BIOCHAR AND ECOSYSTEM RECOVERY:
EVALUATING ARTIFICIAL REGENERATION OUTCOMES AND UNDERSTORY PLANT COMMUNITY DYNAMICS IN RESPONSE TO TOP-DRESS AMENDMENTS IN NORTHERN MINNESOTA

A THESIS SUBMITTED TO THE FACULTY OF THE UNIVERSITY OF MINNESOTA BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE
NATURAL RESOURCE SCIENCE AND MANAGEMENT

Marcella Windmuller-Campione

May 2024

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Acknowledgements

First and foremost, I am profoundly grateful to my advisor, Dr. Marcella Windmuller-Campione, for taking the time three years ago to speak with a technician who aspired to become a silviculturist. Thanks to her patience, support, and guidance, I have accomplished that goal. Her mentorship and commitment to both her students and the field of silviculture will remain with me and serve as a model throughout my professional journey. I'd also like to express my gratitude to my committee members, Dr. Chris Edgar and Dr. Mike Dockry, for their valuable insights and thoughtful feedback over the course of this project.

There are countless number of people from the silva lab who made this research possible, many of whom were involved in the project long before I even knew what biochar was. To Mat Lochner, for his guiding the design and installation of this project from its inception, and for revisiting to witness its progress; to Laura Reuling, for her unwavering enthusiasm and indispensable assistance both in the field and in the office; and to Alan Toczydlowski, for his invaluable insight and advice in analyzing data and interpreting our findings. To my lab mates, spanning two cohorts of students, I am eternally grateful for your support, inside jokes, and wonderful company through the years: Ella Gray, Amy Shaunette, Auste Eigirdas, Quentin Ikuta, Kira Pollack, Abby Daniels, Chelsea Semper, Holly Francart, and many, many others.

This project would not have been possible without the invaluable support of the staff at the Cloquet Forestry Center, including Kyle Gill, Rachael Olesiak, and Lane Johnson. Your contributions were essential to the success of this project, and I thank you wholeheartedly. To my incredible field technicians, Ethan Engel, Rachel Stepp, and Mary Ulrich. I am forever grateful for all of the hard work, laughter, and unwavering support offered up while confronting every challenge, from pouring rain or blistering sun to gophers, spiders, and swarms of mosquitoes and ladybugs. Your dedication is truly invaluable, and I am deeply thankful for the opportunity to learn and lead alongside you.

Outside of the University, I have been incredibly blessed with a strong support system, without whom none of this would have been possible. To my parents, Jean and Jon, you have encouraged and believed in me from day one, and I will never be able to fully put to words my gratitude for all that you have done for me. To my sister, Ella, you've always been there for me, a constant source of encouragement and belly laughs. To my partner Adam, I am deeply grateful for your companionship and unconditional support as we

navigated this journey together. To my cat Baloo, for courageously weathering multiple cross-country moves to make this thesis possible, and for never failing to find my lap when I need comfort the most. To my roommates Jack, Erin, Ian, and Gimli for being the most wonderful little community and support group I could ever ask for. And to my best friends, Sophie and Cassidy, for your reassurance and giggles when I needed them the most.

Dedication

This thesis is dedicated to the Land on which this research was conducted.

Presently known as the University of Minnesota Cloquet Forestry Center, this land is located on Nagaajiwanaang, the homelands and unceded territory of the Fond du Lac Band of the Lake Superior Chippewa.

Abstract

Recurring and severe droughts present significant obstacles to successful forest regeneration. Biochar soil amendments have emerged as a promising possible solution, providing the dual benefit of mitigating climate change through carbon sequestration while enhancing forest soil health in areas facing regeneration challenges. These amendments improve seedling drought resilience through enhanced cation exchange capacity, water retention, and nutrient availability. However, existing research in forested systems is limited, and primarily focuses on short-term impacts of the amendment on soil nutrients, physical properties, and microbial communities. Researchers have identified a need for long-term, site-specific research regarding how biochar affects forests' growth and stand dynamics. Over a five-year period, we investigated the effects of two different doses of top-dressed biochar soil amendments on tree growth across four different species. Our study did not reveal significant positive effects of biochar on seedling growth or survival over this period. However, it also did not indicate any negative influence on seedling survival or growth. Notably, each species exhibited distinct response patterns, suggesting potential trends deserving further investigation and emphasizing the importance of temporal scale in such studies. Additionally, our analysis of vegetative community composition and structure in response to the biochar amendments revealed distinct species-community responses, indicating complex underlying mechanisms warranting further investigation. In summary, this study contributes to a deeper understanding of biochar’s role in post-disturbance vegetation recovery and sheds light on the variability in seedling response patterns influenced by biochar soil amendment. Emphasizing the significance of species-specific responses, as well as the potential ecosystem-wide cascading effects, our findings highlight the complexity of biochar applications and their interactions with both tree species and understory vegetation over time. This research adds to the growing body of knowledge on biochar applications in forestry, underscoring its potential benefits in reforestation and carbon sequestration efforts. Furthermore, it enhances our understanding of the potential long-term effects of biochar soil amendments on forest health, stand dynamics, and sustainable forest management practices in a changing climate.

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Introduction

Headlines across the globe noted summer 2023 as the hottest on record (Levitt & Shao 2023; Sommer & Hersher 2024). Locally within the Great Lakes Region (GLR) in the US (defined as Minnesota, Michigan, and Wisconsin), climatic models predict increased temperatures as well as heightened precipitation, resulting in a general forecast of warmer and wetter spring and summer seasons (Liess et al. 2022). However, despite these warming and wetting trends in the models, climate science also indicates that the GLR will experience greater variability and more extreme weather patterns, including increased incidence of extended dry periods and potential drought occurrences (Handler et al. 2017). The historically unprecedented drought of 2021 and 2023 vividly illustrated the unpredictability and potentially devastating consequences of these predicted conditions, particularly concerning reforestation efforts. Federal, state, tribal, and private forest lands across Minnesota were severely impacted by the drought. The MN DNR estimated that 72 percent of conifer plantings less than four years old died; field monitoring conducted the following summer revealed seedling mortality rates as high as 100 percent in some stands (MN DNR: 2022 Supplemental Budget: Drought Relief). Fluctuating conditions, coupled with uncertainties inherent in climate modeling, frame a multifaceted scientific narrative that outlines the challenges Minnesota forests may face in the future—linked to water scarcity and tangible threats of future droughts.

Drought-induced tree mortality arises from an imbalance in water and carbon dynamics, where the depletion of these resources and their fluxes fails to meet the demands of living tissues (Ryan 2011). The interdependence of these pools and fluxes underpins plant defenses against pathogens and pests, with death often resulting from inadequate soil-to-root water conductance, increased vulnerability to hydraulic failure, excessive cuticular water loss, and low dehydration tolerance (Vose et al. 2015). Such stresses might be intensified by dwindling carbon supplies, essential for cellular survival or averting carbon starvation (McDowell et al. 2022). Studies have identified several mechanisms behind individual tree mortality during drought periods, including vascular damage, carbon starvation, and increased herbivory (Brodribb et al. 2020).

Integrating insights from recent studies bridges the gap between the response of individual trees and the broader landscape-level effects of drought. Gleason et al. (2017) utilized extensive dendrological data to illustrate how forest growth, particularly at a stand scale, declines during periodic droughts. The diminished productivity was found to be more pronounced under severe drought conditions, especially in drier climates, and is further exacerbated by higher stand density (D'Amato et al. 2011, Magruder et al. 2012). Similarly, Gustafson and Sturtevant (2013) highlighted the detectability of drought-induced mortality signals using Forest Inventory and Analysis (FIA) data from the upper midwest suggesting that individual trees are more sensitive to drought duration than severity; there were measurable differences in species' drought tolerance, suggesting that future drought conditions could alter forest compositions significantly. The overall shifts in these communities may be induced by a variety of factors interacting synergistically with drought conditions (Frelich and Reich 2010), including the compounded damage of forest tent caterpillar (*Malacosoma disstria* Hübner) outbreaks during dry spells (Itter et al. 2017), or the alteration of soil moisture profiles in forests undergoing earthworm invasions (Frelich et al. 2019; Alexander, Almendinger, and White 2022). The threat of drought-induced die-offs and forest cover conversion suggests risks to critical ecosystem services, including the loss of sequestered forest carbon and associated atmospheric feedbacks, potentially compounding the effects of climate change (Allen et al. 2010).

Understanding forest health from a regeneration perspective becomes particularly critical when forests, especially long-lived woody vegetation, are out of sync with the climate—such as during periods of climate change (Bertrand et al. 2016). Martinez-Vilalta and Lloret (2016) observed in a meta-analysis of case studies focused on drought induced vegetation shifts that overall, potential vegetation transitions were consistent with more drought-resistant species replacing less resistant ones. Mature forest stands may continue to survive at a location long after local conditions have exceeded the growth limits of seedlings, given their deeper groundwater access and capacity to endure higher temperatures. In contrast, seedlings of the same species may be more vulnerable than their mature counterparts, as they require a narrower range of temperatures and are more sensitive to water availability (Dobrowski et al. 2015; Rank et al. 2022). Major changes in understory species may also occur (Rich et al. 2008), as well as the possible development of novel ecosystems due to new combinations of species that may eventually repopulate the overstory (Clark et al. 2016). Many forests in the GLR are especially vulnerable to additional strains during intense drying episodes due to their nutrient-poor, excessively well drained soils (Janowiak et al. 2014). The impact of drought stress on these forests is predicted to be severe; recent modeling indicates that some MN tree species may decline up to 50% due to the expected high mortality rates from previous droughts, depending on the species and site conditions (2023 Forest Health Annual Report, MN DNR). Recent literature underscores the impact of drought stress on northern Minnesota forests, where multiyear droughts and insect defoliation led to widespread aspen decline (Worrall et al. 2013). Drought's effects on trees can worsen due to several factors, primarily influenced by soil type. Levesque et al. (2016) demonstrated that water-rich, nutrient-dense soils can potentially safeguard tree growth against escalating droughts. In contrast, sandy soils, with low available water-holding capacity and their inability to moderate soil moisture during rainfall fluctuations, present significant challenges for seedling establishment and survival—even for species deemed drought-tolerant (Handler et al. 2017).

The projected increase in extended droughts in upper midwest forest ecosystems may escalate a range of cascading threats to resilience and long-term stability, including increased wildfire risk, amplified deer browsing, and pest and disease proliferation (Vose et al. 2015). As managers continue to combat diminished seedling establishment and growth amidst ongoing or intensifying droughts, the research and development of novel site level treatments such as soil amendments has become even more important (Clark et al. 2016).

Biochar soil amendments have emerged as a promising silvicultural tool with the potential to protect young trees and their associated ecological communities from the adverse effects of future droughts. With proven efficacy in mitigating drought conditions in agricultural settings (J. Lehmann et al. 2006; J. Lehmann & Joseph 2009; Jeffery et al. 2015; Palansooriya et al. 2019), researchers are exploring biochar's applications in forestry, with particular focus on forest restoration and regeneration. Biochar soil amendments promise to enhance forest soil conditions, improving seedling drought resilience through improved cation exchange capacity, water retention, and nutrient availability (Liang et al. 2005; Basso et al. 2003). These benefits, combined with the promise of long-term carbon sequestration, make biochar an attractive option for natural resource managers looking to enhance regeneration outcomes and improve long-term forest health. However, research on biochar's efficacy as a drought amelioration tool in forest ecosystems is still emerging, with operational-scale studies lacking and outcomes varying significantly. Biochar's influence on soil properties depends on its physical and chemical composition, determined by feedstock and pyrolysis conditions during production (Lehmann and Joseph 2009, Ippolito et al. 2020; Toczydlowski et al. 2023), in combination with how existing soil properties mediate these changes (Edeh et al. 2020, Joseph et al. 2021). In addition, the application rate and method employed play a critical role in determining amendment outcomes (Blackwell et al. 2009; Houben et al. 2013a; Gale and Thomas 2019).

The application of biochar in forest management contexts introduces unique challenges absent in agriculture, such as limited accessibility of sites, the need to minimize treatment activity entries (in order to mitigate compaction and herbaceous layers), complex and often rough terrain, as well as heterogeneously distributed above ground vegetation (Ashton and Kelty 2018). Operational constraints often make it impractical to fully integrate amendments like biochar into forest soils on a stand scale, prompting exploration of alternative methods such as top dressing. Top dress applications (applying the amendments directly on the surface of the soil) have been found to be effective in other studies (Edenborn et al. 2015). However, this method may come with its own drawbacks, such as inadvertent material loss due to erosion (Major et al. 2010). Several research findings indicate that while biochar amendments may enhance water retention in soils, methods of application differ among experiments and may influence the outcomes. For example, Page-Dumroese et al. (2015) found that biochar incorporated into the soil was less likely to repel water compared to biochar applied on the surface, whereas Palviainen et al. (2020) found that biochar only promoted seedling survival when it was applied at the soil surface; its integration into the soil had no effect.

In addition to the chosen application method for biochar soil amendments, the properties of the soils and the specific sites where biochar is applied can exert considerable influence on the efficacy of these amendments, particularly with regard to soil texture. It has been proposed that the beneficial effects of biochar may be more pronounced in areas with similar poor soil conditions than in those where soils are richer (El-Naggar et al. 2019). A 2020 meta-analysis showed that the positive effects of biochar are more pronounced in areas with poor soil conditions, such as coarse textured soils (Edeh et al. 2020). Researchers hypothesize that the enhanced water retention provided by the amendment will lead to increased plant productivity, reduced nutrient leaching rates, and ultimately, improved forest management outcomes—particularly in areas where drought impacts impede regeneration efforts (Juno & Ibáñez 2021; Mansoor et al. 2021; Mulcahy et al. 2013).

A comparison of two recent field studies in lake states, Richard et al. (2018) and Slesak et al. (2022), illustrates some of the challenges researchers contend with while investigating seedling response to biochar application. In both studies, biochar and biochar-compost mixtures were integrated into sandy, well-drained soils during planting, and the growth of jack pine and/or red pine seedlings was monitored for several years. While both studies found increased water retention and cation exchange capacity in amended soils, limited evidence supported the hypothesized enhanced initial growth benefits of the treated seedlings. Although biochar-compost amendments enhanced early height and diameter growth of planted seedlings in both studies, the similarity in growth response between biochar-compost and compost-only treatments indicated that the primary effect could be attributed to compost, with biochar exerting a minor influence. In both studies, shading and inherent microsite variations of forest field settings exert more significant limitations on seedling growth and establishment compared to the short-term changes in soil structure or nutrient availability that would be contributed by the biochar treatment (Richard et al. 2018, Slesak et al. 2022). This theory suggests that biochar's impact on seedling growth might intensify over time as seedlings' resource requirements increase, allowing seedlings to eventually leverage the beneficial soil property effects of biochar, complemented by its enduring presence in the soil (Spokas et al. 2012). Other studies have also proposed that the nuances of biochar's influence on seedling growth may only become apparent over longer durations, as seen in boreal forests where Grau-Andrés et al. (2021) reported a 19% growth enhancement in *Pinus sylvestris* over nine years. The potential for biochar to aid forest regeneration in drought-prone areas remains a promising field of study, albeit one that necessitates further exploration and nuanced application (Thomas and Gale 2015; Richard et al. 2017).

In the GLR, recurrent and severe droughts present substantial challenges to successful forest regeneration efforts. The overarching goal of this research is to explore the potential for biochar to reduce impacts of drought on seedling survival and growth in northern Minnesota. Specifically, our objectives are: evaluate the viability of operational-scale biochar application, specifically top-dressed biochar, as an opportunity for enhancing seedling growth in drought-prone sandy soils; assess the influence of two different application rates of top-dressed biochar on the growth and survival of four common tree species facing regeneration challenges in the region; and exploring longer-term regeneration results that relate to 5-year regeneration results that many forest managers organizations use. This study will add to this expanding area of research, providing useful insights for GLR managers trying to understand the potential long-term effects of biochar soil amendments on forest health, stand dynamics, and sustainable forest management practices in a changing climate.

Methods

Study Area

The experiment was installed at the University of Minnesota's Cloquet Forestry Center (hereafter CFC), which is located 27 miles southwest of Duluth, Minnesota, USA in Carlton County. The Cloquet Forestry Center contains 3,471 acres of land located to the east of the town of Cloquet, and is located within the boundaries of the Fond du Lac Band of Lake Superior Chippewa Reservation (FDL). The center also falls within the bounds of the 1854 Treaty Area, within which the treaty-reserved hunting, fishing, and gathering rights of the Grand Portage and Bois Forte bands are enabled and protected (Norrgard 2014).

The CFC property is located about 20 miles east of Lake Superior; this proximity introduces a slight climatic influence which has resulted in slightly warmer annual temperatures compared to the surrounding area (Reinikainen et al. 2015). The CFC has an onsite weather reporting station which has had daily records reported daily since 1911.

These historical records indicate a mean annual temperature of 39.1°F (3.9°C), with fluctuations between 34.4 and 44.5°F (-1.3°C to -6.9°C). Winter means tend to range from 4°F to 21°F (-16°C to -6°C), while summer means range from 61°F to 80°F (16°C to 27°C) (University of Minnesota Cloquet Forestry Center Daily Weather Observation Data, 1914-2023). Drought conditions in the area have varied significantly over the course of the historic records, with decadal scale wetting and drying trends. Broad region level model-based predictions of climate change have affirmed these trends (Nagel et al. 2017). As of 2024, the region is experiencing its third consecutive year of moderate to severe drought conditions across much of its area.

Site Preparation

The experiment was conducted in an 11.9 acre stand, clearcut during the winter of 2018/19. The timber harvest was conducted on frozen ground utilizing a full tree harvest system with complete biomass removal. Prior to harvest, the stand was composed of conifer-hardwood species, dominated by red pine (*Pinus resinosa* Ait. (Pinaceae)), eastern white pine (*Strobus strobus* (L.) Small), jack pine (*Pinus banksiana* Lamb.), black spruce (*Picea mariana* (Mill.) B.S.P. (Pinaceae)), quaking aspen (*Populus tremuloides* Michx. (Salicaceae)), and paper birch (*Betula papyrifera* (Regel) Fern.). The Minnesota Ecological Classification System (MN ECS) system defined the stand as a fire dependent ecosystem, specifically the FDn33a community type (*Field Guide to the Native Plant Communities of Minnesota: The Laurentian Mixed Forest Province,* 2003). However, due to post-colonial fire suppression, this area had not experienced fire on the ground within the past 100+ years. The stand soils are composed of Omega loamy sand, on primarily 0 to 2 percent slopes, with roughly a quarter of the study area in moderately hilly terrain, with slopes between 2 and 12 percent, and variable aspect.

Prior to initiating the study, site preparation activities were conducted to ensure a conducive environment for tree seedling planting and establishment. This included the removal of any remaining logging slash and implementation of mechanical competition control measures within the designated plots in order to prepare the sites for planting.

Experimental Design

The study employed a split-plot design comprising 15 square plots, each approximately 0.3 acres in size. Each plot measured 114 feet by 114 feet, with a 10 x 10 ft spacing for seedlings. The center of the plot contained a 10 ft wide strip designated for soil sampling and vegetation monitoring. Four species were selected for planting using the MN DNR's tree suitability index for the FDn33 community: red pine, paper birch, jack pine, and northern red oak (*Quercus rubra* (Michx. f.) Farw.). While the initial intention was to use a top dress application of biochar prior to planting, delayed delivery and logistical constraints related to the biochar acquisition required the seedlings to be planted prior to application.

Planting occurred in June of 2019. A total of 270 seedlings of jack and red pine were planted but due to shortages related to seed stock only 248 and 254 seedlings of paper birch and northern red oak were planted, respectively. Immediately after planting, initial seedling measurements including basal diameter (two readings per seedling 90º from each other directly above basal flare, measured with calipers to the nearest 1/10 millimeter) and height (unstraightened, measured in centimeters to the bud tip, and for conifers also to the furthest extended needle), were recorded.

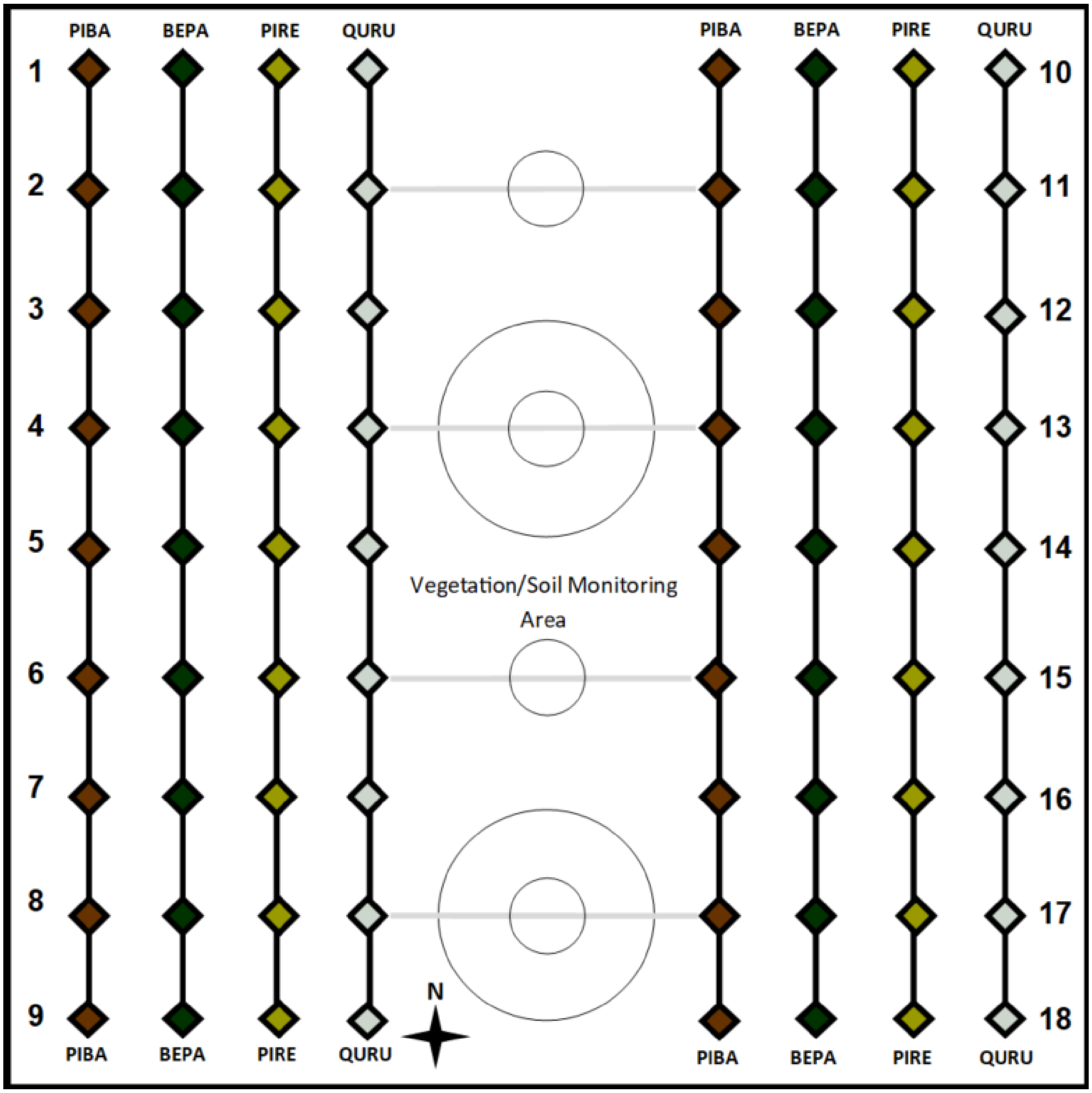

Figure 1.1: Plot design, 4 rows of 9 planted seedlings on each side (10 x 10 ft spacing), with 10 ft gap in the middle for vegetation sampling.

Treatments

The biochar utilized in this study was produced at the Biomass Conversion Lab, located at the Natural Resources Research Institute site in Coleraine, Minnesota. This experiment employs two levels of biochar concentrations to be tested as broadcast applied soil amendments at a low and high rate (Gale and Thomas 2019). Treatments were randomly assigned to each of the 15 0.3 acre plots, with four plots designated for the "high" treatment, receiving 4000 lbs. of biochar each, evenly distributed across the soil surface, equivalent to approximately 13,333 lbs. per acre. Another four plots were assigned to the "low" treatment and received 2000 lbs. of biochar each, approximately 6667 lbs. per acre. The remaining seven plots were designated as controls and did not receive any biochar application. Following the June planting, in July of 2019, the two levels of biochar treatments were weighed and applied to the soil surface of the treatment plots. Biochar distribution across the plots was conducted manually due to operational feasibility, hand rakes were used to evenly distribute the material and loosely incorporate it into the top layer of the soil without disturbing the seedlings.

Annual mechanical suppression of vegetative competition around seedlings was conducted during summer months using brush saws within plots to manage suckering quaking aspen until 2023. Protective tree tubes (Rigid Seedling Protector Tubes from Forestry Suppliers) were installed around seedlings upon planting to mitigate deer browse. Tubes were removed at the end of the third growing season in 2021 as seedlings outgrew them.

Annual survival monitoring and growth measurements

Annual end of season growth measurements were collected in either late September or early October of each year, as well as at the time of planting to capture the initial sizes of the seedlings. Two diameter measurements (90 degrees from each other) were taken using calipers above the root flare for all living seedlings, measured to the nearest mm. For height, the seedlings were left unstraightened, and measured to the nearest cm. For broadleaf species, only terminal bud height was taken. For conifers, height measurements were taken for both the terminal bud as well as the highest point of needle growth. Seedling health was assessed on a scale of 0, 1, or 2, with 0 indicating mortality, 2 indicating that the seedling was live and vigorous, and 1 indicating that the seedling was showing signs of stress.

Data Analysis

The Cloquet Forestry Center weather station has maintained daily records dating back to 1911, which were analyzed to provide historical weather data. The analysis focused on comparing two distinct 55-year intervals within this dataset: 1914-1968 and 1969-2023. The dataset was structured by year, and included various meteorological parameters, including the annual overall mean, annual mean minimum and annual mean maximum temperatures (degree Fahrenheit), total annual precipitation in inches, and cumulative Growing Degree Days (GDD) base 50. GDD base 50 refers to the cumulative sum of daily temperatures exceeding 50°F, indicating favorable conditions for plant growth. To assess the disparities between the two periods, paired t-tests were employed.

To ensure the accuracy and consistency of our data on seedling growth over multiple years, data were examined for measurement anomalies. For trees that showed a small decrease in diameter (less than 0.1 mm) from the beginning to the end of a single growing season, values in the latter year were adjusted to reflect no change. Seedlings that showed a diameter reduction of more than 0.1 mm within a season were removed from the dataset. An additional standardization challenge was recognized during data cleaning procedures, particularly affecting the non-conifer species, paper birch and northern red oak. Seedlings were occasionally misclassified as deceased when they were actually dormant, a recurring incident particularly during drought stress periods. Their status was rectified in subsequent years upon confirmation of their survival, with red oaks being more straightforward to confirm compared to paper birches. This difficulty arose due to the presence of naturally regenerating paper birch in the study area, which complicated the identification of the study cohort's planted seedlings. Consequently, any seedlings not definitively attributable to the planted cohort were excluded from the analysis. Cumulatively, data cleaning led to the exclusion of 1.5% of jack pine, 3.6% of paper birch, 2.4% of northern red oak, and 1.1% of red pine seedlings that were originally planted.

All statistical analyses were conducted using R (version 4.3.0, R Core Team 2017). Summary statistics for initial measurements and each subsequent end of growing season measurement were calculated for survival, diameter, and height by species and treatment. Treatment effects on seedling diameter and height growth were assessed at 5 years (as this represents the common interval in which natural resource managers would assess regeneration following planting) (*National Forest Management Act* 1976, sec. 219.27) using the mean annual relative growth of the measurements. For each seedling, the two diameter values measured were averaged, as were the bud height and needle height of the conifer species, while bud height alone was used for the non-conifer species height measurement. The relative annual growth rates were calculated for each seedling that survived to the fifth year of the study using the following formula:

$$\frac{2023\ measurement - \ initial\ measurement}{initial\ measurement} \div 5\ years$$

Data were inspected visually and using Shapiro, Bartlett, and Levene tests to check for normality and variance. To compare the effects of the biochar treatments, statistical analysis of seedling height and diameter growth (the 5-year mean annual relative growth rate) was completed using one-way Analysis of Variance (ANOVA). Prior to conducting the ANOVA, linear mixed effects models that included plot as a random effect were utilized to determine if plot had a significant effect on the analysis. When plot was found to be insignificant, the term was dropped, and an ANOVA was utilized to assess treatment effects. Initial seedling diameter and/or height were used as covariates when they were found to improve the fit of the models. The square root of the annual diameter and height growth data was used to meet assumptions of variance normality; values were back transformed for presentation and are therefore estimates of the median. When ANOVA results indicated significant treatment effects, multiple comparisons with

Tukey’s adjustment were conducted to determine what treatments differed. An alpha level of 0.1 was used to determine significance due to high variability within ecosystems.

Treatment effects on seedling survival were analyzed using Generalized Linear Mixed Effects Models (GLMM) to conduct repeated measures analysis, using the glmer function from the lme4 package in R (Bates et al. 2015) The response variable of “live” was binary (0=dead, 1=alive), indicating survival status at each sampling interval. Sampling period (year) and treatment (high, low, control) were both used as predictors, as well as their interaction, in addition to initial height or initial diameter of the seedlings. Individual tree identification code was specified as a random factor, in order to conduct repeated measures. Logistic odds of survival were plotted against either initial height or initial diameter of the seedlings if it was found to be a significant variable influencing growth for each individual species. In addition, generalized linear mixed effects models were used for analysis using the individual plot means for each treatment type, with plot coded as a random effect, and each level of biochar treatment coded as fixed effects.

Results

Climate Trends

The analysis of two distinct 55-year intervals, 1914-1968 and 1969-2023, revealed statistically significant changes at the CFC. In recent decades, the average annual temperature has increased by 1.42°F ($p < 0.01$), with increases in both the mean annual minimum (1.89°F, $p < 0.01$) and maximum temperatures (0.94°F, $p < 0.05$) relative to the earlier period. Additionally, mean annual rainfall increased by 3.11 inches ($p < 0.01$) between the two periods. With rising temperatures, the length of the growing season has expanded: the latter half-century shows a higher count of growing-degree-days (GDD base 50) (+173.34, $p < 0.01$) relative to the pre-1968 records.

Table 1.1: Summary of weather data from Cloquet Forestry Center Weather Station; Comparison of climate trends for the periods 1914-1968 and 1969-2023. Paired t-tests were conducted to compare pre-1968 and post-1969 periods at the Cloquet Forestry Center weather station. Differences in variable means are within parentheses next to the 1969-2023 means.

	Time Period Means			
Variable	1914 - 1968	1969 - 2023	Conf. Int. (95%)	p value
Mean Annual Temperature (°F)	38.92	40.34 (+1.41)	(0.75, 2.08)	8.77e-05
Mean Max Temperature (°F)	93.51	93.07 (-0.44)	(-1.78, 0.9)	0.5167
Mean Min Temperature (°F)	-33.55	-28.93 (+4.62)	(2.71, 6.53)	1.09e-05
Annual Precipitation (in)	28.45	31.56 (+3.12)	(1.25, 4.99)	1.52e-03
Degree Growing Days (base 50)	1677.91	1851.25 (+173.35)	(92.37, 254.32)	7.40e-05

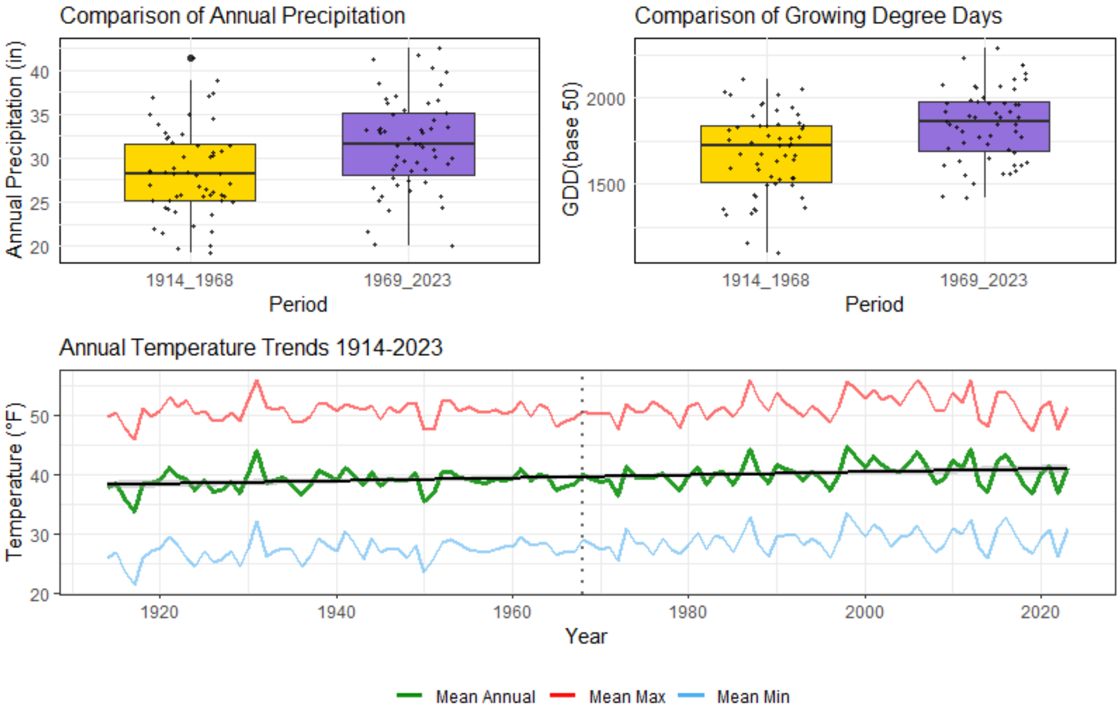

Figure 1.2: Comparison of climatic trends at the CFC for the periods 1914-1968 and 1969-2023; top left figure is a box plot depicting annual precipitation, top right figure is a box plot depicting GDDb50, bottom figure is a line graph depicting the temperature trends over the entire period of record from 1914-2023, with a smoothed regression line depicting the slight upward trend over the past 110 years.

Seedling Survival

Survival was variable between species, with red pine seedlings having the highest proportion of seedlings surviving to the end of the 5 years (75.9% across all treatments), and paper birch seedlings exhibiting the lowest survival rates (16.3% across all treatments). Jack pine and northern red oak had 48.47% and 52.99% survival rates across all treatments, respectively.

All species experienced mortality in 2019, following planting. Of particular note is paper birch, which experienced heavy mortality (44% survived over all treatments), with the seedlings in the low treatment group faring the worst (36%). Jack pine also suffered heavy mortality at the beginning of the study, with only 55% survival over all in 2019. While the low and control groups experienced similar mortality rates at that time (51% and 50% respectively) the high group maintained a notably higher survival rate (65%).

Table 1.2: Seedling survival over 5 years: This table displays the mean proportion of live seedlings for four study species over a five-year period (2019-2023), accompanied by their respective standard errors. The mortality status of seedlings was evaluated at the conclusion of each growing season. The table illustrates the percentage of live seedlings of each species within various treatment types employed during the study duration.

Species	Treatment	*End of Season Sampling Periods (% Live (se %))*				
		2019	2020	2021	2022	2023
Red pine	Control	83.5% (3%)	76.9% (4%)	76.9% (4%)	75.2% (4%)	70.2% (4%)
	Low	88.7% (4%)	84.5% (4%)	80.3% (5%)	78.9% (5%)	77.5% (5%)
	High	91.4% (3%)	84.3% (4%)	82.9% (5%)	81.4% (5%)	81.4% (5%)
Jack pine	Control	50.4% (5%)	48.8% (5%)	45.5% (5%)	45.5% (5%)	45.5% (5%)
	Low	51.4% (6%)	48.6% (6%)	47.1% (6%)	45.7% (6%)	44.3% (6%)
	High	64.8% (6%)	64.8% (6%)	62.0% (6%)	59.2% (6%)	57.7% (6%)
Northern red oak	Control	65.8% (5%)	62.2% (5%)	57.7% (5%)	54.1% (5%)	54.1% (5%)
	Low	70.0% (6%)	66.7% (6%)	60.0% (6%)	58.3% (6%)	58.3% (6%)
	High	57.1% (6%)	52.4% (6%)	49.2% (6%)	46.0% (6%)	46.0% (6%)
Paper birch	Control	46.8% (5%)	41.3% (5%)	21.1% (4%)	15.6% (3%)	13.8% (3%)
	Low	36.1% (6%)	32.8% (6%)	14.8% (5%)	13.1% (4%)	13.1% (4%)
	High	46.0% (6%)	39.7% (6%)	25.4% (6%)	23.8% (5%)	23.8% (5%)

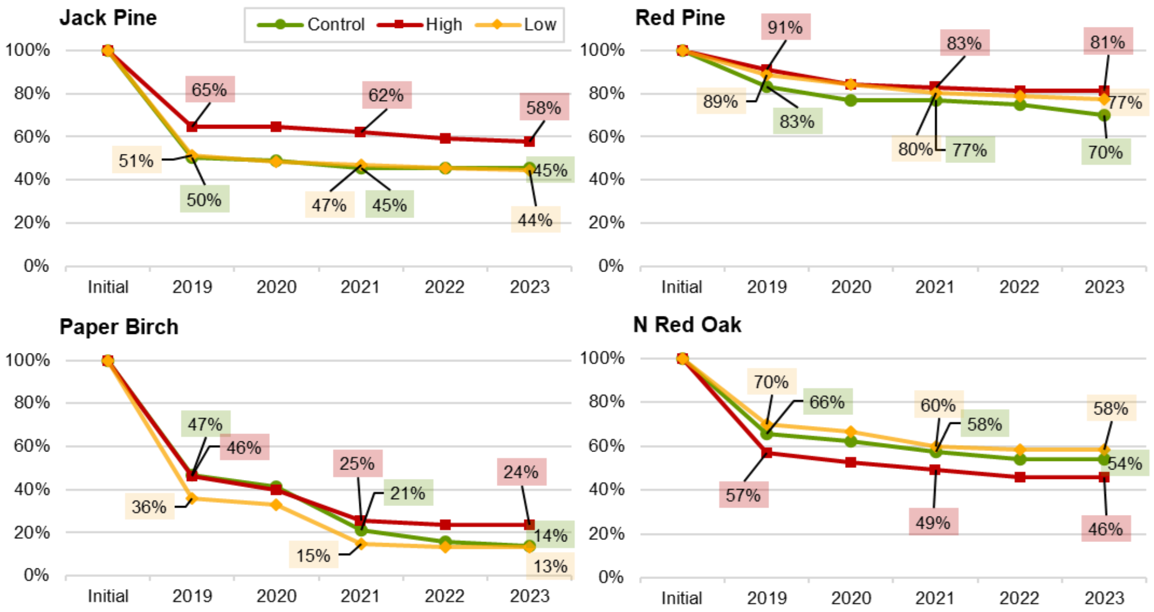

Figure 1.3: Mortality trends of four study species over five-year period (2019-2023). Treatment groups (control, low biochar amendment, high biochar amendment) are plotted against the x-axis representing the sampling interval (year), while the y-axis denotes the mean percentage of mortality. Data callouts indicate the percentage of live seedlings for each treatment and species at specific years: 2019 (reflecting initial planting stress), 2021 (highlighting mortality during a severe drought), and 2023 (depicting the percentage of surviving seedlings at the end of the five-year study period).

Survival rates of jack pine exhibited variability across treatment groups. Notably, in the high biochar treatment, 58% of seedlings survived until the study's conclusion, contrasting with a survival rate of 44% in the low and 45% in the control groups. Paper birch survival mirrored this trend, albeit with smaller margins between the rates; while the low and control treatment groups showed similar survival (14% and 13%, respectively), the high treatment groups survival was comparatively higher (24%) at the end of the study. Red pine survival was greatest in the high treatment plots (81%), with the low treatment group close behind (77%), and the control group a little behind that (70%). Red oak was the only species in which the highest survival rate was not within the high treatment group, rather, the low treatment showed the highest rate (58%), followed by the control (54%), with the high treatment notably lower (46%).

Regression analysis for jack pine found significant interaction effects between sample period and the high treatment across all sample intervals ($p < 0.1$), indicating that the effect of treatment varies over time and with treatment level. The estimates range from 0.12 to 0.16, indicating that high treatment had a positive effect on jack pine survival (Table 4).

The regression analysis also indicated significant interaction effects between sample interval and treatment for paper birch and red pine. For paper birch, the interaction between sample period and treatment was generally not significant. However, immediately following planting in 2019 during the first end of season measurement period, the interaction between sample period and the low treatment resulted in a decrease in survival odds (p = 0.088), reflecting the notable mortality in that treatment group in 2019. For red pine, the interaction between sample period and treatment was also not significant for most combinations, however in the final sample period in 2023, the high treatment resulted in a significant positive effect on survival odds (p = 0.056).

The model also reported a significant negative effect of the sample period across all species, indicating a decline over time. Treatment alone was not found to be a significant factor influencing survival for any of the species, treatments were only found significant when interacting with sample period.

Table 1.3: Treatment Effects on Seedling Survival: Summary of Generalized Linear Mixed Effects Models (GLMM) analysis for four study species over study period. Predictors included sampling period (year), treatment (high biochar, low biochar, control), and their interaction, along with seedlings initial height or diameter. Individual tree identification code was specified as a random factor to account for repeated measures. The coefficients or estimates, along with their standard errors (SE) and p-values are provided for each predictor in the model.

	Red pine		**Jack pine**		**Northern red oak**		**Paper birch**	
Coefficient	*Estimates (SE)*	*p*	*Estimates (SE)*	*p*	*Estimates (SE)*	*p*	*Estimates (SE)*	*p*
Intercept	**1.00***** (-0.04)	**<0.001**	**1.00***** (-0.06)	**<0.001**	**1.00***** (-0.05)	**<0.001**	**1.00***** (-0.04)	**<0.001**
2019	**-0.17***** (-0.03)	**<0.001**	**-0.49***** (-0.04)	**<0.001**	**-0.34***** (-0.03)	**<0.001**	**-0.52***** (-0.04)	**<0.001**
2020	**-0.23***** (-0.03)	**<0.001**	**-0.51***** (-0.04)	**<0.001**	**-0.38***** (-0.03)	**<0.001**	**-0.58***** (-0.04)	**<0.001**
2021	**-0.23***** (-0.03)	**<0.001**	**-0.54***** (-0.04)	**<0.001**	**-0.42***** (-0.03)	**<0.001**	**-0.78***** (-0.04)	**<0.001**
2022	**-0.25***** (-0.03)	**<0.001**	**-0.54***** (-0.04)	**<0.001**	**-0.46***** (-0.03)	**<0.001**	**-0.84***** (-0.04)	**<0.001**
2023	**-0.30***** (-0.03)	**<0.001**	**-0.54***** (-0.04)	**<0.001**	**-0.46***** (-0.03)	**<0.001**	**-0.86***** (-0.04)	**<0.001**
Treatment Low	0 (-0.07)	1	0 (-0.09)	1	0 (-0.08)	1	0 (-0.07)	1
Treatment High	0 (-0.07)	1	0 (-0.09)	1	0 (-0.08)	1	0 (-0.07)	1
2019 × Low dose	0.05 (-0.06)	0.345	0.01 (-0.06)	0.844	0.04 (-0.06)	0.422	**-0.12*** (-0.07)	**0.088**
2020 × Low dose	0.08 (-0.06)	0.181	0 (-0.06)	0.996	0.05 (-0.06)	0.416	-0.1 (-0.07)	0.172
2021 × Low dose	0.03 (-0.06)	0.558	0.02 (-0.06)	0.782	0.02 (-0.06)	0.703	-0.07 (-0.07)	0.317
2022 × Low dose	0.04 (-0.06)	0.512	0 (-0.06)	0.954	0.04 (-0.06)	0.468	-0.03 (-0.07)	0.691
2023 × Low dose	0.07 (-0.06)	0.206	-0.01 (-0.06)	0.872	0.04 (-0.06)	0.468	-0.01 (-0.07)	0.916
2019 × High dose	0.08 (-0.06)	0.157	**0.14**** (-0.06)	**0.031**	-0.08 (-0.06)	0.166	-0.01 (-0.07)	0.921
2020 × High dose	0.07 (-0.06)	0.208	**0.15**** (-0.06)	**0.017**	-0.09 (-0.06)	0.106	-0.01 (-0.07)	0.853
2021 × High dose	0.06 (-0.06)	0.326	**0.16**** (-0.06)	**0.014**	-0.08 (-0.06)	0.169	0.05 (-0.07)	0.48
2022 × High dose	0.06 (-0.06)	0.293	**0.13**** (-0.06)	**0.041**	-0.07 (-0.06)	0.209	0.09 (-0.07)	0.187
2023 × High dose	**0.11*** (-0.06)	**0.056**	**0.12*** (-0.06)	0.067	-0.07 (-0.06)	0.209	0.11 (-0.07)	0.109
σ^2	0		0.01		0		0.01	
τ_{00}	0.01_{plot}		0.02_{plot}		0.01_{plot}		0.01_{plot}	
ICC	0.66		0.76		0.74		0.46	
N	15_{plot}		15_{plot}		15_{plot}		15_{plot}	
Observations	90		90		90		90	
Marginal R^2 / Conditional R^2	0.406 / 0.798		0.636 / 0.913		0.661 / 0.911		0.883 / 0.937	

Diameter and Height Growth

Initial diameter and height were variables for the four species planted (Table 1.4). Average diameter ranged from 3.23 mm for jack pine in the high biochar treatment to 6.44 mm for paper birch planted in the control treatments; height ranged from 18.8 cm for red pine the high biochar treatment to 77.63 cm for paper in the high biochar treatment. There were no significant differences for paper birch, jack pine, or northern red oak for initial diameter or height. There were significant differences in initial measurements for red pine between treatments for both height and diameter ($p < 0.001$ and $p < 0.0001$, respectively).

Table 1.4: Initial seedling sizes, diameter and height at time of planting, and total sample size of treatment group for each species. Mean diameters(in mm) and mean heights (in cm) reported with associated standard error in parentheses. Significant differences between treatment groups are denoted by lowercase letters.

		Mean Initial Diameter		*Mean Initial Height*		*Sample Sizes*
Species	Treatment	mm	SE	cm	SE	n
Paper birch	Control	6.07	(0.214)	69.87	(2.914)	109
	Low	6.44	(0.253)	73.76	(2.846)	61
	High	5.71	(0.222)	77.63	(1.975)	63
Jack pine	Control	4.31	(0.190)	26.23	(0.931)	121
	Low	3.88	(0.199)	24.61	(1.410)	70
	High	3.92	(0.144)	24.22	(0.838)	71
Red pine	Control	3.67 a	(0.099)	20.91 a	(0.484)	121
	Low	3.82 a	(0.125)	21.76 a	(0.563)	71
	High	3.23 b	(0.106)	18.8 b	(0.580)	70
Northern red oak	Control	5.74	(0.193)	38.42	(0.662)	111
	Low	5.52	(0.247)	37.64	(1.123)	60
	High	5.28	(0.309)	37.9	(1.283)	63

Over the five-year period, biochar had a limited statistical impact on height or diameter growth for the majority of the species (Table 1.4). Biochar amendment had significant effects on the relative annual diameter growth of only one species, red pine ($p = 0.046$). Both low and high doses of biochar amendment resulted in reduced relative annual diameter growth compared to the control. In pairwise comparisons, high treatment growth was statistically significant from the low treatment growth ($p = 0.09$) and control ($p = 0.017$).

No significant differences in relative annual diameter growth were found between the two biochar concentration treatments for the other three species. In general, for the seedlings that survived there was limited effect of the treatments over the five-year study period on height and diameter growth (see Table 1.5). When employing Type III ANOVA to investigate main effects (treatment against control), the comparative analysis of relative mean annual seedling diameter growth across the four species over five years did not demonstrate significant differences among the treated and control seedlings ($p > 0.1$) (Table 1.5). Additionally, the assessment of relative mean annual height growth over the five-year span, via Type III ANOVAs, did not show significant differences across treatments ($p > 0.1$) (Figure 1.4). The effect of the treatments on mean annual height growth were also variable, but none were found to be statistically significant.

Table 1.5: Mean annual growth of seedling diameter (mm/mm) and height (cm/cm) over the study period, by species and treatment type. Means and standard errors are depicted.

Species	Treatment	*Mean Diameter Growth*		*Mean Height Growth*	
		mm/mm	SE	cm/cm	SE
Paper birch	Control	0.91	(0.347)	0.23	(0.108)
	Low	0.65	(0.474)	0.19	(0.123)
	High	1.1	(0.628)	0.2	(0.11)
Jack pine	Control	1.63	(0.917)	1.13	(0.545)
	Low	1.8	(0.82)	1.29	(0.475)
	High	2.08	(0.734)	1.23	(0.509)
Red pine	Control	1.56	(0.595)	0.89	(0.315)
	Low	1.44	(0.545)	0.79	(0.23)
	High	1.59	(0.678)	0.9	(0.382)
Northern red oak	Control	0.44	(0.229)	0.2	(0.165)
	Low	0.48	(0.445)	0.16	(0.184)
	High	0.56	(0.348)	0.24	(0.15)

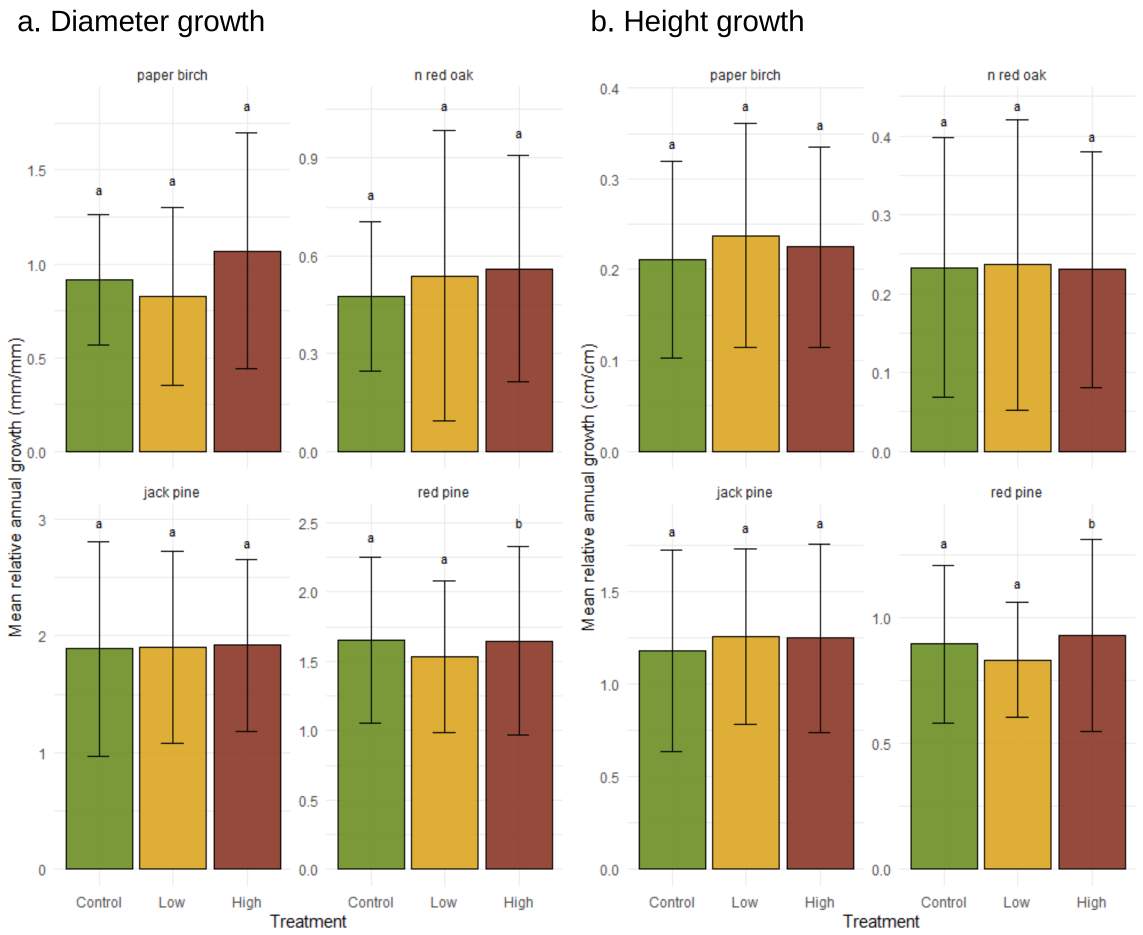

Figure 1.4: a.) Change in seedling relative annual diameter growth and b.) relative annual height growth, by treatment after five years; means are plotted ± standard deviations. Data is separated by treatment: green bars are the control group; yellow bars are low dose biochar treatment group and red bars are the high dose biochar treatment group. Means denoted by a different letter indicate significant differences between treatments ($p < 0.1$). Sample size per treatment was dependent upon survival to the end of the study, and was variable between species. Sample sizes used for analysis can be found in Table 1.6 under observations.

Table 1.6: Linear regression models for seedlings height and diameter growth. Coefficients of linear regression and mixed linear models (with plot as a random effect if found to be significant) for mean relative annual diameter growth (mm/mm) and height (cm/cm) of main effects (standard errors in parentheses below) over total study period. Significance between treatments and control within species are denoted as * =significant.

	Jack pine		Red pine		Paper birch		Northern red oak	
Effect	height	diameter	height	diameter	height	diameter	height	diameter
	linear mixed model			*simple linear regression*				
Treatment High	0.0002 (-0.002)	0.001 (-0.006)	0.001 (-0.002)	**0.004***** (-0.001)	**0.007*** (-0.004)	0.00004 (-0.008)	0.0001 (-0.002)	0.0003 (-0.002)
Treatment Low	0.00001 (-0.002)	0.004 (-0.007)	0.0003 (-0.002)	0.001 (-0.001)	0.003 (-0.004)	0.0001 (-0.011)	0.00002 (-0.001)	0.0002 (-0.002)
Initial Height	**0.001***** (-0.00001)		**0.0005***** (-0.00001)	**0.00003*** (-0.00001)	**0.00004***** (-0.00001)		**0.0002***** (-0.00001)	
Initial Diameter	0.00003 (-0.0003)	**0.032***** (-0.0002)	0.00003 (-0.0002)	**0.022***** (-0.0004)	0.001 (-0.001)	**0.020***** (-0.002)	**0.001***** (-0.0001)	**0.003***** (-0.0001)
Intercept	**3.021***** (-0.005)	**4.441***** (-0.007)	**1.859***** (-0.004)	**2.910***** (-0.006)	**0.558***** (-0.049)	**3.217***** (-0.095)	**0.716***** (-0.013)	**1.011***** (-0.005)
Observations	128	128	199	199	30	37	106	120
R^2				0.27	0.161	0.215	0.168	0.195
Adjusted R^2				0.255	0.027	0.144	0.135	0.174
Log Likelihood	28.209	9.325	96.82					
Resid. Std. Err.				0.205 (df = 194)	0.119 (df = 25)	0.234 (df = 33)	0.164 (df = 101)	0.183 (df = 116)
F Statistic				17.946*** (df = 4; 194)	1.199 (df = 4; 25)	3.016** (df = 3; 33)	5.105*** (df = 4; 101)	9.369*** (df = 3; 116)
AIC	-42.418	-6.65	-179.639					
BIC	-22.454	10.462	-156.586					

*p<0.1; **p<0.05; ***p<0.01

Discussion

Detailed meteorological data over the past century have revealed significant shifts in Minnesota's climate. Analysis from the CFC weather station aligns with these documented trends, indicating increases in mean annual temperature and precipitation alongside a noticeable lengthening of the growing season by several days, as compared to the previous 55-year interval.

The five-year span of this study further highlighted patterns of warming and drying conditions, especially marked in 2021 and 2023. These years experienced a pronounced elevation in daily mean, minimum, and maximum temperatures by approximately 2 to 3 degrees Fahrenheit, primarily during the June to September growing season. Concurrently, 2021 and 2023 reported the lowest levels of cumulative precipitation within the study's timeframe. These observations are supported by the National Oceanic and Atmospheric Administration’s (NOAA) Palmer Z Index, which ranks short-term drought conditions on a monthly scale. According to this index, both 2021 and 2023 are among the top five driest years since 1885, with 2021 sharing the fourth driest spot with 1910, and 2023 standing as the driest year recorded.

The broad shifts in climate described, coupled with the dramatic periods of drought experienced over the course of this study, represent significant challenges for forest regeneration and management, and underscores the importance of exploring potential avenues to enhance seedling resilience. The impacts of these drought conditions undoubtedly played a significant role in shaping the outcomes of this study, particularly evident in the seedling mortality observed during 2021. By the end of the study's five-year timeframe, tree survivorship exhibited marked variability across species, with paper birch showing a low survival rate of 16% across all treatments, while red pine demonstrated a high survival rate of nearly 80%. Analysis of tree seedling growth across various species at the end of the study period also revealed distinct responses to different treatments impacting both mean diameter and height growth. For diameter growth, a general trend of increased growth from control to high treatment was discernible. Jack pine and northern red oak displayed modestly enhanced growth at the low treatment level, whereas paper birch and red pine experienced slight decreases. However, these differences were not statistically significant. As for height growth trends, these varied by species. High treated jack pine, red pine, and northern red oak demonstrated the highest growth rates out of all treatment types. Interestingly, the paper birch control group outperformed the low and high treatment groups in height growth. Conifer species—red pine and jack pine—exhibited more prominent growth compared to deciduous species, paper birch and northern red oak.

This contrast likely arises from the inherent differences in their life histories and growth allotment strategies. Conifers typically prioritize early above-ground growth to outcompete surrounding vegetation and access sunlight, a strategy intended to optimize survival and reproduction. On the other hand, deciduous species often allocate more resources to underground biomass, capitalizing on their ability to produce and shed leaves rapidly, providing a survival advantage in the initial establishment period during unfavorable conditions such as drought and its cascading effects including increased herbivory. In fact, much of the height growth in non-conifer species occurred in the latter two years of the study, while conifers showed a more linear growth rate over the course of the study. Overall, findings suggest that the effects of biochar treatments on growth parameters were species-specific and treatment-dependent. Nonetheless, the observed variation in growth outcomes reveals potential differential impacts of biochar treatments on conifer and deciduous species.

In 2019, all tree species experienced mortality shortly after planting. Notably, paper birch exhibited significant mortality, with only 44% surviving across all treatments; jack pine also experienced substantial mortality, with only 55% surviving overall treatment types. These results reflect the findings of a 2017 survey of Minnesota natural resource managers, who identified jack pine as the species they struggled most with regenerating (Windmuller-Campione et al. 2019). The possible drivers of planting mortality could include factors such as poor planting techniques (bareroot seedlings experience higher planting mortality due to j rooting), unfavorable weather conditions (seedlings were planted in June, fairly late for planting, exposing seedlings to drier soils), site-specific conditions such as soil type, drainage, and exposure to environmental stressors like drought or excessive heat, as well as genetic predisposition of the seedlings.

In addition, it is crucial to acknowledge paper birch's vulnerability to drought. In 2021, birch experienced marked declines across all treatment types, with 18% of all planted seedlings dying during this period. Paper birch has been described as drought sensitive, and studies have found that the species tends to experience water stress conditions sooner than oak species (Federer, 1980). In "Guide to Trees and Shrubs of Minnesota," author Welby Smith notes that the statewide drought experienced in 1988-89 led to a significant spike in the annual mortality rate of paper birch in the subsequent three years, estimated at a staggering 450% increase—a surge greater than that observed for any other tree species. These declines were experienced across all age classes (Smith, trees and shrubs). The drought conditions observed during this study, though not as prolonged as the event documented in 1988-89, provide valuable insights into the potential impacts of drought on regeneration outcomes in the future. If the projected trend of prolonged drought periods is coupled with the heightened severity witnessed during this study, it is likely to have significant consequences for regeneration dynamics.

Red pine seedlings also have been found to grow best in above average precipitation years (Cook 1941), and may have their growth inhibited by certain allelopathic species (Norby & Kozlowski 1980). They are however the most successful at establishing in this study site, reflected by their relatively high survival and annual growth rates. This may be attributable to red pine’s affinity for full sun, additionally, past research has found that red pine transplants typically have faster initial growth than naturally seeded in regeneration (Horton and Bedell 1960). However, red pine is sensitive to depth of planting, so care must be taken not to plant seedlings too deep or too shallow (Benzie 1977b; Chapeskie et al. 1988).

Similarly, young, transplanted jack pine seedlings often are vulnerable to moisture stress, affecting their physiological processes and subsequently, growth and survival. Research by Grossnickle (1988) showed a direct correlation between changes in plant water potential and leaf conductance in recently out-planted jack pine, and found that seedling survival improved in microsites with less than 4 hours of direct sunlight daily. Unsurprisingly and reflected in our findings, the highest mortality of jack pine seedlings occurs within the first two growing seasons, with 53% live seedlings at the end of the second year of our study. Typically, planted seedlings grow approximately 30 inches (140 cm) per year, influenced by site, health, and climate. Soil conditions such as compaction which hinder root development can also limit growth. Seasonality of growth may also be relevant considering the extreme dry summer months seedlings experienced over the course of this study—Belyea et al. (1951) found 80% of seedling height growth happened from mid-May to end-June, potentially followed by a second growth spurt in late summer if moisture is sufficient.

An intriguing finding from our study was the improved survival of jack pine under high treatment biochar amendments. Jack pine, a species well-adapted to fire-prone environments, may potentially thrive in association with the pyrogenic residues presented by biochar amendments. Pyrochemical compounds that survive the combustion process, such as biochar, often mimic the post-fire soil conditions, fundamentally changing the soil's chemical environment. This alteration may influence multiple soil properties and processes, including water retention, nutrient availability, and pH levels, potentially creating a favorable environment for jack pine regeneration.

Moreover, this phenomenon hints at a deeper ecological synergy. Jack pine seedlings have several documented biotic interactions which may also play a role in determining their growth and survival outcomes. A diverse group of ectomycorrhizal fungi are associated with jack pine, which benefit tree growth by enhancing nutrient absorption, producing growth regulators, and increasing disease resistance (Kropp and Langlois 1990). Several ectomycorrhizal inocula have shown beneficial effects on the growth of planted jack pine stock (Navratil et al. 1981; Gagnon et al. 1987; McAfee and Fortin 1989). The introduction of biochar amendments, rich in pyrochemical compounds, might be stimulating these mycorrhizal networks, thereby fostering the potentiation of these symbiotic interactions. This symbiosis, combined with the soil alterations resulting from biochar amendments, could collectively explain why jack pine displayed enhanced survival rates under high biochar treatments. Therefore, the interplay between the fire-adapted traits of jack pine, the pyrochemical-infused changes in soil profile brought about by biochar, and the interactive mycorrhizal networks uniquely position this species to exploit the potential benefits of biochar amendments in forest regeneration and management. However, more research is necessary to confirm and deepen our understanding of these interactions and their potential implications for future forest management strategies.

The role of herbivory as a biotic stressor was demonstrated to be substantial among the non-conifer species within the study, with particular impact on the paper birch early on. Even with the use of protective tree tubes, around 50% of the birch seedlings exhibited signs of herbivory just three months post-planting . The red oak showed a similar trend, facing browse pressure with 18% of surviving seedlings displaying some form of herbivory by 2023. Vulnerability of both oak and birch seedlings extends to multiple species, this includes white-tailed deer (*Odocoileus virginianus*), rabbits (*Sylvilagus* spp.), and voles (*Microtus* spp.) (Irwin 1985; Meiners and Martinkovic 2002), along with many insects (Linit et al. 1986). Especially in regions where habitat modifications and reduced predator populations caused deer numbers to increase dramatically, deer have become of particular concern (Waller & Alverson 1997). The deer's impact on herbivory can drastically affect regeneration (Rooney & Waller 2003), and frequent browsing often leads to mortality or impaired development of birch seedlings in the United States and Canada. The impact extends to both naturally regenerated and intentionally planted seedlings (Jordan & Rushmore 1975).

Despite these interesting variations in growth strategies among species, none of the observed differences in growth responses to biochar treatment were statistically significant, neither negatively nor positively. The noted variability in growth responses echoes the findings of other studies. Two studies examining conifers *Pinus thunbergii* Parl. And *P. ponderosa* found enhanced water retention during drought with biochar treatment compared to controls (Matt, Keyes, and Dumroese 2018; Fujita et al. 2020), and similar protective effects against drought were observed for hardwood species like *Pyrus ussuriensis* Maxim and *Tilia* × *europaea* L. due to increased soil moisture availability (Lyu et al. 2016; Lo Piccolo et al. 2022). Nonetheless, studies on *Quercus* species and *P. banksiana* revealed mixed and sometimes null results, emphasizing the complexity and species-specific responses to biochar amendments (Zoghi et al. 2019; Heydari et al. 2023; Reuling et al. 2023). This variability underscores the need for continued research, particularly given the long lifespans and variable life history traits of trees, as well as the inherent complexity of forest ecosystems compared to crop species and agricultural systems (Luo et al. 2016, Bruckman & Pumpanen 2019). The intricacies of how biochar interacts with its environment stresses the need for extended, localized studies to better understand its impact on forest growth patterns and ecosystem dynamics (Ippolito et al. 2020; Amonette et al. 2021). Expanding our knowledge is vital not only for improving growth and survival rates of tree seedlings but also for understanding how these treatments affect the wider ecosystem. This includes the relationship with soil microbes, mycorrhizal fungi, other plant species, and the long-term physical and chemical properties of forest soils. Given the novelty of biochar in forestry, there is a significant amount of foundational research still to be done to fully harness its potential benefits.

Conclusions and Management Implications

Our investigation into the effects of biochar soil amendments on tree growth spanned a five-year period and entailed a study of four different tree species. The research sought to uncover broad patterns of biochar's influence, yet what emerged was a complex narrative of species-specific responses, and an understanding that the temporal scope of study greatly matters. Ultimately, while no significant positive effects of biochar amendment on seedling growth were uncovered over the course of this 5-year study, no findings indicated that the biochar treatments had a negative influence on the survival or growth of the planted seedlings. Each species did, however, exhibit varied responses that, while not statistically significant at this juncture, suggest a discernible trend warranting further examination. The enhanced survival of jack pine in response to the high dose biochar amendments represents a notable finding and one which again, merits future research, and provides a possible link between biochar amendments and future avenues of study, with an emphasis on fire adapted species.

Forests operate on long timescales that require long-term studies with repeated visits. Effects such as improved water retention and nutrient cycling may emerge slowly as biochar integrates into the soil ecology, influencing tree physiology and forest structure incrementally. It is imperative that future research extends beyond the confines of standard short-term studies to longitudinal investigations that can capture the lagging effects of biochar amendments. As such, the intricacies and potential delayed benefits of biochar in mixed conifer-hardwood forests, as indicated by the divergent growth responses in our study, must be tracked over a timeline that aligns with the lifespans and turnover rates of forest ecosystems. In summary, our findings point to the complexity of its interactions with tree species over time and the necessity for in-depth, long-term studies. Delving into these prolonged ecological exchanges will provide invaluable insights — allowing us to refine biochar application techniques and fulfill its promise as a tool for sustaining forest health and supporting the regeneration efforts within our invaluable forested landscapes.

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Chapter 2: Biochar's Impact on Understory Vegetative Community Composition in Fire-Dependent Forests in Northern Minnesota

Introduction

Understory plant communities play a crucial role in forest ecosystems. Although this layer constitutes less than 1% of a forest's total biomass, it is a source of immense biodiversity, containing up to 90% or more of a forest's plant species (Gillam 2007). These plants contribute to nutrient cycling by efficiently absorbing, storing, and releasing nutrients from the soil. They contribute up to 20% of the foliar litter that accumulates on the forest floor; this leaf litter is generally richer in nutrients compared to trees, contributing an outsized influence on nutrient cycle of forest ecosystem. Species within the herb layer, particularly spring ephemerals, can help mitigate the potential loss of essential nutrients during seasonal shifts (Gillam & Roberts 2003).

During understory reinitiation after a regeneration harvest, the herbaceous layer greatly influences stand development processes. These plants influence the microclimatic conditions of the forest floor and seedbed; by providing shade and altering temperature and humidity levels, herbaceous plants can induce the germination of some tree species' seeds. Conversely, the presence of specific understory plant species can hinder the growth of seedlings and saplings by competing for resources such as light, nutrients, and moisture (George & Bazzaz 1999; Gillam & Roberts 2014). Additionally, human-driven disturbance through forest management activities can influence understory vegetative communities and the ecological processes they support. These impacts depend on many factors, including the land use history of a site, its proximity to viable seed sources, as well as the type and intensity of the disturbance, and can occur in a spectrum of forms and intensities (Gachet et al. 2007; Swanson et al. 2011). Many site preparation treatments can be considered "double disturbances," where multiple entries into the stand are required to complete the treatment activities. Aubin et al. (2014) found that clearcut harvesting followed by mechanical site preparation treatments led to increased abundance of early successional species. The compounding effect of multiple treatment entries will hinge not only on the availability of seed sources following a disturbance but also on the specific life cycle attributes of the regenerating plants. Species with traits such as wind seed dispersal or adaptations to early successional conditions (disturbed mineral soils, increased soil temperature, etc.) will be more successful at establishing within recently disturbed areas—in contrast, species adapted for later successional conditions (where an intact forest floor impedes seed dispersal) will be less likely to establish within heavily disturbed areas (Tucker and Kashian 2018). Forest ecosystems without mechanized harvests tend to host more functional and taxonomic diversity, including ant-dispersed spring-flowering herbs, as well as more disturbance sensitive species, such as saprophytic plants. Additionally, clearcut harvesting without retaining logging slash can elevate soil temperatures immediately after harvest, particularly in the initial growing season. However, this effect tends to diminish over time as vegetation rapidly regrows, leading to transient impacts on soil temperature and associated processes (Slesak 2013). Conversely, certain differences have been observed to persist long after the initial treatment. For example, Ramovs and Roberts (2005) demonstrated that in a comparison of naturally and artificially regenerated stands, the differences in understory communities could endure for at least 60 years post-treatment. The complex interplay between the over and understory vegetative communities in forest ecosystems is important to understand not only in the context of stand reestablishment but also in terms of the taxonomic and functional diversity of a forest—several studies have raised concerns that forest management practices, both historic and those used at present, have led to declines in overall diversity when compared to naturally regenerating stands (Biswas and Mallik 2010).

Concerns regarding forest community shifts have been especially concerning in fire dependent communities. resulting in shifts in successional patterns (Zackrisson et al. 1996), reduced productivity (Wardle et al. 1998), and changes in species dominance (Mallik 2003, Friedman & Reich 2005). In particular, the fire adapted forests of the Upper Lake States have undergone significant transformations in the absence of fire (Gilmore & Palik 2006). These communities historically experienced a fire regime consisting of large-scale, high-severity fires ignited by lightning during dry conditions (Palik & D’Amato 2019; Stambaugh et al. 2021), alongside more frequent, low-severity surface fires typically started intentionally by humans for ecosystem stewardship (Johnson et al. 2022; Kipfmueller et al. 2017). The arrival of European settlers to the region led to the suppression of cultural fire practices among Indigenous communities, with many instances of outright criminalization (Kimmerer & Lake 2001). These fire suppression policies, aimed at protecting timber resources and settled areas, have disrupted the natural fire regime and led to lasting impacts within the collective fire-dependent communities. Without periodic fires, the forests have become homogeneous in structure—transitioning to shade-tolerant species while failing to regenerate and recruit species such as jack and red pine as seedlings and midstory saplings (Aakala et al. 2012; Fraver & Palik 2012; Silver et al. 2013). In addition to impacting the overstory and regeneration of these forests, fire exclusion has led to significant changes in the understory composition of these stands. The transition from open-canopy to closed-canopy conditions in the absence of fire has led to the development of dense understories comprised of woody shrubs and infiltration of fire intolerant tree species such as red maple (*Acer rubrum*) and paper birch (*Betula papyrifera*) in the stands, which have outcompeted shade-intolerant, fire-adapted species once dominant in the understory (Scherer et al. 2018), potentially reducing biodiversity across these landscapes (KC Ryan et al. 2013).

Managers are now exploring strategies and treatments to enhance the resilience of these ecosystems. Drawing from both traditional, locally informed ecological knowledge and evidence-based western science, the reintroduction of low-intensity prescribed burns has emerged as a central focus of forestry research in the Upper Midwest (Booth et al. 2023). In the absence of natural fires, prescribed fires are employed to reduce fuel loads, open canopies, and mitigate competition. However, there remains a tension between the recognized benefits and the associated risks of prescribed burning. Several factors limit the implementation of prescribed fires, such as restricted burn windows, regulations on air quality, insufficient funding and staff, and various environmental laws (Ryan, Knapp, and Varner 2013). These challenges are compounded by high-profile incidents, such as the escape of the Cerro Pelado Fire in New Mexico in 2022, which prompted the Forest Service to implement a national 90-day pause on all prescribed burning activities ("USDA Forest Service National Prescribed Fire Program Review, Fire Research and Management Exchange System", 2022). Such events not only temporarily disrupt prescribed fire operations but are also responsible for fueling public fear and intensifying skepticism towards these controlled burns. Facing these limitations to prescribed burning, many managers have turned to mechanical surrogates, such as thinning and pile burning for fuel reduction, or herbicide applications for site preparation before planting.

However, managers are quick to acknowledge the limitations of these treatments. The increased exploration of biochar in the United States is providing an opportunity for managers to consider if this treatment could better emulate the soil conditions following low to moderate intensity wildfires (Licht & Smith 2020). Much like biochar, the charcoal produced by wildfires represents a critical reservoir of long-term carbon storage in forest soils, and have been found to enhance soil moisture retention and plant physiological functions, in some ways identical to the benefits reaped from anthropogenically produced biochars (DeLuca & Aplet 2008; Alcañiz et al. 2018).

Biochar’s chemical and physical properties become all the more crucial to consider as researchers and managers explore its uses as a management tool. Biochar has been found to enhance plant growth across various ecosystem types by inducing changes in soil chemical, physical, and biological properties (Biederman & Harpole 2013; Thomas & Gale 2015). These changes include increased soil pH, primarily due to a liming effect of biochar (Palviainen et al. 2018), augmented nutrient availability, encompassing elements like nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg), achieved through enhanced ion exchange capacity (Sackett et al. 2015) and the stimulation of microbial activity (Pluchon et al. 2015; Wardle et al. 2008; Xu et al. 2021), as well as heightened water retention capacity (Laird et al. 2010). Natural chars are also far from uniform, varying in forms from partially charred biomass to black carbon and graphitic carbon, and can influence tree regeneration and fire-adapted plant growth in forest soils through mechanisms such as enhanced nutrient availability and adsorption of toxins. However, optimal conditions can be exceeded, impacting vegetation response (Thomas & Gale 2015). Similarly, the application of biochar to soils demonstrates significant variability, affecting both plant growth and understory plant communities, depending on several environmental factors. These encompass the type of plant species involved (Gale et al. 2017), the properties of both the soil and the biochar itself (Pluchon et al. 2014), the study's characteristics, such as whether it is conducted in a greenhouse or a field and its duration (Thomas & Gale 2015), and the specific environmental conditions of the study site (Li et al. 2018).

Only a limited number of studies have explored the response of native plant communities to biochar amendments (Gale et al. 2017; Gundale et al. 2016). Even fewer field studies exist have explored the effects of biochar on the species composition, diversity, and structure of understory communities (Thomas & Gale 2015). Within mixed boreal and sub boreal forest systems, there have been only a handful of studies which concerned biochar and the herbaceous layer. In a study following a clearcut stand of white spruce (*Picea glauca*) in northern Ontario, Bieser and Thomas (2019) reported shifts in the abundance of several species three years after the introduction of biochar—in particular, they found that treatments led to an abundance of *Rubus idaeus*, which in turn inhibited the success of the regenerating tree species; the authors attributed these changes were a result of modifications in soil nutrient availability and potential toxicity effects arising from the biochar feedstock source (in this case, trembling aspen (*Populus tremuloides* Michx.)). In contrast, in a study conducted in a clearcut Scots pine (*Pinus sylvestris*) stand in northern Sweden, Gundale et al. (2016) observed no alterations in community composition three years post-biochar amendment.

Consideration should be given not only to the attributes of the biochar itself, but also to the rate of biochar amendment application when comparing study outcomes. In the mentioned studies, biochar amendments were applied at rates ranging from 5 to 10 tons per hectare. Recent research in jack pine systems examined the response of ground cover vegetation to various treatments, including two types of biochar— one highly torrefied and the other less torrefied— applied at rates of 10 and 20 tons per hectare. The authors noted that the rate and type (level of torrefaction) of the treatments did not significantly impact native plant community composition after one year. However, differences between treatment types emerged when data was analyzed across the growing season (Kelso 2019). The authors suggest that this discrepancy may be attributed to differences in hydrophobicity and light availability between coarser and finer biochar particles.

The disparity in vegetation response in short term studies led to interest in studies which examined the influence over longer periods—the authors of another study in a similar system to Gundale et al. found that over a period of nine years post treatment, biochar amendments applied at a rate of 10 tons per hectare encouraged the development of a more resource-conservative vegetative community, with increased presence of resource conservative ericaceous shrubs and naturally regenerating pine species within treatment plots. They also found that the treatment plots exhibited reduced abundance of resource-acquisitive graminoids and forbs, which are often characterized as the "disturbance loving" early successional species most likely to inhibit the growth and establishment of target tree species, either artificially or naturally regenerating (Grau-andres et al. 2021). These results suggest that the effects of biochar soil amendment on understory vegetative community composition may require several years to manifest (Bieser & Thomas 2019; Gundale et al. 2016).

An additional source of variability in vegetation's response to biochar amendments may stem from differences in application method. Top dress or broadcast applications of biochar are the most feasible in forest settings, due to operational constraints, and differ from methods that more thoroughly incorporate biochar into the soil, often seen in agricultural settings. Short-term effects of top-dressed biochar include shading and increased soil surface temperature. However, these effects diminish over a few years as biochar weathers, breaks down, and integrates into the soil naturally. The application of biochar via topdressing presents several challenges, notably the risk of significant loss of the amendment. Major et al. (2010) have estimated that up to 53% of biochar applied on the soil surface could be lost due to wind displacement and infiltration into the soil, thus diminishing its effectiveness and carbon storage capability. The research conducted by Kelso (2019) identified two primary mechanisms of biochar loss: wind dispersion during application and washout during intense rain events, especially on sloped terrains.

Application method could also contribute to the variability in responses that exist within the current literature. Intuitively, differences exist between top dress applications (in which biochar is spread over the soil surface) and other approaches that will incorporate biochar into soil more completely, as has been more extensively studied in agricultural settings (Richard et al. 2018; Page-Dumroese et al. 2017). The short-term effects of biochar applied in this method include a combination of both shading the soil surface and increasing the temperature of the soil surface as the dark color of the biochar absorbs sunlight. These short-term effects of biochar would likely diminish over just a couple of years due to the combination of the aforementioned losses, and as the material is weathered, broken down, and incorporated through natural processes into the soil.

Additional considerations for vegetation studies include vegetation control (VC) and other forms of site preparation, such as the presence or lack thereof logging debris in the stand. Razzaghi, Obour, and Arthur (2020), Dai et al. (2020) and Farhangi-Abriz et al. (2021) conducted meta-analyses revealing heightened biochar effects on coarse-textured soils, with the enhancement in growth attributed to changes in pH, cation exchange capacity, and organic matter content. This underscores the need to consider not only biochar properties but also the initial conditions of the site when assessing the potential benefits of biochar amendment.

Biochar treatments influence soil properties, nutrient availability, and microbial communities, potentially leading to long-lasting effects on the abundance and health of understory vegetation. This broader research aims to provide a more holistic understanding of the long-term impacts of these treatments within a stand. Specifically, this study seeks to address research gaps by identifying differences in the composition, structure, and species diversity within the herbaceous layer of plots treated with two different doses of biochar amendment compared to an untreated control. By linking biochar's effects on tree species with the understory plants they coexist with, the study aims to enhance understanding of how these treatments might influence overall forest communities.

Methods

Study Area

The experiment was installed at the University of Minnesota's Cloquet Forestry Center (hereafter CFC), which is located twenty-seven miles southwest of Duluth, Minnesota, USA in Carlton County. The Cloquet Forestry Center contains 3,471 acres of land located to the east of the town of Cloquet, and is located within the boundaries of the Fond du Lac Band of Lake Superior Chippewa Reservation (FDL). The center also falls within the bounds of the 1854 Treaty Area, within which the treaty-reserved hunting, fishing and gathering rights of the Grand Portage and Bois Forte bands are enabled and protected (Norrgard 2014)

The CFC property is located about 20 miles east of Lake Superior; this proximity introduces a slight climatic influence which has resulted in slightly warmer annual temperatures compared to the surrounding area (Reinikainen et al. 2015). The CFC has an onsite weather reporting station which has had daily records reported daily since 1911. These historical records indicate a mean annual temperature of 39.1°F (3.9°C) , with fluctuations between 34.4 and 44.5°F (-1.3°C to -6.9°C). Winter means tend to range from 4°F to 21°F (-16°C to -6°C), while summer means range from 61°F to 80°F (16°C to 27°C) (University of Minnesota Cloquet Forestry Center Daily Weather Observation Data, 1914-2023). Drought conditions in the area have varied significantly over the course of the historic records, with decadal scale wetting and drying trends. Broad region level model-based predictions of climate change have affirmed these trends (Nagel et al.

2017). At the time of writing, the region is experiencing its third consecutive year of moderate to severe drought conditions across much of its area.

Fire Dependent Disturbance Dynamics

The Minnesota DNR ecological classification system characterizes the study site as a fire dependent community, specifically within the FDn33 community (MN DNR, *Field Guide to the Native Plant Communities of Minnesota: The Laurentian Mixed Forest Province,* 2003). The natural wildfire disturbances that these conifer-hardwood communities evolved alongside were primarily low-moderate intensity wildfires, which reoccurred roughly every 77 years, and maintained the mixture of paper birch, red pine, and eastern white pine by regenerating patches of paper birch, leaving red and eastern white pine residuals as seed trees, and killing all fire-sensitive species. Stand-replacing fires were significantly more rare, only occurring every 220 years or so. The reinitiated stand would typically initially be occupied primarily by early successional species such as quaking aspen, paper birch, and jack pine. These intermittent severe crown fires would kill the majority of canopy trees in FDn33 woodlands, resulting in the creation of open habitats. These higher intensity fires trigger several adaptive responses to reinitiate the stand: first and foremost, they favored the survival of fire-resistant mature red pine and eastern white pine trees, with their thick fire-resistant bark, and killed off the advance regeneration of fire-sensitive and tolerant trees in the subcanopy, such as red maple and balsam fir. While these fires have profound effects on the aboveground portions of trees, several species have developed adaptations that allow them to quickly respond due to their roots and underground carbohydrate stores—quaking aspen are prolific at suckering, and paper birch and red maple are able to rapidly regenerate via stump sprouts. Some species rely on their seeding capabilities, such as jack pine, with their serotinous cones, whose establishment is further facilitated by the prepared mineral seedbeds, where red pine and big-toothed aspen seeds are also able to establish (Smirnova et al. 2008). Furthermore, these fires substantially influence soil conditions post-disturbance, perhaps most notably resulting in significant depletion of organic matter within the stand as leaf litter and other woody materials have been converted to char and ash. This depletion leads to a reduction in overall nitrogen available within the system, as well as a decrease in the cation-exchange capacity of the soils (MN DNR, *Field Guide to the Native Plant Communities of Minnesota: The Laurentian Mixed Forest Province*, 2003).

Site Preparation

The experiment was conducted in an 11.9 acre stand, clearcut during the winter of 2018/19. The timber harvest was conducted on frozen ground utilizing a full tree harvest system with complete biomass removal. Prior to harvest, the stand was composed of conifer-hardwood species, dominated by red pine (*Pinus resinosa* Ait. (Pinaceae)), eastern white pine (*Strobus strobus* (L.) Small), jack pine (*Pinus banksiana* Lamb.), black spruce (*Picea mariana* (Mill.) B.S.P. (Pinaceae)), quaking aspen (*Populus tremuloides* Michx. (Salicaceae)), or paper birch (*Betula papyrifera* (Regel) Fern.). The Minnesota Ecological Classification System (MN ECS) system defined the stand as a fire dependent ecosystem, specifically the FDn33a community type (*Field Guide to the Native Plant*

Communities of Minnesota: The Laurentian Mixed Forest Province, 2003). However, due to post-colonial fire suppression, this area had not experienced fire on the ground within the past 100+ years. The stand soils are composed of Omega loamy sand, on primarily 0 to 2 percent slopes, with roughly 25% of the study area in moderately hilly terrain, with slopes between 2 and 12 percent, and variable aspect.

Prior to initiating the study, site preparation activities were conducted to ensure a conducive environment for tree seedling planting and establishment. This included the removal of any remaining logging slash and implementation of mechanical competition control measures within the designated plots in order to prepare the sites for planting.

Experimental Design

The study employed a split-plot design comprising fifteen square plots, each approximately 0.3 acres in size. Each plot measured 114 feet by 114 feet. The center of the plot contained a 10 ft wide strip designated for soil sampling and vegetation monitoring, and received the same biochar treatments as the surrounding regions where seedlings were planted. Annual mechanical suppression of vegetative competition was conducted during summer months using brush saws within plots to manage suckering quaking aspen until 2021. From 2022 to 2023, suppression in the 10-foot middle strip of each plot was halted to facilitate vegetation sampling, while suppression continued annually for seedlings. See figure 2.1 for timeline of treatments and sampling periods.

In the summer of 2022, four permanent subplots (A, B, C, and D, arranged from north to south) were installed within each unplanted strip, each covering 1/5000th of an acre, running parallel to the rows of planted seedlings (figure 2.2). In addition, two regeneration subplots measuring 1/300th of an acre were added into this strip, sharing plot centers with subplots A and C.

Within the nested 1/5000th acre subplots (radius of 1.67 feet), detailed data was collected on understory vegetation in June of 2022 and 2023. Data collection included frequency, percent cover and height classification of each species identified, including all understory trees (< 5" DBH), shrubs, forbs, (all identified to species) as well as for ferns, moss/lichen, and graminoids, following the scientific nomenclature of the PLANTS Database (USDA, NRCS. 2024). Additionally, ground cover data was documented for litter, large woody debris (diameter ≥ 3"), small woody debris (diameter < 3"), bare ground, and any identifiable biochar within the plot radius. Stem counts for all tree and shrub species within the plots were also recorded. Total percent cover of plants, litter, woody debris, bare ground, vegetative functional groups (forbs, graminoids, shrubs, trees, ferns) and individual species cover were averaged over each subplot.

In the larger 1/300th acre plots centered upon subplot A & C (radius of 6.8 feet), data collection specific to natural regeneration was conducted in July of 2023. For each living seedling, two diameter measurements (at 90-degree angles from each other) were measured using calipers, measuring to the nearest millimeter. Height measurements were taken for both broadleaf and conifer species, with broadleaf species assessed for terminal bud height, and conifers measured for both the terminal bud and the highest point of needle growth. Seedling health was also assessed on a three-point scale: 0 indicated recent mortality, 2 indicated that the seedling was live and vigorous, and 1 indicated that the seedling was displaying signs of distress.

Treatments

The biochar utilized in this study was produced at the Biomass Conversion Lab, located at the Natural Resources Research Institute site in Coleraine, Minnesota. This experiment employs two levels of biochar concentrations to be tested as broadcast applied soil amendments (Gale & Thomas 2019). Treatments were randomly assigned to each of the 15 0.3 acre plots, with four plots designated for the "high" treatment, receiving 4000 lbs. of biochar each, evenly distributed across the soil surface, equivalent to approximately 13,333 lbs. per acre. Another four plots were assigned to the "low" treatment, and received 2000 lbs. of biochar each, approximately 6667 lbs. per acre. The remaining seven plots were designated as controls, and did not receive any biochar application. Following the June planting, in July of 2019 the two levels of biochar treatments were weighed and applied to the soil surface of the treatment plots. Biochar distribution across the plots was conducted manually due to operational feasibility, hand rakes were used to evenly distribute the material and loosely incorporate it into the top layer of the soil.

Data Analysis

Species richness, diversity indices (Shannon's & Simpsons), and evenness were all calculated in R (version 4.3.0, R Core Team 2017) using functions from the package vegan (Oksanen 2022). Treatment differences were evaluated using ANOVA, and where treatment was found to be significant, Tukey tests were used to assess the differences between treatments. Species frequency was summarized within each treatment type to estimate the overall abundance of the species within each, whereas percent cover by species was averaged at the plot level (n = 4), and plot means were then used to calculate summaries of cover by species, functional groups, broken down by treatment type across the two years. The effect of biochar treatments on tree species natural regeneration was analyzed similarly to the understory vegetation data, and their growth analyzed using ANOVA, in which plot will be treated as a random effect, and treatment as a fixed effect.

To future explore potential community dynamics, a non-metric multidimensional scaling (NMS) ordination was run using percent cover (PC-Ord Version 6.255) (McCune and Mefford 2011). The environmental matrix (secondary matrix) included location, elevation, treatment, distance from edge, and year. NMS was used due to relaxed assumptions of normality (McCune et al. 2002). Autopilot mode (slow and thorough) was selected using Sørensen (Bray–Curtis) distance measurement and a random starting configuration. Two hundred and fifty runs were completed for both the real and randomized data to determine dimensionality. Species and environmental variables with an r^2 (squared correlation coefficient) greater than 0.2 were considered meaningful

(Wilson et al. 2013). A multi-response permutation procedures (MRPP) was run using Sørensen (Bray–Curtis) distance was used to explore if significant differences existed between treatments.

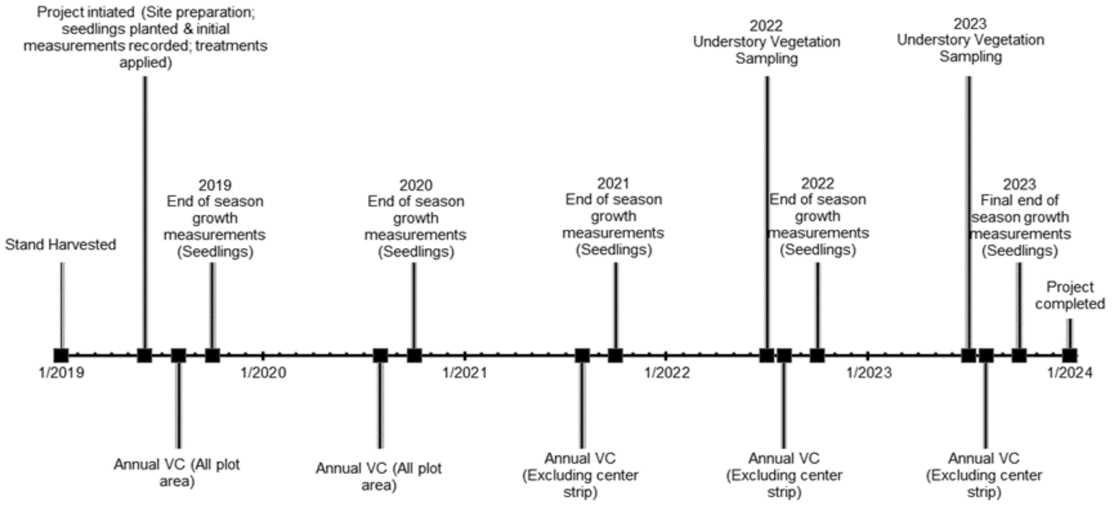


Figure 2.1: Timeline of project actions (including sampling periods, vegetation control, and study initiation and completion dates)

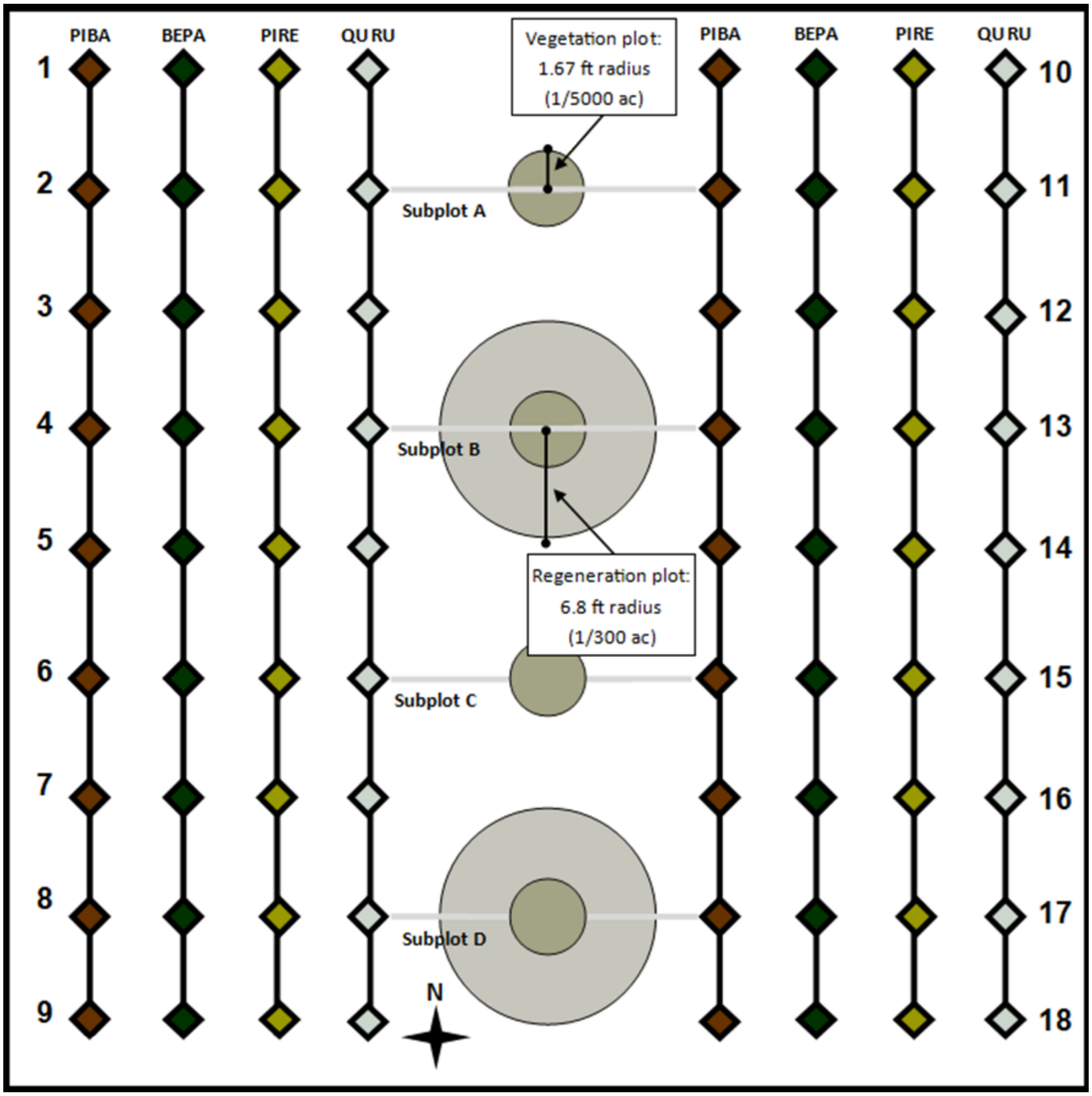


Figure 2.2. Plot design, four rows of nine planted seedlings on each side (10 x 10 spacing), with 10 ft gap in the middle for vegetation sampling. Subplots A, B, C, D (1/5000 acre) were used for vegetation survey, while plots B & D have larger macro plots (1/300 acre) used for natural regeneration survey in 2023.

Results

Species Composition

A total of 57 species were observed over the course of the study, with 55 species identified in 2022, and 41 in 2023.

Grasses (graminoids) were the dominant cover type over all other cover types in 2022 (Control 37.43%, Low 49.1%, High 40.22%) and 2023 (Control 39.57 %, Low 49.44%, High 40.19%). Grasses were also the most commonly occurring species in all treatment groups in both years, occurring in nearly all subplots. Woody shrubs made up the second most dominant functional cover group; the control plots contained 32% and 38% shrub cover in 2022 and 2023 respectively, the low treated plots had 24% and 30% cover, while the high demonstrated cover of 24% and 39% over the two years. Tree species were a minor component with less than 10% cover across treatments and years.

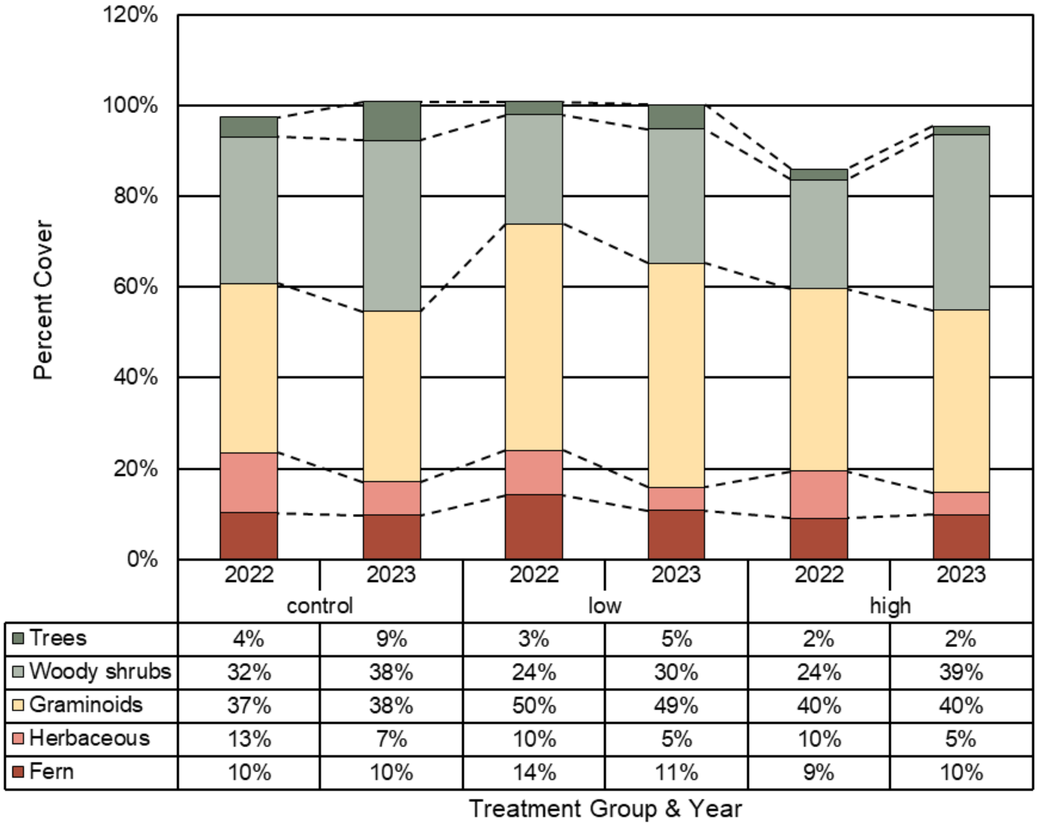
The most commonly occurring individual species was *Rubus idaeus*, or red raspberry, which was found in 72% of the low and high subplots over both years, and in 70% of the control group plots. Mean percent cover was much more variable, with 14.33% in control, dipping to 7.77% cover in the low, and peaking at 15.08% in the high treatment. Bracken fern, *Pteridium aquilinum,* was found in over half of all subplots for all treatment groups as well (54% control, 63% low, 56% high), and cover ranged from 9.41% in the high to 12.41% in the low treated group, with the control cover falling on the lesser end of the range at 9.91% on average over the two year study.

Several species decreased in frequency or cover as the dosage of biochar amendment increased. Multiple species of herbaceous plants common within mixed conifer hardwood forests were identified over the course of the study, and many followed this trend, such as *Anemone quinquefolia* (wood anemone), *Aralia nudicaulis* (wild sarsaparilla), and *Fragaria vesca* (woodland strawberry) (Table 2.1). *Viola sp.* (violet species) were the most frequently occurring species that displayed this trend, which were found in 80% of the control plots, 63% of the low and 44% of the high treated plots. The percent cover of the species decreased accordingly, dropping from 3.46% within the control to 0.61% in the high treatment group, a difference found to be statistically significant (p = 1.49E-04). Similarly, *Trientalis borealis* (starflower) declined in frequency from 48% in control plots to 13% in high treated plots. Significant differences were detected in percent cover for this species as well (p = 0.014), which reduced from 0.58% in control to 0.16% in low and 0.13% in high biochar plots.

For woody shrubs and trees, *Salicaceae* (willow) species were also found to have significant differences in percent cover by treatment type (p = 0.031), and were identified exclusively in control plots (14% occurrence, 1.04% cover). Similarly, other woody species such as *Vaccinium angustifolium* (lowbush blueberry) followed the observed trend of declining frequency and abundance when biochar amendment rates increased. The species was present but variable, occurring within 41% of the untreated plots, 34% of the low, and 13% of the high treatment plots, with percent cover dropping as well, from 2.71% to 2.43% and 0.47%, respectively. *Populus tremuloides* (Quaking aspen) exhibited a parallel decline, with its presence reduced from 38% (control) to 16% (low) and 9% (high), with cover dropping from 1.66% to 1.02%, and then to 0.44%, respectively, as biochar dosage increased. *Acer rubrum* (red maple) frequency decreased steadily as amendment rates increased (36% control, 25% low, 16% high), however the species abundance response notably departed from that of others, as percent cover dropped dramatically from 4.39% in the control group to just 1% in the low treated group, before rebounding to 3.39% in the high treatment group.

Not all species experienced declines as the amendment rate increased, however. Several species increased in frequency and abundance in the treated plots as compared to their counterparts in the untreated plots, including *Cirsium arvense* (Canadian thistle), a species known for its affinity for recently disturbed sites. The species was identified in only the low and high treated plots, occurring in 3% of the low and 9% of the high plots over the two years of the study, with percent cover of 0.03% in the low, but increasing to 0.44% in the high amendment plots, a difference which was found statistically significant ($p = 0.062$). Other disturbance affiliated herbaceous species, such as *Conyza canadensis* (Canadian horseweed or fleabane) and *Taraxacum officinale* (Common dandelion), displayed similar trends. *Conyza canadensis* increased in occurrence and cover alongside amendment dosage, nearly doubling in frequency from the control to the high plots (20% control, 53% high) and increasing by over seven fold in mean percent cover (0.29% control, 2.09% high), demonstrating resilience and rapid establishment in disturbed settings. *Taraxacum officinale* differed slightly, with frequency peaking in the low treated plots, but cover increased tenfold in the high treated plots compared to the controls (0.02 control, 0.2% high).

Certain shrub species were found to respond similarly, such as *Corylus cornuta* (beaked hazel), a species highly affiliated with fire dependent ecosystems—its frequency increased from 41% in the control to 56% under low and 69% of high biochar plots. However, its cover response was more varied, peaking in the low treated plots at 9.8%, suggesting potentially significant effects on vegetation density or individual growth with biochar amendments. Additional species of note include *Prunus pensylvanica* (pin cherry) and *Rubus allegheniensis* (Allegheny blackberry), both of which exhibited marginal increases in frequency as amendment rate rose, but varied in their cover response, with a drop in cover in the low treated group for pin cherry, and notably higher cover in the high treatment group for blackberry.



	control 2022	control 2023	low 2022	low 2023	high 2022	high 2023
■ Trees	4%	9%	3%	5%	2%	2%
■ Woody shrubs	32%	38%	24%	30%	24%	39%
■ Graminoids	37%	38%	50%	49%	40%	40%
■ Herbaceous	13%	7%	10%	5%	10%	5%
■ Fern	10%	10%	14%	11%	9%	10%

Figure 2.3: Percent cover of functional groups by treatment group and year. Lines depict changes between treatments and sampling years.

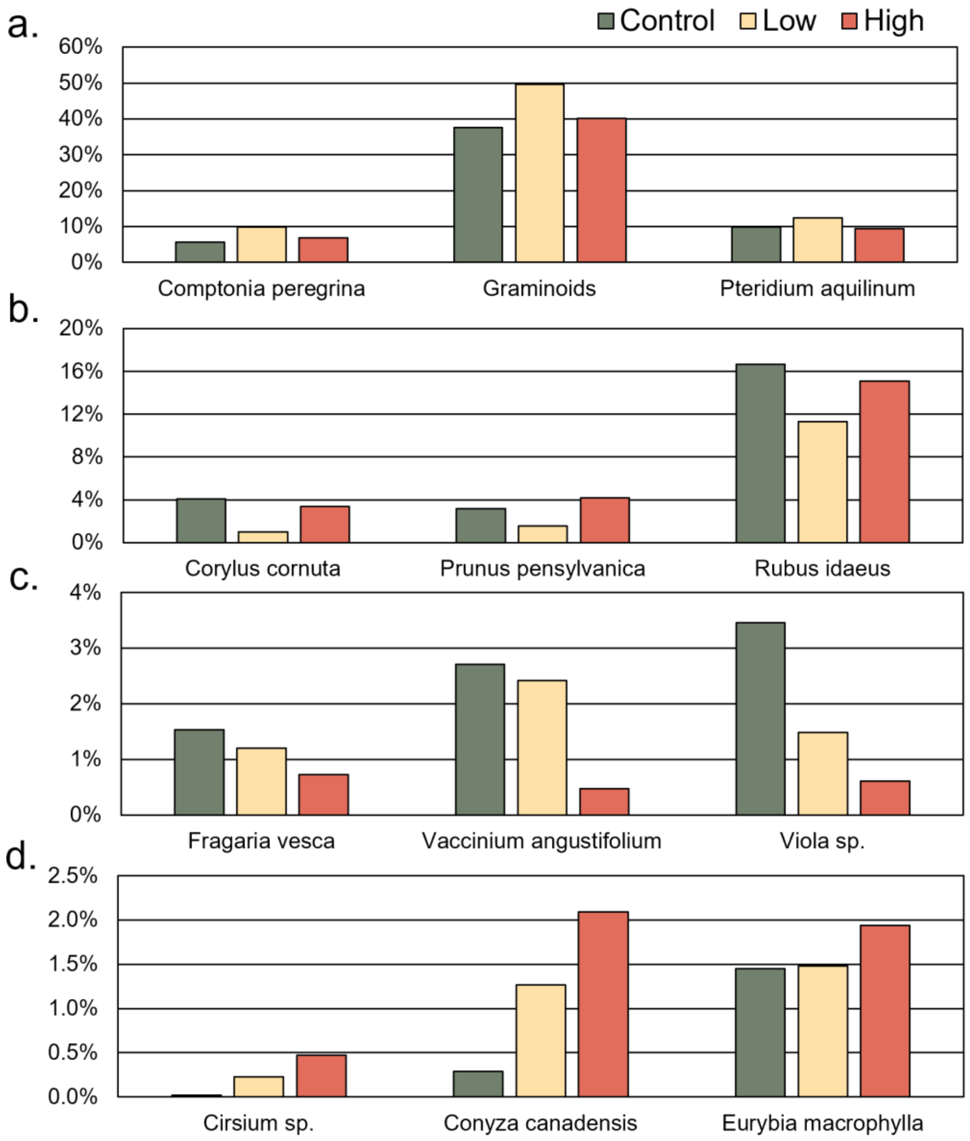


Figure 2.4: Mean percent cover of selected species identified over course of 2022 & 2023 sampling. Cover was calculated for each species at the plot level, averaging the four subplots, before pooling these means to calculate treatment level means, which pool both years of cover data. Species included: a.) *Comptonia peregrina* (Sweetfern), *Graminoids* (grasses), *Pteridium aquilinum* (bracken fern) b.) *Corylus cornuta* (beaked hazelnut), *Prunus pensylvanica* (pin cherry), and *Rubus idaeus* (red raspberry) c.) *Fragaria vesca* (wild or woodland strawberry), *Vaccinium angustifolium* (lowbush blueberry), and *Viola* species (violet family) d.) *Cirsium* species (thistle family), *Conyza canadensis* (Canadian horseweed, or Canadian fleabane) and *Eurybia macrophylla* (Big leafed aster).

Table 2.1. Mean species percent cover (and standard error) over 2022 and 2023 sampling periods for select species by treatment group, as well as frequency of species occurrence within plots. ANOVA was conducted to assess differences in treatment group using plot means for each sample year (Control n = 28, Low n = 14, High n = 14). F statistic and p value reported, with significant p values (>0.1) bolded. For complete table of species means and frequencies, see appendix Table A.2.1.

	Control		Low		High		ANOVA	
		% CC		% CC		% CC		
species	Freq.	mean (se)	Freq.	mean (se)	Freq.	mean (se)	*F*	p value
Acer rubrum	36%	4.39% (34%)	25%	1% (46%)	16%	1.20% (43%)	2.19	0.131
Anemone quinquefolia	38%	0.57% (46%)	25%	0.42% (50%)	19%	0.36% (69%)	0.19	0.83
Aralia nudicaulis	9%	0.29% (79%)	6%	0.06% (67%)	3%	0.03% (100%)	0.59	0.563
Cirsium arvense	0%	0% —	3%	0.03% (100%)	9%	0.44% (61%)	3.09	**0.062**
Cirsium sp.	2%	0.02% (100%)	6%	0.23% (83%)	13%	0.47% (55%)	2.09	0.143
Corylus cornuta	41%	5.55% (32%)	56%	9.80% (27%)	69%	6.81% (20%)	1.11	0.344
Conyza canadensis	20%	0.29% (48%)	22%	1.27% (86%)	53%	2.09% (37%)	1.96	0.16
Comptonia peregrina	34%	3.49% (29%)	13%	1% (72%)	31%	3.39% (58%)	1.03	0.371
Eurybia macrophylla	25%	1.45% (52%)	28%	1.48% (43%)	38%	1.94% (39%)	0.11	0.897
Fragaria vesca	23%	1.53% (29%)	16%	1.20% (43%)	22%	0.73% (60%)	0.65	0.529
graminoids	98%	37.50% (8%)	97%	49.67% (13%)	94%	40.20% (15%)	1.57	0.226
Populus tremuloides	38%	1.66% (24%)	16%	1.02% (39%)	9%	0.44% (55%)	2.47	0.104
Prunus pensylvanica	34%	3.15% (35%)	34%	1.55% (19%)	50%	4.19% (29%)	1.19	0.319
Pteridium aquilinum	54%	9.91% (17%)	63%	12.41% (22%)	56%	9.41% (33%)	0.36	0.7
Rubus allegheniensis	9%	0.38% (47%)	13%	0.30% (70%)	13%	1.53% (61%)	1.7	0.202
Rubus idaeus	70%	14.33% (26%)	72%	7.77% (27%)	72%	15.08% (20%)	1.13	0.339
Salicaceae sp.	14%	1.04% (38%)	0%	0% —	0%	0% —	3.97	**0.031**
Taraxacum officinale	2%	0.02% (100%)	6%	0.06% (67%)	3%	0.20% (95%)	0.97	0.39
Trientalis borealis	48%	0.58% (24%)	16%	0.16% (38%)	13%	0.13% (31%)	4.99	**0.014**
Vaccinium angustifolium	41%	2.71% (32%)	34%	2.42% (28%)	13%	0.47% (62%)	2.08	0.145
Viola sp.	80%	3.46% (14%)	63%	1.48% (24%)	44%	0.61% (34%)	12.43	**1.49E-04**

Table 2.2: Mean stems per acre (and percent standard error) by sample year of the seven most abundant woody shrub and tree species identified in vegetation plots. Paired t test comparisons conducted, p values depicted here, significant values are bolded (p > 0.1).

	Control			Low			High		
species	2022	2023	p val	2022	2023	p val	2022	2023	p val
Acer rubrum	4,643 (±51%)	3,393 (±53%)	0.403	2,188 (±49%)	938 (±100%)	0.391	2,188 (±82%)	938 (±64%)	0.423
Comptonia peregrina	7,500 (±45%)	19,643 (±50%)	0.113	13,125 (±40%)	18,125 (±34%)	**0.068**	14,375 (±45%)	27,500 (±29%)	0.323
Corylus cornuta	16,071 (±41%)	16,250 (±39%)	0.736	1,875 (±100%)	2,500 (±100%)	0.65	5,313 (±51%)	7,188 (±83%)	0.391
Populus tremuloides	3,750 (±41%)	2,857 (±43%)	0.253	1,250 (±71%)	313 (±100%)	0.182	1,250 (±71%)	625 (±100%)	0.215
Prunus pensylvanica	4,107 (±41%)	5,179 (±45%)	**0.055**	5,000 (±37%)	4,063 (±26%)	**0.014**	5,938 (±59%)	6,250 (±37%)	**0.002**
Rubus idaeus	115,536 (±27%)	138,571 (±23%)	0.141	95,938 (±29%)	129,063 (±24%)	**0.017**	86,250 (±34%)	124,063 (±18%)	**0.027**
Vaccinium angustifolium	16,964 (±43%)	22,857 (±45%)	**0.055**	8,750 (±46%)	15,625 (±51%)	0.391	3,750 (±100%)	2,500 (±100%)	0.124

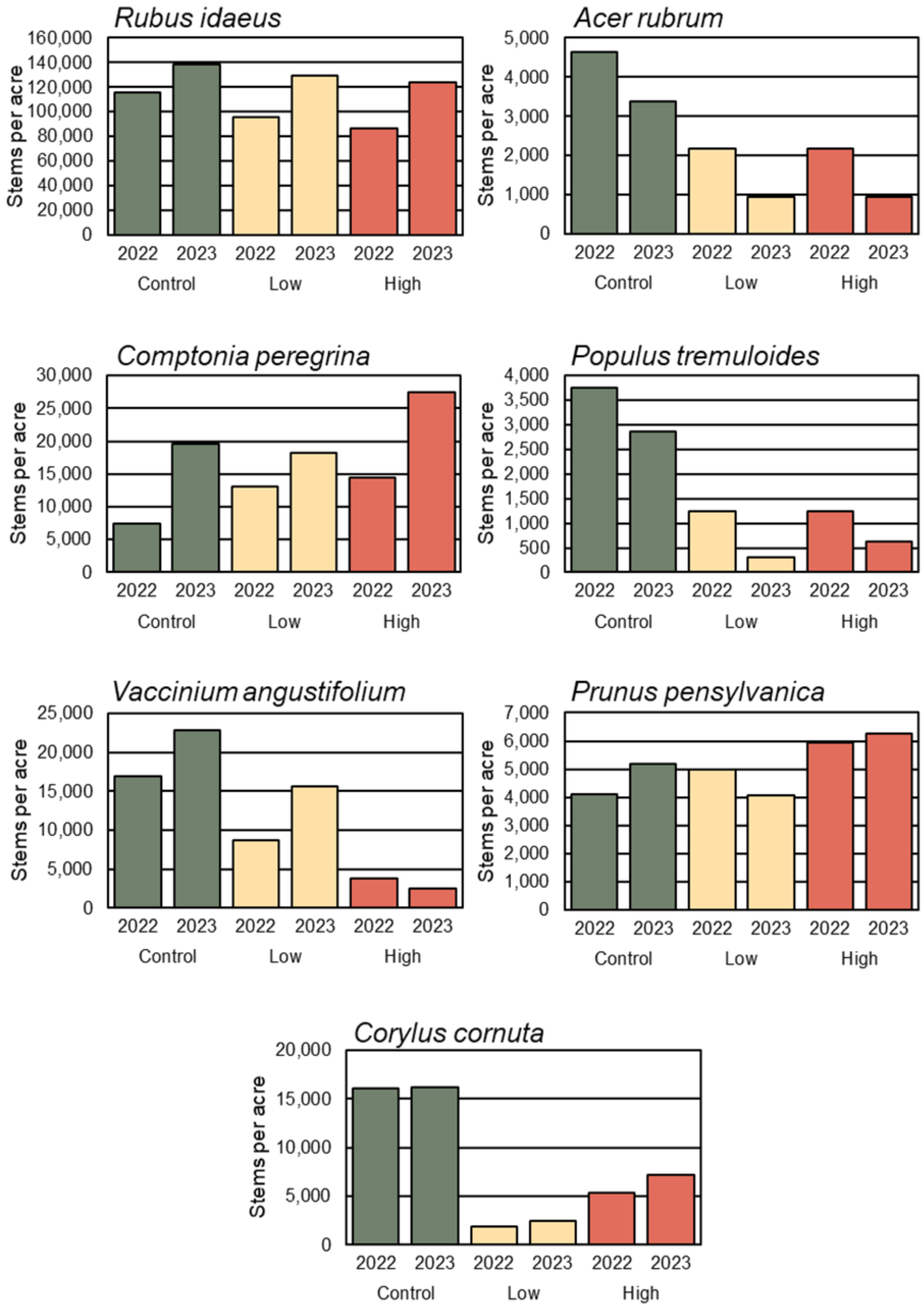


Figure 2.5: Stems per acre of most common woody shrubs by treatment and year sampled.

Diversity analysis

Species Richness

In 2022, an analysis of species richness revealed significant differences among treatment types (F(2, 57) = 2.905, p = 0.0629). Post-hoc tests resulted in no significant differences. However, a trend suggested lower species richness in both Low and High treatments compared to Control. The mean species richness for Control, Low, and High treatments were 9.04, 7.13, and 7.25, respectively, with Control treatments exhibiting the highest mean species richness, followed by High treatments, and Low treatments.

Similarly, in 2023, an analysis of species richness indicated a marginally significant difference among treatment types (F(2, 57) = 2.782, p = 0.0703) with significant differences between High and Control treatments (difference of 1.964286) which was found to be significant (p = 0.0931). No other treatment combination was significant. Consistent with the previous year, a trend suggested lower species richness in both Low and High treatments compared to Control. The mean species richness for Control, Low, and High treatments were 8.71, 7.13, and 6.75, respectively, mirroring the patterns observed in 2022.

Shannon’s diversity

The analysis of Shannon's diversity index (H’) in 2022 revealed important insights into treatment effects. A marginally significant difference in the index among treatment types was observed (F(2, 57) = 2.563, p = 0.0859). No significant differences were observed in the post-hoc tests. Control treatments exhibited the highest mean Shannon's H' (2.0074), followed by Low treatments (1.7842) and High treatments (1.7565). Control treatments also displayed the highest standard deviation (0.3904) compared to Low (0.3254) and High (0.4961) treatments, consistent with the 2022 findings.

In 2023, a significant difference in the index among treatment types was observed (F(2, 57) = 2.579, p = 0.0847), subsequent Tukey multiple comparisons did not identify any significant differences between treatment types. The mean values in 2023 showed a similar pattern to that which emerged in 2022, with Control treatments exhibiting the highest mean Shannon's H' (1.9668), followed by Low treatments (1.7737) and High treatments (1.7198). Control treatments also displayed the highest standard deviation (0.3952) compared to Low (0.3563) and High (0.3764) treatments, indicating greater variability within the Control group.

Simpson's diversity

In 2022 and 2023, the analysis of Simpson's diversity index (Simpson's D) suggested minor potential differences among treatment types, although the one-way ANOVA test yielded a non-significant result (2022: F(2, 57) = 2.181, p = 0.122; 2023: F(2, 57) = 2.187, p = 0.122). Examining the mean values, Control treatments exhibited the highest mean Simpson's D (0.8385 in 2022, 0.8317 in 2023), followed by Low treatments (0.8014 in 2022, 0.797 in 2023) and High treatments (0.7873 in 2022 and 0.7881 in 2023). High treatments also displayed the highest standard deviation in both years (0.1145 in 2022, 0.0808 in 2023) , indicating greater variability within this group compared to Low (2022: 0.0716, 2023: 0.0788) and Control (2022: 0.0688, 2023: 0.0652) treatments.

Evenness

In 2022, the analysis of Pielou's Evenness index did not yield statistically significant differences among treatment types (F(2, 57) = 1.528, p = 0.226). Similarly, in 2023, the analysis of the evenness index did not reveal significant differences among treatment types (F(2, 57) = 1.257, p = 0.292).

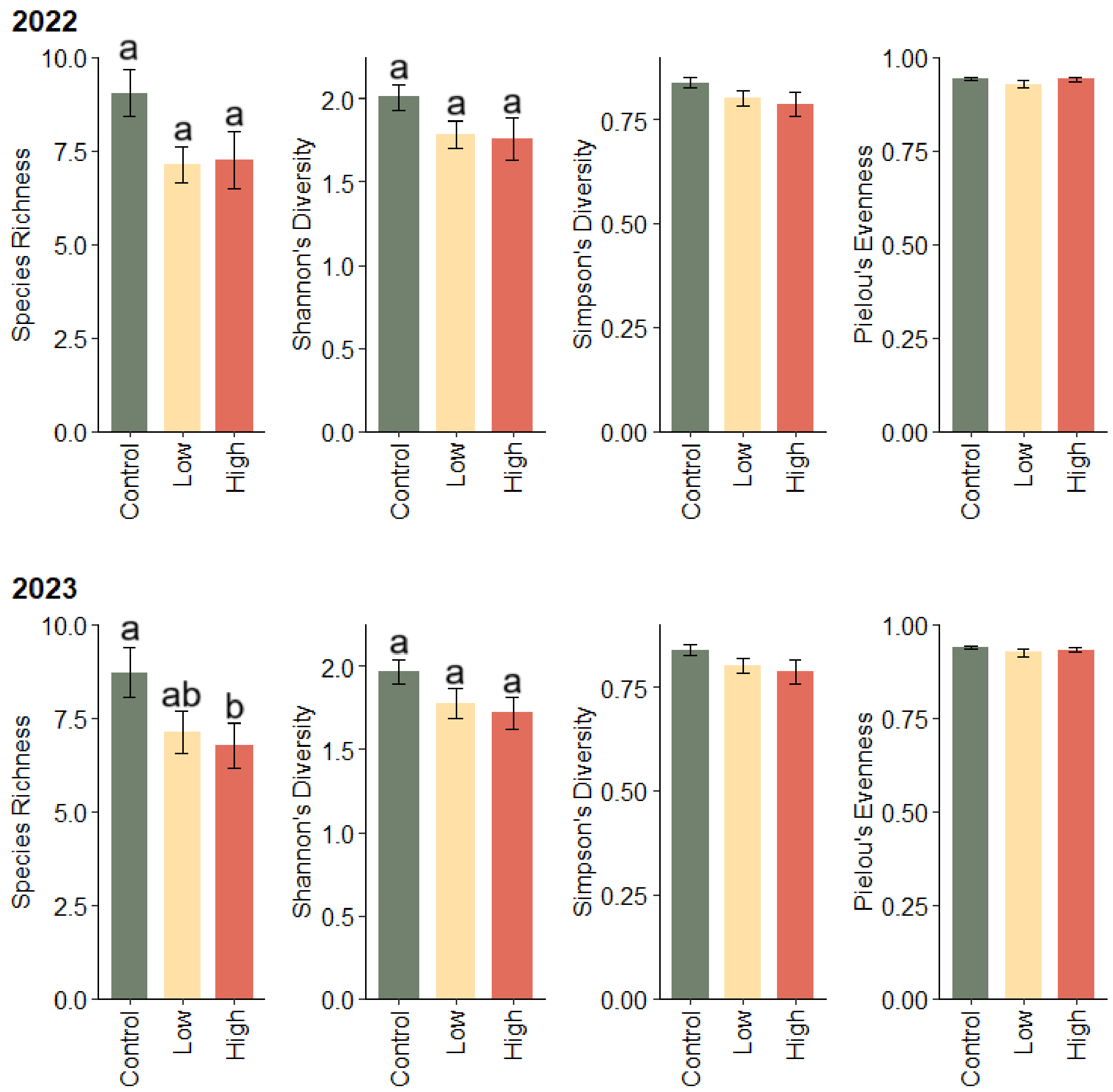


Figure 2.6: Mean Shannon & Simpson diversity indices, as well as species richness and evenness by treatment group and sampling year. When treatment was found to be significant, post-hoc Tukey tests were performed, differences noted by letters (p > 0.1)

Table 2.3: Comparison of vegetation characteristics (across control, low, and high treatment groups over 2022 and 2023). It includes percent cover, species richness, Shannon diversity index, Simpson diversity index, and evenness. ANOVA was used to compare treatment types within each sampling year, and when the p-value was significant, Tukey post hoc tests were conducted to evaluate differences between treatment types, denoted by lowercase alphabetical letters next to means.

		2022				2023			
Index	Value	Control	Low	High	p val	Control	Low	High	p val
Percent Cover	Mean	97.90%	101%	86.50%	0.852	101%	100.30%	95.60%	0.942
	Range	70 - 134.625%	84 - 115.125%	55.625 - 124.5%		60.75 - 130.75%	68.875 - 114.5%	62 - 123.75%	
Richness	Total species	43	30	28		33	28	24	
	Mean se	9.04 a 0.631	7.13 a 0.499	7.25 a 0.766	**0.063**	8.71 a 0.664	7.13 ab 0.554	6.75 b 0.602	**0.07**
Shannon Diversity Index	Mean se	2.01 a 0.074	1.79 a 0.081	1.76 a 0.124	**0.086**	1.97 a 0.075	1.77 a 0.089	1.72 a 0.094	**0.085**
Simpson Diversity Index	Mean se	0.84 0.013	0.8 0.018	0.79 0.029	0.122	0.83 0.014	0.8 0.018	0.79 0.018	0.122
Evenness	Mean se	0.94 0.004	0.93 0.01	0.94 0.006	0.226	0.94 0.004	0.93 0.01	0.93 0.006	0.292

Community Composition

A three-dimensional NMS ordination explained 86% of the variation with axis 1 and axis 2 explaining 58.5% and 17.7%, respectively (Figure 2.7). The ordination had a final stress of 12.6 and a final instability of 0.000. There were neither plots nor species that were considered outliers. Significant differences among treatments were detected with multi-response permutation procedures (MRPP) (p = 0.03). Pairwise comparisons observed significant differences between the control and the high biochar treatments (p =

0.01) which is also observed within the graphed ordination (Figure 2.7). No other treatment combinations were significantly different.

Multiple species were found to be correlated with the ordination axes (Table 2.4). Raspberry species (*Rubus idaeus & Rubus sp.*, r2 = 0.589 & 0.489 respectively) and low bush blueberry (*Vaccinium angustifolium,* r2 = 0.565) were strongly correlated with axis 1. On axis 2, bracken fern (*Pteridium aquilinum*) displayed the strongest correlation out of any species in the ordination (r2 = 0.722).

Table 2.4: R-squared values for each axis of the NMDS ordination (Axis 1, Axis 2, and Axis 3) of strongest correlated species. R-squared values above 0.2 indicate a strong relationship between the species abundance and the respective ordination axis, and are bolded in this table.

Species	Axis 1 R^2	Axis 2 R^2	Axis 3 R^2
Achillea millefolium	0.019	**0.209**	0.013
Anemone quinquefolia	**0.217**	0.025	0.183
Corylus cornuta	0.009	**0.218**	0.115
Eurybia macrophylla	0.051	**0.228**	**0.235**
Lamiaceae sp.	**0.2**	0.073	0.001
Maianthemum canadense	**0.213**	0	0.166
Populus balsamifera	0.092	0.125	**0.209**
Prunus pensylvanica	**0.341**	0.057	0.144
Pteridium aquilinum	0.002	**0.722**	0.024
Rubus idaeus	**0.589**	0	0.105
Rubus sp.	**0.489**	**0.256**	0.029
Vaccinium angustifolium	**0.565**	0.016	0.011
Vaccinium myrlitoides	**0.232**	**0.234**	0.001

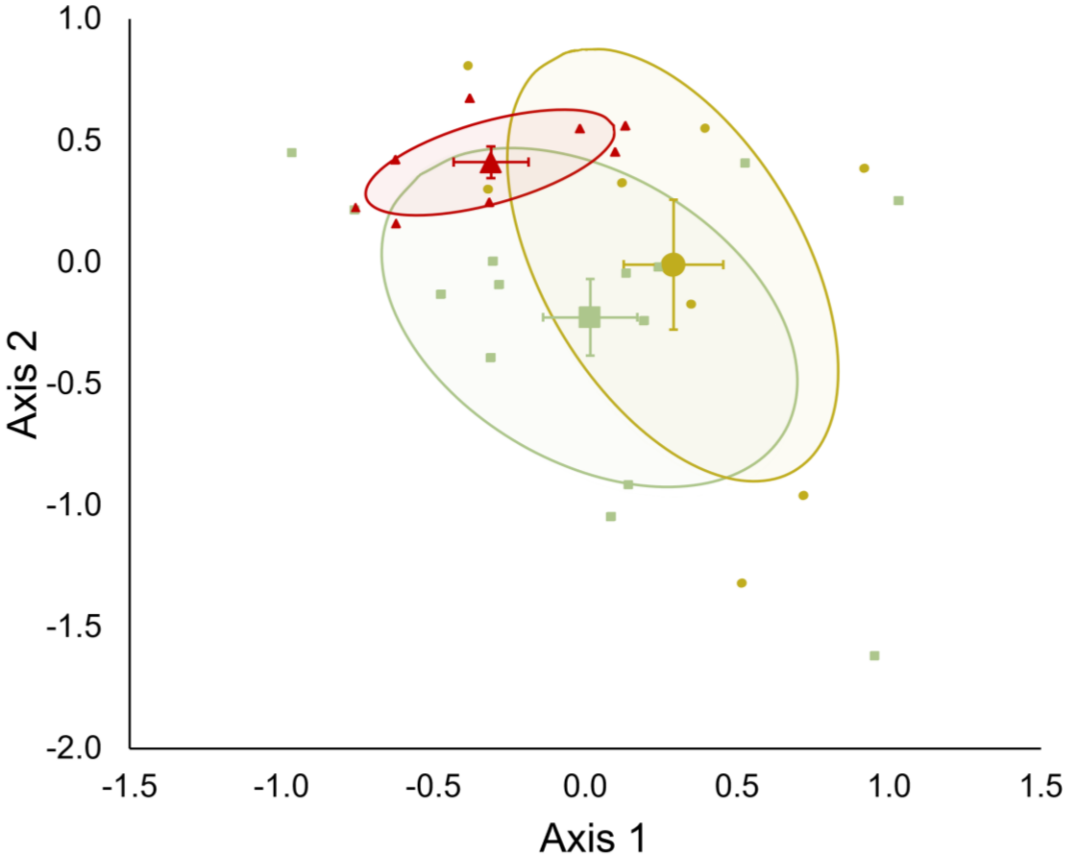


Figure 2.7: Non-metric multidimensional scaling (NMS) ordination displaying the relationship among plots across sampling time period ordination space. Axis 1 and Axis 2 are displayed as they explained the greatest amount of variation, 58.5% and 17.7%, respectfully. Individual treatment values within ordination space were averaged with associated standard errors for each axis graphed.

Discussion

Species trends in percent cover and abundance

This study aimed to investigate the impacts of biochar addition on understory vegetation following clearcut harvesting in 2019. Throughout the two-year study, distinct patterns emerged in the associations between species and treatment types, likely influenced by individual species' ecological characteristics, particularly their responses to disturbance. The varying relationships with fire and disturbance regimes offer insights into potential successional trajectories and management strategies for forested ecosystems post-disturbance. Environmental attributes within a stand, including natural disturbance history, function as environmental filters. When altered by human-driven activities such as silvicultural treatments, these conditions can lead to significant differences in ecological function and species composition, as observed in our study.

Fire- and disturbance-adapted species were prominent in the regenerating site. In the context of fire ecology, several species exhibit distinct adaptations to fire. These adaptations may enable these species to survive and persist following the disturbances, which often grants a competitive edge during the reestablishment process. Tree species *Populus balsamifera*, *Populus grandidentata* (bigtooth aspen), and *Populus tremuloides* were all found within the plots. These early successional species are often prevalent in fire-disturbed or otherwise recently disturbed areas, with their role in fire-adapted communities well-noted (DeByle 1985). Fast growth and prolific root sucker regeneration are key traits for the aspens, enabling them to rapidly regenerate within recently burned or disturbed areas. *Prunus pensylvanica* is notable for its capability to establish rapidly following fire disturbance, so much so that it is commonly called fire cherry. Its ability to resprout from the root collar when above-ground stems are damaged ensures successful regeneration in post-fire environments (Marks 1974). However, other known fire adapted species displayed variable responses to biochar amendment. *Corylus cornuta*, another species highly affiliated with these fire dependent ecosystems, exhibited increased frequency but varied in its cover response, ultimately not exceeding that of the control plots. *Vaccinium angustifolium* thrives in post-fire environments, with fire acting as a mechanism to reduce the competitive overstory and recycle nutrients, ensuring its continued prevalence in these habitats (Vander Kloet & Hall 1981). However, the species declined in both frequency and cover under biochar treatments, signifying an adverse effect or competition dynamics unfavorable to its growth in amended soils.

Rubus idaeus and *Rubus allegheniensis*, both shrubs thriving in disturbed areas, presented interesting responses. *R. idaeus* slightly increased in frequency with biochar treatment, indicating a possible preference or resilience to biochar conditions. However, its cover declined under low biochar treatment but increased in high biochar conditions, suggesting nuanced interactions with biochar amendments that could relate to its disturbance-adapted traits. This species is highly represented in recently disturbed areas in the GLR, and is particularly adept at dominating disturbed sites within forested ecosystems. Following a disturbance such as fire or logging, *R. idaeus* quickly resprouts from buds located on the root crown, effectively allowing it to become one of the first species to establish in these settings (DeGraaf & Yamasaki 2001). This species thrives in the increased light availability and reduced competition that disturbance events create, establishing extensive patches via clonal growth (Whitney 1986). In addition, studies have shown that *R. idaeus* exhibits high seed longevity, with seed banks remaining viable under several inches of duff that is consumed during intense fires. Post-disturbance, these seeds get exposed to light and the favorable conditions stimulate germination, further enhancing the species' rapid establishment within disturbed areas (Johnston & Woodward 1985; Lieffers & Stadt 1994). *R. allegheniensis* experienced decreased frequency but increased percent cover in high biochar treatments, possibly indicating a competitive advantage in denser stand formation post-disturbance with high biochar levels.

Cirsium arvense and *Conyza canadensis*, species known for their ability to rapidly establish following disturbances, showed similar responses to biochar amendments. *C. arvense* displayed a statistically significant affinity for the higher dosage of biochar, while *C. canadensis* flourished in the high treatment plots, nearly doubling in frequency from control to high treatment and also showing sevenfold increase in cover, which underscores its flexible adaptation to soil amendments likely paralleling its disturbance resilience. These species both tend to dominate in fields and roadsides post-disturbance, and often the presence of *C. arvense* is indicative of recent soil disruption (Allen & Hansen 1999). *Taraxacum officinale* and *Fragaria vesca* are known to exploit niches in forest edges and meadows, exhibiting distributive reproductive strategies, seen in their frequencies and covers, increased in frequency with higher rate of biochar. However, *F.*

vesca, while known for opportunistic establishment post-disturbance, experienced a reduction in individual plant coverage despite its increased presence, whereas *T. officinale* showed proportional increases in both presence and density with higher biochar levels, underlining the distinctive interactive effects of biochar on these species.

However, not all species identified throughout the study were affiliated with frequent disturbances, and made up a greater percentage of the cover within the control plots. *Anemone quinquefolia* is a notable understory species, indicative of long-term forest stability (Zenner and Berger 2008). *Maianthemum canadense* echoes this trend, typically found under a forest canopy that buffers it from extremes in moisture and temperature variability (Cain 1950). *Trientalis borealis* and *Viola* sp. fall on the same spectrum, as these species flourish in microenvironments offset from the influences of fire and substantial physical disturbance, thriving in areas with consistent ecological conditions (Braun 1950). These species collectively underscore the diversification present in a stable mesic forest microhabitat, often absent in stages dominated by disturbance-affiliated counterparts.

Previous field studies examining the impact of biochar on plant community composition have been limited, and their findings have been inconsistent. For instance, Rhoades et al. (2017) conducted a study in a lodgepole pine forest in Colorado and reported no noticeable effects of biochar addition on total forb cover or the cover of fireweed (*Chamerion angustifolium*), a prolific early pioneer species within recently burned areas.

In contrast, Bieser et al. (2023) conducted a field study in Ontario and found that biochar significantly increased the cover of opportunistic/disturbance favoring species *Solidago canadensis* (Canada goldenrod) and *Vicia cracca* (Tufted Vetch) compared to the control over a three-year period. Additionally, they observed a significant increase in the cover of the ruderal species *Rubus ideaus* with biochar application. The authors concluded that biochar may foster a microenvironment favorable for disturbance-adaptive species through the alteration of soil properties, thus giving these disturbance adapted species a competitive edge. In addition, Bieser et al. (2023) also evaluated a high carbon wood ash amendment in their experiment, and found that it decreased the cover of *Symphyotrichum cordifolium* (Blue wood-aster) and increased the cover of *Ranunculus acris* (Meadow buttercup) compared to both the biochar and control treatments, parallelling the trends seen among many of the forb species identified in our study.

One striking consistency with prior research is the observed increase in disturbance-adapted species like *R. idaeus* and *Conyza canadensis*, similar to findings Bieser et al. (2023) noted in their study, where biochar significantly increased the coverage of species with opportunistic ecological strategies. However, the study's findings also noted some deviations from established expectations, chiefly the adverse impact on *Vaccinium angustifolium*. This species, hailed for its ability to flourish in post-fire environments, surprisingly declined in frequency and cover under biochar treatments. Such observation contradicts some expectations around biochar's role in fostering post-fire vegetation recovery. For instance, Biederman and Harpole's biochar meta-analysis in 2013 reported increased survival and growth rates among fire-affected species after biochar application. The noted decline of *Vaccinium angustifolium* in this study might be attributed to competitive dynamics induced by biochar that are unfavorable to its growth or certain bio-physiological factors. Thomas and Gale's meta-analysis in 2015 hinted that biochar's effects on vegetation might be species-specific and could be mediated by changes in soil properties and microbial communities exclusive to certain species. Therefore, while *Rubus idaeus* and *Conyza canadensis* might prosper under biochar treatment, *Vaccinium angustifolium* may not experience the same favorable conditions, leading to its decline in both frequency and cover. In conclusion, the exploration of biochar's influences on vegetation reveals a complex network of reactions that vary depending on the specific ecological traits of different species, as well as the characteristics of the biochar applied. This knowledge further reinforces the need for a more nuanced understanding of biochar's multi-faceted effects on vegetation, particularly in post-disturbance environments.

Diversity indices

Our study revealed a negative correlation between the dosage of biochar amendment and both species richness and Shannon's diversity index. Habitats rich in diversity often feature a spectrum of environmental conditions that support a multitude of ecological niches. Species typically gravitate to niches along this spectrum that best suit their survival strategies, enabling them to outcompete others within the same context. Fundamental soil characteristics—such as nutrient availability, moisture content, and pH—directly influence plant diversity. Our findings suggest that biochar amendments alter these soil properties, thus shifting the habitat's range of ecological niches and, consequently, affect plant establishment, growth, and species interactions like competition.

Despite these alterations in habitat characteristics, species evenness showed surprising stability across the gradient of biochar treatment levels. This stability indicates that while biochar amendments may have affected the community’s species composition and richness overall, they did not disproportionately advantage or disadvantage individual species regarding their relative abundance within the community population.

The discrepancy in the ANOVA results between Simpson's Diversity Index (SDI) and Shannon's Diversity Index (SHI) despite similar levels of evenness within and between treatment groups warrants careful consideration of the underlying mechanisms driving community dynamics. While both indices aim to quantify the diversity of species within a community, they emphasize different aspects of diversity. Simpson's Diversity Index primarily reflects the dominance of species within a community, while Shannon's Diversity Index considers both species richness and evenness. These differences in emphasis can lead to variations in the detection of treatment effects and the significance of ANOVA results. The sensitivity of Simpson's Diversity Index to changes in the dominance structure of a community makes it particularly responsive to alterations in the abundance of dominant species, such as the variability displayed within multiple species in our study. Conversely, Shannon's Diversity Index, with its incorporation of an evenness component, is more sensitive to shifts in the distribution of species abundances across the community. As a result, treatment effects that primarily influence the dominance structure of the community may be more readily detected by Simpson's Diversity Index, leading to significant ANOVA results, whereas treatment effects that impact species evenness without altering species richness may be more apparent in Shannon's Diversity Index.

In the context of this study, where evenness was similar across treatments and within each year sampled, the significant findings for SHI but not for SDI in 2022 may suggest that treatment effects primarily influenced species richness rather than the dominance structure of the community. SHI, which accounts for both species richness and evenness, may have detected significant differences in response to treatments due to variations in the number of species present in each treatment group. In contrast, SDI, which is more sensitive to changes in species dominance, may not have detected significant treatment effects if alterations in species abundances did not substantially impact the dominance hierarchy within the community.

Overall, these findings highlight the importance of considering the specific attributes and sensitivities of different diversity indices when interpreting ecological data. While SHI and SDI provide valuable insights into community diversity, their distinct emphases on species richness and dominance, respectively, underscore the need for a multifaceted approach to community ecology that integrates multiple diversity metrics and considers the broader ecological context in which communities are situated.

Community response to biochar soil amendments

The non-metric multidimensional scaling (NMS) ordination in this study, which accounted for a significant portion of the variation (86%), has nuanced implications for understanding species responses to biochar treatment. Both *Rubus idaeus, Rubus sp*. and *Vaccinium angustifolium* are associated with axis 1, and were found to have positive correlations, suggesting these species might thrive or prefer conditions promoted by biochar application.

However, the disparity in responses among these species, especially the decline of *Vaccinium angustifolium* in response to biochar treatments, underscores the complexity hidden within these data. Species-specific effects appear to interplay with biochar application, with certain species (*Rubus* spp.) showing resilience or even a preference for such conditions, while others (*Vaccinium angustifolium*) present an adverse relationship. This further suggests that in the context of sites and ecosystems similar to ours, biochar might foster a microenvironment that benefits disturbance-adapted species; the disturbance resilience of these species gains them a competitive edge against other flora impacted negatively by the application of biochar.

Axis 2, showing a strong correlation with *Pteridium aquilinum* (bracken fern), also presents significant implications. Despite no significant variability of this species cover or frequency in response to biochar amendments in the current study, its strong correlation flags up a potential avenue for future study. If *Pteridium aquilinum* behavior also mimics disturbance-resilient species in response to biochar, it could signal broader trends of biochar application stimulating the resurgence of disturbance-associated species within the community, possibly acting on a different temporal scale than the species currently displaying differing responses to treatments.

The multi-response permutation procedures revealed significant differences among treatment types, providing a robust statistical basis supporting the differences in vegetative communities' response to biochar amendment shown by the graphed ordination, specifically the differentiation that occurs in response to biochar applied at the high rate used in this study. This again affirms that varied responses to biochar amendment exist among species, but also for communities, and further prompts a comprehensive understanding and more in-depth research focusing on the underlying mechanisms of such variation.

Overall, these ordination results furnish valuable insight into the complex interplay between species and biochar application, and suggest that biochar amendment may foster a microenvironment that influences species' competitive strategies by altering soil properties. Consequently, disturbance-adapted species such as *R. idaeus* and *Conyza canadensis*, which were observed to thrive under biochar treatment, might gain an edge over species like *Vaccinium angustifolium*. The outcome bring to light a complex network of interactions and the subtlety necessary when examining biochar effects on vegetation, calling for further research to enhance understanding in this area.

Conclusions and Management Implications

Given the substantial impact that understory layers exert on the composition and growth of tree regeneration, resource managers are becoming increasingly interested in the assessment of conventional and innovative site preparation methods and their ability to alter competitive conditions during regeneration efforts. Understanding the specific influences of site preparation treatments on the understory vegetative community response in a broad spectrum of conditions and contexts will ensure that managers will be able to tailor their treatments to the individual sites in which they are working, and create favorable regeneration conditions despite increasingly unpredictable or unfavorable climatic conditions (Damato et al. 2011; Ashton & Kelty 2018, Zhang et al. 2018).

A comparison with findings from similar studies reveals both consistency and deviation from our analysis of biochar's effect on vegetation. The increase in disturbance-adapted species like *Rubus idaeus* and *Conyza canadensis* aligns with Bieser et al. (2023), highlighting biochar’s potential to favor species with certain ecological traits. However, the adverse impact observed on *Vaccinium angustifolium* contrasts with expectations around biochar's benefits to post-fire vegetation recovery. Notably, the findings underscore the critical need to consider species-specific responses when understanding the implications of biochar amendments, as evidenced by the varied responses in plant species percent cover and diversity.

The shifts observed in vegetation response to biochar amendments are likely driven by a variety of factors, reflecting the complexity of interactions between plants and their environment. One potential driver is the influence of biochar on soil microbial communities and mycorrhizal interactions. Biochar can alter soil microbial populations, which in turn may affect nutrient cycling and availability, thereby impacting plant growth and composition. Additionally, changes in mycorrhizal associations, which play a crucial role in nutrient uptake and plant health, could influence plant responses to biochar. Another factor to consider is the possibility of allelopathic effects, where biochar compounds leach into the soil and affect the growth of neighboring plants. Furthermore, direct physical effects of biochar, such as altering soil structure and water retention, could affect growing space availability and soil surface temperature, influencing plant establishment and competition. Understanding the interplay of these factors is essential for unraveling the mechanisms behind the observed shifts in vegetation dynamics in response to biochar amendments, and must be considered holistically in the context of specific ecosystems. Any of these factors could differentially benefit certain plant species over others, influencing competitive dynamics and succession trajectories post-disturbance. Furthermore, the negative correlation between biochar dosage and species richness, coupled with stable species evenness, underscores the complex interactions between soil properties and species composition. The NMDS ordination reveals distinct species responses to biochar treatment, emphasizing the need for further research to elucidate underlying mechanisms. Overall, our findings contribute to a more comprehensive understanding of biochar's role in shaping post-disturbance vegetation recovery and ecosystem resilience, highlighting the importance of considering species-specific responses and ecosystem complexities in ecosystem management strategies.

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Appendix

A.2.1 Mean species percent cover (and standard error) over 2022 and 2023 sampling periods for all species by treatment group, as well as frequency of species occurrence within plots. ANOVA was conducted to assess differences in treatment group using plot means for each sample year (Control n = 28, Low n = 14, High n = 14). F statistic and p value reported, with significant p values (>0.1) bolded.

	Control			Low			High			ANOVA	
		% CC			% CC			% CC			
species	Freq.	mean	(se)	Freq.	mean	(se)	Freq.	mean	(se)	*F*	p value
Acer rubrum	36%	4.39%	(34%)	25%	0.01	(46%)	16%	1.20%	(43%)	2.19	0.131
Achillea millefolium	0%	0%	—	6%	0.06%	(67%)	0%	0%	—	3.3	**0.052**
Amelanchier sp.	5%	0.25%	(64%)	6%	0.41%	(61%)	0%	0%	—	1.08	0.353
Anemone quinquefolia	38%	0.57%	(46%)	25%	0.42%	(50%)	19%	0.36%	(69%)	0.19	0.83
Apocynum androsaemifolium	2%	0.02%	(100%)	3%	0.03%	(100%)	0%	0%	—	0.47	0.629
Aralia nudicaulis	9%	0.29%	(79%)	6%	0.06%	(67%)	3%	0.03%	(100%)	0.59	0.563
Arctostaphylos uva-ursi	0%	0%	—	3%	0.03%	(100%)	0%	0%	—	1.41	0.261
Asarum canadense	2%	0.12%	(100%)	0%	0%	—	0%	0%	—	0.55	0.581
Asteraceae sp.	11%	0.30%	(57%)	6%	0.23%	(83%)	6%	0.06%	(67%)	0.53	0.593
Betula papyrifera	4%	0.13%	(92%)	0%	0%	—	0%	0%	—	0.74	0.488
Calystegia spithamaea	7%	0.17%	(76%)	6%	0.23%	(83%)	6%	0.06%	(67%)	0.29	0.748
Cirsium arvense	0%	0%	—	3%	0.03%	(100%)	9%	0.44%	(61%)	3.09	**0.062**
Cirsium sp.	2%	0.02%	(100%)	6%	0.23%	(83%)	13%	0.47%	(55%)	2.09	0.143
Clintonia borealis	2%	0.02%	(100%)	0%	0%	—	0%	0%	—	0.55	0.581
Corylus cornuta	41%	5.55%	(32%)	56%	9.80%	(27%)	69%	6.81%	(20%)	1.11	0.344
Conyza canadensis	20%	0.29%	(48%)	22%	1.27%	(86%)	53%	2.09%	(37%)	1.96	0.16
Corylus americana	7%	0.57%	(79%)	0%	0%	—	0%	0%	—	0.9	0.417
Comptonia peregrina	34%	3.49%	(29%)	13%	1%	(72%)	31%	3.39%	(58%)	1.03	0.371
Cystopteris sp.	2%	0.02%	(100%)	0%	0%	—	0%	0%	—	0.55	0.581
Diervilla lonicera	4%	0.13%	(100%)	0%	0%	—	0%	0%	—	0.55	0.581
Eurybia macrophylla	25%	1.45%	(52%)	28%	1.48%	(43%)	38%	1.94%	(39%)	0.11	0.897
Fabaceae sp.	0%	0%	—	3%	0.03%	(100%)	0%	0%	—	1.41	0.261
Fragaria vesca	23%	1.53%	(29%)	16%	1.20%	(43%)	22%	0.73%	(60%)	0.65	0.529
Frangula alnus	2%	0.02%	(100%)	0%	0%	—	0%	0%	—	0.55	0.581
Galium sp.	5%	0.05%	(80%)	0%	0%	—	0%	0%	—	1.06	0.36
Galium triflorum	4%	0.04%	(50%)	0%	0%	—	0%	0%	—	1.2	0.317
Graminoids	98%	37.50%	(8%)	97%	49.67%	(13%)	94%	40.20%	(15%)	1.57	0.226

Hieracium sp.	4%	0.04% (50%)	0%	0% —	0%	0% —	1.2	0.317
Hylotelephium sp.	4%	0.04% (100%)	0%	0% —	0%	0% —	0.55	0.581
Lactuca canadensis	0%	0% —	3%	0.20% (95%)	0%	0% —	1.41	0.261
Lactuca sp.	5%	0.05% (60%)	0%	0% —	0%	0% —	1.96	0.16
Lamiaceae sp.	2%	0.02% (100%)	0%	0% —	0%	0% —	0.55	0.581
Lonicera canadensis	2%	0.12% (100%)	0%	0% —	0%	0% —	0.55	0.581
Lonicera dioica	4%	0.34% (94%)	0%	0% —	0%	0% —	0.62	0.545
Lonicera sp.	0%	0% —	3%	0.03% (100%)	0%	0% —	1.41	0.261
Maianthemum canadense	38%	0.77% (34%)	9%	0.09% (44%)	25%	0.25% (36%)	2.87	**0.074**
Pinus resinosa	4%	0.23% (70%)	0%	0% —	0%	0% —	1.2	0.317
Populus balsamifera	2%	0.12% (100%)	0%	0% —	0%	0% —	0.55	0.581
Polygonum cilinode	5%	0.25% (64%)	6%	0.23% (83%)	0%	0% —	0.7	0.503
Populus grandidentata	7%	0.07% (57%)	0%	0% —	3%	0.03% (100%)	1.02	0.373
Polygala paucifolia	4%	0.04% (50%)	9%	1.78% (67%)	9%	0.44% (61%)	2.26	0.124
Populus tremuloides	38%	1.66% (24%)	16%	1.02% (39%)	9%	0.44% (55%)	2.47	0.104
Prenanthes alba	0%	0% —	3%	0.03% (100%)	0%	0% —	1.41	0.261
Prunus pensylvanica	34%	3.15% (35%)	34%	1.55% (19%)	50%	4.19% (29%)	1.19	0.319
Prunus sp.	5%	0.15% (80%)	0%	0% —	3%	0.03% (100%)	0.73	0.49
Pteridium aquilinum	54%	9.91% (17%)	63%	12.41% (22%)	56%	9.41% (33%)	0.36	0.7
Pyrolaceae sp.	2%	0.02% (100%)	0%	0% —	3%	0.20% (95%)	1.24	0.306
Rubus ablatus	2%	0.02% (100%)	0%	0% —	0%	0% —	0.55	0.581
Rubus allegheniensis	9%	0.38% (47%)	13%	0.30% (70%)	13%	1.53% (61%)	1.7	0.202
Rubus idaeus	70%	14.33% (26%)	72%	7.77% (27%)	72%	15.08% (20%)	1.13	0.339
Rubus sp.	20%	2.36% (50%)	28%	3.55% (61%)	0%	0% —	1.28	0.294
Salicaceae sp.	14%	1.04% (38%)	0%	0% —	0%	0% —	3.97	**0.031**
Salix Humilis	2%	0.12% (100%)	0%	0% —	0%	0% —	0.55	0.581
Salix interior	2%	0.12% (100%)	0%	0% —	0%	0% —	0.55	0.581
Salix bebbiana	2%	0.12% (100%)	0%	0% —	0%	0% —	0.55	0.581
Streptopus sp.	0%	0% —	0%	0% —	3%	0.03% (100%)	1.41	0.261
Symphyotrichum lanceolatum	2%	0.12% (100%)	0%	0% —	0%	0% —	0.55	0.581
Taraxacum officinale	2%	0.02% (100%)	6%	0.06% (67%)	3%	0.20% (95%)	0.97	0.39
Taraxacum sp.	2%	0.02% (100%)	0%	0% —	0%	0% —	0.55	0.581
Trientalis borealis	48%	0.58% (24%)	16%	0.16% (38%)	13%	0.13% (31%)	4.99	**0.014**
Vaccinium angustifolium	41%	2.71% (32%)	34%	2.42% (28%)	13%	0.47% (62%)	2.08	0.145
Vaccinium myrlitoides	2%	0.02% (100%)	3%	0.03% (100%)	0%	0% —	0.47	0.629
Viola sp.	80%	3.46% (14%)	63%	1.48% (24%)	44%	0.61% (34%)	12.43	**1.49E-04**