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Negative Interactions between Tree Seedlings and Non-Native Grasses in a Restoration Setting: Getting to the "Root" of the Problem

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Jennifer A. Franklin, Major Professor

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(Original signatures are on file with official student records.)
Negative Interactions between Tree Seedlings and Non-Native Grasses in a Restoration Setting: Getting to the “Root” of the Problem

A Thesis Presented for the Master of Science Degree
The University of Tennessee, Knoxville

Matthew Stephen Philip Aldrovandi
August 2018
DEDICATION

I would like to dedicate this thesis to my wife and my three daughters. Without their love and support, I never would have finished this. Thank you for always being there to bolster me in hard times, and keep my spirits up.
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Dr. Jennifer Franklin, my mentor and my friend, deserves the lion’s share of this acknowledgement. It was she who got me hooked on all things reclamation back in late 2013, then set me loose in 2014 to help her with research. She has advised me well since 2012, and I will always be grateful for the opportunities she has provided for me. Thank you, Dr. Franklin, for allowing me to try to make a difference while doing what I love, as well as answering an innumerable amount of my questions.

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Finally, I would like to thank my family for their love, support, and ridiculous outpouring of encouragement in this whole process. I really appreciated the big deal that was made of every small step of this that I completed. Thank you.
ABSTRACT

Aggressive herbaceous plants are often planted on reclaimed mine sites, but these can inhibit the establishment of woody vegetation on the site. The objective of this study was to determine which of nine ground cover species [ragweed (*Ambrosia artemisifolia*), annual ryegrass (*Lolium multiflorum*), partridge pea (*Chamaecrista fasciculata*), black mustard (*Brassica nigra*), lablab (*Lablab purpureus*), sorghum (*Sorghum bicolor*), German millet (*Setaria italica*), smartweed (*Polygonum pensylvanicum*), sunn hemp (*Crotalaria juncea*)] would outcompete tall fescue without outcompeting shortleaf pine (*Pinus echinata*), chinkapin oak (*Quercus muehlenbergii*), and northern red oak (*Quercus rubra*) tree seedlings on a legacy mine site. It was predicted that groundcover species that do not have a shallow root system with an extensive surface area will be less competitive with tree seedlings than species that have a shallow root system with an extensive surface area. Competition was determined by tree growth, transpiration rate, and chlorophyll content over the period of about 1 year.

Results from this experiment showed no significant effect on height or root collar diameter (RCD) growth by treatment or seedling species. There was no effect on transpiration rate or chlorophyll content by treatment. Some effect was found on transpiration rate and chlorophyll content by cover. Intermediate levels of groundcover were found to be the most beneficial.

Despite treatment species being seeded at high rates, results did not indicate effective competition between treatments and the vegetation already present on site. Even if seeding rates were increased to outlandish levels, the cost of seed would outweigh any benefit that might exist.
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CHAPTER ONE: INTRODUCTION

Interactions between plants are perhaps some of the most complex found in life. Circumstances influencing the growth, fitness, and survival of plants are nearly endless, and although these factors have been studied for centuries, new information and a constantly changing environment have introduced novel questions.

As complicated as these interactions are, understanding them is becoming increasingly more important, especially as we move into an age in which we are trying to mitigate environmental damage caused by current and past generations. The field of environmental restoration is rapidly expanding as time goes on; by knowing how different species of plants will relate positively or negatively with each other will enable us to more effectively restore degraded, damaged, or destroyed ecosystems.

In the United States, our forests were estimated at 414 million hectares in 1630 (46% of total land area); by 1910 that area had decreased to 305 million hectares (34% of total land area). As of 2012, forested land stands at 310 million hectares (33% of total land area), remaining relatively stable since 1910 (Oswalt et al., 2014). However, just because the forest area has remained stable does not mean that the character has not changed. Urban development and reversion to and from agriculture have taken their toll on domestic forests over the years, as have aging and other natural processes. As populations increase, so does the fragmentation (from developments outside yet in close proximity to urban areas) and degradation (from ill-managed runoff, industrial processes, resource extractions, etc.) of forested lands. Additionally, forest health continues to decline from invasive species of plants and animals introduced by the expansion of global trade and travel; areas with the highest rates of introduction tend to be along the coasts or major inland waterways. Human disruption of ecosystems such as suppression of natural disturbance regimes, soil alteration, and removal of vegetative cover promote the introduction and spread of invasive species (Smith et al., 2014).

The challenge that restoration ecologists ultimately face is in identifying unique qualities within an ecosystem, and then using those qualities to assist the area into becoming nearly self-sustainable. Developing a systematic approach to this venture becomes of utmost importance, as monitoring progress and using adaptive management skills would ultimately be impossible without such an approach. Forest restoration in particular often involves the manipulation or establishment of understory vegetation in addition to trees. Although there are many things one must learn in order to restore an ecosystem to a healthy condition, the keystone to the entire process is building a self-sustaining baseline of vegetation to re-establish a desirable successional trajectory; in order to do that, one must understand plant interactions.

Direct interactions between plants can be described as interference, where interference refers to the proximity of one plant (Plant 1) altering the growth, reproduction, or survival of another plant (Plant 2) (Newman, 1983). The alteration of Plant 2 can be either beneficial or harmful; it should be noted that Plant 2 will not necessarily also affect Plant 1. Competition, therefore, can be described as a type of interference. Competition occurs when two plants compete for the same
limited resource. The supply of this resource to one plant may be diminished by the presence of the other, and thus the reduced supply results in an alteration to the growth, reproduction, or survival of the other plant (Newman, 1983). Competition is not necessarily reciprocal, such as in the case of a taller plant shading out a shorter plant.

Competition and other forms of interference can occur in many different forms. When planting trees and ground cover together to restore a damaged or destroyed area of the environment, it becomes critical to understand the processes affected.

However, not all interactions between plants are negative. The beneficial effects on crop yield of planting trees or other plants in hedges or as shelterbelts are well known in agricultural practice (Carlsson & Callaghan, 1991), but not as well studied in other instances. In arctic and alpine environments it has been proposed that plant interactions may be positive because plants of these environments are often aggregated into islands surrounded by bare ground (Aleksandrova, 1988). Seedling establishment and survival in arid environments is dependent on the shelter provided by “nurse plants” which protect seedlings from temperature extremes or predation (Carlsson & Callaghan, 1991). In temperate ecosystems, the study of ecological interactions between plants has been dominated by competition (Bertness & Callaway, 1994; Callaghan & Emanuelsson, 1985).

Typically, positive plant interactions take place in environmentally extreme locations. Environmental factors constraining plant growth or survival tend to be alleviated by the physical presence of another plant. This means that the positive interaction does not usually occur through the exchange of resources but rather through an amelioration of the conditions of the external environment (Hunter & Aarssen, 1988). As a result of this, the strength of the interaction is often density dependent (Callaghan & Emanuelsson, 1985). Increased plant density can lead to increased litter accumulation (Wilson & Keddy, 1986), improved water uptake via hydraulic lift (Okland & Okland, 1996), increased soil stability (Heilbronn & Walton, 1984), and/or decreased evaporative water loss (Bertness & Hacker, 1994).

Positive interactions, therefore, should also be considered when formulating a restoration plan, particularly in an extreme environment. By pairing species that facilitate one another, success can be more easily achieved. The careful consideration of positive and negative interactions between chosen species will result in a better outcome.

Plant growth and physiology is influenced by many factors, but it is important to first be able to differentiate between the growth characteristics of annual and perennial plants. The early stages of annual plant growth are characterized by the full direction of all photosynthetic compounds into the formation of roots and leaves (Schulze, 1982). Maximum stem growth occurs just before anthesis; it is this period of time that the upper and lower leaves’ carbohydrate production are reassigned to the seed head formation and food for the stem and roots, respectively. Once the flower is formed, all growth except for that put into seeds declines sharply. At the end of an annual plant’s life cycle, all energy produced by the plant is directed into increasing seed weight.
Herbaceous and woody perennial plants have a very similar growth cycle to annuals, except that they are able to shed leaves and roots continuously or seasonally without mortality (Schulze, 1982). This means that during arid or cold conditions, perennial plants can become dormant, with perhaps only a single main bud and a few roots containing the energy reserves needed to initiate new growth after dormancy. While the annual gain in biomass may be low, the seasonal production can be very high. Additionally, perennial plants can commence vegetative growth and photosynthesis before annuals have germinated, continuing through the time in which annual plants have dedicated all their resources to seed production. Hence, woody perennials have an even greater advantage over herbaceous perennials in that they are able to produce a large amount of permanently functional biomass. These advantages all stem from perennial plants possessing the capability to store resources in perpetuity to initiate later growth.

Trees differ from annuals and other perennials in that their primary production is at their youngest age when leaf biomass is proportionally far higher than woody biomass (Schulze, 1982). As woody tissues increase in trees, leaf biomass decreases or remains constant even though respiratory requirements increase. Trees generally outcompete other vegetation for light at older ages, giving them the ability to make do with less leaf biomass. Thus it can be said that competitive interactions are most critical in the early years of a tree’s growth.

The many elements that must come together to ensure a successful outcome to a restoration project can make the planning and execution stages quite difficult. Species of both herbaceous vegetation and trees must be chosen carefully to match the site conditions of a given area. Factors such as water and nutrient availability, soils, plant chemistry, and microbial presence must all be considered when planning a revegetation effort. Failure to consider all aspects of the venture may doom an otherwise potentially successful project to mediocrity or failure.

Plants, like all other living beings, require water not just for hydration, but for most biochemical reactions of metabolic events. Water also affects the physical structures of organic molecules; acting as a solvent or assisting in biochemical reactions such as hydrolysis and dehydration. The transport of nutrients and organic compounds is also assisted by water; maintenance of leaf temperature in direct sun is regulated by water as well (Fagerstedt, 2009).

There are many ways during ecological succession that plants facilitate subsequent organisms in water acquisition. Soil surface coverage by herbaceous plants and their litter has the potential of increasing soil water content by increasing infiltration and decreasing evaporation from the soil surface (Evans et al., 1981; Knoop & Walker, 1985). The addition of organic matter also increases the water holding capacity of the soil. The deep roots of woody plants can transport water to shallower soil layers using hydraulic lift where it may be utilized by shallow-rooted plants (Caldwell, 1990; Dawson, 1993).

Stiff competition for water can affect plant physiology. In a study done in the early 1980s, controlling weeds in a Pinus radiata planting increased water uptake in the trees and was reflected in almost double the height gain in the weed-free plots as compared to the weedy plots (Sands & Sadanandanam Nambar, 1983). Stem volume was also similarly affected. It was found that as the trees aged, the water loss affected them less and less, due to the fact that they
obtained water from a different part of the soil profile as they aged. When developing a restoration plan for an area, one could easily mismatch organism types, leading to a catastrophic failure when trees and herbaceous vegetation begin to compete for water resources.

In a series of plant removal experiments performed in arid regions, it was found that when water availability was low, leaf water potential was affected by the presence of neighbors (Fonteyn & Mahall, 1981; Fonteyn & Mahall, 1978). In subsequent studies, measurements of leaf conductance to water vapor taken with a leaf porometer, or stomatal conductance, has been found to be affected as well (Ehleringer, 1984). Although the response of stomata to environmental and physiological factors is complex, it is well known that stomatal conductance varies with leaf irradiance, leaf temperature, atmospheric water vapor pressure deficit, and CO₂ concentration. Additionally, stomatal conductance depends on guard cell and epidermal turgor; regulation of turgor in these cells requires metabolic energy. Leaf turgor also depends on the balance between loss of water through transpiration and supply of water to the leaf from the soil (Tuzet et al., 2003). Using a leaf porometer to measure stomatal conductance is a simple and quick way to test transpiration and therefore stress in plants.

In most terrestrial ecosystems, plant growth is limited by nitrogen, but phosphorus limitations also occur frequently (Aerts & Chapin III, 1999). Nutrient deficits can be caused by lack of nutrients in the soil itself, as well as by competition from other plants in soils without nutrient deficiencies; the combined effects will work to intensify the outcome. The significance of competition for water versus nutrients is difficult to establish in environments where both are limited because water has an overriding effect on the availability of nutrients in soil and therefore uptake by plants (Sadanandan Nambiar & Sands, 1993).

Nutrient availability to plants can be greatly affected by soil pH. Nitrogen mineralized from organic matter, such as that found in a forest setting, takes the form of ammonium. In alkaline soils, ammonium becomes ammonia, and can be volatilized, preventing uptake by plants (Miller, 2016). In acidic soils, the additional hydrogen helps maintain ammonium concentrations. Uptake of nitrate by plants is best at a lower pH, while ammonium is absorbed more efficiently at a neutral pH. Denitrification, which transforms nitrate into gaseous nitrogen in waterlogged soils, occurs at lower rates in acidic soils (pH < 5).

Optimum phosphorous availability is at a pH of 6.5. Below 6.5, phosphorous becomes insoluble aluminum/iron minerals or absorbs to oxides and clay (Miller, 2016). Above 6.5, phosphorous bonds with calcium to form solid minerals similar in structure to calcium-phosphate fertilizers. Potassium, calcium, and magnesium are usually less available in acidic soils because they have been leached out, not necessarily due to solubility issues. Aluminum can also limit potassium availability by dominating soil cation exchange capacity (CEC). Compared to potassium, calcium and magnesium are more competitive with aluminum for CEC sites. Unfortunately, high levels of manganese and aluminum may damage plants’ roots, preventing uptake of calcium, magnesium, and potassium.

While alkaline soils are associated with greater concentrations of calcium, this can be in the form of precipitated lime (Miller, 2016). Conversely, acidic soils tend to retain the ionic form of sulfur better. When elemental sulfur is added to soil, it creates sulfuric acid (lowering pH).
However, compounds containing gypsum (such as certain sulfur containing fertilizers) do not have the same ability to lower pH.

Most micronutrients decrease in availability as pH rises, with the exception of molybdenum (Miller, 2016). Zinc, copper, and manganese decrease 100-fold in concentration with every one-unit increase in pH. These nutrients are not lost, but rather precipitated into solid minerals, rendering them unavailable to plant roots. Severe micronutrient deficiencies will cause obvious visual symptoms in the field such as chlorotic leaves. If a micronutrient deficiency is observed in an acidic soil, it is probably related to lower concentrations and the leached nature of the soil.

High diversity of species in a forest ecosystem is often related to low soil nutrient levels; low nutrient soils exhibit low plant growth rates and therefore longer periods of coexistence between organisms, leading to richer diversity. Higher levels of soil nutrients result in faster growing plants becoming dominant, therefore outcompeting slower growing plants and resulting in lower species diversity (Huston, 1979; Van der Moezel & Bell, 1984). Interestingly enough, species from nutrient-poor habitats do not necessarily allocate more biomass to their roots than species from more fertile environments. Instead of adding biomass to the roots, these plants adapt by having a high specific root length (SRL: root length per unit root mass) (Aerts & Chapin III, 1999). Plants adapted to infertile soils normally have a high capacity to absorb mobile ions such as potassium (Veerkamp & Kuiper, 1982), but a relatively lower capacity to absorb immobile ions like phosphate (Chapin III et al., 1986; Raab et al., 1999).

Weedy plants present the greatest source of competition to young tree seedlings, for both water and nutrients, during the first year after planting (Sadanandan Nambiar & Sands, 1993). Nutrients are especially hard to compete for because unlike water, which can be gained by growing deeper roots, nutrients are generally restricted to the top soil layer. A study in the late 1980s on pine plantings showed that 90% of nitrogen mineralized in the upper 30cm of a soil profile was produced by the top 15 cm of soil (Smethurst & Nambiar, 1989). Tree roots were outcompeted by weed roots in this thin layer of soil by 50 to 100-fold.

The very presence of vegetation can facilitate subsequent organisms in succession. Elevated concentrations of nutrients in the soil under woody plants, as compared to that in open areas between woody plants, have been found in arid ecosystems. These areas of relative fertility appear to be due to accumulation of wind-borne organic material and litter deposition (Holzapfel & Mahall, 1999).

The mass flow of water and dissolved nutrients is driven by plant transpiration and is a function of both the rate of water flow to the root and the concentration of dissolved nutrients in the soil solution. Diffusion of nutrients toward the root occurs when plant nutrient uptake exceeds nutrient supply by mass flow, creating a concentration gradient. Diffusion is important for nutrients with large fractions bound to the solid soil matrix, such as potassium and phosphate; mass flow is important for nitrogen, particularly nitrate. The supply of nitrogen, phosphorous, and potassium largely depends on diffusion and mass flow working together; the two processes can be nearly impossible to separate experimentally in the field (Nye & Tinker, 1977).
Competition between plants for nutrients can be measured by quantifying the role of root interactions in reducing resource uptake, biomass production, or fecundity. Measurements at a large scale (population or community level) generally estimate competition directly from biomass increases when interactions with neighboring roots are prevented through the use of root exclusion tubes, trenching, or neighbor removal. Regrettably, these methods often alter the soil environment, possibly affecting the availability of resources for which the plants are competing in the process. Resource-based measurements normally involve less manipulation of the environment, but the integrated consequences of competition for plant performance are often unknown or require assumptions of scaling. Combining large-scale and resource-based methods of measurement is necessary to document both the resource intermediary and the ecological significance of belowground competition (Casper & Jackson, 1997).

The extent to which plant size offers a competitive advantage can be an important difference between belowground and aboveground competition for nutrients. The advantage of plant size in competing for light occurs because larger plants shade smaller ones; it is this suppression of smaller plants that results in the development of size hierarchies that become more notable over time. Plants that are larger aboveground do not seem to possess an advantage in competing for belowground resources (Weiner, 1990). Belowground competitive ability appears to be size-symmetric; root interactions with neighbors hamper plant growth but do not increase size variation among competing individuals. A study performed in the 1990s examining nitrate and ammonium uptake as a function of plant size showed that within crowded populations of yellow birch seedlings grown in tubs, individuals acquired nitrate and ammonium in direct proportion to several measures of root system size (Casper & Jackson, 1997).

A study performed in the 1980s in the northern taiga showed that trees perform most of their nutrient uptake in the spring and summer months. Leaf samples taken in the autumn suggested that about half of the nitrogen and phosphorous retranslocation occurred during the few days of color change, immediately prior to abscission. (Chapin & Kedrowski, 1983). The seasonal patterns of leaf and stem growth reported in this study for taiga trees are typical of both northern and temperate trees (Chapin, 1980; Kozlowski & Keller, 1966; Tilton, 1977) and shrubs (Grigal et al., 1976; Johnson & Tieszen, 1976). Deciduous species break bud in early spring and gain leaf mass until mid- or late summer, after which time there is a variable loss in leaf mass associated with senescence and retranslocation prior to leaf abscission. This means that trees, unlike herbaceous vegetation, can store nutrients during dormancy and use it in the spring with minimal nutrient uptake from the soil pool.

Resorption of nutrients from senescing leaves by trees is an important adaptation because it enables plants to reuse nutrients, thereby leading to a higher nutrient retention (Aerts, 1990; Chabot & Hicks, 1982; Chapin, 1980). This process has significance at both the population level and the ecosystem level. At the ecosystem level, nutrient resorption from senescing leaves has a profound influence on element cycling. Nutrients that are resorbed during senescence are directly available for further plant growth, which makes species less dependent on immediate nutrient uptake (Aerts & Chapin III, 1999).

Nutrients that are not resorbed will be circulated through litterfall. The litter must be decomposed and the nutrients contained in that litter must be remineralized to become
available for plant uptake again. Compared with the resorption pathway, this dependency of plants on the decomposition pathway has the disadvantages that each plant must compete for the mineralized nitrogen with micro-organisms (Kaye & Hart, 1997) and with neighboring plants, and that part of the nitrogen can be incorporated in stable soil organic nitrogen pools and become unavailable for plant uptake (Aerts, 1997). However, low molecular organic nitrogen compounds can be taken up by mycorrhizal (Read, 1991) and non-mycorrhizal plants and provide a large part of the annual N requirement (Kielland, 1994). A repeated suggestion of early studies based on comparison of a few plant species was that plants from nutrient-poor environments have a higher nutrient resorption efficiency than those from more nutrient-rich environments.

Most of the nutrients garnered by annual plants are devoted directly to vegetative growth with minimal reserve storage. When reproduction commences, the senescence of roots and leaves begins, with nutrients being recycled from vegetative to reproductive tissues. Generally, 50-90% of nitrogen and phosphorous but less than 5% of carbon is recycled from vegetative to reproductive tissues (Chapin III & Wardlaw, 1988). Annuals also show relatively modest short-term nutrient accumulation in response to increases of nutrient supply; their rapid growth allows increases in growth to match increases in supply (Chapin III et al., 1990).

Most perennial plants depend strongly on storage, but the type of storage differs among species. Biennials and species adapted to frequent disturbances develop large nutrient storage reserves, even under conditions of nutrient limitation (Aerts & Chapin III, 1999). It can be said that evergreen species depend less on reserve storage than deciduous species as much of leaf senescence occurs with new growth. This allows for the direct recycling of nutrients from old to new leaves (Chapin III & Shaver, 1989; Nambiar & Fife, 1991).

When restoring areas with poor soils, it becomes increasingly important to choose a proper mixture of plants to establish nutrient availability in soils for both the long and short term. If the soil needs to be covered immediately in vegetation, then the choice of plants should ideally be ones that will contribute to the soil nutrition once they die (such as short-lived annuals) or contribute while they live with minimal influence on the establishment of other species. Additionally, subsequent vegetation or trees also need to be carefully chosen in order to coexist in harmony in this situation.

Plant hormones can best be described as naturally occurring substances which can influence physiological processes at even low concentrations (Davies, 2010). Influenced processes include growth, development, and differentiation; other processes such as stomatal movement can also be affected.

Developmental plasticity, the expression of variability amongst individuals of an identical genotype triggered by different input signals, is a hormonal response that is frequently overlooked in modern ecology teachings (Aphalo et al., 1999). Details of the mechanisms which allow plants to sense inputs and react to them tend to be left out in favor of more in-depth discussions of competition in the traditional sense. The inherent problem with this oversight is that less intricate competition models cannot account for the active morphological plasticity of plants, which in many situations is critical to the outcome of survival.
It is well established that plants attacked by herbivores or pathogens can communicate via chemical signals through the air to other plants to “warn” them of imminent attack (Song et al., 2010). These signals allow neighboring plants to anticipate damage and therefore utilize direct defense, by increasing levels of repellents and toxins (Farmer & Ryan, 1990), or by indirect offense, by attracting natural enemies (Baldwin et al., 2006; Bruin et al., 1995).

The detection of neighboring plants can be accomplished indirectly through changes in resource availability, or directly through above or below ground chemical signals. Hormonal interactions, therefore, are not typically plant-plant interactions, but rather reactions to the outside environment within a plant. Nevertheless, they certainly can be, as many volatiles (such as ethylene) are plant-plant communication signals.

When one plant produces a chemical that influences another plant, this process, which can be harmful or beneficial, is known as allelopathy (Newman, 1983). Allelopathy can also include chemical interactions mediated by microorganisms. With respect to plant interference, allelopathy has been reported in the literature for over 2000 years (Weston & Duke, 2003). Allelopathic chemicals are present in almost all plants; leaves, stems, buds, bark, roots, seeds, and flowers can all possess allelopathic compounds.

Many studies have been done on allelopathy and competition, but not very many have been done on the two combined effects (Ridenour & Callaway, 2001). Besides the Ridenour paper just mentioned, there was a study in the 90s that determined leaf leachates of *Empetrum hermaphroditum* and resource competition both contributed to the suppression of *Pinus sylvestris* seedling growth (Nilsson, 1994).

In an in-pot study, increasing the abundance of *Centaurea maculosa*, a noxious weed found in western North America, was found to be correlated with a decrease in the abundance of *Festuca idahoensis*, a native bunchgrass. However, the balance of competition shifted in favor of *Festuca* when the effects of *Centaurea* root exudates, as well as potential leaf leachates working through the soil medium, were ameliorated with activated carbon. The results of these experiments provide experimental evidence that allelopathy may play an important role in the overall competitive balance between these species. In this case, the contribution of allelopathy to interference was estimated under greenhouse-like conditions (readily available water, nutrients, and light). Under natural conditions, these species would have fewer available resources; the importance of resource competition would therefore increase (Ridenour & Callaway, 2001).

What this means to us as restoration ecologists is that we must consider all aspects of such relationships when choosing seed mixes and trees to plant. Even if a tree or herbaceous plant is only mildly allelopathic, it could become vastly more so given the constraints of a nutrient-deficient environment.

Mycorrhizae are another feature in the vast and complex web of plant interactions. Plants in both natural ecosystems and agricultural settings are mycorrhizal and acquire a large proportion of their nutrients via mycorrhizae. Mycorrhizae are one of the most important groups of fungi within the forest flora, and are thought to acquire nutrients at a lower carbon cost than roots because of their smaller diameter and greater surface to volume ratio (Aerts & Chapin III, 1999). In temperate ecosystems, the ability to take up organic nitrogen sources is
restricted mainly to plants with ectomycorrhizae (ECM) and hardly occurs in species with vesicular-arbuscular mycorrhize (VAM) and in non-mycorrhizal (NM) plants (Smith & Read, 1997). While most temperate trees form a symbiosis with ECM fungi, VAM fungi are generally associated with herbaceous plants or tropical trees (Smith & Read, 1997). All mycorrhizae act as a sink for mineral ions in the surrounding soil, forming an important mutualistic symbiosis with their host plant to assist with nutrient cycling (Dighton & Mason, 1985).

A relatively significant part of fixed carbon is lost to soil through tree roots (Lynch & Whipps, 1990); it has been shown that more bacteria and fungi are found in the soil directly surrounding the roots than in bulk soil (Newman, 1985). Mycorrhizae also produce vitamins and hormones which appear to enhance root size and longevity (Slankis, 1973). In turn, these fungi depend on their hosts for their carbon and energy requirements, demonstrating a unique mutualistic symbiotic biotrophy between fungus and higher plant (Lewis, 1973). The uptake of organic nitrogen compounds by both mycorrhizal and NM plants is a unique aspect of the terrestrial nitrogen cycle. This ability may be of adaptive significance in nitrogen-poor habitats; lending some plants access to a nitrogen source of which other species are deprived (Aerts & Chapin III, 1999). Mycorrhizae expand the physical area of tree roots, not only enabling the plant to have enhanced nutrient cycling capabilities, but also to draw water during times of drought (Egerton-Warburton & Querejeta, 2007).

Ectomycorrhizal fungi play an important role in both tree nutrition and carbon balance, supplying soil resources to their plant hosts in exchange for sugars (Smith & Read, 1997). ECM trees dominate nitrogen-limited forest ecosystems; the ECM themselves vary in their nitrogen uptake physiology (Chalot & Brun, 1998; Smith & Read, 1997). ECM fungi are the prevailing plant root symbionts in boreal coniferous and mixed forests (Allen et al., 1995; Dahlberg, 2001), which cover massive areas of the northern hemisphere. Most fine roots and ECM of trees are aggregated in the uppermost 20 cm of soil (Schenk & Jackson, 2002), where nutrient circulation is most rapid.

Approximately 80% of terrestrial plants establish a mutualism with VAM fungi (Smith & Read, 1996). VAM symbiosis can alter rates of water movement into, through and out of host plants, consequentially affecting tissue hydration and leaf physiology. VAM and NM plants often display different transpiration rates and stomatal conductances. Although these rates differ in VAM and NM plants, with only a few exceptions rates have been higher in VAM plants (Augé, 2001).

Mycorrhizae can assist in plant-plant communications, as well. Communication via volatile signals through the air is at the whim of atmospheric conditions (Song et al., 2010). However, mycorrhizal fungal mycelia have adapted to extend from one plant’s roots to another, forming what is referred to as common mycorrhizal networks (CMNs). They are able to form CNMs due to the lack of specificity of mycorrhizal fungi (Chiariello et al., 1982; Selosse et al., 2006). Different plants and even different species are able to be interconnected via CNMs, allowing plant-plant communication throughout these networks.

It would seem that the presence or absence of mycorrhizae adds another significant level to the success of a restoration project. If plants can and will communicate or share substances through CNMs, then this could be yet another element in the restoration equation that will need to be
considered. Since mycorrhizae are not the most well understood organisms, this may prove to be quite difficult.

Forest productivity and species composition differ highly among sites that differ in soil properties (Binkley & Giardina, 1998). Parent material, soil texture, and structure all contribute to these differences either together or as separate entities. Additionally, trees themselves can change soils differently, depending on species, using biogeochemical and evolutionary mechanisms (Binkley, 1994).

Forest soils differ significantly from soils found in other vegetation types in that the trees contribute to typically developing surficial O horizons, modifying the microclimate at the surface, and altering the biological, chemical, and physical features of the soil. Effects on soil by trees differ between species of trees; the mechanisms that species employ to change soils include rates of nutrient cycling, inputs, and outputs. It can be said that these differences may have developed from direct trait selection that increase fitness, such as rates of nitrogen fixation or rates of the chemical weathering of parent material (Binkley & Giardina, 1998).

In Michigan, USA, the presence of long term stands of sugar maple (*Acer saccharum*) or eastern hemlock (*Tsuga canadensis*) appear to be directly controlled by the effects of the respective species on soil nitrogen availability (Frelich et al., 1993). Long term stands of western hemlock (*Tsuga heterophylla*) or western red cedar (*Thuja plicata*) in Vancouver Island, Canada, demonstrate the perpetuation of dominant species via patterns of nitrogen and phosphorous availability in addition to long-term disturbance patterns, which subsequently lead to massive differences in soil development (Keenan et al., 1993; Prescott et al., 1993).

Trees affect nutrient, light and water availability of understory vegetation simultaneously (Scholes & Archer, 1997). By increasing nutrient availability, trees facilitate the growth of understory vegetation; higher soil fertility underneath tree canopies has been reported (Ludwig et al., 2013). It is thought that trees act as a nutrient pump, pulling nutrients from deeper soil layers or from soil outside the canopy and depositing them under their canopy via litterfall or leaching (Kellman, 1979; Scholes, 1990). Other possibilities are that trees are an effective trap for atmospheric dust or attract mammals which defecate underneath the canopies (Belsky, 1994; Bernhard-Reversat, 1982; Georgiadis, 1989).

The effects of trees on water availability are less clear than the effects on nutrient availability. There are reports of increased soil moisture content under trees as compared to open grassland, which is presumably caused by either decreased transpiration of understory plants or hydraulic lift. Hydraulic lift is the process of water movement from deep, relatively wet to dry and shallow soil layers through the roots of plants that have access to both deep and shallow soil layers (Ludwig et al., 2013). Other studies showed reduced soil water availability under trees due to a high water uptake (Amundson et al., 1995; Anderson et al., 2001). Shade can have both positive and negative effects on understory plant production. Reduced light availability limits plant growth but lowers temperatures, resulting in improved water availability and potentially increased growth (Anderson et al., 2001; Belsky, 1994).
Soil changes brought about by trees is yet another factor that could be used to an ecologist’s advantage when mitigating an area. By pairing trees that are known to change soil in certain ways with ground cover that flourishes from those changes, we can more easily establish a baseline of vegetation that can act as a keystone for an ecosystem.

The interactions between plants is a particular problem in the reforestation of coal mines. The Industrial Revolution drove coal extraction efforts in Southern Appalachia to a new high in the late 1800s (Shifflett, 1995), promoting massive growth in the southern United States. Prior to the Surface Mining Control and Reclamation Act of 1977 (SMCRA), reclamation was not required, but following 1977 reclamation mostly consisted of planting invasive, fast growing trees and ground covers (Franklin et al., 2012). Historically, mine soils and overburden were often graded smoothly to prevent erosion, causing severe soil compaction. Sites were seeded with aggressive ground covers in an attempt to provide rapid establishment, but these dense herbaceous covers compete strongly with tree seedlings. On many of these sites the typical successional sequence, one that results in the establishment of hardwood forest, has failed to develop. These areas, characterized by shrubby and primarily non-native vegetation, are referred to as “legacy mine sites”.

Former mine sites have severe abiotic limitations to forest restoration such as poor soil chemical and physical properties. However, there are many sites that actually have soil and spoil materials with properties favorable to trees (Zipper et al., 2011). Soils dictate a reclaimed mine site’s productivity long term, but herbaceous vegetation strongly influences reforestation success short term.

Factors to consider when selecting ground cover species for planting on mine sites are germination, vigor, suitability for the site, non-invasiveness, and mature leaf area. These species must be able to tolerate disturbed areas, full sunlight, intense competition, rocky and inconsistent soil, and generally poor conditions. Additional favorable characteristics of groundcover species are early germination, rapid growth and an annual life cycle. These characteristics may help them to compete with tall fescue (*Festuca arundinacea*), a common non-native grass widely planted on legacy mines that has dense, fibrous roots that interfere with tree seedlings. Among the many species for which seeds are commercially available, nine may be particularly well suited for legacy mine restoration, based on the above criterion along with their value for pollinators or wildlife.

Ragweed (*Ambrosia artemisifolia*) is an annual forb native to Tennessee (USDA, 2018a). It releases pollen during late summer or early fall, after which numerous seeds are produced, which can remain viable for 5 years or more. The extensive root system is fibrous. Ragweed prefers full sun and average to slightly dry conditions; it will thrive in soil containing high amounts of clay, gravel, or sand (Hilty, 2017b). Drought resistance is excellent. This plant thrives in a variety of disturbed sunny sites, especially where the topsoil has been exposed (such as legacy mine sites). It has allelopathic properties that can inhibit the growth and development of neighboring plants.

Ragweed is very valuable to many kinds of wildlife. Honeybees have been observed collecting pollen from the male flowers (Hilty, 2017b). The caterpillars of several moths eat the foliage,
flowers, or seeds, including the ragweed flower moth (*Schinia rivulosa*), wavy-lined emerald (*Synchlora aerata*), small bird-dropping moth (*Tarachidia erastrioides*), olive-shaded bird-dropping moth (*Tarachidia candefacta*), and others. Many upland gamebirds and granivorous songbirds are attracted to the oil-rich seeds; because the seed spikes often remain above the snow, they are especially valuable to these birds during winter. Ragweed seeds are also eaten to some extent by the thirteen-lined ground squirrel, meadow vole, and prairie vole. The seeds are semi-digestible, thus are likely distributed by these animals. Ragweed foliage is quite bitter; therefore mammalian herbivores do not often consume it.

Annual rye (*Lolium multiflorum*) is a non-invasive, annual grass naturalized in Tennessee (Peterson, 2002). This grass is usually an annual, although sometimes it is a biennial or short-lived perennial. The blooming period can occur from late spring to fall, but lasts only 2 weeks; the florets are wind-pollinated (Hilty, 2017c). The root system is fibrous; however, annual rye spreads by reseeding itself. It prefers full or partial sun, moist to dry-mesic conditions, and fertile loam, although other kinds of soil are tolerated. Growth and development are rapid, but ryegrass tends to be short-lived. Naturalized habitats include disturbed meadows (such as legacy mine sites), roadsides, fields, and waste areas. Annual rye is cultivated as a source of quick vegetative cover where the ground has been exposed by anthropogenic activities. It is also used as a source of forage, particularly in the southern United States.

Insects that feed on rye grass include the flea beetle (*Chaetocnema plicaria*), bird cherry or oat aphid (*Rhopalosiphum padi*), bronzed cutworm (*Nephelodes minians*), and larger sod worm (*Pediaisia trisecta*) (Hilty, 2017c). Cattle, sheep, and other domesticated farm animals readily graze on the foliage, particularly while it is still young and immature. When this grass is planted near bodies of water, Canada geese will browse the foliage.

Partridge pea (*Chamaecrista fasciculata*) is an annual legume native to the eastern and midwestern portions of the United States (Row, 2002). The blooming period is from mid-summer to fall; during the fall, seed pods develop. The root system consists of a central taproot and smaller auxiliary roots (Hilty, 2017d). Partridge pea prefers full sun and average to dry conditions. The soil can contain sand, loam, gravel, or clay. The plant tends to favor poor soil due to reduced competition from other plants. Partridge pea can spread readily in dry, open situations; habitats include mesic to dry black soil prairies, sand prairies, savannas, limestone glades, abandoned fields, open areas along railroads and roadsides. It can be planted to stabilize banks around ditches and other areas; it favors disturbed areas (such as legacy mine sites).

Long-tongued bees are responsible for pollination of the flowers, which include honeybees, bumblebees, long-horned bees (*Melissodes* spp.), and leaf-cutting bees (*Megachile* spp.) (Hilty, 2017d). They are initially attracted to the food pollen of the purple anthers, and are subsequently dusted by the reproductive pollen of the yellow anthers. Two species of bees, *Anthophora walshii* and *Svastra atripes atripes*, are oligoleges of partridge pea. Leaf-cutting bees have been observed cutting off portions of the petals for their brood chambers. Flowers are cross-pollinated by insects, but sometimes self-pollinate. The petiolar nectaries attract a completely different assortment of insects, which includes halictid bees; wasps, flies, ants, and
velvet ants (Mutillidae). The caterpillars of several sulfur butterflies feed on the foliage of this plant, including little sulfur (Eurema lisa), sleepy orange (Eurema nicippe), and cloudless sulfur (Phoebis sennae cubule). Other insects that feed on partridge pea include the bean leaf beetle (Cerotoma trifurcata) and partridge pea seed beetle (Sennius cruentatus). The seeds are an important food source for the bobwhite quail (Colinus virginianus) and greater prairie chicken (Tympanuchus cupido). The foliage of the partridge pea is strongly cathartic and thus usually avoided by grazing animals; however, white-tailed deer (Odocoileus virginianus) occasionally browse on the foliage in limited amounts.

Black mustard (Brassica nigra) is a non-invasive, annual forb introduced to most of the United States (USDA, 2018b). The blooming period occurs primarily during the summer and lasts about 1-2 months. A few plants may bloom during the fall. Each flower is replaced in the fall by a slender silique containing dark brown or black seeds. The root system consists of a thick taproot; this plant spreads by reseeding itself. Black mustard prefers full or partial sun, fertile soil, and mesic conditions. On less fertile ground, it tends to be smaller in size. Habitats include weedy meadows, thickets, areas along railroads and roadsides, fallow fields, vacant lots, and miscellaneous waste places. Disturbed areas are preferred, making this plant ideal for mine sites; black mustard has not been found to invade high quality natural areas to any significant degree.

The nectar and pollen of the flowers attract primarily small bees and flower flies; less common visitors include white butterflies and wasps (Hilty, 2017a). The foliage is occasionally eaten by the caterpillars of various white butterflies, including cabbage white (Pieris rapae) and checkered white (Pontia protodice). The foliage is usually avoided by mammalian herbivores as it is somewhat toxic to them.

Lablab (Lablab purpureus) is a non-invasive legume that has been introduced to parts of the eastern United States (Sheahan, 2012a). It is an herbaceous, climbing, warm season annual or short-lived perennial with a vigorous taproot. Lablab is a short-day annual, flowering in response to longer nights in late-summer and fall. It is mainly self-fertilizing, and will set seed within the first year after planting. Flowering and seedpod production is sporadic. The plant can grow in a variety of soils, from sand to clay (such as soils found on legacy mine sites), but it does not grow well in saline or poorly-drained soils. Lablab can continue to grow in drought or shady conditions, and is more drought resistant than other similar legumes due to its ability to access soil water up to 1.8m deep. Lablab is used as a nitrogen-fixing green manure to improve soil quality. It not only produces nitrogen through fixation, but returns nitrogen through leaf decay. Initially growth is slow, but once established, it competes well with weeds. It has an extensive root system that improves the physical condition and function of the soil. Although not much is known about lablab in the wild, pollinating insects such as bees and wasps have been known to frequent the flowers, and mammals such as deer and rodents will eat the mature seeds.

Sorghum (Sorghum bicolor) is a non-invasive, annual grass introduced to the United States and parts of Canada (USDA, 2018j). It is a short-day, summer annual. Sorghums can exhibit different heights and maturity dates, but generally flower from Aug to October, with seeds
ripening from September to October (Duke, 1983). The flowers are hermaphroditic and wind pollinated. Sorghum is adapted to wide range of soils varying from light loams to heavy clays (such as those found on legacy mine sites), but it thrives best on light, easily worked soils of high fertility, with moderate to high available water, with negligible erosion. Moderately well-drained soils are suitable for sorghums, but it is not tolerant of frost, shade, or sustained flooding.

Sorghum is used as a drought tolerant, summer annual rotational cover crop either alone or seeded in a warm season cover crop mixture (Dial, 2012). All sorghum species have the potential to smother weeds, suppress nematode species, and penetrate compacted subsoil. Sorghum has an extensive root system that can penetrate up to 2.5m into the soil and extend more than 1m away from the stem. These aggressive root systems can help alleviate subsoil compaction. Sorghum is a quick growing grass that has the potential to shade out and/or smother weed populations when planted at a high density. In addition, root exudates of sorghum have been shown to reduce the growth of weeds such as velvet leaf, thorn apple, redroot pigweed, crabgrass, yellow foxtail, nutsedge, and barnyardgrass.

Some cultivars of sorghum harbor beneficial insects such as seven-spot lady beetles and lacewings (Dial, 2012). It also provides an excellent seed source for birds. Despite being planted during spring and summer, sorghum can provide a food source for deer during the fall and winter. Sorghum is also a highly attractive and digestible source of nutrition for deer that provides a source of carbohydrates during the winter months. In the southern US, where late-summer can be a nutritional stress period for deer, deer often consume sorghum seed heads as soon as they are mature in August or September.

German millet (Setaria italica) is a non-invasive, annual grass introduced to much of the United States (USDA, 2018i). It is a warm-season crop, self-pollinated, and will produce seed in 75–90 days from germination (Sheahan, 2014). German millet experiences a very quick transition period from vegetative growth to flower development. It can grow in sandy to loamy soils, and will grow rapidly in warm weather. Millet can grow in semiarid conditions; however, it has a shallow root system that does not easily recover from drought. German millet mostly occurs as a facultative upland plant that usually occurs in non-wetlands, but it may occasionally grow in wetlands.

German millet is similar to other warm-season grasses in terms of forage quality and can be used as a weed-suppressing smother crop (Sheahan, 2014). It can be useful as a dead-standing winter cover, or to help suppress weeds when used in rotation after winter wheat. Due to its characteristic as a fast-growing grass that produces more biomass than annual rye, it is sometimes the preferred choice for restoration of mine lands or steep slopes (Burger et al., 2009). Many granivorous birds are attracted to millet and it is often included in finch and exotic birdseed mixes. It is also planted in food plots for deer, turkey, quail, and dove.

Smartweed (Polygonum pensylvanicum) is an annual forb native to most of the United States and parts of Canada (USDA, 2018e). The blooming period occurs during the summer or early autumn, lasting about 1 month (Hilty, 2017e). The root system consists of a taproot with smaller and shallower lateral roots. Smartweed prefers full or partial sun, moist conditions, and rich
loamy soil. Standing water is tolerated if it is temporary; it also has a reasonable tolerance to drought. Habitats include wet prairies, prairie swales, swamps, low areas near ponds or rivers, edges of marshes, degraded seasonal wetlands, abandoned fields, low areas along railroads, roadside ditches, vacant lots, fence rows, and waste areas (such as legacy mine sites). Smartweed thrives on the reduced competition resulting from disturbance.

Smartweed flowers attract many kinds of insects, including honeybees, bumblebees, cuckoo bees (*Epeolus* spp., *Tripeolus* spp.), digger bees (*Melissodes* spp.), leaf-cutting bees (*Megachile* spp.), halictid bees, andrenid bees, sphecid wasps, vespid wasps, spider wasps (*Anoplius* spp.), syrphid flies, tachinid flies, flesh flies (*Ravinia* spp.), and miscellaneous butterflies and moths (Hilty, 2017e). Most of these insects feed on the nectar and assist in cross-pollination of flowers. Other insects feed on the leaves, roots, plant juices, and other parts of smartweed. These species include the red-headed flea beetle (*Systena frontalis*) and other leaf beetles; larvae and adults of various weevils, the polygonum aphid (*Capitophorus hippophaes*) and other aphids; larvae of the dock sawfly (*Ametastegia glabrata*) and other sawflies; larvae of the bent-line carpet (*Orthonama centrostrigaria*) and other moths, and larvae of the bronze copper (*Lycaena hyllus*) and other butterflies.

Among vertebrate animals, smartweed seeds are an important source of food to many bird species, including waterfowl, upland game birds, and granivorous songbirds (Hilty, 2017e). Seeds are also eaten by small rodents, including the white-footed mouse and wild house mouse. Some turtles feed on smartweed; this includes the snapping turtle (*Chelydra serpentina*), painted turtle (*Chrysemys picta*), eastern box turtle (*Terrapene carolina*), and slider (*Trachemys scripta*). Mammalian herbivores usually avoid smartweed foliage because their leaves are somewhat bitter and peppery.

Sunn hemp (*Crotalaria juncea*) is a non-invasive legume introduced to the United States (USDA, 2018c). It is a shrubby, herbaceous, sub-tropical annual with a long tap root and vigorous lateral roots; root nodules are lobed (Sheahan, 2012b). Sunn hemp is a short-day annual with vegetative growth primarily occurring during the longer days of summer. Blooming occurs in early fall, with seeds appearing shortly after, however it will not reproduce in the US anywhere north of central Florida. It is adapted to a wide range of soils, and is well-suited for sandy soils. Sunn hemp grows well on marginal soils (such as those found at a legacy mine site) and under droughty conditions. In most of the United States, it can only be grown as a summer annual. It is well adapted to, and more productive in high humidity, but is not winter hardy, and has a low to moderate tolerance to saline soils. Sunn hemp is grown in tropical, subtropical, and temperate locations from sea level to approximately 1524m elevation, but is not found in the wild in the United States.

Sunn hemp is crosspollinated by bees and self-pollination occurs only if stigmas are manipulated by insects or humans (Sheahan, 2012). It can be used as a nitrogen-fixing green manure to improve soil quality; to reduce soil erosion, conserve soil moisture, suppress weeds and nematodes, and recycle plant nutrients. Sunn hemp produces allelopathic compounds that suppress sedentary plant-parasitic nematodes such as root-knot (*Meloidogyne* sp.), soybean cyst
(Heterodera glycines), and reniform nematodes (Rotylenchulus reniformis). Deer will browse plants and turkey and quail will use it for shelter and food.

Tree seedlings undergo significant amounts of stress when they are transplanted. Commonly, bareroot seedlings utilized for reforestation come from a nursery, where they receive water and nutrients at regular intervals and in specified amounts. Many seedlings grown in a nursery have the added benefit of mycorrhizae, which can quickly die during transplantation.

Transplantation is generally conducted during the winter months, when the seedlings are dormant, in order to reduce the stress associated with planting. Seedlings are thrust into an often unforgiving environment where they lack the regularity of water and nutrients they have become accustomed to receiving; many will also inevitably lose mycorrhizal relationships they possessed prior to transplant. Therefore, an initial decline in health of transplanted seedlings is to be expected; methods to quantify seedling health are critical to successful reforestation activities.

It is well known that stomatal conductance varies with leaf irradiance, leaf temperature, atmospheric water vapor pressure deficit and CO2 concentration (Tuzet et al., 2003). Stomatal conductance depends on guard cell and epidermal turgor; regulation of turgor in these cells requires potassium as well as metabolic energy. Leaf turgor also depends on the balance between loss of water through transpiration and supply of water to the leaf from the soil. These facts establish transpiration rate as an excellent indicator of water and nutrient stress; as well as stresses caused by other factors.

Leaf chlorophyll content may also be used to assess tree health and function; leaf nitrogen content (Papasavvas et al., 2008) as well as the severity of leaf chlorosis associated with iron deficiency (Peryea & Kammerick, 1997) have both been evaluated using chlorophyll content. When compared statistically, there exists a high degree of correlation between readings taken by chlorophyll meters in the field and laboratory chemical tests, even at higher chlorophyll content levels (Gitelson et al., 1999). Long- or medium-term changes in chlorophyll content have been found to be related to plant photosynthetic capacity (and thus, productivity), developmental stage, and canopy stresses (Gitelson et al., 2005).

Reclaimed mine sites have a high potential to support productive forests. At ground coverages of more than approximately 60%, tree seedling survival drops significantly (Franklin et al., 2012). At low levels of ground cover, the balance of facilitative versus competitive effects is likely determined by other factors. Current reclamation guidelines call for the planting of non-competitive ground cover to facilitate seedling establishment and survival, but non-native grasses are still widely planted. Negative interactions with trees are more frequently reported for non-native cool-season grasses than native warm- or cool-season grasses.

Tall fescue (Festuca arundinacea) is a widely planted, non-native cool season grass that is frequently used for the revegetation of legacy mine sites despite its aggressiveness. It has been and still is grown extensively (12-14 million ha) in the central and southcentral United States for livestock feed, turf, and conservation purposes (Burns & Chamblee, 1979). Tall fescue is the dominant grass seeded in Conservation Reserve Program plantings in the central and
southcentral United States (Osburn et al., 1992). Tall fescue’s aggressive domination of open areas may reduce plant species diversity, decreasing opportunities for native plants and wildlife to thrive (Barnes et al., 1995). Additionally, many post-SMCRA mine sites remain in a state of arrested succession covered by grasses such as tall fescue (Groninger et al., 2007).

Therefore, the purpose of this study is to determine which of nine ground cover species will outcompete tall fescue without outcompeting tree seedlings on a legacy mine site. It is hypothesized that ground cover species that do not have a shallow root system with an extensive surface area will be less competitive with tree seedlings than species that have a shallow root system with an extensive surface area. Competition in this case will be determined by tree growth, transpiration rate, and chlorophyll content.
CHAPTER TWO: MATERIALS AND METHODS

The study site is a legacy strip mine on the lower part of Horseshoe Mountain, located in Claiborne County, TN at 36°31’21.11”N, 83°51’39.85”W. This area is owned and managed by Molpus Woodlands Group, LLC, a timberland investment company out of Jackson, MS. The site is located at an elevation of about 579m, and has a maximum of 30% slopes. The location maintains a south-southwest aspect; it consists of about 10 contiguous hectares.

Prior to site preparation, a vegetation survey was conducted during May of 2016. The survey quantified cover within a 1m² quadrat by the dominant 5 species in each sampling point, the ratio of the most dominant to least dominant species, and the total number of species. The amount of bare ground was also quantified during this time.

Out of 60 sample plots surveyed, 25 plots contained sericea lespedeza (Lespedeza cuneata) as the dominant species, 22 plots contained tall fescue as the dominant species, and the remaining 13 plots contained an unidentified, non-native grass as the dominant species (Fig. 1). 55 out of the 60 plots had 60% cover or more by the top 5 dominant species, yet the highest number of total species in any plot was only 12 (and only in one of the plots), indicating a serious lack of vegetative biodiversity (Fig. 1; all figures and tables located in Appendix).

The study site was deep ripped to a depth of 1.2m in late fall of 2016. Ripping is the process of relieving subsoil compaction mechanically, usually by pulling a large iron hook through the ground behind a bulldozer. Mine reclamation regulations under SMCRA required that mined areas be returned to approximate original contour (AOC); this meant that general practice was to backfill these areas with spoils and topsoil, then compact and grade with bulldozers. This caused heavy compaction that resulted in the inhibition of successful tree growth; therefore, ripping is necessary for successful reforestation efforts.

Ripping was conducted in this instance using two 1.2m ripping shanks spaced 2.5m apart, fully immersed in the soil, pulled behind a D-9 bulldozer. All slopes were cross ripped; the shanks were first pulled perpendicular to the slope, then parallel. Cross ripping prevents surface water from running down furrows during storm events, thus preventing erosion of hillsides prior to revegetation. Ripping at this location was conducted in the fall, ensuring that the ground is dry which maximizes fracturing of the soil and allows for steeper slopes to be ripped safely.

Soil samples were collected subsequent to ripping operations. Forty-one samples were collected in a grid pattern across the site and tested for pH. Soil pH was found to be related to topography, with higher pH soils found at lower elevations. On this basis, the study site was divided into two planting zones (Fig. 4): an “upland” area, to be planted with shortleaf pine (Pinus echinata) and American chestnut (Castanea dentata), and a “lowland” area, to be planted with mixed mesophytic hardwoods and shrubs. Soil samples from each zone were combined, and sent to Clemson University for analysis (Fig. 2 and 3). Soil pH was found to be high in both zones, with excessive amounts of phosphorous, potassium, and calcium.
Trees were planted in early 2017 on a 2.5m grid. Seedlings were placed into the rip intersections, about halfway up the side of the furrows. Species were composed of 49% shortleaf pine; the rest were mixed mesophytic hardwoods and shrubs (Table 1) appropriate for provenance and site conditions.

In late April 2017, 50 FIA-style (Forest Inventory Analysis) plots (a circular plot with a radius of 7m) (Burrill et al., 2018) were established subsequent to tree planting. These plots were laid out in 120° from center tetrad formation, ensuring easy location of plots using a compass once vegetation obscured marking flags and tape in later years. Twelve tetrads and two extra plots were installed in order to evenly sample the entire area (Fig. 5).

Nine annual ground cover species were chosen to compete with tall fescue (Table 2) on the basis of germination, vigor, suitability for the site, non-invasiveness, and mature leaf area. Each of the 50 FIA-style plots had 20, 1m² subplots with a tree seedling in the middle established within them using a calibrated circular hoop to create a uniform seeding area. These tree seedlings were either shortleaf pine, northern red oak (Quercus rubra), or chinkapin oak (Quercus muehlenbergii); these were the top three most abundant tree seedling species (Table 1). Subplots had one species of ground cover each for a total of 18 subplots and 2 unseeded control plots within each plot. A flag with a number designating treatment was placed into each subplot; subplots were then seeded by hand in early May of 2017 based on the numbered flags. All legumes (lablab, partridge pea, and sunn hemp) were inoculated using a rhizobium powder (provided by the seed vendor) mixed with water; a plastic spray bottle filled with the solution was used to spray seeds in the field before sowing.

Seeding rate was calculated prior to field work using germination rates given by vendors; weighed amounts for 1m² areas were parceled into individual numbered envelopes for planting. All tree seedlings in plots were measured for initial height, RCD, vigor, and browse in May 2017. Transpiration rate using the steady state porometer LI-1600 (LI-1600, LI-COR Biosciences, Lincoln, NB) and chlorophyll content using the Opti-Sciences CCM-300 (CCM-300, Opti-Sciences, Inc., Hudson, NH) were measured only on the top three most abundant tree species during the late spring and early summer of 2017. This was due to the narrow daily window available for transpirational testing. Chlorophyll measurements were taken from August 29th to August 31st, 2017; transpiration was measured August 9th, 10th, 14th-17th, 2017. While chlorophyll could be measured at any time of day, transpiration was only able to be measured from 10am until 2pm, as this is the time of day when transpiration rates are least variable (vapor pressure deficit is relatively stable in the middle of the day). A second set of measurements of seedling height, RCD, and browse were taken from January to April 2018. Mortality was quantified in addition to height and RCD measurements.

After the transpiration and chlorophyll content measurements were complete, the cover by treatment species and amount of bare ground were visually quantified. The above-ground portion of herbaceous vegetation within the quadrat of each tree was cut to ground level using a cordless hedge trimmer and collected from September 11th-13th, 2017. Vegetation was then transported back to the lab and stored at 4°C until it could be processed. Vegetation was separated into categories: “forb”, “legume”, “grass”, and “treatment species”. Plant matter that
was too diminutive for morphological identification was categorized as “chaff”. Biomass was oven dried at 50°C for 48 hours and weighed. For every ten bags of chaff, one was weighed and used as a comparison against total plot biomass weight for error. Only plots receiving transpiration and chlorophyll content measurements were utilized for biomass quantification.

A further greenhouse study was conducted in order to quantify the rooting depth and root surface area of the planted ground cover species, as there currently exists nothing on this subject in the literature. Three pots each, for a total of 27 pots, of the nine ground cover species were planted in May 2017 in the greenhouse in 30.5 cm length by 15.3 cm diameter PVC pots. Soil was obtained from the study site and pots were filled to within 2.5 cm of the top. No artificial light or fertilizer were used; plants were on an automatic watering system that watered at a rate of approximately 25 GPH for about 1 hour each morning at 4 am. Temperature in the greenhouse was maintained at 18°C at night and 25°C during the day. Once well established, the plants were weeded down to one individual per pot. These plants were grown until they flowered in September 2017, then carefully removed from the pots. The roots were washed with a root washer and photographed next to a ruler to establish a reference for root depth; root surface area was determined using an image area quantifying software (ImageJ; https://imagej.nih.gov/ij).

Statistical analysis of transpiration and chlorophyll as affected by vegetative biomass were conducted using linear regression (R Studio Version 1.1.383, R Foundation for Statistical Computing, Vienna, Austria). A simple ANOVA was used to determine transpiration and chlorophyll content differences among tree species. Biomass differences were quantified using an ANOVA test followed by a Tukey’s Honest Significant Difference Test. Dry grass weight and dry forb weight differences were tested using a linear mixed effects model. Correlation was also checked for these two variables. A linear mixed effects model was conducted to test transpiration rate as affected by visual bare ground and visual cover by treatment. The same model was used to test chlorophyll content compared to biomass and visual cover/bare ground. All analyses were considered significant at an alpha level of 0.05.
CHAPTER THREE: RESULTS

Survival rate was reported as 85.6% amongst treated seedlings (Fig. 6). Mortality rate was 14.4% amongst treated seedlings. Shortleaf pine showed 80.3% survival; oaks had 91.1% survival. A linear model showed no significant difference in average height growth amongst treatments, despite average height growth appearing to be different (Fig. 7). The effect on tree species’ height growth was not constant across groundcover treatments. When an ANOVA test was conducted, the interaction term of treatment and species was significant (p=0.0002011; Equation 1, Table 3); however, even if the data was separated by seedling species, no significant pattern was found to indicate that treatment affected tree height growth.

There was also no significant difference in average RCD growth amongst treatments using a linear model, despite average RCD growth appearing to be different (Fig. 8). The effect on tree species’ RCD growth was not constant across groundcover treatments. When an ANOVA test was conducted, the seedling species term was significant (p=6.345e-13; Equation 2, Table 3); however, even if the data was separated by seedling species, no significant pattern was found to indicate that treatment affected tree RCD growth.

Average height growth among tree seedling species appeared to vary widely (Fig. 9). A simple ANOVA test showed a significant difference (p=< 2.2e-16; Equation 3, Table 3) between height growth and tree seedling species. Average RCD growth among tree seedling species also appeared to vary (Fig. 10). A simple ANOVA test showed a significant difference (p= 4.885e-13; Equation 4, Table 3) between RCD growth of different tree seedling species.

Further testing investigating the effects of treatment, species, and the interaction effects of treatment and species on height and RCD showed inconsistencies. Significance was found using an ANOVA (p=0.0002011; Equation 1, Table 3) when testing height growth as affected by treatment, species, and the interaction effects of treatment and species. Likewise, significance was found using an ANOVA (p=6.35E-13; Equation 2, Table 3) when testing RCD growth as affected by treatment, species, and the interaction effects of treatment and species. However, when individual tree seedling species were examined statistically for effects of treatment, black mustard was found to affect northern red oak height growth (p=0.0413, R²=0.1308; Equation 5, Table 3), but German millet was found to affect northern red oak RCD growth (p=0.021, R²=0.08488; Equation 6, Table 3). No other effects of treatments on tree seedling species were found.

As mentioned in the methods section of this document, only the top three most abundant tree species were measured for chlorophyll and transpiration. This was due to the narrow window available for transpirational testing. The following results for transpiration rates, chlorophyll content, and biomass will reflect that.

Transpiration rates measured on different days cannot be analyzed as a single dataset as they differ too greatly, therefore the readings taken from two separate days were analyzed separately. An ANOVA test showed no significant difference in transpiration rate among tree seedling species, despite average transpiration rates appearing to be different (Fig. 11).
Transpiration rates were found to not be affected by groundcover biomass of any individual category (grass, legume, forb, or treatment species), or by total biomass at any alpha level when tested using linear regression. Treatment species were then distributed into appropriate categories and tested again for a relationship between transpiration and biomass of grass, legumes, and forbs, but no significant effect on transpiration was found.

An ANOVA test showed no significant difference in chlorophyll content among tree seedling species, despite average chlorophyll content readings appearing to be different (Fig. 12). Chlorophyll content was found to not be affected by groundcover biomass at any alpha level when tested using linear regression. Treatment species were then distributed into appropriate categories and tested again for a relationship between chlorophyll content and biomass of grass, legumes, and forbs, but no significant effect on chlorophyll content was found.

All biomass was separated into four categories before being dried: “Forbs”, “Grasses”, “Legumes”, and “Treatment Species” (Fig. 13). Forbs had the highest average weight at 93 g/m², followed by legumes at an average of 55 g/m². Grasses and treatment species were quite low in average dry weight, at 21 g/m² and 5 g/m², respectively.

Treatment species of groundcover were quantified with biomass measurements. Sunn hemp (Crotalaria juncea), had the highest dry weight per meter squared at 16.68g/m². Ragweed (Ambrosia artemisifolia) and sorghum (Sorghum bicolor) were close in biomass production at 13.71 g/m² and 10.05g/m², respectively (Fig. 14). Smartweed was never observed to have germinated in the field or greenhouse; the possibility exists that the weight in this figure is due to purely natural occurrence as this plant is native to east Tennessee. An ANOVA test followed by a Tukey’s Honest Significant Difference Test showed significant differences (p=0.02643; Equation 7, Table 3) in the treatment weights by treatment (Fig. 14). To test the efficacy of the seed treatments as competition for tall fescue, a linear mixed effects model was run testing all variables against dry grass weight. A weak relationship was found between dry grass weight and dry forb weight (p=0.03033, R²=0.04675; Equation 8, Table 3), but no correlation or significance was found between dry treatment weight and dry grass weight.

The amount of bare ground and cover by species in each plot was quantified visually before removing the biomass. Plots with German millet had the most bare ground at an average of 42%, followed by lablab and annual rye, which both had an average of 35% (Fig. 15). Plots with ragweed had the highest visual cover by treatment at an average of 14%, followed by German millet and sunn hemp at an average of 11% and 9%, respectively.

A linear mixed effects model showed transpiration rate to be affected positively (p=0.0238, R²=0.1931; Equation 9, Table 3) by visual bare ground at the 30% level. When run in the same model by itself, the result was the same. When transpiration was compared to visual cover by treatment, it was found that lablab had a significant effect on transpiration rate (p=0.0232, R²=0.1005; Equation 10, Table 3).

Percent cover by treatment and bare ground were recategorized into three main categories: 0-24%, 25-59%, 60-100%. Although the data appeared to show differences in transpiration rates (Fig. 16 and 17), when the model was run again using the new categories no significance was found in any category for either transpiration or chlorophyll content. When the same model
was run using just cover by treatment and transpiration, 25-59% cover by treatment was found to have a positive effect on transpiration (p= 0.047895, R²=0.0381; Equation 11, Table 3). The same model using just bare ground and transpiration showed no significance; cover by treatment and chlorophyll showed no significance either.

Significance was found when running a linear mixed effects model on chlorophyll content compared to biomass and visual cover/bare ground. At 40% visual cover by treatment, there was a significant (p=0.00257, R²=0.4131; Equation 12, Table 3) effect on chlorophyll content. Visual bare ground percentage was significant at 65% (p=0.02371, R²=0.4131; Equation 12, Table 3) and at 80% (p=0.00268, R²=0.4131; Equation 12, Table 3). However, when visual cover by treatment was taken out of the model, the results changed, with visual bare ground only being significant (p=0.0163, R²=0.2817 Equation 13, Table 3) at 80%.

Groundcover species grown in the greenhouse were measured for length using a ruler and photographed on a reference board (Fig. 18). There was a great variety in root morphology; the legumes used in the study (lablab, partridge pea, and sunn hemp) tended to have smaller root systems, and the grasses (annual rye, millet, and sorghum) tended to have larger, more fibrous root systems. Ragweed and black mustard had an equal mix of morphologies in their roots; each had lengthy roots with a fibrous texture.

Lablab had the longest roots out of all groundcover species grown in the greenhouse at an average of 43 cm, followed by sorghum and ragweed at an average of 39 cm and 32 cm, respectively (Fig. 19). Smartweed (Treatment 8) never germinated. Sorghum had the largest root area out of all groundcover species grown in the greenhouse at an average of 59 cm², followed closely by lablab at 58 cm² (Fig. 20). Smartweed never germinated.
CHAPTER FOUR: DISCUSSION

In Appalachia, the vast majority of strip-mined land was originally covered by eastern deciduous forest. Early reclamation laws prescribed reforestation after backfilling and levelling land with soil, subsoil, and overburden; conifers and some hardwood tree species would then be planted (Skousen et al., 2009). This reclamation methodology was chosen because the land had been originally forested; reforested sites provide long-term site stabilization, wildlife habitat, and future economic value when trees are harvested (Torbert & Burger, 2000).

One of the most important factors in the establishment of productive forests on reclaimed mines is the physical and chemical properties of the rooting medium (Skousen et al., 2009). Soils found at the study site contain significant amounts of unweathered “gray” material, which was located at lower depths in the geologic profile prior to ripping. These unweathered materials have an alkaline pH, variable sized particles, adequate supplies of nutrients, and enough water for grass and tree growth (Burger & Torbert, 1992); however, they tend to favor forages. By contrast, the weathered brown sandstone found immediately underlying the soil profile in many other areas is usually lower in pH and composed of fine materials and weathered rock fragments; this material is generally more conducive to hardwood tree growth (Emerson & Skousen, 2008; Skousen et al., 2006).

Poor survival and growth of hardwood species occurs frequently on reclaimed mine sites, especially when tree seedlings are planted into heavy herbaceous ground cover (Skousen et al., 2009). Trees planted into introduced aggressive forages (especially tall fescue and sericea lespedeza) are often overtopped by the grasses or legumes, and are unable to break free through the coverage. The seedlings are pinned to the ground and have little chance for survival.

Therefore, given the study site’s vegetation survey and soil tests, measuring seedling mortality was critical to evaluating the success of this reforestation project. Results in this case were surprising in that the survival rate of seedlings was unusually high at 86.5%; survival rates have been known to vary widely on reclaimed mine sites in Appalachia from 14% to 77% (Casselman et al., 2006). The survival rate of shortleaf pine on our site was 80.3%; this was especially fascinating given that the site had high pH (<7.0), calcium, and phosphorous; and is fairly moist in several areas. Shortleaf pine is not known to be able to tolerate above a maximum pH of 6.0, has low calcium tolerance, and cannot tolerate moist soils (USDA, 2018d).

A study done in 1989 by Hallgren and Tauer showed that lifting date and storage length greatly affected shortleaf seedling survival rate. Seedlings lifted in December and January, and not stored, had a 90% survival rate after the first year (Hallgren & Tauer, 1989). This contrasted with a March lift, which had only 42% survival after one year. Perhaps the timing of the lift and planting in our experiment influenced survival; it remains to be seen whether these results will hold up in later years.

Oaks in the experiment had a survival rate of 91.1%, which is excellent, and has been seen before, such as in the study conducted by Skousen et al. (2009). In that same study, however, the
survival rates declined heavily after year 1; in fact, first year oaks in high pH soils exhibited just 12% survival. Northern red oak, chinkapin oak, and black oak can tolerate higher pH levels (USDA, 2018f, 2018g, 2018h), although in this case black oak is above its maximum pH range of 6.5. Despite exhibiting low levels of tolerance for calcium, these seedlings do have medium tolerances for moisture levels.

Tree seedling height and RCD are excellent indicators of seedling health. They are almost universally used within the reclamation community as indicators of overall seedling condition over time. Although many of the seedlings in this experiment did increase in height and RCD, no significant differences were found to indicate that treatment affected tree height growth. Likewise, no significant difference was found in height or RCD growth amongst species.

Decreases in height and RCD are explainable. Tree seedlings on legacy mine sites, especially those in the northeast Tennessee region, are browsed heavily. The location of the experimental site is close to Royal Blue, an area boasting a large herd of elk. These elk have been observed browsing the tree seedlings on the study site, leading to dieback and/or mortality of seedlings; decreases in measured heights in some of the seedlings occur as well. Decreases in RCD can be explained by water uptake; seedlings do not take up nearly as much water when dormant in the winter months.

An ANOVA test showed the interaction term of treatment and species to be significant for both height and RCD; however, no significant pattern was found indicating that treatment affected tree growth. This was evidenced by finding that treatment 4 (black mustard) had an effect on northern red oak height, but treatment 7 (German millet) had an effect on northern red oak RCD. No other effects of treatments on species were found.

Moreover, when a statistical test such as an ANOVA lacks balance amongst experimental units, such as in the case of this study, it reduces the power of the test. Our study consisted of close to 50% shortleaf pine; this was reflective of the numbers of seedlings planted across the site. Furthermore, it is well known that seedlings of different species do not exhibit identical growth rates. This leads to an imbalance in statistical testing when attempting to compare growth rates across species as equal; the imbalance confounds the tests and can lead to false positives.

All of the seedling species are preformed, meaning that foliar growth is predetermined before bud break in the spring (unless multiple flushes are experienced). Any growth exhibited during the 2017-2018 measurement interval could be due to the nutrients they received while in the nursery; measurements should be taken in years two and three to obtain a more accurate portrayal of seedling growth.

Root growth and seedling establishment are inextricably linked factors that lead to a successful reforestation effort (Grossnickle, 2004). Site environmental conditions and seedling quality at time of planting are crucial to the success of seedling establishment (Burdett, 1990; Rietveld, 1989). A newly planted seedling can be coupled to the reforestation site only if it has access to available soil water to meet transpirational demand. This coupling is important because reforestation sites, especially those on legacy mines, can present extreme environmental conditions that alter site heat exchange processes and soil water relations (Miller, 1983). The
ability of a seedling to take up water is affected by its root system size and distribution, root-soil contact, and root hydraulic conductivity. The shoot system has transpirational water loss from leaves which is directly determined by the amount of stomatal opening and leaf area.

Typically, newly planted seedlings have restricted root placement, low root system permeability and/or poor root-soil contact, which can limit water uptake from the soil (Burdett, 1990; Kozlowski & Davies, 1975; Rietveld, 1989). Additionally, it has been shown that the external mycelium of ectomycorrhizal fungi transports water to the host plant (Dudridge et al., 1980); it has also been shown that water taken up by these mycelium can be sufficient enough to make a difference between the survival and death of a tree seedling (Boyd et al., 1986).

Ectomycorrhizal fungi may account for at least some of the frequently observed enhancement of root hydraulic conductance (Muhsin & Zwiazek, 2002). Hence, the external mycelia function as extensions of the root systems in water uptake, as they do in phosphorus and nitrogen uptake (Smith & Read, 2008); thus ectomycorrhizal symbiosis can be an advantage for water uptake of tree seedlings.

Seedlings can be exposed to stress just after planting because they are not fully coupled into the transpirational hydrologic cycle. This cycle consists of water flowing from the soil to plant roots, through the plant and into the atmosphere. Planting stress can lead to root growth being limited by the lack of water and photosynthates, and in turn photosynthesis being limited by water stress due to a lack of root growth (Burdett, 1990; Grossnickle, 2000). These factors are easily measured via transpiration rate and chlorophyll content. Seedlings that develop a root system after planting establish a proper water balance and respond to field site atmospheric conditions without limitations that occur when seedlings do not have access to soil water (Margolis & Brand, 1990). Thus, seedlings that are able to balance access to soil water with transpirational water loss can have a cycle of root growth supported by photosynthesis, and photosynthesis supported by root growth (Burdett, 1990). Seedlings enter the establishment phase when they are fully coupled into the site hydrological cycle and begin to respond to silvicultural practices that have been used to create favorable site conditions (Grossnickle, 2000; Rietveld, 1989).

The average transpiration rates of northern red oak seedlings in this study were found to be outside the normal range of about 82 mmol/m²/s (Naidu & DeLucia, 1997), despite this reference rate being reported for transplanted seedlings. The red oak seedlings in this study were recently planted; the low average readings (1.9 mmol/m²/s for Day 1; 2.2 mmol/m²/s for Day 2) were most likely due to severe planting stress.

The average transpiration rates of chinkapin oak seedlings were also below reported averages of 176 mmol/m²/s (Abrams & Knapp, 1986), however this average was reported for unstressed seedlings in a forest setting. Again, the seedlings in this study had been recently planted; the low average readings (1.4 mmol/m²/s for Day 1; 2.0 mmol/m²/s for Day 2) were most likely due to severe planting stress.

The average transpiration rates of shortleaf pine were below reported averages for the genus in the forest of 290 mmol/m²/s (Elliot & Vose, 1993), but above averages reported for the genus under drought conditions of 3 mmol/m²/s (Tolley & Strain, 1985). While the averages reported by Elliot & Vose (1993) seem high, they were located in a forest, growing in rich soils in North
Carolina, which receives plenty of rainfall. The averages by Tolley & Strain (1985), however, were reported from seedlings in a growth chamber under drought. This study saw reasonable, albeit variable, readings (48.4 mmol/m²/s for Day 1; 16.5 mmol/m²/s for Day 2) for site conditions and considering planting stress.

The amount of solar radiation absorbed by a leaf is a function of the photosynthetic pigment content. Therefore, chlorophyll content can directly determine photosynthetic potential and primary production (Curran et al., 1990; Filella et al., 1995). In addition, chlorophyll gives an indirect estimation of nutrient status because much of leaf nitrogen is incorporated into chlorophyll (Filella et al., 1995; Moran et al., 2000). Furthermore, leaf chlorophyll content is closely related to plant stress (Gitelson et al., 2003).

Traditionally, leaf extraction with organic solvents and spectrophotometric determination in solution has been required for pigment analysis. Recently, alternative solutions of leaf pigment analysis with non-destructive optical methods (such as the CCM-300 used in this experiment) have been developed. These newer methods are non-destructive, inexpensive, quick, and possible in the field (Gitelson et al., 2003).

Chlorophyll content has been reported as low as 200 mg/m² in low density forest canopy situations, and as high 400 mg/m² (Yoder & Pettigrew-Crosby, 1995) for mixed species forests. In conifers, including *Pinus* sp., chlorophyll content has been reported from 200 mg/m² up to 900 mg/m² in freshly regenerated seedlings (Leverenz, 1987). This study showed shortleaf pine to have an average reading of 303.5 mg/m², which is well within the given range by Leverenz (1987) for this genus. The averages for northern red and chinkapin oak were just above the low threshold as given by Yoder and Pettigrew-Crosby (1995) for chlorophyll content at 211.6 mg/m² and 236.5 mg/m², respectively. This fairly low value may indicate planting stress; seedlings dedicate most of their resources to root development in their first year in the field. Additionally, initial nutrient uptake in seedlings can be quite low due to lack of mycorrhizal networks and in this case, high soil pH that prevents nitrogen from being uptaken (Miller, 2016).

Current and past theories of plant community organization make different predictions about the relationship between competition and biomass (Bonser & Reader, 1995). Some theories (Grime, 1979; Keddy, 1990) predict that competition should increase with an increase in biomass. The basis of this prediction is that plants are more likely to interact and to compete for resources at sites with higher biomass. In contrast, other theories (Taylor et al., 1990; Tilman, 1988) predict that competition should not increase with an increase in biomass. Taylor et al. (1990) argue that competition reflects the ratio of resource demand to supply and that this ratio could be equally high at sites with low or high biomass. Tilman (1988) and others (Grubb, 1985; Newman, 1973) feel that competition for light may be greater where biomass is high but that competition for soil resources may be greater where biomass is low; consequently total competition could be about equal at sites with low vs. high biomass.

Competition can be an important factor in reducing growth of forest trees (Gjerstad et al., 1984). Despite the importance of competition, little is known about morphological and physiological characteristics that influence the establishment and early growth of tree seedlings in highly...
competitive environments. Grime (1979) stressed that characteristics of biomass partitioning influence a plant's ability to compete with other vegetation. In particular, he hypothesized that highly competitive plants exhibit greater plasticity in the amount of growth partitioned to leaves and roots than less strongly competitive plants. Most comparisons of plasticity in biomass partitioning between tree species in response to competition have emphasized effects of shading (Kolb & Steiner, 1990), while effects of competition for soil resources have been less studied.

Seedlings of northern red oak are often suppressed by interfering herbaceous and woody vegetation. In a study done in the 1990s (Kolb & Steiner, 1990), grass root competition was found to significantly reduce oak biomass (-61%). Biomass relative growth rate for oak was negative during the first flush of stem growth (days 0-59 after planting). Oak relative growth rates were positive over almost all remaining intervals; seedlings responded to grass root competition by increasing biomass partitioned to the root at the expense of the shoot. This change in partitioning for oak was the result of decreased leaf growth and increased root growth. Grass root competition reduced leaf area proportion in all seedlings.

In another study done in the 2000s (Marshall et al., 2009), the competitive effects of Japanese stiltgrass (*Microstegium vimineum*) on seedlings of three native hardwood species were studied. Growth and survival of red maple (*Acer rubrum*), tulip poplar (*Liriodendron tulipifera*), and northern red oak seedlings were compared in plots with and without stiltgrass in three planting beds under 50% shade. Red maple and tulip poplar seedlings experienced growth reduction in leaf biomass and leaf number in the presence of stiltgrass. Northern red oak did not exhibit any differences in foliar characteristics between plots with and without stiltgrass, however there was a reduction in stem weight resulting from the presence of stiltgrass. Reductions in the growth of red maple and tulip poplar suggest that the presence of Japanese stiltgrass in forest understories may reduce the rate at which seedlings of these species are recruited into larger size classes.

Biomass in this study was found to be the highest in the “Forbs” category, followed by “Legumes”, then “Grasses”, and “Treatment Species”. Interestingly enough, none of these had an effect on transpiration or chlorophyll content in any of the tree seedlings. This could suggest that in the first year of seedling growth on a mine site, the seedlings are too stressed to react to competition.

Treatment species of groundcover in this study were quantified with biomass measurements. Sunn hemp had the highest dry weight per meter squared followed by ragweed and sorghum. This follows what Sheahan (2012) had stated about the vigor, growth, and size of sunn hemp. Ragweed has been observed both by Hilty (2017) and this author (anecdotally) to do well in poor conditions. However, the treatment species still had nowhere near the growth of the species that were present prior to treatment, despite being selected for rapid biomass production and being seeded at high rates.

As stated in the materials and methods section of this document, the study site was primarily vegetated with sericea lespedeza and tall fescue prior to ripping. Although ripping is one of the ways groundcovers can be temporarily extinguished (Michler & Rathfon, 2006), it has been
shown that total vegetative cover will not necessarily be reduced by ripping, even years later (Burger & Evans, 2010). In spite of the treatment species having been seeded heavily, the fact remains that the seed bank already present at the site was robust due to having been there for decades. In addition, the vegetative cover on site was not only invasive, but tenacious and fast-growing, with many perennial species that are able to grow rapidly from the root system, even after disturbance. This suggests that even at heavy seeding rates, the treatment species had little chance of being successful without a more complete site preparation regime. Seed treatments were sown when the ground was fairly bare; possible seed predation could also explain the lack of biomass. Birds and rodents could easily have decimated even heavy seed amounts before they had the chance to germinate.

Although greater amounts of herbaceous biomass may exert a greater degree of competition with planted trees for resources, there is not always a direct correlation between total ground coverage by herbaceous vegetation and tree growth. On mine sites with rapid and dense ground cover growth, competition for light may be a primary factor causing poor growth and survival of some planted tree seedlings. The study conducted by Rizza et al (2007) found that ground cover of 20–50 % and approximately 0.5 m tall reduced photosynthetically active radiation (PAR) at 5 cm above ground by approximately 50 %, while cover greater than 75 % allowed for the transmission of only 20% of PAR, shading small seedlings and the lower foliage of larger seedlings. Also found was a clear trend of decreasing diameter growth with increasing ground cover percentage was seen for eastern redbud (Cercis canadensis) and Virginia pine (Pinus virginiana) after two growing seasons. Northern red oak growth was significantly reduced by the presence of any ground cover over 25%.

On the contrary, a study at a mine site in Tennessee where surface soil temperatures can reach 50°C, intermediate ground cover levels were found to be associated with the greatest growth of planted tree seedlings (Franklin & Buckley, 2006). This was attributed to an improvement in subsurface water relations. Seedling growth on a wide variety of sites in relation to groundcover competition has been found to be related to the duration of competition and tree species’ suitability to site characteristics, rather than groundcover percentage (Berkowitz et al., 1995).

A metanalysis study done in the last decade (Franklin et al., 2012) suggested that a very dense ground cover is detrimental to tree establishment. At ground coverage of less than approximately 60% however, tree survival varies widely. At low levels of ground cover, the balance of facilitative versus competitive effects is likely determined by numerous factors including soil properties, resource availability, planting stock, and selection of tree and ground cover species.

In this study, the amount of bare ground and cover by species in each plot was quantified visually before removing the biomass. Transpiration rate was affected positively by visual bare ground at the 30% level; when transpiration was compared to visual cover by treatment, it was found that only lablab had a significant effect on transpiration rate. These levels appear to coincide with the metanalysis done by Franklin et al (2012). Percent cover by treatment and bare ground were recategorized into three main categories: 0-24%, 25-59%, 60-100%. Although the
data appeared to show differences in transpiration rates, when the model was run again using the new categories no significance was found in any category for either transpiration or chlorophyll content. When the same model was run using just cover by treatment and transpiration, 25-59% cover by treatment was found to have a positive effect on transpiration. The same model using just bare ground and transpiration showed no significance; cover by treatment and chlorophyll showed no significance either. These levels also appear to coincide with the metanalysis done by Franklin et al (2012).

Significance was found when comparing chlorophyll to biomass and visual cover/bare ground. At 40% visual cover by treatment, there was a significant effect on chlorophyll content. Visual bare ground percentage was significant at 65% and at 80%. However, when visual cover by treatment was taken out of the model, the results changed, with visual bare ground only being significant at 80%. This significance is quite interesting; again, earlier studies have suggested that at low levels of ground cover, the balance of facilitative versus competitive effects is likely determined by numerous factors. Also suggested is that intermediate groundcover levels are most beneficial (Franklin et al., 2012); results in the case of this study appear to bear that out.

Groundcover species grown in the greenhouse showed a great variety in root morphology; the legumes used in the study (lablab, partridge pea, and sunn hemp) tended to have smaller root systems, and the grasses (annual rye, millet, and sorghum) tended to have larger, more fibrous root systems. Ragweed and black mustard had an equal mix of morphologies in their roots; each had lengthy roots with a fibrous texture. This could be expected based on existing knowledge of these types of plants; however, no study in the literature so far has attempted to quantify root structure of groundcovers in a reference-minded framework.
CHAPTER FIVE: CONCLUSIONS

This study did not have the desired outcome of using planted groundcover to outcompete fescue. However, it did substantiate Franklin et al. (2012) findings that intermediate levels of ground cover are the most beneficial. Likewise, we were able to explain seedling mortality using physiological characteristics. This study was an excellent example of observing subsurface competition and rhizospheric interactions *in situ*. It remains to be seen whether the results will hold up in later years, as the trees will reach a certain point of growth where groundcover will no longer pose a competitive risk to them.

Although this study was effective in demonstrating the importance of belowground competition in damaged land reclamation, it would not be cost-effective to repeat this experiment. The fact is that even though the treatment species were seeded at high rates, the results did not indicate effective competition between treatments and the vegetation that was already present. Even if the seeding rate of treatment species was increased to massive levels, the cost of seed would outweigh any benefit that might exist.

However, in forthcoming ventures that require transpiration and/or chlorophyll content readings, a larger sample size could be obtained by employing several porometers and chlorophyll content meters with two or three crews of people. Time was a huge sampling inhibitor in this study; there is only so much ground one can cover during the allotted transpirational testing period.

It was observed during normal data collecting activities that browse is present in many spots on this site, and that there are two different forms that appear to come from two separate species: rodent browse (rabbits, wood rats, etc.) and ungulate browse (deer and elk). Browse from ungulates occurs at the top of the seedling and affects meristematic tissues, leading to dieback but not usually death of seedlings. Moreover, ungulates often consume the aluminum tree marking tags, leading to the loss of accurate mortality counts and a resulting smaller sample size. Conversely, rodent browse occurs at the root collar or just above on tree seedlings, effectively girdling them, which can often be deadly. These observations lead to the conclusion that perhaps a study quantifying the effects of ungulate and rodent browse should be conducted to ascertain the effects on seedling mortality.

Ripping assists in diversification of the overall landscape. It serves many purposes in mine reclamation, such as soil compaction relief, improvement of water infiltration, breakup of mat-forming grasses, and erosion control. Interestingly enough, it also creates microhabitats that assist in diversification of the overall landscape. Wetlands are inadvertently created in depressions by cross rips, assisting in water retention; only months later frogs, water beetles, hydric plants, and other organisms can be observed thriving where a dry area existed prior. Where ripping shanks snag large boulders or slabs of rock in the soil, small caves and burrows are created, which ground nesting animals quickly take advantage of. Therefore, a study involving wildlife experts might be embarked upon examining the habitat creation advantages of ripping a previously compacted area.
Removing the biomass from plots was an effective way to reduce the normal bias associated with a field vegetation survey. Being able to quantify all vegetation via visual identification and weight in a laboratory setting definitely provided a more robust dataset. However, now that the seedlings are exposed, wildlife could decimate the sampled areas. It remains to be seen in future travels to the site for data collection whether or not this will be the case.

Despite the experiment not meriting repetition, there are ways the results of this study can be applied to improve restoration practices on legacy mines. Site preparation is of the utmost importance when the goal is to rid an area of unwanted vegetation. Methods similar to those described in Michler and Rathfon (2006) for killing grasses and deep-rooted perennials (such as tall fescue and sericea lespedeza) could be used to ensure a more complete elimination of the vegetation prior to groundcover application. Mowing the site twice in midsummer, then broadcast applying glyphosate herbicide in early fall has been shown to be quite effective at the eradication of vegetation. Although the cost of herbicide and equipment use/transport is initially high, the results would certainly be better than doing without.
REFERENCES


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APPENDIX
Figure 1: Pre-ripping vegetation survey of site at Horseshoe Mountain. Numbers within pie charts represent the number of plots out of 60.
Figure 2: "Upland" area soil test results

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil pH</td>
<td>7.4</td>
</tr>
<tr>
<td>Buffer pH</td>
<td>7.75</td>
</tr>
<tr>
<td>Phosphorus (P)</td>
<td>Low</td>
</tr>
<tr>
<td>Potassium (K)</td>
<td>Medium</td>
</tr>
<tr>
<td>Calcium (Ca)</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>High</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>Excessive</td>
</tr>
<tr>
<td>Manganese (Mn)</td>
<td></td>
</tr>
<tr>
<td>Boron (B)</td>
<td></td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td></td>
</tr>
<tr>
<td>Sodium (Na)</td>
<td></td>
</tr>
<tr>
<td>Sulfur (S)</td>
<td></td>
</tr>
<tr>
<td>Soluble Salts</td>
<td></td>
</tr>
<tr>
<td>Nitrate Nitrogen</td>
<td>ppm</td>
</tr>
<tr>
<td>Organic Matter</td>
<td>% (LCI)</td>
</tr>
</tbody>
</table>

**Calculations**

<table>
<thead>
<tr>
<th>Carbon Exchange Capacity (CEC)</th>
<th>Activity</th>
<th>Ca</th>
<th>Mg</th>
<th>K</th>
<th>Na</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>31.7 mg/100 g</td>
<td>2 mg/200 g</td>
<td>88%</td>
<td>5%</td>
<td>1%</td>
<td>0%</td>
<td>94%</td>
</tr>
</tbody>
</table>
Figure 3: "Lowland" area soil test results
Figure 4: Zone map of Horseshoe Mountain
Figure 5: Map of tetrads and plots at Horseshoe Mountain
Figure 6: Seedling mortality from 2017 to 2018

Figure 7: Average height growth of tree seedlings in each of 10 ground cover treatments from 2017 to 2018
Figure 8: Average root collar diameter (RCD) growth of all tree species, in each of 10 ground cover treatments from 2017 to 2018.

Figure 9: Average height growth amongst tree seedling species from 2017-2018, across all ground cover treatments.
Figure 10: Average root collar diameter (RCD) growth amongst tree seedling species from 2017-2018, across all ground cover treatments.

Figure 11: Average transpiration rate of tree seedlings, across all treatments, on two different dates.
Figure 12: Average chlorophyll concentration of foliage in three species of tree seedlings, across all treatments.

Figure 13: Average dry weight of herbaceous vegetation within treatment plots, in four different biomass categories, across all treatments.
Figure 14: Average dry weight of planted ground cover species within treatment plots. Different letters indicate a significant difference between species at α=0.05 using Tukey’s HSD; all differences are in log weight to meet assumptions.
Figure 15: Average percentage of bare ground, cover by the planted treatment species, and non-planted vegetative cover within treatment plots.
Figure 16: Average transpiration rates for tree seedlings in three cover categories

Figure 17: Average transpiration rate for tree seedlings in three bare ground categories
Figure 18: Photographs of groundcover species grown in the greenhouse. Photos are numbered by species: 1= ragweed, 2= annual rye, 3= partridge pea, 4= black mustard, 5= lablab, 6= sorghum, 7= German millet, and 9= sunn hemp.
Figure 19: Average root lengths in cm of planted groundcover species. All species were grown in the greenhouse.

Figure 20: Average root area in cm² of planted groundcover species. All species were grown in the greenhouse.
Table 1: Seedlings planted on Horseshoe Mountain. Tree species selection was based on availability and on soil tests.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Scientific Name</th>
<th>Number of Seedlings Planted</th>
<th>% of Total</th>
<th>Trees per Hectare</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shortleaf pine</td>
<td><em>Pinus echinata</em></td>
<td>11,000</td>
<td>49</td>
<td>178</td>
</tr>
<tr>
<td>Northern red oak</td>
<td><em>Quercus rubra</em></td>
<td>3,200</td>
<td>14</td>
<td>52</td>
</tr>
<tr>
<td>Chinkapin oak</td>
<td><em>Quercus muehlenbergii</em></td>
<td>3,000</td>
<td>13</td>
<td>49</td>
</tr>
<tr>
<td>American chestnut</td>
<td><em>Castanea dentata</em></td>
<td>2,000</td>
<td>9</td>
<td>32</td>
</tr>
<tr>
<td>Black oak</td>
<td><em>Quercus velutina</em></td>
<td>1,600</td>
<td>7</td>
<td>26</td>
</tr>
<tr>
<td>Hazelnut</td>
<td><em>Corylus americana</em></td>
<td>600</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Elderberry</td>
<td><em>Sambucus nigra</em></td>
<td>400</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Buttonbush</td>
<td><em>Cephalanthus occidentalis</em></td>
<td>300</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Staghorn sumac</td>
<td><em>Rhus typhina</em></td>
<td>300</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td></td>
<td><strong>22,400</strong></td>
<td><strong>100</strong></td>
<td><strong>363</strong></td>
</tr>
</tbody>
</table>

Table 2: Key to seeding treatments. Seeding rates were calculated based on manufacturer’s recommendations.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Common Name</th>
<th>Scientific Name</th>
<th>Seeding rate (kg/ha)</th>
<th>Seeding Rate per Tree (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ragweed</td>
<td><em>Ambrosia artemisifolia</em></td>
<td>8.4</td>
<td>6.6</td>
</tr>
<tr>
<td>2</td>
<td>annual ryegrass</td>
<td><em>Lolium multiflorum</em></td>
<td>28</td>
<td>21.9</td>
</tr>
<tr>
<td>3</td>
<td>partridge pea</td>
<td><em>Chamaecrista fasciculata</em></td>
<td>16.8</td>
<td>13.1</td>
</tr>
<tr>
<td>4</td>
<td>black mustard</td>
<td><em>Brassica nigra</em></td>
<td>16.8</td>
<td>13.1</td>
</tr>
<tr>
<td>5</td>
<td>lablab</td>
<td><em>Lablab purpureus</em></td>
<td>16.8</td>
<td>13.1</td>
</tr>
<tr>
<td>6</td>
<td>sorghum</td>
<td><em>Sorghum bicolor</em></td>
<td>28</td>
<td>21.9</td>
</tr>
<tr>
<td>7</td>
<td>German millet</td>
<td><em>Setaria italica</em></td>
<td>28</td>
<td>21.9</td>
</tr>
<tr>
<td>8</td>
<td>smartweed</td>
<td><em>Polygonum pensylvanicum</em></td>
<td>16.8</td>
<td>13.1</td>
</tr>
<tr>
<td>9</td>
<td>sunn hemp</td>
<td><em>Crotalaria juncea</em></td>
<td>44.8</td>
<td>35.0</td>
</tr>
<tr>
<td>10</td>
<td>control</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</table>
Table 3: Summary table of statistical tests, equations, and associated significance values

<table>
<thead>
<tr>
<th>Reference Number</th>
<th>Test</th>
<th>Equation</th>
<th>P-value</th>
<th>R²</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>ANOVA</td>
<td>height~trt+sp+subplot+trt*sp</td>
<td>0.0002011</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>ANOVA</td>
<td>rcd~trt+sp+subplot+trt*sp</td>
<td>6.35E-13</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>ANOVA</td>
<td>height ~ sp</td>
<td>&lt; 2.2e-16</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>ANOVA</td>
<td>rcd ~ sp</td>
<td>4.89E-13</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>LM</td>
<td>height ~ trt</td>
<td>4.13E-02</td>
<td>0.1308</td>
</tr>
<tr>
<td>6</td>
<td>LM</td>
<td>rcd ~ trt</td>
<td>2.10E-02</td>
<td>0.08488</td>
</tr>
<tr>
<td>7</td>
<td>ANOVA</td>
<td>trt_wt~trt</td>
<td>0.02643</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>LME</td>
<td>grass_wt ~ forb_wt + trt_wt + leg_wt</td>
<td>0.03033</td>
<td>0.04675</td>
</tr>
<tr>
<td>9</td>
<td>LME</td>
<td>trans ~ vis_bare + vis_cov_trt</td>
<td>0.0238</td>
<td>0.1931</td>
</tr>
<tr>
<td>10</td>
<td>LME</td>
<td>trans ~ vis_cov_trt</td>
<td>0.0232</td>
<td>0.1005</td>
</tr>
<tr>
<td>11</td>
<td>LME</td>
<td>trans ~ vis_cov_trt</td>
<td>0.047895</td>
<td>0.0381</td>
</tr>
<tr>
<td>12</td>
<td>LME</td>
<td>conc ~ grass_wt + total_wt + forb_wt + trt_wt + leg_wt + factor(vis_cov_trt) + factor(vis_bare)</td>
<td>0.02371</td>
<td>0.4131</td>
</tr>
<tr>
<td>13</td>
<td>LME</td>
<td>conc~grass_wt+total_wt+forb_wt+trt_wt+leg_wt+factor(vis_bare)</td>
<td>0.0163</td>
<td>0.2817</td>
</tr>
</tbody>
</table>
VITA

Matthew Aldrovandi was born in Plymouth, Massachusetts. He attended The University of Tennessee Knoxville, where he received his Bachelor of Science degree in Forestry: Natural Resource Management in December 2015. Matt’s previous work experiences include research positions with Dr. Jennifer Franklin, Dr. Scott Schlarbaum, Dr. Karen Hughes, and Dr. Mark Windham at the University of Tennessee Knoxville. In January of 2016 he accepted a graduate research assistantship at the University of Tennessee under the advising of Dr. Jennifer Franklin. While at the University of Tennessee, Matt graduated twice summa cum laude, was an active member of the American Society of Mining and Reclamation, the Society of Ecological Restoration, and the Lion’s Club. He was inducted into the Xi Sigma Pi forestry honor society and received the Cornell and Frances Bradshaw Houston Fellowship. He received his Master of Science degree in Forestry in August, 2018.