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FACTORS INFLUENCING THE ESTABLISHMENT AND SURVIVAL OF NATIVE HARDWOOD TREE SEEDLINGS OF THE KENTUCKY INNER BLUEGRASS BLUE ASH-OAK SAVANNA-WOODLAND

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FACTORS INFLUENCING THE ESTABLISHMENT AND SURVIVAL OF NATIVE HARDWOOD TREE SEEDLINGS OF THE KENTUCKY INNER BLUEGRASS BLUE ASH-OAK SAVANNA-WOODLAND

THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the College of Arts and Sciences at the University of Kentucky

By

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2013

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FACTORS INFLUENCING THE ESTABLISHMENT AND SURVIVAL OF NATIVE HARDWOOD TREE SEEDLINGS OF THE KENTUCKY INNER BLUEGRASS BLUE ASH-OAK SAVANNA-WOODLAND

Historically, the Kentucky Inner Bluegrass blue ash-oak savanna-woodland was the primary ecosystem of the Inner Bluegrass Region (IBR) of Kentucky. After European settlement, the majority (>99%) of Bluegrass savanna was converted to agricultural and urban land uses. Currently remnant savanna tree species are failing to recruit. Therefore, a long-term restoration ecology project researching competition and disturbance on seedling establishment, survival, and growth has been established at Griffith Woods (the largest remaining savanna in Kentucky) in Harrison Co., KY. Fourteen native hardwood tree species (a total of 6,168 seedlings) have been experimentally planted. Light, soil, surrounding vegetation, and herbivory, factors thought to influence seedling survival, have been initially assessed. Results show that soils differed spatially in P, Ca, Mg, Zn, pH, N percent and soil organic matter percent. Light was significantly reduced by diffusive filtering through vegetation. Vegetation biomass was influenced by pH and Mg. Initial seedling survival was high, but significantly differed by species type, location, and soil pH, Mg, and Zn. This research demonstrates that under a similar range of conditions, native hardwood tree seedling establishment is possible. Therefore, the potential exists to restore Bluegrass savanna-woodland in order to return proper ecological functioning into a degraded landscape.

KEYWORDS: Kentucky Inner Bluegrass blue ash-oak savanna-woodland, plant community ecology, hardwood tree seedling establishment, restoration ecology, plant-soil relationships

James D. Shaffer

July 27, 2013
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July 27, 2013
This manuscript is dedicated to

my family and friends

but most of all

Mimi, Poppo, Mama DJ, and Papa John

and my brother Andy
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Preface

The long-term research goals inherent in this project are to determine how the ecological factors of competition and disturbance regulate the formation of Kentucky Inner Bluegrass blue ash-oak savanna-woodland, and to understand how these have been altered through human disruption of natural ecological functioning which has resulted in a recruitment failure of native hardwood trees into the Bluegrass savanna-woodland. The specific aims of this project report are to describe the initial experimental setup, place the experiment into the historical and ecological context of the Bluegrass savanna, quantify on-site environmental variables, assess initial survival of planted seedlings, and interpret connections between the environmental variables and their potential influence on initial seedling survival. To do this, in chapter one I will first describe the background knowledge of the Bluegrass savanna-woodland ecosystem, the conservation and restoration implications and ecological problems of the system, characterize the ecological traits of the primary savanna tree community members, and hypothesize what initial factors may be influencing establishment at the seedling stage. Next, in chapter two I will characterize the site where the long-term research project will be conducted with primary emphasis in describing the topography, watersheds, and soil types, followed by a complete description of the long term experimental project that has been installed, and then specifically detailing the data sets that have been initially gathered as they pertain to our hypotheses about initial factors and seedling establishment. In chapter three, I will present the results of the many abiotic and biotic components I have assessed, focusing on the factors of soil parameters, available light, surrounding vegetation community biomass, initial survival of experimentally planted native tree seedlings, and the relationships that exist between the various biotic and abiotic parameters. Finally, in chapter four I will conclude with how the interrelationships between soil, light, vegetation, and seedlings have resulted in the specific quantifications made in the experiment, how this knowledge relates to the ecological functioning of Kentucky Bluegrass savanna-woodland, how the initial experimental setup phase relates to the long-term experiment and the potential for Bluegrass savanna-woodland to persist on the future landscape.
Chapter 1: Introduction

Historical Background of Bluegrass Region

The Inner Bluegrass Physiographic Region (IBR) is a 6,216km² (2,400mi²) area of central Kentucky historically characterized by a unique plant community, the Kentucky Inner Bluegrass blue ash-oak savanna-woodland (Bryant et al, 1980). The Inner Bluegrass Region (also known as the Trenton outcrop or Lexington Plain) (Figure 1.1) is signified by its gently rolling topography and underlying low acidity calcium and phosphorus rich soils of the Maury silt-loam type, derived from calcium phosphate rich Middle Ordovician limestone parent material (Figure 1.2) (Wilson 1941; Braun, 1950; McInteer, 1952; Bryant et al., 1980; Wharton & Barbour, 1991). An aspect of the underlying limestone bedrock is rapid drainage, typical of karst topography, where surface waters are rare due to the rapid percolation through bedrock (Bryant et al., 1980).
Figure 1.1: Kentucky map illustrating 6 physiographic provinces. The light yellow-green section is the Inner Bluegrass Region (IBR) which is typified by the Bluegrass savanna-woodland. Notice how the physiographic regions closely correspond to the underlying geologic strata in Figure 1.2 (Kentucky Geologic Survey, 2001).
Figure 1.2: Geologic map of Kentucky. Note that the Bluegrass Region is underlain by Ordovician limestone parent material (shaded in purple) (Noger & Dever, Jr., 2000).
André Michaux, who in 1793 was one of the first European explorers to categorize the botanical diversity of Kentucky, described the rolling uplands of the Bluegrass Region as having rich soils and there being a dominance of blue ash, walnut, buckeye, Kentucky coffeetree, multiple hickory species and sugar maple with an understory dominated by cane, ironweed, and buffalo grass (Bryant, 2004). His account coincides with observations of an open canopy habitat, also described by other explorers at the time of European settlement. Collectively, these observations describe a central Kentucky that was primarily a savanna ecosystem, with bison and elk herds grazing in a grassland under large, open grown oak (Quercus spp.) and blue ash (Fraxinus quadrangulata) trees (McInteer, 1952; Bryant et al., 1980). High incidences of bur oak (Quercus macrocarpa), blue ash, honey locust (Gleditsia triacanthos), and black locust (Robinia psuedoacacia) documented during the time of initial settlement of the region suggest a high light, open canopy environment, as these are all classified as shade intolerant species (McInteer, 1952; Bryant et al., 1980). Additionally, the understory was described as being dominated by cane (Arundinaria gigantea), pea-vine (Amphicarpaea bracteata), bluegrass (Poa sp.), wild rye (Elymus sp.), buffalo grass, and white clover (McInteer, 1952). Many times the region was characterized as having a meadow and park like structure and one early explorer was quoted as saying that “the fertile region of Kentucky is the land of cane and clover- spontaneously growing to feed the buffalo, elk and deer” (McInteer, 1952).

All of these anecdotal floristic descriptions indicate a savanna habitat, which is defined by a low density of large, open grown trees scattered throughout a grassland dominated understory that lacks any subcanopy layer (Braun, 1950; Weltzin & Coughenour, 1990; Dettman, 2009). Specifically, Wharton & Barbour (1991) define the savanna-woodland as “open forests in which trees are dominant but with a well-developed grassy undergrowth”, which is contrasted with their definition of a true savanna as an area where “the tree density is so low that the actual dominants of the community are the grasses and other herbaceous vegetation”.

Confounding these initial descriptions of the IBR savanna-woodland habitat is a recent study of tree ring widths, which seems to indicate a canopy release event that increased the light environment which coincided with European settlement. This suggests forest clearing which refutes the anecdotal evidence of a savanna system in central Kentucky (McEwen and McCarthy, 2008). Additionally, conflicting anecdotal accounts of early settlement report areas of dense forest growth but also an understory composed of clover and grass. Yet, in these descriptions of species composition, high abundances of locust and other shade intolerant species seem to indicate that the canopy must not have been as dense as in some accounts (McInteer, 1952). One interpretation could be that the bluegrass landscape would have been very mosaic like with a gradient of dense stands interspersed in typical savanna all the way to grassland meadows. Braun (1950) concludes that “the forest of the Bluegrass could not have been dense” after taking in all of the floristic description accounts.

Because the Inner Bluegrass Trenton outcrop soils are considered to be very productive and fertile for cultivated crops, conversion of native Bluegrass savanna to croplands, pastures and horse farms has removed the vast majority of the Bluegrass savanna-woodland (Wilson,
Currently native Bluegrass savanna remnants occur on private lands and in a few nature preserves, of which Griffith Woods (38°19′48″N, 84°21′01″W) in Harrison Co., KY is one of the best examples. Wharton & Barbour (1991) described the Griffith Woods savanna tract as “the most remarkable of these woodland pastures.” The reason for Griffith Woods being considered as the preeminent example of Inner Bluegrass savanna is that the land was held by the same family for seven generations and the savanna tract had never been used for row crops, leaving the ground unplowed. The savanna-woodland nature of this particular field was maintained by cattle grazing and mowing every one to two years. To meet agricultural needs, savanna-woodlands were converted from the native grass assemblage to fescue (*Poa pratensis*) and other forage species for the grazing of stock animals (Wharton & Barbour, 1991).

**Ecological factors influencing savanna maintenance**

The current factors that may limit or promote the recruitment of Bluegrass savanna hardwood trees are not well understood, and the few stands of Bluegrass savanna that are left have been experiencing a lack of seedling recruitment for many years (Bryant et al., 1980). Many factors are hypothesized to influence the ability for the various species that comprise this community to recruit subsequent age classes including, but not limited to, the following: herbivory by deer, rabbit, and voles, competition with herbaceous vegetation, periodic droughts, and fire tolerance/disturbance. Research in other savanna systems and old field habitats has linked these factors to regeneration of the tree species component and the succession of plant communities. In these systems, small mammals have been shown to effect seed germination and seedling establishment (Ostfeld et al., 1997; MacDougall et al. 2010) and large ungulates have been implicated in slower growth rates and preferential species mortality in *Fraxinus* and *Acer* species through overbrowse (Kupferschmid & Bugman 2008). Grass cover has been linked to suppressed growth of seedlings, especially through light interception (MacDougall et al., 2010; Flory & Clay, 2010) and biomass accumulation is greater in both tree seedlings and grasses in the absence of competition, but lower when in direct competition (Kambatuku et al., 2010). Griscom et al (2011) found that both competition from the herbaceous layer and herbivory by white-tailed deer (*O. virgiana*) influenced the growth of tree seedlings, but that herbivory seemed to be the greater controller of survival in this growth stage. As a control for herbivory, many studies have evaluated the effects of herbivore browse prevention. Research has shown that excluding herbivores with mesh fencing significantly increased survival and growth of oak seedling from acorns (Adams, Jr. et al, 1992). Furthermore, by utilizing individual seedling protectors (aka tree shelters) herbivore browse damage was reduced in sugar maple (*A. saccharum*) and yellow birch (*Betula alleghaniensis*) (Pinna et al., 2012) and protectors prevented browsing in cherrybark oak (*Quercus pagoda*) seedlings (Dubois et al., 2000). Finally, seedling protectors were shown to increase the survival in ten hardwood species by 35% as compared to unprotected seedlings and additionally allowed increased height accumulation in seven of these species (West et al., 1999).
In addition to the previously mentioned biotic influences, multiple lines of evidence suggest that abiotic factors affect the development and maintenance of Bluegrass savanna-woodland. One such factor that may be influencing the development of Inner Bluegrass savanna is the fact that the region is susceptible to droughts and the soils drain quickly (McInteer, 1952). Furthermore, soil properties and nutrient limitations can influence the accumulation of biomass and therefore determine the successional trajectory of plant communities (Tilman, 1984). Charcoal ash, pollen records, and archaeological evidence suggest anthropogenic fires were a dominant disturbance regime in eastern forests and savannas following Pleistocene glaciations, as naturally occurring fires are rare in the region due to high precipitation amounts (Delcourt et al., 1998; Guyette et al., 2002). Some insight has been gained through a unique pollen study in Eastern Kentucky which helped to show that as glaciers retreated after the most recent ice age, a shift from boreal to temperate species increased the dominance of *Quercus*, *Castanea*, *Carya*, and *Juglans nigra* (Delecourt et al., 1998). Additionally, the data shows a spike in ragweed (*Ambrosia* sp.) that coincided with the settlement of the region by Europeans, which suggests further the effects of land clearing of native vegetation and conversion to agriculture, as this is a species that opportunistically follows European land use change (Delecourt et al., 1998). Even though not specific to the Bluegrass Region, early explorations by Michaux in the 1790s described the Bluegrass adjacent barrens region of Kentucky as being “burned every year” (Bryant, 2004). The barrens region, although not exactly the same, is similar to the IBR in having limestone as its predominant bedrock and similar soil qualities (Bryant, 2004). Currently it is suggested that in the IBR many of the areas occupied by remnant savanna stands are consistently mown, which may not allow the seedling crop to establish (Bryant et al., 1980). This practice might mimic disturbance originally attributed to fire and grazing by large herbivores which have long since been extirpated from the region (Bryant et al., 1980).

*How and why bluegrass savanna has been lost: Conservation, preservation, and restoration efforts*

The conversion to agriculture and urban development has left only remnant parcels of intact native Bluegrass savanna-woodland. There have only been a few instances of preservation and restoration efforts of these remnants by public entities and the majority of remnant parcels remain in private land holdings, which are predominately thoroughbred horse farms (Wharton & Barbour, 1991). Percent cover is not specific, but throughout Kentucky 88% of forested lands are in private holdings (Oswalt, 2012) which would roughly correspond to the approximate acreage of private lands in the Bluegrass Region, although this could be an underestimate. Other figures have stated that >99% of oak savannas in the Midwest region of the US have been lost due to the conversion of land to agriculture or urban uses (King & Magnusson, 2002). Specifically for the Bluegrass Region of Kentucky, 100% of Bluegrass savanna has been degraded or entirely lost, primarily due to land use changes promoting forage and pasture lands (Braun, 1950; Barnes, 1999; Thompson III & DeGraaf, 2001). These numbers demonstrate the fact that oak savannas, and principally the Kentucky Inner Bluegrass blue ash-oak savanna-woodland, have
been drastically affected by human impacts and therefore the need for restoration of these systems is obvious.

Early in European settlement of the IBR, land use changes had already impacted much of the herbaceous layer of vegetation by removing cane and clover through overgrazing and trampling (McInteer, 1952). Furthermore, even though the tree component of remnant savanna-woodland stands exists in old agricultural fields and on private homesteads, intact herbaceous and ground cover vegetation has long since been converted to fescue (Festuca sp.) and Kentucky Bluegrass (P. pratensis), both of which are non-native species (Braun, 1950). Therefore, since no Bluegrass savanna-woodland has an unaffected ground layer, there is not a remnant stand left that could be considered intact or undegraded (Bryant et al., 1980). Altogether, land clearing and conversion to agriculture combined with urbanization and development has left the native plant assemblage in central Kentucky in an imperiled state (Wilson, 1941; Wharton & Barbour, 1991).

The fact that the majority of native grasses have been removed in favor of forage species (e.g. fescue) and the remaining 1% of native Bluegrass savanna stands left have lacked recruitment of oak, ash, hickory and Kentucky coffeetree seedlings for many years (Bryant et al., 1980; Wharton & Barbor, 1991) leaves the need to restore all aspects native bluegrass vegetation readily apparent. Concerning the tree regeneration failure, one hypothesis is that fescue is inhibiting seed to soil contact for many of the savanna tree species, preventing any natural germination and recruitment from occurring. Therefore, through restoration of native grasslands and removal of non-native vegetation one may be able to promote natural recruitment of savanna tree species; however, as this may take some time to occur naturally, restorative plantings of seedlings may be necessary to sustain savanna stands until natural regeneration can occur. Moreover, the restoration of natural disturbance regimes may be necessary to promote seedling regeneration as well. Until one can restore fire, large herbivore impacts (i.e. grazing and trampling), and native vegetation structure it will be essential to plant native seedlings to ensure Bluegrass savanna stands will continue to exist, even if in only small and isolated remnants. If ecological restoration efforts are established and carried to fruition, essential ecosystem services (i.e. wildlife habitat and forage) will be reinstated, a resilience to natural disturbances and stresses will be returned, and an improved likelihood for biodiversity increases will follow (SER Primer, 2004).

Other than the project that will be described further in the paper, the “Reforest the Bluegrass” campaign spearheaded by the Lexington-Fayette Urban County Government’s Urban Forestry Program has been the impetus of one of the largest bluegrass restoration efforts (Reforest the Bluegrass, 2013). The motivation of the program is to “recreate pre-settlement, streamside forests that were once native to the Inner Bluegrass Region of Kentucky”. The ecological restoration goals for the project are explicit and they include filtering pollutants, shading streams, stabilizing stream banks, slowing flood waters, lowering city temperatures by reducing reflective heating, increasing wildlife habitat, and controlling mosquito populations (Reforest the Bluegrass, 2013). There is no explicit reference system of Bluegrass savanna-
woodland to make comparisons with, but the restoration goals are achieved by planting native species of the Bluegrass Region along impaired public streams. This impairment has been described as “the Bluegrass aesthetic”, a practice of mowing and maintaining grass fields up to the edges of streambanks (Buranen, 2007a; Buranen 2007b). Their project boasts considerable success with over 100,000 tree seedlings planted with a first year success rate of 75% and a total restored floodplain coverage of greater than 175 acres (Reforest the Bluegrass, 2013).

Therefore, project goals of Reforest the Bluegrass help to reinforce the necessity of Bluegrass savanna-woodland restoration. Their primary target is to stabilize impeded streambanks due to the loss of natural forest communities. My focus is on the restoration of savanna-woodland habitat which is usually confined more to the drier ridges of land away from riparian zones, but obviously a topographical gradient exists between these two habitats (McEwan & McCarthy, 2008). Because the goals and outcomes of “Reforest the Bluegrass” are different from my research, it demonstrates the necessity to first understand what constitutes a native Bluegrass savanna-woodland and the ecological factors involved for natural savanna-woodland regeneration and maintenance. Addressing these questions is my project’s focus, which also serves as the other main savanna restoration project currently being implemented.

**Defining the Inner Bluegrass blue ash-oak savanna-woodland reference system**

According to the Society for Ecological Restoration (SER) International Primer on Ecological Restoration (2004) “Ecological restoration is an intentional activity that initiates or accelerates the recovery of an ecosystem with respect to its health, integrity, and sustainability.” Due to the fact that the majority of Bluegrass savanna has been lost as a result of human land use changes (Wharton & Barbour, 1991; McEwan & McCarthy, 2008), the need to preserve remaining tracts while also increasing the coverage of Bluegrass savanna habitat underscores the need for ecological restoration of this ecosystem. One of the first tasks to beginning a restoration project is to identify a reference system, defined as the model ecosystem one would like to convert the existing landscape back towards in order to return ecosystem function.

To define a reference system evaluations of typical, unaffected habitats combined with historical descriptions of the landscape are generally used (SER Primer, 2004). Unfortunately, the only reference systems we have for Bluegrass savanna come from anecdotal evidence of early European explorers, as the only remaining remnant stands of Bluegrass savanna cannot be considered ecologically intact due to the loss of native species assemblages and historical disturbance regimes (Wharton & Barbour, 1991; McEwan & McCarthy, 2008). Therefore, one must utilize the original floristic surveys as the only form of evidence for creating a bluegrass savanna reference system. This is not without controversy, as even when European explorers first documented the area it is likely that original maintenance of the landscape by Native Americans had been altered through the progression of European diseases prior to pioneers actually setting foot in Kentucky (Mann, 2005). Because certain diseases probably eliminated the original abundance of Native Americans, their disturbance activities were reduced. Following this human population decline and subsequent decrease in hunting pressures, game populations
likely increased in abundance which potentially allowed for a higher grazing disturbance in the region (Mann, 2005).

Braun (1950) recognized that due to the impacted nature of remnant stands one could not accurately describe what species would have occupied an intact savanna. As stated before, the bluegrass savanna community is failing to regenerate naturally. Continual mowing and grazing keeps the woodland structure in remnant stands, but this also clips the few seedlings attempting to naturally recruit. Within the few remaining remnant woodlands, mature savanna trees are nearing climax ages and are being subjected to lightning strikes. Furthermore, the likelihood for mature trees to be toppled by windthrow in strong weather events has been increased due to physical undermining of the soil structure by groundhog (Marmota monax) (Bryant et al. 1980). Finally, even though oak savannas exist throughout the Midwest region of the US, the importance and abundance of the somewhat unique and specially adapted blue ash (F. quadrangulata) to the distinctive soils of the region distinguish the Kentucky Inner Bluegrass savanna-woodland from all others (Bryant et al., 1980).

Through experimental reconstruction of savanna, by placing in potential community members and subjecting them to the hypothesized disturbance regimes, the emergent members of the community should help return original species compositions and ecological services. This research is an attempt to replicate the initiation of a native Bluegrass savanna-woodland stand, based on descriptions of historical floristic surveys and various lines of evidence as to what constituted a natural disturbance regime while also completing the goal of reintroducing and restoring native tree species that are not naturally recruiting seedling age classes.

Life history description and ecological function of Bluegrass savanna tree species

Below I describe some of the basic life history and ecology of the constituent Bluegrass savanna tree species that are being used in my research project. These species were selected from a known species pool and are commonly found at Griffith Woods. Additionally, the selected species represent a categorized gradient of shade tolerance classes (J. Cox, pers. comm.). These descriptions are by no means exhaustive, and much more information can be gained by thoroughly reading the sources that were drawn upon. In some instances basic biological and ecological information is depauperate, especially for the unique and range restricted Bluegrass savanna type species such as blue ash (Fraxinus quadrangulata) and Kentucky coffeetree (Gymnocladus dioicus). Through my research I hope to fill in some informational gaps on these species while also evaluating the previous evidence for the more thoroughly understood species. A summary of the important ecological traits for these species in relation to the Bluegrass savanna-woodland are presented at the end of this section in Table 1.1.

Bitternut Hickory Carya cordiformis (Wangenh.) K. Koch. Juglandaceae: Walnut Family

One of the most widely distributed eastern hickories, but one of the shorter lived (~200 years), it can be found on moist bottomlands but also on drier sites poor in nutrients. Bitternut
fruit ripens about September and can be dispersed through December, and is limited to successful fruit production after 30 years of age until about 175 years, with mast crops occurring every 3-5 years. Seeds are approximately 70-85% viable, maturation is rather slow and must be stratified to germinate. Bitternut hickory produces a healthy root system with a large taproot and is known to produce root and stump sprouts. Saplings and mature trees can be harmed by fire due to a lowered ability for insulation due to its hard bark which can leave fissures susceptible to a few rot diseases (Burns & Honkala, 1990; Coladonato, 1992). Nuts are susceptible to weevils reducing establishment by seed and during drought years stems are attacked by bark beetles. As with many hickories, the wood is prized for lumber, fuel wood, and furniture. Bitternut is considered intermediate to intolerant of shade (Burns & Honkala, 1990; Coladonato, 1992). Empirical research has shown that bitternut is highly affected by mammalian browse during the seedling stage causing increased mortality to the point that effects of competition are unclear, although sprouting from whole plant browse is possible (Myster & McCarthy, 1989).

**Black Walnut Juglans nigra** L. Juglandaceae: Walnut Family

Black Walnut ranges over most of the eastern and central U.S. on many different site types, but is most commonly found on deep, well drained neutral pH soils derived from limestone. It is found in many different forest types, but never in great abundance, but a common associate with good sites for walnut is Kentucky coffeetree (*Gymnocladus dioicus)*. Black walnut is known for the toxin Juglone, which can inhibit the growth of competing plants and is thought to lower its palatability. Flowering and leafing occurs fairly early, usually in April, and fruit is produced in September to October with mast crops occurring every 2-5 years. Though fruiting can occur in small amounts at a young age, heavy masts do not occur until about 20 years and can produce up until 130 years. Seeds must be stratified to germinate. Black walnut seedlings are intolerant of shade and usually germinate from squirrel cached nuts that are not eaten in April with rapid growth in initial years. Black walnut has large taproots but also spreading shallow roots, are known to sprout from stumps, and have been shown to grow best when competition is reduced in the first few years. It can reach heights up to 37m (120ft) and can be damaged by a variety of insects, but the recently discovered introduced and invasive thousand cankers disease (*Geosmithia morbida*) in east Tennessee is of grave concern for this species (Grant *et al*., 2011). Herbivore damage is common from deer browse and rubs, bark gnawing by rabbit and rodent, and yellow-bellied sapsuckers commonly drill holes in the bark, and frost damage is common in varying weather patterns. It is prized for many uses, most notably for furniture and gunstocks and the nuts are valuable as well for food and the shells are ground for a variety of industrial uses (Burns & Honkala, 1990; Coladonato, 1991). Black walnut is considered to be very adapted to fire when mature, as the thick nature of the bark prevents scarring and the very solid inner heartwood layers are resistant to rot. In younger trees it is likely to be topkilled, but will readily resprout from the stump (Coladonato, 1991).
Bur Oak *Quercus macrocarpa* Michx. Fagaceae: Beech Family.

Widely distributed in the central U.S. in the Ohio, Mississippi, and Missouri river basins, bur oak is a drought resistant species of dry uplands and fertile limestone soils and has the largest acorns of native *Quercus* species. These acorns are important food for deer, rabbit, and various rodents including is primary disperser, squirrels. Acorns drop between August and November with germination soon afterwards. Seed bearing begins at 35 years and can continue up to 400 years, with mast crops every 2-3 years. Successful germination has been shown in areas low in leaf litter, due to a lowered susceptibility to rodent, fungus and insect attack. Initial growth is focused underground with rapid taproot development and efficient water usage lends to its successfulness on dry sites, though it can be found in bottomlands as well. Sprouting can occur from stumps and burned individuals. Bur oak is sometimes classed as intermediate with respect to shade tolerance, but generally it is considered to be a shade intolerant species. It is susceptible to a variety of insects, wilts, and rots. Bur oak is considered to be fire adapted and mature trees survive due to the thick bark and dense inner wood; however, seedlings are usually topkilled and resprout (Burns & Honkala, 1990). Frequent fires are thought to be necessary for this species to remain on sites as succession to closed forest would impede its growth due to shade intolerance (Gucker, 2011). Drought and fire resistance combined with shade intolerance make it a common species in oak savannas and openings (Burns & Honkala, 1990). There is an interesting interpretation as to the abundance of bur oak in the Inner Bluegrass Region where it is thought that small groups of oaks probably occupied fractional forest openings due to its intolerance of shade, dry sites unsuitable for agriculture due to its drought tolerance, and wet sites also unsuitable for crops due to its potential to tolerate lower oxygenated soils (McInteer, 1952).

Chinquapin Oak *Quercus muehlenbergii* Engelm. Fagaceae: Beech Family.

Chinquapin oak is a species of well drained, weakly acidic, upland soils of limestone origin. Petrides (1978) further emphasized this specific site affinity in his description, which states that *Q. muehlenbergii* is found in “dry woods especially on limestone soils”. It is distributed over a rather large range west of the Appalachians through the central plains, south of the Great lakes to the Gulf of Mexico where it grows in association with many different oak species. It flowers in April to May and fruits in September to October utilizing gravity and rodent dispersal, but knowledge on its mast years is lacking. Seeds can germinate even if they fall in moderate leaf litter and sprouting is common. They can grow up to 24m (80ft) and are intolerant of shade. Seedlings and saplings are commonly topkilled by fire but usually resprout, and chinquapin oak is adapted to moderate to low intensity fires at 10-20 year intervals. They can be attacked by fungi, oak wilt, and are susceptible to gypsy moth defoliation as well as other insects. The acorns are important wildlife food for deer, rodents, and turkey and it is an important browse species. The lumber is graded in the white oak category (Burns & Honkala, 1990; Tirmenstein, 1991). Of additional interest with *Q. muehlenbergii* is that the national champion tree (i.e. the largest specimen) is located on the study site for this research, Griffith Woods, Harrison Co. KY, where this individual has a circumference of 790cm (311in), a height of
23.2m (76ft), and a canopy spread of 21m (69ft) giving it a total score of 404 points (Kentucky Division of Forestry, 2011; American Forests, 2013)

**Shumard Oak** *Quercus shumardii* Buckl. Fagaceae: Beech Family.

This species has a more southerly distribution than the other oaks considered here, with the northern range extending into Indiana and most of the range encompassing the southern coastal plain over to the lower central basin. It is most common on moist, well drained soils with a pH near 7.5 but can tolerate droughts, as evidenced by its range into central Texas, and can handle nutrient deficient alkaline soils. Shumard oak generally flowers in March to April and acorns drop in September to October, with healthy fruit production starting at 25 years and continuing for about 50 years. Acorns of this species can be multiseeded (an infrequent and peculiar trait) and are internally dormant, needing a cold stratification in moist conditions for about 3 months before germinating the following spring (Sullivan, 1993). It can attain heights of 30.5m (110ft) and is intolerant of shading, needing ample light for successful reproduction. As it is common to bottomlands, Shumard oak appears tolerant of inundation, but can persist on dry sites as well. It is susceptible to wilt and rot and a variety of generalist insects and borers. Acorns are important wildlife food (waterfowl, turkey, deer, and squirrel), it is a browse species for deer, and wood is used in the red oak type (Burns & Honkala, 1990). Shumard oak can have a long lifespan, and the oldest individual was aged at 480 years in a remnant blue ash savanna (Bryant et al., 1980). No specifics on fire tolerance are known, and given that Shumard oak is found in the fire prone IBR along with fire resistant bottomland forests it is hard to deduce its tolerance. It is likely moderately tolerant of low intensity fires and can readily stump sprout if top killed (Sullivan, 1993). However, experimentation on Shumard oak seedlings suggest that belowground competition and fire significantly affect survival in this growth stage (Myster, 2009), so frequent burns could reduce the ability of Shumard oak to recruit in the IBR.

**Blue Ash** *Fraxinus quadrangulata* Michx. Oleaceae: Olive Family.

Common indexes used for species description in this document do not include blue ash in their descriptions, possibly hinting at the uniqueness of this species. Blue ash has a rather restricted distribution, in a horseshoe shape that is characterized by underlying limestone bedrock across the upper Midwest in the Ohio and Mississippi river basins, but most notably in the Inner Bluegrass Region of Kentucky and the Nashville Basin of Tennessee (USGS 1999). Blue ash can attain heights of 25m (80ft), it flowers in April to May, and fruits in October releasing a wind dispersed samara. Its habitat is described as being moist limestone soils. The best identifying characteristic is the 4 corky wings on small branches and twigs, lending to its specific epithet *quadrangulata*. The wood is similar to white ash (*F. Americana*) and is used for furniture and baseball bats, but historically extracts from the bark were utilized for blue dye, lending to its common name (Johnson & Hoagland, 1999). Ash seed weevils (*Lignyodes bischoffi*) attack the seeds of all ash species and reduce their viability (Dix, 1986; Barger & Davidson, 1967) and the possibility of infection by anthracnose (*Gnomoneilla fraxini*) is possible, but rare in blue ash (Jacoby & Danielson, 2002). The most common current threat to blue ash is the introduced invasive emerald ash borer (*Agrilus planipennis*), a threat to all of the native ash species.
However, experimental feeding assays suggest that although *A. planipennis* is able to complete its life cycle on blue ash, it is the least preferred of North American *Fraxinus* species for feeding and oviposition (Pureswaran & Poland, 2009). As has been stated before in this paper, the increased abundance and dominance of blue ash in the Inner Bluegrass Region (IBR) of Kentucky distinguish the Bluegrass savanna-woodland from all other oak savanna habitats in the Midwestern US (Bryant *et al*., 1980) which may be indicative of this species’ preference for calcareous parent material, but atypical from the literature in the droughty nature of the IBR soils.

**Common Hackberry *Celtis occidentalis* L. Ulmaceae: Elm family**

Ranging across the U.S. from the East Coast to the central plains and from the great lakes south to Tennessee, common hackberry is a medium sized softwood tree common across a variety of habitats. It occupies a range of soil types, it is most common on limestone soils and in bottomland areas, although it is rather drought resistant while also moderately flood tolerant. Hackberry flowers and sets leaves in April to May and the fruit, a cherry like drupe, ripens in September to October. Abundant seed is produced in most years and seedlings develop in previously established hardwood stands, although in the IBR of Kentucky it is common along fencerows. Height is generally around 15m (50ft) but on high quality sites it can reach 40m (130ft). Hackberry can reach ages of 200 years, stump sprouting is restricted to smaller individuals, and it develops a deep root system. Hackberry is intermediate in its shade tolerance, and individuals growing in heavy shade are usually less healthy. It is a host to a few gall producing insects and has many fungi species that attack its leaves. The fruit and seed of hackberry are eaten by a variety of wildlife, especially game birds such as turkey, pheasant, and quail but also for some songbirds and small mammals. Furthermore, deer are known to browse hackberry heavily in some areas. Fire generally topkills seedlings and saplings but these will resprout, and mature trees are somewhat protected by their thick and warty bark, however, other sources imply that hackberry can respond detrimentally to fire. Seeds have been demonstrated as to withstand low intensity fires and hackberry can colonize burned areas (Burns & Honkala, 1990; Gucker, 2011).

**Kentucky Coffeetree *Gymnocladus dioicus* (L) K. Koch Fabaceae: Pea Family**

A unique, dioecious tree with a rather restricted distribution compared to the other species described in this report, Kentucky coffeetree is found predominately in the Midwestern U.S. in E. Ohio, the Bluegrass Region of Kentucky, Indiana, Illinois, the Nashville Basin of Tennessee, Missouri, and Eastern Kansas and Northeastern Oklahoma (USGS, 1999). Information about this species is not available in the primary sources utilized for most of this report (USDA Silvics manual and USDA FEIS) giving credence to its uniqueness and rarity. This species is a medium to large tree reaching heights of 30.5m (100ft), with deep and irregularly furrowed bark and large bipinnately compound leaves, the largest leaves of any N. American species (up to 0.9m/3ft in length). It is most commonly found on moist soils and bottom lands, but also open woodlands in rocky soil and commonly on limestone soils. It can tolerate drought and inundation and is known to stump sprout and produce root suckers. Kentucky coffeetree is
considered to be intermediate in its shade tolerance, though it grows best in full light and some sources deem it as intolerant of shade. Flowers appear shortly after the leaves in May to June and the fruit, a large leguminous pod with 4 to 8 dark seeds, appear in September to October. Although a legume, it does not fix nitrogen. The seeds will not germinate until the tough pod husk decomposes and the seed coat, impermeable to water, breaks down, which can take up to two years (USDA, NRCS, 2013). This reproductive strategy is perplexing and natural germination is difficult with water being the only currently recognizable dispersal agent, leading to some researchers speculating that historically coffeetree would have been most likely dispersed by now extinct Miocene and Pleistocene megafauna, where digestion through the intestinal tract would have been the only way to release the seeds from their impermeable seed coat (Zaya & Howe, 2009). Further complicating this stage in coffeetree’s life history is that the seeds and pod, though containing a sweet pulp, are considered extremely toxic to humans and livestock, and Native Americans were even known to fill ponds and streams with the pods to poison fish for easier harvesting. Even now, farmers are recommended to not let livestock feed on seedlings or drink from ponds contaminated by coffeetree seeds or leaves. As toxic as it is, it was known to be historically used as a coffee substitute by early settlers and as a medicine by Native Americans to treat various stomach ailments, but all of these uses were only after being boiled or roasted to break down the cytisine toxin. Currently this species is planted as an ornamental, replacing ash and elm trees which have been devastated by disease and pests, as coffeetree has no current threats, and additionally as a species for mine reclamation as it is able to tolerate a range of soil conditions. Historically pioneers utilized coffeetree’s strong wood as useful timber for fenceposts and cabinets causing it to be planted around farmsteads, which is one thought as to why it is fairly well distributed even though it has a difficult natural and unique dispersal and germination strategy (USDA, NRCS, 2013). No information is available regarding its fire tolerance, but due to its ability to root and stump sprout any topkilled individual would likely resprout.

White Ash *Fraxinus americana* L. Oleaceae: Olive family.

Also known as Biltmore white ash, this species has an extensive range from the central plains east to the Atlantic coast, and from Ontario to the Gulf of Mexico. Burns & Honkala (1990) state that this species “has demanding soil fertility and soil moisture requirements” with limestone or shale parent material (among others) and abundant nitrogen or calcium concentrations being a necessity. Flowers and leaves appear in April/May and, starting at 20 years of age, wind dispersed samaras are distributed between September to December, although these seeds require about a three month stratification period before germinating. Root competition has been shown to impair seedling/sapling growth and these growth stages show high apical dominance yet will readily stump sprout. The taproot is associated with additional vertically growing roots and lateral rooting depends on soil conditions. Considered a rather early successional species, *F. americana* easily invades old field habitats, but is also known to grow under light suppressed forest canopies, leading to its intermediate shade tolerance category. White ash seedlings respond poorly to browsing by deer, the bark is eaten by rabbit and beaver, and the seeds are utilized by fox squirrel (*Sciurus niger*) and a variety of birds including wood
ducks (*Aix sponsa*), quail, and finches. The wood is prized for tool handles and baseball bats and it is commonly planted in urban areas (Burns & Honkala, 1990). Although fire will usually kill all aboveground parts in white ash, it will usually resprout from the stump, and some research indicates that in burned areas white ash saplings will increase in stem per acre abundance (Griffith, 1991). Air pollution is known to be detrimental to white ash, and it can be susceptible to ash decline (Burns & Honkala, 1990), but studies indicate that it is tolerant of anthracnose (*G. fraxini*) (Jacobs & Daneilson, 2002). Although susceptible to a variety of pests such as the ash seed weevil (*L. bischoffi*) (Dix, 1986; Barger & Davidson, 1967), the largest current threat is the emerald ash borer (*Agrilus planipennis*) (EAB), a highly invasive insect pest decimating eastern North America ash populations. Unlike blue ash (*F. quadragulata*) which isn’t preferred by EAB, white ash appears to be highly susceptible and one of the more preferred species of EAB, which has contributed to its drastic decline in EAB invaded areas (Pureswaran & Poland, 2009).

**Box elder *Acer negundo* L. Aceraceae: Maple family**

The most widely ranging maple species of North America, box elder can be found from the Atlantic to the Pacific coasts and from Canada south to Florida/Texas, but its primary distribution is through the central plains and watersheds of the Mississippi, Ohio, and Tennessee rivers in association with bottomland hardwoods (USGS, 1999). Box elder can tolerate a wide variety of climatic conditions, wide array of soil types, and can persist in extended xeric and mesic soil conditions. Box elder is a completely dioecious tree and depending on location, due to its wide range, flowers and leaves appear anywhere between March through May and its seeds, samaras, are annually wind dispersed between August and October beginning at about 10 years of age. Seeds germinate in a variety of conditions, from forest to field, but unless openings are provided many seedlings die off by age two. A medium sized, fast growing, short lived tree, box elder can attain heights reaching upwards of 23m (75ft) and can live up to 100 years, but on average usually only 75 years. In younger trees it is common for them to stump or root sprout, and the root system is usually shallow, with mostly fibrous roots and occasionally a small taproot. Box elder is classified as a shade tolerant species, but is can also be an early successional colonizer of recently disturbed or created habitats. A variety of threats exist for box elder including various fungi species that cause rot damage, staining of wood, or the more serious Verticillium wilt (*Verticillium albo-atrum*), and rarely will insects cause detrimental damage (Burns & Honkala, 1990). Physically, box elder is easily susceptible to wind, ice, and fire damage due to its soft wood and thin bark and although likely topkilled by severe fires, low to moderate fires will probably still allow box elder to root or stump sprout and its abundant seed source allows it to recolonize recently disturbed habitats (Rosario, 1988). It has a variety of uses, from ornamental plantings due to its cosmopolitan nature resulting from its cold and drought resistance, to erosion control from its high surface area root system, and lastly as a good wildlife food source from its prolific seeding and tender foliage and twigs for browse (Burns & Honkala, 1990).
Ohio Buckeye *Aesculus glabra* Willd. Hippocastanaceae: Horsechestnut family

The natural range of Ohio buckeye is primarily in the Ohio River and Upper Mississippi watersheds, while also extending somewhat into the central plains and south through central Kentucky and Middle Tennessee, but has been planted in the Eastern U.S. and Europe. Generally disregarded for commercial uses and somewhat poisonous to livestock, it is planted as an ornamental. Mostly confined to moist soils of river banks, it can be found on drier sites where it usually is slower growing and not a major component of the forest. However, Ohio buckeye is also found on the limestone derived soils of the Kentucky Bluegrass where it is in association with Bur oak, chinquapin oak, white ash, Kentucky coffeetree and the other dominants of the IBR. Considered an early phenology species it is often one of the first species to leaf and flower in early March through May, and the fruit, a capsule containing up to three seeds, is dispersed by gravity, animals, or water in September to October starting at 8 years of age. Seeds overwinter and usually germinate the next spring developing a large taproot first and followed by a short stout stem. It is a shade tolerant species commonly found in maple-beech understories and it has few insect pests or diseases, however, because of its early phenology young flowers and leaves can be damaged by late frosts. A toxin found in the seeds, a narcotic glucoside, tends to make most animals shy away from eating them, especially cattle; however, it does seem that fox squirrels may consume the seed but not enough to limit germination (Burns & Honkala, 1990). No information is given about its browse preference or fire tolerance, and it is not listed as a species in the USDA fire effects information system (FEIS).

Red Mulberry *Morus rubra* L. Moraceae: Mulberry family

Widespread throughout the central and Eastern U.S. to the gulf, but absent from New England, red mulberry is not valued commercially, but the fruits are highly prized by wildlife and humans alike. Generally found on moist soils, and growing best in flood plains or wet coves, it is widely dispersed by birds so can be found on almost any suitable site with enough moisture and as well is common along fence rows in more heavily impacted areas. Mulberry flowers in April to May and the drupelet fruit is ripe from June to August on trees as young as 10 years but as old as 125 years. Seeds will germinate without being stratified in the fall but sometimes will wait until spring but it also commonly sprouts from roots, ultimately reaching heights of 21m (70ft). Red mulberry is susceptible to a few insects and a bacterial disease is thought to be causing declines in the central part of is range, but of most concern is genetic dilution and introgression due to hybridization with White Mulberry (*Morus alba*) an introduced, invasive species from China (Stone, 2009). As stated earlier, red mulberry is a good food source for wildlife from waterfowl and songbirds, up to small mammals, and deer are known to browse both leaves and twigs. It is considered to be tolerant of shade as it grows under canopy cover but attains optimum growth in open habitats. Due to its shallow roots and thinner bark, red mulberry is considered very sensitive to fire and in fire prone habitats it is more common in areas where fire has been suppressed but will colonize recently burned sites (Burns & Honkala, 1990; Sullivan, 1993).
**Shellbark Hickory Carya laciniosa (Michx. F.) Loudl. Juglandaceae: Walnut family.**

Also known as kingnut hickory due to it having the largest nuts of the hickories, it is most readily identifiable by the flaky nature of its bark, which Petrides (1972) described as being “very shaggy, loosening in long strips”. Shellbark hickory ranges mostly through the Ohio River and middle region of the Mississippi River watersheds, it is not considered common in any part of its range. This species grows best on moist, deep, fertile soils of a silt-loam texture and slightly basic pH and is mainly thought of as a bottomland species, although in Kentucky it is found in association with bur oak on drier sites. It produces flowers in April to June and the fruit will mature in September to November. The large seeds need cold stratification before germinating, and trees are usually 40 years old before setting seed but will continue to reproduce up to 200 years. Main dispersal agents are squirrels and gravity. Shellbark is a prolific stump sprouter, produces a deep taproot and is considered to be very shade tolerant. Many insects are known to damage this species, but impacts from diseases are not often documented. Fire can damage its trunk which then serves as an introduction point for fungus rots but one would imagine that its stump sprouting ability prevents complete dieback. Another mast species, its nuts are food for many forest species such as deer, fox, raccoon (*Procyon lotor*), rodents and gamebirds. The lumber is used as other hickories for furniture, tools, and fuel but the nuts, although sweet, are difficult to break on a commercial scale (Burns & Honkala, 1990). Though not specifically documented in *C. laciniosa*, the related species shagbark hickory (*Carya ovata*) with morphologically similar bark structure that produces flaky strips that cling to the tree, described as “exfoliating bark”, has been shown to be important hibernacula habitat for roosting Indiana bats (*Myotis sodalis*), a federally endangered species (Britzke *et al.*, 2006).

**Sugar Maple Acer saccharum Marsh. Aceraceae: Maple Family.**

Sugar maple ranges across the northern U.S. from Minnesota across the Great Lakes through Ontario and New England, but stretches south through the Appalachians and Tennessee, but is absent along the Piedmont and Coastal Plain. It is generally found in cooler climates with more moisture, and is found on loamy soils that can be acidic, but avoids extremely dry or wet sites and because of its importance it is the type species for many forests. Flowers and leaves appear in late March through May, and the double samara fruit ripen in late summer and begin dispersal via wind in early fall. Trees usually need to be about 20-25 years old before reproducing, and can continue for to set seed for another 100 years and the seeds require a moist stratification period before which about 95% will germinate. Sugar maple is highly shade tolerant, and in intense light it has been shown that development of seedlings can fall drastically. High abundances of seed and high germination rates create large seedling cohorts, but the survival rate for these seedlings is only about 50%. One thought on why *A. saccharum* does so well in forest understories compared to open habitats is its rather shallow root system, which creates high competition for soil moisture and nutrients which it is outcompeted for in ample light environment, however, in low light conditions it is able to gain ample water and nutrition as most species cannot tolerate the poor light environment. It is an ample stump sprouter in younger trees, and can grow to heights of 37m (120ft) and live up to 400 years. Although subject to attack by a variety of insects these are usually minor and there
are no current serious infestation threats but sugar maple can be susceptible to browse by deer, and squirrels feed on many portions of the tree (Burns & Honkala, 1990). The largest commercial use of sugar maple is for the production of maple syrup, which was historically one of the only sources of sugar for many of the pioneers of Kentucky and over-tapping of this species could be why old individuals are rare in remnant savanna-woodland stands (Wharton & Barbour, 1991). It is generally thought that sugar maple, which has a thin bark, is sensitive to fire and even though physically damaged trees will stump sprout, those damaged by fire rarely resprout. Therefore, it is anomalous to think that the Bluegrass, thought to be fire maintained, would have sugar maple consistently listed as a common tree by the early botanical explorers to Kentucky (Tirmenstein, 1991; Wharton & Barbour, 1991).
Recapitulation of species summaries

Table 1.1: Summary of important biological and ecological traits that pertain to the constituent Inner Bluegrass savanna-woodland tree species used in this study. Emphasis has been placed on traits that are hypothesized to influence persistence of the species and the overall community structure.

<table>
<thead>
<tr>
<th>Species</th>
<th>Shade Tolerance</th>
<th>Limestone derived soils</th>
<th>Fire Tolerance</th>
<th>Stump Sprouting</th>
<th>Wildlife benefit</th>
<th>Drought tolerance</th>
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</thead>
<tbody>
<tr>
<td>Bitternut Hickory (<em>Carya cordiformis</em>)</td>
<td>intolerant</td>
<td>unknown</td>
<td>moderate</td>
<td>yes</td>
<td>Hard mast</td>
<td>moderate</td>
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<tr>
<td>Black Walnut (<em>Juglans nigra</em>)</td>
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<td>yes</td>
<td>high</td>
<td>yes</td>
<td>Browse and hard mast</td>
<td>moderate</td>
</tr>
<tr>
<td>Bur Oak (<em>Quercus macrocarpa</em>)</td>
<td>intolerant</td>
<td>yes</td>
<td>high</td>
<td>yes</td>
<td>Hard mast</td>
<td>high</td>
</tr>
<tr>
<td>Chinquapin Oak (<em>Quercus muehlenbergii</em>)</td>
<td>intolerant</td>
<td>Unknown, tolerates alkaline soils</td>
<td>Unknown but likely tolerant</td>
<td>yes</td>
<td>Hard mast</td>
<td>high</td>
</tr>
<tr>
<td>Shumard Oak (<em>Quercus shumardii</em>)</td>
<td>intolerant</td>
<td>yes</td>
<td>moderate</td>
<td>yes</td>
<td>Unknown, but likely tolerant</td>
<td>high</td>
</tr>
<tr>
<td>Blue Ash (Fraxinus quadrangularis)</td>
<td>intermediate</td>
<td>yes</td>
<td>unknown</td>
<td>unknown</td>
<td>Low</td>
<td>Possibly high</td>
</tr>
<tr>
<td>Common Hackberry (<em>Celtis occidentalis</em>)</td>
<td>intermediate</td>
<td>yes</td>
<td>moderate</td>
<td>yes</td>
<td>Unknown but likely tolerant</td>
<td>high</td>
</tr>
<tr>
<td>Kentucky Coffeetree (<em>Gymnocladus dioicus</em>)</td>
<td>Intermediate to intolerant</td>
<td>yes</td>
<td>Unknown but likely tolerant</td>
<td>yes</td>
<td>Poisonous seed pods</td>
<td>high</td>
</tr>
<tr>
<td>White Ash (Fraxinus americana)</td>
<td>intermediate</td>
<td>yes</td>
<td>moderate</td>
<td>yes</td>
<td>Unknown but likely tolerant</td>
<td>low</td>
</tr>
<tr>
<td>Boxelder (<em>Acer negundo</em>)</td>
<td>tolerant</td>
<td>no</td>
<td>moderate</td>
<td>yes</td>
<td>Hard mast</td>
<td>moderate</td>
</tr>
<tr>
<td>Ohio Buckeye (<em>Aesculus glabra</em>)</td>
<td>tolerant</td>
<td>sometimes</td>
<td>unknown</td>
<td>unknown</td>
<td>Low, toxic seeds</td>
<td>moderate</td>
</tr>
<tr>
<td>Red Mulberry (<em>Morus rubra</em>)</td>
<td>tolerant</td>
<td>no</td>
<td>low</td>
<td>yes</td>
<td>Unknown but likely tolerant</td>
<td>high</td>
</tr>
<tr>
<td>Shellbark Hickory (<em>Carya laciniosa</em>)</td>
<td>tolerant</td>
<td>unknown</td>
<td>low</td>
<td>yes</td>
<td>Hard mast and bat roosts</td>
<td>moderate</td>
</tr>
<tr>
<td>Sugar Maple (<em>Acer saccharum</em>)</td>
<td>tolerant</td>
<td>Unknown, but on many types</td>
<td>low</td>
<td>yes</td>
<td>Unknown but likely tolerant</td>
<td>low</td>
</tr>
</tbody>
</table>

Motivating factors, long-term objectives and overall goals

One conceptual idea ecologists believe is that plant communities emerge from two main forces, resource competition (for water, light, and/or nutrients) and disturbance (e.g., herbivory and/or fire). I am testing this concept by applying it to the intriguing problem of the
experimental reconstruction of a native Bluegrass savanna community. Savanna systems are particularly interesting because they involve the coexistence of two contrasting life forms (grasses and trees) and are thought to require a delicate balance between the forces of competition and disturbance. This study is focused on the key sub-problem of the regeneration of individual trees in a grassland habitat. Fourteen hardwood species native to the Central Kentucky region have been planted as seedlings in a grassland environment to assess the relative importance of competition and herbivory on species survival and growth. A variety of traits are being measured in order to explain species differences in response to the treatments. Ultimately, these results will be used to help predict a successful tree component of a reconstructed savanna system.

Through experimental planting of a variety of common hardwood tree species of the Bluegrass savanna community we wish to determine how the specific factors of soil nutrient availability, light intensity, grass competition and herbivore browse influence the composition of the Bluegrass savanna tree community. Information related to these factors and various physiological traits of the particular species will help to determine the proper techniques that would be utilized in the restoration and management of remnant bluegrass savanna parcels. This knowledge would allow proper decisions to be made when concerned with how one should properly manage a land tract in the Bluegrass savanna for a healthy, self-sustaining community that is beneficial to the constituent organisms.

Specific aims and questions to be addressed

In the primary phase of this long-term study I wish to address how soil conditions on the site initially influenced seedling survival and surrounding vegetation parameters (biomass, diversity) in the early stages of a Bluegrass savanna restoration project. We will hopefully gain insights into how microsite differences in soil nutrients and particle size parameters may or more likely, may not have, influenced seedling survival. Additionally, light availability could have also influenced vegetation and seedling survival during the seedling establishment stage as well. This research will give needed information into some of the basic biological and ecological traits for understudied native hardwood tree species, quantify the variability of soil parameters to give base line data going forward with the long-term goals of this restoration project, quantify how restoration of the vegetation component was influenced by soil variability, and draw connections between light, soil, vegetation, and seedling survival. Finally, I hope to give potential management and restoration advice to those who are interested in reconstructing savanna stands, for both aesthetic and ecosystem services objectives. Ultimately, by replicating these practices, one can then hope to attain similar success in Kentucky Inner Bluegrass blue ash-oak savanna-woodland restoration.

Hypotheses and Predictions

Because the ultimate long-term aim of this study is to determine the growth and survival of hardwood tree seedlings native to the Inner Bluegrass blue ash-oak savanna-
woodland, specific hypotheses and predictions have been made concerning the biotic and abiotic environmental conditions of the initial experiment setup and how these may relate to seedling survival during the establishment phase. For abiotic conditions, soil nutrient parameters are hypothesized to differ between blocks and fields. Light availability and intensity will differ between different heights in and above surrounding vegetation, with the lowest values at ground level and the highest values above the vegetation. Vegetation biomass, species richness, and diversity will differ between blocks, and higher vegetation indices will correlate with higher quality soil sites (i.e. locations with higher nutrient availability). Next, seedling survival will differ between blocks, treatments, and species with seedlings in protectors having higher survival. Additionally, seedlings in higher quality soil will have higher survival, and those species that are considered savanna specialists will show higher survival rates than more generalist species. Finally, multiple correlations are predicted to exist between abiotic and biotic responses with seedling survival showing correlations with vegetation biomass and higher biomass indicating higher quality soil which may or may not result in higher seedling survival.

Flow charts of my hypotheses and predictions and the directions of relations are shown below in Figures 1.3-1.5.

Figure 1.3: Flowchart of major components (biotic and abiotic) of the Inner Bluegrass savanna-woodland tested in this experiment. Note the arrows and symbols showing directions of influence. Indirect effects are not detailed, such as the replenishment of soil nutrients by decaying vegetation.
Figure 1.4: Flowchart of major components (biotic and abiotic) of the Inner Bluegrass savanna-woodland, including the biotic pressures of herbivores. Note the arrows and symbols showing directions of influence. Indirect effects are not detailed, such as the shading effect of vegetation increasing soil moisture, which indirectly benefits tree seedlings.

Figure 1.5: Flowchart of major components (biotic and abiotic) of the Inner Bluegrass savanna-woodland, including the biotic pressures of herbivores and abiotic disturbance of fire. Note the arrows and symbols showing directions of influence. Indirect effects are not detailed, such as the benefits of vegetation clearing by fire which can increase available light for seedlings and return essential nutrients back to the soil pool.
Chapter 2: Methods

Study Site

Griffith Woods (formerly Silver Lake Farm) is a 748 acre farm located at the intersection of Kentucky Highway 353 (Russell Cave Rd.) and US Highway 62, approximately 8.5km (5.3mi) southwest of Cynthiana, KY in Harrison County (38°19′48″N, 84°21′01″W). The site is located within the Inner Bluegrass Region (IBR) of Kentucky and contains what has been described as the best remaining example of Kentucky Inner Bluegrass blue ash-oak savanna-woodland (colloquially referred to as Bluegrass savanna). On the property exists a variety of old farm fields in various stages of succession, some advanced succession woodlots, and a large tract of remnant savanna for which the site has been preserved. Historically owned by the same family for 7 generations since the time of European pioneer settlement, then acquired by the University of Kentucky and The Nature Conservancy with assistance from the Kentucky Heritage Land Conservation Fund, Griffith Woods is now under the direction of the Kentucky Department of Fish and Wildlife Resources and operated as a Wildlife Management Area as of May 2012.

Griffith Woods is typical of the IBR, with karst topography, rolling hills, ephemeral creeks, and intermittent springs and seeps. The soils are overall of the Maury silt loam type (Wharton & Barbour, 1991), but the soils in the experimental fields will be described in further detail later in this paper. IBR weather annually averages 12.78°C (55°F) in temperature and 111.76cm (44in) in precipitation, with most precipitation occurring during the spring and early summer (Wharton & Barbour, 1991). Specific weather data for Griffith Woods is given later in this chapter. The elevation of the Griffith Woods ranges from 274-287m (900-940ft), with the savanna tract lying on the highest point on the property at 287m (940ft).

Research for this project has been primarily conducted in 2 former agricultural fields, both approximately 8.1ha (20ac) in size. The elevation of experimental Field 1 is at around 280m (920ft) and experimental Field 2 is about 277m (910ft). The savanna tract and field 1 sit at the top of a ridge that divides the watersheds of Edgewater Branch and Huskens Run of the South Fork of the Licking River (SFLR). Specifically, Huskens Run joins Townsend Creek before joining the SFLR. Field 2 lies entirely in the Edgewater Branch watershed. Highway 62 divides Griffith Woods and the north side of Griffith Woods/Highway 62 drains into Grays Run before joining the SFLR. The confluence of Grays Run and the SFLR is in downtown Cynthiana, KY, the nearest large town. Due to this topography, Griffith Woods is affixed on top of a ridge that is some of the highest elevation in the immediate area (USGS. “Shawhan Quadrangle, Kentucky” 1:24,000). Please refer to Figure 2.1-2.2 for better understanding of these descriptions.
Figure 2.1: USGS US topo 7.5- minute map for Shawhan, KY. The Major Drainage running south to north on the eastern edge of the map is the South Fork of the Licking River (SFLR). Griffith Woods is located in the area outlined in the red square. Scale: each yellow square is 1000mx1000m (3280’x3280’) (USGS. “Shawhan Quadrangle, Kentucky” 1:24,000.)
Figure 2.2: Close-up imagery of Griffith Woods taken from the USGS US topo 7.5-minute map for Shawhan, KY. This close-up map corresponds to the area outlined in red in Map 1. The approximate Griffith Woods boundary is outlined in thick black (see Fig 2.5 for actual boundary). Notice the Savanna tract (outlined in blue), Field 1 and Field 2 (outlined in green and red, respectively) and their locations atop a ridge that divides the watersheds of Huskins Run and Edgewater. BranchScale: each yellow square is 1000mx1000m (3280’x3280’) (USGS. “Shawhan Quadrangle, Kentucky” 1:24,000.)
Figure 2.3: NRCS Web Soil Survey Map of Griffith Woods. The approximate Griffith Woods boundary is outlined in thick black (see Fig 2.5 for actual boundary) while Field 1 in outlined in green, Field 2 is outlined in red and the Savanna tract is outlined in blue. Yellow lines denote boundaries of different soil types. Notable soil types: FwB- Faywood silt loam, LoB- Loradale silt loam 2-6% slope, LoC2- Loradale silt loam 6-12% slope (NRCS Web Soil Survey, 2013).
Figure 2.3 depicts a soil map generated from the NRCS Web Soil Survey. In this map it can be noted that in both of the experimental fields there exist 3 soil types. The most dominant is the Faywood silt loam (FwB), characterized as mainly clay residual particles derived from limestone parent material which constitutes approximately 23% (about 105ha/260ac) of all of Griffith Woods soils. FwB is typical of upland ridge summits from 165-305m (540-1000ft) on slopes of 2-6% and is well drained and has bedrock at depths of 51-102cm (20-40in). The average depth to the water table for FwB is usually more than 203cm (80in) and has a low available water capacity for plant roots (12.2cm/4.8in), meaning that the soils are unable to retain ample amounts of water in the root zone for plants (Chapin III et al, 2002). The typical profile for FwB has a silt loam A horizon from 0-15cm (0-6in) and a clay rich B horizon from 15-76cm (6-30in). The next major soil represented in the experimental fields is the Loradale silt loam (LoB), another clay rich soil weathered from phosphate rich limestone and/or calcareous shale. LoB covers about 17.2% (about 79ha/195ac) of all of Griffith Woods and is most commonly found in the same elevation as the FwB and on 2-6% slopes as well. LoB is another well-drained soil and bedrock is usually located at depths of 102-203cm (40-80in). Finally, the last soil type represented in the experimental fields is another Loradale silt loam (LoC2), but is differentiated from LoB in that it occurs on 6-12% slopes and is more heavily eroded. Another soil of upland ridges in the same elevation range as the other two soil types, it is more commonly found on the shoulders and side slopes of these ridges and constitutes about 9% (41ha/101.4ac) of the Griffith Woods soil types. Derived from the same phosphatic limestone or calcareous shale as LoB, it is also well drained and bedrock lies as the same depth as LoB. The depth to the water table for the two Loradale soil types is usually 36-72in and they have a high available water capacity for plant roots (25cm/9.9in). Additionally, LoB and LoC2 have similar profiles with an A1 horizon of silt loam from 0-30.5cm (0-12in), an A2 horizon with silty clay from 30.5-86cm (12-34in), and a B horizon of clay particles from 86-183cm (34-72in). None of the three soil types represented in the experimental fields are prone to flooding or ponding and all are classified as being prime for use as farmland (NRCS Web Soil Survey, 2013). The relationship of these soil types to the topography and parent material from which they are derived is illustrated in Figure 2.4, where one can note that the Faywood type soils are on the highest elevation areas and the Loradale types are usually on the toe-slopes of the ridges. It can also be noted that the Maury type soils which are generally considered the dominate soil type of the IBR are located in association with the Faywood/Loradale series, but at lower elevations. This further emphasizes the droughty, higher elevation areas of Griffith woods where the Bluegrass savanna-woodland is the primary vegetation community. Whether this soil type influences the community dominate species will be part of the focus of this paper.
Figure 2.4: The major soil types located at Griffith Woods, their locations in relation to topography, and underlying geologic parent material strata from which they are derived. Note that Faywood/Loradale soils are located at higher elevations and it is these soils and elevations that are associated with Griffith Woods. From USDA-SCS (1968) Soil Survey of Harrison Co., KY.

Figure 2.5 shows an aerial photo of Griffith Woods detailing the location of the experimental fields with the locations of experimental blocks within. Note that each field has 6 blocks each and that each field is composed of both soil types (Faywood & Loradale). Comparisons between Figure 2.3 and Figure 2.5 can be made to understand the locations of soil types in relation to the experimental blocks.
Figure 2.5: Aerial photo of Griffith Woods, KY. This figure includes the border to Griffith Woods (red line), the location of the two experimental fields utilized for the tree seedling experiment (yellow lines), and the location of blocks within the experimental fields (green squares).
Site conditions in the two experimental fields were originally hypothesized to be apparently the same, but I investigated these areas to determine if any drastic differences exist in the microhabitats of these fields. The experimental fields have a rolling topography, the relief is gentle, and the slope and aspect are most likely not influencing the planted seedlings. Overall, the slopes of the blocks within the fields are relatively similar, but there are some variations in the aspects of the gentle slopes, but aspect differences should be minor. However, for reference, slope and aspect data is presented in Table 2.1 and as can be seen, although the aspect varied, the slope variation was relatively minor and in some instances could not be determined due to there being level ground (i.e. slope equals zero). Aspect was assessed by visually determining general direction of slope and measuring this direction with a hand held compass (Sunto Co., Model A-10). Slope was determined at the 100’ interval and was measured in degrees using a handheld clinometer (Sunto Co., Type PM-5 360 PC). Slope and aspect data were collected on 2/25/13.

Table 2.1: Slope and aspect of 12 experimental blocks measured on site at Griffith Woods, KY. Notice that in general all blocks contain level to gently rolling slopes but aspect varies greatly between blocks.

<table>
<thead>
<tr>
<th>Block</th>
<th>Aspect</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>314° WNW</td>
<td>4-6° or 7-10%</td>
</tr>
<tr>
<td>B2</td>
<td>310° WNW</td>
<td>2-6° or 4-10%</td>
</tr>
<tr>
<td>B3</td>
<td>293° WNE</td>
<td>2-6° or 4-10%</td>
</tr>
<tr>
<td>B4</td>
<td>353° N</td>
<td>2-6° or 4-10%</td>
</tr>
<tr>
<td>B5</td>
<td>Level</td>
<td>Level</td>
</tr>
<tr>
<td>B6</td>
<td>94° E</td>
<td>4-5° or 7-9%</td>
</tr>
<tr>
<td>B7</td>
<td>Mostly level, P27 dips 147° SE</td>
<td>Level</td>
</tr>
<tr>
<td>B8</td>
<td>Level, P29 dips 90° E</td>
<td>Level</td>
</tr>
<tr>
<td>B9</td>
<td>P33 46° ENE, P34 6° N</td>
<td>3-5° or 5-9%</td>
</tr>
<tr>
<td>B10</td>
<td>Level, P37 dips 350° N</td>
<td>3-5° or 5-9%</td>
</tr>
<tr>
<td>B11</td>
<td>170° S</td>
<td>2-5° or 3-9%</td>
</tr>
<tr>
<td>B12</td>
<td>142° SSE</td>
<td>2-4.5° or 3.5-8%</td>
</tr>
</tbody>
</table>

**Site History**

For many years the experimental fields were fescue dominated pastures. As recently as late 2001-02 field 2 was utilized for tobacco production (John Cox, pers. comm.). In the summer of 2008 both fields were cropped for corn and then followed by wheat. In 2009 the fields had herbicide applied to suppress the growth of non-native grasses (*P. pratensis* & *Festuca* spp.) and other invasive species (i.e. Poison hemlock *Conium maculatum* L.) and a native grass, forb, and wildflower mixture was planted (Habitat mix, Roundstone, Inc) (Table 2.2 & 2.3). In the late winter of 2011 (~February) the fields were cleared with a Bushhog to make for ease of planting and setting up of the block/plot design.
Table 2.2: Detailed list of grass species planted in experimental blocks at Griffith Woods, KY.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific Name*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Big Bluestem</td>
<td>Andropogon gerardii</td>
</tr>
<tr>
<td>Canadian Wild Rye</td>
<td>Elymus Canadensis</td>
</tr>
<tr>
<td>Fall Panicum</td>
<td>Panicum anceps</td>
</tr>
<tr>
<td>Indian Grass</td>
<td>Sorgahastrum nutans</td>
</tr>
<tr>
<td>Little Bluestem</td>
<td>Schizachyrium scoparium</td>
</tr>
<tr>
<td>Lopsided Indiangrass</td>
<td>Sorgahastrum secundum</td>
</tr>
<tr>
<td>Honeywood Drop Seed</td>
<td>**Sporobulus sp.</td>
</tr>
<tr>
<td>Side Oats Gramma</td>
<td>Bouteloua curtipendula</td>
</tr>
<tr>
<td>Switchgrass (variety not specified)</td>
<td>Panicum virgatum</td>
</tr>
<tr>
<td>Tall Dropseed</td>
<td>Sporobulus composites</td>
</tr>
<tr>
<td>Toothache Grass</td>
<td>Ctenium aromaticum</td>
</tr>
<tr>
<td>Virginia Wild Rye</td>
<td>Elymus virginicus</td>
</tr>
<tr>
<td>Wiregrass</td>
<td>Aristida stricta</td>
</tr>
</tbody>
</table>

*Scientific names came from:  [http://www.roundstoneseed.com/productshowcase.html](http://www.roundstoneseed.com/productshowcase.html)

**Scientific name not identifiable by this common name, Genus inferred.

Table 2.3: Detailed list of forb species planted in experimental blocks at Griffith Woods, KY.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Scientific Name*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bergamot</td>
<td>Monarda fistulosa</td>
</tr>
<tr>
<td>Blackeyed Susan</td>
<td>Rudbekia hirta</td>
</tr>
<tr>
<td>Browneyed Susan</td>
<td>Rudbekia triloba</td>
</tr>
<tr>
<td>Butterfly Milkweed</td>
<td>Asclepias tuberos</td>
</tr>
<tr>
<td>False Sunflower</td>
<td>Heliopsis helianthoides</td>
</tr>
<tr>
<td>Grayheaded Coneflower</td>
<td>Ratibida pinnata</td>
</tr>
<tr>
<td>Illinois Bundleflower</td>
<td>Desmanthus illinoensis</td>
</tr>
<tr>
<td>Lance Leaved Coreopsis</td>
<td>Coreopsis lanceolata</td>
</tr>
<tr>
<td>Maximilian Sunflower</td>
<td>Helianthus maximilianii</td>
</tr>
<tr>
<td>New England Aster</td>
<td>Aster novae-angliae</td>
</tr>
<tr>
<td>Prairie Dock</td>
<td>Silphium pinnatifidum</td>
</tr>
<tr>
<td>Purple Coneflower</td>
<td>Echiniacea purpureum</td>
</tr>
<tr>
<td>Purple Prairie Clover</td>
<td>Dalea purpureum</td>
</tr>
<tr>
<td>Rigid Goldenrod</td>
<td>Solidago rigida</td>
</tr>
<tr>
<td>Rough Blazing Star</td>
<td>Liatris aspera</td>
</tr>
<tr>
<td>Roundheaded Lespedeza</td>
<td>Lespedeza capitata</td>
</tr>
<tr>
<td>Korean Lespedeza</td>
<td>**Kummerowia stipulacea or Lespedeza stipulacea</td>
</tr>
<tr>
<td>Smooth Aster</td>
<td>Aster laevis</td>
</tr>
<tr>
<td>Spiked Blazing Star</td>
<td>Liatris spicata</td>
</tr>
<tr>
<td>Whorled Rosinweed</td>
<td>Silphium trifoliatum</td>
</tr>
</tbody>
</table>


** Scientific names came from:  [http://plants.usda.gov/factsheet/pdf/fs_kust.pdf](http://plants.usda.gov/factsheet/pdf/fs_kust.pdf)
**Experimental Species**

A variety of tree seedlings native to the bluegrass savanna of Kentucky have been planted in this experiment, and they range from dominant canopy species to minor components and are considered natural and native to the Bluegrass savanna. A description of their basic ecology is included in Chapter One of this manuscript, and Table 1.1 summarizes the important ecological information. The species and their shade tolerance class are listed in Table 2.4.

Table 2.4: Fourteen hardwood tree seedling species experimentally planted at Griffith Woods, KY.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Scientific name</th>
<th>Shade Tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Bitternut Hickory</td>
<td><em>Carya cordiformis</em> (Wangenh.) K. Koch</td>
<td>Intolerant</td>
</tr>
<tr>
<td>Black Walnut</td>
<td><em>Juglans nigra</em> L.</td>
<td>Intolerant</td>
</tr>
<tr>
<td>Bur Oak</td>
<td><em>Quercus macrocarpa</em> Michx.</td>
<td>Intolerant</td>
</tr>
<tr>
<td>Chinquapin Oak</td>
<td><em>Quercus muehlenbergii</em> Engelm.</td>
<td>Intolerant</td>
</tr>
<tr>
<td>Shumard Oak</td>
<td><em>Quercus shumardii</em> Buckl.</td>
<td>Intolerant</td>
</tr>
<tr>
<td>Blue Ash</td>
<td><em>Fraxinus quadrangulata</em> Michx.</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Common Hackberry</td>
<td><em>Celtis occidentalis</em> L.</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Kentucky Coffeetree</td>
<td><em>Gymnocladus dioicus</em> (L) K. Koch</td>
<td>Intermediate</td>
</tr>
<tr>
<td>White Ash</td>
<td><em>Fraxinus americana</em> L.</td>
<td>Intermediate</td>
</tr>
<tr>
<td>*Box Elder</td>
<td><em>Acer negundo</em> L.</td>
<td>Tolerant</td>
</tr>
<tr>
<td>Ohio Buckeye</td>
<td><em>Aesculus glabra</em> Willd.</td>
<td>Tolerant</td>
</tr>
<tr>
<td>Red Mulberry</td>
<td><em>Morus rubra</em> L.</td>
<td>Tolerant</td>
</tr>
<tr>
<td>Shellbark Hickory</td>
<td><em>Carya laciniosa</em> (Michx. F.) Lould.</td>
<td>Tolerant</td>
</tr>
<tr>
<td>Sugar Maple</td>
<td><em>Acer saccharum</em> Marsh.</td>
<td>Tolerant</td>
</tr>
</tbody>
</table>

*denotes not fully replicated at Griffith Woods

**Experimental Setup**

In the spring of 2010, 6,168 hardwood tree seedlings comprised of 14 different species were planted at Griffith Woods, KY. Table 2.4 lists the experimental species studied and their associated shade tolerance classes.

Seedlings were provided by the Forrest Keeling Nursery in Elsberry, Missouri. All species planted in the experiment were provided by this nursery other than two exceptions. A total of 548 seedlings of hackberry (*C. occidentalis*) were germinated and grown for two years by the nursery from seed stock gathered on site at Griffith Woods in the summer and fall of 2008. An attempt was made to utilize seed gathered from the Inner Bluegrass Region for the other experimental species, but this was not accomplished by the nursery. Blue ash (*F. quadrangulata*) was the only species not provided by the nursery. Therefore, blue ash seedlings were dug on site at Griffith Woods during the interval of March 25th-27th, 2011. Blue ash not dug on site was provided by the on-site nursery, Griffith Woods Nursery. All blue ash seedlings were planted during the period of March 25th-27th, 2011. Delivery of seedlings provided by the nursery occurred on March 15th, 2011 and planting began immediately. Eleven species were planted between March
15th and April 15th, with the majority of planting occurring on March 31st, April 2nd, and April 10th, 2011. Bitternut hickory (C. tomentosa) and box elder (A. negundo) were planted in the interval between April 15th and May 5th, 2011.

A 2x2 factorial treatment was installed into the planting design. It was composed of seedling protectors to control for herbivory, herbicide and mowing to control for grass competition, both protectors and herbicide, and neither to serve as a negative control. Seedling protectors are approximately 60cm (2ft) tall and 10cm (4in) in diameter and are composed of a polypropylene diamond mesh that inhibits herbivory by mammalian browsers, but not insect herbivory (Rigid Seedling Protector Tubes, Forestry Suppliers Product #17405, forestrysuppliers.com). They were placed around seedlings immediately after planting and held in place by a 0.9m (3ft) bamboo stake. Mowing occurred around the trees to begin the vegetation competition elimination treatment during the interval from 6/20- 6/29. At this point the vegetation was already rather tall and it was difficult to find the trees. Field 1 was re-mowed on 7/23/11 and Field 2 was re-mowed on 7/27/11.

After mowing, herbicide treatments were applied. A 1.5% solution of Accord® herbicide (active ingredient glyphosate) was applied to a 0.6-0.9m (2-3ft) diameter circle around every stem in the competition removal plots. Herbicide application generally occurred on windless days without any precipitation that would wash the herbicide off the vegetation. Spraying occurred between 7/6/11 and 7/15/11.

Planting occurred in a block/plot method with 12 total blocks and 4 plots per block, totaling 48 plots (Figure 2.6). Individual trees were planted by hand using tree planting bars (aka dibble bars), with the majority of work performed by graduate and undergraduate student volunteers. Trees were planted 10 stems per row, with trees spaced at 3.05m (10ft) intervals. Rows were spaced 3.05m (10ft) apart. On average there were 12 rows per plot (but up to 14 rows in some plots). An additional gap of 6.1m (20ft) was added between plots to serve as a delineating border. This created a total plot size of 33.5m (110ft) by 39.6m (130ft) and a total block size of 67.1m (220ft) by 79.2m (260ft). Six blocks were planted in a field, and 2 fields were used (Figures 2.7, 2.8, & 2.9). These fields were located ~0.4km (~0.25mi) from each other, and the site quality was originally assumed to be similar, but has been assessed in this paper. The reason for this particular design was to also accommodate a prescribed burn treatment built into the experimental setup, with 4 blocks received a 5 year burn interval, 4 blocks receiving a 10 year burn interval, and the 4 remaining blocks receiving no burn treatment to act as a control. Additionally, blocks were spaced 12.2m (40ft) apart in order to accommodate fire break lines for the planned burn treatments.

Species rows were randomized to ensure the same species were not always planted next to each other. Treatments were also randomized to ensure that the same treatments were not always in the same plot position in the blocks. Complete randomization of individual seedlings and treatments was not possible due to the logistical constraints that would be involved in planting in this way, along with keeping track of individuals and with the
maintenance of treatments. Therefore, only treatment plots and species rows were randomized which resulted in a split-plot experimental design.

An addendum was made to planting protocol after the 12 primary species were planted. Because bitternut hickory (C. tomentosa) and box elder (A. negundo) were added after initial block/plot setup they are not fully replicated and have a slightly different experimental treatment allocation and distribution. To accommodate the herbivore removal treatment, every other tree in the row for these species was surrounded by a protector. Their planting rows were necessarily on the edges of the blocks and could not be randomly distributed as with the other species. Because they are planted on edge rows, the vegetation removal treatment is not fully replicated for these species, although every attempt logistically feasible was made to accommodate this treatment.
Figure 2.6: Example of block/plot layout showing dimensions, locations of treatments, and locations of seedling species rows. Also depicted are the four locations (black squares) where samples for soil, light, and surrounding vegetation were collected. This format was replicated 12 times, but one should note that treatment locations (i.e. quadrants) and species rows were randomized for each particular block.
Figure 2.7: Aerial imagery with blocks in experimental fields as referenced by onsite GPS data. Notice the savanna tract located southwest of field 1 (blocks 1-6) and the residential development that borders the Griffith Woods property to the east of field 2 (blocks 7-12) (Google Earth, 2012).
Figure 2.8: Close-up aerial photo of Field 1, with Blocks 1-6 depicted. Notice the adjacent savanna remnant to the southwest of Field 1 with the large, open spaced savanna trees. Also of note is the layout of Blocks 5 & 6 which were offset to avoid the old farm road that cuts diagonally between them running from the midway point on the northeast border to the S corner of Field 1 (Google Earth, 2012).
Data collection

Initial survival assessment of seedlings

After initial mowing, data was gathered to determine the success at establishment of planted seedlings. All trees in mowed plots were assessed for leaf/no-leaf as a correlate for survival of planting. As all trees up to this point were exposed to the same treatments (prior to mowing) only mowed plots were assessed due to ease of data gathering and the difficulty to find trees in un-mowed plots. This sampling occurred on 6/30/11.

Vegetation harvesting

Surrounding vegetation was assessed by harvesting all stems (grass and forb) in 0.25m² sampling frames at 4 sample sites per block (30’, 70’, 30’#2, 70’#2) along un-mowed center dividing row 1m in from edge (i.e. where vegetation was mowed) (See Figure 2.6). All stems within the 0.25m² (0.5m X 0.5m) frame were cut and then divided into grass or forb. Distinction between grass or forb vegetation was made by visual assessment of growth form. Vegetation
was stored in feed bags and dried at 60°C for ~1 week (10-14 days B1-B4; 7-9 days B5-B12). Vegetation was harvested between 9/12/11 and 9/17/11. Dried vegetation was weighed on 9/30/11 with the grass and forb components weighed separately. These two weights were then summed for a total vegetation biomass weight.

**Soil samples**

Soil Samples were collected between 9/12/11 and 9/17/11. Soil samples of approximately 15cmx15cmx6cm obtained in 4 sample sites per block (30’, 70’, 30’#2, 70’#2) (i.e. same sample site as harvested vegetation; see Figure 2.6) and one homogenized sample was made for each block from the 4 sample sites. Therefore a total of 5 samples were obtained per block. The homogenized sample was created by taking equal representative amounts from the 4 sample site soil samples and these were physically combined and hand mixed on site prior to soil testing in order to give a single representative sample per block. Each individual site sample was analyzed with the “Routine Soil Test” and the “Organic Matter & Nitrogen” test. The Routine Soil Test analyzes for Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg) and Zinc (Zn) using the Mehlich III extraction method and determines their amount in pounds per acre (lb/ac). It also tests for soil pH using a 1M KCl analysis as opposed to using water. The Organic Matter & Nitrogen test determines the percent composition of a soil sample for soil organic matter (SOM%) and total Nitrogen (totN%). One homogenized sample from the four sampling sites was analyzed for the “Particle Size Analysis” and “CEC Base Saturation” tests. The Particle Size Analysis test samples for percent composition of sand, silt, and clay particles and classifies the soil texture (i.e. silt-loam vs. clay-loam, etc.). The CEC Base Saturation test analyzes for base saturation percentage, cation exchange capacity (CEC) in meq/100g units, and the exchange rate of cations which include Postassium (exchK), Calcium (exchCa), Magnesium (exchMg) and Sodium (exchNa) in meq/100g units. In addition, the tests on the homogenized samples also return data for the Routing Soil Test and the Organic Matter & Nitrogen test as well. Soil samples air dried for approximately one month before analysis. The soil samples were submitted to Regulatory Services at the University of Kentucky for analysis and the nutrient assessment was made by the Lexington Soil Testing Laboratory. Their statement with regard to how they test is as follows:

“We determine soil pH in the routine soil test using 1 M KCl rather than water. For producer reports, we calculate a soil-water pH using the following equation based on analysis of 240 soil samples in March 2009: soil-water pH = 0.91 x 1 M KCl soil pH + 1.34. Soil-water pH is now considered an optional test. ”

**Light samples**

Light samples were taken at 0m, 0.5m and above vegetation (i.e. full light). Three light readings were taken per height and averaged. The light meter was allowed to stabilize for about 10 seconds, and then each data point was collected by glancing at meter, then looking away for 3 seconds before glancing again. The average of the three data points was taken as the representative data point for that location due to the innate variability of the light meter. Data
was collected at 40’, 80’, 40’#2, 80’#2 along the un-mowed center row of each block. These sample sites were 10’ from the original soil and vegetation sample sites, but these could not be reused due to the harvesting of vegetation (see Figure 2.6). The instrument used was the Licor Model LI-189 Quantum/Radiometer/Photometer. Light samples were collected on 9/24/11 during the afternoon.

Vegetation Identification and Percent cover

Field vegetation was identified and an estimate of percent cover of each species was made in a 0.25m$^2$ sampling frame at 40’, 80’, 40’#2, 80’#2 along the un-mowed center row of each block. These sample sites were shifted 10’ up from the original soil and vegetation sampling sites due to that vegetation previously being harvested (See Figure 2.6). Some species were not able to be identified and were given a morphospecies classification. Surrounding vegetation community data was collected during the period of 10/11/11 and 10/25/11.

Vegetation species diversity and evenness were calculated from the percent cover and species richness data and used for statistical analysis. Both the Shannon-Weiner Diversity Index ($H'$) and Simpson’s Diversity Index ($D$) were calculated from this data. Species evenness was calculated utilizing the Shannon-Weiner $H'$ index value.

Weather patterns over time of initial planting and study

![Mean Temperature and Precipitation over course of experiment](image)

Figure 2.10: Mean temperature and precipitation of Harrison Co., KY over 9 month timespan of experiment in 2011. Approximate location: (38.5°N, -84.35°W; elevation 200.25m/657ft). Weather data provided by KY Mesonet (2013).
Weather patterns over the course of the experimental timespan were within the averages for the Inner Bluegrass Region (IBR), however it should be noted that the cumulative precipitation for April 2011 of 28.5cm (11.2in) far exceeded the state average of 5.77cm (2.3in) for this month and additionally exceeded the average rainfall amount for Harrison Co. in April of 2010 & 2012 (7.59cm/2.99in and 7.77cm/3.06in, respectively) (Figure 2.10). The maximum summer temperature recorded in 2011 for Harrison Co. was 36.3°C (97.4°F) which is slightly lower than average maximum temperatures (37.8°C/100°F) (KY Mesonet, 2013).

**Statistical Analysis**

This experiment utilized a randomized complete block design with the species row considered as the experimental unit, therefore for survival data an average survival data point was obtained for each species row per plot. Statistical analysis was performed in JMP v10.0.0 (©SAS 2012) and all tests were considered significant at the $\alpha=0.05$ level.

**Light Data**

For Light data a two way ANOVA was performed with average $\mu$mol ($\mu$mol m$^{-2}$s$^{-1}$ is a measure of available light/photon flux) as the response variable and block as a random effect and height of measurement a fixed effect (see “Seedling Survival” subsection below for description and justification of random and fixed effects).

**Soil Data**

To analyze soil data to determine if statistical differences existed in abundances of the various soil parameters, a one-way ANOVA was performed, where block was nested within field. To accommodate the nature of percentage data as a response variable, an arcsine transformation of the square root of the percent values for Soil Organic Matter and total Nitrogen was performed (see “Seedling Survival” subsection below for description and justification for using an arcsine transformation on percentage data). If the overall ANOVA model and the individual effect were significant, a Tukey’s Honestly Significant Differences (HSD) post-hoc test was performed to determine which pairwise comparisons significantly differed. Tukey’s HSD test is the preferred method for pairwise comparisons as it controls the probability of a Type I error better than a simple Student’s t test (Quinn & Keough, 2002).

To summarize block variations in all soil parameters, the value for each block was compared to the overall mean for a given parameter, which gave a ratio for how each block varied from the mean in a given parameter. These ratios for each parameter were then averaged to see the overall variation in each block in soil parameters. By doing this it is easier to see whether each block was higher or lower than the mean in all block parameters so one can understand if the block has “rich” or “poor” soil characteristics.

A principal component analysis was performed on the primary soil nutrients to determine colinearity in these parameters and two principal component axes were generated and utilized as covariates in the statistical models to determine survival.
A paired t-test was utilized to determine if differences between the mean value of the 4 soil samples per block differed from the single homogenized soil sample per block (n=12).

**Vegetation Data**

**Vegetation Biomass:** To analyze vegetation biomass data to determine if statistical differences existed in total vegetation weight, forb weight, and grass weight, a one-way ANOVA was performed, where block was nested within field. If the overall ANOVA model and the individual effect were significant, a Tukey’s Honestly Significant Differenced (HSD) post-hoc test was performed to determine which pairwise comparisons significantly differed.

**Vegetation Community:** Species richness and percent cover data were used to calculate Shannon-Weiner Diversity index values. Again, a one-way ANOVA was performed, where block was nested within field.

**Soil influence on Vegetation:** To determine what soil factors influenced vegetation total biomass, grass biomass, and forb biomass a backwards selection stepwise linear regression model was utilized. Multiple soil factors were initially included in the model, and those that were not significant were subsequently removed until only significant predictor parameters were included. A test for normality of the predicted values and of the residuals was performed and also a test of the slope of the line through the predicted values and the residuals was performed to determine that the model was an appropriate fit.

**Seedling survival**

For seedling survival data, multi-factor mixed model ANOVA using the Restricted Maximum Likelihood (REML) method was performed with treatment and species as fixed effects and field and block as random effects. Percent survival of tree seedlings served as the response and this data was arcsine transformed as an attempt to normalize the data, but due to the high survival the data remained skewed after the transformation. An arcsine transformation is the typical method employed for percentage data where the data set has upper and lower limit boundaries (i.e. 0% and 100%). Most power transformations would have different effects on the ends of the distribution, whereas the arcsine transformation is efficient at redistributing the ends of the data similarly but has little effect on the centrally distributed data (Quinn & Keough, 2002).

Due to the nature of using both fixed and random effects it was deemed necessary to utilize a mixed model (Zar, 1999). The factors of treatment and species were considered to be fixed effects as the treatment levels (of seedling protector or no seedling protector) were imposed on the chosen set of seedling species, which again are fixed as it was determined prior to the experiment what species would be used (Quinn & Keough, 2002). Block and field were determined to be random effects as the variability in these locations could not be controlled and were not manipulated prior to experimental setup. According to Quinn & Keough (2002), “[r]andom factors in biology are often randomly chosen spatial units like sites or blocks”.

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Furthermore, the random factor nature of blocks and fields is due to the fact that these represent a subset of possible site conditions that may exist across the entire Kentucky Inner Bluegrass blue ash-oak savanna-woodland, but I would like to utilize the responses gained from this experiment to extrapolate to the entire IBR landscape (Quinn & Keough, 2002). Furthermore, if we were to set up this experiment again or repeat this experiment, the variable nature of environmental conditions could not specifically be replicated as any microsite chosen would be different from the original experiment (Quinn & Keough, 2002). The experimental design deemed it necessary to utilize a split-plot model, with the whole plot factor being the protector/no protector treatment and the split-plot factor being the species type (Figure 2.11). This design was necessary as species type was distributed between plots (i.e. “split”) but the protector treatment was applied to whole plots (Quinn & Keough, 2002).

![Figure 2.11](image)

Figure 2.11: Statistical unit for consideration in the seedling survival models. Each block was replicated 12 times, with 6 blocks each distributed into two different fields. The protector treatment represents the whole-plot factor and the species type represents the split-plot factor.

This model was reanalyzed with field and block designated as fixed effects in order to determine their potential influence on survival. This contrasts with the original analysis in that when field and block are designated as random effects, their influence of their variation on the model effects are minimized; however, in this experiment I was interested in determining if blocks did influence survival and if so, how much and in what ways.

As it was quickly seen that Ohio buckeye (*A. glabra*) was an outlier in the sense that its survival was significantly different than that of other species, both models were performed a second time without this species included. Similarly, the model was performed an additional time with just *A. glabra* survival as the response to look into finer differences with this species performance.

Finally it was determined in the fixed model that because blocks influence survival, and through previous testing that blocks significantly varied in certain environmental parameters...
(soil, light, vegetation), that block could be dropped from the model. Survival was then reanalyzed with the various environmental parameters as main effects via a linear regression analysis to determine whether they can explain the variations in survival.

In all three seedling survival models various soil, light, and vegetation biomass parameters were utilized as covariates to determine if they influence the differential response in survival. After it was determined that some do exhibit an influence, a correlation analysis between certain parameters and mean seedling survival was performed to determine the direction of influence (either positive or negative). All three models utilized Tukey’s HSD test to determine post-hoc pairwise differences.
Chapter 3: Results

Overview

In this chapter I will first present a detailed analysis of generated data, starting with the abiotic factors of light, followed by soil parameters and a soil description and how these factors varied between blocks and fields. This will be followed by a look at the surrounding vegetation biomass and community and if variations within vegetation existed between blocks and fields. Next I will look into the role of the soil parameters and their influence on the vegetation community in order to determine which parameters may have influenced vegetation. Last I will present seedling survival by utilizing three different statistical models, with emphasis on how the location (i.e. block and/or field), treatment (herbivory protector), seedling species, and environmental parameters (biotic and abiotic) may have contributed to differential survival.
Section 1: Light

Light levels varied significantly at different heights in and above vegetation (F=285.2184, df=2, p<0.0001) (Figure 3.1). As expected, above the vegetation light was most intense (1375.38 ± 60.25 µmol m⁻²s⁻¹) compared to at ground level (i.e. 0m) where light was the least intense (86.19 ± 10.34 µmol m⁻²s⁻¹). At 0.5m, roughly the height that at which most tree seedlings currently have their leaves, light levels were in between the two other measurement heights (414.48 ± 41.94 µmol m⁻²s⁻¹). When the analysis was conducted with height as the main effect, but grouped by block, the variations for each height within each block were not significant. The output from this analysis is shown in Table 3.1.

Table 3.1: Significant differences between blocks at each measured level for light.

<table>
<thead>
<tr>
<th>Height</th>
<th>F-stat</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Above</td>
<td>1.6278</td>
<td>11</td>
<td>0.1324</td>
</tr>
<tr>
<td>0.5m</td>
<td>1.8727</td>
<td>11</td>
<td>0.0772</td>
</tr>
<tr>
<td>0m</td>
<td>1.5199</td>
<td>11</td>
<td>0.1671</td>
</tr>
</tbody>
</table>
As shown before, light varied significantly between heights in and above surrounding vegetation. However, light levels within each height did not vary significantly between blocks (0m: p=0.1671; 0.5m: p=0.0772; above: p=0.1324), indicating light levels between blocks were consistent. Although there were some variations in light levels above vegetation, this is probably due to measurements being collected on a partly cloudy day and sun flecks potentially added variability to this level. Variations in the 0.5m level, although not significant, were potentially due to variations in density of surrounding vegetation. How this may have influenced seedling survival will be addressed later in this paper. Within each block light levels did continue to vary significantly between heights, although in some instances light did not vary significantly between the 0m and 0.5m levels. This is detailed in Table 3.1.

Figure 3.2: Mean light intensity (µmol m⁻²s⁻¹) at three different heights in and above vegetation in 12 different blocks. Light varied significantly by height as seen in Fig 3.2, but light did not significantly differ in the three different height levels between blocks.

As can be seen in Figure 3.2, light was dramatically reduced by the diffusive filtering effects of the surrounding vegetation. The majority of filtering occurred between the “above” vegetation level to the 0.5m level, which is roughly the height of the growing tree seedlings. This helps to demonstrate the differences in growing environment between vegetated and unvegetated experimental units. However, up until the assessment of survival for this paper all seedlings were growing in a reduced light, competitive environment. What should also be noted
is that although light was further reduced from the 0.5m to 0m level, the reduction was
generally the same percent wise as the original reduction from above to 0.5m (Figure 3.3);
however, by reducing the light further, only about 10% of the original amount of light remains,
as can be seen in Figure 3.4.

Figure 3.3: Percent reduction in light intensity at three different heights in 12 different
experimental blocks. Note that although each block varied in the above light level, this was
standardized by setting the above level to 100% and reductions in light were compared to the
absolute value.
Figure 3.4: Percentage light was diffused by filtering through vegetation. Notice that the light levels were reduced about 85-95% from above vegetation to ground level (i.e. 0m), showing that surrounding vegetation can reduce available light. However, for tree seedlings growing at approximately 0.5m, the reduction in light is still large, between 50-90%, but this is variable showing that although a competitive environment exists for light in all blocks, blocks can vary in the amount of light available to seedlings.

When looking at the percentage light is reduced by surrounding vegetation it becomes apparent that light levels are changed when moving from the open, above environment to that of the ground level, 0m environment. Light is reduced by an average of %93.5±0.01 when moving from the above level to the 0m level. This helps to show how growing environment is changed and the competition for light can be strong underneath the vegetation. How this may correlate with surrounding vegetation biomass and the influence it has on seedling survival will be assessed later in the paper. Also of interest is that from above vegetation to the 0.5m level, light is reduced by %69.4±0.03, but then when going from the 0.5m level to the ground level it is further reduced by another %77.8±0.02. The levels of light at 0.5m roughly correspond to the light environment experienced by the tree seedlings as they are all approximately within this height during this growth stage.
Section 2: Soils

Differences in soil parameters in blocks and fields

Experimental blocks were shown to significantly differ in the soil parameters of Phosphorus (P), Calcium (Ca), Magnesium (Mg), Zinc (Zn), Soil Organic Matter percent (SOM%), Total Nitrogen percent (totN%), and pH, but blocks did not statistically differ in Potassium (K). Experimental fields were shown to significantly differ in Potassium (K), Ca, Mg, Zn, SOM%, Total N%, and pH, but did not statistically differ in Phosphorus (P). All differences were significant at the $\alpha=0.05$ level and can be seen in Figures 3.5-3.12.

Figure 3.5: Soil Phosphorus (P) concentrations (lb/ac) in different blocks and fields. Phosphorus varied significantly between blocks ($F=9.7719$, df=10, $p<0.0001$) but not between fields ($F=1.4264$, df=1, $p=0.2402$). Note that data for field 1 is the mean from blocks 1-6 and data for field 2 is the mean from blocks 7-12.
Figure 3.6: Soil Potassium (K) concentrations (lb/ac) in different blocks and fields. Potassium did not vary significantly between blocks (F=0.3718, df=10, p=0.9510) but it did significantly differ between mean field concentrations (F=9.7255, df=1, p=0.0036). Note that data for field 1 is the mean from blocks 1-6 and data for field 2 is the mean from blocks 7-12.

Figure 3.7: Soil Calcium (Ca) concentrations in different blocks and fields. Calcium varied significantly between blocks (F=2.5019, df=10, p=0.0214) and also between fields (F=7.8141, df=1, p=0.0083). Note that data for field 1 is the mean from blocks 1-6 and data for field 2 is the mean from blocks 7-12.
Figure 3.8: Soil Magnesium (Mg) concentrations (lb/ac) in different blocks and fields. Magnesium varied significantly between blocks ($F=2.8537$, $df=10$, $p=0.0101$) and also between fields ($F=12.9747$, $df=1$, $p=0.0009$). Note that data for field 1 is the mean from blocks 1-6 and data for field 2 is the mean from blocks 7-12.

Figure 3.9: Soil Zinc (Zn) concentrations (lb/ac) in different blocks and fields. Zinc varied significantly between blocks ($F=2.3490$, $df=10$, $p<0.0297$) and also between fields ($F=20.8797$, $df=1$, $p<0.0009$). Note that data for field 1 is the mean from blocks 1-6 and data for field 2 is the mean from blocks 7-12.
Figure 3.10: Percentage of soil organic matter (SOM%) in different blocks and fields. SOM% varied significantly between blocks ($F=2.6396$, df=10, $p=0.0159$) and also between fields ($F=10.6003$, df=1, $p=0.0025$). Note that data for field 1 is the mean from blocks 1-6 and data for field 2 is the mean from blocks 7-12. Also note that although the overall ANOVA test and the individual effects test showed significant differences between blocks, the post-hoc Tukey’s HSD test could not determine significant differences between blocks. A student t’s post-hoc test did reveal significant differences in the pairwise comparisons, but these results are not reported as that diverges from the standard protocol used and it is not as conservative of a test. Although, by looking at the distribution of means and standard error’s it is probably safe to assume that there are differences that exist between blocks. Also of note is that graphically I have portrayed the percent concentrations of SOM in the blocks, but analyses were conducted on arcsine transformed proportion data. Equivalent analysis was performed on the percentage data, and although the $p$ values were slightly higher, they were still significant (Block: $p=0.0205$; Field: $p=0.003$)
Figure 3.11: Percentage of total Nitrogen (N%) in different blocks and fields. Total N% varied significantly between blocks (F=2.9499, df=10, p=0.0083) and also between fields (F=18.8785, df=1, p<0.0001). Note that data for field 1 is the mean from blocks 1-6 and data for field 2 is the mean from blocks 7-12. Also note that graphically the percent concentrations of N in the blocks are depicted, but analyses were conducted on arcsine transformed proportion data. Equivalent analysis was performed on the percentage data, and they were still highly significant (Block: p=0.0002; Field: p=0.0131).
As can be seen in Figures 3.5-3.12, the different blocks and fields varied significantly in most important soil nutrients. Of most significance is the overall trend of field 1 having higher available nutrients and field 2 having lower soil nutrients overall. This trend will be explored further to see if variations in blocks and fields related to variations in vegetation biomass and seedling survival.
Figure 3.13: Average deviation from mean in 5 soil nutrients in 12 different blocks.

Figure 3.14: Average deviation from mean in 8 soil parameters in 12 different blocks.
Figures 3.13 & 3.14 summarize the variation in blocks in the multiple soil parameters that were monitored. Each block was compared against the mean in a certain soil parameter, and the ratio for each block was then averaged. These graphs then basically show, when compared to other blocks at this site, whether they were below or above average in all abiotic soil parameters measured. Looking at the data this way one notices first that Block 1 was richest on average in all soil parameters, but Block 10 was the poorest. The other main trend to notice is that all of the blocks in Field 2 (i.e. B7-B12) were all below average in mean soil parameters (excluding B7 which was about average), but only one block, B4, was below average in Field 1. This further illustrates the potential growing environment differences between the two fields, in addition to the variations in growing environments between blocks.

Table 3.2: Mean Cation Exchange Capacity (CEC) and exchange potential for various elements of homogenized soil samples.

<table>
<thead>
<tr>
<th></th>
<th>mean</th>
<th>se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cation Exchange Capacity (CEC) (meq/100g)</td>
<td>18.79583</td>
<td>0.480622</td>
</tr>
<tr>
<td>Exchange K (meq/100g)</td>
<td>0.286667</td>
<td>0.007521</td>
</tr>
<tr>
<td>Exchange Ca (meq/100g)</td>
<td>7.668333</td>
<td>0.239336</td>
</tr>
<tr>
<td>Exchange Mg (meq/100g)</td>
<td>1.033333</td>
<td>0.03412</td>
</tr>
<tr>
<td>Exchange Na (meq/100g)</td>
<td>0.164167</td>
<td>0.023851</td>
</tr>
<tr>
<td>Base Saturation (%)</td>
<td>48.84</td>
<td>1.184637</td>
</tr>
</tbody>
</table>

Table 3.2 above is used for descriptive purposes due to the sampling procedure preventing any statistical analysis. Of note is the high available exchange rate of Calcium compared to the other cations, a nutrient that is commonly poor in IBR soils, especially the Faywood and Loradale soils found at Griffith Woods, and has necessitated the addition of lime and potash to most agricultural soils in the area (USDA-SCS, 1968).
In Figure 3.15 we can see the distribution of the various particle sizes from the homogenized soil samples. This data help to confirm that the soil type is a silt-loam with approximately 70% of the soil dominated by silt particles, and roughly even distribution of the remaining sand and clay particles. As described earlier in this paper, the silt-loams of the IBR are deep and well drained.

Table 3.3: Comparison of mean values for soil parameters using two different soil sampling techniques. All means are in lb/ac units except for pH. (n=12)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>se</th>
<th>mean diff</th>
<th>1 tail paired t</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>4 separate mean 33.896</td>
<td>3.51</td>
<td>2.229</td>
<td>p=0.009</td>
</tr>
<tr>
<td></td>
<td>Homogenized 31.667</td>
<td>3.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>4 separate mean 185.125</td>
<td>6.40</td>
<td>10.375</td>
<td>p=0.0115</td>
</tr>
<tr>
<td></td>
<td>Homogenized 195.500</td>
<td>5.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>4 separate mean 2989.417</td>
<td>96.67</td>
<td>88.167</td>
<td>p=0.0121</td>
</tr>
<tr>
<td></td>
<td>Homogenized 3077.583</td>
<td>94.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>4 separate mean 243.646</td>
<td>8.08</td>
<td>9.688</td>
<td>p=0.0012</td>
</tr>
<tr>
<td></td>
<td>Homogenized 253.333</td>
<td>7.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>4 separate mean 2.265</td>
<td>0.11</td>
<td>0.060</td>
<td>p=0.0962</td>
</tr>
<tr>
<td></td>
<td>Homogenized 2.325</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>4 separate mean 4.470</td>
<td>0.04</td>
<td>0.030</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Homogenized 4.500</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3.3 shows the mean response for six different monitored soil parameters from the experimental blocks at Griffith Woods. This table compares the calculated average value for four different soil samples as opposed to the “in the field” homogenized soil sample. In most instances the homogenized sample estimated the response higher than the average for each separate sample. This is not the case for phosphorus (P) and pH, where the homogenized sample was lower in its value than the average of the 4 separate soil samples. All showed significant differences based on a paired t-test. In other words, the homogenized soil sample was usually statistically different than that of the 4 separate samples, and in most cases the homogenized sample was larger. The only value that was not statistically different was Zinc (Zn), but it should be noted that 7 of the homogenized data points were larger than their corresponding 4 separate sample data points.

**Soil Principal Components**

A principal component analysis was conducted to determine if colinearity existed between any of the main soil parameters of interest (P, K, pH, Ca, Mg, Zn, SOM%, TotalN%) and the calculated principal axes were then utilized as covariates in the analyses of models (1), (2), & (3) in Section 5 of this chapter. Output from that analysis is seen in Table 3.4.

Table 3.4: Loading matrix of Principal Component Analysis (PCA) of soil parameters.

<table>
<thead>
<tr>
<th></th>
<th>Prin1</th>
<th>Prin2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MehP(lb/ac)</td>
<td>0.41013</td>
<td>-0.70565</td>
</tr>
<tr>
<td>MehK(lb/ac)</td>
<td>0.69808</td>
<td>0.03052</td>
</tr>
<tr>
<td>pH</td>
<td>0.69763</td>
<td>0.60494</td>
</tr>
<tr>
<td>BufpH</td>
<td>0.34215</td>
<td>0.85820</td>
</tr>
<tr>
<td>MehCa(lb/ac)</td>
<td>0.39082</td>
<td>0.71100</td>
</tr>
<tr>
<td>MehMg(lb/ac)</td>
<td>0.83831</td>
<td>0.11361</td>
</tr>
<tr>
<td>MehZn(lb/ac)</td>
<td>0.85585</td>
<td>-0.12459</td>
</tr>
<tr>
<td>SOM%</td>
<td>0.76058</td>
<td>-0.46428</td>
</tr>
<tr>
<td>Tot_N%</td>
<td>0.78561</td>
<td>-0.45943</td>
</tr>
</tbody>
</table>

As can be seen in Table 3.4 of the PCA loading matrix table, the calculated axes of PC1 is primarily explained by K, pH, Mg, Zn, SOM%, and Total N%, all with loading matrix values of ±0.69 or greater. The PC1 axis explains 44.9% of the data. Next, PC2 is primarily explained by P, Buffered pH, and Ca all with loading matrix values of ±0.70 or greater and the PC2 axis further explains 28.5% of the data. With that said, PC1 and PC2 together explain 73.4% of the soil data.
Section 3: Surrounding Vegetation

Figure 3.16: Surrounding vegetation biomass in various blocks and fields showing variations in grass, forb, and total vegetation biomass. Differences in blocks and fields were not significant. Only a significant difference in amount of forb biomass when comparing fields was observed, but note that the error bars for field refer to the total biomass mean standard error (F=4.4915, df=1, p=0.041).

Although vegetation biomass did vary from block to block, overall the total weights did not significantly differ (F=0.9567, df=12, p=.4961). Additionally, biomass of forb or grass vegetation did not statistically differ between blocks (Forb: F=1.6134, df=10, p=.1422; Grass: F=1.3810, df=10, p=.2282). Total vegetation biomass did not vary significantly for fields (f=0.5486, df=1, p=.4637) and neither for grass (F=.163, df=1, p=0.6888), but a significant difference did appear in forb biomass between the two fields (F=4.4915, df=1, p=0.041) (Figure 3.16).
Community indices of Shannon-Weiner $H'$ diversity, species Evenness, and Simpson’s D diversity did not show significant differences between blocks or fields. However, species richness did significantly differ between blocks ($F=2.4713$, $df=10$, $p=0.0228$) which can be seen in Figure 3.18, but differences did not exist between fields ($F=0.1888$, $dr=1$, $p=0.667$). One thing to note is that Simpson’s D diversity showed a trend toward significance between blocks, with $p=0.0866$ suggesting that additional sampling should be made about diversity differences (Table 3.6).
Table 3.5: Summary data for species richness, evenness and diversity values.

<table>
<thead>
<tr>
<th>Block</th>
<th>Species Richness</th>
<th>se</th>
<th>H' diversity</th>
<th>se</th>
<th>Species Evenness</th>
<th>se</th>
<th>Simpson's D diversity</th>
<th>se</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>4.75</td>
<td>1.031</td>
<td>1.105</td>
<td>0.379</td>
<td>0.694</td>
<td>0.213</td>
<td>0.626</td>
<td>0.102</td>
</tr>
<tr>
<td>B2</td>
<td>3.25</td>
<td>0.479</td>
<td>0.846</td>
<td>0.123</td>
<td>0.763</td>
<td>0.085</td>
<td>0.486</td>
<td>0.065</td>
</tr>
<tr>
<td>B3</td>
<td>3.25</td>
<td>0.250</td>
<td>0.956</td>
<td>0.092</td>
<td>0.819</td>
<td>0.075</td>
<td>0.557</td>
<td>0.062</td>
</tr>
<tr>
<td>B4</td>
<td>3.25</td>
<td>0.629</td>
<td>1.020</td>
<td>0.189</td>
<td>0.905</td>
<td>0.038</td>
<td>0.590</td>
<td>0.066</td>
</tr>
<tr>
<td>B5</td>
<td>5.75</td>
<td>0.479</td>
<td>1.505</td>
<td>0.091</td>
<td>0.864</td>
<td>0.018</td>
<td>0.739</td>
<td>0.022</td>
</tr>
<tr>
<td>B6</td>
<td>4</td>
<td>0.408</td>
<td>1.287</td>
<td>0.098</td>
<td>0.939</td>
<td>0.008</td>
<td>0.700</td>
<td>0.029</td>
</tr>
<tr>
<td>B7</td>
<td>4.75</td>
<td>0.250</td>
<td>1.355</td>
<td>0.138</td>
<td>0.865</td>
<td>0.062</td>
<td>0.686</td>
<td>0.069</td>
</tr>
<tr>
<td>B8</td>
<td>4.5</td>
<td>0.289</td>
<td>1.202</td>
<td>0.097</td>
<td>0.799</td>
<td>0.040</td>
<td>0.636</td>
<td>0.053</td>
</tr>
<tr>
<td>B9</td>
<td>4.25</td>
<td>0.479</td>
<td>1.221</td>
<td>0.128</td>
<td>0.857</td>
<td>0.054</td>
<td>0.659</td>
<td>0.049</td>
</tr>
<tr>
<td>B10</td>
<td>3.75</td>
<td>0.479</td>
<td>1.077</td>
<td>0.162</td>
<td>0.822</td>
<td>0.083</td>
<td>0.585</td>
<td>0.083</td>
</tr>
<tr>
<td>B11</td>
<td>3.75</td>
<td>0.250</td>
<td>0.935</td>
<td>0.106</td>
<td>0.713</td>
<td>0.070</td>
<td>0.505</td>
<td>0.065</td>
</tr>
<tr>
<td>B12</td>
<td>4</td>
<td>0.408</td>
<td>1.290</td>
<td>0.079</td>
<td>0.945</td>
<td>0.017</td>
<td>0.704</td>
<td>0.017</td>
</tr>
</tbody>
</table>

As can be noted in Table 3.5, variations existed in species richness, but there were very low variations in H' diversity, species evenness and Simpson's D diversity values. Overall block 5 had the highest species richness and H' diversity and D diversity, however, block 6 showed the highest species evenness values. Of note is that only species richness is significantly different and can be better seen in Figure 3.17. ANOVA output on vegetation community parameters can be seen in Table 3.6 showing that only species richness differed significantly (p=0.0228) at the field level and forb biomass differed significantly at the field level (p=0.041).

Table 3.6: ANOVA output for vegetation biomass and community data. Note that block was nested within field for this analysis. Significant differences are denoted by an asterisk (*0).

<table>
<thead>
<tr>
<th>Block, Field</th>
<th>F</th>
<th>df</th>
<th>p</th>
<th>F</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forb(g)</td>
<td>1.6134</td>
<td>10</td>
<td>0.1422</td>
<td>4.4915</td>
<td>1</td>
<td>*0.041</td>
</tr>
<tr>
<td>Grass(g)</td>
<td>1.381</td>
<td>10</td>
<td>0.2282</td>
<td>0.163</td>
<td>1</td>
<td>0.6888</td>
</tr>
<tr>
<td>Total(g)</td>
<td>0.9567</td>
<td>10</td>
<td>0.4961</td>
<td>0.5486</td>
<td>1</td>
<td>0.4637</td>
</tr>
<tr>
<td>Species richness</td>
<td>2.4713</td>
<td>10</td>
<td>*0.0228</td>
<td>0.1888</td>
<td>1</td>
<td>0.6665</td>
</tr>
<tr>
<td>Species evenness</td>
<td>1.0683</td>
<td>10</td>
<td>0.4107</td>
<td>0.0027</td>
<td>1</td>
<td>0.9585</td>
</tr>
<tr>
<td>H' diversity</td>
<td>1.5632</td>
<td>10</td>
<td>0.1578</td>
<td>0.41493</td>
<td>1</td>
<td>0.5214</td>
</tr>
<tr>
<td>Simpson's diversity</td>
<td>1.8496</td>
<td>10</td>
<td>0.0866</td>
<td>0.13</td>
<td>1</td>
<td>0.7205</td>
</tr>
</tbody>
</table>
Table 3.7: Grass and forb species occupying various blocks at Griffith Woods, KY. Classification was made via a morphospecies description and should not be considered an exhaustive survey of vegetation. This table displays all the different species identified in each block, but does not take into account their relative abundances and ranking is not in any particular order.

<table>
<thead>
<tr>
<th>Block</th>
<th>Species</th>
<th>Species</th>
<th>Species</th>
<th>Species</th>
<th>Species</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>Goldenrod</td>
<td>Aster</td>
<td>Aster</td>
<td>Aster</td>
<td>Aster</td>
<td>Goldenrod</td>
</tr>
<tr>
<td></td>
<td>Aster</td>
<td>Indian Grass</td>
<td>Indian Grass</td>
<td>Indian Grass</td>
<td>Goldenrod</td>
<td>Aster</td>
</tr>
<tr>
<td></td>
<td>Bee Balm</td>
<td>Switchgrass</td>
<td>Goldenrod</td>
<td>Goldenrod</td>
<td>Indian Grass</td>
<td>Switchgrass</td>
</tr>
<tr>
<td></td>
<td>Indian Grass</td>
<td>Sunflower #1</td>
<td>Switchgrass</td>
<td>Switchgrass</td>
<td>Flower #1</td>
<td>Bee Balm</td>
</tr>
<tr>
<td>Coneflower</td>
<td>Goldenrod</td>
<td>Bee Balm</td>
<td>Bee Balm</td>
<td>Switchgrass</td>
<td>Flower #1</td>
<td>Aster</td>
</tr>
<tr>
<td></td>
<td>Flower #1</td>
<td>Aster</td>
<td>Indian Grass</td>
<td>Ragweed</td>
<td>Coneflower</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Switchgrass</td>
<td>Big Bluestem</td>
<td>Ragweed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B7</td>
<td>Goldenrod</td>
<td>Goldenrod</td>
<td>Aster</td>
<td>Aster</td>
<td>Indian Grass</td>
<td>Indian Grass</td>
</tr>
<tr>
<td></td>
<td>Sunflower #1</td>
<td>Indian Grass</td>
<td>Goldenrod</td>
<td>Goldenrod</td>
<td>Switchgrass</td>
<td>Switchgrass</td>
</tr>
<tr>
<td></td>
<td>Aster</td>
<td>Switchgrass</td>
<td>Switchgrass</td>
<td>Indian Grass</td>
<td>Bee Balm</td>
<td>Coneflower</td>
</tr>
<tr>
<td></td>
<td>Bee Balm</td>
<td>Aster</td>
<td>Indian Grass</td>
<td>Switchgrass</td>
<td>Wheat</td>
<td>Aster</td>
</tr>
<tr>
<td></td>
<td>Switchgrass</td>
<td>Sunflower #1</td>
<td>Sunflower #1</td>
<td>Bee Balm</td>
<td>Aster</td>
<td>Wheat</td>
</tr>
<tr>
<td></td>
<td>Coneflower</td>
<td>Coneflower</td>
<td>Goldenrod</td>
<td>Goldenrod</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Indian Grass</td>
<td>Big Bluestem</td>
<td>Bee Balm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As can be seen in Table 3.7, the species represented in the various blocks at Griffith Woods were fairly similar across blocks and only slight variations were noticed. As stated before, diversity values were not significantly different. The only significant differences noted were in species richness with Block 5 having the highest mean species richness (Table 3.6). This differs from the block with the highest absolute species richness, which was Block 1 with 10 different species ultimately identified.

Section 4: Soil influences on vegetation

To determine if soil parameters influence total vegetation biomass, grass biomass, and forb biomass a backwards selection stepwise linear regression model was constructed. Initially the parameters of P, K, pH, Ca, Mg, Zn, SOM%, Total N%, 0m light, 0.5m light, and Above light
were included in the model. The non-significant predictor variables were dropped until only significant variables were included in the model. For total vegetation biomass, after dropping the non-significant predictor variables a model with just pH (p=0.0099), Mg (p=0.028), and Ca (p=0.1055) remained to explain total surrounding vegetation biomass, which is displayed in Figure 3.18. The overall model was significant (p=0.0442) but only if you left in the three soil parameters even though Ca was not significant. If Ca was dropped, the overall model lost its significance. The residuals and the predicted values were normally distributed and the slope of the line between the residuals and the predicted values was equal to zero, which meant this met the assumption of the linear regression model. However, the one critique of this model is that the $r^2$ value is very low ($r^2=0.17$). These variables left a linear regression equation of:

$$ (Eq1) \quad \text{Total Vegetation Biomass}= -829.68 + 371.2 \times \text{pH} - 1.328 \times \text{Mg} - 0.0779 \times \text{Ca} $$

For grass biomass, once non-significant variables were dropped it left a significant model (p=0.0001) with just pH (p=0.0039), Mg (p=0.0010), and Above light (p=0.0021). Ca was dropped from this model even though borderline significant (p=0.0844), but unlike for total vegetation biomass dropping Ca did not reduce the significance of the overall model. As before, this model met the assumption of normality and the $r^2$ value was much higher in this iteration ($r^2=0.37$). Figure 3.19 shows the linear regression plot and the predicted equation is listed below:

$$ (Eq2) \quad \text{Grass Biomass}= -1163.337 + 366.79 \times \text{pH} - 2.153 \times \text{Mg} + 0.142 \times \text{Above Light} $$

Figure 3.18: Total vegetation biomass explained by the soil variables of pH, Mg, and Ca. Each point is 1 of 4 sample points per block (n=48).

For grass biomass, once non-significant variables were dropped it left a significant model (p=0.0001) with just pH (p=0.0039), Mg (p=0.0010), and Above light (p=0.0021). Ca was dropped from this model even though borderline significant (p=0.0844), but unlike for total vegetation biomass dropping Ca did not reduce the significance of the overall model. As before, this model met the assumption of normality and the $r^2$ value was much higher in this iteration ($r^2=0.37$). Figure 3.19 shows the linear regression plot and the predicted equation is listed below:

$$ (Eq2) \quad \text{Grass Biomass}= -1163.337 + 366.79 \times \text{pH} - 2.153 \times \text{Mg} + 0.142 \times \text{Above Light} $$
For forb biomass, the only model with just significant predictor variables left just “Above light” (p=0.0028), however the $r^2$ value for this model was rather low ($r^2=0.178$). Instead it was determined that the best model (p=0.0005, $r^2=0.42$) contained K (p=0.064), pH (p=0.0202), Mg (p=0.0695), Zn (p=0.0226), 0.5m light (p=0.0369), and above light (p=0.0054). A critique is that this model included two slightly less than significant variables (K & Mg). Residuals met the assumption of normality, however, the predicted values were slightly off of a normal distribution but when these were plotted against each other the mean of the line was equal to zero, so for the most part linear regression assumption were met. The graph for forb biomass can be seen in Figure 3.20 and the equation is as follows:

\[
\text{Forb biomass} = 724.75 + -0.59\times K + -154.96\times \text{pH} + 0.775\times \text{Mg} + 62.17\times \text{Zn} + -0.075\times 0.5\text{mLight} + -0.068\times \text{Above Light}
\]
Section 5: Seedling survival

To test for differences in species survival, first a linear mixed model was utilized. In the first iteration of the model, the most conservative version was analyzed (1). It is as follows:

1. Survival= Field&Random + Block[Field]&Random + Treatment + Species + Field&Random*Species + Block[Field]&Random*Species + Treatment*Species

Notation for this and subsequent equations are as follows: “&Random” denotes a random effect (terms without this notation are considered fixed effects), “Block[Field]” specifies that experimental blocks are nested within fields, and an asterisk (*) symbol specifies an interaction term.

Figure 3.21: Seedling survival for 12 species of the Inner Bluegrass Region. Treatment effect (i.e. protection from herbivory) was not significant [F=2.3895, df=1, p=0.1246], but Species [F=14.7529, df=11, p<0.0001] and Treatment*Species [F=2.3, df=11, p=0.0131] were significant effects on seedling survival. This model could not test for Field or Block effects.

As one can see in Figure 3.21, the treatment of herbivory protection did not influence initial survival (p=0.1246). However, species was a significant effect (p<0.0001) and there was a significant treatment by species interaction (p=0.0131) indicating that survival was slightly different for certain species depending on treatment. Overall however it can be noticed that survival was high for all species, with white ash having 100% survival, and the protector & herbicide treatment for bur oak having 100% survival. All other species and treatment combinations had >90% survival, except for blue ash herbicide (i.e. no protector) having 89.2%
survival and Ohio buckeye having the lowest overall survival, regardless of treatment. In fact, Ohio buckeye survival was the overall poorest, with the herbicide (i.e., no protector) treatment the lowest at 58.3% and the protector & herbicide treatment the second lowest at 67.5%.

Multiple covariates were then tested in this model across the range of soil, light, and vegetation parameters and none were significant in explaining overall species survival. However, it should be noted that the covariates of homogenized pH and homogenized Zn removed the significant species effect of survival, raising the p-value to 0.7801 and 0.0591, respectively. Also of note is the borderline significant covariate of clay particle percentage at $p=0.0639$. Furthermore, this model was reanalyzed with shade tolerance category as a main effect instead of species, and shade tolerance also significantly influenced survival ($p=0.0369$) with those species categorized as “shade tolerant” having lower survival than those categorized as “intermediate or shade intolerant”.

Because Ohio buckeye seemed to be the main driver of survival variation in model (1), the data were reanalyzed removing this species from the data set. The base model (1) without any covariates or Ohio buckeye’s survival response caused species to no longer be a significant main effect ($p=0.0923$), implying that Ohio buckeye is the main driver in the species response of survival. However, the treatment by species interaction is still significant ($p=0.0159$) showing that variations still exist in how each individual responded in survival based on treatment. Treatment was still not significant ($p=0.3374$). Additionally, when Ohio buckeye is removed there appear to be no covariates that are significant or any covariate that caused species to become a significant effect. What is of interest, is that when buckeye is removed and shade tolerance category replaces species as a main effect, then shade tolerance category is no longer a significant effect on survival ($p=0.4430$). The explanation for this would be that the Ohio buckeye’s shade tolerance category (shade tolerant) was the main driver for this being a significant effect.

To understand the particular effect of Ohio buckeye further, model (1) was analyzed only on this species’ survival data. In this model, species is no longer used as a main effect since only one species (Ohio buckeye) is analyzed. In this single species iteration of model (1) treatment is not a significant effect ($p=0.1088$), unlike in the full model (1) where there did appear to be a difference between the two treatments for Ohio buckeye which indicated the greatest species*treatment interaction. This suggests another factor must be driving the survival differences in Ohio buckeye other than treatment alone. Surprisingly though none of the multiple environmental covariates tested in the Ohio buckeye model (1) were significant effects.

To understand the influence of a block or field effect on explaining variations in survival, a second model was analyzed but this time block and field were considered fixed effects to determine their influence on survival. The model tested was as follows:

\[
(2) \quad \text{Survival} = \text{Field} + \text{Block}[\text{Field}] + \text{Treatment} + \text{Species} + \text{Field}*[\text{Species} + \text{Block}[\text{Field}] * \text{Species} + \text{Treatment} * \text{Species}
\]
In this model Field was a significant effect (p=0.0002), with higher overall survival in field 1. Block was also a significant effect (p=0.0046), with differential survival in the various blocks. These results can be seen in Figure 3.22. Treatment was still not significant (p=0.1246) and species was still significant (p<0.0001), so the results of model (1) can still be considered as the best explanation for the species effect. There were also significant interactions, with Field*species (p=0.0133), Block*species (p=0.0005), and Treatment*Species (p=0.0131) all being significant in their explanation of survival. What is of note is that the p-values of treatment, species, and Treatment*Species are the exact same as in model (1). Unfortunately, in model (2) covariates were untestable due to the degrees of freedom being used up when block and field are considered fixed effects.

Figure 3.22: Mean survival of seedlings in blocks and fields regardless of species or treatment. These are results from model (2) where Block [F=2.7135, df=10, p=0.0046] and Field [F=214.3246, df=1, p=0.0002] were significant effects on overall survival.

Model (2) was tested a second time, with the removal of Ohio buckeye. In this iteration some interesting differences from model (1) without buckeye appeared. As in the original model (2), field was a significant effect (p=0.0005) and treatment was not a significant effect (p=0.3374). However, block is no longer a significant effect (p=0.0925) but species remains a significant effect (p<0.0001). The various interactions remained significant [field*species (p=0.0059); Block*species (p=0.0028); treatment*species (0.0159)]. This shows that buckeye may be driving the block differences, but not the species differences. This is an opposite result from model (1) where buckeye was driving the species difference. As before, model (2) was tested on the Ohio buckeye survival response alone, and this shows an interesting relation with field (p=0.2176) and treatment (p=0.1088) not being significant, but block remaining significant (p=0.0364). This further supports the notion that in model (2) Ohio buckeye was driving the significant differences in blocks, but not in fields. Similar as before, model (2) did not allow an analysis with covariates due to a loss of degrees of freedom.
Because it has been demonstrated that blocks are a significant effect on survival in model (2) and that blocks differed significantly in various soil, light, and vegetation parameters (See sections 1, 2, & 3 of this chapter) a third model was constructed that no longer utilizes field or block as main fixed effects. Instead various environmental parameters are incorporated as main effects. The third model is as follows:

\[
\text{Survival} = \text{Treatment} + \text{Species} + \text{Treatment}^*\text{Species} + \text{Covariate} + \text{Covariate}^*\text{Species}
\]

When this analysis was utilized, as before treatment \((p=0.2086)\) was not significant and the species effect was highly significant \((p<0.0001)\). However, in model (3) most treatment*species interactions were not significant \((p=0.1211)\) but this did change with some of the covariate main effects. A summary of main effect parameters is presented in Table 3.7.

Table 3.8: Environmental covariates utilized as main effects in model (3). Emphasis has been placed on covariates that were significant or trending (*). If a treatment*species interaction was significant it has been detailed.

<table>
<thead>
<tr>
<th>Effect</th>
<th>F statistic</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>9.3168</td>
<td>1</td>
<td>0.0025</td>
</tr>
<tr>
<td>pH Homogenized</td>
<td>8.3471</td>
<td>1</td>
<td>0.0042</td>
</tr>
<tr>
<td>Buffered pH</td>
<td>5.6743</td>
<td>1</td>
<td>0.0180</td>
</tr>
<tr>
<td>Buffered pH Homogenized</td>
<td>5.7652</td>
<td>1</td>
<td>0.0171</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>7.5020</td>
<td>1</td>
<td>0.0066</td>
</tr>
<tr>
<td>Mg Homogenized</td>
<td>4.0955</td>
<td>1</td>
<td>0.0441</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>15.2136</td>
<td>1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Zn*species</td>
<td>2.0897</td>
<td>11</td>
<td>0.0215</td>
</tr>
<tr>
<td>Zn Homogenized</td>
<td>11.1685</td>
<td>1</td>
<td>0.0010</td>
</tr>
<tr>
<td>Zn Hom*Species</td>
<td>1.8769</td>
<td>11</td>
<td>0.0427</td>
</tr>
<tr>
<td>Clay %</td>
<td>9.1647</td>
<td>1</td>
<td>0.0027</td>
</tr>
<tr>
<td>Clay%*species</td>
<td>2.2137</td>
<td>11</td>
<td>0.0143</td>
</tr>
<tr>
<td><em><strong>CEC</strong></em></td>
<td>3.8396</td>
<td>1</td>
<td>*0.0512</td>
</tr>
<tr>
<td>Base Saturation</td>
<td>7.1108</td>
<td>1</td>
<td>0.0082</td>
</tr>
<tr>
<td>Base Sat*Species</td>
<td>1.9948</td>
<td>11</td>
<td>0.0294</td>
</tr>
<tr>
<td>Forb(g)</td>
<td>4.0341</td>
<td>1</td>
<td>0.0457</td>
</tr>
<tr>
<td>Forb(g)*Species</td>
<td>2.0812</td>
<td>11</td>
<td>0.0222</td>
</tr>
<tr>
<td>Total Vegetation Weight(g)</td>
<td>4.5261</td>
<td>1</td>
<td>0.0344</td>
</tr>
<tr>
<td>PC1</td>
<td>7.5333</td>
<td>1</td>
<td>0.0065</td>
</tr>
</tbody>
</table>

As you can see in Table 3.8 multiple parameters were significantly associated with species survival, and some parameters had an interaction with species. Most notably, pH homogenized and Zinc homogenized appeared as significant effects in model (3), but were also significant effects in model (1) indicating possibly that these are some of the more important
soil parameters in influencing seedling survival. Also of note is clay percentage which was borderline significant in model (1), which is significant in model (3) as well.

Because Ohio buckeye has been previously shown to influence the outcomes of models (1) & (2), these data points were again removed and model (3) was retested. For the most part the same parameters that influenced survival in the first iteration of model (3) were again significant. The outcome of this test is presented in Table 3.9.

Table 3.9: Environmental covariates that influenced seedling survival in model (3). Note that these results were obtained without including Ohio buckeye data points. Emphasis has been placed on those that were significant or trending(*) and if a treatment*species interaction was significant it has been detailed.

<table>
<thead>
<tr>
<th>Effect</th>
<th>F statistic</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium (K)</td>
<td>4.2240</td>
<td>1</td>
<td>0.0410</td>
</tr>
<tr>
<td>pH</td>
<td>9.1175</td>
<td>1</td>
<td>0.0028</td>
</tr>
<tr>
<td>pH Homogenized</td>
<td>8.9037</td>
<td>1</td>
<td>0.0032</td>
</tr>
<tr>
<td>Buffered pH</td>
<td>5.9172</td>
<td>1</td>
<td>0.0158</td>
</tr>
<tr>
<td>Buffered pH Homogenized</td>
<td>6.1268</td>
<td>1</td>
<td>0.0140</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>5.4411</td>
<td>1</td>
<td>0.0205</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>12.0985</td>
<td>1</td>
<td>0.0006</td>
</tr>
<tr>
<td>Zn*species</td>
<td>2.3068</td>
<td>10</td>
<td>0.0134</td>
</tr>
<tr>
<td>Zn Homogenized</td>
<td>8.5457</td>
<td>1</td>
<td>0.0038</td>
</tr>
<tr>
<td>Zn Hom*Species</td>
<td>2.0573</td>
<td>10</td>
<td>0.0288</td>
</tr>
<tr>
<td>Clay %</td>
<td>4.0110</td>
<td>1</td>
<td>0.0464</td>
</tr>
<tr>
<td><strong>CEC</strong></td>
<td>3.8206</td>
<td>1</td>
<td>*0.0518</td>
</tr>
<tr>
<td>CEC*Species</td>
<td>1.9438</td>
<td>10</td>
<td>0.0405</td>
</tr>
<tr>
<td><strong>Base Saturation</strong></td>
<td>2.9630</td>
<td>1</td>
<td>*0.0865</td>
</tr>
<tr>
<td><strong>Total Vegetation Weight(g)</strong></td>
<td>3.3095</td>
<td>1</td>
<td>*0.0702</td>
</tr>
<tr>
<td>PC1</td>
<td>7.8269</td>
<td>1</td>
<td>0.0056</td>
</tr>
<tr>
<td><strong>PC1*Species</strong></td>
<td>1.7567</td>
<td>10</td>
<td>*0.0696</td>
</tr>
</tbody>
</table>

Because the covariates showed relationships with survival a closer analysis to determine if there were any relationships between certain parameters and overall survival, a correlation analysis was conducted. This analysis can be considered somewhat redundant, but it further reinforces the covariate analysis and is included for additional discussion. Correlations between covariates existed with the overall average survival per block and are detailed in Table 3.10.
Table 3.10: Significant soil parameters that influenced the overall survival of tree seedlings. Note that this data has been pooled over species.

<table>
<thead>
<tr>
<th>Overall Survival</th>
<th>Correlation (r)</th>
<th>Significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>0.6176</td>
<td>0.0324</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>0.5776</td>
<td>0.0492</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>0.728</td>
<td>0.0073</td>
</tr>
<tr>
<td>Homogenized pH</td>
<td>0.5804</td>
<td>0.0479</td>
</tr>
<tr>
<td>Homogenized Zn</td>
<td>0.6297</td>
<td>0.0282</td>
</tr>
</tbody>
</table>

Overall survival, regardless of species, was most correlated with soil pH, magnesium, and zinc. This was further supported by the fact that the homogenized soil samples (representing a field average response, as opposed to a data average response) of pH and zinc were correlated with overall survival as well. This additional correlation analysis emphasizes the role that pH, Mg, and Zn exhibited in the initial survival of tree seedlings in this experiment. What is also interesting is that a correlation analysis on only Ohio buckeye does not include these soil parameters, but indicates that clay particle percentage, base saturation and the exchange of Calcium cations are negatively correlated with buckeye survival (Table 3.11).

Table 3.11: Soil parameters correlated with only Ohio buckeye survival.

<table>
<thead>
<tr>
<th>Ohio Buckeye (Aesculus glabra)</th>
<th>Correlation (r)</th>
<th>Significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Clay</td>
<td>-0.5408</td>
<td>0.0064</td>
</tr>
<tr>
<td>Soil Base Saturation</td>
<td>-0.5014</td>
<td>0.0126</td>
</tr>
<tr>
<td>Exchange Calcium (Ca)</td>
<td>-0.4605</td>
<td>0.0235</td>
</tr>
<tr>
<td>Surrounding Forb biomass (g)</td>
<td>0.4283</td>
<td>0.0368</td>
</tr>
</tbody>
</table>
Chapter 4: Discussion

Survival and growth of tree seedlings and vegetation are closely influenced by environmental factors such as light, soil nutrients, topography, and moisture, but also by biotic factors such as competition and herbivory (Tilman 1984; Adams, Jr. et al., 1992; Ostfeld et al., 1997; Flory & Clay, 2010; Kambatuku et al., 2010; Kupferschmid & Bugman 2008; MacDougall et al., 2010; Griscom et al., 2011). All of these factors will be addressed in the long-term goals of this multi-year research project, but the focus of this document is on a subset of the factors. Specifically for this document, my hypotheses predicted that we would see variations in available soil nutrients and light and that these factors would influence the aboveground biomass of surrounding vegetation and also the survival of tree seedlings native to the Kentucky Inner Bluegrass blue ash-oak savanna-woodland and utilized in this experiment.

Soils: Block differences and field differences

During the original setup of this experiment it was hypothesized that there could possibly be variations in soil conditions at Griffith Woods, and thus a blocked setup was utilized, but in order to incorporate potential variations for the long term aspect of this experiment soil characteristics were assessed. It was determined that soils differed significantly on a rather fine spatial scale (i.e. blocks within fields and between fields) in various soil parameters that are important for health and growth of plants. This could be due to the two major soil types, the Faywood and the Loradale series, being derived from slightly different parent materials and located in slightly different topographical locations but existing in both experimental fields (see Figure 2.3 and Figure 2.4 in Chapter 2). Most notable in the soil data is that consistently field two tested lower for all soil parameters. Looking at Figures 3.13 & 3.14 (Chapter 3) again illustrates that Field 2 was consistent in its pattern of lower soil nutrients and lower in all measured soil parameters. This information will have to be utilized going forward for the long term aspects of the experiment and a realization that we do in fact have soil differences could impact the long term growth of tree seedlings, depending on the block in which they are planted.

Interactions between light and vegetation

It was further hypothesized that if nutrient variations occurred between areas at Griffith Woods then there may also be differences in vegetation composition and biomass. Overall blocks varied in vegetation biomass but this did not result in statistical differences. At every scale of analysis, either looking only at forb or grass component, or looking at the total vegetation biomass, statistically blocks were not different. The only difference noted was in forb biomass between fields. However, we did see statistical differences in species richness between blocks. It is not certain that species richness in and of itself could be impacting seedling growth or the available amount of light, as light was hypothesized to fluctuate concordantly with variations in block vegetation biomass. Results indicate that, as was hypothesized, light amounts
did statistically differ between heights. This demonstrates a potential biotic influence (i.e. diffusive filtering by surrounding vegetation) on available light at the height of growing seedlings and it is assumed then that at these heights (approximately 0.5m/1.6ft) competition for light is high between grass/forb vegetation and tree seedlings. Additionally, this shows that the growing environment for the tree seedlings is different, when referencing available light for photosynthesis, between areas with vegetation and areas where vegetation has been removed. However, light levels were constant at this growing height, and there was not a statistical difference between light levels at the 0.5m or the 0m height between blocks, respectively. Therefore, even though diffusive filtering is occurring, it is rather uniform between blocks indicating a similar light environment for and by vegetation, and to another extent for seedlings.

**The relationship between vegetation biomass and soil components**

It was hypothesized that if soil variations existed this would result in differences in vegetation biomass and composition. Overall composition parameters did not respond to most soil parameters but moderate correlations between species richness and H’ diversity with Phosphorus was demonstrated (r²=0.3147 & 0.4283, respectively). Previously it was shown that species richness was statistically different between blocks. Of more importance are the results from the linear regression analysis for biomass. All three responses, forb, grass, and total vegetation biomass showed that pH and Magnesium were influential soil nutrients in determining their biomass. This data also suggests that soil Calcium is important for vegetation biomass as well. Previous research by Tilman (1984) demonstrated that, although in his experiment Nitrogen seemed most limiting for plant growth, when N was supplemented Mg became the next most limiting soil nutrient. This helps to support my findings that Mg concentrations can be a determining factor in vegetation biomass. Although statistically no differences in vegetation biomass between blocks existed, there were large variations recorded. Statistical differences did exist with pH and Mg, and this varied by block and was shown to influence the subsequent vegetation biomass in all three categories. Therefore, one can conclude that pH and Mg are likely strong influences on determining vegetation biomass. What is unclear then is how these nutrients vary statistically, but vegetation biomass did not vary significantly between blocks. This could be a remnant of sampling protocol and greater differences in vegetation biomass between blocks could be determined with additional study which would further support the connection between biomass, pH and Mg.

**Seedling survival and treatment effects: Influences of field, block, herbivory protection and species**

It was hypothesized that survival should not be influenced by location (i.e. field or block) but would be influenced by herbivory protector treatment (to prevent herbivory by deer, rabbit, and rodent) and seedling species. Differential survival was demonstrated in different fields, blocks, and seedling species, but survival was overall not influenced by the seedling protector treatment. It is my conclusion that first, statistical evidence showing differential survival by seedling species (and their associated shade tolerance category) was primarily driven by the low
survival of Ohio buckeye (*Aesculus glabra*) which had only about 58-68% survival success rate. These low survival numbers influenced the overall survival rate of species in statistical analyses and when *A. glabra* was removed from analysis, species no longer was an influential factor. All species in all treatments other than *A. glabra* had >89% mean survival. However, it should be noted that even when *A. glabra* is removed from analysis there is still a statistical difference in survival depending on field, with field 2 showing lower survival overall. Whether this can be explained by nutrient differences will be discussed later in this chapter.

As for the lack of a protector treatment influence, what is most likely is that there was not enough time for the protector treatment to take effect from planting time to survival evaluation. An alternate hypothesis would be that herbivore browsing pressure is not exerted at this time of year since during the growing season ample food may be available elsewhere for herbivores. What will be interesting to see is if this changes on a seasonal basis, as herbivory is going to be closely monitored over the course of the longer experiment. It may be that the protector treatment was confounded by the vegetation treatment. Up until the point of survival estimation the seedlings were growing in a vegetated habitat and this could obscure the seedlings from being located by herbivores. The interaction between vegetation removal and protector treatment will be very interesting to follow up on in the future. Finally, rather than true treatment effects determining survival, the seedling species by protector treatment interaction may have been drive by pure randomness (see Figure 3.21 in Chapter 3). In other words, bad planting methods or poor seedling stock could have determined survival differences, and the effects of a protector treatment were negligible. Because these poorly planted individuals were randomly distributed between species and treatments this surfaced by chance in the data as an interaction, as opposed to a truly ecologically driven response. However, future research into herbivory in this experiment will help to determine if in actuality browse preference for certain species would in fact influence seedling survival and growth, thus resulting in a species by treatment interaction. Conversely, there may be an actual impact by the protectors on some species as a result of their growth-form and architecture, where the protectors inhibit growth in some way by physical impedance and this could cause some species to do poorer in the protector treatment. All of this is slightly speculative, and as stated, more in depth research into browse preference and damage will elucidate differential species responses and potential differences by browser type and the pressures they exact on different species.

**Seedling survival and growing environment: Influences of soil, light, vegetation, and shade tolerance as ecological filters**

A complicated interaction between biotic and abiotic factors was hypothesized to influence survival of seedlings, with higher quality soils imparting higher vegetation biomass and higher seedling survival. Additionally, those species that are considered savanna specialists (i.e. those species that currently occupy remnant savanna tracts as very old, mature trees such as blue ash, chinquapin oak, bur oak, Kentucky coffeetree and shellbark hickory) were hypothesized to show the highest survival rates due to their evolutionary adaptations to the soil nutrient availability, the light environment, and the competitive interactions with surrounding
vegetation all somewhat manifested in their categorization as shade intolerant species. What is shown in my results is that survival varied by species, but this was primarily determined by the low survival rates of Ohio buckeye. What seems common is that soil pH, Zinc, and Magnesium show relations either as covariates or by correlation analysis with seedling survival, whether or not buckeye was included in analysis. Also because the mean pH, Zn, and Mg amounts (i.e. mathematical average amount) along with the homogenized pH, Zn, and Mg samples (i.e. the “in the field” average amounts) were both shown to be statistically influencing survival, these nutrients may be the most important in the establishment phase of seedlings in the Bluegrass savanna-woodland. Additionally it is pH, Zn, and Mg that are strongly related to total vegetation biomass further emphasizing their importance. Therefore, one would assume that these soil parameters are influential for the growth and survival of both vegetation and seedlings in the bluegrass savanna ecosystem.

To a lesser extent my research has shown, by covariation, that clay particle percentage, cation exchange capacity (CEC), and base saturation also explained variations in survival. As these three parameters are related to how tightly the soil can hold onto nutrients it makes sense that they could be influencing survival as well. As is shown by the particle size distribution, clay particles make up about 16% of the soil, but the main particle is silt (70%). Clay particles help to bind and hold onto nutrients as they have the most surface area and polarity potential to hold onto cations. Low clay amounts, combined with available clay particles becoming saturated with cations, decrease the potential for excess cations to be exchanged, which results in leaching of these nutrients from the soil (Corey, 1990; Haby et al., 1990). This leads to the necessity for many agricultural areas in the Inner Bluegrass Region to supplement soils with potash (K) or lime (Ca), as they can be limiting to plant growth (USDA-SCS, 1968). Although we didn’t see much influence by these nutrients on survival, the exchange of Ca was correlated with Ohio buckeye survival, along with clay percentage and base saturation, lending credence to these parameters determining the lower survival for buckeye (See Table 3.11 in Chapter 3). Additionally, in the covariate model (3) without buckeye, K was determined to be influential for other seedling species survival. Finally, as was previously stated, pH was consistently shown to influence seedlings, and the buffering ability by these various nutrients that can be leached can lead to the fairly low pH soil conditions observed (mean experimental soil pH=\~4.5, acidic) as is common in limestone derived soils (Corey, 1990; Haby et al., 1990).

When shade tolerance categories were examined, as opposed to species, at first they showed significance, but when A. glabra was removed this was no longer a variable that could explain survival variations. Going forward in the long-term project, it is hypothesized that the vegetation removal treatment will show growth and survival differences based on a high light versus a low light growing environment. However, at this early stage survival was not dramatically influenced by shade tolerance category.

When one considers the influence of species shade tolerance differences and aboveground versus belowground biomass accumulation of seedlings, shading by vegetation will likely result in differential growth and survival because of the high competition for this limited
resource. In high competition environments those species able to tolerate low light levels (i.e. maple, buckeye, etc.) are likely to persist better than those who are obligated to grow in high light environments (oaks primarily). Still, light is not the ultimate controlling factor but one of the many biological filters in the Bluegrass savanna habitat. Some species may have advantages by allocating growth to roots first, disadvantageous when only considering light availability, but potentially more adaptive given the various disturbance regimes (i.e. fire, herbivory, drought) thought to influence savanna community structure.

Weather Patterns

After looking at weather patterns for the growing season of 2011, the timespan of when this experiment was initiated and data collection occurred, it appears that overall weather was typical for the Inner Bluegrass Region (IBR) of Kentucky. Recorded temperatures were within average limits and most monthly precipitation amounts were within average ranges. However, it should be noted that 2011 was a wetter than average year for this region of Kentucky, especially the month of April which exceeded the state average precipitation by 22.73cm (8.9in). Excluding one species (*Aesculus glabra*), tree seedling survival was very high during the initial phase of this long-term project. This may have given us an additional advantage for seedling survival as they were well hydrated that year. The following year in 2012 one of the harshest droughts in recent history occurred, with only 2.5cm (0.99in) falling in June. My inference is that high precipitation amounts during April 2011, just after and during the timespan when the majority of seedlings were planted, favored their establishment. If planting had occurred in 2012 instead the outcome could have been drastically different.

Potential reasons that growth was favorable in these “wetter” conditions could be greater soil moisture, which softens soils, allowed for unimpeded root growth. At this early life stage, root establishment in extremely important, because it is commonly known that soil moisture can easily influence root growth (Westfall *et al.*, 1990). Furthermore, having ample water available provides favorable conditions for cell growth and division which would improve the ability of seedlings to grow new roots to establish. Moister conditions would also favor new leaf growth and additional above ground biomass accumulation, facilitating survival at this growth stage. Contrast these weather patterns to one year later wherein survival could have been much lower simply by the fact of being water stressed. Water stress at this age for a seedling would impede rapid and easy root growth, preventing establishment below ground, which would entirely impact aboveground growth as well. As Kentucky fluctuates in weather patterns between favorable and stressful years for plant growth, this could very well be an influential factor in native savanna regeneration. For instance, if drought conditions one year occurred during a mast year (because the previous year was moister and encouraged seed production), germination and establishment of seedlings could be severely hampered and therefore whole cohorts could fail to establish. Additionally, if only drought tolerant species are Inner Bluegrass Region dominants (as seems likely based on the descriptions of species found in the Bluegrass savanna-woodland, see Table 1.1 in Chapter 1), then these species once
established during favorable conditions would be able to persist in the unfavorable conditions
that can occur seasonally and/or yearly. Because of the stochastic droughty nature of Kentucky’s
climate and the topography, with the presence of “dry ridges”, it could be that savanna
formation and maintenance may be due to the drought prone nature of the soils of the area, the
karst topography with little surface water, and rapid water percolation through underlying rock
strata. Therefore, the combination of drought prone climates and soils along with the
topographical nature of dry ridges probably influences how and why savannas communities can
be maintained. Further research should be conducted on the individual species to understand
better their drought tolerances during the seedling establishment and the mature life stages of
these species, and also whether the distribution of prototypical savanna stands are limited to
primarily droughty upland ridges.

Conversely, an abnormal weather year, as was observed in 2011, may reduce the ability
to generalize any conclusions reached in these initial survival results. Because this wet year
potentially favored the establishment of seedlings, any conclusions about natural establishment
or restorative plantings conducted during an average year cannot be made. Seedlings
establishing in a normal or drought year may experience lower survival rates than observed in
this initial phase during a wet year. However, for the long term goals of this research, which are
to determine how competition and disturbance influence growth and survival of native tree
seedlings, having favorable establishment for the long-term project has been beneficial and will
allow us to gather more data on the influence of these major ecological pressures on seedlings.
Although, as stated previously, it may be this fluctuation between wet and dry years that is a
determining factor in natural establishment and persistence of the drought tolerant Bluegrass
savanna species.

**Critiques**

As was presented before, vegetation species richness did vary significantly between
blocks, although this data set is low resolution due to morphospecies classes utilized for
identification purposes. A more in depth analysis of the exact species that occupy these fields
should be conducted in order to determine true species richness and diversity and to further
understand the success of utilizing a commercially available native grass/forb mixture. In fact, a
comparison between the species that compose the commercially available “habitat” mixture
used to re-vegetate these fields and the native species list offered in Wharton & Barbour (1991),
probably the most definitive species list for native organisms of the IBR, shows that many of the
species sold are not traditionally considered part of this region’s vegetation community (Table
4.1 & 4.2). In fact, Wharton & Barbour (1991) do not list 5 of the grass species planted, although
one is listed in the USDA-NRCS database as within the native distribution, and it is likely that the
two dropseed (*Sporobulus* sp.) varieties are also within their native distribution, although this is
unclear. For forb species, this list more reflects the native species in the area than the grass
species. Many are not listed in Wharton & Barbour (1991) but are listed in the native range by
the USDA-NRCS plant database. The only forb species of concern is Korean Lespedeza 
(*Lespedeza stipulaceae*) an introduced, although not very invasive, species. Why this species is 
included in a native grass/forb seed mixture is perplexing and should probably be discontinued, 
although the nitrogen fixing capability and wildlife benefits provided by this species are probably 
why it is included.

**Table 4.1: Detailed list of grass species planted in experimental blocks at Griffith Woods, KY and whether they are considered species native to the region.**

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific Name*</th>
<th>Found in Wharton &amp; Barbour (1991) IBR Species list</th>
</tr>
</thead>
<tbody>
<tr>
<td>Big Bluestem</td>
<td><em>Andropogon gerardii</em></td>
<td>Yes</td>
</tr>
<tr>
<td>Canadian Wild Rye</td>
<td><em>Elymus Canadensis</em></td>
<td>Yes</td>
</tr>
<tr>
<td>Fall Panicum</td>
<td><em>Panicum anceps</em></td>
<td>Yes</td>
</tr>
<tr>
<td>Indian Grass</td>
<td><em>Sorghastrum nutans</em></td>
<td>Yes</td>
</tr>
<tr>
<td>Little Bluestem</td>
<td><em>Schizachyrium scoparium</em></td>
<td>Yes</td>
</tr>
<tr>
<td>Lopsided Indiangrass</td>
<td><em>Sorghastrum secundum</em></td>
<td>No##</td>
</tr>
<tr>
<td>Honeywood Drop Seed</td>
<td><strong>Sporobulus sp.</strong></td>
<td>No</td>
</tr>
<tr>
<td>Side Oats Gramma</td>
<td><em>Bouteloua curtipendula</em></td>
<td>No#</td>
</tr>
<tr>
<td>Switchgrass (variety not specified)</td>
<td><em>Panicum virgatum</em></td>
<td>Yes</td>
</tr>
<tr>
<td>Tall Dropseed</td>
<td><em>Sporobulus composites</em></td>
<td>No</td>
</tr>
<tr>
<td>Toothache Grass</td>
<td><em>Ctenium aromaticum</em></td>
<td>No##</td>
</tr>
<tr>
<td>Virginia Wild Rye</td>
<td><em>Elymus virginicus</em></td>
<td>Yes</td>
</tr>
<tr>
<td>Wiregrass</td>
<td><em>Aristida stricta</em></td>
<td>No##</td>
</tr>
</tbody>
</table>

*Scientific names came from: [http://www.roundstoneseed.com/productshowcase.html](http://www.roundstoneseed.com/productshowcase.html)

**Scientific name not identifiable by this common name, Genus inferred.

#NRCS plant database includes Kentucky in native distribution (USDA-NRCS, 2013).

## NRCS plant database does not include Kentucky in native distribution (USDA-NRCS, 2013).
Table 4.2: Detailed list of forb species planted in experimental blocks at Griffith Woods, KY and whether they are considered species native to the region.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Scientific Name*</th>
<th>Found in Wharton &amp; Barbour (1991) IBR Species list</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bergamot</td>
<td>Monarda fistulosa</td>
<td>Yes</td>
</tr>
<tr>
<td>Blackeyed Susan</td>
<td>Rudbekia hirta</td>
<td>Yes</td>
</tr>
<tr>
<td>Browneyed Susan</td>
<td>Rudbekia triloba</td>
<td>Yes</td>
</tr>
<tr>
<td>Butterfly Milkweed</td>
<td>Asclepias tuberose</td>
<td>Yes</td>
</tr>
<tr>
<td>False Sunflower</td>
<td>Heliopsis helianthoides</td>
<td>Yes</td>
</tr>
<tr>
<td>Grayheaded Coneflower</td>
<td>Ratibida pinnata</td>
<td>Yes</td>
</tr>
<tr>
<td>Illinois Bundleflower</td>
<td>Desmanthus illinoensis</td>
<td>Yes</td>
</tr>
<tr>
<td>Lance Leaved Coreopsis</td>
<td>Coreopsis lanceolata</td>
<td>No#</td>
</tr>
<tr>
<td>Maximilian Sunflower</td>
<td>Helianthus maximiliani</td>
<td>Yes (probable cultivar escape)</td>
</tr>
<tr>
<td>New England Aster</td>
<td>Aster novae-anglia</td>
<td>Yes</td>
</tr>
<tr>
<td>Prairie Dock</td>
<td>Silphium pinnatifidum</td>
<td>No#</td>
</tr>
<tr>
<td>Purple Coneflower</td>
<td>Echinacea purpurea</td>
<td>Yes</td>
</tr>
<tr>
<td>Purple Prairie Clover</td>
<td>Dalea purpureum</td>
<td>No#</td>
</tr>
<tr>
<td>Rigid Goldenrod</td>
<td>Solidago rigida</td>
<td>No#</td>
</tr>
<tr>
<td>Rough Blazing Star</td>
<td>Liatris aspera</td>
<td>No#</td>
</tr>
<tr>
<td>Roundheaded Lespedeza</td>
<td>Lespedeza capitata</td>
<td>No#</td>
</tr>
<tr>
<td>Korean Lespedeza</td>
<td>**Kummerowia stipulacea or Lespedeza stipulacea</td>
<td>Yes (Introduced)</td>
</tr>
<tr>
<td>Smooth Aster</td>
<td>Aster laevis</td>
<td>No#</td>
</tr>
<tr>
<td>Spiked Blazing Star</td>
<td>Liatris spicata</td>
<td>No#</td>
</tr>
<tr>
<td>Whorled Rosinweed</td>
<td>Silphium trifoliatum</td>
<td>Yes</td>
</tr>
</tbody>
</table>

** Scientific names came from: http://plants.usda.gov/factsheet/pdf/fs_kust.pdf
#NRCS plant database includes Kentucky in native distribution (USDA-NRCS, 2013).
## NRCS plant database does not include Kentucky in native distribution (USDA-NRCS, 2013).

I recommend that a more specific assemblage of seed be utilized for re-vegetating areas in the Inner Bluegrass Region (IBR), and those not typically found in the region and especially those that are non-native to Kentucky, let alone the United States, should not be distributed at all. This mainly is targeted at *L. stipulacea*, *Sorghastrum secundum*, *Ctenium aromaticum*, and *Aristida* stricta found in the mix, however, our rough scale morphospecies identification did not recognize these species as becoming established in these fields, although differentiation between the two species of Indian grass (*Sorghastrum* sp.) was not made. At the very least though, this vegetation assemblage is more representative in species and physical structure to the lost native IBR grasslands than was the previous forage grass species that dominated the agriculture pastures (i.e. *Poa pratensis* and *Festuca* sp.) and are therefore a better grassland component for determining competitive interactions with the experimentally planted seedlings.
Assessments of vegetation biomass and composition were not statistically different and no differences in light levels were measured between blocks; however, personal observations suggest that our sampling method potentially missed some of the variation in vegetation biomass and composition that exists in these fields. I suggest more sample sites in each block be assessed for vegetation biomass and composition. By looking at a finer scale, both in sampling and in identification, better answers as to how the blocks and fields may actually vary in surrounding vegetation could be determined. Certain unmeasured areas that could lead to clearer determinations of vegetation variations are the appearance of low density vegetation strips running across the fields. These areas are probably remnants of old farming machinery paths. These strips are visually different in their structure and composition as the vegetation that lies within them is much shorter and less dense (pers. obs.) which would indicate a lower overall biomass. Specific sampling in these strips should be conducted to determine if soil characteristics are drastically different in these areas as well.

The greatest critique one could make of these results, when looking at the overall picture, is how can a single soil parameter, correlated with survival, be implicated as the ultimate cause of seedling mortality if most species experienced a >89% survival rate? Is there really a clear connection between pH, Zn, Mg and the survival of tree species or is this simply an artifact of the statistical procedures? Maybe these parameters can influence survival, but it must not be by much, as we see high survival at this stage of the project. These conclusions lead to several possible avenues to follow up on. Primarily, as the survival analysis was conducted to determine planting success after the initial setup of a long-term study, maybe not enough time had passed to see true survival rates. However, subsequent survival estimates will certainly be influenced by the experimental treatments, therefore the initial survival estimates presented here are the only information we have pertaining to the planting success without a treatment influence. The mortality rates we do see are more likely a remnant of bad seedling stock or poor planting practices, as the majority of seedlings were planted at a rapid rate by volunteer help and therefore one could conservatively assume that about 10% of seedlings would not establish simply because of these variations in planting. Concerning Ohio buckeye, a species with early leaf phenology, it could have been the fact that planting occurred at a time when this species normally would have already mobilized tissue nutrients and started the process of leaf out. Because buckeye was not it the ground early enough it may never have had a chance to establish as well as it could have. An even more simple explanation could be that the Ohio buckeye seedlings that were provided were in poor condition prior to ever being placed in soil.

To truly determine if the soil parameters do influence the survival of these tree species, manipulated experiments should be conducted, as opposed to this observational study that attempts to correlate natural variations in soil parameters with survival. If one was to establish an experiment with extremes of high and low soil parameters, then a better determination of the filtering threshold by these parameters on seedling survival could be made. It is only by a strict experimental method that precise determinations of how individual soil nutrients can impact each species’ survival. With that said, due to the simple fact that pH, Zn, and Mg (both
average values and homogenized samples) consistently correlated with survival this lends strong support to research this topic, and these specific elements, further.

All of the environmental parameters (soil, light, and vegetation) in the sense that they can vary between blocks, have potentially influenced seedling survival on some level. Though this may be thought of as somewhat unlikely given the closeness and similarities of the site in the ecological sense, slight variations on local scale could affect the survival of the seedlings more than one might think. If minor variations can influence survival, what does this tell us ecologically? Shouldn’t these species be somewhat tolerant of this variation? Surely these species have encountered comparable sites such as this before? Is it due to the fact that (unfortunately) Missouri genotype trees were planted in a unique Kentucky habitat? Additional studies should be conducted at alternative sites in the IBR, although the availability of these sites is severely lacking due to the previously mentioned land use changes and lack of intact savanna-woodland habitat.

**Future Directions**

As for hardwood tree species native to the Bluegrass savanna-woodland, an obvious recruitment failure phenomenon has been ongoing for some time in this ecosystem. As is justified in the introduction, restoration of this community is necessary in order for the species to persist on the landscape. A major obstacle for Bluegrass savanna-woodland restoration is the fact that no un-impacted or degraded reference system remains. Our only source for habitat structure and constituent species comes from historic floristic surveys and anecdotal accounts. None of these were conducted in a systematic fashion, so data is somewhat questionable and is further confounded by the fact that many of the species names utilized varied by explorer. What does seem certain is that at least some form of open woodland habitat existed when pioneers first settled the region. Whether this is a remnant of pure climax community assemblage or a snapshot of succession after multiple years of habitat manipulation by Native American cultures is debatable, but it is most likely the latter. In my opinion, whether the Bluegrass savanna-woodland constitutes a “natural” community is merely a semantics argument, but the ecosystem services provided by having the community on the landscape is obvious. In the species descriptions it is apparent that most are drought tolerant, limestone soil species that provide essential wildlife benefit primarily through a browse or mast food resource. Combine this with the fact that the majority of the Bluegrass region of Kentucky has been impacted by some form of land use change, the benefits of habitat heterogeneity as refugia for all organisms (be they plantae, animalia, *et al.*) gives ample motivation for the necessity to re-establish Bluegrass savanna-woodland habitat.

What my research will hopefully determine in the future is what species likely occupy this community, what disturbance regimes determine the structure of the community, and finally, how the interaction between ecological filters and intrinsic traits of the species combine to create the ecosystem. The initial phase of this study is unable to answer these questions, although it has provided insight to the fact that at the seedling stage, all but one of these
species is highly successful at establishing themselves. The interrelationships between competition (for light, nutrients, and water) and disturbance (by drought, herbivory, and fire) will be the impetus of my research in the future stages of this project in restoration ecology. To do this I intend to follow the impact of the experimental treatments of competition removal and herbivory removal installed in the design of this project. Furthermore I wish to better identify the specific mammalian species that are utilizing these seedlings as browse food, quantify their abundances, and assess their movement patterns. To better understand the adaptations of the plant community I intend to conduct fine scale physiological analyses. One would hypothesize that physiological differences exist between the savanna specialists and the more generalist species, but also within species based on the competitive environment treatment in which they are growing. Additionally, better understanding of how the role of soil moisture influences seedling growth needs to be determined, either by field observation of soil moisture content during the growing season or by experimentally controlled moisture experiments either in a common garden or greenhouse experiment. This information will help to elucidate how drought conditions can determine savanna seedling success. Finally what may be of most importance is to understand the response at the early seedling life stage of these tree species when exposed to fire. Native American burning of the Bluegrass Region of Kentucky is thought to be a major factor in what has determined the structure and composition of this community, but there is a complete lack of empirical research on how fire affects the survival and growth of these species in this ecosystem. As data is collected over the coming years to address these questions management and restoration practices will be determined which will help to predict a successful tree component to the Kentucky Inner Bluegrass blue ash-oak savanna-woodland.

Conclusions: Implications for Savanna Restoration and Regeneration

As has been stated, even though variations is soil and light existed, we had high survival rates overall. This seems to show that for this life stage (that of a seedling) these species are able to survive the initial establishment stage in the savanna regeneration process. Therefore we can confidently state that soil parameters and light environment can slightly influence, but will not overall determine the success of these species (although this interpretation may not apply to Ohio buckeye). What this does suggest is that there must be a filter at some other life stage that is inhibiting the regeneration of Bluegrass savanna-woodland. As is suggested in the species accounts, many of the seeds of these species are readily consumed by mammalian herbivores, but also predated by various pests, diseases and rot or simply impeded by a lack of soil contact by heavy leaf litter. Furthermore, field observations in the remnant savanna tract at Griffith Woods suggest that the presence of a groundcover of fescue, with its dense matting growth form, may inhibit soil contact for many of these species. All of this combined implies the regeneration failure of Bluegrass savanna-woodland occurs at the seed germination stage, because as is shown in this research, if the seed is able to germinate then it is highly likely that these species will be able to at least establish at the seedling stage. What will be researched in the future of this experiment is how after the seedling is established what factors may inhibit successful growth to be recruited into older age classes. As was previously indicated,
competition with surrounding vegetation, herbivory by various mammals, fire, drought, or the combination of all of these, are the biological filters that exist once seedlings establish. A mixture of suppressed growth by competition, inhibited biomass accumulation through removal by herbivory, slow growth due to limited soil water availability, and suppression and topkill by fire may be too much pressure for seedlings to escape this life stage. However, it may be the combination of these factors that certain species typical of the savanna remnants (i.e. bur oak, chinquapin oak, shellbark hickory, Kentucky coffeetree and blue ash) are able to tolerate and recruit into the mature age class. However, these disturbances and filters have been severely altered by human activities (i.e. fire suppression, mowing, and higher mammalian populations due to the lack of an apex predator). Without a natural disturbance regime, natural regeneration will not be possible. This implies that if humans cannot let natural disturbances occur then they must restore or mimic lost disturbance practices. Therefore, a use of prescribed fire to eliminate competition, and the use of managed hunting practices, or better yet the reintroduction of extirpated apex predators to suppress overpopulated mammalian browsers, will help to return the savanna to its original ecosystem function and trajectory.

As we continue to monitor survival, further effects of drought and experimental treatments (of competition and herbivory removal) are predicted to influence seedling survival. Additional monitoring of mammalian herbivore populations, the role of prescribed fire, and the role of the once abundant canebrakes in the Bluegrass savanna-woodland are all important aspects of future research. Additionally, comparisons and monitoring of the “Reforest the Bluegrass” program should be initiated to help draw more conclusions from additional sites across the Inner Bluegrass Region of Kentucky. To the best of my knowledge, this experiment represents the first, and largest, experimental reconstruction of Kentucky Bluegrass savanna-woodland and the first to research the roles of competition and disturbance on this species assemblage, especially those that are considered savanna specialists. I have attempted to describe the initial phase of this project, interpret how seedlings experienced the environmental conditions of soil, light and vegetation and imply how the high planting survival can be interpreted as being a sound restorative practice. Conversely, the true ecological questions we are interested in which pertain to natural regeneration cannot be addressed just yet, but with continued monitoring we will understand how different disturbance and competition factors will influence natural savanna-woodland formation.

The need to restore Bluegrass savanna-woodland is apparent and this study attempts to not only restore, but to better understand what conditions and factors would contribute to successful natural regeneration of the community. Because all intact Kentucky Inner Bluegrass blue ash-oak savanna-woodland has been lost or degraded, it is our duty to restore this system not just to reinstate natural ecological functioning, but also for its unique intrinsic value, which Braun (1950) so eloquently described as the “most anomalous of all vegetation areas of [the] eastern United States”.

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REFERENCES


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Assistant Manager, Mountain Outfitters, Monteagle, TN
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2012- Graduate Student Research Grant, Kentucky Native Plant Society
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2012- UK Biology Ribble Summer Research Grant, University of Kentucky
2011- Graduate School Student Support for Travel, University of Kentucky
2011- UK Biology Ribble Summer Research Grant, University of Kentucky
2011- G. Flora Ribble Conference Travel Enrichment Grant, University of Kentucky

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2013- Factors influencing the establishment and survival of native hardwood tree seedlings of the Kentucky Inner Bluegrass blue ash-oak savanna-woodland. Center for Ecology, Evolution, and Behavior Symposium. University of Kentucky, Lexington, KY

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