EVALUATING PRESCRIBED FIRE AS A TOOL FOR BLIGHT RESISTANT B_3F_3 HYBRID AMERICAN CHESTNUT SEEDLING INTRODUCTION

A Thesis
by
FELIX STITH

Submitted to the School of Graduate Studies
at Appalachian State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

May 2023
Department of Biology

EVALUATING PRESCRIBED FIRE AS A TOOL FOR BLIGHT RESISTANT B_3F_3 HYBRID AMERICAN CHESTNUT SEEDLING INTRODUCTION

A Thesis
by
FELIX STITH
May 2023

APPROVED BY:

Dr. Mike Madritch
Chairperson, Thesis Committee

Dr. Matt C. Estep
Member, Thesis Committee

Dr. Howard Neufeld
Member, Thesis Committee

Dr. Ava Udvadia
Chairperson, Department of Biology

Marie Hoepfl, Ed.D.
Interim Dean, Cratis D. Williams School of Graduate Studies

Abstract

EVALUATING PRESCRIBED FIRE AS A TOOL FOR BLIGHT RESISTANT B_3F_3 HYBRID AMERICAN CHESTNUT SEEDLING INTRODUCTION

Felix Stith
B.S., University of North Carolina Asheville
M.S., Appalachian State University

Chairperson: Mike Madritch

The functional extinction of American chestnut (*Castanea dentata*) from the invasive chestnut blight (*Cryphonectria parasitica*) and wildland fire suppression have reduced the diversity and resiliency of southern Appalachian forests. Breeding programs have created Chinese-American B_3F_3 hybrid chestnut trees with some resistance to chestnut blight and physically resemble American chestnut. This hybrid chestnut may assist American chestnut restoration. A knowledge gap exists regarding the responses of hybrid chestnuts to various environmental factors. American chestnut has several fire-adapted traits. We established two research sites in western NC. Each site had a prescribed fire and control plot. Each plot was planted with two- and three-year old B_3F_3 hybrid chestnuts. Soil C and N samples were collected from the plots. Understory light, diameter, height, and leaf C and N measurements were collected for each chestnut in the study. After one year for one site, and two years at the other site, B_3F_3 hybrid chestnuts in the burned plots were taller and had larger stem diameters than in unburned plots. Understory light was higher in the burned plots. Hybrid chestnut leaf C/N ratios positively correlated to light availability. Prescribed fire may be an effective tool for introducing B_3F_3 hybrid chestnuts into certain forest communities.

Acknowledgments

I am very grateful for my adviser Dr. Mike Madritch and my committee members Dr. Matt C. Estep and Dr. Howard Neufeld for their expertise and guidance. I also want to thank Woodlands Management Inc and Blue Ridge Resource Conservation & Development for their support of this project. I appreciate grant funding from the Appalachian State Office of Student Research. Without these people and organizations, this project would not have been possible.

Table of Contents

List of Tables

List of Figures

Introduction

The American chestnut (*Castanea dentata* (Marsh.) Borkh.) was once one of the most common trees in eastern hardwood forests until invasive pathogens killed ~4 billion, or 90%, of American chestnut trees throughout its entire native range in the early 20th century (Saucier 1973; Dalgleish et al. 2016). The heart of its range was in the southern Appalachians, where roughly one in four trees was an American chestnut (Saucier 1973). American chestnuts were large, very quick growing, overstory trees with diameters that could reach two meters (Saucier 1973; Latham 1992) and often formed pure stands on ridges and mountain tops. Historical accounts exist where the ridges appeared "covered in summer snow" when all the chestnut trees would bloom with white flowers in June and July (personal accounts from regional elderly people). They were economically and ecologically important and are considered to have been foundational species in the southern Appalachians (Saucier 1973; Jacobs et al. 2013).

Chestnut blight (*Cryphonectria parasitica* (Murr.) Barr) is an invasive pathogen that killed 3-4 billion American chestnut treesand is native to East Asia (Saucier 1973). Chestnut blight is dispersed by wind and is now widespread throughout the entire historical range of the American chestnut. Root rot (*Phytophthora cinnamomi* Rands.) is another invasive pathogen that kills American chestnuts in the southern part of its range in areas with poorly drained soils and likely arrived in the southern Appalachian Mountains before 1824 (Anagnostakis 2012).

It is now rare for American chestnuts to reach maturity. Most surviving American chestnuts exist as small understory trees that sprout from rootstock instead of regenerating from seed and the densest American chestnut population is still located in the southern Appalachians but is not reproductively viable (Dalgleish et al. 2016). The American chestnut is listed as critically endangered by the International Union for Conservation of Nature (Stritch 2018).

The loss of American chestnut had widespread community- and ecosystem-level effects. The trees provided large and consistent nut mast crops which were a very important food source for a diversity of animals, including humans and their livestock (Diamond et al. 2000; Orwig 2003). The demise of American chestnut severely disrupted the food-web and population dynamics within its native range (Diamond et al. 2000; Orwig 2003; Ellison et al. 2005). This resulted in the possible extinction of five species of moths and decreased predator and prey animal populations (Orwig 2003). Losing American chestnut has also very likely impacted the fire regimes and fire ecology throughout its range (Mitchell et al. 2009; Dickinson et al. 2016, Kane et al. 2019, 2020). The loss of American chestnut along riparian corridors resulted in an increase in ericaceous shrubs and mesic tree species which further altered fire dynamics (Vandermast and van Lear 2002). The demise of American chestnut altered decomposition rates, productivity, nutrient cycling, and terrestrial and aquatic ecological functions (Ellison et al. 2005; Jacobs et al. 2013). The change in leaf litter along streams likely impacted the trophic input and aquatic macroinvertebrate community ecosystems (Ellison et al. 2005). American chestnut leaves have a relatively low carbon to nitrogen ratio, while the wood is very high in tannins and decomposes slower than co-occurring hardwoods. The replacement of American chestnut by other trees probably altered the cycling of nutrients within soils (Ellison et al. 2005) while its rapid growth rate possibly impacted carbon sequestration (Ellison et al. 2005). In addition, forest stand dynamics throughout its wide range would have changed as other tree species replaced the American chestnut. American chestnut may have been allelopathic (Vandermast et al. 2002; Vandermast and van Lear 2002), potentially allowing it to outcompete mesic species such as big leaf rhododendron (*Rhododendron maximum* L.) and eastern hemlock (*Tsuga canadensis* L.) along riparian corridors (Vandermast and van Lear 2002; Ellison et al.

2005). The community-and ecosystem-level impact of American chestnut functional extinction highlight the importance of forest-scale restoration efforts.

Until the past few decades wildland fire was historically understudied, and a knowledge gap still exists regarding the role of fire in eastern deciduous forests (Ellison et al. 2005; Nowacki and Abrams 2008; Christensen 2014). Most of the American chestnut range (~88%) had a fire return interval of <20 years, and much of the southwestern portion of the historical range of American chestnut had a fire return interval of <5 years prior to the mid-19th century (Guyette et al. 2012; Kane et al. 2019, 2020). Although fire suppression has led to a large decrease in wildland fire frequency, wildland fire still occurs throughout the historical range of the American chestnut. A change in forest structure and composition, and high fuel loads from decades of fire suppression, paired with climate change, are leading to an increase in fire intensity when wildland fires do occur (James et al. 2020).

Given the functional extinction of American chestnut, a knowledge gap exists surrounding the management, silvics, natural reproduction, successional pathways, and the fire ecology of American chestnut (Jacobs et al. 2013; Varner et al. 2016a; Belair et al. 2018; Kane et al. 2019, 2020). Despite this poor understanding of American chestnut silvics, there is evidence that American chestnut is fire-adapted and potentially fire-dependent (Vandermast and van Lear 2002; Kane et al. 2019, 2020). Historical accounts suggest that American chestnut co-occurred in stands with other fire-loving or "pyrophytic" species including oak (*Quercus* spp. L.) and hickory (*Carya* spp. Nuttall.) in xeric to sub-mesic sites, but occasionally in mesic sites within deciduous forests (Foster et al. 2002; Vandermast and van Lear 2002; Varner et al. 2016b; Belair et al. 2018). The American chestnut exhibits common pyrophytic traits including highly flammable leaves, the ability to resprout repeatedly, a large taproot, and extensive carbohydrate storage abilities, thick bark, a relatively high ability to compartmentalize and heal wounds, a very high growth response to increased light, and understory sapling banking (in which the tree "banks" saplings in the understory that wait for a disturbance to be released – the banked saplings require some light in the understory) (Paillet and Rutter 1989; Wang et al. 2006; Joesting et al. 2007; Collins et al. 2017; Belair et al. 2018; Kane et al. 2019, 2020; Varner et al. 2021). Pyrophytic species such as oaks, hickories, and likely American chestnut, depend on increased light and disturbance from fire to outcompete shade tolerant mesic species and ericaceous shrubs (Vandermast and van Lear 2002; Kane et al. 2008, 2020; Belair et al. 2014; Flatley et al. 2015; Varner et al. 2016b). American chestnut may devote less growth to roots and concentrates more energy on faster above ground growth when provided the opportunity than many co-occurring oaks (Wang et al. 2006; Belair et al. 2014, 2018; Brown et al. 2022).

Plantings will be critical for successful restoration efforts (Clark et al. 2016, 2022). There are three primary efforts underway to reintroduce this foundational species (Jacobs et al. 2013). These include creating genetically modified American chestnuts that are blight resistant, biocontrol of chestnut blight via hypovirulence where the chestnut blight fungus is weakened or killed by being infected with a virus, and selective breeding (Jacobs et al. 2013). The third effort works by breeding blight-susceptible American chestnut trees with blight-tolerant Chinese chestnut trees (*Castanea mollissima* Blume.) to create blight resistant hybrids (Jacobs 2007; Hebard 2012; Jacobs et al. 2013). This breeding program is led by the American Chestnut Foundation and has produced sixth generation, B_3F_3, hybrid chestnuts (Diskin et al. 2006; Clark et al. 2016). B_3F_3 hybrid chestnuts are the most advanced hybrid chestnuts and exhibit medium to high levels of blight tolerance, though more research is needed to assess the level of resistance in different environments and with different genetic backgrounds (Jacobs 2007; Hebard 2012;

Jacobs et al. 2013; Clark et al. 2014, 2019; Skousen et al. 2018). B_3F_3 hybrid chestnut seeds were first harvested in 2005 (Diskin et al. 2006).

Organisms with novel genotypes may express novel functions compared to wild type organisms (Jacobs 2007; Skousen et al. 2018; Goldspiel et al. 2019; Kane et al. 2019). Ideally, B_3F_3 hybrid chestnuts (referred to henceforth as "hybrid chestnuts") will function ecologically as American chestnut trees within an ecosystem, have blight tolerance levels of Chinese chestnuts, be able to reproduce in natural settings, and confer genetic resistance to wild populations of American chestnut (Burnham et al. 1986; Jacobs 2007; Hebard 2012). Hybrid chestnuts have to date been shown to provide similar ecosystem services and possess comparable phenotypic traits as American chestnut (Diskin et al. 2006; D'Amico et al. 2015; Goldspiel et al. 2019; Cipollini et al. 2020; Coughlin et al. 2021). However, little understanding exists of how these hybrid trees function within a forest ecosystem once they are reintroduced (Jacobs et al. 2013, Kane et al. 2020).

American chestnut responds positively to being planted in burned areas (McCament and McCarthy 2005) whereas hybrid chestnuts possess a different genetic makeup and a distinct evolutionary history from both their 15/16th American and 1/16th Chinese chestnut ancestries. Although both American and Chinese chestnut trees have a similar climate envelope, preliminary evidence shows that the leaves from Chinese and B_3F_2 hybrid chestnut trees are less flammable than American chestnut (Fei et al. 2012; Kane et al. 2019). Consequently, hybrid chestnuts may not respond to environmental conditions, including fire and changes in light, in the same manner as pure American chestnuts, and they may not fill the same historical niche (Kane et al. 2019, 2020).

The effect of prescribed fire as a silvicultural site preparation tool for planting hybrid chestnut seedlings is unknown. Our goal was to evaluate the influence of prescribed fire as a site preparation tool for hybrid chestnut seedling introduction in forested areas. We hypothesized that hybrid chestnuts would have higher growth rates when planted in plots that had been treated with prescribed fire than in adjacent unburned plots.

Methods

Research sites were established on private forested property in Yancey and McDowell Counties in North Carolina in November 2021. At each site there was a plot that received prescribed fire (burned) and a control plot that received no treatment. Each plot was ~0.20 hectares. Burned and control plots were selected ~300 m apart at the McDowell site in areas with best matched site characteristics as possible. Prescribed fire was implemented in March 2021 at the McDowell burned plot. The prescribed fire treatment was low in intensity and resulted in a scorch height of up to 2 m, with ~95% of fine fuels removed. The fire top-killed ~75% of ericaceous shrubs and ~10% of the hardwood midstory but 0% of the overstory. Two-year-old hybrid chestnut saplings were planted in rows using an auger on a 3.6 m spacing in late March 2022. Due to land and project constraints, the trees located at the McDowell site did not have cages to protect them from deer herbivory. Tree ID tags were hung on flagged metal stakes located 0.3 m away from each chestnut seedling.

The McDowell control plot was at an elevation of 655 – 677 m above sea level, with an aspect of 160°, and a slope of 7%. The McDowell control plot was in a transitional area near the bottom of a slope with elements of both rich-cove and montane oak-hickory forest type with an overstory of mixed oak with hickory, red maple (*Acer rubrum* L.), tulip poplar (*Liriodendron tulipifera* L.), black gum (*Nyssa sylvatica* Marshall.), and some white pine (*Pinus strobus* L.).

The McDowell burned plot was at an elevation of 627 – 659 m, an aspect of 150^{o}, and a slope of 15%. Because the McDowell burned plot was located ~300 m from the McDowell control plot, it had a slightly different forest community. The McDowell burned plot was located on a slope in a montane oak-hickory forest type with an overstory like the control plot but with some Virginia pine (*Pinus virginiana* Mill.), but less tulip poplar. The midstory in both plots was almost completely absent of oak and primarily consisted of red maple, tulip poplar, white pine, big leaf rhododendron, mountain laurel (*Kalmia latifoilia* L.), and some American chestnut sprouts.

The Yancey burned plot received a prescribed fire treatment in March 2022. The prescribed fire treatment was relatively low in intensity which resulted in a scorch height of 1.5 m and the removal of ~90% of fine fuels. The fire top-killed ~35% of the ericaceous shrubs, and damaged the remaining live ericaceous shrubs to a scorch height of 1.5 m, resulting in all the ericaceous shrubs dropping their leaves to a height of 1.5 m. The fire also resulted in ~10% midstory mortality, but 0% overstory mortality.

The Yancey site was planted with 60 two-year-old hybrid chestnut seedlings and 6 three-year-old hybrid chestnut seedlings in April 2022. Half of the two- and three-year-old seedlings were planted in the burned plot, with the other half planted in the control plot. Following the planting methods from Belair et al. (2018), hybrid chestnut seedlings were planted using an auger in a 2 m x 2 m grid in 9 - 12 tree blocks. Each chestnut was given a 0.4 m diameter and 1.2 m high, welded wire cage to prevent deer herbivory. Tree ID tags were hung from each cage.

The Yancey plots were adjacent to each other in a montane oak-hickory forest type with an overstory dominated by scarlet oak (*Quercus coccinea* Wangenh.) with occurrences of other co-occurring oaks, hickory, red maple, tulip poplar, and occasional species such as Fraser magnolia (*Magnolia fraseri* Walter.) and black gum. The Yancey site had an elevation of 858 -

869 m above sea level, an aspect of ~140°, and a slope of 22%. The Yancey plot was selected in an area with significant slope and well drained soils to reduce the chance of mortality in hybrid chestnut seedlings from root rot. As with the McDowell site, the midstory and understory of both Yancey plots contained very little oak or hickory and primarily consisted of ericaceous shrubs and mesic species including white pine, red maple, tulip poplar, and some American chestnut sprouts. Climate measurements were recorded 0.69 km from the Yancey study site at a NOAA weather station. Care was taken while planting to avoid disturbing the leaf litter in both unburned plots. Hybrid chestnut seedlings were not watered or given care of any kind after planting.

Hybrid chestnut height was measured in cm with a measuring stick, and diameter was measured in mm with digital calipers 5 cm above ground level. Pre-growing season height and diameter measurements were taken in April 2022 at the Yancey site and post-growing season measurements were taken in November 2022. We had less access to the trees located at the McDowell site where height and diameter were measured only at the end of the growing season in November 2022.

Understory light was measured in µmol $m^{-2}s^{-1}$ 1 m above each hybrid chestnut with a 15 second average reading using a LI-COR LI-250 light meter. Light measurements were taken on September 2022 near solar noon on partly cloudy days to measure ambient understory light and avoid inconsistencies from sun flecks.

Soil samples were collected in February 2022 for the Yancey site prior to the prescribed fire treatment. Hybrid chestnut leaves and post-fire soil samples were collected during peak growing season for both sites in August 2022. Soil samples were collected with three 2 x 10 cm cores taken one meter apart within a 10 x 10 m grid covering each study plot. All three cores were then mixed and pooled into one sample and transported on ice. Soil samples were then sieved at 2 mm, placed in scintillation vials, lyophilized, and then pulverized using a ball mill before analysis. Three to five fully emerged leaves were handpicked from each hybrid chestnut in the study. The leaves were lyophilized, placed in scintillation vials, pulverized using a ball mill, and lyophilized again before analysis. Soil and leaf samples were stored at -20° C before analysis. Soil and leaf samples were analyzed for total carbon and nitrogen (C and N) percent and C/N ratio using combustion analyses on a FlashEA 1112 NC Analyzer (Thermo Fisher Scientific, Waltham, MA).

Statistical Analysis

Statistical analyses were done using jamovi (R Core Team 2021; The jamovi project 2022). Shapiro-Wilk tests were used to check data normality. The prescribed fire effects on burned plots, and the growth responses of hybrid chestnuts, were analyzed by comparing variables from the burned and control plot at each site using Welch's t-tests for normal data and Mann-Whitney U tests for nonnormal data. Nonnormal data were not transformed. Results were significant when $p \leq 0.05$. Control and burned treatment post-growing season hybrid chestnut diameter and height values were analyzed for the McDowell site. Relative diameter and height growth values were examined for the Yancey site where pre-growing season data were collected. Leaf C/N ratios were compared to determine the growth response to control vs. burned treatments. Site characteristics of light $m^{-2}s^{-1}$, soil total C and N, and soil C/N ratio were compared between burned and control plots at each site, while simple linear regression was used to analyze the impact of individual site characteristics impacts on hybrid chestnut growth. Relative growth and relative soil C/N ratio change values were calculated with the following formula: (final – initial) / initial.

Results

Hybrid chestnuts at the McDowell site had more understory light availability in the burned plot than in the control plot (Figure 2a). As with the McDowell site, hybrid chestnuts at the Yancey site had more understory light in the burned plot than in the control plot (Figure 2b).

The soil C/N ratios were higher in the burned plot than the control plot at the McDowell site, while total soil C and N were higher in the control plot (Table 1). Given the lack of pre-burn treatment data, it is difficult to discern why these differences exist. There were no differences in total soil C and N, as well as C/N ratio between the burned and control plots before or after the prescribed fire treatment at the Yancey plot (Table 1). The relative soil C/N ratio at the Yancey plot did slightly increase in the burned plot while it slightly decreased in the burn plot, these differences were not significant (Table 1).

Two growing seasons after being planted, the stem diameters of hybrid chestnuts planted at the McDowell burned plot were larger than hybrid chestnuts planted in the control plot (Figure 1a). Additionally, hybrid chestnuts planted in the burned plot were taller than those planted in the unburned plot at the McDowell site (Figure 1b). After one growing season, the Yancey site hybrid chestnuts planted in the burned plot had higher stem diameter relative growth rates than those planted in unburned plots (Figure 1c). Although the stem height relative growth of hybrid chestnuts trended toward being taller in the burned plot compared to the unburned plot, there was no significant difference (Figure 1d).

Leaf total C was not different between the burned and control plots at both sites. There was no difference in total leaf N between the burned and control plots at the Yancey site while at the McDowell site leaf total N was higher in the burned than the control plot (Table 1). Hybrid chestnuts leaf C/N ratios from McDowell in the burned plot were higher than those in the unburned plot (Table 1). The leaf C/N ratios from hybrid chestnuts in the Yancey burned plot were not higher than hybrid chestnuts in the unburned plot (Table 1).

Light was not a good predictor for hybrid chestnut diameter and height for the McDowell site (Table 2). But at the Yancey site, light positively predicted diameter and height relative growth (Table 2 and Figure 3). At both sites, increased light led to increased leaf C/N ratio (Table 2) and soil C/N ratio predicted leaf C/N ratio at the McDowell site (Figure 4). Otherwise, no relationships were found between the soil C/N ratio and hybrid chestnut characteristics at both sites.

Discussion

In general hybrid chestnuts grew faster when planted in plots that had been treated with prescribed fire than in adjacent unburned control plots (Figures 1). This increased growth was likely in response to increased light in burned plots compared to unburned plots (Figure 2). The effect of fire on soil characteristics was minimal for the Yancey plot and difficult to assess for the McDowell site.

The general positive relationship between light availability and growth agrees with previous research that has demonstrated an increase in American chestnut stem and height growth with high light conditions (Wang et al. 2006; Joesting et al. 2007; Belair et al. 2014; 2018). In fact, McCament and McCarthy (2005) found light to be the most important factor for young American chestnut growth compared to soil texture and chemistry. Despite a strong dependence on light for rapid growth, American chestnut exhibits a significant degree of shade tolerance and can survive in the understory by reducing vertical growth and increasing horizontal growth and leaf surface area (McCament and McCarthy 2005; Wang et al. 2006; Belair et al.

2014). With an increase in light, it can rapidly adjust its morphology and allocate resources for greater vertical growth (Wang et al. 2006; Belair et al. 2014, 2018).

Deciduous tree seedlings grown with more light generally have higher leaf C/N ratios (Giertych et al. 2015) and we found this to be the case for the hybrid chestnuts in the burned McDowell plot (Table 1). There was a similar but nonsignificant trend in the Yancey burned plot (Table 1). Light positively predicted leaf C/N ratios at both sites (Table 2). Quick growing deciduous seedlings, including the American chestnut, allocate C for growth with increased light conditions (Imaji and Seiwa 2010; Giertych et al. 2015; Belair et al. 2018) and the higher leaf C/N ratio in the burned plot may indicate that hybrid chestnuts are be prioritizing C for growth instead of storage in the root or as defense chemicals (Imaji and Seiwa 2010).

Transplant shock, in which a plant undergoes a period of intense stress, impaired growth, or decline after planting from unestablished root systems (Close et al. 2005; Jacobs et al. 2009) likely affected the hybrid chestnuts in our experiment. A slightly negative diameter growth rate in the Yancey unburned plot reflects symptoms of transplant shock, in which stems die then resprout from rootstock (Figure 1c). Transplant shock at the Yancey site was likely exaggerated as record high temperatures were paired with only 29 mm of precipitation from May 27 – July 3, 2022, compared to the average accumulation of 181 mm (Eggleston and NOAA 2013). Precipitation remained below normal for the remainder of 2022 (Eggleston and NOAA 2013).

The growth response to prescribed fire treatment may have been pronounced at the McDowell plot because the hybrid chestnuts had two growing seasons to respond to transplant shock and the postfire growing conditions (Close et al. 2005; Jacobs et al. 2009). Removal of rhododendron has been shown to increase soil moisture content and improve conditions for seedlings (Dharmadi et al. 2022). Although not tested here, it is possible that hybrid chestnuts in burned plots with less rhododendron had higher soil moisture contents which could have contributed to the faster growth rate in burned plots.

The prescribed fire treatment had little effect on soil total C and N in this experiment (Table 1). Total soil C and N loss may occur after wildland fire but varies depending on the fire intensity, with low intensity fires usually contributing little to nutrient loss and changes in the C/N ratio (Hatten et al. 2005; Homann et al. 2011; Pellegrini et al. 2015, 2018). Given the lack of pre-burn soil data for the McDowell site, it is difficult to assess the fire's effect on soil characteristics between the McDowell burned and unburned plot. The higher soil total C and N values at the McDowell control plot can likely be attributed to the different locations of the burned and control plots. The control plot is situated close to the bottom of a hill with a slope of ~7%, while the burned plot is located on a steeper ridge with a slope of ~15%. Soil erosion carries nutrients downslope, resulting in higher amounts of nutrients in flatter areas at the bottom of slopes (el Kateb et al. 2013). The difference in soil between both plots is reflected in the plant communities present at both plots, in which the control plot has some characteristics of a rich cove plant community. Long term monitoring of plant, soil, and hybrid chestnut leaf characteristics would be beneficial to determining the exact relationship between these site variables in relation to hybrid chestnut growth.

Herbivory, primarily deer browse, impacts the population growth of deciduous species, including American chestnut (Elwood et al. 2018; Blossey et al. 2019). Young seedlings, such as in most reforestation plantings, are very susceptible to herbivory and are one of the most critical life stages for a population (Blossey et al. 2019). A higher growth rate of planted restoration seedlings shorten the time to maturity and sexual reproduction; this would decrease the level of stocking needed and time until natural regeneration, leading to quicker population growth (van Drunen et al. 2017).

With widespread fire suppression throughout eastern North American forests for the last ~100 years, mesic, fire-intolerant trees are colonizing areas historically dominated by pyrophytic trees and outcompeting them in a process coined "mesophication" (Nowacki and Abrams 2008). This process is a positive feedback loop that alters forest succession in which the flammability of the forest is decreasing as mesic trees dominate, further reducing the chance for fire-tolerant species to reproduce and recruit (Nowacki and Abrams 2008; Flatley et al. 2015). With mesophication, the forest will be less flammable and less diverse in the future (Nowacki and Abrams 2008). Within the eastern North American deciduous forest, most fire behavior is influenced by continuous fuels made up of leaves and needles on the forest floor (Kane et al. 2008, 2019; Dickinson et al. 2016). Introducing hybrid chestnuts into the forest may increase the flammability of continuous fuels within the forests of the future which could help offset the effects of mesophication (Kane et al. 2019).

Given the response exhibited by American chestnut to disturbance and the ecological importance of wildland fire in the southern Appalachian region, it is necessary for ecologists and land managers to understand the relation between fire and hybrid chestnuts for restoration with hybrid chestnuts to succeed long-term on a large spatial scale (Wang et al. 2006; Jacobs 2007; Kane et al. 2020). In our one year project, planted B_3F_3 hybrid chestnut seedlings grew faster in burned plots, suggesting that prescribed fire is an effective silvicultural tool for introducing hybrid chestnuts in certain forest communities. However, large-scale restoration of the American chestnut through hybrid planting has several unknown implications for ecological processes. These include restoring flammability to forests through the reintroduction of a fire promoting tree, the potential impacts associated with introducing a novel genotype, and how light and soil characteristics change in response to hybrid chestnuts establishment.

There is little known about the long-term survival of hybrid chestnuts. Given this knowledge gap, it is difficult to predict the future growth of the hybrid chestnut seedlings in our study. If our hybrid chestnut seedlings have similar long-term growth strategies as American chestnut, then further disturbance may be needed to maintain the necessary understory light levels for continued survival and competition against species with higher shade-tolerance. Additional canopy level disturbance events may be needed to allow midstory hybrid chestnut saplings the light needed to recruitment to the overstory.

Literature Cited

Anagnostakis, S. L. 2012. Chestnut breeding in the United States for disease insect resistance. Plant Disease 96:1392–1403.

Belair, E. D., M. R. Saunders, and B. G. Bailey. 2014. Four-year response of underplanted American chestnut (Castanea dentata) and three competitors to midstory removal, root trenching, and weeding treatments in an oak-hickory forest. Forest Ecology and Management 329:21–29.

Belair, E. P., M. R. Saunders, and S. M. Landhäusser. 2018. Growth traits of juvenile American chestnut and red oak as adaptations to disturbance. Restoration Ecology 26:712–719.

Blossey, B., P. Curtis, J. Boulanger, and A. Dávalos. 2019. Red oak seedlings as indicators of deer browse pressure: Gauging the outcome of different white-tailed deer management approaches. Ecology and Evolution 9:13085–13103.

Brown, S. P., S. L. Clark, E. Ford, A. Jumpponen, A. M. Saxton, S. E. Schlarbaum, and R. Baird. 2022. Comparisons of interspecies field performance of Fagaceae (Castanea and Quercus) planted in the southeastern United States with attention to soil fungal impacts on plant performance. Forest Ecology and Management 525 (doi: 10.1016/j.foreco.2022.120569).

Burnham, C. R., P. A. Rutter, and D. W. French. 1986. Breeding Blight-Resistant Chestnuts. Plant Breeding Reviews 4:347–397.

Christensen, N. L. 2014. An historical perspective on forest succession and its relevance to ecosystem restoration and conservation practice in North America. Forest Ecology and Management 330:312–322.

Cipollini, M., N. Wessel, J. P. Moss, and N. Bailey. 2020. Seed and seedling characteristics of hybrid chestnuts (Castanea spp.) derived from a backcross blight-resistance breeding program. New Forests 51:523–541.

Clark, S. L., S. E. Schlarbaum, C. C. Pinchot, S. L. Anagnostakis, M. R. Saunders, M. Thomas-Van Gundy, P. Schaberg, J. McKenna, J. F. Bard, P. C. Berrang, D. M. Casey, C. E. Casey, B. Crane, B. D. Jackson, J. D. Kochenderfer, R. F. Lewis, R. MacFarlane, R. Makowski, M. D. Miller, J. A. Rodrigue, J. Stelick, C. D. Thornton, and T. S. Williamson. 2014. Reintroduction of American Chestnut in the National Forest System. Journal of Forestry 112:502–512.

Clark, S. L., S. E. Schlarbaum, A. M. Saxton, and R. Baird. 2019. Eight-year blight (Cryphonectria parasitica) resistance of backcross-generation American chestnuts (Castanea dentata) planted in the southeastern United States. Forest Ecology and Management 433:153–161.

Clark, S. L., S. E. Schlarbaum, A. M. Saxton, and F. V. Hebard. 2016. Establishment of American chestnuts (Castanea dentata) bred for blight (Cryphonectria parasitica) resistance: influence of breeding and nursery grading. New Forests 47:243–270.

Close, D. C., C. L. Beadle, and P. H. Brown. 2005. The physiological basis of containerised tree seedling “transplant shock”: a review. Australian Forestry 68:112–120.

Collins, R. J., C. A. Copenheaver, M. E. Kester, E. J. Barker, and K. G. DeBose. 2017. American Chestnut: Re-Examining the Historical Attributes of a Lost Tree. Journal of Forestry 116:68–75.

Coughlin, E. M., R. P. Shefferson, S. L. Clark, and N. Wurzburger. 2021. Plant–soil feedbacks and the introduction of Castanea (chestnut) hybrids to eastern North American forests. Restoration Ecology 29 (doi: 10.1111/rec.13326).

Dalgleish, H. J., C. D. Nelson, J. A. Scrivani, and D. F. Jacobs. 2016. Consequences of shifts in abundance and distribution of American chestnut for restoration of a foundation forest tree. Forests 7 (doi: 10.3390/f7010004).

D'Amico, K. M., T. R. Horton, C. A. Maynard, S. v. Stehman, A. D. Oakes, and W. A. Powell. 2015. Comparisons of ectomycorrhizal colonization of transgenic American chestnut with those of the wild type, a conventionally bred hybrid, and related Fagaceae species. Applied and Environmental Microbiology 81:100–108.

Dharmadi, S. N., K. J. Elliott, and C. Ford Miniat. 2022. Larger hardwood trees benefit from removing Rhododendron maximum following Tsuga canadensis mortality. Forest Ecology and Management 516 (doi: 10.1016/j.foreco.2022.120234).

Diamond, S. J., R. H. Giles, and R. L. Kirkpatrick. 2000. Hard Mast Production Before and After the Chestnut Blight. Southern Journal of Applied Forestry 24:196–201.

Dickinson, M. B., T. F. Hutchinson, M. Dietenberger, F. Matt, and M. P. Peters. 2016. Litter species composition and topographic effects on fuels and modeled fire behavior in an oak-hickory forest in the Eastern USA. PLoS ONE 11 (doi: 10.1371/journal.pone.015999).

Diskin, M., K. C. Steiner, and F. v. Hebard. 2006. Recovery of American chestnut characteristics following hybridization and backcross breeding to restore blight-ravaged Castanea dentata. Forest Ecology and Management 223:439–447.

van Drunen, S. G., K. Schutten, C. Bowen, G. J. Boland, and B. C. Husband. 2017. Population dynamics and the influence of blight on American chestnut at its northern range limit: Lessons for conservation. Forest Ecology and Management 400:375–383.

Ellison, A. M., M. S. Bank, B. D. Clinton, E. A. Colburn, K. Elliott, C. R. Ford, D. R. Foster, B. D. Kloeppel, J. D. Knoepp, G. M. Lovett, J. Mohan, D. A. Orwig, N. L. Rodenhouse, W. v Sobczak, K. A. Stinson, J. K. Stone, C. M. Swan, J. Thompson, B. von Holle, and J. R. Webster. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. Frontiers in Ecology and the Environment 3:479–486.

Elwood, E. C., N. I. Lichti, S. F. Fitzsimmons, and H. J. Dalgleish. 2018. Scatterhoarders drive long- and short-term population dynamics of a nut-producing tree, while pre-dispersal seed predators and herbivores have little effect. Journal of Ecology 106:1191–1203.

Fei, S., L. Liang, F. L. Paillet, K. C. Steiner, J. Fang, Z. Shen, Z. Wang, and F. v. Hebard. 2012. Modelling chestnut biogeography for American chestnut restoration. Diversity and Distributions 18:754–768.

Flatley, W. T., C. W. Lafon, H. D. Grissino-Mayer, and L. B. LaForest. 2015. Changing fire regimes and old-growth forest succession along a topographic gradient in the Great Smoky Mountains. Forest Ecology and Management 350:96–106.

Foster, D. R., S. Clayden, D. A. Orwig, B. Hall, and S. Barry. 2002. Oak, chestnut and fire: climatic and cultural controls of long-term forest dynamics in New England, USA. Journal of Biogeography 29:1359–1379.

Giertych, M. J., P. Karolewski, and J. Oleksyn. 2015. Carbon allocation in seedlings of deciduous tree species depends on their shade tolerance. Acta Physiologiae Plantarum 37 (doi: 10.1007/s11738-015-1965-x).

Goldspiel, H. B., A. E. Newhouse, W. A. Powell, and J. P. Gibbs. 2019. Effects of transgenic American chestnut leaf litter on growth and survival of wood frog larvae. Restoration Ecology 27:371–378.

Guyette, R. P., M. C. Stambaugh, D. C. Dey, and R.-M. Muzika. 2012. Predicting fire frequency with chemistry and climate. Ecosystems 15:322–335.

Hatten, J., D. Zabowski, G. Scherer, and E. Dolan. 2005. A comparison of soil properties after contemporary wildfire and fire suppression. Forest Ecology and Management 220:227–241.

Hebard, F. V. 2012. The American Chestnut Foundation breeding program. Proceedings of the fourth international workshop on the genetics of host-parasite interactions in forestry: Disease and insect resistance in forest trees. General Technical Report PSW-GTR-240.

Homann, P. S., B. T. Bormann, R. L. Darbyshire, and B. A. Morrissette. 2011. Forest soil carbon and nitrogen losses associated with wildfire and wrescribed Fire. Soil Science Society of America Journal 75:1926–1934.

Imaji, A., and K. Seiwa. 2010. Carbon allocation to defense, storage, and growth in seedlings of two temperate broad-leaved tree species. Oecologia 162:273–281.

Jacobs, D. F. 2007. Toward development of silvical strategies for forest restoration of American chestnut (Castanea dentata) using blight-resistant hybrids. Biological Conservation 137:497–506.

Jacobs, D. F., H. J. Dalgleish, and C. D. Nelson. 2013. A conceptual framework for restoration of threatened plants: The effective model of American chestnut (Castanea dentata) reintroduction. New Phytologist 197:378–393.

Jacobs, D. F., K. Francis Salifu, and A. S. Davis. 2009. Drought susceptibility and recovery of transplanted Quercus rubra seedlings in relation to root system morphology. Annals of Forest Science 66:504–504.

James, N. A., K. L. Abt, G. E. Frey, X. Han, and J. P. Prestemon. 2020. Fire in the Southern Appalachians: Understanding impacts, interventions, and future fire events. e-General Technical Report SRS-249.

Joesting, H. M., B. C. McCarthy, and K. J. Brown. 2007. The photosynthetic response of American chestnut seedlings to differing light conditions. Canadian Journal of Forest Research 37:1714–1722.

Kane, J. M., J. M. Varner, and J. K. Hiers. 2008. The burning characteristics of southeastern oaks: Discriminating fire facilitators from fire impeders. Forest Ecology and Management 256:2039–2045.

Kane, J. M., J. M. Varner, and M. R. Saunders. 2019. Resurrecting the lost flames of American chestnut. Ecosystems 22:995–1006.

Kane, J. M., J. M. Varner, M. C. Stambaugh, and M. R. Saunders. 2020. Reconsidering the fire ecology of the iconic American chestnut. Ecosphere 11 (doi: 0.1002/ecs2.3267).

el Kateb, H., H. Zhang, P. Zhang, and R. Mosandl. 2013. Soil erosion and surface runoff on different vegetation covers and slope gradients: A field experiment in Southern Shaanxi Province, China. Catena 105:1–10.

Eggleston, K., and NOAA. 2013. SC ACIS. NOAA Northeast Regional Climate Center.

Latham, R. E. 1992. Co-Occurring Tree species change rank in seedling performance with resources varied experimentally. Ecology 73:2129–2144.

McCament, C. L., and B. C. McCarthy. 2005. Two-year response of American chestnut (Castanea dentata) seedlings to shelterwood harvesting and fire in a mixed-oak forest ecosystem. Canadian Journal of Forest Research 35:740–749.

Mitchell, R. J., J. K. Hiers, J. O'brien, and G. Starr. 2009. Ecological forestry in the Southeast: Understanding the ecology of fuels. Journal of Forestry 107:391–397.

Nowacki, G. J. , and M. D. , Abrams. 2008. Nowacki and Abrams., 2008, The demise of fire and mesophication of forests in the eastern US. BioScience 58:123–138.

Orwig, D. A. 2003. Ecosystem to regional impacts of introduced pests and pathogens: historical context, questions and issues. Journal of Biogeography 29:1471–1474.

Paillet, F. L., and P. A. Rutter. 1989. Replacement of native oak and hickory tree species by the introduced American chestnut (Castanea dentata) in southwestern Wisconsin. Canadian Journal of Botany 67:3457–3469.

Pellegrini, A. F. A., A. Ahlström, S. E. Hobbie, P. B. Reich, L. P. Nieradzik, A. C. Staver, B. C. Scharenbroch, A. Jumpponen, W. R. L. Anderegg, J. T. Randerson, and R. B. Jackson. 2018. Fire frequency drives decadal changes in soil carbon and nitrogen and ecosystem productivity. Nature 553:194–198.

Pellegrini, A. F. A., L. O. Hedin, A. C. Staver, N. Govender, and H. A. L. Henry. 2015. Fire alters ecosystem carbon and nutrients but not plant nutrient stoichiometry or composition in tropical savanna. Ecology 96:1275–1285.

R Core Team. 2021. R: A Language and environment for statistical computing.

Saucier, J. R. 1973. American chestnut an American wood. USDA Forest Service Publication 230.

Skousen, J. G., K. Dallaire, S. Scagline-Mellor, A. Monteleone, L. Wilson-Kokes, J. Joyce, C. Thomas, T. Keene, C. DeLong, T. Cook, and D. F. Jacobs. 2018. Plantation performance of chestnut hybrids and progenitors on reclaimed Appalachian surface mines. New Forests 49:599–611.

Stritch, L. 2018. Castanea dentata, American Chestnut. The IUCN Red List of Threatened Species 2018 (doi 10.2305/IUCN.UK.2018-1.RLTS.T62004455A62004469.en).

The jamovi project. 2022. jamovi. (Version 2.3) [Computer Software].

Vandermast, D. B., and D. H. van Lear. 2002. Riparian vegetation in the southern Appalachian mountains (USA) following chestnut blight. Forest Ecology and Managment 155:97–106.

Vandermast, D. B., D. H. van Lear, and B. D. Clinton. 2002. American chestnut as an allelopath in the southern Appalachians. Forest Ecology and Management 165:173–181.

Varner, J. M., M. A. Arthur, S. L. Clark, D. C. Dey, J. L. Hart, and C. J. Schweitzer. 2016a. Fire in eastern north American Oak ecosystems: Filling the gaps. Fire Ecology 12:1–6.

Varner, J. M., J. M. Kane, J. K. Hiers, J. K. Kreye, and J. W. Veldman. 2016b. Suites of fire-adapted traits of oaks in the Southeastern USA: Multiple strategies for persistence. Fire Ecology 12:48–64.

Varner, J. M., J. M. Kane, J. K. Kreye, and T. M. Shearman. 2021. Litter Flammability of 50 Southeastern North American Tree Species: Evidence for Mesophication Gradients Across Multiple Ecosystems. Frontiers in Forests and Global Change 4 (doi: 10.3389/ffgc.2021.727042).

Wang, G. G., W. L. Bauerle, and B. T. Mudder. 2006. Effects of light acclimation on the photosynthesis, growth, and biomass allocation in American chestnut (Castanea dentata) seedlings. Forest Ecology and Management 226:173–180.

Tables

Table 1. Soil and hybrid chestnut leaf C and N statistical descriptives for burned vs. unburned plots at both study sites.

	McDowell					**Yancey**				
Parameters	***n***	**Burned *M, SD***	**Control *M, SD***	**Statistic**	***p* value**	***n***	**Burned *M, SD***	**Control *M, SD***	**Statistic**	***p* value**
Soil C/N ratio	36	27.0, 4.31	18.3, 1.66	$U = 1.00$	<.001	12	23.8, 1.58	22.4, 2.60	$t = -1.12$	.294
Soil total C	36	6.64, 2.41	14.1, 3.51	$t = 7.49$	<.001	12	8.17, 1.59	8.07, 0.993	$t = -0.130$	.900
Soil total N	36	0.245, 0.0762	0.776, 0.197	$t = 10.8$	<.001	12	0.341, 0.0488	0.363, 0.0545	$t = 0.721$	.488
Soil C/N ratio†						10	22.4, 1.39	23.4, 2.25	$t = 0.833$	.429
Soil total C†						11	10.7, 6.16	9.27, 3.02	$t = -0.461$	.662
Soil total N†						11	0.502, 0.326	0.410, 0.176	$U = 15.0$	1.00
Soil relative C/N†						10	0.0857, 0.0960	-0.0359, 0.111	$t = -1.84$	.107
Leaf C/N ratio	125	36.4, 3.49	26.0, 5.91	$U = 193$	<.001	47	18.2, 2.45	17.4, 2.71	$t = -0.0981$	.332
Leaf total C	125	50.5, 3.80	53.0, 1.95	$U = 1666$	.171	47	47.1, 0.730	46.7, 0.982	$U = 191$	.072
Leaf total N	125	1.42, 0.235	2.08, 0.283	$t = 14.03$	<.001	47	2.64, 0.406	2.74, 0.434	$U = 242$	.479

†Pre-burn treatment soil data was collected in February 2022 for the Yancey site only.

Table 2. Simple linear regression results for light vs. hybrid chestnut characteristics at both study sites.

	McDowell					Yancey				
Parameters	***n***	**Slope**	**Intercept**	r^2	***p* value**	***n***	**Slope**	**Intercept**	r^2	***p* value**
Diameter (relative growth for Yancey)	123	0.00721	4.09	0.0161	.169	49	0.00931	-0.0586	0.120	.015
Height (relative growth for Yancey)	123	0.0595	36.4	0.00495	.447	48	0.0248	-0.0159	0.0844	.045
Leaf C/N Ratio	125	.249	23.8	0.183	<.001	47	0.310	14.6	0.202	.002

Figure Legends

Figure 1. Hybrid chestnut physical growth values for the unburned and burned plots at both study sites.

Box and whisker with violin plots and data distribution represent hybrid chestnut mensuration values from burned vs. control (unburned) plots: (a) McDowell diameter, (b) McDowell height, (c) Yancey diameter relative growth, and (d) Yancey height relative growth. The dark band in each box plot represents the median of the data, the square dot is the mean, and the violin plot shows the density distribution of data. Statistical results are from Welch's t-tests.

Figure 2. Understory light availability for the unburned and burned plots at both study sites.

Descriptives plots for light measurements taken at the (a) McDowell and (b) Yancey sites. The hollow circle represents the mean, and the hollow square shows the mode. The "whiskers" demonstrate the standard deviation of each data set. Statistical results are from Welch's t-tests.

Figure 3. Light vs. Diameter relative growth simple linear regression line, Yancey study site.

The positive linear relationship of light as a predictor for hybrid chestnut seedling diameter relative growth at the Yancey site.

Figure 4. Soil C/N ratio vs. leaf C/N ratio simple linear regression line, McDowell study site.

The positive linear relationship between soil C/N ratio and hybrid chestnut leaf C/N ration at the McDowell plot. No other significant relationships were found between soil C/N ratio and hybrid chestnut characteristics at either site.

Figures

Figure 1. Hybrid chestnut physical growth values for the unburned and burned plots at both study sites.

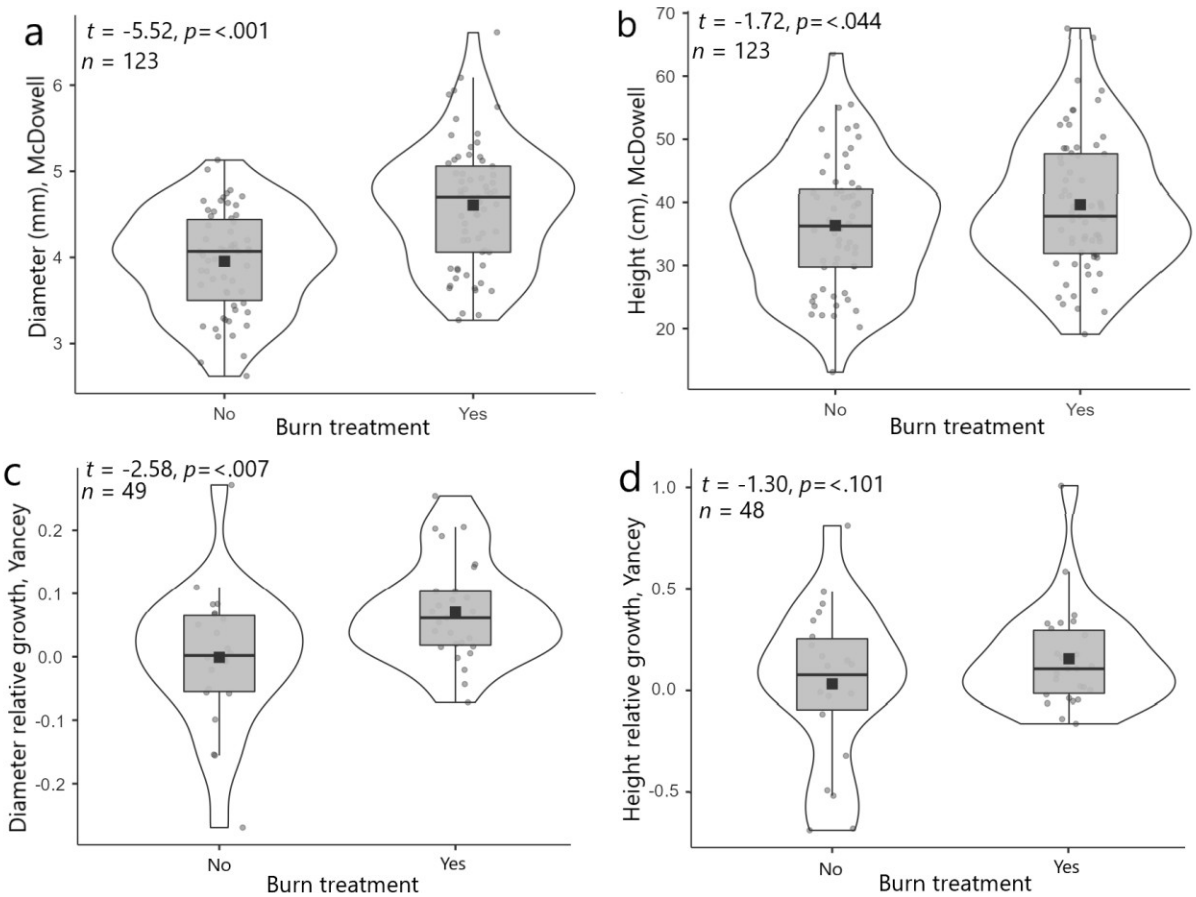


Figure 2. Understory light availability for the unburned and burned plots at both study sites.

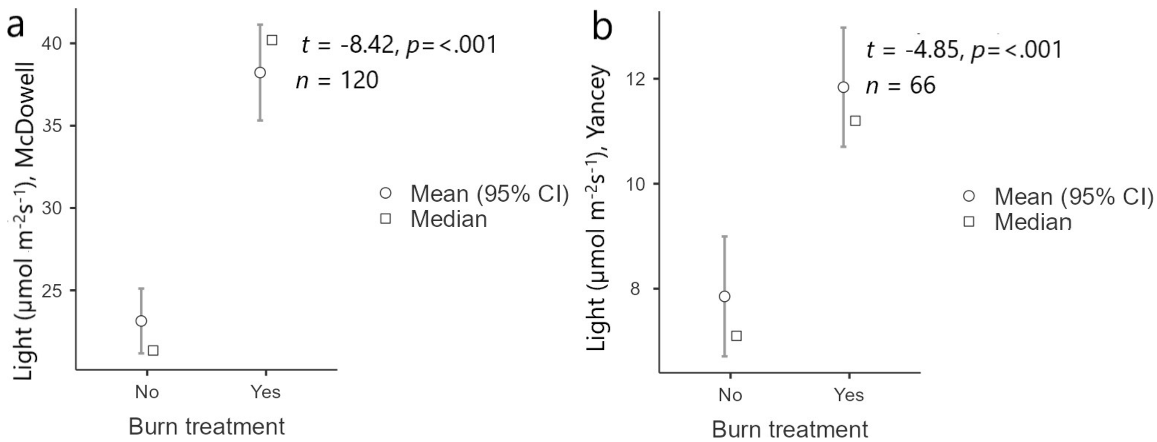


Figure 3. Light vs. Diameter relative growth simple linear regression line, Yancey study site.

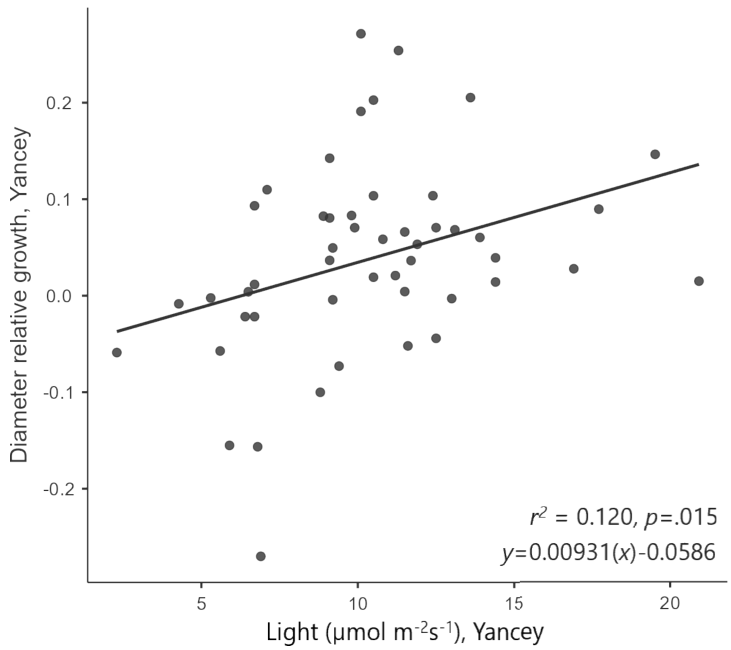
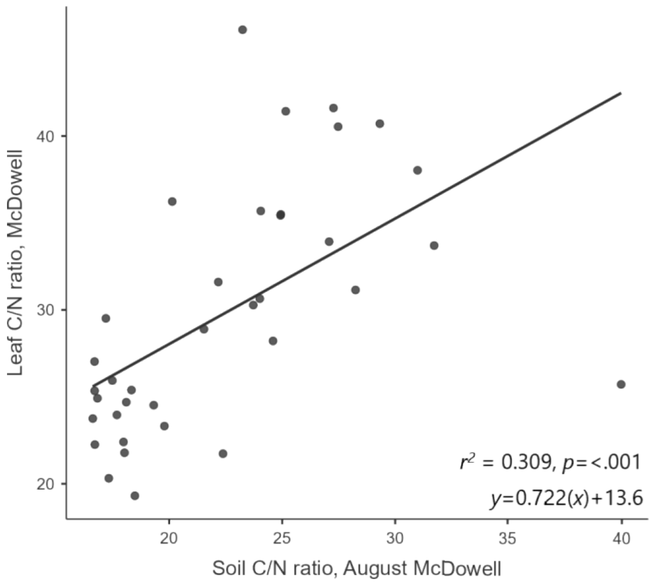


Figure 4. Soil C/N ratio vs. leaf C/N ratio simple linear regression line, McDowell study site.



Vita

Felix Stith was born and raised in western NC. He earned a BS in Environmental Studies from UNC Asheville in 2018. While in college he worked for the US Forest Service mapping streams in the Nantahala National Forest and as a hydrochemical lab technician for the Environmental Quality Institute in Asheville NC. After graduating from UNC Asheville, he started working for Blue Ridge Resource Conservation & Development as the Toe-Cane Watershed Coordinator. He enrolled in Appalachian State University in the fall of 2021 to earn his MS in Biology with a concentration in Ecology and Evolutionary Biology. In the summer of 2022, he started his own ecological restoration and forestry company, Black Mountain Field & Forest LLC. He plans to continue to live and work in western NC after graduating from ASU in spring 2023.