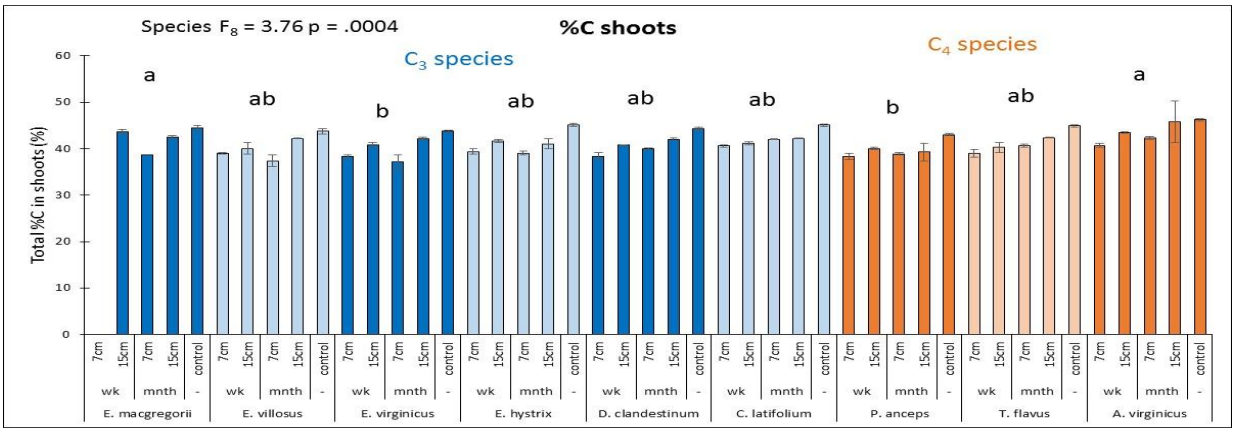
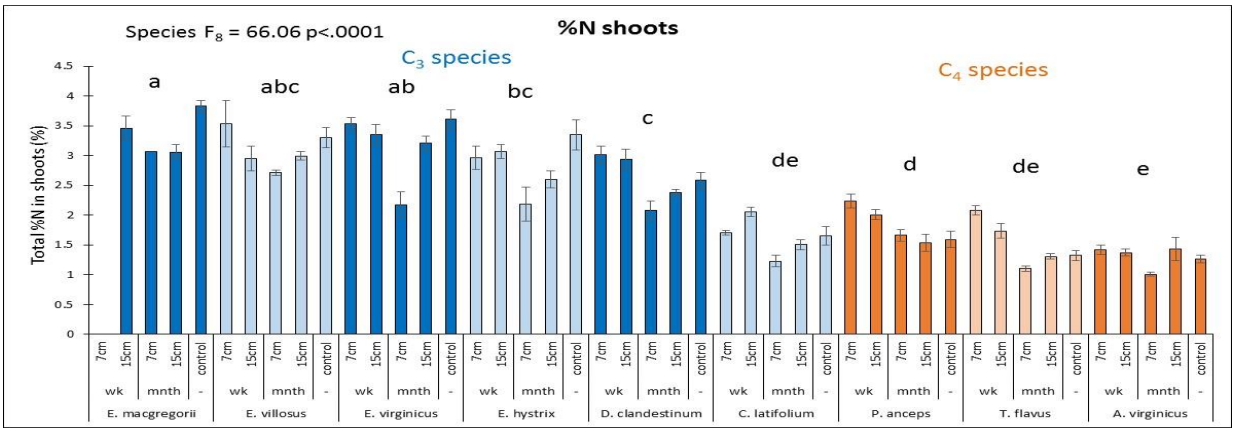
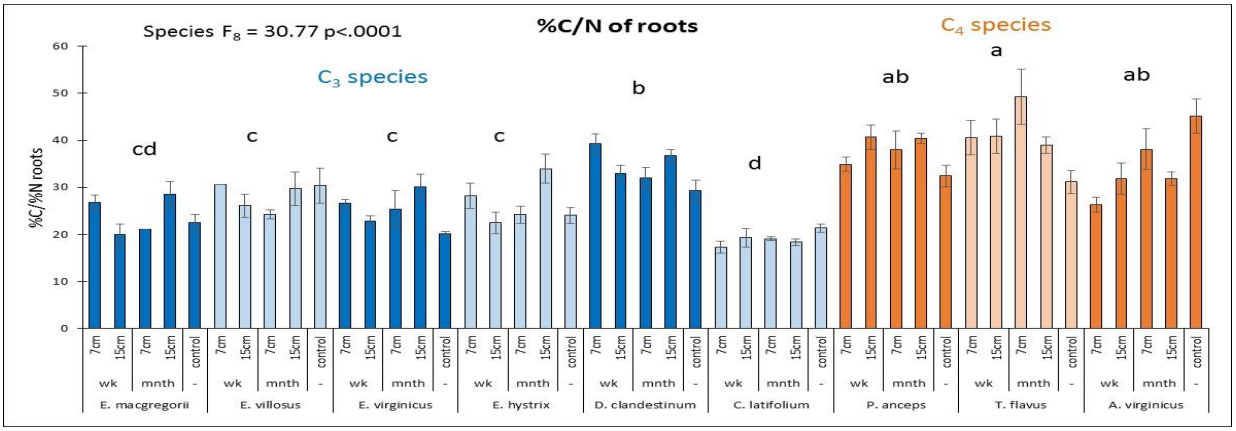


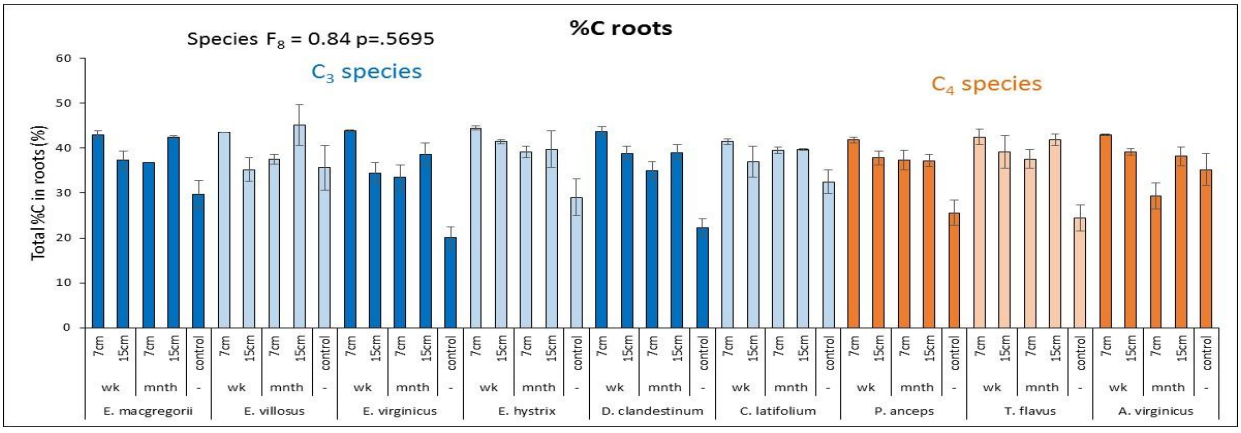
Figure 4.5: Macroscopic variables with treatment means (±SE) for each species. The species are listed on the x-axis in order of their flowering times. F and p values for the overall species comparisons over all treatments are included with different letters signifying significant differences between species overall mean (P value ≤ 0.05) determined by adhoc Tukeys.

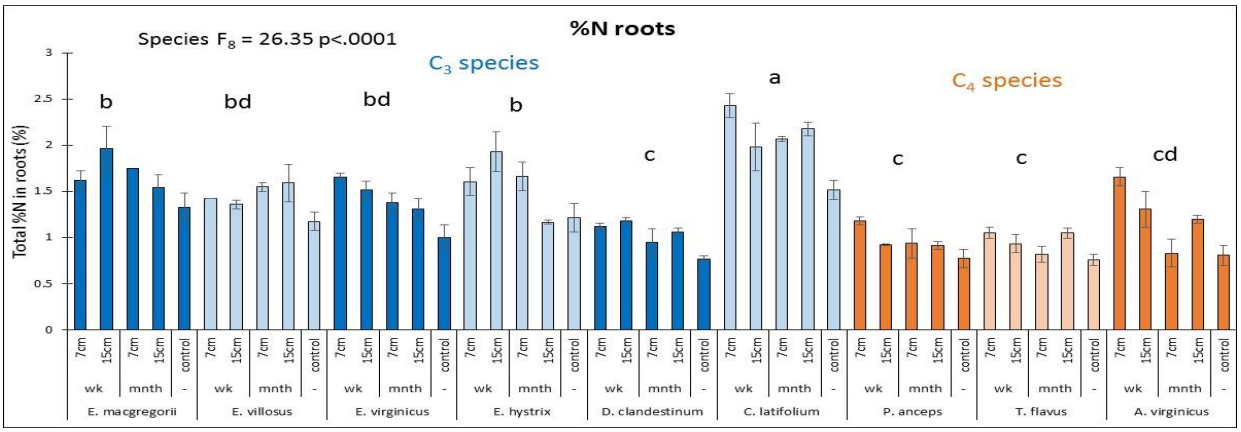


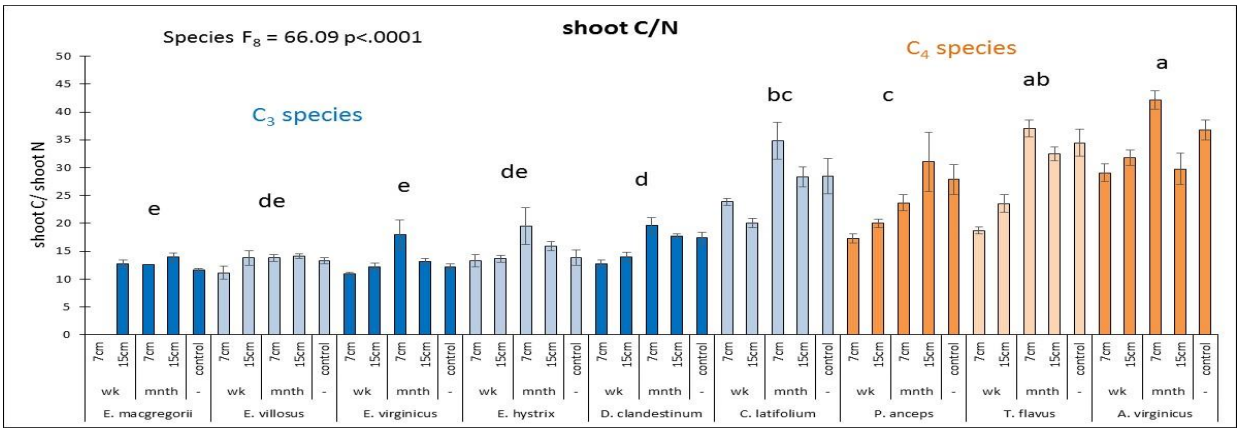

Species F_8 = 3.76 p = .0004
%C shoots
C_3 species
C_4 species
Total %C in shoots (%)
a
ab
b
ab
ab
ab
b
ab
a
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus

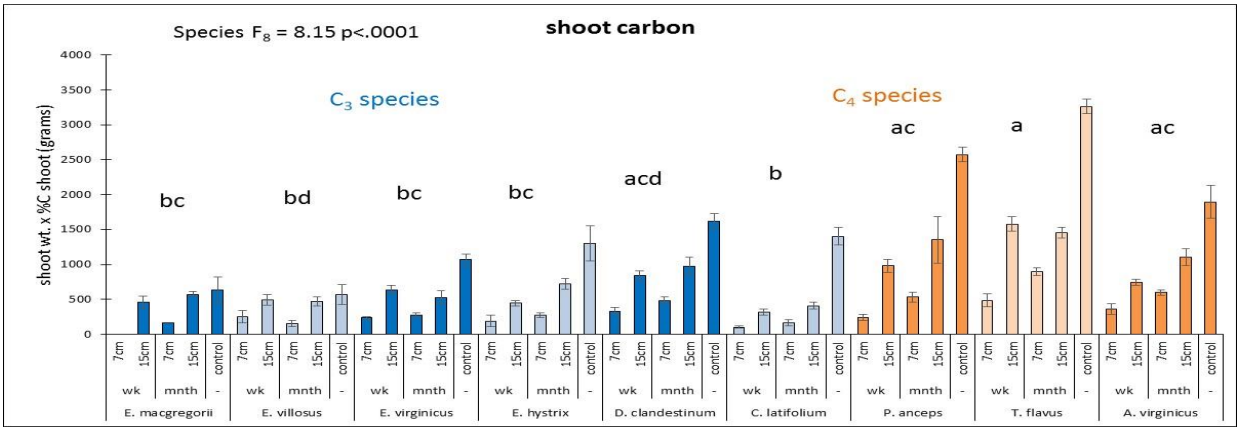

Species F_8 = 66.06 p<.0001
%N shoots
C_3 species
C_4 species
Total %N in shoots (%)
a
abc
ab
bc
c
de
d
de
e
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus

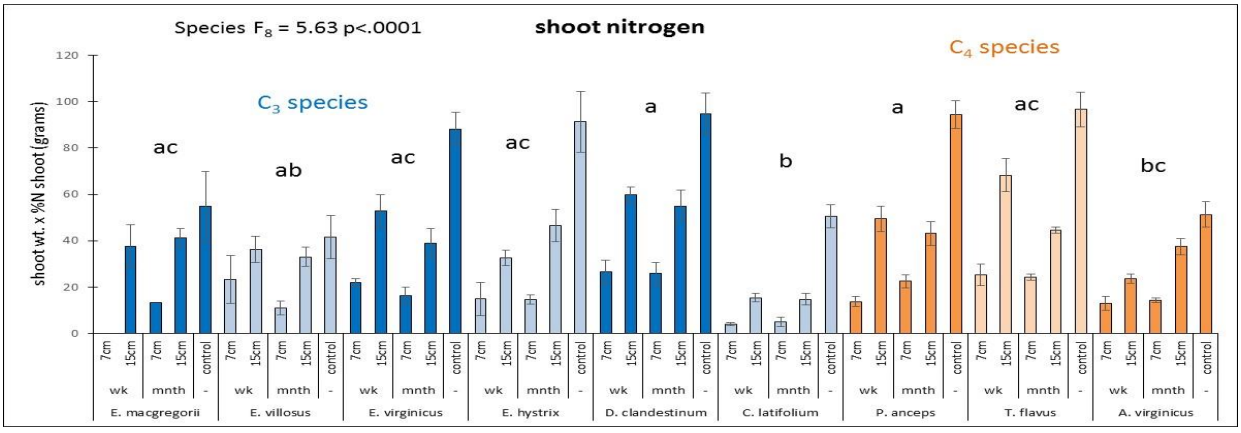

Species F_8 = 30.77 p<.0001
%C/N of roots
C_3 species
C_4 species
%C/%N roots
cd
c
c
c
b
d
ab
a
ab
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus


Species F_8 = 0.84 p=.5695
%C roots
C_3 species
C_4 species
Total %C in roots (%)
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus


%N roots
Species F_8 = 26.35 p<.0001
C_3 species
C_4 species
Total %N in roots (%)
b
bd
bd
b
c
a
c
c
cd
7cm
15cm
wk
mnth
control
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus


shoot C/N
Species F_8 = 66.09 p<.0001
C_3 species
C_4 species
shoot C/ shoot N
e
de
e
de
d
bc
c
ab
a
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus


shoot carbon
Species F_8 = 8.15 p<.0001
C_3 species
C_4 species
shoot wt. x %C shoot (grams)
bc
bd
bc
bc
acd
b
ac
a
ac
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus


shoot nitrogen
Species F_8 = 5.63 p<.0001
C_3 species
C_4 species
shoot wt. x %N shoot (grams)
ac
ab
ac
ac
a
b
a
ac
bc
E. macgregorii
E. villosus
E. virginicus
E. hystrix
D. clandestinum
C. latifolium
P. anceps
T. flavus
A. virginicus

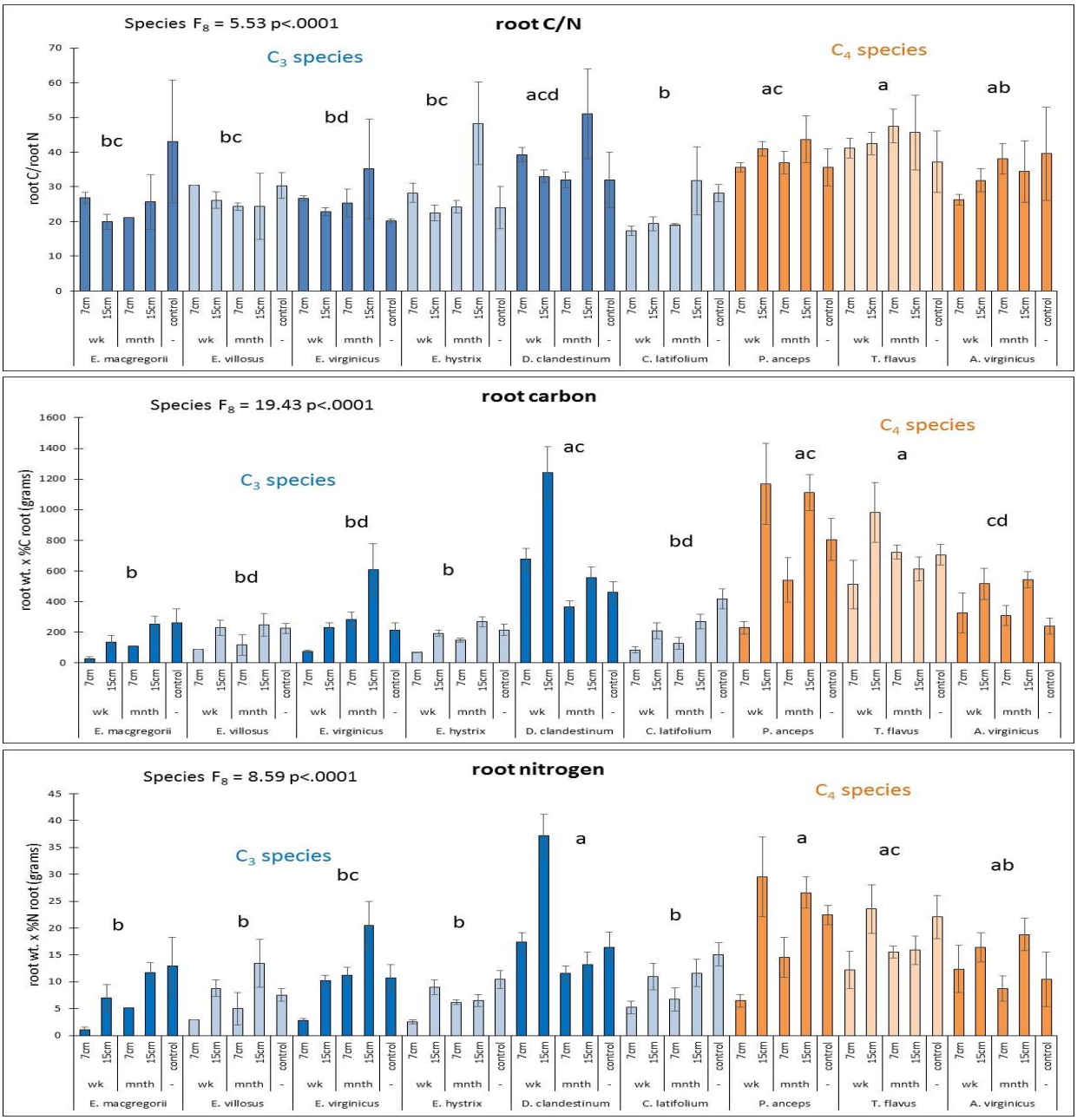
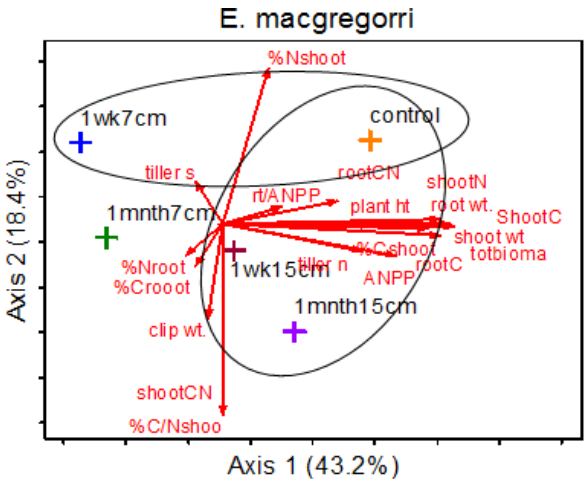
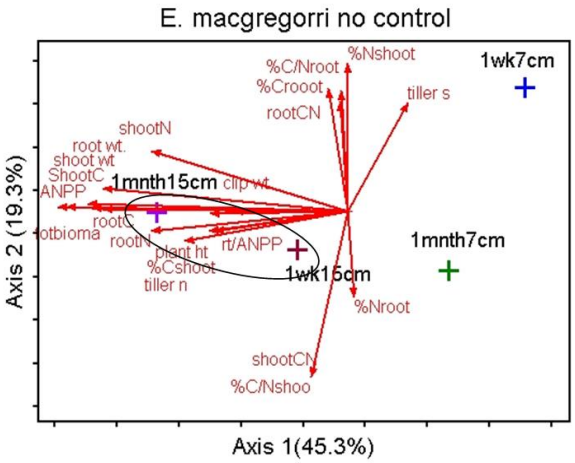
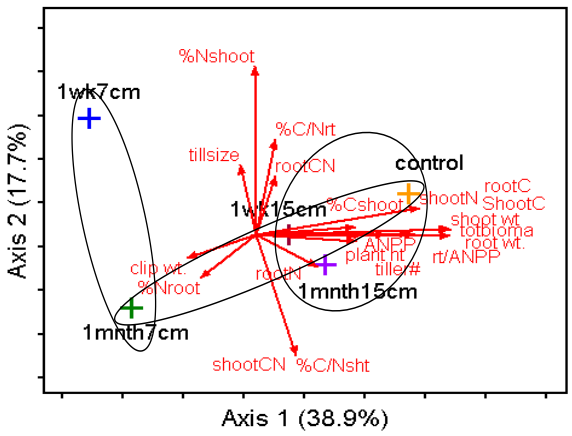
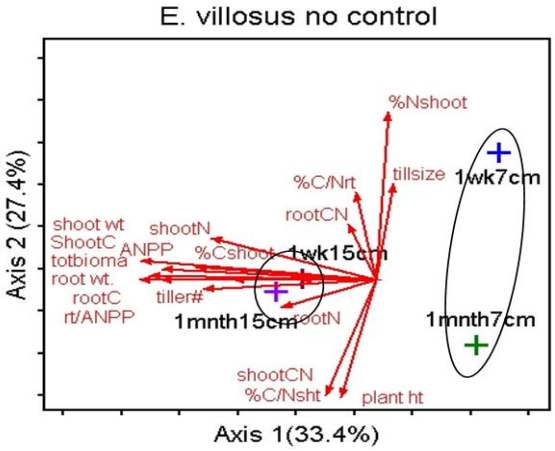


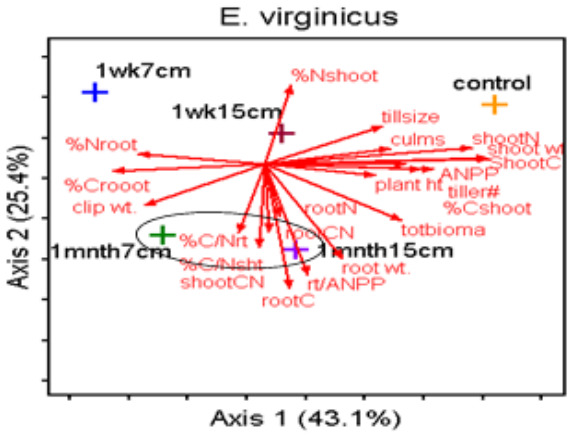
Figure 4.6: Microscopic variables with treatment means (±SE) for each species. The species are listed on the x-axis in order of their flowering times. F and p values for the overall species comparisons over all treatments are included with different letters signifying significant differences between species overall mean (P value ≤ 0.05) determined by adhoc Tukeys.

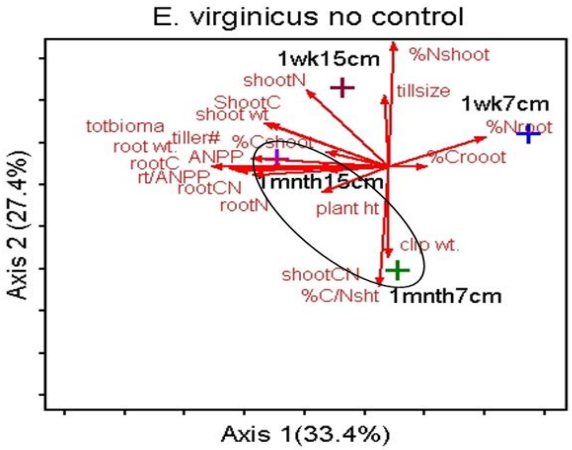

E. macgregorri
%Nshoot
1wk7cm
control
tiller s
rootCN
shootN
rt/ANPP
plant ht
root wt.
ShootC
1mnth7cm
shoot wt
%Cshoot
totbioma
%Nroot
1wk15cm
tiller n
rootC
%Crooot
ANPP
1mnth15cm
clip wt.
shootCN
%C/Nshoo
Axis 2 (18.4%)
Axis 1 (43.2%)

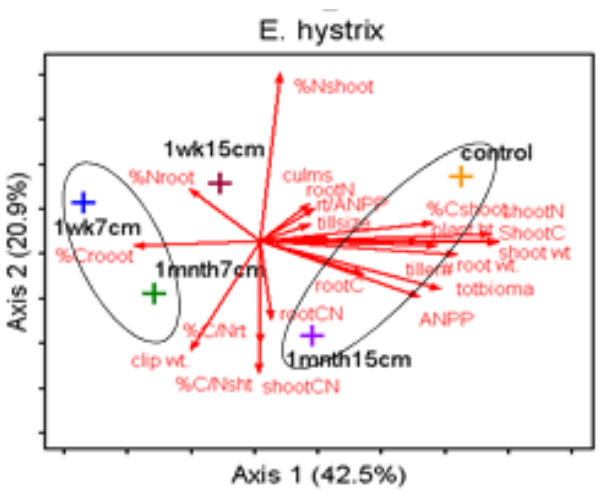

E. macgregorri no control
%Nshoot
%C/Nroot
1wk7cm
%Crooot
tiller s
rootCN
shootN
root wt.
shoot wt
ShootC
ANPP
1mnth15cm
clip wt.
totbioma
rootC
rootN
rt/ANPP
plant ht
%Cshoot
tiller n
1mnth7cm
1wk15cm
%Nroot
shootCN
%C/Nshoo
Axis 2 (19.3%)
Axis 1(45.3%)

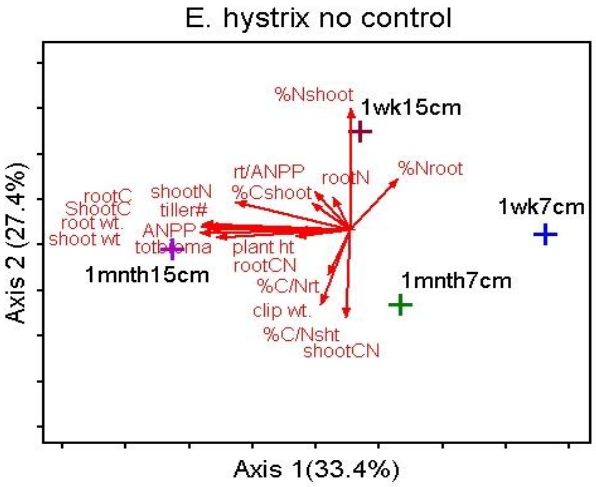

E. villosus
%Nshoot
1wk7cm
%C/Nrt
tillsize
rootCN
control
rootC
ShootC
shootN
1wk15cm
%Cshoot
shoot wt
totbioma
root wt.
ANPP
plant ht
rt/ANPP
clip wt.
%Nroot
rootN
tiller#
1mnth15cm
1mnth7cm
shootCN
%C/Nsht
Axis 2 (17.7%)
Axis 1 (38.9%)

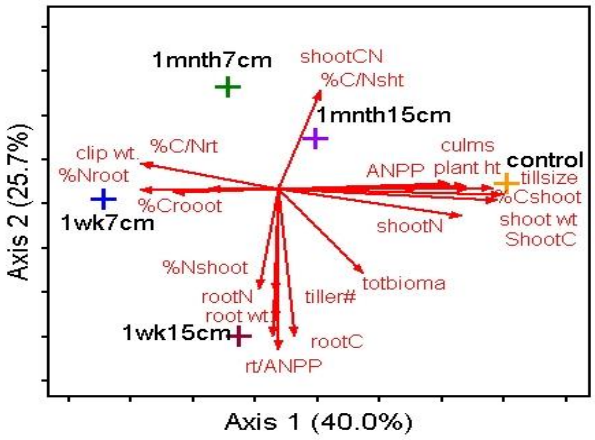

E. villosus no control
%Nshoot
tillsize
1wk7cm
%C/Nrt
rootCN
shoot wt
ShootC
shootN
totbioma
ANPP
%Cshoot
1wk15cm
root wt.
rootC
tiller#
rt/ANPP
1mnth15cm
rootN
1mnth7cm
shootCN
%C/Nsht
plant ht
Axis 2 (27.4%)
Axis 1(33.4%)

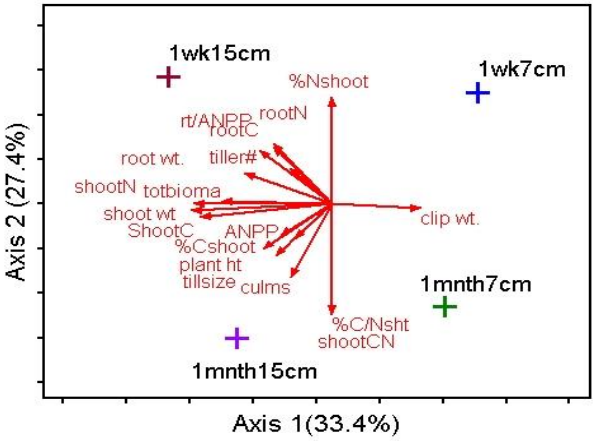

E. virginicus
1wk7cm
%Nshoot
control
1wk15cm
tillsize
culms
%Nroot
shootN
shoot wt
ShootC
%Crooot
ANPP
plant ht
clip wt.
tiller#
rootN
%Cshoot
totbioma
1mnth7cm
%C/Nrt
rootCN
%C/Nsht
1mnth15cm
root wt.
shootCN
rt/ANPP
rootC
Axis 2 (25.4%)
Axis 1 (43.1%)

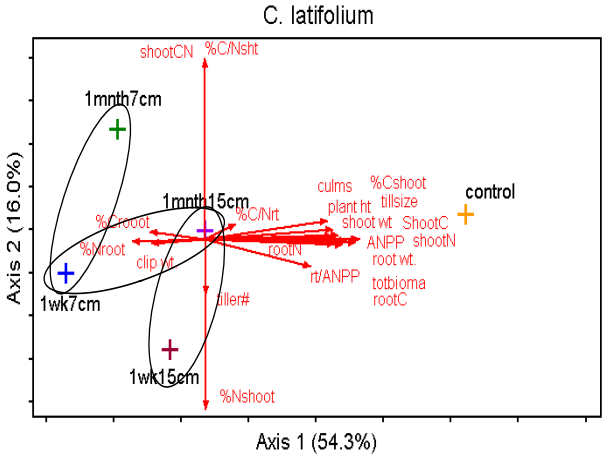

E. virginicus no control
1wk15cm
%Nshoot
shootN
tillsize
ShootC
1wk7cm
shoot wt
%Nroot
totbioma
tiller#
%Cshoot
root wt.
ANPP
%Crooot
rootC
rt/ANPP
1mnth15cm
rootCN
rootN
plant ht
clip wt.
shootCN
%C/Nsht
1mnth7cm
Axis 2 (27.4%)
Axis 1(33.4%)

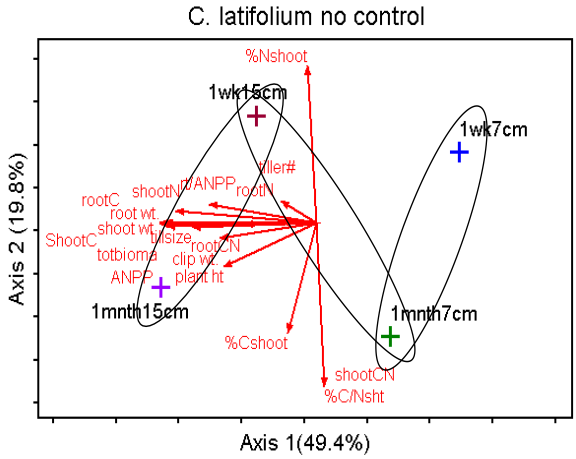

E. hystrix
%Nshoot
1wk15cm
control
%Nroot
culms
rootN
rt/ANPP
%Cshoot
shootN
tillsize
plant ht
ShootC
shoot wt
1wk7cm
%Crooot
1mnth7cm
tiller#
root wt.
rootC
totbioma
rootCN
%C/Nrt
ANPP
clip wt.
1mnth15cm
%C/Nsht
shootCN
Axis 2 (20.9%)
Axis 1 (42.5%)


E. hystrix no control
%Nshoot
1wk15cm
%Nroot
rt/ANPP
rootN
rootC
shootN
%Cshoot
ShootC
tiller#
root wt.
ANPP
shoot wt
totbioma
plant ht
1wk7cm
rootCN
1mnth15cm
%C/Nrt
1mnth7cm
clip wt.
%C/Nsht
shootCN
Axis 2 (27.4%)
Axis 1(33.4%)


D. clandestinum
1mnth7cm
shootCN
%C/Nsht
1mnth15cm
clip wt.
%C/Nrt
culms
ANPP
plant ht
control
%Nroot
tillsize
%Cshoot
1wk7cm
%Crooot
shootN
shoot wt
ShootC
%Nshoot
totbioma
rootN
tiller#
root wt.
1wk15cm
rootC
rt/ANPP
Axis 2 (25.7%)
Axis 1 (40.0%)


D. clandestinum no control
1wk15cm
1wk7cm
%Nshoot
rt/ANPP
rootN
rootC
root wt.
tiller#
shootN
totbioma
shoot wt
clip wt.
ShootC
ANPP
%Cshoot
plant ht
tillsize
culms
1mnth7cm
%C/Nsht
shootCN
1mnth15cm
Axis 2 (27.4%)
Axis 1(33.4%)


C. latifolium
shootCN
%C/Nsht
1mnth7cm
culms
%Cshoot
plant ht
tillsize
control
1mnth15cm
%C/Nrt
%Crooot
shoot wt
ShootC
%Nroot
clip wt
ANPP
shootN
rootN
root wt.
rt/ANPP
totbioma
1wk7cm
tiller#
rootC
1wk15cm
%Nshoot
Axis 2 (16.0%)
Axis 1 (54.3%)


C. latifolium no control
%Nshoot
1wk15cm
1wk7cm
tiller#
rootC
shootN
rt/ANPP
rootN
root wt.
shoot wt
tillsize
ShootC
rootCN
totbioma
clip wt.
ANPP
plant ht
1mnth15cm
1mnth7cm
%Cshoot
shootCN
%C/Nsht
Axis 2 (19.8%)
Axis 1(49.4%)

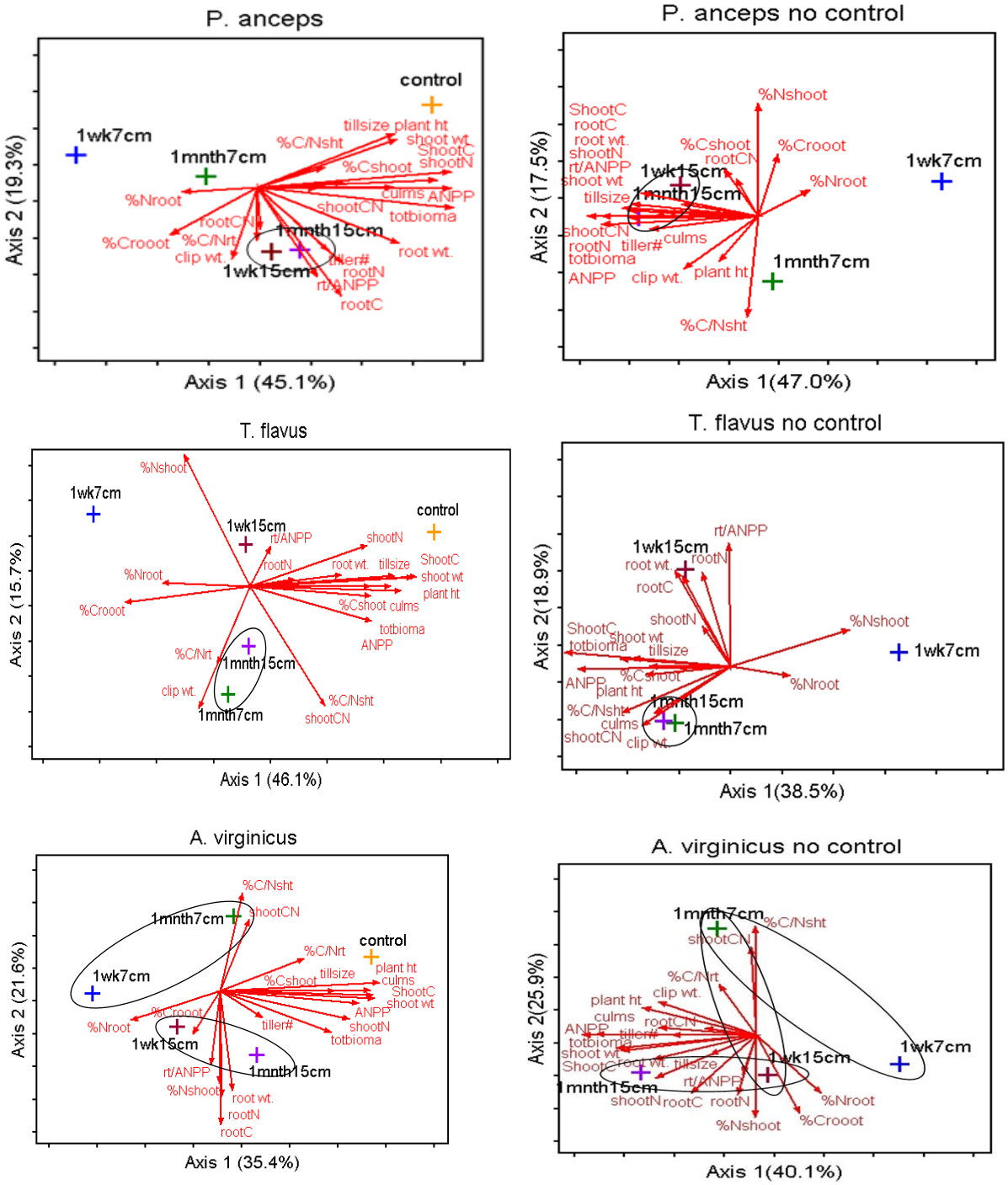
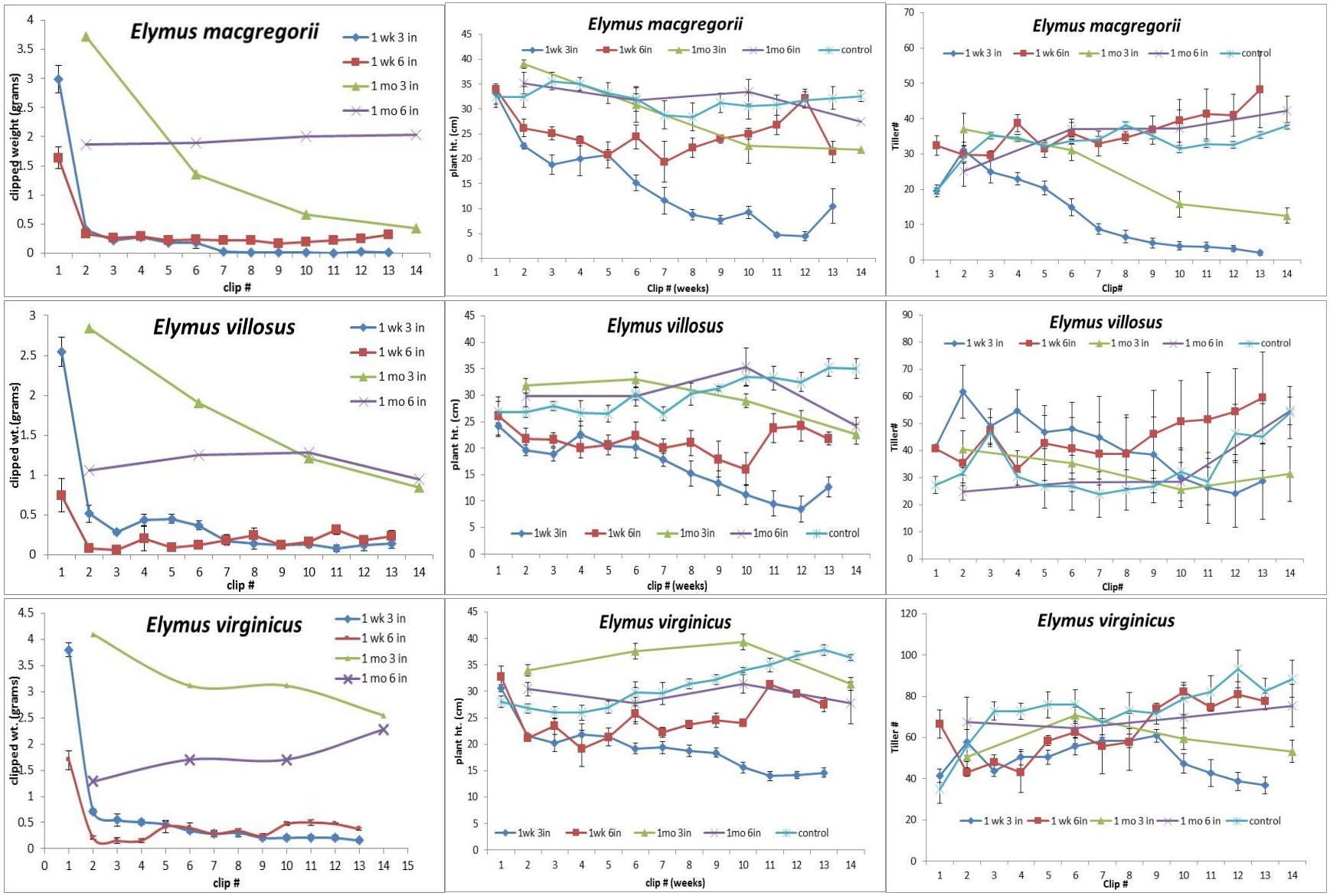
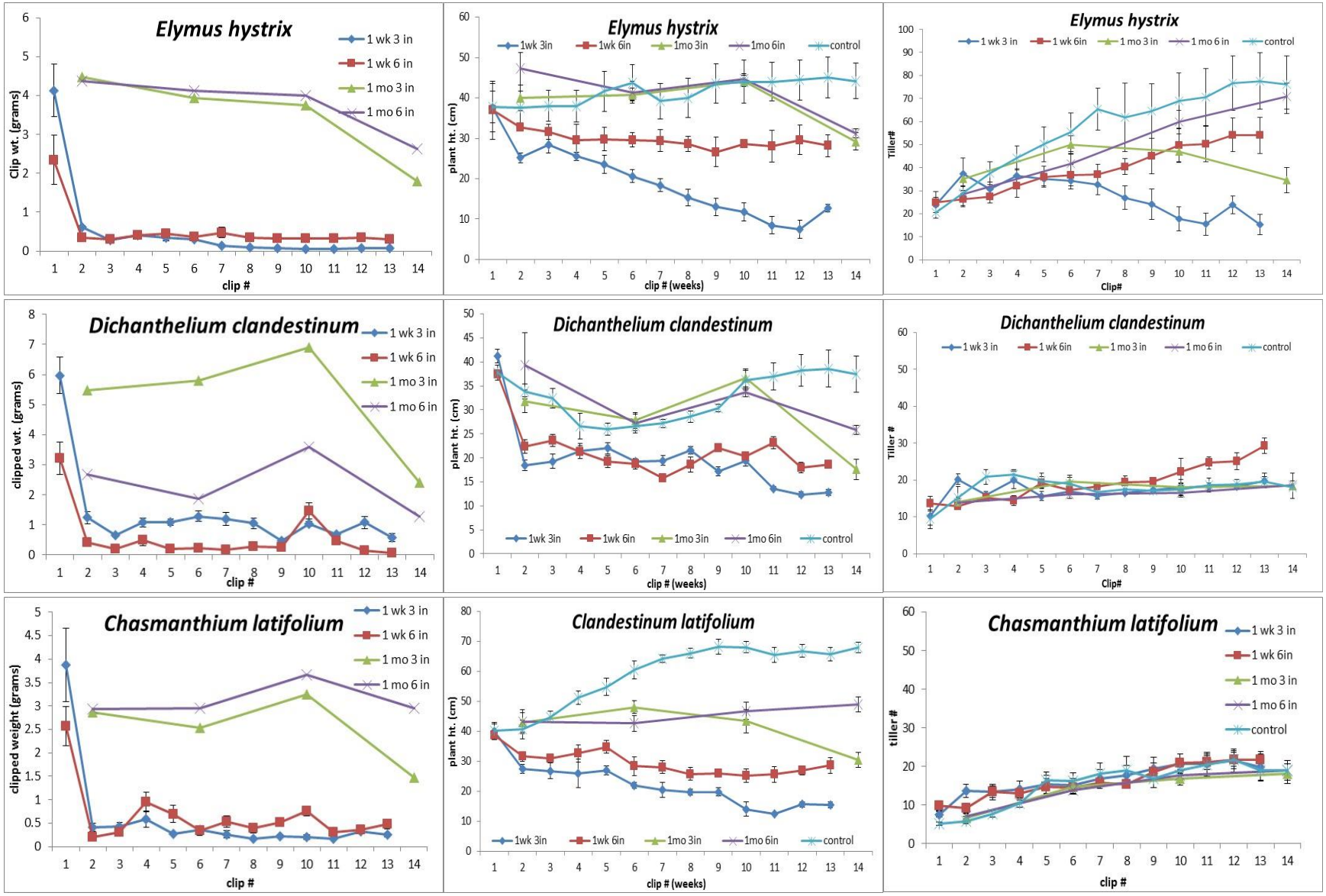


Figure 4.7: PCA results for each species using all traits and grouped by treatment. The circles represent the treatment means that are not significantly different in pairwise comparisons using MRPP ($p<.025$). The percent of variance explained for each axis is reported.

Supplemental




Elymus hystrix
Clip wt. (grams)
clip #
1 wk 3 in
1 wk 6 in
1 mo 3 in
1 mo 6 in
Elymus hystrix
plant ht. (cm)
clip # (weeks)
1wk 3in
1wk 6in
1mo 3in
1mo 6in
control
Elymus hystrix
Tiller#
Clip#
Dichanthelium clandestinum
clipped wt. (grams)
Dichanthelium clandestinum
plant ht. (cm)
Dichanthelium clandestinum
Tiller #
Chasmanthium latifolium
clipped weight (grams)
Clandestinum latifolium
plant ht. (cm)
Chasmanthium latifolium
tiller #

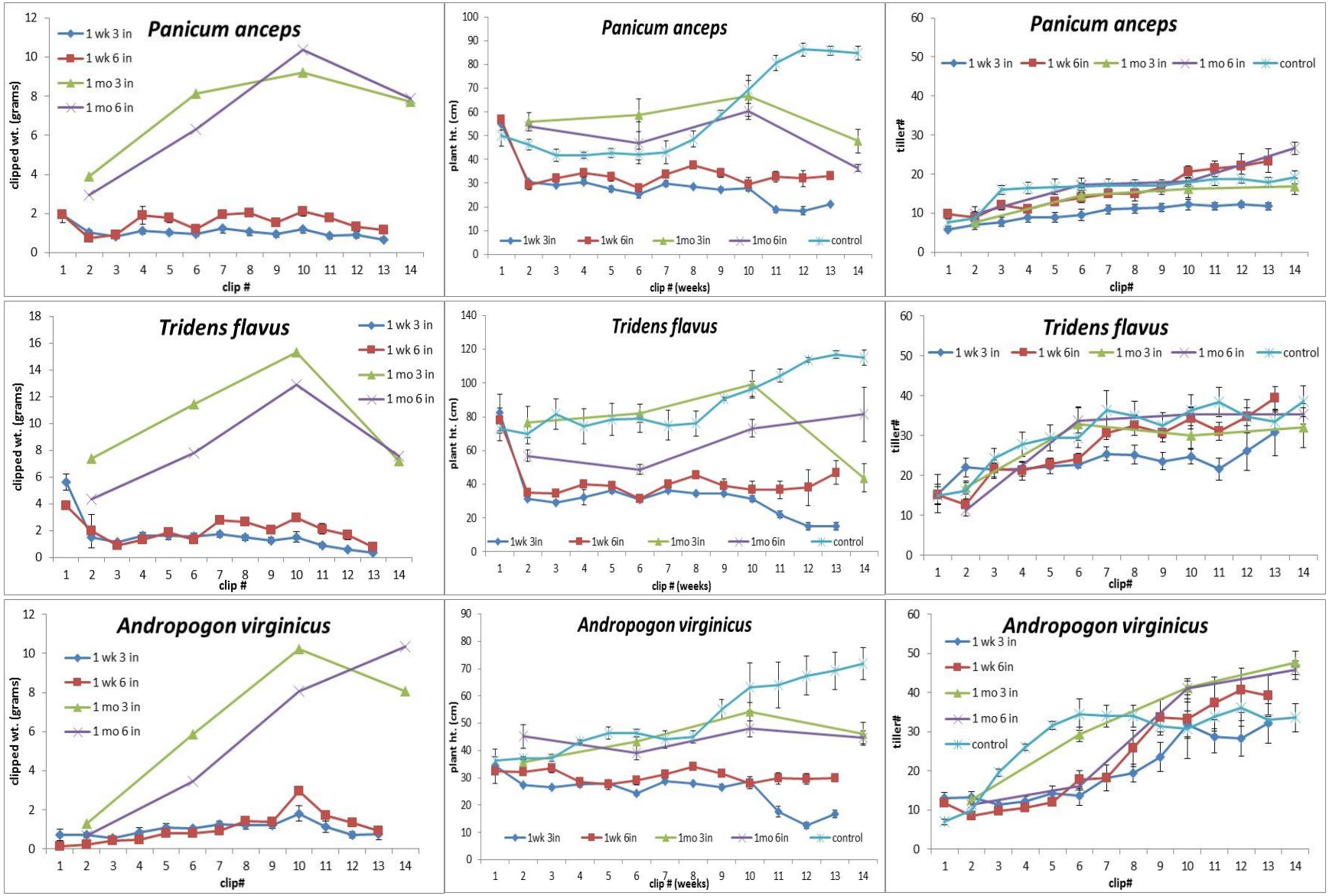


Figure 4.8: For each species, clipped wt., plant ht. and tiller number taken at each clipping treatment.

Chapter 5: Conclusion

The goal of this study was to use the plant trait method to evaluate the ability of native grasses to restore functionality of the grassland component of the oak savanna-woodland in central KY. According to the response-and-effect framework (Garnier and Navas 2012), this study used ANPP as the performance trait, response traits according to the abiotic habitat filter of drought and the biotic habitat filters of competition and grazing. N and C cycling, and soil nutrient concentrations were the effect traits measured. These traits were measured in a field monoculture experiment and a greenhouse clipping experiment. This information was used to help predict how these nine species would perform in a community setting according to these response and effect traits.

In the monoculture experiment, the C_3 and C_4 grasses differed in how they performed, which was generally explained by the trade-off of allocating biomass to more but smaller tillers or fewer but bigger tillers. In general, the C_3 species produced more smaller tillers with a lower C:N ratio that allocated more C to cell solutes than the C_4 species. In general, the C_4 species produced bigger but fewer tillers with a high C:N and allocated more C to lignin and cellulose than the C_3 species. The three top performing species used different strategies to produce ANPP. *T. flavus* grew the tallest plants with fewer but larger tillers that were supported by high amounts of recalcitrant C. *C. latifolium* grew more but smaller tillers than *T. flavus* with high amounts of recalcitrant C. *E. virginicus* was the most prolific producer of tillers, which were shorter and smaller and had high amounts of lignin and cell solutes compared to those of *C. latifolium* and *T. flavus*. The other three *Elymus* species were similar to *E. virginicus* but produced less tillers. *D. clandestinum* generally grew the shortest plants.

In response to interannual rainfall variability, only four C_3 species were plastic in the performance trait, and plant height was the most affected macroscopic trait whereby all species except for *T. flavus* grew shorter plants in the dry year. Generally, the microscopic traits were more affected by drought than the performance trait and macroscopic traits. As expected, the C_3 species generally had more macroscopic trait value differences in response to drought than the C_4 species macroscopic trait values, and the C_4 species had more microscopic trait values differences than macroscopic trait differences. Thus, as predicted, in response to drought, C_3 species trait values differed in the performance trait and macroscopic traits which is consistent with the plasticity strategy, and C_4 species trait values differed in the microscopic traits which is consistent with the stress tolerant strategy. However, the C_3 species microscopic trait values also differed which was not consistent with the plasticity strategy. In response to inter- vs. intra-specific competition, *E. virginicus* was the best inter-specific competitor in both the wet and dry year which was most likely due to life history traits that gave it a head start over the other species. Differences in ANPP were found for all five species between inter- vs. intra-specific

competition. Consistent with the plasticity strategy, the trait values of the C_3 species were different in the performance trait and the macroscopic traits. However, traits values of the C_3 species also differed in the microscopic traits which is inconsistent with the plasticity strategy. The C_4 did not respond to competition as predicted as their trait values only differed in the performance trait and the macroscopic traits which is inconsistent with the stress tolerant strategy. My last prediction that C_3 species will be more competitive in the wet year and the C_4 species will be more competitive in the dry year was not supported. *E. virginicus* competed better in the species mixture treatment for both years and the two C_4 species competed better in the species mixture treatment in the wet year.

My prediction that the four *Elymus* species would be the least affected by drought as their plant traits were measured before the summer drought was not supported. *E. macgregorii* and *E. virginicus* were plastic in ANPP which may have been caused by the winter drought. My prediction that the two C_3 species that were actively growing during the drought would be plastic in response to drought was supported. Both *D. clandestinum* and *C. latifolium* species were plastic in ANPP, and *D. clandestinum* was the only species that was plastic in all four macroscopic traits. My prediction that the C_4 species would be the least plastic and stress tolerant in response to drought was supported. All this evidence supports the idea that the C_3 species may be better adapted to the Bluegrass Savanna-Woodland's mesic heterogeneous environment. The *Elymus* species may be at a particular advantage because they overwinter their tillers which then begins growing early in the spring. This early growth may give them a competitive advantage in both light and space over the later growing species. Also, the *Elymus* species are actively growing before the canopy closes on the Bluegrass Savanna-Woodland. All these factors would make them good candidate species in the restoration of this savanna-woodland.

The results of N and C cycling experiment found that C_3 species had plant traits that promoted fast N cycling and both C_3 and C_4 species had plant traits that promoted slow N cycling. *E. virginicus* had the most plant traits that supported the fast N cycling strategy with high quality litter that rapidly decomposed, and was efficient at taking up both NO_3-N and NH_4-N. After *E. virginicus*, *E. villosus* and *E. macgregorii* had the most plant traits that promoted fast N cycling. *D. clandestinum* had traits that promoted both fast N cycling and slow N cycling. *C. latifolium* and *E. hystrix* were the two C_3 species that tended to have slow N cycling traits with lower litter quality than the other C_3 species. The C_4 species had traits that promoted only slow N cycling. My data did not support the second prediction that slow N cycling species will have a positive feedback loop where poor litter quality will promote immobilization, and limit plant available N. For fast N cycling species as well as slow N cycling species, similar levels of resin NO_3-N and NH_4-N were observed. Also, decomposition of litter was not limited by N as all species except for *E. villosus* and *E. virginicus* increased percent litter N over the course of the

experiment. Thus, similar to other litter decomposition studies (Melillo, Aber et al. 1982, Hobbie 1996) the litter was losing mass and C but retaining N. Also species with initially high litter C:N reduced litter C:N by over 70 % over the course of the experiment which again suggests no N limitation. Knops et al. (2002) suggests that the slow N cycling feedback loop does not limit plant available N because species differences in litter quality have a limited impact on plant available N compared to the N in the soil organic pool which accounts for 90 % of total ecosystem N. Most N gained from the decomposing litter is retained and incorporated into the soil organic matter, which prevents immediate feedbacks to the plants. Thus, the soil organic matter has a bigger impact on mineralization and immobilization and ultimately plant available N compared to plant and litter characteristics (Knops, Bradley et al. 2002).

The results of the N and C cycling experiment suggest that these nine species did not differentially deplete soil N as was predicted by the resource-competition theory. At the ecosystem level, the soil data does not suggest that the species differentially depleted soil nutrients over the four years this experiment was conducted. This data suggests that N may not be the primary limiting nutrient for the Bluegrass Savanna-Woodland which is opposite of what has been found to be true for many temperate grasslands (Polley and Detling 1988, Schlesinger 1991, Vitousek and Howarth 1991).

The results of the N and C cycling experiment are consistent with the reported species distribution in the field. The fast N cycling species were expected to have traits that make them better adapted for habitats that are not limited by N and water. The four fast N cycling C_3 species, *E. macgregorii*, *E. villosus*, *E. virginicus* and *D. clandestinum* do frequent the Bluegrass savanna-woodlands with mesic eutrophic soils as well as the more open woods (Wharton and Barbour 1991, Campbell 2004). The *Elymus* species may also be best adapted at taking up plant available N because the time they are actively growing and plant N demands are high coincides with the Bluegrass Region's wet spring. Also, the *Elymus* species produces high quality litter during the summer months when soil microbes are most active. My data also supports the prediction that the slow N cycling species will be best adapted for N limited habitats. The C_4 grasses had more conservative N traits that promote slow N cycling which would explain why they are found in local openings on poorer soils in the Bluegrass savanna-woodland or openings created by disturbance such as fire or bison trails (Campbell 2004). The C_4 species actively grow during the summer months which was during the summer drought when N uptake may have been limited by water availability.

For the greenhouse clipping experiment, my hypothesis that clipping frequency would have a bigger effect than clipping intensity that was reported by Augustine and McNaughton (1988) was not supported. I found a significant clipping intensity effect but no significant frequency effect. However, a

significant frequency effect was detected for the macroscopic trait analysis but only at the most intense (7 cm) clipping treatment. Thus, frequency became an important factor only when the grasses were more intensively clipped. My prediction that the C_4 species will be better adapted to grazing than the C_3 grasses was partially supported as the C_4 species and *D. clandestinum* had better overall performance than the other five C_3 species. In response to clipping, *T. flavus* was the most productive of the C_4 grasses, and the *Elymus* species, particularly *E. macgregorii*, were the least productive. *P. anceps* performed well at the 15 cm treatments, and *D. clandestinum* performed well at the 1 week 7 cm treatment. While the *Elymus* species and *C. latifolium* were the poorest performers at all treatment levels, *E. macgregorii* had the lowest performance at the 7 cm intensity treatments.

My hypothesis that the nine grasses would have different grazing strategies at different frequency and intensity treatments was supported for all the species except for *E. macgregorii* and *P. anceps*. *D. clandestinum* had the most plastic traits in response to grazing, and it was the only species to display traits for the three grazing strategies of tolerance, avoidance and deterrence. The most obvious grazing strategies were at the 1/week 15 cm treatment where *D. clandestinum* displayed clear avoidance traits, *T. flavus* displayed clear tolerance traits, and *P. anceps* optimized sexual reproduction through the production of flowering culms. In general, the strategies of tolerance and deterrence were determined by clipping treatment, and the avoidance grazing strategy was species specific. The grazing tolerance strategy was found at the 1/week frequency treatments and the grazing deterrence strategy was found at only the 1/month 7 cm clipping treatment. Only *D. clandestinum* and *A. virginicus* had trait values that supported the avoidance strategy. No grazing strategies were detected for the 1/month 15 cm treatment except for *A. virginicus*.

Greenhouse conditions also had an effect on the performance of the species which in turn, may have affected their response to clipping. For the control treatment, the C_4 species performed the best, followed by *D. clandestinum* and *C. latifolium* with the four *Elymus* performing the worst. While the C_4 species were positively correlated to all the macroscopic traits in the control treatment, the C_3 species were positively correlated to only a few microscopic traits. The *Elymus* species were probably the least adapted to the high light and heat environment of the greenhouse, and the timing of the clipping experiment coincided with the time the *Elymus* species would have been dormant under field conditions.

The results of the greenhouse clipping experiment suggests that the Bluegrass Savanna-Woodland was not intensively grazed at least for long periods of time. The fact that clear grazing strategies were found at the 1/week 15 cm clipping treatment suggests that the Bluegrass Savanna-Woodland may have been historically frequently but less intensely grazed. At high frequency and intensity grazing regimes,

the *Elymus* species would most likely be eliminated from the community. However, the *Elymus* species may use an effective avoidance grazing strategy that was not assessed in this experimental design. In the field, the aboveground biomass the *Elymus* species goes dormant during the summer months when the other species are active growing and subject to herbivory. In response to grazing, these results suggest that the C_4 species particularly *T. flavus* are at a competitive advantage over the *Elymus* species. While *C. latifolium* was not well adapted to grazing, *D. clandestinum* was well adapted to grazing. Less intense mowing regimes would be recommended to maintain these grasses in a community setting. The frequency of mowing regimes may also be important for the control of woody growth particularly in the absence of fire.

Using the response-and-effect framework was an effective tool to detect differences between species that can then be used to predict how these species will function in a community setting. This plant trait-based approach produced valuable information about the species that can be used to guide ecological restoration at Griffith Woods WMA and the Bluegrass Region of Kentucky in general. The two habitat filters of fire and light availability may also be important factors in determining community assembly of the Bluegrass Savanna-Woodland that were not included in this study. This methodology could be tailored for other restoration sites to assess the response and effect traits according to the important habitat filters of the study system. This methodology is particularly useful where limited information is known of the oak savanna being studied.

For this study, *E. virginicus* was one of the top performers in the monoculture and was the best inter-specific competitor for both the dry and wet year. *E. virginicus* was also the species that was most effective at cycling N. Under normal environmental conditions, I predict that *E. virginicus* would be the best competitor. I think that the other three *Elymus* species would be good competitors under normal environmental conditions as well which may be partially due to their life history traits. In the species mixture treatment, *E. virginicus* had a competitive advantage of both light and space as this species began actively growing and flowered before the other species. At the time the other species were actively growing, the plants of *E. virginicus* were dying back which then lodged and further shaded out neighboring plants. For this reason, I think that the life history traits of *E. virginicus* had a bigger effect on competitive ability than plasticity of traits. In response to clipping, the *Elymus* species were not well adapted to intense clipping regimes. Less intense and frequent mowing regimes may be important to maintain these grasses in a community setting.

For the other two C_3 species, I predict that *C. latifolium* would be a better competitor than *D. clandestinum* under normal conditions. I also predict that, with more plastic traits, *D. clandestinum* is

better adapted to a heterogeneous environment and grazing than *C. latifolium*. *C. latifolium* was a top performer and *D. clandestinum* was one of the lowest performing species in the monoculture. While both of these species were plastic in ANPP in response to drought, *D. clandestinum* was plastic in all macroscopic traits and fewer microscopic traits, and *C. latifolium* was plastic in only macroscopic traits. In response to competition, both species were plastic in ANPP and all macroscopic traits and less plastic in microscopic traits. Both *D. clandestinum* and *C. latifolium* competed better in the monoculture than the species mixture treatment in both the dry and wet year. *C. latifolium* had slow N cycling traits with lower litter quality than the other C_3 species, and *D. clandestinum* had traits that promoted both fast N cycling and slow N cycling. *C. latifolium* as not well adapted to clipping particularly at the most intense clipping treatments. Of the C_3 grasses, *D. clandestinum* performed the best in response to clipping. It was the most plastic of all species in response to grazing and was the only species to exhibit all three grazing strategies.

For the C_4 species, *T. flavus* was a top performer in the monoculture where it produced a low number of tillers with big and taller tillers than the other species. *P. anceps* and *A. virginicus* produced the same number of tillers but smaller and shorter tillers than *T. flavus*. All species grew well in monoculture except for *A. virginicus*. *A. virginicus* was the last species to begin in the growing season and generally remained in a rosette until it bolted in late summer to produce flowering culms. *A. virginicus* did not show the ability to bolt through the established weedy layer of plants like the other two C_4 species. For this reason, I conclude that *A. virginicus* was not a good competitor for light, which may explain in part why it is found on poor disturbed sites where competition for nutrients may be stronger than competition for light. This may also explain why *A. virginicus* was the only species to increase ANPP, tiller number, and the number of flowering culms in the dry year when the plots were less weedy and light competition may have been reduced compared to the wet year. For these reasons, *A. virginicus* is not a good prospective species to use in the restoration of the Bluegrass Savanna-Woodland. *P. anceps* and *T. flavus* were generally stress tolerant in response to drought with *P. anceps* being more plastic in both macroscopic and microscopic traits compared to *T. flavus.* *T. flavus* was the only species that was not plastic in plant height in response to drought. While *T. flavus* and *P. anceps* competed better in the monoculture than the species mixture treatment, these two species (particularly *T. flavus*) competed better in species mixture treatment in the wet year compared to the dry year. The C_4 species had traits that promoted only slow N cycling. All C_4 species were well adapted to clipping with *T. flavus* being the most productive species in the clipping experiment. All three C_4 species had plant traits that were the most negatively affected in the 1/week 7 cm treatment. I predict that *T. flavus* and *P. anceps* may be better competitors for light but may be outcompeted by the earlier developing *Elymus* species. The three C_4

may be at a selective advantage under extended droughts conditions and more intense grazing frequency and intensity regimes compared to the C_3 species. Thus, for the Bluegrass Region of Kentucky, the C_3 species (particularly the *Elymus* species) would be selected for under normal environmental conditions of the Bluegrass Region of KY, and the C_4 species (particularly *T. flavus*) would be selected for under extended drought conditions and more intense grazing frequencies and intensities. I conclude that management of disturbance levels is important for the community setting of these nine species.

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Curriculum Vitae

Jann Elise Fry

Place of Birth – Harrisburg, PA

Education:

University of Kentucky, Lexington, Kentucky
Ph.D. May 2014
Major: Biology (Plant Ecology)

University of Kentucky; Lexington, Kentucky
M.S. 1992-1995
Major: Biology (Plant Ecology)
GPA: 3.9 on a 4.0 scale

University of Kentucky; Lexington Kentucky
B.S. with Honors, 1992
Major: Biology
GPA: 3.3 on a 4.0 scale

Professional position held:

•University of Kentucky; Lexington, Kentucky.

Teaching Assistant: 1992 - 1995 (during Masters work)
2007 -2014 (during PhD work)

Ecology Lab – Bio 325
Plant Kingdom Lab – Bio 351
General Biology Lab – Bio 151 and Bio 153
Ecology Lab – Bio 452G
Plant Anatomy Lab
Plant Biology Lab
Algology

•Bluegrass Community and Technical College; Lexington, Kentucky

Adjunct Faculty Position Fall 2001 to Spring 2006
Courses Taught - Human Ecology and General Biology
Biological Sciences Department

•United States Census Bureau

Supervisor of Field Operations January 1999 to July 2000

•Natural Resources and Environmental Protection Commission (NREPC)
Kentucky Division of Water
(Actual employer was University of Kentucky through a grant from Kentucky Water Resources)

- **Water Quality Branch, Water Quality Certification Section**
  Database manager, field assistant — October 1995 to March 1997

- **Kentucky Watershed Management Project**
  GIS Technician — March 1997 to October 1998

Scholastic and Professional honors:

Teaching Assistantship for UK Biology Dept.	Fall 2007 to Spring 2013.
Lyman T. Johnson Fellowship	Fall 2013
University of Kentucky Woman's Club Fellowship	Fall 2013

Professional publications

Gleeson, S. K. and J. E. Fry (1997). "Root proliferation and marginal patch value." Oikos **79**(2): 387-393.