EFFECTS OF PRESCRIBED FIRE ON OAK REGENERATION, MIDSTORY RETENTION, AND MYCORRHIZAL SYMBIOSIS IN THE CENTRAL HARDWOOD REGION

by

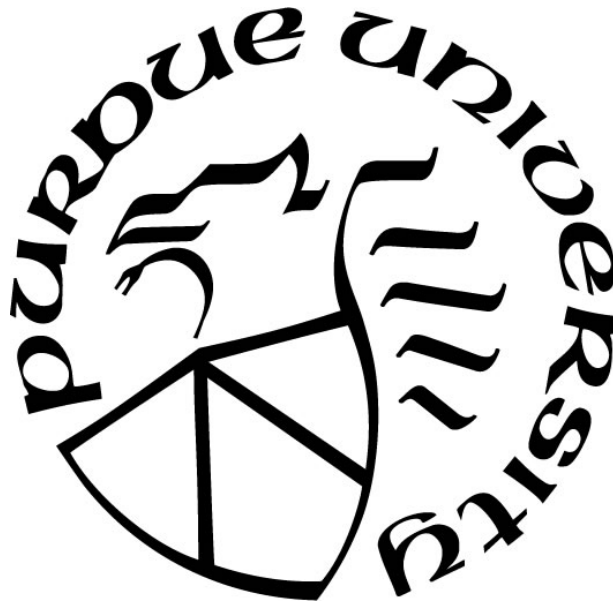**Sarah A. Cuprewich**

A Thesis

Submitted to the Faculty of Purdue University
In Partial Fulfillment of the Requirements for the degree of

Master of Science

Department of Forestry and Natural Resources

West Lafayette, Indiana

December 2021

THE PURDUE UNIVERSITY GRADUATE SCHOOL
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To my parents, Georgette and Paul, who have given me endless love and support

ACKNOWLEDGMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Oak (*Quercus*) is being replaced by maple (*Acer*) and other mesic species across the Central Hardwood Region (CHR) and other eastern deciduous forests. This replacement is due to a self-reinforcing process called mesophication, in which the environment becomes increasingly mesic and hinders both oak retention and the disturbances which aid in the maturity of an oak-dominated forest. Within the last couple decades, forest managers have been prescribing surface fire in an attempt to mimic the historic disturbance regime that likely created these forests centuries ago. There is limited guidance on how many fires are needed and the temporal frequency with which they should be applied, leaving room for confusion and potentially detrimental burn practices. Fire is used for both seedling establishment and midstory tree retention, which may be conflicting goals with how surface fire is usually carried across the CHR landscape. Even less is known about the effects of prescribed surface fire on mycorrhizal communities and their dispersal agents, which are necessary parts of the oak ecosystem and enhance seedling establishment and maturity through their symbiosis. Fungal spore dispersal by small mammal is an important step in recovery after a disturbance such as fire, but the fires themselves may be endangering this relationship.

To better understand the relationship between immature trees, prescribed fire, topography, and stand structure, I surveyed 63 stands both with (n = 47) and without (n = 16) a known prescribed fire since 1990 in the Hoosier (IN) and Wayne (OH) National Forests. Here I quantified tree seedlings (height > 15 cm, DBH < 10 cm) and midstory saplings and poles (10 cm < DBH < 25 cm) in two concurrent studies. Across all sites, white oak (section *Quercus*) comprised 47.8% of all seedlings, red oak (section *Lobatae*) comprised 8.2%, and the common interfering species maple and American beech (*Fagus grandifolia*) comprised 5.4%. Mid- and overstory basal area, percent slope, years since the last burn, and total number of burns all significantly affected oak seedling density, but site characteristics like slope and midstory basal area were just as important of predictors as number of burns. However, all oak and hickory (*Carya*) midstory trees averaged 0.65 ± 0.003 m^2 ha^{-1} basal area of stocking, while sugar maple (*A. saccharum*) midstory averaged 0.87 ± 0.001 m^2 ha^{-1} basal area. Furthermore, overstory stand structure variables were often more important than burn variables when predicting midstory basal area and composition. Prescribed fire does favor oak and hickory over mesophytic species, but mesophication may be preventing

fire alone from significantly reducing midstory maple. Likewise, prescribed fire may condition a site for regeneration of oak seedlings, but if an established midstory already exists or fire has not been used on the landscape for almost a decade, repeated applications of prescribed fire will likely have a negligible effect on oak regeneration unless the site is burned more than three times with less than four years between burns.

Finally, I extracted fungal spores from eastern chipmunk (*Tamias striatus*) scat collected across the Hardwood Ecosystem Experiment from plots in oak-dominated stands without a recent burn history (n = 33 trap stations) and with one burn since 2015 (n = 32 trap stations). I identified 17 fungal taxa in 64 samples; 70.6% of taxa were found in at least 10% of all samples. Arbuscular mycorrhizal taxa richness was not affected by any measured variable, but ectomycorrhizal taxa richness decreased after burning. However, environmental variables like leaf layer depth, EM tree and AM tree basal areas each helped explain some variation in spore composition and were correlated with fungal community composition in scat. Therefore, prescribed surface fire appears to differentially affect the truffles of mycorrhizal functional groups, but due to the nature of this pilot study, additional research is needed to more closely examine the relationship between mycorrhizal fungi, their dispersal agents, and prescribed fire.

CHAPTER 1.   INTRODUCTION

1.1   History of Oak and Prescribed Fire in the Central Hardwood Region

Oak (*Quercus* spp.) are distributed across almost 600 species found mostly in the northern hemisphere (Little 1979). Over 50 species are native to North America east of the 100th meridian, dominating over 191 million acres of hardwood forest (Stein et al. 2001). They are the United States' largest tree genus, are genetically diverse, and hold significant ecological and economic value to those in contact with them (Smith 1993). Since the Holocene, oak have been especially prevalent in the temperate deciduous forests of the eastern part of the country known as the Central Hardwood Region (CHR; Shumway et al. 2001). They have even been declared by Congress as America's national tree for their beauty on a landscape and production of high-quality wood. Oak is also a valuable crop, for in as little as three decades it produces both higher- quality timber and a larger volume of growth than almost any other hardwood species found in the U.S. (Clark 1993).

The importance of oak is not purely symbolic or economic, however. The oak bark is unique with its rough texture, providing a home to insects and therefore giving a habitat to birds close to their preferred food (Brose et al. 2014). They are long-lived and produce acorns, a nutritious food source, for many of those years. Over 100 forest vertebrates regularly consume acorns. In some areas of the country the acorn crop is so important that it can even have a direct influence on white-tailed deer reproductive rates and antler characteristics (Wentworth et al. 1992). Oak also has a special connection with the people of the Midwest. Since the 1930s, this region has been used as oak nursery grounds, exporting the wood to other parts of the country, and creating a sense of pride for the citizens of the CHR (Clark 1993).

Many CHR forests are dominated by oak in the overstory, but have little understory oak saplings and regeneration. On many sites, acorn germination is not failing, but rather mortality between seedling and maturity is up to 90 percent, especially on the more mesic sites (Lorimer 1993). This, along with a lack of disturbance, creates a positive feedback loop known as mesophication, where the site continually shifts to more mesophilic species until more xeric species, like oak and hickory, disappear from the canopy (Nowacki and Abrams 2008). This relatively disturbance-free environment also creates the closed canopy that most CHR forests currently have, with relatively few gaps and without much light reaching the understory. Shade

intolerant species, like oak, simply cannot compete with the shade tolerant trees and they perish in these highly competitive conditions.

In the last few decades, more shade tolerant species, like maple (*Acer*) and beech (*Fagus*), have begun to dominate the understory by growing faster than oak and outcompeting them for light and, therefore, extraditing oak from growth (Smith 1993). Sugar maple is described as one of the most shade tolerant hardwood species, producing plentiful, wind-dispersed seeds ready to take advantage of any amount of light they receive (Abrell and Jackson 1977). This problem, first realized over 50 years ago (Boggess and Bailey 1964, Carvell and Tyron 1961, Clark 1993, Lafer and Wistendahl 1970), is not isolated to one region or forest structure. Rather, it is seen throughout oak-dominated forests of many sizes, successional stages, and levels of human contact (Aldrich et al. 2005, Lowney et al. 2015).

After European settlement, the CHR was plundered for its resources. Trees were removed in vast quantities to make room for towns, railroads, and coal furnaces associated with the charcoal iron industry (Albrecht and McCarthy 2006). After timber-dominant industries declined in the 20th century, what was left of the oak forests was mostly unmanaged except for occasional fire suppression (Brose et al. 2014). Oaks are also susceptible to arthropod and mammal herbivory, further harming their chance of natural regeneration (Kellner and Swihart 2017, Adams and Rieske 2001). Insects feed upon the leaves of both young and mature trees, and as many as 92% of fallen acorns can become completely infested with arthropods (Galford et al. 1991). Herbivory is dangerous to a tree of any age, but especially for immature saplings and seedlings that do not yet have a large store of carbon; plants utilize their carbon reserves when repairing herbivore damage and to produce substances to keep their predators away (Adams and Rieske 2001). White-tailed deer are especially detrimental to regenerating oak stands, as they regularly consume acorns and prefer oak seedlings over most other vegetation (Waller and Alverson 1997).

Researchers agree that one major change to the oak ecosystems across the country over the past century is the change in fire regime, and some claim that the oak-dominated forests of the recent past were only made possible through this disturbance pattern (Aldrich et al. 2005, Crow 1988, Lorimer 1993, Shumway et al. 2001). These occasional, low-intensity understory fires opened up the canopy by removing fire-sensitive vegetation and allowing light to reach the forest floor (Shumway et al. 2001). Periodic human-induced fire in North America has been part of the landscape since Native American tribes burned land for management, hunting, and other purposes

(Day 1953). Upon European settlement, the forests were described as being park-like without much undergrowth impeding foot travel (Greeley and Ashe 1907). Using mean fire intervals, or time between successive fires, researchers have determined that before European settlement, a fire burned on average once every 13 years. Upon settlement, that number increased to 7 years. With the advent of modern fire exclusion policy, fire return intervals have increased to every 46 years (Brose et al. 2014).

Oaks are not only adapted to surface fires, but also have a competitive advantage, especially in their youth, over other less fire-adapted species (Clatterbuck 1998). Most of their photosynthate is preferentially stored in the roots, rather than devoted to shoot growth, early in life so they are able to sprout more quickly from these carbohydrate reserves if they suffer aboveground damage in a fire (Lorimer 1993). A large number of dormant buds usually reside on the root collar, ready to flush if the main stem is destroyed (Larsen and Johnson 1998). The newly opened canopy space created by dead, non-fire adapted trees gives the shade intolerant oaks the light they need to make considerable advancements in their growth and development (Dolan and Parker 2004). Oak are comprised of about 5.0 – 6.5 percent bark tissue, while maple are only about 3.5 percent. This difference reinforces how fire resistance is directly correlated with tree size and bark thickness (Spalt and Reifsnyder 1962). If a mature oak does become injured in fire, their superior wound compartmentalization prevents rot and decay from spreading to other healthy tree regions (Smith and Sutherland 1999). Regardless of their injury mitigation techniques, oak saplings with a diameter at breast height of over ten centimeters generally have bark thick enough to protect against most surface flames (Brose et al. 2014).

However, the same qualities that give oak their fire resistance are part of the reason why maple have been so successful replacing them. The shade tolerant species have a lower light compensation point and saturation level, meaning they can achieve a higher level of photosynthesis with less sunlight and are hindered by constant direct light (Barton & Gleeson 1996). Maple generally have higher photosynthetic rates, a higher yield of light, and faster stomatal opening and closing as conditions change (Wuenscher and Kozlowski 1970). This in turn leads to higher carbon sequestration, faster growth, and therefore higher survival.

1.2 Summary of Mycorrhizal Fungi in relation to Oak and Fire

The greater ecosystem as a whole should be taken into consideration when looking for answers to a problem of this magnitude. Oaks live in obligate symbiosis with ectomycorrhizal (EM) fungi, so the presence of fungal associates is considered a necessary component of oak ecosystems (Frank et al. 2009, Morris et al. 2008, Walker et al. 2005). Although arbuscular mycorrhizae have associations with up to 200,000 plant species, they comprise one phylum and have less than 350 known species (van der Heijden et al. 2015). Ectomycorrhizal fungi, conversely, make associations with mostly woody species and comprise several phyla and over 200 genera (Tedersoo et al. 2010, Tedersoo et al. 2013). Arbuscular mycorrhizal fungi colonize cells directly (Marks and Foster 1973, Strullu-Derrien et al. 2017), while EM do not penetrate cell walls and instead form a hyphal covering ('Hartig net') on plant roots. Therefore, an EM tree could form associations with hundreds of fungal species, but some mycorrhizal taxa are only able to form symbioses with a single tree species (Courty et al. 2010). The high level of niche partitioning allows for possibly dozens of EM species to live within a few meters of each other without much direct competition of resources (Koide et al. 2011).

Mature trees with EM associations, like oak, have better seedling recruitment than AM trees, like maple; this is possibly due to the superior ability of EM fungi to transfer nutrients in a nutrient-starved environment and the protective properties of the Hartig net on fine roots (Bennett et al. 2017, Valverde-Barrantes et al. 2018). Trees invest more carbohydrates into EM relationships than AM relationships, so the quality of the relationship improves as a result and trees usually receive more water and nutrients from their EM symbionts in return.

Many of the mycorrhizal fungi in the Central Hardwood Region are hypogeous and form fruiting bodies containing their spores, commonly known as truffles, underground (Watson et al. 1990). Truffles are thought to have originally evolved to give the mycorrhizae a form of drought tolerance, and today truffles can be found and are eaten all across the globe (Frank et al. 2009). The famous Périgord black truffle (*Tuber melanosporum*), well known for its pleasing aroma and flavor, grows in association with oak trees mainly in Spain, Italy, and France (Büntgen et al. 2015).

Hypogeous fungi are consumed by animals (mycophagists), usually mammals, and the fungal spores are dispersed elsewhere. Mycophagy exists because of the relatively rare, when compared to seeds, vitamins and minerals obtained upon consumption (Fogel and Trappe 1978). Mycophagy is seasonal in many regions of the country, as EM fruit only during brief periods of

the year. So, many mycophagists are nearly exclusive fungi consumers during these periods. An Australian study found that a mycophagous marsupial favored fungal sporocarps over all other food sources, and only showed preference for the other foods when sporocarp production was low (Johnson 1994), however fungal sporocarps are often consumed year-round (North et al. 1997). Insectivorous, omnivorous, herbivorous, and carnivorous mammals have all been documented to consume fungi, but the most common mycophagists are rodents such as chipmunks and squirrels (Fogel and Trappe 1978). Because of the importance of fungi as a food source, it is currently recommended to take special care when burning areas of high truffle production value (Trappe et al. 2006).

Even though truffles are anywhere between 70 to 94 percent water by weight, squirrels have been known to dry out their truffle stash, suggesting that they utilize the fungus as more than just a supply of water and count on these fungi as a source of food (Fogel and Trappe 1978). Compared to the usual diet of squirrels, like nuts and other seeds, dried mushrooms are excellent sources of vitamins and minerals, and protein and carbohydrates to a lesser extent. Interestingly, hindgut fermentation, which many small mammals like the eastern chipmunk utilize, likely cannot extract most nutritional value from truffles, so consumption of hypogeous fruiting bodies may only contribute modestly to the nutrient load in the overall diet (Claridge et al. 1999).

The cell cytoplasm is easily digested, the cell walls are occasionally digested, but the spores are never fully digested which allows the consumer to become a dispersal agent for the fungus (Fogel and Trappe 1978). As the spores travel through the mycophagist, dormancy is usually broken before they are deposited in a new location. Due to the nature of fruiting underground, hypogeous fungi must use an external agent for spore dispersal. Fortunately, 75 percent of small mammal mycophagy occurs with mycorrhizal fungi (Fogel and Trappe 1978). Mature forests can contain a diverse array of fungi with various ecological functions, so the preference for consuming mycorrhizal fungi is especially interesting. There is evidence these small mycophagous mammals evolved to eat fungi, as there have been no reports of poisoning by mushrooms usually dangerous to humans (Fogel and Trappe 1978).

While not much is known about the fate of soil fungi after prescribed fires, it has been postulated that soil bacteria thrive in a post-fire environment while fungi suffer a decline, although it can be quite difficult to obtain accurate measures of soil microbial biomass (Vázquez et al. 1993). The real effect fire has on soil-dwelling microorganisms is held in the soil moisture content, as

that provides insulation to the organisms and sustains their populations in the harsh post-fire underground environment (Certini 2005). The post-fire soil dryness may, nevertheless, aid in the establishment of new ectomycorrhizal colonies due to the difficulty of hyphal strands travelling through waterlogged soil. Ectomycorrhizal root tips are more commonly found in upland, xeric sites, which highlights their need for at least some decreased level in soil moisture (Watson et al. 1990). Mycorrhizae also tend to favor more basic soil conditions, so the increase in soil pH after a fire may benefit their production (García-Montero et al. 2007). Furthermore, fungi may escape the disturbance, as it is well known that some plant seeds are stimulated to germinate by smoke or heat (Brown and van Staden 1997, Wicklow 1988), but fungi can utilize the same protective mechanism (Baar et al. 1999).

Most of the existing knowledge of effects of fire on truffle communities is from non-hardwood ecosystems, namely eucalyptus (Johnson 1994a, 1994b, 1995) and conifer (Baar et al. 1999, Cowan et al. 2016, Harvey et al. 1976, Makoto et al. 2010, Oliver et al. 2015) forests. Conifer forest soils are usually much more acidic, due to the input of pine needles (Hızal et al. 2013), and therefore support different microbial and fungal communities than broadleaf forests (Rasmussen et al. 2007). Microbial communities in hardwood forests may have differential survival after a fire, as some taxa may be more adapted to extreme environments than others, so when comparing across forests the forest types should be as similar as possible as to not confound results in this manner. The difference in leaf morphology between forests types directly impacts fuel load, which helps to characterize the fire. Pine needles are more flammable than broad leaves, and can increase the duration and intensity of a fire (Ellair and Platt 2013).

Research in conifer systems or greenhouse studies prove difficult to apply to the CHR. One study in northwestern Montana found that the soil organic layer actually contained no mycorrhizae, suggesting that if a prescribed fire were to burn through this region, the EM community would remain relatively unscathed (Harvey et al. 1976). In fact, Harvey et al. also found that charcoal was the most suitable substrate for EM colonization, suggesting that if fire does have any impact on the fungal community, it may be a positive one. However, DeBano (1990) asserted that EM most commonly live in the organic matter near the surface and would be consumed by the fire. A later greenhouse experiment confirmed that charcoal is a superior growth medium for mycorrhizal growth, and supports the greatest amount of aboveground biomass produced by the tree the fungi has an association with (Makoto et al. 2010). Charcoal benefits mycorrhizae in a multitude of ways:

increasing soil aeration with greater pore space, capturing toxic substances potentially released by fire, and absorbing and retaining water and nutrients for both fungal and plant use (Makoto et al. 2010, Warnock et al. 2007). Another study in the Pacific Northwest has proposed that prescribed fire alters only the first foot of soil, so if organisms are living past the first foot of topsoil they may remain unscathed by direct fire effects (Cowan et al. 2016).

It is difficult to determine what exactly occurs underground after a fire, and that is why researchers often use mycophagous mammals to gauge the fate of hypogeous fungi. So where then is the connection between small mammal mycophagy and fire? The results may be self-confounding. Does fire really induce an increase in hypogeous fungi production, or do mycophagous mammals simply feed more often in burned sites due to the lack of excess vegetation limiting their access? The animals may also search for and consume more fungi after a fire because the fire may have removed other food sources. Even if the mycophagists migrate away from the fire site in search for a better habitat to make a home, they often still return to the burn site to forage for truffles, occasionally directly following the extinguishing of flames, suggesting that this may be the best or only food source nearby (Johnson 1995). Mycorrhizal fungi in a boreal Alaskan forest were found to significantly decline in richness as available nitrogen increased in the soil (Lilleskov et al. 2002). This could suggest that fungal richness may in fact increase after a fire due to the immobilization of available soil nitrogen. Researchers also stress being familiar with the primary spore dispersal method of local EM species, so the effects of disturbance can be more efficiently studied and succession can be aided when necessary (Cowan et al. 2016).

1.3   Direct and Indirect Effects of Prescribed Fire in the Central Hardwood Region

Many processes may take place in the soil as it is heated by burning vegetation, depending on the duration and intensity of the fire. In turn, duration and intensity are controlled by interactions between fuel load (the combination of aboveground biomass and dead organic material), fuel type, fuel moisture, local weather, and the surrounding topography (Boerner 1982, Cairney and Bastias 2007). Because of the many factors that determine fire characteristics, there can be significant local variation in intensity for a single fire (Boerner 1982). These local differences can make average temperatures, of either air or soil, unable to describe what really occurred during the fire. There can be many direct and indirect effects of fire on the edaphic environment. Examples of direct effects include loss of vegetation, deposition of charcoal and ash, combustion of the organic layer

and therefore loss of nitrogen and carbon to the atmosphere, and increased soil temperature (Cairney and Bastias 2007, Monleon and Cromack 1996, Neary et al. 1999). Indirect effects include changed soil pH and moisture, increased erosion, and the physical and chemical effects of charcoal deposition (Amaranthus and Trappe 1993, Cairney and Bastias 2007, Neary et al. 1999). The effect of fire is more pronounced with a higher burning frequency, no matter how intense the burn.

Prescribed surface fire (hereafter referred to as prescribed fire) practitioners are usually aware of these variables, so they can account for them when planning their fire to have the optimal management result. Prescribed fires in a hardwood ecosystem very rarely reach over 1000º C at the surface and such high temperatures are usually localized to microsites on the landscape (e.g., consumed piles of dry coarse woody debris; Peter et al. 2011). More likely, the average surface temperature hovers around 150º C for hardwood ecosystems, with temperatures remaining above 30º C for only about 10 minutes (Iverson et al. 2004). Due to air mixing, temperatures rapidly cool as one moves away from the ground surface. At a height of 2.6 m, the surrounding temperature can be expected to only be around 100º C, a tenfold decrease from the soil surface temperature (Peter et al. 2011). Temperatures within the soil are usually more conserved than the air, due to the insulative properties of moist soil. Even at a depth of nearly a foot in dry conditions, the soil rarely exceeds 30º C and the top 15 cm is the only location with a real possibility of reaching temperatures at or above 100º C (Cowan et al. 2016).

The immediate physical fire effects are as follows: understory vegetation will suffer mortality, litter will be consumed, and the organic layer will be reduced (Ahlgren and Ahlgren 1960). The water-holding capacity of the soil decreases, while potential for damaging runoff and surface erosion increases along with the soil pH (but ash and charcoal can later neutralize the soil; Certini 2005). Higher-intensity fires can convert most organic nitrogen to inorganic forms, as ammonium is a product of combustion, while organic phosphorus is converted to orthophosphate, the only form of bioavailable phosphorus (Certini 2005). While the effects of fire on soil quality have been studied for decades (Lewis Jr. 1974, Boerner 1982, Certini 2005), the recorded impact on mycorrhizal communities has been varied. The one conclusion is that repeated burns do not only have an amplified impact on physical and chemical soil properties, but on soil fungal communities as well (Cairney and Bastias 2007).

1.4 Thesis Objectives

The primary objective of this thesis was to better understand the relationship between prescribed fire and oak-dominated forests in the Central Hardwood Region. I expanded upon studies centered on the effects of prescribed fire on overstory trees by instead focusing on seedling regeneration, midstory composition, and oak-chipmunk-fungi relationships. This holistic approach offers a clearer view of the direct and indirect ways in which oak regeneration may be impacted by use of prescribed fire. I first investigated if oak seedling regeneration may be benefitted by repeat prescribed burns in the Hoosier and Wayne National Forests. On those same sites, I then quantified midstory trees to see how prescribed fire is affecting non-merchantable timber species composition. Local topography and stand structure were each also considered for these two projects to ensure that prescribed fires are the event that is actually driving seedling and midstory compositional changes. Lastly, on sites throughout the Hardwood Ecosystem Experiment, I assessed how hypogeal fungal spore density and composition found in eastern chipmunk scat are affected by a single prescribed fire.

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CHAPTER 2.   OAK REGENERATION RESPONSE TO THE USE OF PRESCRIBED FIRE IN THE CENTRAL HARDWOOD REGION

2.1   Abstract

Oak (*Quercus*) is being replaced by maple (*Acer*) across the eastern deciduous forest, and forest managers have been using prescribed surface fires to remedy the decline. However, there is limited guidance on how many repeat applications of prescribed fire may be needed to accumulate enough oak regeneration to successfully re-establish stands, particularly in the Central Hardwood Region. Several stands, roughly half with north-facing aspects and half with south-facing, with (n = 47) and without (n =16) a prescribed fire since 1990 were surveyed in the Hoosier and Wayne National Forests in southern Indiana and Ohio, respectively. On 15 plots spread throughout each stand, tree seedlings (height > 15 cm, DBH < 10 cm) and midstory saplings and poles (10 cm < DBH < 25 cm) were quantified. Averaged across all sites, seedlings in the white oak group (section *Quercus*) comprised 47.8% of all seedlings, and seedlings in the red oak group (section *Lobatae*) comprised 8.2%. In comparison, common interfering species (maples and American beech) comprised 5.4% of all seedlings. Per one-way ANOVA, mid- and overstory BA, slope, years since last burn, and total number of burns significantly affected oak seedling density. Random forest analysis indicated that total burn number is an important predictor of oak seedling density; however, site characteristics like slope and amount of midstory basal area are more important predictors of oak seedling density than time since the last burn. Midstory species composition was not as influential on oak seedling density as total midstory basal area. My results suggest that prescribed fire may condition a site for natural regeneration of oak species, but if an established midstory already exists or fire has not been used on the landscape for almost a decade, repeated applications of prescribed fire will likely have a negligible effect on oak regeneration unless the site is burned repeatedly ($\geq$ 3 times) with high temporal frequency ($\leq$ 4 years between burns).

2.2   Introduction

Oaks (*Quercus* spp.), a group of foundational species in many eastern North American forest ecosystems, are slowly being successionally replaced by maples (*Acer* spp.) and other mesic species throughout their range. This self-reinforcing process, termed mesophication (Nowacki and

31

Abrams 2008), fundamentally shifts the disturbance regimes of these oak-dominated forests from fire-mediated (oak) to self-replacing (maple). Correspondingly, this regime shift and compositional change alters the ecosystem services that are provided. For example, there are 96 known species of forest-dwelling birds and mammals that consume acorns, providing a critical energy source during the dormant season for many vertebrate species (Martin et al. 1961). High acorn production is directly linked to increased body mass, population density, or home ranges for animals like white-footed mice (Jones et al. 1998, Ostfeld et al. 1996), ruffed grouse (Whitaker et al. 2005), and eastern chipmunks (McShea 2000). Maple seeds, on the other hand, contain a higher percentage of protein, but have much less fat and are less digestible to animals when compared to most acorns (Kirkpatrick and Pekins 2002, Tissier et al. 2019).

This shift in forest composition from mesophication also changes forest floor communities. Maples contain less lignin in their leaves than oaks, allowing maple litter to decompose more quickly and alter soil formation dynamics (Palus et al. 2018). Moisture is more easily trapped in the litter and aeration is minimized, increasing the speed of decomposition even further. Trapped forest floor moisture coupled with a dense, packed litter alters the fuel dynamics to such an extent that surface fires become exceedingly rare and largely ineffective to affect regeneration dynamics, even when prescribed. Therefore, through mesophication, maples and other mesic species are creating an ever-increasing mesic environment in which these trees outcompete oak seedlings, grow into the midstory, and eventually self-replace (Abrams and Nowacki 1992, Abrams 2005, Nowacki and Abrams 2008).

Prescribed fire has been increasingly used by land managers in the past few decades to establish oak regeneration. Managers are attempting, in effect, to mimic natural disturbance regimes that produced the oak-dominated forests of the recent past (Aldrich et al. 2005, Crow 1988, Crow et al. 1994, Lorimer 1993). Research has shown, however, that no single fire prescription consistently establishes oak regeneration on all sites; in fact, there are reports of positive, negative, and neutral results from use of prescribed fire over the past few decades (Brose et al. 2014). A single fire commonly provides either negative or negligible outcomes for oak seedlings (Albrecht and McCarthy 2006, Collins and Carson 2003, Dolan and Parker 2004, Loftis 1990), but dual fires provide mixed responses as well (Arthur et al. 1998, Merritt and Pope 1991, Schuler et al. 2013). Other previous work (Arthur et al. 1998) has shown that stopping at just one burn may in fact create more favorable conditions for less fire-resistant species like maple, or shrubs and

herbaceous species. By topkilling part of the midstory canopy, quick-growing maple can infill the holes, creating even shadier conditions for oak regeneration that develops (Brose et al. 2005). Without another fire or other disturbance to suppress midstory competitors, the mesophication cycle continues.

Studies monitoring long-term effects of multiple fires are fairly rare, but on the whole, these studies describe positive benefits to oak reproduction (Huddle and Pallardy 1996, Stratton 2007). This is particularly true on more xeric sites; oaks are better adapted to these environments than other species, so oak seedlings may be recruited with more success than on mesic sites where conditions favor other species (Hodges and Gardiner 1993, Lorimer 1993). However, if sites are burned too frequently, mortality of oak species can greatly increase as the incessant disturbance becomes too stressful for young oak seedlings and they perish alongside their competitors (Dey and Hartman 2004).

The interval between multiple prescribed fires is extremely important. At a Tennessee site, Stratton (2007) reported that even a five-year fire return interval was too short a timespan to allow for successful oak reproduction and time to mature to a fire-resistant size. Historically, the surface fire return interval in the Central Hardwood Region (CHR) before European settlement averaged ~13 years, but decreased to 7 years as industry increased in the region (Brose et al. 2014). This historic return interval may, on first glance, provide strong guidance for a return interval between modern prescribed fires; however, many oak forests have fundamentally changed since the time of settlement. While undocumented oak regeneration issues may have historically occurred, most CHR forests certainly cannot currently be described as they were upon settlement (Greeley and Ashe 1907). Mesophication creates a positive feedback loop that prescribed fire alone may not mitigate.

The main objective of this study is to determine the relationship between prescribed fire, existing stand characteristics, and competitive oak regeneration in the CHR. I inventoried regeneration and mid-/overstory interference data from 63 sites that differed in prescribed fire history, ranging from no fires to six fires since 1990. I hypothesized (H1) that prescribed fire, with repeat applications, will increase density of competitive oak regeneration, defined as seedlings over 30 cm tall, and decrease density of mesic species (e.g., maple and beech). I also hypothesized (H2) that the regeneration composition will shift from oak-dominated toward a mix of more mesic species as the time since the last burn increases. Lastly, I hypothesized (H3) that topographic and

stand structural variables will strongly mediate prescribed fire effects on oak seedling density and composition.

2.3   Methods

2.3.1   Study Sites

This study was located on the Hoosier National Forest (HNF) in southern Indiana and the Wayne National Forest in southern Ohio (WNF). The HNF comprises over 80,000 hectares of the unglaciated portions of southern Indiana; specifically, the Shawnee Hills and Highland Rim Natural Regions (Woodall et al. 2007; Homoya et al. 1985). The area mainly consists of a sandstone-shale bedrock, with several deep stream valleys and wooded hillsides to the east, and stony outcrops of rock bluffs with more arable land to the west (Ponder 2004). Sandstone-derived soils, like Crider silt loam and the Wellston-Zanesville-Berks Association are most common (Homoya et al. 1985). Sinkholes produced by the breakdown of limestone bedrock are also an important geologic feature of the region. Average yearly precipitation is 112 to 137 centimeters and the average temperature is between 13 and 16º C across the HNF (Ponder 2004).

Prior to settlement the HNF was mainly deciduous, dominated by oak (*Quercus*), hickory (*Carya*), beech (*Fagus*), and aspen (*Populus*) (Ponder Jr. 2004). The forest was heavily cutover in the 1800s and early 1900s prior to the establishment of the HNF in 1935. Common second-growth overstory species today include white oak (*Q. alba*), northern red oak (*Q. rubra*), shagbark hickory (*C. ovata*), and pignut hickory (*C. glabra*) in the upland forests and sugar maple (*Acer saccharum*) and American beech (*F. grandifolia*) on more mesic sites (Homoya et al. 1985). Fire, either through natural ignitions or deliberately set by Native Americans, can be assumed to have been a part of this landscape for centuries, according to the written observations of the first European settlers to the region (Homoya et al. 1985, Jenkins 2013). These settlers described open and park-like forests, similar to most eastern hardwood forests, without much understory or midstory present (Greeley and Ashe 1907). In more recent years, however, prescribed fire has been used to promote oak and hickory regeneration, reduce fuel loads, and restore barren habitats (Mann et al. 2020, Stanis et al. 2019).

The ~100,000 ha WNF is located in the unglaciated Allegheny Plateau in southern Ohio (Palus et al. 2018). Its topography consists of high hills, sharp ridges, and narrow valleys and the

bedrock is comprised mostly of sandstone, siltstone, and shale, while the soils can be described as acidic and well-drained loams or silt loams (Sutherland et al. 2003). Gilpin and/or Steinsburg series complexes or associations dominate the region (Boerner and Sutherland 2003). Average yearly precipitation is between 81 to 96 cm and annual temperatures range from around 10 to 13º C (Palus et al. 2018)

Similar to the HNF, this oak-dominated forest was also described as open and park-like before European settlement. Even after the arrival of settlers, but before fire suppression policies, the fire return interval was between 5 and 15 years (Sutherland et al. 2003). After settlement, the forests were harvested for the charcoal iron industry; most forests have been undergoing secondary succession since the decline of the industry in the early 1900s. Currently, the sapling layer is dominated by shade tolerant trees like red and sugar maple, blackgum (*Nyssa sylvatica*), and beech (Hutchinson et al. 2003). The most abundant overstory species include white, chestnut (*Q. montana*), and black (*Q. velutina*) oak, various hickories, and red (*A. rubrum*) and sugar maple. Overstory white oak, especially in size classes under 30 cm, are less abundant on mesic sites and sugar maple of all sizes were rare on xeric sites (Yaussy et al. 2003). As in the HNF, prescribed burns are currently used by managers to promote oak regeneration in the understory. Burns in both national forests are usually low-intensity and patchy, producing a heterogeneous landscape without large swaths of litter consumption and mineral soil exposure is rare.

2.3.2 Plot Selection

From a population of stands previously inventoried for prescribed fire damage to overstory timber (Mann et al. 2020, Stanis et al. 2019), I selected 47 stands that had prescribed fire history and 16 unburned control stands across the two national forests. All stands were dominated (> 50% basal area [BA]) by oak, usually white oak per national forest fire prescription preferences, and hickory species. Each had merchantable timber with an average diameter at breast height (DBH) of at least 25 cm. Each stand had a relatively homogeneous species composition, aspect, and age structure, and was usually between 4 and 13 ha (Mann et al. 2020, Stanis et al. 2019). Stands were further classified using burn class, i.e., the number of prescribed burns that the stand had received since 1990, and by predominant aspect. Aspect, in particular, has repeatedly been shown to influence the intensity of prescribed burns, with slopes on more xeric, south- and west-facing aspects usually

hosting more intense fires than their more mesic counterparts on easterly or northerly aspects (Estes et al. 2017, Pyne et al. 1996).

Each stand was sampled with 15 points, using the same locations as Stanis et al. (2019) and Mann et al. (2020). Their point selection procedures used the fishnet or point ArcGIS tool to randomly select points until at least 8 points were on slopes with aspects corresponding to the predominant stand aspect (e.g., 8/15 points on north-facing slopes for a stand with a northerly aspect). Points had to be at least 30 m away from each other in order to avoid double-sampling of overstory trees. Although care was taken to represent as many total burn numbers and years since the last burn as possible, weather and personnel ultimately dictate when burns can happen, resulting in an unbalanced design. For example, the three-burn category contained 19 stands while the two-burn category contained five, and 20 stands were burned one year prior to sampling but the two-, seven-, and ten-year categories each contained only one stand (Tables 1 & A2).

2.3.3   Data Collection

Field sampling methods were loosely based on the SILVAH protocol for collecting data on oak regeneration in the Allegheny Mountain region (Brose et al. 2008). I measured aspect and percent slope at the center of each sample point. One midstory plot, three reduced-midstory subplots, and three regeneration subplots were installed at each point (Fig. 2.1). The midstory plot captured all potential regeneration interferences by quantifying both midstory trees and groundcover, but the reduced-midstory plots just captured interferences in the form of midstory trees, hence the inclusion of "reduced".

The 3.8 m^2 regeneration subplots were placed with their centers 3.5 m from the plot center, 120º away from each other (i.e. one subplot 3.5 m from plot center at 0º azimuth, one subplot at 120º, and another subplot at 240º). Within each regeneration subplot, I measured all woody stems greater than 15 cm tall with a DBH less than 10 cm. I recorded species, height to the nearest cm, and root collar diameter (RCD) to the nearest 5 mm for each individual.

The three reduced-midstory subplots, 10.2 m^2 each, were overlain on each regeneration subplot. I quantified midstory trees in these subplots, measuring the species, DBH, and which regeneration subplot (0º, 120º, or 240º) the midstory was associated with for each species with a DBH between 10 and 25 cm.

The 201 m^2 midstory plot was also centered on the sample plot center, completely engulfing both the reduced-midstory and regeneration subplots. I quantified all midstory trees (i.e., DBH between 10 and 25 cm) and groundcover within this plot. I recorded the species and DBH of each midstory tree and the percent cover, to the nearest 5%, for each taxa group comprising the groundcover of the entire midstory plot (i.e. briar/thorny shrubs, shrubs, forbs, grasses, herbaceous vegetation, moss, sedges, tree seedlings, and rocks/bare ground).

2.3.4 Data Preparation

Total midstory basal area (BA), oak midstory BA (oak BA), and mesic species midstory BA (mesic BA) were all calculated for each stand. Oak BA was defined as total midstory BA of all oak species found on the study sites: white, chestnut, northern red, black, bur (*Q. macrocarpa*), chinkapin (*Q. muehlenbergii*), post (*Q. stellata*), blackjack (*Q. marilandica*), and shingle (*Q. imbricaria*). Mesic BA was defined as the midstory BA of the species likely to most interfere with the growth and/or survival of oak regeneration: American beech, sugar maple, and red maple. Overstory BA, trees per acre, and site index were estimated based on Stanis et al. (2019).

Site index, trees per acre, and percent slope were classified into "low", "medium", and "high" categories based on the first quartile, median, and third quartile of their respective distributions, as the absolute values associated with these variables for any given plot were far less important than their values relative to all other plots in the study. The observed ranges for all stand structural and environmental variables can be found in Appendix A. Aspect was also reduced into north (north and east aspects) and south (south and west aspects) based on the stand classification system of Stanis et al. (2019).

Due to the highly unbalanced design, stands were aggregated into year classes that corresponded to the time since burn; these were: one year, two to three years, four to seven years, and eight to ten years. These classes captured immediate, short-, medium-, and long-term regeneration lags to fire disturbance. Results for unaggregated data can be found in Appendix A.

The likelihood of survival for short seedlings is inherently low (Brose et al. 2008), so I completed the statistical analyses using only the seedlings that were ≥ 30 cm in height; descriptive data summaries include all seedlings. I summarized seedling density (seedlings per hectare) per sub-plot, then aggregated the data to the plot-level so local environmental variables like slope and aspect could be used as predictors without averaging across an entire stand.

2.3.5 Statistical Analysis

To test the effect of repeated burns (H1) and time since last burn (H2) on regeneration density, I used one-way ANOVA and post-hoc Tukey's Honest Significant Difference multiple comparison test (hereafter, Tukey HSD) with burn number and year class as treatments, respectively. These analyses were conducted using densities of all competitive seedlings, of competitive oak seedlings, and of competitive mesic seedlings (maple and beech) for both H1 and H2.

The effects of topographic and structural variables on the relationship between prescribed fire and regeneration (H3) were tested two ways. First, I used random forest regression trees to predict oak seedling densities as a function of stand characteristics, environment, and burn variables. I used number of burns, years since the last burn class, aspect, slope, trees per acre, site index, overstory BA, midstory BA, oak BA, and mesic BA to predict densities of all oak species seedlings. Regression trees are widely used in ecology due to their ease of use, high accuracy, and success with classifying complex relationships among many variables (Cutler et al. 2007). Random forest is a nearly nonparametric approach; the only two defined parameters are the number of variables at each node and the total number of "trees" in the forest (Liaw and Wiener 2002). Random forest incorporates bootstrapping to improve classification. Out-of-bag observations, those observations that occur in the real data but not in the bootstrap sample, have a misclassification rate associated with them to better measure the importance of each predictor variable (Cutler et al. 2007). Relative variable importance is also calculated via a measurement of increase in node purity and mean square error (MSE). Predictors that have a higher MSE, the most commonly used indicator of random forest variable importance, are more important for successfully classifying the model, and the accuracy of the model would suffer if this variable was removed. A higher increase in node purity value is given to a variable that is more useful at predicting the response at each node split, leading to higher purity in the decision tree overall. Then, from those variables deemed important by the random forest analysis, I used one-way ANOVA and Tukey HSD to more specifically investigate their impacts on oak seedling density within different burn numbers and year classes.

I used R 3.6.1 (R core team 2019) and the "randomForest" package (Liaw and Wiener 2002) specifically for these analyses. All analyses used $\alpha = 0.05$.

2.4   Results

2.4.1   Summary Regeneration Patterns

A total of 14,200 seedlings were measured in 63 stands. White oak, black oak, chestnut oak, and northern red oak were among the most prevalent seedlings counted (Fig. 2.2). Sassafras (*Sassafras albidum*), hickory species, eastern redbud (*Cercis canadensis*), American beech, and tulip poplar seedlings were the next most prevalent species encountered. Sugar and red maple seedlings were both found on plots, but at low densities.

2.4.2   Effect of Repeated Burns on Regeneration

Stands that were burned six times had the highest overall seedling density ($21,100 \pm 57$ seedlings ha^{-1}) (mean $\pm$ SE) and stands burned only once had the lowest seedling density ($9,930 \pm 40$ seedlings ha^{-1}) (Table 2.1). Burn number significantly affected overall seedling density ($F_{5, 8401} = 351$, $p < 0.001$). Control plots had fewer seedlings than plots burned at least three times ($p < 0.001$ for all). Plots burned once had fewer seedlings than plots burned three ($p = 0.025$), four ($p < 0.001$), and six ($p < 0.001$) times, plots burned twice had a lower seedling density than plots burned four ($p = 0.025$) and six ($p < 0.001$) times, and plots burned six times had a higher density of seedlings than plots burned three and four times ($p < 0.001$ for both).

Oak seedling density was also significantly affected by burn number ($F_{5, 4768} = 327$, $p < 0.001$). Control plots had a higher oak seedling density than plots burned once ($p=0.037$), but lower density than plots burned four ($p < 0.001$) and six ($p < 0.001$) times. Plots burned once had a lower oak seedling density than plots burned at least three times ($p < 0.001$ for all), plots burned three times had fewer oak seedlings than plots burned four and six times ($p < 0.001$), and plots burned six times also had a higher density than plots burned two and four times ($p < 0.001$ for all).

The density of mesic species was significantly impacted by burn number ($F_{5, 452} = 5.55$, $p < 0.001$). Control plots had a higher mesic seedling density than plots burned one ($p < 0.001$) and three times ($p = 0.014$). No other burn numbers differed.

The white oak group (white, chestnut, bur, chinkapin, and post oak) comprised a majority of seedlings in stands burned four times (55.4% of all seedlings) and six times (54.9%), but was also very common in stands without burns (55.1%) (Table 2.1; Fig. 2.4). Seedlings in the red oak group (northern red, black, blackjack, and shingle oak) comprised the largest proportion in stands

with two (13.7% of all seedlings) and six (14.8%) burns, but any burned stand had at least 10% red oak seedlings (Table 2.1; Fig. 2.4). Mesic species seedlings (American beech, red maple, sugar maple) made up less of the total seedling population as the burn number increased, declining from 11.3% of all seedlings in unburned stands to 1.2% of all seedlings in stands burned 6 times (Table 2.1; Fig. 2.4).

Stands burned six times had the highest proportion of seedlings over 30 cm tall (72.1% of all seedlings) and those burned once had lowest proportion (45.6% of all seedlings) (Table 2.1). Generally, as the number of burns increased, so did seedling density and proportion of seedlings over 30 cm tall (Fig. 2.3).

2.4.3 Effect of Time Since Last Burn on Regeneration

Overall seedling density generally declined for several years after a burn, but started to rebound in the eighth year (Figure 2.5). Stands burned one year prior had the highest seedling density (14,700 $\pm$ 30 seedlings ha^{-1}) and stands burned four to seven years before sampling had the lowest (5,880 $\pm$ 61 seedlings ha^{-1}) (Table 2.2).

Years since burn significantly affected, overall ($F_{4, 8402} = 220.6$, $p < 0.001$), oak ($F_{4, 4769} = 184.4$, $p < 0.001$), and mesic ($F_{4, 453} = 6.12$, $p < 0.001$) seedling density. The density of all seedling species on unburned plots was lower than plots burned within the last three years ($p < 0.001$ for all). The one year post-burn category was significantly higher than all other categories ($p < 0.001$ for all). The two-three years post-burn category was also higher than four-seven years and eight-ten years ($p < 0.001$ for both). The four-seven and eight-ten categories did not differ in seedling density response. Oak seedling densities on control plots were lower than plots burned one ($p < 0.001$) and two-three ($p = 0.005$) years prior. The one year post-burn category was higher than all other categories ($p < 0.001$ for all). Additionally, the two-three year category was higher than the eight-ten category ($p < 0.001$), but no other categories differed. Mesic species seedling density on unburned plots was higher than those burned within the last three years ($p < 0.001$ for all). No other year classes differed.

Mesic species seedlings were proportionality lowest in the first four years after a burn (1.3% to 2.4% of all seedlings present), while oak seedling densities are variable but often an order of magnitude or more higher (Table 2.2). White oak seedlings, in particular, accounted for >30% of the regeneration density at all times, while red oak seedlings decreased in proportion for the

first seven years post-burn and then increased thereafter (Fig. 2.6). Likewise, seedlings of all species over 30 cm tall dominated in the first three years after a burn (63.7% to 72.4 % of all seedlings; Table 2.2).

The density of all seedling species ($F_{10, 8396} = 184.9$, $p < 0.001$) and oak species seedlings ($F_{10, 4763} = 170.8$, $p < 0.001$) were both significantly affected by the interaction between the number of years since the last burn and total burn number. Mesic species seedling density was not affected by this interaction.

2.4.4 Effect of Topography and Stand Structure on Regeneration

The most important variables in the random forest model when predicting oak seedling density were slope, burn number, midstory BA, mesic BA, overstory BA, and oak BA (Fig. 2.8). Trees per acre and years since burn were of moderate importance, and aspect and site index were the least important variables (Fig. 2.8).

All important random forest predictors significantly affected oak seedling density. The relationship between oak density and burn number is described above in Section 2.4.2. Oak seedling density was significantly affected by slope ($F_{2, 4771} = 26.33$, $p < 0.001$), midstory BA ($F_{106, 4667} = 30.82$, $p < 0.001$), mesic BA ($F_{1, 4772} = 100.3$, $p < 0.001$), oak BA ($F_{1, 4772} = 89.16$, $p < 0.001$), and overstory BA ($F_{1, 4772} = 8.04$, $p = 0.005$).

All slope categories differed from each other ($p < 0.001$ for all, except $p = 0.05$ when comparing low and high slopes). Oak seedling density was highest in moderately sloped sites, especially as the burn number increased (Fig. 2.7). As midstory BA, mesic BA, oak BA, and overstory BA increased, oak seedling density decreased.

2.5 Discussion

2.5.1 Emergent Oak Regeneration Patterns

The regeneration layer of the HNF, WNF, and in many oak-dominated stands, is mostly comprised of oak seedlings (Fig. 2.2), corroborating the claim that the Central Hardwood Region does not suffer from an oak regeneration issue, but rather an oak maturation or retention problem (Lorimer 1993). Mature oaks are producing acorns, these acorns are germinating, the seedlings are growing, but then these seedlings fail to reach the midstory (Chapter 3), highlighting the importance of

continued maintenance of even the densest oak seedling patches. This discrepancy would be partially explained by the fact that acorn germination may be enhanced by prescribed surface fire, even if advance regeneration is not present prior to the burn. Surface fire can topkill midstory competition to give acorns more light to germinate, eliminate seed competitors, reduce the physical soil litter barrier (up to 90% of the litter layer can be burned in a surface fire), and make the environment inhospitable for insects, fungi, and the small mammals that feed on acorns (Arthur et al. 2012, Brose et al. 2013, Wang et al. 2005). Therefore, acorns are germinating and growing to seedlings, but topographic and stand structural variables are preventing these seedlings from reaching maturity.

2.5.2   Repeated Applications of Prescribed Fire Favor Oak

My results suggest that prescribed fire increases oak regeneration density with repeated applications. Overall seedling density increased with burn number (Fig. 2.3; Table 2.1), and my results suggest that as long as a site is burned three times, overall seedling density will increase, with additional burns leading to additional increases in density. Arthur et al. (1998) found that seedling density of oak and competitor species increased after two burns of differing intensities, so it appears that repeat prescribed fires benefit the regeneration layer in general.

Sites burned once had a lower density of oak seedlings than control sites, which may be caused by the negative effect that conducting only one burn has on oak regeneration (Albrecht and McCarthy 2006, Collins and Carson 2003, Dolan and Parker 2004, Loftis 1990). A single burn often reduces the density of all seedling species (Dolan and Parker 2004), and depending on other stand characteristics may lead to a recolonization of maple (Albrecht and McCarthy 2006). Miller et al. (2017) found that stands treated with midstory removal or a shelterwood harvest in concert with herbicide application or deer fencing all had better oak seedling regeneration responses than stands with one prescribed surface fire. It appears that at least three additional burns are needed after the first before a significant impact on oak seedling density emerges, with densities remaining high even after six burns.

Mesic species were also affected by burn number, as expected. The regeneration layer never had a large proportion of mesic seedlings (Fig. 2.4, Table 2.1), and density on control plots exceeded those burned one and three times, suggesting that even a lower number of burns can decrease mesic seedling density. Single and repeat burns were previously found to decrease maple

survival (Alexander et al. 2008), so it is unsurprising that mesic seedling density continuously decreased with additional burns. With increasing burn numbers, mature maples and beech will be injured or killed by fires, reducing their reproductive capacity and reducing their numbers in the younger generations. Even mature sugar maples often suffer from open wounds and trunk volume loss after prescribed surface fires (Mann et al. 2020, Saunders et al. in prep), further decreasing their ability to devote resources to reproduction. Maple and beech seedling numbers are also low in the first four years following a burn (Table 2.2, Fig. 2.6), which may be linked to the fact that seedlings of any species rarely survive an intense enough surface fire, and the subsequent injury and death in the mature individuals reduces seed production (Brose et al. 2014).

Increasing the burn number does not directly affect the seedlings that are already present, however, as they are likely just a few years old and were not alive for multiple burns, but increased use of fire can affect the mid- and overstory composition (Chapter 3). Additionally, due to differential survival after a fire event, not all trees respond the same. While oaks, especially in the white oak group, are adapted to surface fires, other species will suffer mortality and create canopy gaps allowing seedlings and other vegetation to grow (Clatterbuck 1998, Dolan and Parker 2004, Lorimer 1993). Therefore, it makes sense that as burn number increases, there are fewer maples and beech in the understory due to their lack of adaptations to survive fire (Fig. 2.4).

2.5.3   Longer Time Lags Favor Mesic Species Over Oak

Regeneration composition also shifts as the time since the last prescribed fire increases. The amount of time since the last burn significantly affected overall, oak, and mesic species seedling densities, but in different ways. Overall seedling densities were higher than control plots only in stands burned within the last three years, and considering stands burned four to seven years before sampling had the lowest seedling density (Fig. 2.5; Table 2.2), it appears that the entire regeneration layer benefits the most within the first three years post-burn. This is surprising considering most seedling species suffer mortality after a burn and take more than a year to re-establish (Dolan and Parker 2004). However, due to the patchiness of surface fires in the CHR, many of these seedlings may not be top-killed as a direct result of the fire, but rather develop injuries or openings for disease to cause mortality years after the disturbance.

However, overall seedling density is not high in the first several years following a prescribed fire because all seedling species equally benefitted. Oak seedling densities were higher

than those found on control plots only within the first three years post-fire, so the high oak density in the regeneration layer is likely heavily weighting the overall seedling density. Mesic seedling densities were opposite those of oak (Fig. 2.6) and were higher than control plots only in stands burned at least four years prior. This switch from oak species to mesic species favored in the regeneration layer occurs around four years post-fire, which is somewhat unexpected. The switch from oak- to mesic-favored regeneration is a common occurrence, but there is a lack of consensus as to when that switch happens. Stratton (2007) found that a five year return interval was too soon for competitive oak regeneration to form before the next fire; but, in another study, oak seedling density did not increase in the first two years post-fire (Apsley and McCarthy 2004). This lack of consensus highlights the importance of prescribed fire studies focusing not just on total burn numbers, but also the spacing between the burns or spacing between the last burn and time of sampling.

2.5.4   Topography and Stand Structure Can Override Fire Effects on Regeneration

The important variables when using a random forest model to predict oak seedling density were a mixture of stand structure, topographic, and burn variables (Fig. 2.8). Each of these variables was also found to significantly affect the density of oak regeneration, suggesting that topographic and stand structural variables affected oak regeneration density as much as burn variables on my study sites.

The only topographic variable of import was slope, which was one of two most influential predictors overall of oak regeneration density (Fig. 2.8). Slope influences fire behavior, which in turn influences species composition. As a surface fire progresses, it pre-heats material upslope leading to faster and potentially more intense combustion on steeper slopes. For example, the upslope side of mature trees are usually more damaged than the downslope side (Barnes and Van Lear 1998), while ridgetops often suffer the most damage because the fire has had time to gain intensity while moving upslope (Elliott et al. 1999). The nature of fire may have caused low oak seedling densities on sites with high slopes (Fig. 2.7). These areas with steeper slopes may have been host to more locally intense burns, spurring the growth of any seedling species.

Aspect, unexpectedly, was not an important predictor variable. Although aspect should influence fire behavior (Pyne et al. 1996), the magnitude of its effect does not always impact mature trees (Stanis et al. 2019). The unimportance of aspect here may simply be due to the light

44

and water preferences of present species. Understory oak are often attempting to establish in areas with insufficient light, so xeric sites that receive more sunlight may be preferred; however, soil moisture can also be a limiting factor in seedling establishment so receiving too much light may be a detriment (Hodges and Gardiner 1993). However, the seedlings measured in this study were still quite young and their presence on a mesic slope at this stage does not necessarily equate to long-term establishment, which may have influenced aspect's the lack of importance.

All measures of mid- and overstory basal area (overstory BA, midstory BA, oak BA, and mesic BA) were important and significant predictors of oak seedling density. This finding is expected due to the shade intolerance of oak seedlings (Burns and Honkala 1990). The shade intolerance of oak, and shade tolerance of maple, is a contributing factor to oak decline in the CHR (Nowacki and Abrams 2008) so it is not surprising that mid- and overstory basal area partially controls oak seedling density. A disturbance like fire breaks open the canopy by causing injury or mortality to more mature trees and allows more light to filter to the regeneration layer, giving shade intolerant species a chance at growth (Ryan et al. 2013, Shumway et al. 2001). Furthermore, an increase in light, caused by gap-creation, in concert with prescribed fire usage has been shown to best benefit the oak regeneration layer (Izbicki et al. 2020). This effect of mid- and overstory BA on oak seedling regeneration has been established (Keyser and Loftis 2015, Paquette et al. 2006), so midstory thinning is often used to specifically promote oak establishment and growth (Lhotka and Loewenstein 2009).

Lastly, the two burn variables, burn number and years since burn, had differing importance in the random forest model but were both found to significantly affect oak regeneration density. Burn number was one of the most important predictors while years since burn was not, but that may be due to the design of this study. The longest some of these stands had gone without fire since 1990 is ten years, which is similar to historic fire return intervals of the region (Brose et al. 2014). This means that some competitive oak regeneration is likely to occur during that extended interval as it has in the past, so perhaps the study interval should be extended to see the full extent of the effect of years since burn on oak regeneration. Additionally, the year classes do not account for the total number of burns, which has been previously noted as an issue with study design (Arthur et al. 2015). The interaction between years since last burn and number of burns significantly affected overall and oak seedling densities, so it is clear that these two variables are working in tandem and neither should be ignored when prescribing burns or managing burned

stands. The two burn variables may have also been as or less important than topographic and structural variables due to the nature of prescribed surface fire. Sending prescribed fire through a stand of mature trees produces patchy and inconsistent results at best, as the one constant seems to be that controlled burns in the Central Hardwood Region rarely if ever cause significant overstory mortality (Chapter 1). The heterogeneous results of surface fire coupled with the retrospective nature of this study (as reviewed in Brose et al. 2005) likely diminished the importance of both burn variables, but especially years since burn.

2.6 Conclusion

Topographic and stand structure variables were relatively as important as predictors of oak seedling species density as the total number of burns, suggesting the importance of removing midstory and overstory volume when also using prescribed burns. Essentially, prescribed fires may be used to condition the site for further work, but they should not be used without knowledge of what else that stand needs to successfully create oak habitat. It is known that due to the many factors that determine fire behavior and the microsite conditions, there is much local variation in intensity for a single fire (Boerner 1982). This variation can make it difficult to generalize across one fire, much less across a whole region's relationship with prescribed burns.

Prescribed burns alter site conditions for oak regeneration either directly through changes in forest floor conditions, or indirectly through differential mortality of regenerating species or mortality of midstory stems. Even if the percent slope has a greater proximal influence over oak seedling density, more prescribed burns ultimately lead to fewer maple and beech seedlings, particularly during the first four years after a burn. Repeated burns would prolong the period of favorable competitive conditions for oak seedlings. Nevertheless, a more efficient approach may be to directly target the midstory first. Midstory removal, for example, has been used previously to enhance oak seedling survival with great success (Craig et al. 2014, Lhotka and Loewenstein 2009, Motsinger et al. 2010, Parrott et al. 2012), so if the midstory has already developed it may be best to use some form of midstory removal in concert with prescribed fire to achieve the most desirable results.

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2.8   Tables and Figures

Table 2-1 Summary statistics categorized by burn number. Stands = number of stands that have been burned that many times; seedlings ha^{-1}= the number of seedlings per ha for that burn count $\pm$ SEM; % tall = percentage of total seedlings that are above 30 cm in height; % white oak = percentage of total seedlings that belong to the white oak species group; % red oak = percentage of total seedlings that belong to the red oak species group; % maple/beech = percentage of total seedlings that are either sugar maple, red maple, or American beech.

Burn count	0	1	2	3	4	6
Stands	16	10	5	19	6	7
Seedlings ha^{-1}	$10,500 \pm 25$	$9,930 \pm 40$	$10,300 \pm 53$	$12,100 \pm 30$	$14,000 \pm 50$	$21,100 \pm 57$
% tall	53.8	45.6	63.4	60.4	57.4	72.1
% white oak	55.1	38.2	42.8	40.7	55.4	54.9
% red oak	9.3	4.0	13.7	11.7	10.6	14.8
% maple/beech	11.3	6.0	4.3	3.1	3.9	1.2

Table 2-2 Summary statistics categorized by time since the last burn classes. Stands = number of stands that have been burned that number of years ago; seedlings ha^{-1} = the number of seedlings per ha for that year count ± SEM; % tall = percentage of total seedlings that are above 30 cm in height; % white oak = percentage of total seedlings that belong to the white oak species group; % red oak = percentage of total seedlings that belong to the red oak species group; % maple/beech = percentage of total seedlings that are either sugar maple, red maple, or American beech.

Years since last burn	1	2-3	4-7	8-10
Stands	20	10	6	11
Seedlings ha^{-1}	14,700 ± 30	13,000 ± 75	5,880 ± 61	11,810 ± 103
% tall	63.7	72.4	44.1	51.2
% white oak	47.3	52.4	45.2	34.0
% red oak	15.3	9.3	4.3	31.2
% maple/beech	2.4	1.8	38.3	8.2
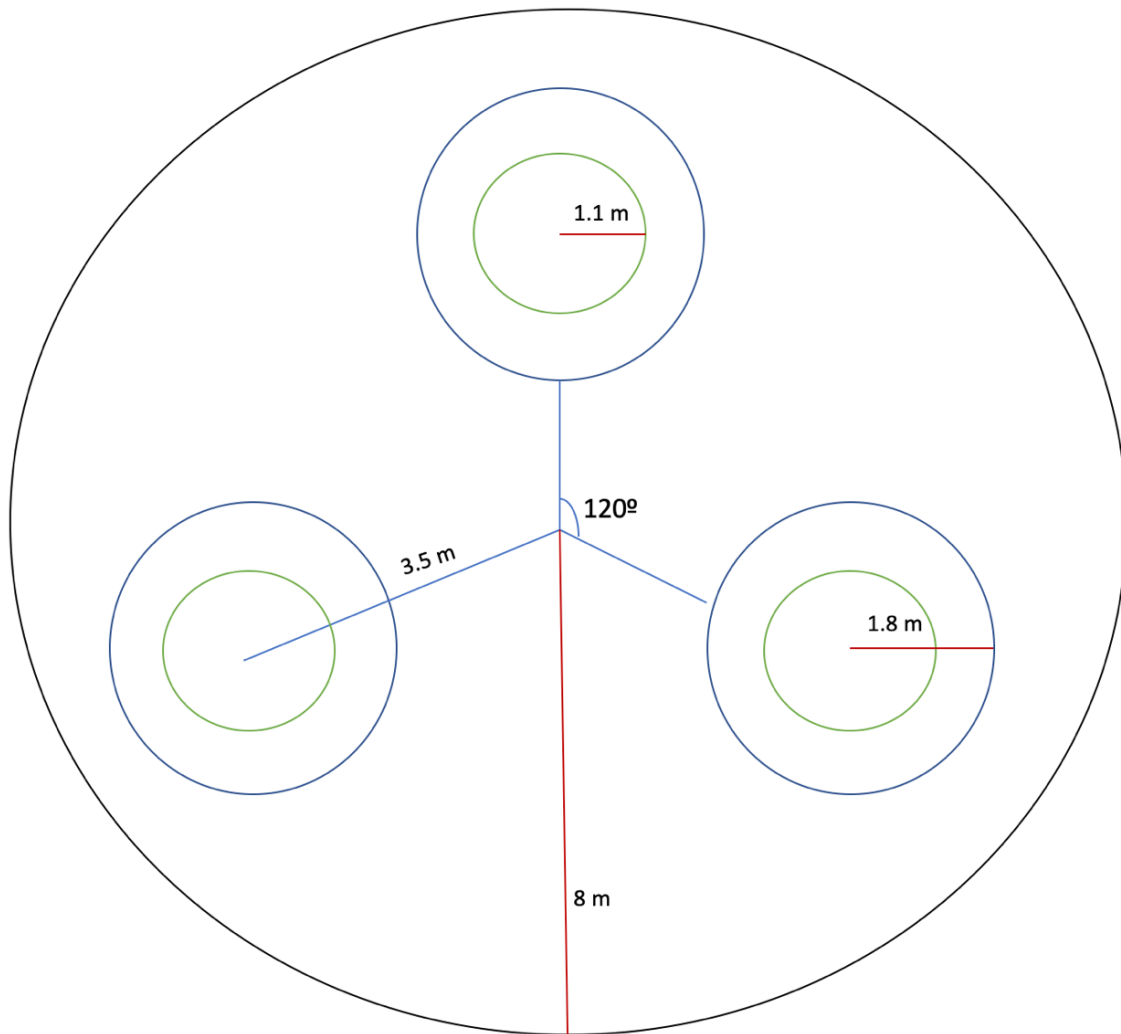
Figure 2-1 Plot design for regeneration surveys. The midstory plot had an 8 m radius, and the three reduced-midstory subplots each had a radius of 1.8 m. The regeneration sampling occurred in a 1.1 m radius circle within each of the three subplots, placed 3.5 m from the center of the plot and 120º away from each other.
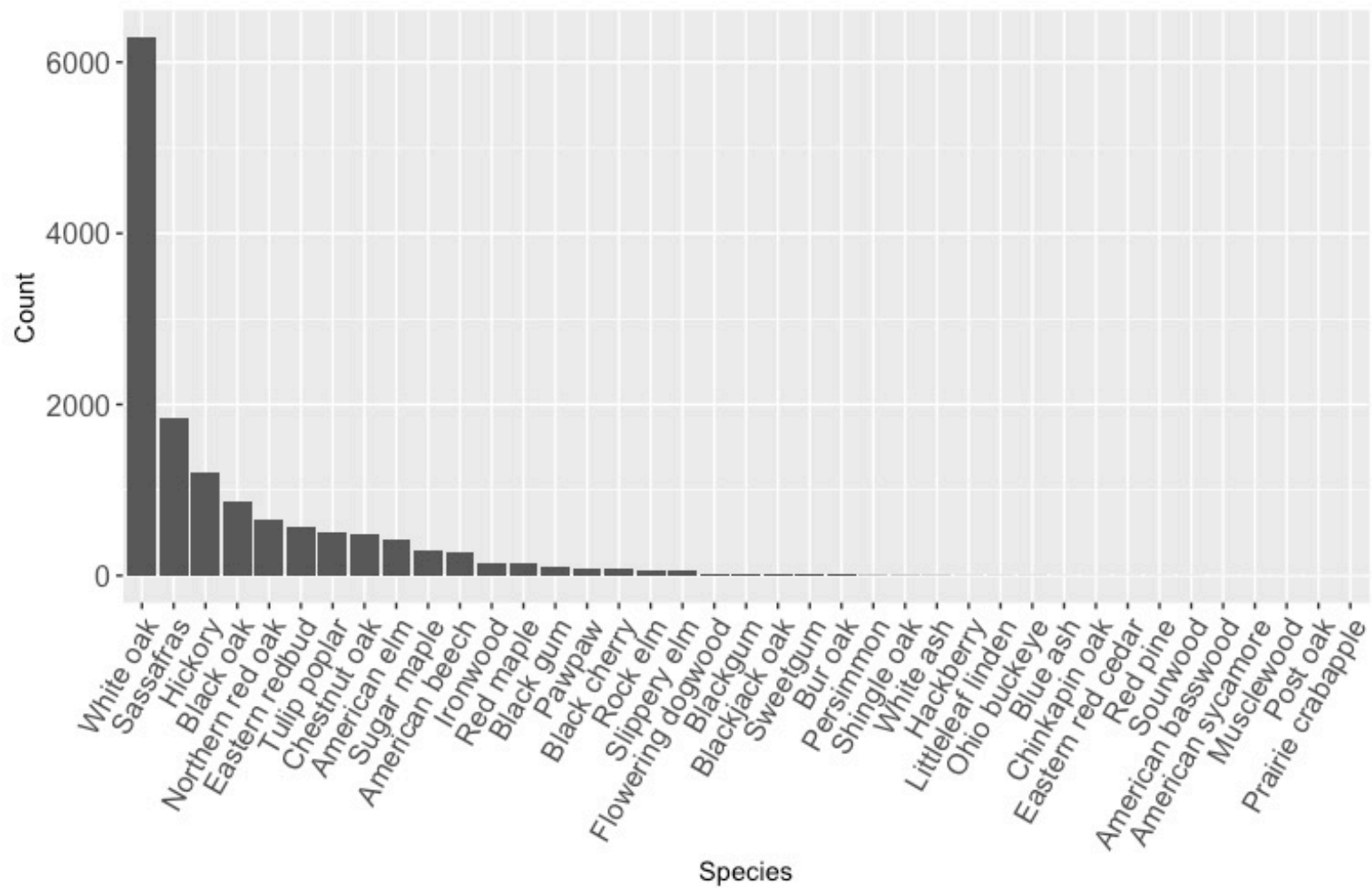
Figure 2-2 The total count of all species found across plots in all 63 stands. 14,200 seedlings were recorded. White oak is by far the most common species (n=6,289), and sassafras is the second most abundant species (n=1,841).
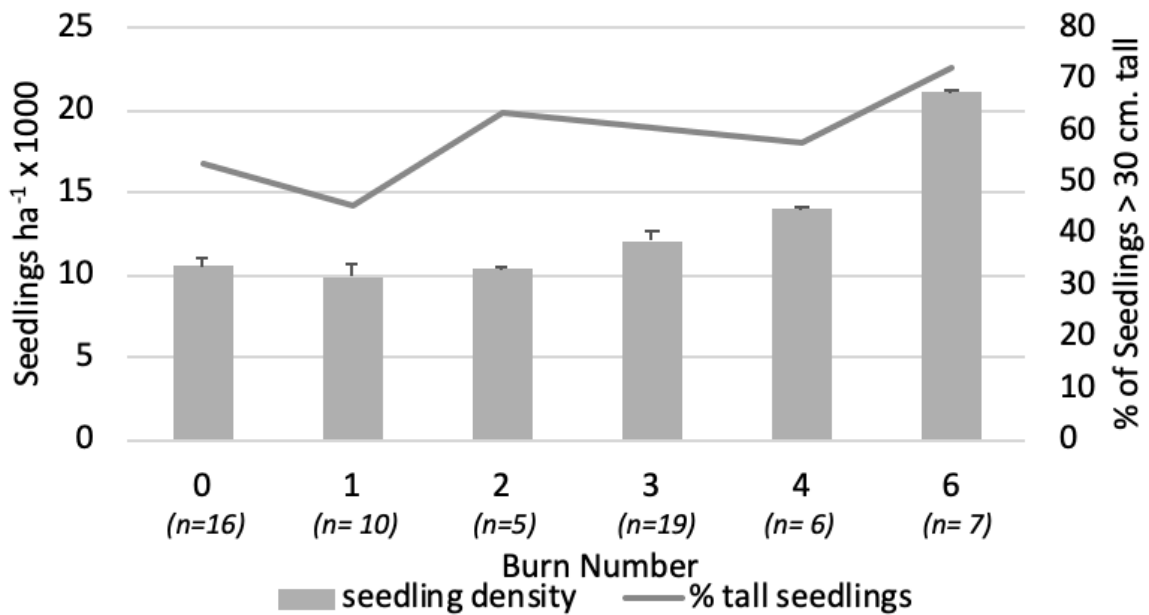
Figure 2-3 Seedling density (seedlings ha^{-1} x 1000) and percentage of total seedlings that were taller than 30 cm displayed by total number of burns. Stand sample sizes for each burn class are shown in italics below the x-axis, and error bars are displaying the standard error of the mean.
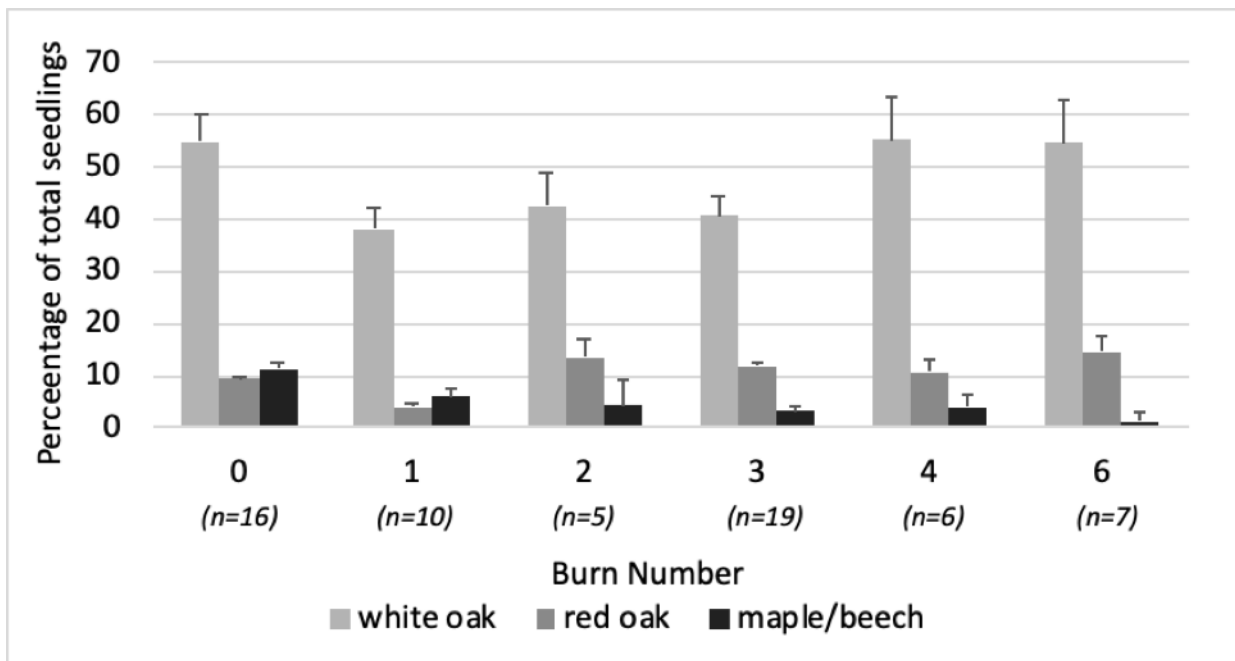
Figure 2-4 Percentage of total seedlings that belong to either the white oak group, red oak group, or maple/beech group. Stand sample sizes for each burn class are shown in italics below the x-axis, and error bars are displaying the standard error of the mean.
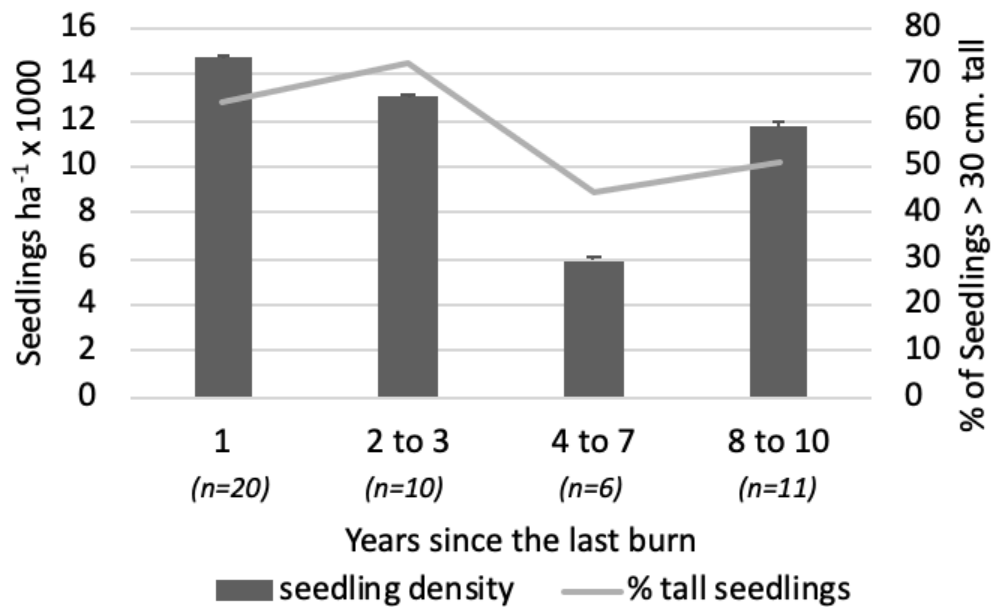
Figure 2-5 Seedling density (seedlings ha^{-1} x 1000) and percentage of total seedlings taller than 30 cm displayed by the number of years since the last burn category. Stand sample sizes for each year category are shown in italics below the x-axis, and error bars are displaying the standard error of the mean.
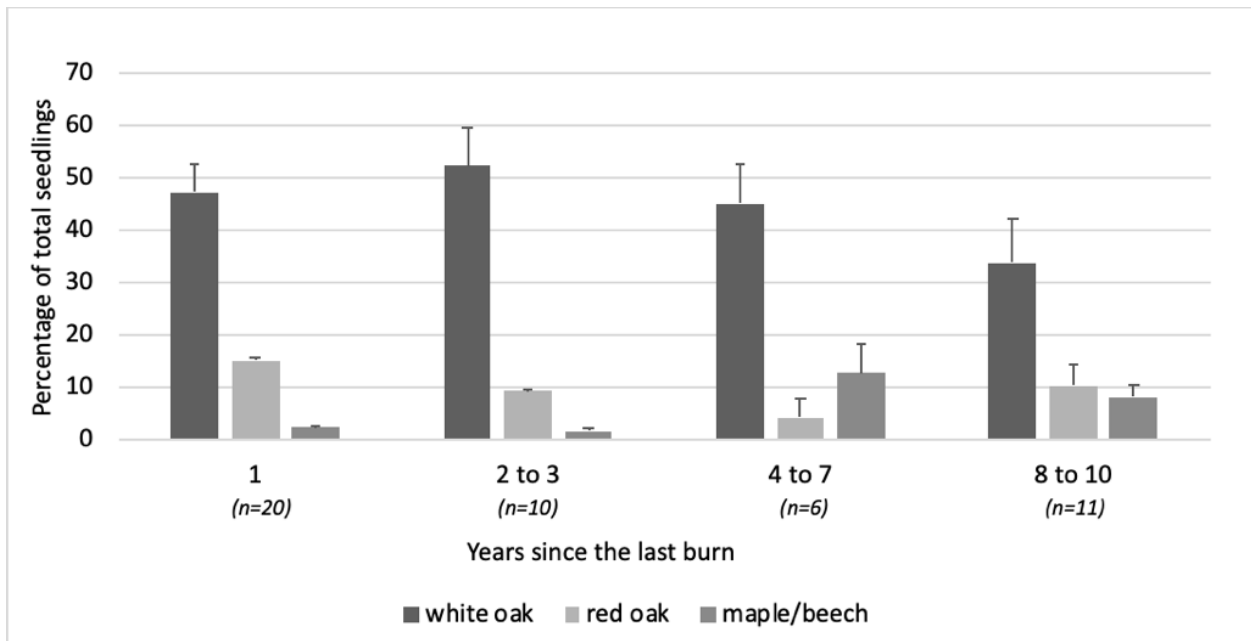
Figure 2-6 Percentage of total seedlings that belong to either the white oak group, red oak group, or maple/beech group displayed by the number of years since the last burn category. Stand sample sizes for each year are shown in italics below the x-axis, and error bars are displaying the standard error of the mean.
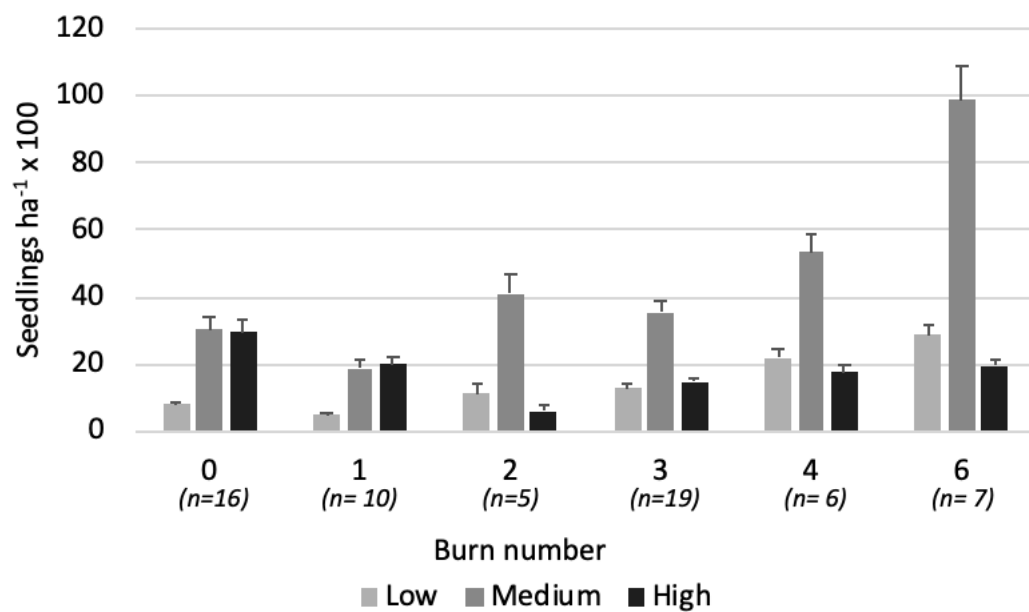
Figure 2-7 All oak species seedling density (seedlings ha^{-1} x 100) for low, medium, and high percent slope categories displayed by the total number of burns. Stand sample sizes for each burn class are shown in italics below the x-axis, and error bars are displaying the standard error of the mean.
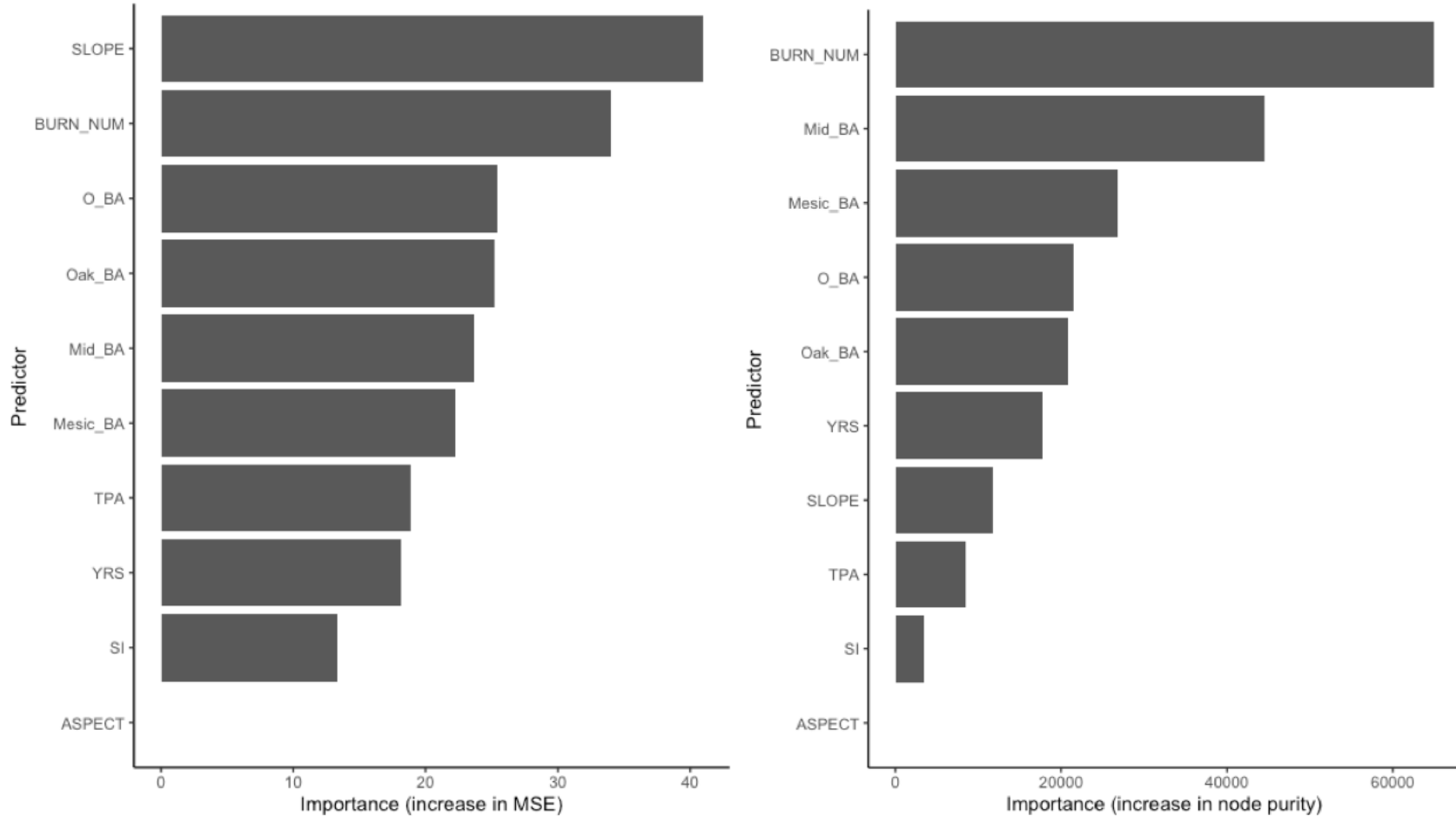
Figure 2-8 The importance of each predictor variable, measured by the increase in mean square error (MSE) on the left and increase in node purity on the right, on the response of oak seedling density. Predictor variables, as described in Section 2.3.4, are: mid_BA = total midstory basal area, SLOPE = slope class, mesic_BA = mesic species midstory basal area, ASPECT = aspect class, oak_BA = oak midstory basal area, O_BA = total overstory basal area, YRS = number of years since the last burn, BURN_NUM = total number of burns, TPA = overstory density class, and SI = site index class.

CHAPTER 3. MIDSTORY COMPOSITIONAL RESPONSE TO THE USE OF PRESCRIBED FIRE IN THE CENTRAL HARDWOOD REGION

3.1 Abstract

Oaks (*Quercus* spp.) are slowly being replaced by maple (*Acer* spp.) and other shade tolerant tree species throughout North America's eastern deciduous forests. With varying results, land managers are implementing prescribed surface fires in an attempt to break this cycle of mesophication, both by promoting oak seedling regeneration and reducing midstory stem density. However, the prolonged absence of fire in many forests has led to the formation of dense midstories, so fire alone may not be a successful management tool. I inventoried oak-dominated stands in the Hoosier and Wayne National Forests in southern Indiana and Ohio, respectively, both without a known burn history (n = 16) and with at least one prescribed fire since 1990 (n = 47). Across all sites, all oak and hickory (*Carya* spp.) midstory trees (10 cm < DBH < 25 cm) averaged 0.65 ± 0.003 m^2 ha^{-1} basal area of stocking, while sugar maple midstory averaged 0.87 ± 0.001 m^2 ha^{-1} basal area. Midstory basal area and composition significantly differed among sites with varying numbers of prescribed burns and years since the last prescribed burn. Random forest analysis indicated that several overstory stand structure variables were often more important than burn variables when predicting midstory basal area and composition. Prescribed fire appears to differentially affect midstory compositional groups, i.e., favoring oak and hickory over mesophytic species, but these effects may not be strong enough to mitigate the effects of mesophication. If prescribed fire is kept off the landscape for more than four years, direct treatments of the midstory and/or overstory thinning may also be needed.

3.2 Introduction

In response to oak (*Quercus* spp.) replacement by maples (*Acer* spp.) and other mesophytic species throughout most of their range in eastern North America, land managers are increasingly prescribing fire to arrest this transition (Dey and Schweitzer 2018). Prescribed burning partially mimics the fire disturbance pattern that likely produced these oak-dominated forests in many areas (Aldrich et al. 2005, Crow 1988, Crow et al. 1994, Lorimer 1993). The lack of fire disturbance in these forests has led to what Nowacki and Abrams (2008) term 'mesophication'. Essentially, many

previously or currently oak-dominated ecosystems are caught in a positive feedback loop in which the local conditions are increasingly favoring the growth and maturity of shade tolerant competitors, including maple and beech (*Fagus*). By withholding periodic surface fire from these ecosystems, these forests are becoming more resistant to reinstatement of fire regimes, even in light of climate change (Nowacki and Abrams 2008, Abrams 2005, Abrams 1992, Lorimer 1985). For example, maple leaves trap higher amounts of moisture and decompose more quickly than those of oak (Palus et al. 2018), making fuelbed conditions challenging to successfully carry a prescribed burn through a maple-dominant forest. Without disturbances to create small canopy gaps, remove midstory competitors, and allow sunlight to reach the seedling layer, shade tolerant maple will continue to outcompete shade intolerant oak, reach maturity and shift oak ecosystems into an alternate "maplized" state (Abrams 2005, Nowacki and Abrams 2008).

The current forest structure of Central Hardwood Region (CHR) forests, with oak dominant in the canopy, but maple dominant in the midstory, is thought to be novel (Nowacki and Abrams 2008) and managers are unsure about the best way to proceed. When wind-damaged overstory trees left large gaps in a similarly structured forest in Alabama, the midstory maple was recruited into the overstory canopy (White et al. 2015). Maple and beech midstory trees can also quickly reorient their stems after a disturbance to take advantage of patches with increased light levels (Collet et al. 2011), so to prevent a higher mesophytic species presence in the overstory it is clear that the midstory itself should be targeted in stands undergoing mesophication. However, a more recent study recommended that multiple fires be conducted in concert with overstory thinning to boost oak recruitment to the midstory (Izbicki et al. 2020). This contradiction is likely due to what is at the core of mesophication: shade tolerance. The amount of light that reaches the forest floor in undisturbed oak forests is insufficient for recruiting oak to maturity (Cunningham et al. 2011), and the heterogeneous stand-level results of prescribed fire may be exactly what is needed to break the cycle of mesophication without releasing maples to the overstory.

While prescribed fire may be the preferred disturbance to target midstory species composition, conflicting results make it difficult to predict exactly how stand structure will be altered. From their meta-analysis, Brose et al. (2013) determined that more than two prescribed surface fires sufficiently decreased overall midstory density on most sites. Blankenship and Arthur (2006) also found that oak and maple midstory basal area and density decreased in stands burned at least twice; however, midstory red maple (*A. rubrum*) recovered within four years, while

midstory oak species did not. More concerningly, Arthur et al. (2015) found that while midstory maples suffered more crown dieback than oak species, the maples also had a higher probability of survival after a prescribed fire and dominated the midstory layer even after four burns over eight years. In contrast to these results, on sites in southeastern Australia, Blair et al. (2016) concluded that logging has a larger effect on midstory species presence than even severe surface fires, supporting the notion that mesophytic species may be too established to be overwhelmingly affected by prescribed surface fire. This leads to a concern that if prescribed fire does not successfully reduce midstory interference, repeated application of fire may only accelerate succession and release midstory maples to the canopy.

This study determines the relationship between prescribed fire, existing stand structure and characteristics, and midstory structure in the CHR. I inventoried mid-/overstory data from 63 sites that differed in prescribed fire history, ranging from no fires to six fires since 1990. I hypothesized: 1) that repeat prescribed fires will decrease overall midstory basal area (BA); 2) that shade tolerance will differentially affect midstory BA as time since the last burn increases; and 3) that topographic and stand structural variables will strongly mediate the effects of prescribed fire on midstory composition.

3.3   Methods

3.3.1   Study Sites

The sites for this study were located on the Hoosier National Forest (HNF) in southern Indiana and the Wayne National Forest in southern Ohio (WNF). The stands used in this study were the same as those described in Chapter 2, as measurements for both objectives were collected simultaneously. Below is a brief description of the study sites, but refer to Section 2.3.1 for a more detailed description.

The ~ 80,000 hectare HNF is located in the unglaciated Shawnee Hills and Highland Rim Natural Regions of southern Indiana (Woodall et al. 2007, Homoya et al. 1985). The bedrock is mostly sandstone-shale, and topographic features include deep stream valleys, wooded hillsides, rock bluffs, and sinkholes produced by the breakdown of limestone bedrock (Ponder Jr. 2004). Prior to European settlement leading the forest to be heavily cut in the 19th and early 20th centuries, the HNF was mainly deciduous and dominated by oak (*Quercus*), hickory (*Carya*),

beech (*Fagus*), and aspen (*Populus*) (Ponder Jr. 2004). The current second-growth forest mostly consists of white oak (*Q. alba*), northern red oak (*Q. rubra*), shagbark hickory (*C. ovata*), and pignut hickory (*C. glabra*) upslope and sugar maple (*Acer saccharum*) and American beech (*F. grandifolia*) on more mesic sites (Homoya et al. 1985). Prescribed fire has been used to promote oak and hickory regeneration, reduce fuel loads, and restore barren habitats (Mann et al. 2020, Stanis et al. 2019).

The WNF comprises ~ 100,000 ha of land in the unglaciated Allegheny Plateau in southern Ohio (Palus et al. 2018). Topographic features include high hills, rocky ridges, and narrow valleys while the bedrock is mostly sandstone, siltstone, and shale (Sutherland et al. 2003). Similar to the HNF, the once oak-dominated forests were heavily harvested in the 1800s to early 1900s and have been undergoing secondary succession. The overstory is currently comprised of white, chestnut (*Q. montana*), and black (*Q. velutina*) oak, various hickories, and red (*A. rubrum*) and sugar maple (Yaussy et al. 2003). Prescribed burns are also used here to promote understory oak regeneration.

3.3.2   Plot Selection

I selected 47 stands with a history of prescribed fire and 16 unburned control stands across the HNF and WNF. Stands had relatively homogeneous species composition, aspect, and age structure, and were generally not larger than 20 hectares. Each stand was dominated (>50% basal area) by oak and hickory species and had merchantable timber with an average diameter at breast height (DBH) of at least 25 cm. Stands were classified both by burn class, i.e. the number of prescribed burns the stand had received since 1990, and by the predominant aspect.

Each stand was sampled with 15 points, as described in Stanis et al. (2019) and Mann et al. (2020). At least 8/15 points were on slopes with aspects corresponding to the predominant stand aspect and points were placed at least 30 m away from each other. More details on plot selection and point placement can be found in Section 2.3.2.

3.3.3   Data Collection

Sampling methods were loosely based on the SILVAH protocol for collecting data on oak regeneration in the Allegheny Mountain region (Brose et al. 2008). At each point, one 201 m^2 midstory plot, three 10.2 m^2 reduced-midstory subplots, and three 3.8 m^2 regeneration subplots

were installed (Fig. 2.1). Data were collected concurrently with the study described in Chapter 2; a more detailed description of data collection methods can be found in Section 2.3.3.

This study utilizes only the data collected in the midstory plot. I recorded the species and DBH of each individual with a DBH between 10 and 25 cm, and estimated the percent cover, to the nearest 5%, for each taxa group comprising the groundcover of the entire midstory plot (i.e. briar/thorny shrubs, shrubs, forbs, grasses, herbaceous vegetation, moss, sedges, tree seedlings, and rocks/bare ground).

3.3.4 Data Preparation

Rare species, i.e., those that occurred less than 10 times in total across all stands, were removed from the dataset. Total midstory basal area (BA), shade tolerant species midstory BA, and shade intolerant species midstory BA were then calculated for each stand (Table B1). Oak BA was calculated as the total midstory BA of oak species found on the study sites; mesic BA was calculated as the total midstory BA of the most common mesophytic species, i.e., American beech, sugar maple, and red maple. Overstory BA, trees per acre, and oak site index (base age 50) were estimated based on Stanis et al. (2019) and Mann et al. (2020).

To simplify analysis, site index, trees per acre, and percent slope were classified into "low", "medium", and "high" categories based on the first quartile, median, and third quartile of their respective distributions. "Low" classes comprised all values up to the first quartile, "medium" classes covered the first to the third quartile, and "high" classes included all values higher than the third quartile (see also Table B1). Aspect was classified into north (north and east aspects) and south (south and west aspects) based on the aspect classification system of the whole stand (Stanis et al. 2019).

Due to the unbalanced study design, sites were reclassified into aggregated classes based on the number of years since the last burn: one year, two to three years, four to seven years, and eight to ten years. These classes represent immediate, short-, medium-, and long-term midstory mortality and basal area losses due to fire disturbance. Results for unaggregated data can be found in Appendix B.

3.3.5   Statistical Analysis

To test the effect of repeated burns (H1) and time since the last burn (H2) on midstory basal area and composition, I used one-way ANOVA and post-hoc Tukey's Honest Significant Difference multiple comparison test (Tukey HSD). The impact of repeat burns was tested by using burn number as a treatment and midstory BA, oak BA, and mesic BA as responses. The impact of the time since the last burn was tested by using years since last burn as a treatment and midstory BA, shade tolerant BA, and shade intolerant BA as responses.

I used two analytical approaches to test the effect of topographic and stand structural variables on the relationship between fire and midstory composition (H3). First, I used random forest regression trees with burn number, years since last burn, aspect, slope, overstory BA, trees per acre, site index as predictors and midstory BA, shade tolerant BA, and shade intolerant BA as response variables. Random forest is a nearly nonparametric method; the two defined parameters are the number of variables at each node and the total number of 'trees' in the forest (Liaw and Wiener 2002). Bootstrapping is used to improve classification, and there is a misclassification rate associated with out-of-bag observations to better measure the importance of predictors. Relative variable importance is also associated with each predictor (Cutler et al. 2007). Here, variable importance was calculated with a measurement of increase in node purity and mean square error (MSE). Mean square error is the most commonly used indicator of variable importance; predictors that have a high MSE value are more necessary for successfully classifying the model, and the accuracy of the model would decrease if this variable was removed. A higher increase in node purity value is assigned to a predictor that is more useful at predicting the response at each node split in the regression tree, leading to higher purity in the decision tree overall. Then, with these important variables, I used one-way ANOVA and Tukey HSD to further investigate the impact of important predictors on midstory BA, shade tolerant BA, and shade intolerant BA.

All analyses were completed using R 3.6.1 (R core team 2019) and the "randomForest" package (Liaw and Wiener 2002). Analyses used $\alpha = 0.05$.

3.4   Results

A total of 2,580 midstory trees were measured. Midstories averaged 139 ± 22.3 trees ha^{-1} (mean $\pm$ standard error) and 2.3 ± 0.009 m^2 ha^{-1} (Table 3.1); this represents 217% and 10.8% of the total

overstory stand density and basal area, respectively. Sugar maple had by far the highest average basal area and stem density in each stand (0.87 ± 0.001 m^2 ha-1 and 57.6 ± 9.8 trees ha^{-1}; Table 3.1). Hickory species (0.29 ± 0.002 m^2 ha^{-1} and 16.5 ± 2.9 trees ha^{-1}) and white oak (0.36 ± 0.002 m^2 ha^{-1} and 16.1 ± 2.6 trees ha^{-1}) were the next two prevalent midstory species, but American beech (*Fagus grandifolia*; 0.11 ± 0.002 m^2 ha^{-1} and 8.0 ± 2.3 trees ha^{-1}), blackgum (*Nyssa sylvatica*; 0.13 ± 0.002 m^2 ha^{-1} and 9.1 ± 3.3 trees ha^{-1}), and other maples (0.11 ± 0.002 m^2 ha^{-1} and 6.8 ± 1.4 trees ha^{-1}) also contributed modestly to both midstory basal area and stem density (Table 3.1).

3.4.1   Effect of Burn, Topographic, and Stand Structural Variables on Midstory BA

Unburned control stands averaged 3.16 ± 0.09 m^2 ha^{-1}, more basal area than any other burn class, while stands burned four times had the least, 1.12 ± 0.29 m^2 ha^{-1} (Table 3.2). Burn number significantly affected overall midstory BA ($F_{5, 2578} = 203.7$, $p < 0.001$). All burn counts differed from each other except four and two burns and four and three burns ($p < 0.001$ for all, except $p = 0.004$ for two and three burns). Average midstory basal area generally decreased as burn number increased (3.16 ± 0.09 to 1.26 ± 0.20 m^2 ha^{-1}, from control plots to plots burned six times respectively), but especially dropped after three burns (Fig. 3.1).

Total midstory basal area was highest in stands burned four to seven years ago (2.68 ± 0.17 m^2 ha^{-1}) and lowest on sites burned one year ago (1.54 ± 0.12 m^2 ha^{-1}; Table 3.3). Years since the last burn significantly affected overall midstory BA ($F_{4, 2579} = 255.1$, $p < 0.001$). Unburned stands had higher values than all year classes ($p < 0.001$ for all); stands burned one year prior to sampling had the least ($p < 0.001$ for all). No other year classes differed significantly. The total basal area gradually increased as the year class increased, then decreased slightly eight to ten years post-fire (Fig. 3.3). The proportion of midstory basal area that was shade tolerant or intolerant stayed relatively constant as the year class increased, as each tolerance class had a range of about 10% (Fig. 3.4).

Random forest analysis identified overstory BA, years since burn, site index, burn number, and trees per acre as important predictor variables for midstory BA; aspect and slope were the least influential both in terms of node purity and reduction in variance (Fig. 3.5). In addition to burn number and years since burn, midstory BA was also significantly impacted by overstory BA ($F_{1, 2582} = 4.691$, $p = 0.030$), site index ($F_{2, 2581} = 6.336$, $p = 0.002$), and trees per acre ($F_{2, 2581} = 114.1$,

p < 0.001). As overstory BA increased, midstory BA generally decreased. Stands with a medium site index differed from those with high (p = 0.004) and low (p = 0.08) values, a high site index usually led to more midstory BA and a low site index lead to less. Stands with low, medium, and high trees per acre all differed from each other (p < 0.001), with midstory BA increasing as trees per acre decreased.

3.4.2 Effect of Burn, Topographic, and Stand Structural Variables on Midstory BA Composition

Mesic species basal area peaked on control sites (47.5%) and was lowest in stands burned twice (34.6%). Both oak species and mesic species basal area followed a similar pattern of decreasing until the third burn, then remained relatively steady (Fig. 3.2). Burn number also significantly affected mesic species midstory BA ($F_{5, 2578}$ = 127.9, p < 0.001). Control sites had more mesic species basal area than all other burn counts (p < 0.001 for all) and sites burned once also had more basal area than sites burned two, three, and six times (p < 0.001 for all). Additionally, stands burned six times had less basal area than stands burned three (p = 0.005) times.

The proportion of shade tolerant basal area was highest eight to ten years (55.3%) and lowest two to three years (45.4%) post-burn (Table 3.3). Years since burn significantly impacted shade tolerant species BA as well ($F_{4, 2579}$ = 156.1, p < 0.001). Unburned stands had the most shade tolerant basal area (p < 0.001 for all). Stands burned one year prior had less basal area than stands burned four to seven and eight to ten years before sampling (p < 0.001 for both), as did stands burned two to three years ago (p < 0.001 for both).

The most important random forest variables for predicting shade tolerant BA were overstory BA, burn number, site index, years since burn, and trees per acre; aspect had very low importance (Fig. 3.6). Each important predictor, except overstory BA, had a significant effect on shade tolerant BA. In addition to years since burn, shade tolerant BA was affected by burn number ($F_{5, 2578}$ = 134.3, p < 0.001), site index ($F_{2, 2581}$ = 74.48, p < 0.001), and trees per acre ($F_{2, 2581}$ = 56.08, p < 0.001). Burning at least once decreased shade tolerant BA: control stands and stands burned once differed from all other burn classes (p < 0.001 for all). However, stands burned six times had more basal area than stands burned two (p = 0.03), three (p < 0.001), and four (p < 0.001) times. Stands with low, medium, and high site index values all differed from each other with an increase in shade tolerant BA as site index increased (p < 0.001 for all). Stands with high trees per

acre supported more shade tolerant BA compared to stands with low and medium values ($p < 0.001$ for both).

Oak species basal area was proportionally highest in stands burned six times (35.2%) and lowest in stands burned twice (14.0%); however, mesic species had more midstory basal area than oak species in every burn number class (Fig. 3.2). Burn number significantly impacted oak species midstory BA ($F_{1, 436} = 262.6$, $p < 0.001$). Control stands had the most oak basal area ($p < 0.001$ for all) and stands burned once had less basal area than stands burned four and six times ($p < 0.001$ for both). Stands burned twice had less oak basal area than stands burned four and six times ($p < 0.001$ for both), as did stands burned three times ($p < 0.001$ for both).

Shade intolerant basal area peaked two to three years after fire (54.6%) and was proportionally lowest eight to ten years (44.7%) after a burn (Table 3.3). Years since burn also significantly affected shade intolerant species BA ($F_{4, 2579} = 40.16$, $p < 0.001$). Unburned stands had more shade intolerant basal area than stands burned one year prior ($p < 0.001$ for both). Stands burned one year ago had less basal area than all other higher year classes ($p < 0.001$ for all), but stands burned two to three years prior to sampling had the most basal area ($p < 0.001$).

Random forest analysis indicated that overstory BA, years since burn, trees per acre, burn number, and site index had the highest importance when predicting shade intolerant BA; aspect and slope again had minimal importance (Fig. 3.7). Each important random forest predictor had a significant effect on shade intolerant BA. In addition to years since burn, shade intolerant BA was significantly affected by overstory BA ($F_{1, 2582} = 26.12$, $p < 0.001$), burn number ($F_{5, 2578} = 14.55$, $p < 0.001$), site index ($F_{2, 2581} = 94.95$, $p < 0.001$), and trees per acre ($F_{2, 2581} = 75.01$, $p < 0.001$). The relationship between shade intolerant BA and overstory BA was negative. Burning at least six times increased shade intolerant BA, as plots burned six times differed from all other burn classes ($p < 0.001$ for all), but no other burn classes differed. Stands with low, medium, and high site index all differed from each other ($p < 0.001$), with a decrease in shade intolerant BA as site index increased. Low, medium, and high trees per acre stands also all differed from each other ($p < 0.001$ for low-high and low-medium, $p = 0.003$ for medium-high), and stands with a higher trees per acre generally supported less shade intolerant BA.

3.5   Discussion

3.5.1   Emergent Midstory Composition Changes

The midstory was mostly comprised of mesophytic species on my study sites (Table 3.1), aligning with the predicted compositional makeup of Nowacki and Abrams (2008). While oak and hickory were prevalent in the midstory, the basal area of all oak and hickory species combined (0.65 m^2 ha^{-1}) was less than that of just sugar maple. Abrams (2005) suggested that such a heavy mesophytic species presence may signal that the stand is past remediation by prescribed fire. Maple may be so heavily entrenched in the midstory that returning fire to these stands, as Abrams and Nowacki (1992) theorized, could actually accelerate succession and quickly allow midstory maple to release to the overstory.

3.5.2   Repeat Burns Decrease Midstory BA

Repeated prescribed fires did lead to a decrease in overall midstory basal area. Unburned stands had the highest total basal area, and basal area especially declined after three burns (Table 3.2, Fig. 3.1). This corroborates results reviewed by Brose et al. (2013); they reported that more than two prescribed burns decreased midstory stem density by 71%. Blankenship and Arthur (2006) also found that two and three prescribed fires reduced overall midstory BA by 86%. In this study, midstory basal area did not differ among stands burned two, three or four times, so it appears that any moderate number of burns may produce similar midstory impacts. Further there may be a cumulative effect of burning to decrease overall canopy cover (Alexander et al. 2008), including the overstory. Overstory losses could offset midstory impacts, leading to comparable results in stands with an intermediate amount of burns.

The overall decline in midstory basal area with additional burns was not dominated by the loss of any single species group, as I did not observe that repeat prescribed burns differentially affect oak species (oak BA) or mesic species (mesic BA). While oak BA was proportionally highest in stands burned six times and mesic BA was highest in control stands (Fig. 3.2), mesic species still had a higher absolute basal area in every burn class. These results may seem to be contradictory to the oak-fire hypothesis (Arthur et al. 2012), but successful oak regeneration is not always affected by absolute midstory density and thus a relative increase in midstory oak can be seen as a departure from mesophication. Likewise, Arthur et al. (2015) found that repeat burns do

71

not necessarily reduce mesophytic species in favor of oak due to the minimal stand structural changes caused by burning. Clark and Schweitzer (2016) also noted that while burning may improve overall forest health, it has rarely been successful in creating a sparse midstory layer. Furthermore, in South Carolina stands that were burned yearly, Waldrop and Lloyd (1991) found that midstory species composition hardly changed throughout the duration of the decades-long study.

Similar to the effect of years since burn on tolerant and intolerant BA, total burn number also differentially impacted midstory basal area. Control stands and stands burned once produced higher levels of shade tolerant basal area when compared to every other burn number, however only stands burned six times lead to higher levels of shade intolerant basal area. This means that stands can be burned only twice to have a negative impact on shade tolerant basal area, whereas stands must be burned six times to decrease shade intolerant basal area. This difference is again likely due to the nature of mesophication (Nowacki and Abrams 2008) working in concert with the disturbance ecology of maple versus oak. Oak, especially when juveniles, are better adapted to fire compared to shade tolerant species (Clatterbuck 1998). And while the current mesophytic, shade tolerant midstory is not adapted to fire, due to the overwhelming presence of these species, four or more burns may be needed before the basal area begins to decline in favor of shade intolerant species. The second most important predictors after overstory BA for shade tolerant species are site index and burn number, so it is apparent that total burn number does have a large effect on shade tolerant species basal area.

3.5.3 Shade Tolerance Differentially Affected by Time Since Last Burn

Overall midstory basal area patterns were driven by differences in shade tolerant and intolerant basal area response to prescribed fire over time. Out of the burned stands, stands burned four to seven years prior to sampling had the highest overall midstory basal area and stands burned one year ago had the lowest (Table 3.3, Fig. 3.3), suggesting that midstory mortality or crown dieback starts to occur soon after the fire disturbance. Arthur et al. (2015) found that midstory maple species suffered crown dieback up to eight years post-burn, which appears to be a more extended timeline than seen here as midstory basal area begins to recover as soon as two to three years post-fire.

The proportion of tolerant to intolerant midstory basal area remained relatively constant as years since last burn increased (Fig. 3.4), however years since burn affected shade tolerant and intolerant basal area differently. Shade tolerant basal area was highest at least four years after a burn, suggesting that shade tolerant midstory species may lose the most basal area in those first four years after fire. These results are similar to the findings of Blankenship and Arthur (2006), where they found that midstory maple stem density recovered from a prescribed fire after four years but a similar rebound was not seen for oak species. Conversely, for shade intolerant species, stands burned two – three years prior had the most basal area, suggesting that the first three years post-burn may be the best opportunity for the growth of intolerant midstory species. This could signal a shift to an environment more conducive to tolerant tree species, as the opposite pattern was described above for shade tolerant species basal area.

3.5.4   Midstory BA Affected by Overstory Structure More Than Burns

The only important variables when using random forest models to predict overall midstory BA, shade tolerant midstory BA, and shade intolerant midstory BA were the stand structure and burn variables; topographic variables were not important. This was somewhat surprising; slope, for example, is known to influence fire behavior, which in turn influences how locally severe a fire becomes (Pyne et al. 1996). As fire moves up a slope, it pre-heats leaf litter and other highly flammable material leading to a faster spread and more intense effects (Barnes and Van Lear 1998). Aspect should also have a similar effect on fire behavior; more xeric ridgetops and south-facing slopes usually host a more intense fire than mesic valleys and north-facing slopes (Pyne et al. 1996). Shade tolerant species common in the CHR are usually found in the more protected, mesic areas and shade intolerant in more exposed, xeric sites. Tolerant species, thereby, escape the most severe prescribed burns by their local environmental preferences (Hodges and Gardiner 1993). However, aspect does not always influence prescribed fire behavior in the CHR enough to damage mature overstory trees (Stanis et al. 2019; Mann et al. 2020; Saunders et al. in prep); it appears this pattern also extends to midstory-sized stems.

Overstory BA was the most important predictor for midstory BA, shade tolerant midstory BA, and shade intolerant midstory BA (Figs. 3.5, 3.6, 3.7); however, overstory BA did not significantly affect shade tolerant midstory BA and was the only important random forest predictor not found to have a significant relationship with a response. Shade tolerant CHR midstory species

can often grow under a closed canopy and have the ability to quickly take advantage of new sources of light (Valladares and Niinemets 2008). Their physiology allows them to survive in low-light environments, so the amount of overstory basal area does not strongly influence the amount of shade tolerant midstory basal area. Overall midstory BA and shade intolerant BA were still significantly affected by overstory BA, however. Willson et al. (2020) found that an increase in canopy openness, leading to more light reaching the forest floor, translates to increased midstory density, which is likely to especially occur with overall and shade intolerant basal area. Site index and trees per acre significantly affected all classes of midstory BA tested, though, so it is clear that mature stand structure does heavily impact midstory BA regardless of shade tolerance status. Generally, as site index increased so did midstory basal area, but as trees per acre increased midstory basal area decreased. This is likely due to stand growth dynamics, as a high site index can help promote establishment and growth but once the trees per acre reaches a threshold then many midstory trees are out-competed for light, water, and nutrients.

3.6   Conclusion

Forest managers are returning prescribed fire to landscapes to mimic natural disturbance patterns and attempt to break the cycle of mesophication (Aldrich et al. 2005, Crow 1988, Crow et al. 1994, Lorimer 1993, Nowacki and Abrams 2008). Surface fire affect midstory basal area differentially by species groups and in variable stand structures. In fact, stand structure is as, or perhaps more, important a predictor of midstory basal area than is the total number of prescribed burns or year since the last burn. The fact that stand structure is so important in influencing midstory basal area suggests that if stands are managed in a one-size-fits-all, instead of holistic, approach the desired results may never be met.

My results suggest that promoting oak regeneration with prescribed fire alone is unlikely to succeed. Many CHR stands have had such a long period of fire exclusion that the forest structure is now resistant to the effects of prescribed fire; for example, mesophytic species became more prevalent in the midstory canopy than oak species even after six burns, and particularly if return intervals between prescribed fires extended beyond 3-4 years. Additional mechanical treatments to the forest floor, midstory and/or overstory may need to be taken to secure oak regeneration and eventual recruitment. For example, scarification has been shown to greatly reduce a maple-dominant midstory and promote oak seedling regeneration (Lhotka and Zaczek 2003); midstory

removal with the purpose of regenerating seedlings has also been used to great success (Craig et al. 2014, Lhotka and Loewenstein 2009, Motsinger et al. 2010, Parrott et al. 2012). Overstory thinning is also commonly used to reach different management objectives (Navarro et al. 2010, Brudvig et al. 2011, Yanai et al. 1998), but care should be taken in the CHR to ensure under- and midstory maple have been cleared before reducing the overstory density or maple may be released to maturity (Chiang et al. 2008).

3.7   References

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3.8   Tables and Figures

Table 3-1 Summary of common (n > 10) midstory (10-25 cm DBH) tree species measured. Shade tolerance was estimated using Burns and Honkala (1990a,b). N is the total number of stems measured in all stands, DEN, DBH and BA represent the mean ± standard error stand-level stem density, diameter at breast height and basal area for each species. The TOTAL line provides a sum of the various structural variables (n, DEN, BA) or stand-level averages for DBH.

Species	Latin name	Shade tolerance	n	DEN (stems ha^{-1})	DBH (cm)	BA (m^2 ha^{-1})
American beech	*Fagus grandifolia*	tolerant	149	8.0 ± 2.3	15.0 ± 0.3	0.11 ± 0.002
American elm	*Ulmus americana*	intolerant	40	2.2 ± 2.0	14.5 ± 0.7	0.03 ± 0.004
American hornbeam	*Carpinus caroliniana*	tolerant	10	0.5 ± 0.5	15.1 ± 1.1	0.01 ± 0.007
Black cherry	*Prunus serotina*	intolerant	11	0.6 ± 0.6	17.1 ± 1.5	0.01 ± 0.010
Black oak	*Quercus velutina*	intolerant	44	2.4 ± 1.8	17.2 ± 0.6	0.04 ± 0.004
Blackgum	*Nyssa sylvatica*	tolerant	169	9.1 ± 3.3	15.2 ± 0.3	0.13 ± 0.002
Bur oak	*Q. macrocarpa*	intolerant	11	0.6 ± 0.6	19.5 ± 0.8	0.01 ± 0.006
Chestnut oak	*Q. prinus*	intolerant	55	3.0 ± 2.1	18.9 ± 0.6	0.06 ± 0.004
Eastern red cedar	*Juniperus virginiana*	intolerant	25	1.3 ± 1.1	17.1 ± 0.9	0.02 ± 0.006
Flowering dogwood	*Cornus florida*	tolerant	22	1.2 ± 1.0	13.4 ± 0.8	0.01 ± 0.005
Hickory	*Carya* spp.	intolerant	307	16.5 ± 2.9	17.1 ± 0.2	0.29 ± 0.002

Table 3-1 continued

Ironwood	***Ostrya virginiana***	**tolerant**	**18**	**1.0 ± 1.0**	**12.9 ± 0.6**	**0.01 ± 0.003**
Northern red oak	*Q. rubra*	intolerant	29	1.6 ± 1.3	20.1 ± 0.6	0.04 ± 0.005
Sassafras	*Sassafras albidum*	intolerant	36	1.9 ± 1.5	14.8 ± 0.7	0.03 ± 0.004
Sugar maple	*Acer saccharum*	tolerant	1,070	57.6 ± 9.8	15.8 ± 0.1	0.87 ± 0.001
Other elms	*U. rubra* and *U. thomasii*	tolerant	14	0.8 ± 0.8	14.2 ± 0.6	0.01 ± 0.003
Other maples	*A. saccharinum* and *A. rubrum*	tolerant	126	6.8 ± 1.4	16.1 ± 0.3	0.11 ± 0.002
Tulip poplar	*Liriodendron tulipifera*	intolerant	72	3.9 ± 1.6	18.7 ± 0.5	0.08 ± 0.004
White ash	*Fraxinus americana*	intolerant	74	4.0 ± 1.6	16.4 ± 0.5	0.07 ± 0.003
White oak	*Q. alba*	intolerant	299	16.1 ± 2.6	19.4 ± 0.2	0.36 ± 0.002
TOTAL			**2,580**	**139 ± 22.3**	**16.5 ± 0.5**	**2.3 ± 0.009**

Table 3-2 Summary statistics categorized by burn number. Stands = number of stands that have been burned that many times; basal area = mean total midstory BA (m^2 ha^{-1}) ± SE for a stand; % oak spp. = percentage of total midstory BA that is oak BA; % mesic spp. = percentage of total midstory BA that is mesic BA.

Burn number	0	1	2	3	4	6
Stands	16	10	5	19	6	7
Basal area (m^2 ha^{-1})	3.16 ± 0.089	2.79 ± 0.131	2.14 ± 0.205	2.19 ± 0.103	1.12 ± 0.291	1.26 ± 0.202
% oak spp.	25.9	15.8	14.0	21.2	25.4	35.2
% mesic spp.	47.5	46.6	34.6	36.8	40.3	37.5

Table 3-3 Summary statistics categorized by time since the last burn category. Stands = number of stands that have been burned that many times; basal area = mean total midstory BA (m^2 ha^{-1}) ± SE for a stand; % shade tolerant = percentage of total midstory BA that is tolerant BA; % shade intolerant = percentage of total midstory BA that is intolerant BA.

Years since last burn	1	2 to 3	4 to 7	8 to 10
Stands	20	10	6	11
Basal area (m^2 ha^{-1})	1.54 ± 0.119	2.45 ± 0.132	2.68 ± 0.172	2.22 ± 0.142
% shade tolerant	49.9	45.4	53.9	55.3
% shade intolerant	50.1	54.6	46.1	44.7
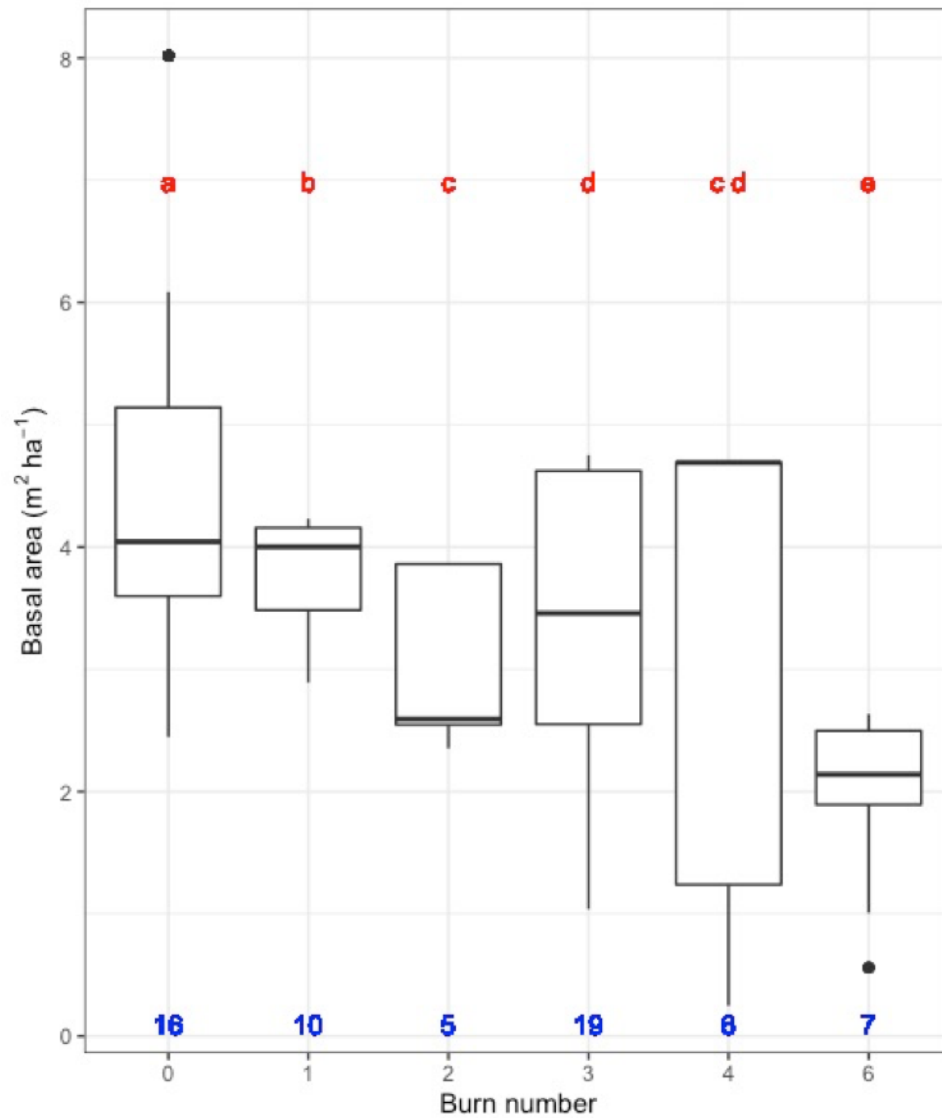
Figure 3-1 Box plot displaying average midstory BA per stand by total number of burns. Stand sample sizes for each burn class are shown in blue below each box, and pairwise comparison statistical significance is represented by red letters above each bar.
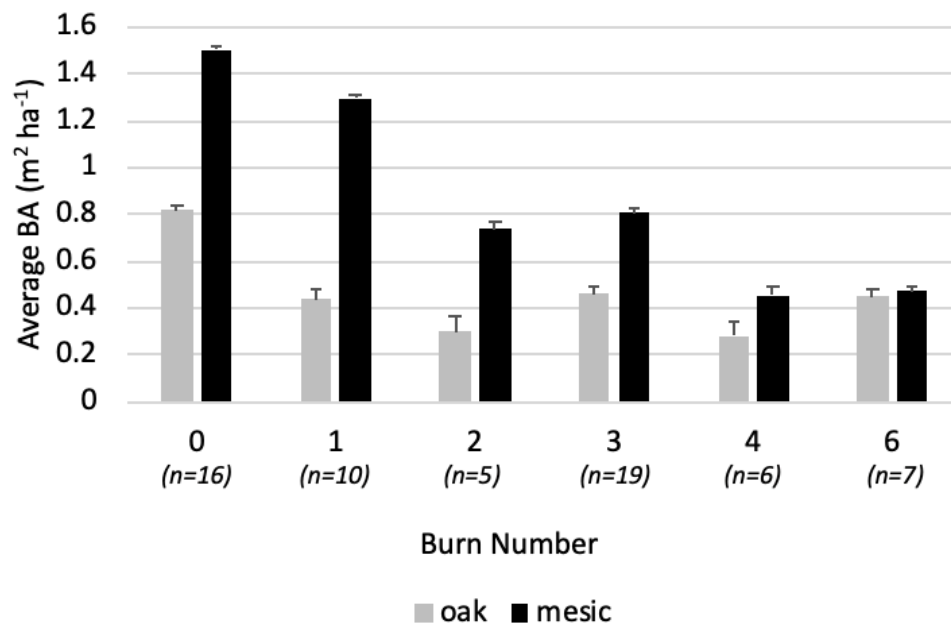
Figure 3-2 Average midstory BA per stand for oak spp. and mesic spp. displayed by total burn number. Stand sample sizes for each burn class are shown in italics below each bar, and error bars are displaying the standard error of the mean.
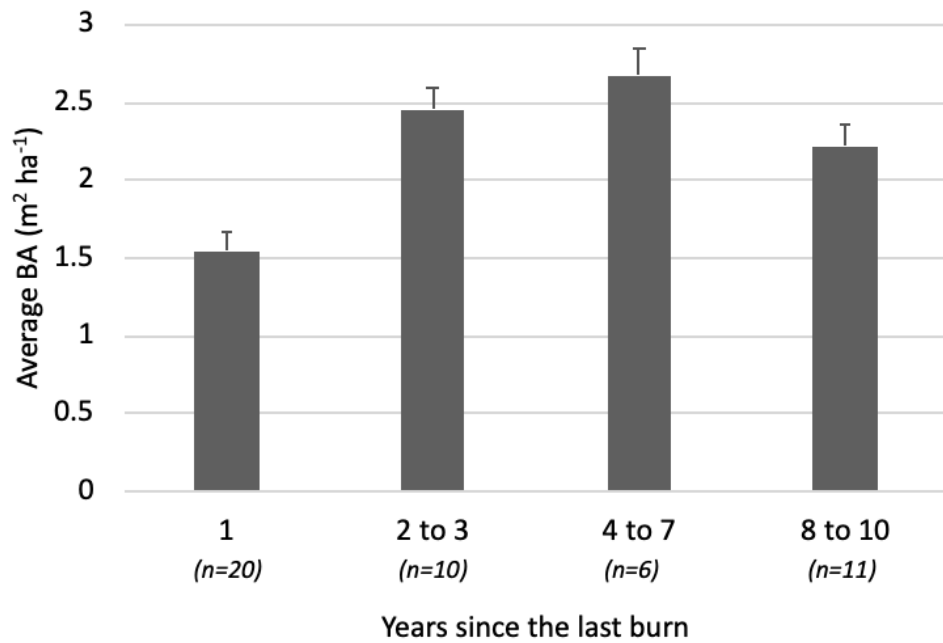
Figure 3-3 Average midstory BA per stand displayed by the number of years since the last burn class. Stand sample sizes for each year category are shown in italics below each bar, and error bars are displaying the standard error of the mean.
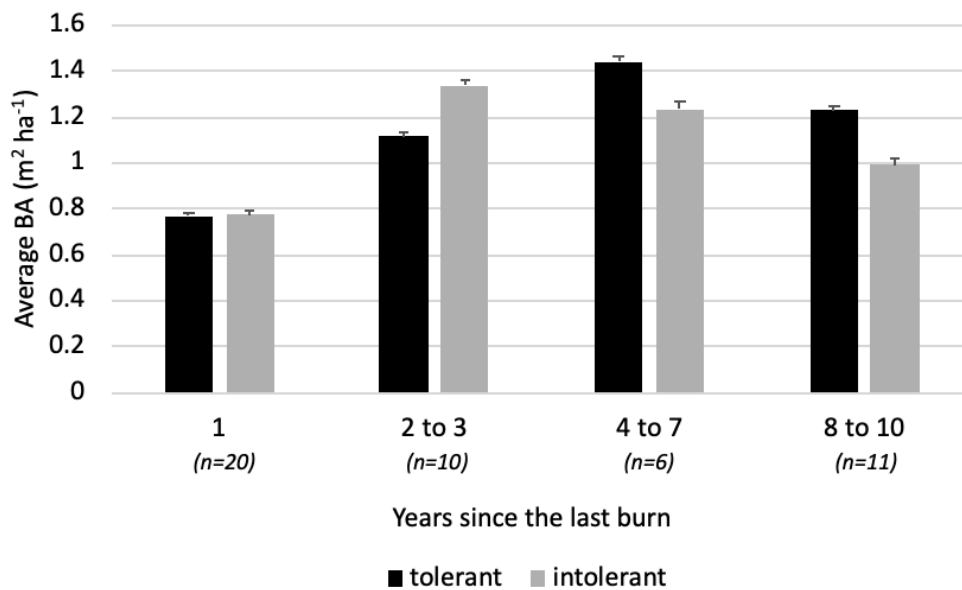
Figure 3-4 Average midstory BA per stand for shade tolerant and intolerant species displayed by the number of years since the last burn class. Stand sample sizes for each year category are shown in italics below each bar, and error bars are displaying the standard error of the mean.
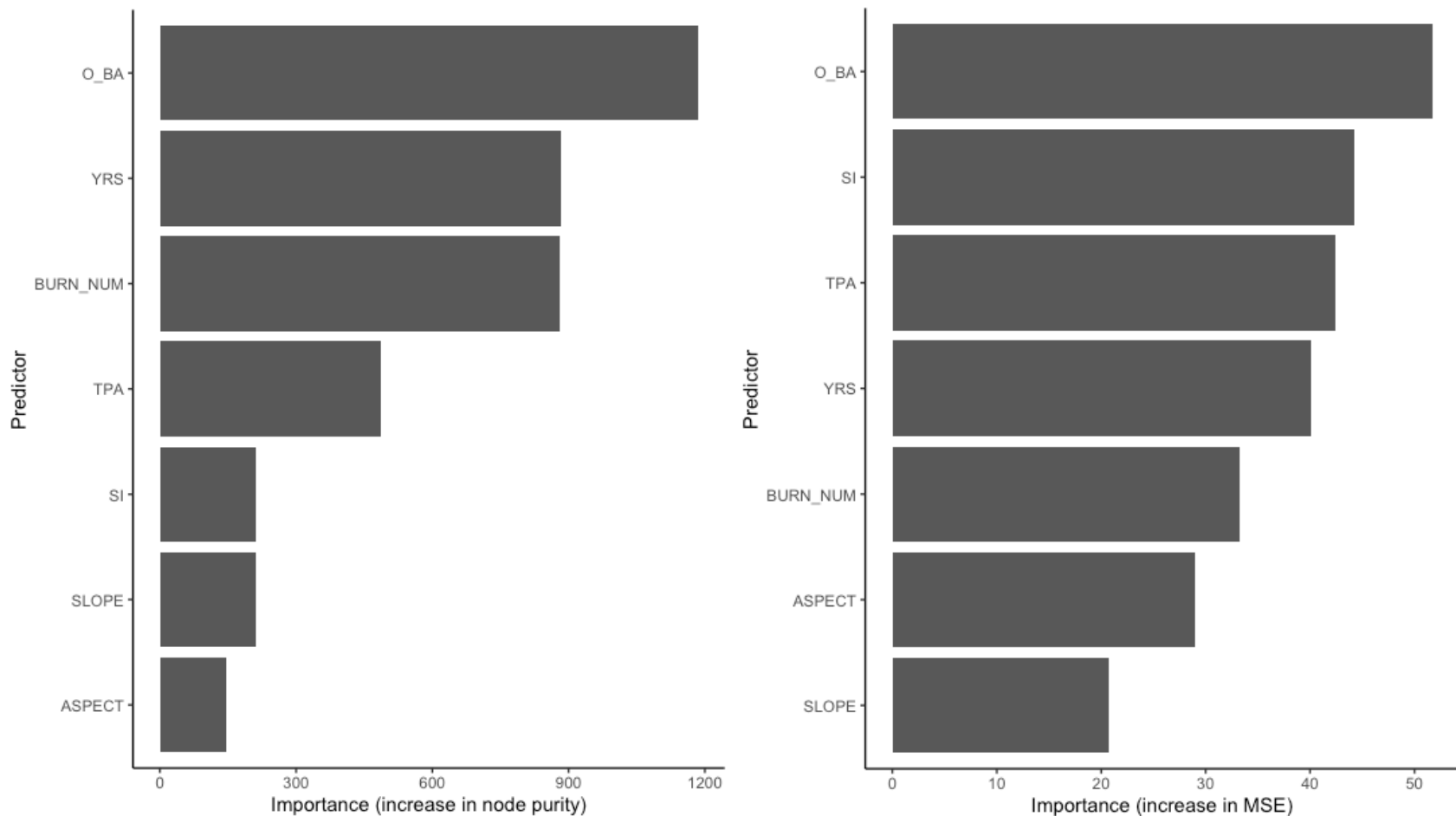
Figure 3-5 The importance of each predictor variable, measured by the increase in node purity on the left and increase in mean square error (MSE) on the right, on the response of total midstory BA. Predictor variables, as described in Section 3.3.4, are: SLOPE = slope class, ASPECT = aspect class, O_BA = total overstory basal area, YRS = number of years since the last burn, BURN_NUM = total number of burns, TPA = overstory density class, and SI = site index class.
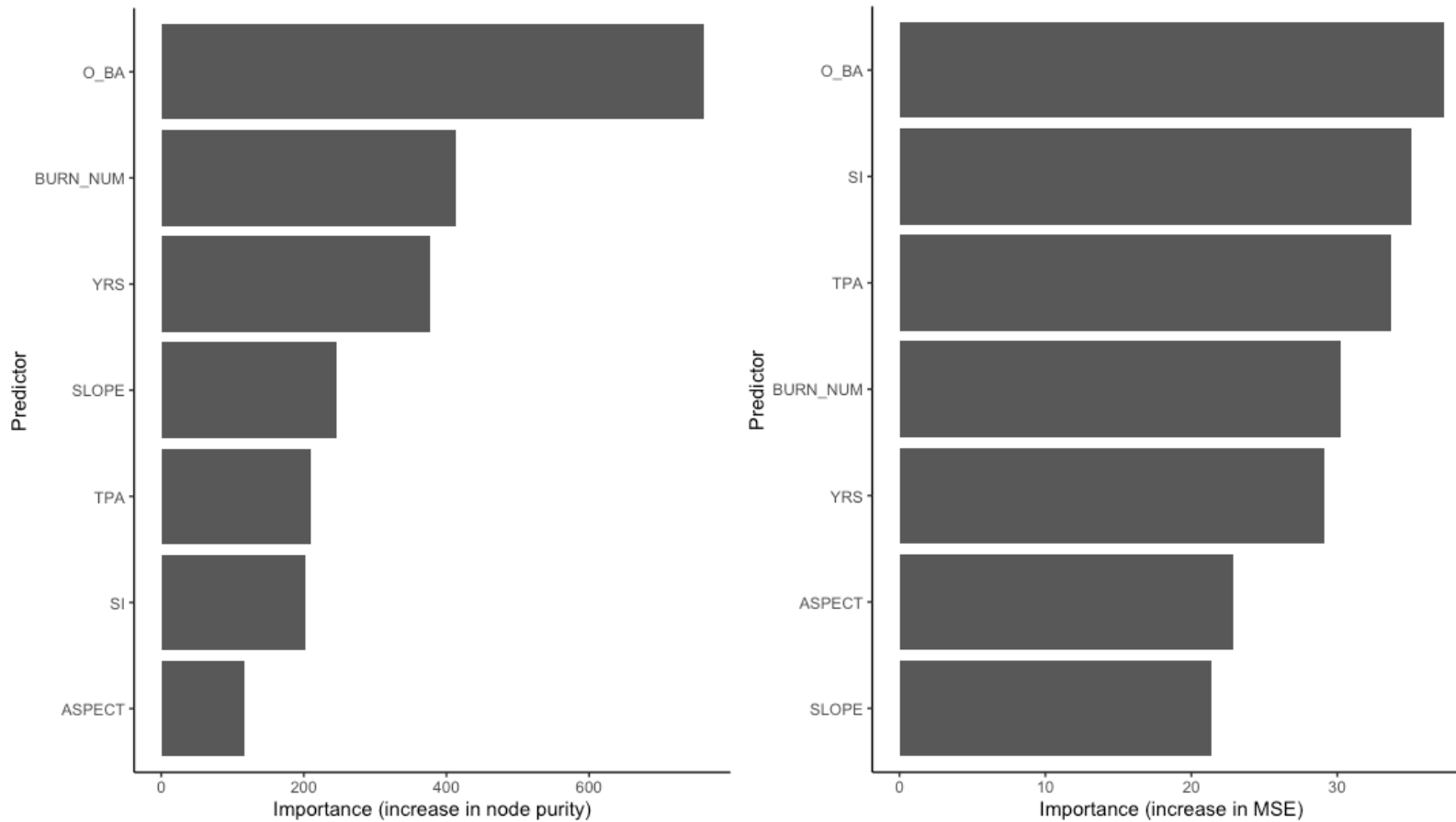
Figure 3-6 The importance of each predictor variable, measured by the increase in node purity on the left and increase in mean square error (MSE) on the right, on the response of shade tolerant midstory BA. Predictor variables, as described in Section 3.3.4, are: SLOPE = slope class, ASPECT = aspect class, O_BA = total overstory basal area, YRS = number of years since the last burn, BURN_NUM = total number of burns, TPA = overstory density class, and SI = site index class.
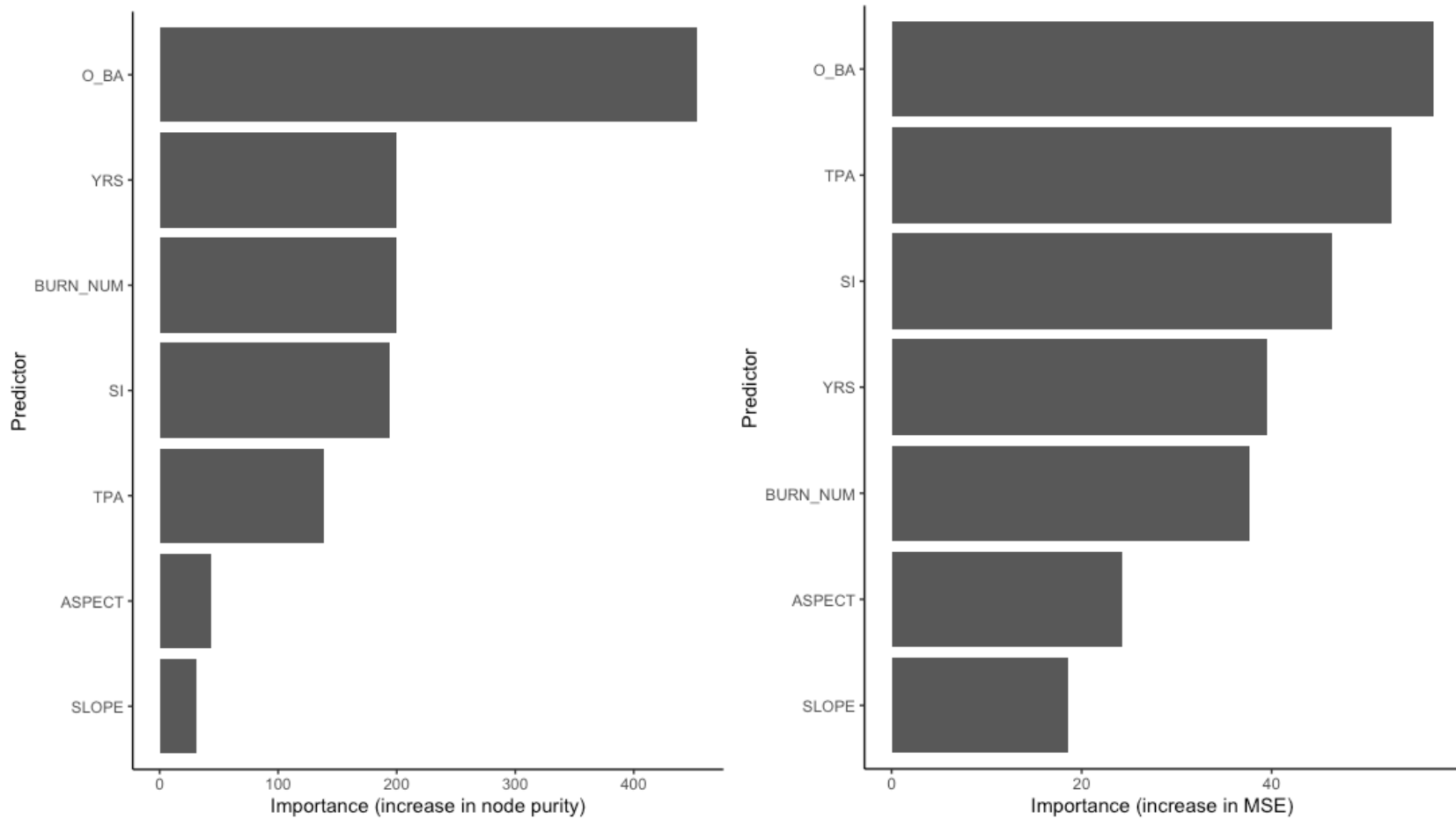
Figure 3-7 The importance of each predictor variable, measured by the increase in node purity on the left and increase in mean square error (MSE) on the right, on the response of shade intolerant midstory BA. Predictor variables, as described in Section 3.3.4, are: SLOPE = slope class, ASPECT = aspect class, O_BA = total overstory basal area, YRS = number of years since the last burn, BURN_NUM = total number of burns, TPA = overstory density class, and SI = site index class.

CHAPTER 4. MYCORRHIZAL HYPOGEOUS FRUITING BODY SPORE ABUNDANCE AND COMPOSITION IN EASTERN CHIPMUNK SCAT RESPONSE TO THE USE OF PRESCRIBED FIRE IN THE CENTRAL HARDWOOD REGION

4.1 Abstract

Mycorrhizal fungi are known to affect composition, productivity, and biodiversity of many forest types, but the effects of forest management on the relationships among mycorrhizae, trees, and small mammal dispersal agents are not well studied. For example, prescribed surface fire and overstory harvests are being conducted in many oak-dominated (*Quercus* spp.) forests in the Central Hardwood Region to favor oak regeneration, yet it is currently unknown how these fires will affect their fungal symbionts, particularly the mycorrhizal dispersal pathways which are often mediated by small mammals. These mammals consume the fruiting body of the fungi, i.e. truffles, and disperse spores elsewhere, allowing the mycorrhizal network to spread and benefitting host trees. I extracted fungal spores from eastern chipmunk (*Tamias striatus*) scat collected across the Hardwood Ecosystem Experiment from trapping grids (n = 12) in oak-dominated stands without a recent burn history (n = 33 trap stations) and with one burn since 2015 (n = 32 trap stations). I identified 17 fungal taxa in 65 samples; the most commonly found taxon was a clear *Glomus* spore morphotype that was found in 56.9% samples, but 70.6% of taxa were found in at least 10% of all samples. Arbuscular mycorrhizal taxa richness was not affected by any measured variable, but ectomycorrhizal taxa richness decreased after burning. However, local environmental variables including litter layer depth, EM tree basal area, and AM tree basal area each helped explain some variation in spore composition and were correlated with fungal community composition in scat. Prescribed surface fire appears to differentially affect the consumed truffles of mycorrhizal functional groups, and more research will be needed to investigate fire's impact on EM fungi and their oak hosts.

4.2 Introduction

Over 80% of terrestrial plant species and 99% of flowering plants are thought to form relationships with mycorrhizal fungi (Brundrett 2009), fungal associates that assist plants in water and nutrient uptake. A mycorrhizal relationship can be more specifically defined as a mutualism in which both

organisms involved in the association benefit (Marks and Foster 1973); fungal hyphae explore soil for nutrients and, in return, receive carbohydrates from plants (however, occasionally the carbon flow is reversed; Beiler et al. 2010). As the earth's most abundant plant mutualist, it has been estimated that kilometers of mycorrhiza hyphal strands can be found in just one gram of many soils (Reinhart et al. 2012). The two most common fungal symbionts worldwide are arbuscular mycorrhizae (AM) and ectomycorrhizae (EM), both of which can produce hypogeous fruiting bodies, colloquially called truffles (Maser et al. 1978). Notably, in the eastern deciduous forest of North American, oak (*Quercus*) species are most commonly EM, while their understory maple (*Acer*) competitors are mostly AM; this may help explain oak's superior ability to thrive in more nutrient-poor environments than maple (Valverde-Barrantes et al. 2018).

Underground fungal diversity of a forest is likely much higher than the diversity of vascular plants, and integral to the functioning of the entire forest ecosystem (van der Heijden et al. 1998). For example, mycorrhizae create an underground hyphal mat; when several trees are connected through the same mycorrhizal association, that is called a common mycorrhizal network (Simard et al. 1997). These networks heavily influence local tree community structure, can help facilitate seedling recruitment, and can determine the survival and establishment rate of seedlings (Courty et al. 2010). Common mycorrhizal networks can also be useful when restoring ecosystems, like oak forests in the Central Hardwood Region, as inoculation can increase similarity between restored plant and reference communities, and may increase plant species richness in those communities by 30% (Neuenkamp et al. 2019).

Truffles remain closed underground until animals (mycophagists) retrieve them, consume the spores, and disperse these spores away from the original site of consumption (Fogel and Trappe 1978). Mycophagy is part of an important ecological phenomenon delivering mycorrhizal inoculum to seedlings otherwise germinating outside the root zone of the nearby mature trees. It would be nearly impossible for many of these seedlings to become established and grow into the overstory if they did not make mycorrhizal associations early in life (Frank et al. 2006). Most mycorrhizae are obligate symbionts, meaning they must be attached to roots to survive; ectomycorrhizae especially are not found in open, isolated soil patches (Dickie and Reich 2005, Strullu-Derrien et al. 2017). Obligate symbiosis influences forest resilience as well, since aboveground recovery after a disturbance is linked directly to the persistence of EM, and recovery

of these EM communities is linked to their ability to survive the disturbance and their recolonization ability (Smith et al. 2004).

Therefore, an important part of the tree-mycorrhiza relationship is the fungal dispersal agent, which is often rodents and other small mammals. Small mammals can be both fungal specialists or generalists, but specialists often consume and disperse a higher diversity and abundance of EM taxa (Nuske et al. 2018). However, when generalists, like the eastern chipmunk (*Tamias striatus*) reach a high abundance, their dispersal importance may surpass that of local specialists (Stephens and Rowe 2020). Furthermore, generalists have the ability to switch between food sources fairly readily depending on environmental cues and conditions (Pauli et al. 2019), so both types of dispersers are important in an ecosystem. In fact, a lack of rodent-dispersed fungal spores may prevent seedlings from establishing (Terwilliger and Pastor 1999), and hypogeous fungi inoculum have been found as far as 72 m from the nearest mature oak, suggesting that small mammals are prominent dispersal agents in these ecosystems (Frank et al. 2006). *Rhizopogon* spores that passed through the gut of a Townsend's chipmunk (*Tamias townsendii*) had higher metabolic activity when compared to undigested spores, so consumption may trigger spore germination (Colgan and Claridge 2002).

While EM fungi are a historic part of oak ecosystems, so are surface fires (Crow 1988, Lorimer 1993). As soil can be heated to high temperatures during a surface fire, these soil-dwelling fungi populations may be in danger of hyphal injury or death. Several studies suggest that burning has either a negative or negligible impact on fungal biomass (Bergner et al. 2004, Bettucci and Alonso 1995, Fonturbel et al. 1995, Stendell et al. 1999), but may induce fruiting and therefore give the appearance of a larger number of fungi being present than there really are (Cairney and Bastias 2007). Some soil fungi found in fire-dependent ecosystems may themselves be fire-tolerant; e.g., Hansen et al. (2019) found that recurrent fires in a pine savanna did not decrease overall fungal abundance. Interestingly, species that are generally tolerant of high temperatures and water stress are found more readily on plots in the months following a fire than on unburned plots, and post-fire fungi tend to be found rarely or not found at all on unburned plots (Bettucci and Alonso 1995, Dahlberg et al. 2001, Stendell et al. 1999). Hence, the fungal response to fire may be extremely varied, with some species thriving and others completely removed from the environment. In the study closest in geographic proximity to mine, on a pine plantation in central Georgia, Oliver et al. (2015) found that diversity did not change after a fire but rather the whole community shifted

toward more fire-resistant EM species. Thus, it should not be automatically assumed that diversity is lost if a species occurring before a fire is no longer present after.

The objective of this study was to estimate the effects of prescribed surface fire on the relationship between mycorrhizal hypogeous fruiting bodies (truffles) and a small mammal dispersal agent in the CHR. I extracted fungal spores from eastern chipmunk (*Tamias striatus*) scat collected from 65 trap stations that differed in prescribed fire history. I hypothesized that 1) prescribed surface fire would affect fungal spore density and community composition found in scat, especially for AM taxa and 2) local environmental variables, such as aspect, litter layer depth, local basal area of AM and EM trees, and total basal area, would mediate the influence of fire.

4.3 Methods

4.3.1 Study Area

The Hardwood Ecosystem Experiment (HEE) lies in southern Indiana within the Morgan-Monroe and Yellowwood State Forests, located within the Brown County Section of the Highland Rim National Region of Indiana (Homoya et al. 1985). Due to the lack of recent glacial activity, the region is characterized by a steep hill and valley system, and was unwelcoming to agriculture at the time of European settlement. The intense topography also influences the range of microsites and mosaic of vegetation types present (Jenkins 2013). The soils are bedrock-derived and mostly in the Wellston-Berks-Gilpin soil complex, meaning the region consists of silty-loams that are very well drained and prone to erosion from the intermittent creeks and several larger rivers located in the area (Jenkins 2013).

Dominant overstory species are oak, especially white oak (*Q. alba*) and chestnut oak (*Q. prinus*), and hickory (*Carya* spp.) in the uplands, and American beech (*Fagus grandifolia*), sugar maple (*A. saccharum*), tulip poplar (*Liriodendron tulipifera*), and red oak (*Q. rubra*) on the mesic slopes and lowlands. The understory mostly consists of various shrubs, like greenbrier (*Smilax* spp.) and black huckleberry (*Gaylussacia baccata*), on more xeric sites and sugar maple seedlings and saplings on mesic slopes (Jenkins 2013). The region was historically burned by Native Americans, which ceased upon European settlement in the early 1800s. Prescribed surface fires have been implemented on some sites within the last three decades, with a focus on both rare

95

habitat maintenance (e.g., barrens) and promoting oak seedling regeneration (Saunders and Swihart 2013, Stanis et al. 2019).

4.3.2   Plot and Environmental Variable Description

Chipmunk scat collection occurred from 2 July to 8 August 2019. My plots were singular baited trap stations that are part of a larger system of small mammal trapping grids (n = 12) for each HEE management stand, and were placed > 20 m apart; only one grid was placed on a management stand and each ranged in area from 2.4 – 3.75 ha (as described in Urban and Swihart 2013). Management stands had similar species composition, age structure, and aspect and were roughly 4 ha (10 ac) in size (Kalb and Mycroft 2013). Scat was collected from livetrapped individuals on plots burned either in the spring or fall two to four years before sampling (n = 33) and unburned control plots (n = 32). All burned plots were burned just once prior to sampling.

Local topography and dominant tree species have been shown to influence underground fungal communities (Claridge et al. 2000). Therefore, predominant aspect (N, S, E, W), burn intensity (mean maximum temperature [ºF] recorded at each burned plot via paint tags), litter layer depth (height of leaf litter and duff layers [cm] averaged across each grid), total overstory (diameter at breast height > 10 in) basal area (ft^2 ac^{-1}), and overstory basal area of both EM trees and AM trees, based on Wang and Qui (2006), were quantified for each plot. Proportion of total basal area, with values ranging from 0 to 1 was also calculated to ensure that plots could still be compared to each other even if their absolute values differed. Because an individual trapped at a plot may not have consumed fungi at that very plot, all three basal area variables were averaged across the plot itself and the four plots directly adjacent (N, S, E, and W) the plot in the livetrapping grid.

Burn intensity and litter layer depth data were collected by others as part of the larger HEE prescribed fire dataset between 13 May 2015 and 7 August 2017; burn intensity data were gathered using paint tags during the fires and leaf litter depth was measured post-burn. Each plot had two paint tags associated with it, so burn intensity was averaged for each burned plot; all unburned plots had a burn intensity value of 0. Additionally, plots were assigned a burn class: 'burned' for burned plots and 'unburned' for control plots.

4.3.3 Scat Collection

Baited Sherman traps (H.B. Sherman Traps, Inc., Tallahassee, FL) were first prebaited for three days near each plot center, then checked twice per day for five consecutive days during the duration of livetrapping; trap bait included sunflower seeds, rolled oats, and mealworms (*sensu* Nelson et al. 2019). The scat sample was collected only if the animal inside the trap was an eastern chipmunk, the individual was the first animal captured in that trap during the current trapping session, and the trap had been cleaned of all debris before the trapping session. After processing and releasing the chipmunk, fecal pellets from each individual were then either extracted directly from the trap with tweezers, or the contents of the trap were first dumped onto a clean surface and then picked through with tweezers. The pellets were then placed in microcentrifuge tubes filled with 95% ethanol, labelled, placed in a resealable plastic bag, and frozen at -18º C / 0º F at the end of each field day. Livetrapping and handling procedures were approved by the Purdue Institutional Animal Care and Use Committee (protocol 1112000404).

4.3.4 Fungal Spore Extraction and Preparation

I extracted fungal spores from fecal pellets using a method similar to Stephens et al. (2017). I first air-dried the scat in a fume hood at ambient temperature, cleaned the sample of foreign material, and then ground the sample to a powder using a plastic micropestle. Approximately 20 mg of the ground scat, weighed to the nearest 0.1 mg, was placed in a 1.5 mL microcentrifuge tube and 1 mL of 10% KOH was added. I shook the tube vigorously for at least 15 s, allowed the sample to rest for 5 min, and then rinsed the sample through a 125 μm paint screen (Gerson Elite paint strainers, Middleboro, MA) with about 40 mL deionized water. I then allowed this solution to rest for 24 hr in a 50 mL centrifuge tube. After this period, I extracted the spore isolate using a glass pipette, placed the isolate in a clean 1.5 mL microcentrifuge tube, and filled the tube with 1 mL 95% ethanol. The ethanol solution was allowed to settle for another 1.5 hr in the microcentrifuge tube, and then I measured the total volume of the spore isolate using a micropipette. Next, I spread 100 μm of spore isolate on a glass slide followed by a drop each of Visikol (Visikol, Inc, Hampton, NJ) and iodine. In 7 to 10 days, after the solution dried, the slide was sealed with Permount mounting medium (Fisher Chemical, Waltham, MA) under a 22 x 22 mm cover slip.

4.3.5   Fungal Spore Identification and Preparation

Fungal spores were identified to the lowest taxonomic unit using Castellano et al. (1989) and were assigned AM or EM status. Spores were quantified by counting the number of each taxon at 400x magnification within 25 fields of view evenly distributed across the slide. Another 25% of the slide was scanned at 100x for larger spores not seen at 400x magnification. The spore counts, isolate volume, and scat weight were used to calculate the total number of spores for each taxon in 1 mg of scat (*sensu* Stephens et al. 2017).

Spore densities were first log_{10} transformed to reduce the influence of outliers. Due to the nature of fungal spore morphology and reproductive strategies, spore counts were then scaled from 0 to 1 for each taxon across all samples (i.e. the sample with the highest density for a taxon received a 1, the lowest a 0) to more easily compare amongst different taxa with different inherent spore loads. To prevent the inflated importance of certain samples when ranking during non-parametric tests, samples with one or fewer taxa present (n = 9) were removed from the dataset before PERMANOVA and NMDS but were included for other analyses.

4.3.6   Statistical Analysis

To determine the effect of prescribed fire and other environmental variables on fungal spore abundance, I first conducted permutational multivariate analysis of variance (PERMANOVA) on a Bray-Curtis dissimilarity matrix to test the effect of burn class, years since the last burn, litter layer depth, burn intensity, aspect, and total basal area on both AM and EM spore densities, proportion of AM basal area on AM spore density, and proportion of EM basal area on EM spore density using the "vegan" package in R (Oksanen et al. 2019). I then used non-metric multidimensional scaling (NMDS) on a log_{10} transformed and scaled, 56 (number of samples with ≥ 2 taxa present) x 17 (number of fungal taxa) matrix to better visualize how EM and AM spore densities were altered by burn and environmental variables using the "vegan" package in R (Oksanen et al. 2019). To test the effect of environmental variables I conducted an additional correlation analysis using the 'envfit' command ("vegan" package, Oksanen et al. 2019), then added vectors representing each significant variable to more easily identify how each sample was impacted by its environmental conditions.

To investigate how burn and environmental variables may influence broad diversity patterns, I used one-way ANOVA to test the effect of burn class, years since the last burn, litter layer depth, burn intensity, aspect, and total basal area on both AM and EM fungal taxa richness, the proportion of AM tree basal area on AM fungal taxa richness, and the proportion of EM tree basal area on EM fungal taxa richness. Additionally, since indicator species can be used to classify sites recovering from a disturbance like fire (De Cáceres et al. 2010), I tested for the presence of indicator taxa between the two burn classes (burned/unburned) and between the four years since the last burn categories (2, 3, 4, unburned) using the 'multipatt' command ("indicspecies" package, De Cáceres and Legendre 2009). All analyses were completed using R 3.6.1 (R core team 2019). Analyses used $\alpha = 0.05$.

4.4 Results

Out of the total of 65 samples (63 of which contained spores), 17 different fungal taxa (6 AM, 10 EM, 1 unknown) were identified. Unburned plots were represented with 33 samples and burned plots had 32 (2 plots burned 2 years prior, 20 burned 3, and 10 burned 4). Overall, the most commonly found taxa were the clear *Glomus* spore morphotype (found in 56.9% of samples), *Tuber* spp. (36.9%), and the yellow (33.8%), orange (27.7%), and brown (23.1%) *Glomus* spore morphotypes (Table 4.1). However, 12 taxa (70.6% of all taxa) were found in at least 10% of samples.

Scat samples from unburned plots generally contained a higher EM spore density than those from burned plots, but the density of AM spores remained roughly constant (Fig. 4.1). Out of the burned plots, EM spore loads were the largest two to three years post-burn and lowest four years after a prescribed fire, while AM spore density was similar for scat collected across all burn sites (Fig. 4.2). Overall, unstandardized and unscaled spore densities for an individual taxon in a sample ranged from 1.5 spores/mg to 9,969.4 spores/mg (Table 4.1).

4.4.1 Fungal Spore Richness and Presence in Scat

I identified 15 taxa in samples from unburned plots (Fig. 4.3), with average per-sample richness of 3.2 taxa. *Tuber* spp. spores had the highest total density, but clear *Glomus*, *Melanogaster*, and

Hysterangium spores were also fairy dense (Fig. 4.3). *Leucogaster*, *Tuber*, and *Boletaceae* spores were unique to scat collected from unburned plots.

Burned plots had an average per-sample richness of 2.7 taxa and a total of 14 identified taxa (Fig. 4.4). *Alpova* and *Melanogaster* spore densities were the highest, especially within the first three years post-burn (Fig. 4.4). The yellow *Glomus* spore morphotype was the next most dense, with roughly equal contribution from plots burned two to three years and four years prior (Fig. 4.4). *Elaphomyces verruculosus* and yellow mushroom spores were unique to scat collected from burned plots.

All scat samples had an average AM fungal spore richness of 1.69 taxa and an EM fungal spore richness of 1.26 taxa. Arbuscular mycorrhizal spore richness did not significantly differ with any burn or environmental variable measured, but EM spore richness in scat significantly decreased with increasing burn intensity ($F_{1, 63} = 4.45$, $p = 0.039$), and samples from west-facing slopes had a lower EM spore richness than south slopes ($p = 0.031$). The effect of prescribed surface fire on EM spore richness in scat was marginally negative ($F_{1, 63} = 3.44$, $p = 0.060$).

The presence of two AM taxa in scat indicated a burned site. The yellow glomus spore morphotype was associated with burned sites overall ($p = 0.024$) and the clear glomus morphotype was specifically associated with sites burned two years prior ($p = 0.035$). Unburned sites had only one indicator species found in scat samples: *Tuber* ($p = 0.001$).

4.4.2   Fungal Spore Composition in Scat

Results from a PERMANOVA suggested that burn class ($F_{1, 52} = 7.2$, $p = 0.001$) and years since last burn ($F_{3, 50} = 3.19$, $p = 0.001$) explained the most variation in fungal spore composition in scat, with each explaining 12.2%. Pairwise comparison revealed that scat samples from unburned plots differed in AM and EM composition from those burned three years prior ($p = 0.01$). Aspect (11.1% variation explained, $F_{3, 50} = 2.08$, $p = 0.001$), litter layer depth (10.9% variation explained, $F_{1, 52} = 6.35$, $p = 0.001$), EM tree basal area (10.2% variation explained, $F_{1, 38} = 4.3$, $p = 0.001$), AM tree basal area (5.8% variation explained, $F_{1, 48} = 2.97$, $p = 0.009$), burn intensity (5.8% variation explained, $F_{1, 52} = 3.19$, $p = 0.002$), and total basal area (4.9% variation explained, $F_{1, 52} = 2.69$, $p = 0.008$) all significantly affected, and contributed to explaining some amount of variation in, fungal composition in scat.

The NMDS ordination (2 dimensions, stress = 0.221) moderately partitioned unburned and burned plots (Fig. 4.5). 'Envfit' revealed that fungal community composition was significantly correlated with litter layer depth ($r^2 = 0.477$, $p = 0.001$), EM tree basal area ($r^2 = 0.306$, $p = 0.001$), AM tree basal area ($r^2 = 0.305$, $p = 0.001$), years since the last burn ($r^2 = 0.282$, $p = 0.001$), burn class ($r^2 = 0.263$, $p = 0.001$), burn intensity ($r^2 = 0.185$, $p = 0.006$), and aspect ($r^2 = 0.131$, $p = 0.025$). Litter layer depth, EM tree basal area, years since the last burn, burn class, and aspect were most associated with unburned plots; burn intensity and AM tree basal area were most associated with burned plots.

NMDS axis 1 appeared to be most influenced by differences in burned and unburned plots. Ectomycorrhizal tree basal area and AM tree basal area influenced NMDS axes 1 and 2 in opposing directions, as did the litter layer depth and burn intensity. NMDS axis 2 also appeared to be somewhat influenced by a difference in burn class, and not a particular environmental variable. Leaf litter depth and EM tree basal area were more associated with unburned plots, and burn intensity and AM tree basal area were more associated with burned plots (Fig. 4.5).

4.5   Discussion

4.5.1   EM and AM Taxa Richness and Presence Differ Across Burn Classes

Most identified taxa (70.6%) were found in at least 10% of samples, and only four taxa were found in fewer than five samples, so the fungal taxa found were fairly commonly consumed and dispersed throughout the study area. As hypothesized, prescribed fire affected fungal spore density and composition in chipmunk scat. However, AM and EM taxa in scat were differentially affected by prescribed fire, and EM composition in scat was more heavily influenced by burn variables.

Arbuscular mycorrhizal taxa richness in scat was not significantly affected by burn or environmental variables, and AM spore densities remained relatively constant across burned and unburned plots (Fig. 4.1). This pattern of uniformity suggests that either AM taxa are producing similar amounts of sporocarps regardless of a single prescribed surface fire, or chipmunks are fairly consistently eating lower amounts of AM sporocarps on burned and unburned plots. Arbuscular mycorrhizal taxa overall had a high incidence of occurrence in scat samples (Table 4.1), so chipmunks were commonly eating these taxa, but the spore densities were low compared to EM densities so individuals were likely not consuming as many AM sporocarps. Arbuscular

mycorrhizal taxa, in general, have been described as cosmopolitan in their distribution pattern, with almost 130 known species in North America, so it is hardly surprising that they also had the highest incidence of occurrence in this study (Stürmer et al. 2018). However, AM fungi density and composition at a landscape scale have been shown to be controlled more by local abiotic factors like precipitation, soil pH, and soil type, rather than land use or distance from inoculum (Hazard et al. 2013). I did not measure these abiotic variables and so cannot assess their contribution to the uniformity of AM taxa richness across all scat samples, independent of measured burn or environmental variables. No AM taxa were unique to scat samples from either burn class, but the only species that indicated a burned plot were AM taxa. However, this difference is likely due to the decrease in overall EM spore density in scat from burned plots when compared to unburned, thereby lessening their importance in burned plots (Fig. 4.1).

Burning affected the consumption and dispersal of EM spores by eastern chipmunks in my study area. Ectomycorrhizal taxa richness in scat was significantly affected by both burn variables and the environment, and EM spore densities were higher in scat samples from unburned plots when compared to burned (Figs. 4.1, 4.2). Ectomycorrhizal spore richness in scat decreased with increasing burn intensity, coupled with the marginal negative effect of burning on EM richness in scat suggested that chipmunks consumed a lower variety of EM taxa in burned sites, especially those that hosted more intense burns. However, across samples from both burned and unburned sites, EM taxa had a lower incidence of occurrence in scat but were present in scat at a higher density than AM spores. Additionally, the sole EM indicator species was *Tuber*, which indicated an unburned scat sample and was only found in samples from unburned plots. The finding that EM fungal taxa in chipmunk scat is affected by burning is similar to the recent results of Marion et al. (2021). They found that most sites had differing dominant species of all root-associated fungi, and the distribution of fungal taxa was mostly influenced by land use on a landscape-level scale. Nevertheless, chipmunk consumption preference of EM taxa may still be driving the observed effect of burning.

Small mammals are known for their ability to transport fungal inoculum throughout a harvest-disturbed forest (Maser et al. 1978); however, a disturbance like fire may impact their movement patterns and local density. Australian small mammals were found to preferentially forage in recently burned areas, and had a higher foraging success in these sites, due to fewer foraging obstacles and a propensity for some truffle species to fruit after a burn (Johnson 1995,

Vernes and Hayden 2001, Vernes et al. 2001). Similarities were observed in responses to burning of Australian small mammals and Central Hardwood Region eastern chipmunks. When trapping small mammals in the same study area as this project, Nelson (2017) found that chipmunk density was between 40-50% higher and movement was greater in the first 12-18 months following a prescribed surface fire. After 12-18 months, density and movement measures returned to control levels; considering my study does not use plots burned within the last two years, we can assume that chipmunk movement and local density in both burned and unburned plots were fairly similar. The timing is important to note, as EM and AM fungal spore densities and communities were different in scat samples collected from burned and unburned plots, which means that if chipmunk movement and density across both burn classes were relatively equal, either fungal relative availability, differential selection by chipmunks, or some other factor is likely driving the fungal consumption patterns of chipmunks.

4.5.2 Local Environment May Mitigate Effect of Burns on Fungal Community in Scat

I found some support for my second hypothesis, environmental mediation of fire effects on fungal communities in chipmunk scat. While burn class and number of years since the last burn explained the most variation in fungal spore composition in scat, EM tree and AM tree basal areas both contributed modestly to spore composition variation. Additionally, fungal community composition in scat samples was significantly correlated with leaf litter depth, EM tree basal area, and AM tree basal area, making it clear that more than just burning is affecting the relationship of chipmunks and fungi. Regardless of EM tree adaptations to fire, like the thick bark and hypogeal sprouting of oak, leaf litter depth and EM tree basal area were most associated with unburned plots and burn intensity and AM tree basal area were most associated with burned plots (Fig. 5). Considering a goal of burns in the Central Hardwood Region is often EM tree regeneration, it is interesting how mature EM tree basal area is associated with unburned rather than burned plots. Nonetheless, local biotic and abiotic conditions alike affected mycorrhizal fungi composition in scat samples.

Saitta et al. (2018) found that host species presence is the main driver of mycorrhizal richness and composition, and concluded that local plant diversity may override other seemingly important abiotic factors, like soil moisture or pH. Relative abundances of host tree species may directly influence composition of different fungal functional groups (Saitta et al. 2018), which in turn influences what chipmunks are able to consume by altering the availability of certain taxa or

entire fungal functional groups. Many abiotic factors, like topography and topographic position, leaf litter later, and time since the last fire, may interact to become the most locally important predictors of truffle presence and composition (Claridge et al. 2000). It is likely these interactions of biotic and abiotic factors most influence which fungal species are present, eventually determining which taxa chipmunks can consume and disperse. To highlight the uncertainty of the nature of ecological interactions, Vašutová et al. (2018) found that host species presence is an unimportant predictor of mycorrhizal composition, and concluded that as management intensity increased, fungal richness decreased. Chipmunk density, too, is affected by non-burn habitat variables. Nelson et al. (2019) found that the forest matrix housed the lowest density (~3.54 individuals ha^{-1}) of eastern chipmunks in my study area when compared to edge and opening habitats. My study plots mostly utilized the forest matrix, so chipmunk density and therefore fungal consumption may have been lower on my plots due to a habitat variable not accounted for in this study. These contradictory results of the importance of certain elements of the environment when studying the relationship between mycorrhizae and their dispersal agents warrant additional work on this topic, especially focusing on the foraging preferences of eastern chipmunks in forests burned at least two years prior.

4.5.3   Management Implications

Overall, the composition all taxa in chipmunk scat was impacted by burn and environmental variables including burn class, years since last burn, litter layer depth, and basal area of AM and EM trees. More specifically, AM and EM taxa found in scat were differentially affected by fire. Arbuscular mycorrhizal taxa richness in scat was not significantly impacted by any variable we measured, and two AM taxa indicated samples from burned plots. In contrast, EM taxa richness in scat was affected by both burn intensity and burn class and only an EM taxon indicated a sample from an unburned plot. These AM-EM differences may influence decisions on forest management, if, for instance, an EM or AM host tree species is favored or discouraged from establishment. The consumption and dispersal of fungal spores by small mammals will impact which seedlings successfully establish, so maintaining preferred dispersal pathways for these animals will help ensure regeneration for a desired tree species.

The consumption and composition of truffle taxa found on my study sites were most influenced by litter layer depth and AM and EM tree basal area, which may create an issue when

managing these stands with prescribed surface fire. While AM taxa consumed by chipmunks were seemingly unaffected by a single prescribed burn, EM spore density and richness in scat were negatively affected by burning. Evidence of a long-term negative relationship between burning and eastern chipmunk movement patterns and population density has yet to be found, so this pattern may be caused by less EM fungi fruiting. The observed decrease in EM fruiting body consumption at burn sites may be due to the habitat preferences of EM fungi, as many taxa live in and near the leaf litter layer, which is frequently consumed or reduced after a prescribed fire (Yang et al. 2019, Claridge et al. 2000).

Of greatest concern, in stands where fire has been excluded for several decades, the return of surface fire may actually preferentially injure fire-adapted EM trees due to the location of EM fungal hyphae, which I may have some evidence for as EM tree basal area was most associated with unburned stands. Carpenter et al. (2021) found a positive relationship between EM tree abundance and the soil organic horizon, probability of crown decline, probability of basal sprouting, and probability aboveground biomass mortality. Additionally, stands with the most fire-adapted, EM tree species often had the most negative impacts after a fire that consumed over 70% of the leaf litter layer, likely partially caused by EM hyphal exploration in duff (Carpenter et al. 2021). Whether the damage to EM trees is driving EM fungi compositional changes, or the destruction of EM fungal networks is allowing hosts trees to become more damaged aboveground, using prescribed fire as a management tool to promote the regeneration of EM trees like oak and hickory may be even more difficult than expected, especially in areas in which fire has been absent for long periods.

In stands where fire has been suppressed for several decades, both the above and belowground components of mature oak forests could best be supported by a slow return to prescribed burns. Until the system has had time to adjust to the reintroduction of this disturbance by allocating more tree fine roots and fungal hyphae into the organic layer of mineral soil, fires should be more frequent and lower intensity than the more common litter layer-depleting burns. The short-term positive response of chipmunk density and movement to prescribed surface fire (Nelson 2017) may promote the transport of EM inoculum into recently burned areas to ensure EM tree regeneration is supported, especially considering the decline in EM spore density and richness I found in chipmunk scat collected from burned sites in this study. However, current stand management and fire prescription methods, along with monetary and logistical constraints, do not

allow managers to easily transition to even patchier burns, so this remains impractical for now. Small, patchy burns may be too difficult to implement, in which case prioritizing fires of lower intensity may better support oak maturation via chipmunk dispersal of mycorrhizal inoculum than traditional prescriptions.

4.6   References

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4.7   Tables and Figures

Table 4-1 Table displaying fungal taxa, assigned environmental function (either AM or EM), number of samples that taxon was identified in, the percentage of unburned, burned, and total samples the taxon was located in, and mean spore density (spores mg scat⁻¹) found in a single sample for unburned and burned plots. Total n = 65, unburned n = 33, and burned n = 32 samples.

Taxa	Function	% unburned	% burned	% samples	Density – unburned	Density – burned
Glomus_clear	AM	69.7	43.8	56.9	10.7	2.1
Tuber	EM	72.7	0	36.9	70.6	0
Glomus_yellow	AM	18.2	50	33.8	1.0	10.2
Glomus_orange	AM	24.2	31.3	27.7	0.9	1.5
Glomus_brown	AM	27.3	18.8	23.1	1.0	0.7
Glomus_dark_orange	AM	24.2	15.6	20	0.9	0.6
unknown	-	9.1	31.3	20	0.4	0.8
Melanogaster	EM	12.1	18.8	15.4	50.8	466.0
Leucophleps	EM	18.2	6.3	12.3	4.3	0.6
Alpova	EM	3.0	18.8	10.8	54.0	749.1
Hysterangium	EM	12.1	9.4	10.8	22.8	0.2
Elaphomyces americanus	EM	9.1	12.5	10.8	0.2	0.4
Glomus_large_yellow	AM	3.0	12.5	7.7	0.2	0.3
Leucogaster	EM	6.1	0	3.1	38.9	0
Boletaceae	EM	6.1	0	3.1	22.5	0
E. verruculosus	EM	0	3.1	1.5	0	0.1
yellow_mushroom	EM	0	3.1	1.5	0	0.6
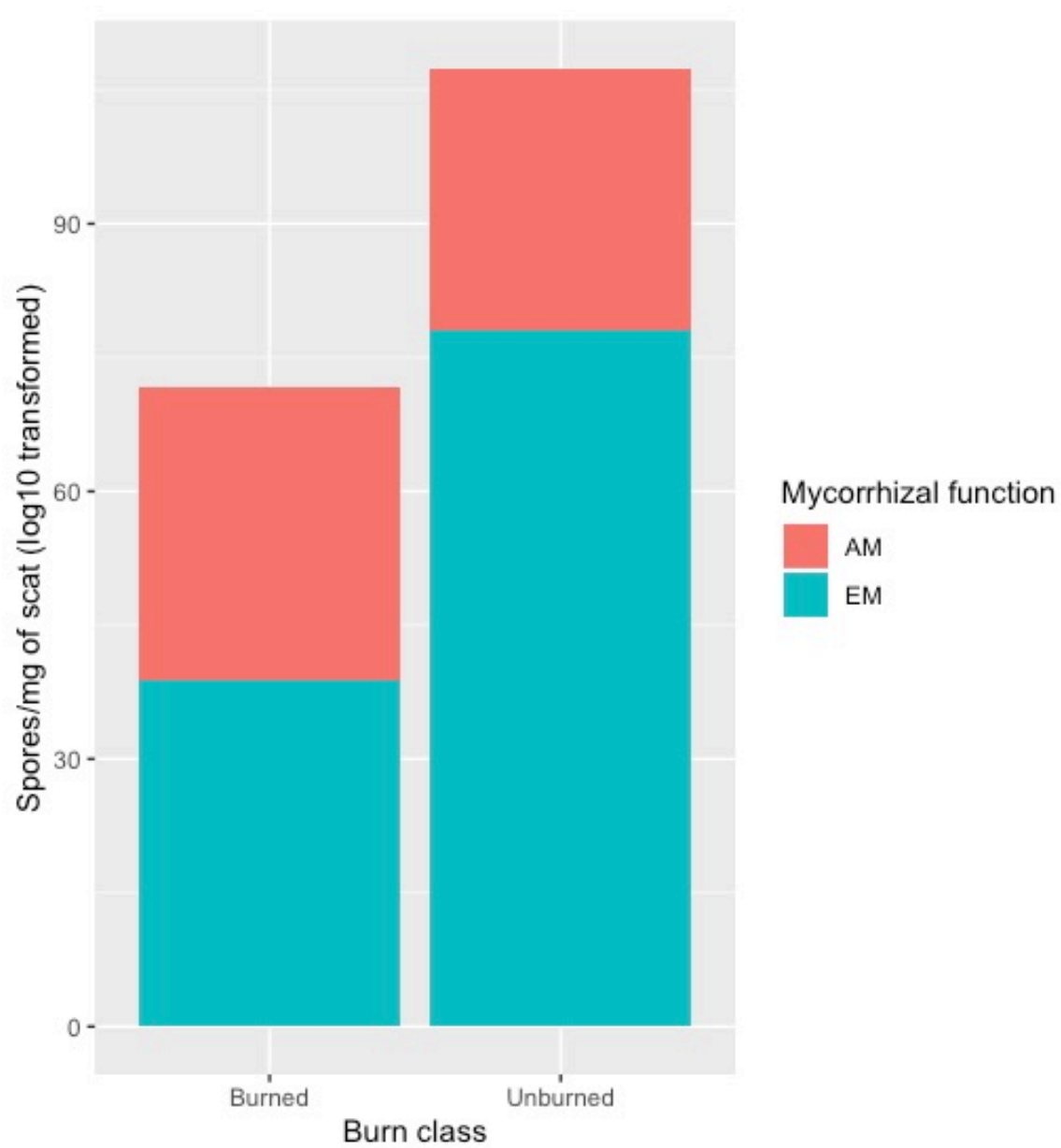
Figure 4-1 Total spore density for burned (n = 32) and unburned (n = 33) $\log_{10}$ transformed samples, colored by fungal environmental function of AM or EM.
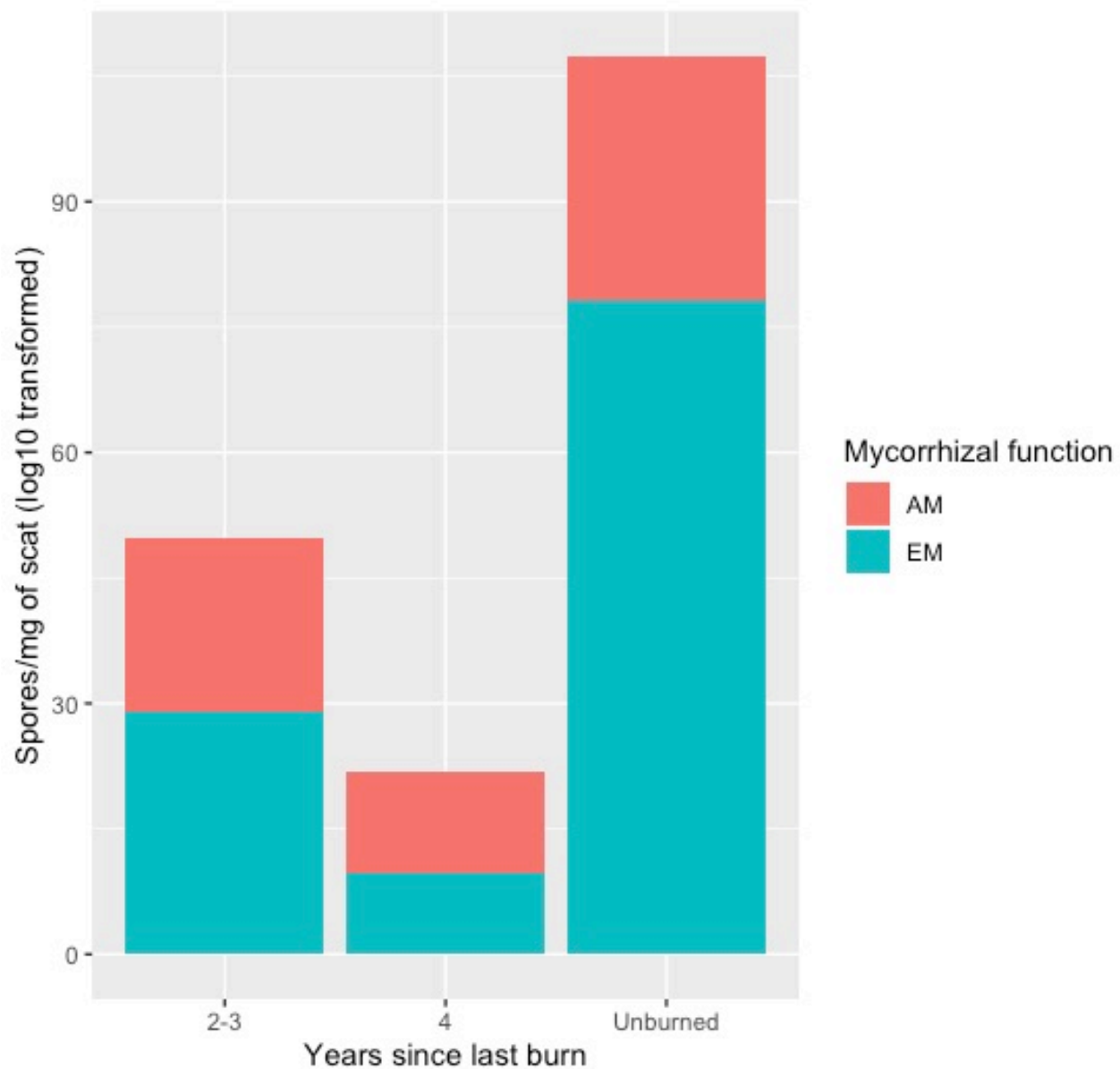
Figure 4-2 Total spore density for unburned (n = 33) sites and sites burned 2-3 (n = 22) or 4 (n = 10) years prior for log_{10} transformed samples, colored by fungal environmental function of AM or EM.
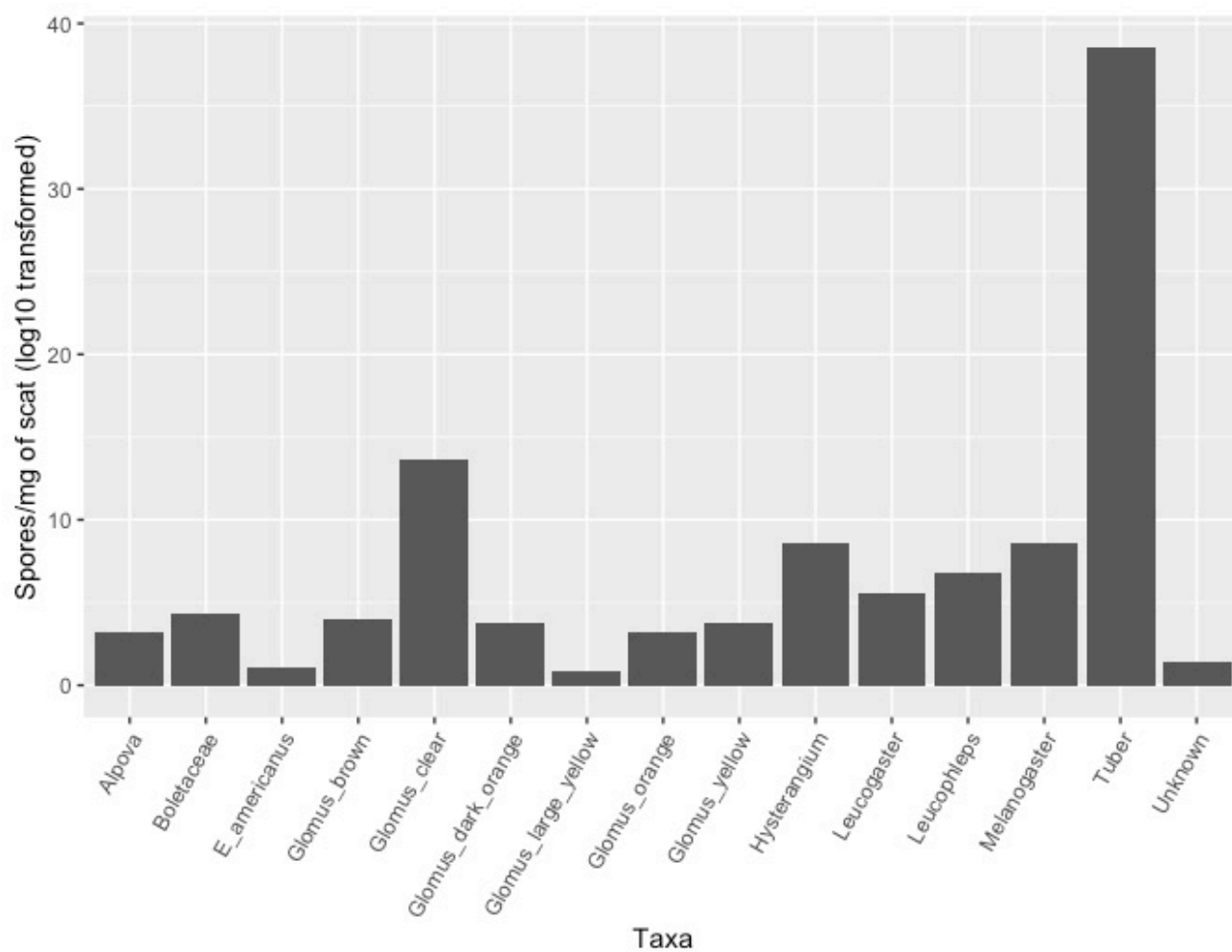
Figure 4-3 Total spore density for each taxon found in samples from unburned (n = 33) plots, log_{10} transformed.
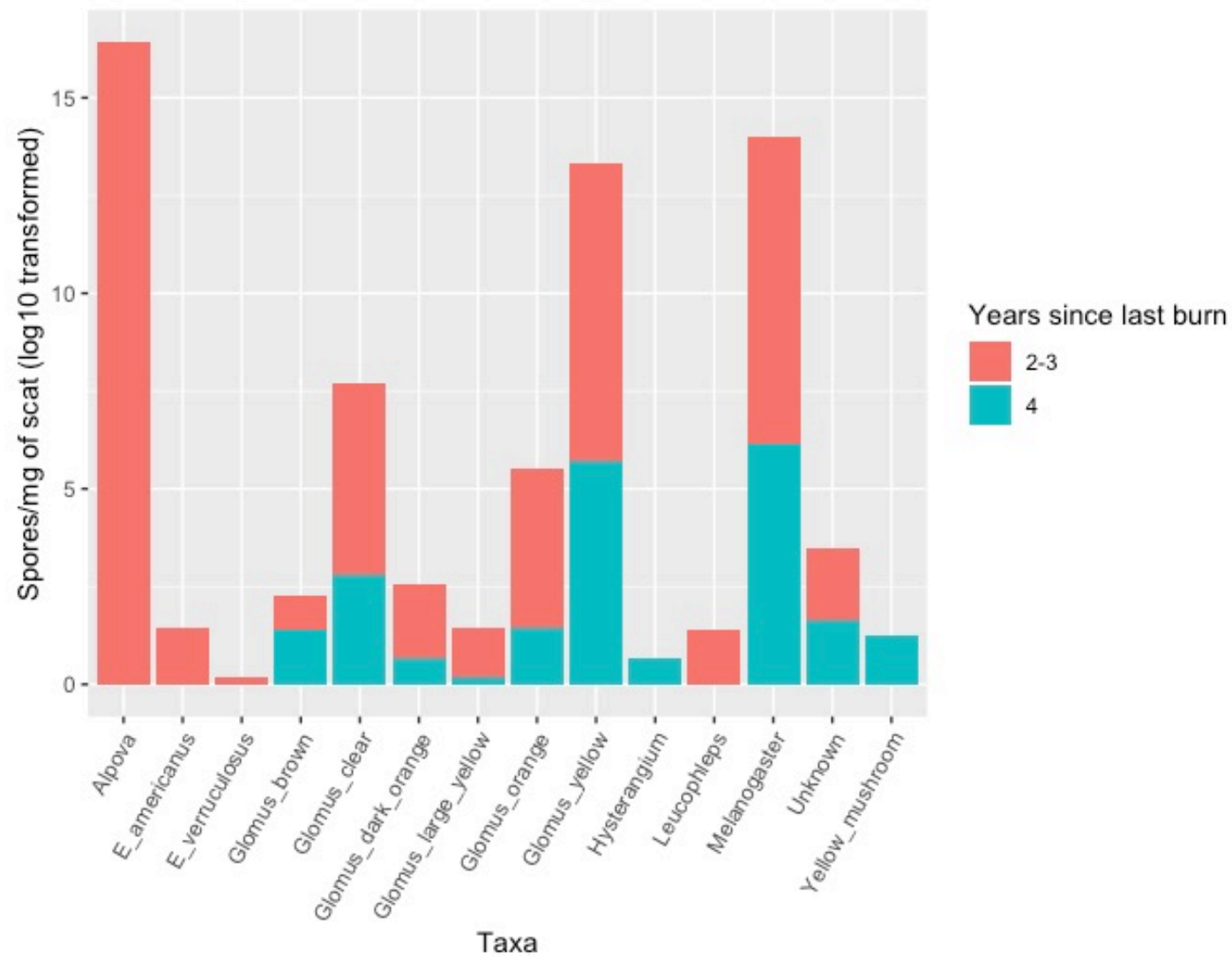
Figure 4-4 Total spore density for sites burned 2-3 (n = 22) or 4 (n = 10) years prior, $\log_{10}$ transformed. Note difference in scale of vertical axis compared to Figure 4-3.
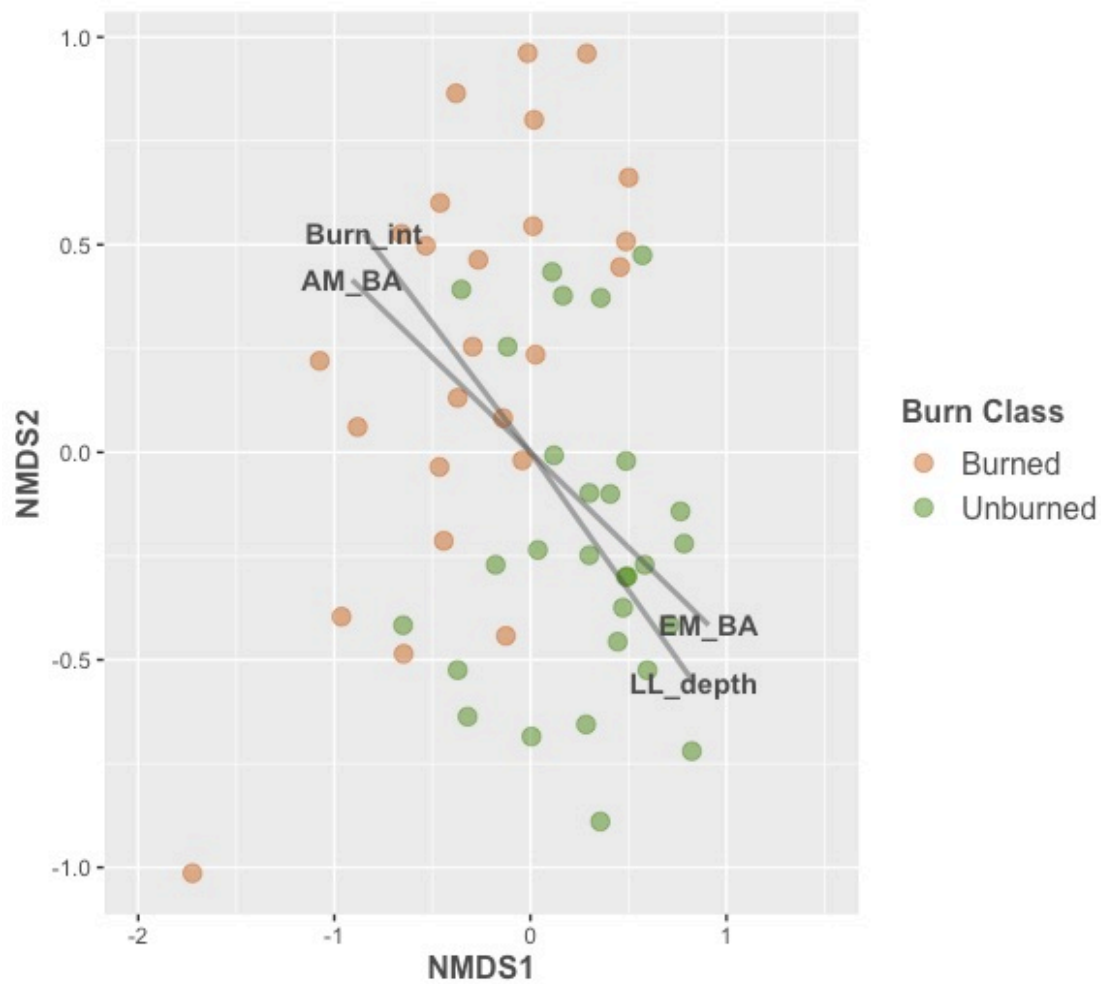
Figure 4-5 NMDS ordination (2 dimensions, stress = 0.221) of scaled and $\log_{10}$-transformed spore density on burned (n = 27) and unburned (n = 29) plots. 'Envfit' environmental variable correlations are shown via vectors overlain on the ordination points. Arbuscular mycorrhizal tree basal area (AM_BA) and burn intensity (burn_int) are the vectors most associated with burned plots; ectomycorrhizal tree basal area (EM_BA), and leaf litter depth (LL_depth) are the vectors most associated with unburned plots.

CHAPTER 5.   CONCLUSIONS

The objectives of my thesis research shared the goal of increasing knowledge of the effects of prescribed surface fire on oak regeneration in the Central Hardwood Region (CHR). Oak (*Quercus*) is being replaced by maple (*Acer*) and other mesic species across their range in the eastern deciduous forest, which has led to a positive feedback loop called mesophication (Nowacki and Abrams 2008). Mesophication alters the disturbance regime, making it increasingly difficult to return surface fire to historically fire-adapted landscapes, allowing non-adapted species to outcompete oak and self-replace (Abrams and Nowacki 1992, Abrams 2005). One way to break the cycle is to return fire to the landscape, which is becoming an increasingly popular method, to attempt to mimic the historic disturbance regime that produced these oak-dominated forests (Aldrich et al. 2005, Crow 1988, Crow et al. 1994, Lorimer 1993). However, research shows varied results with differing burn counts and intervals (Albrecht and McCarthy 2006, Collins and Carson 2003, Merritt and Pope 1991, Stratton 2007). Studies on the belowground effects of fire on mycorrhizal fungi communities produce results that are just as varied (Bettucci and Alonso 1995, Stendell et al. 1999, Cairney and Bastias 2007).

The establishment of oak in the seedling layer (Chapter 2), maturation into the midstory (Chapter 3), and the symbiotic relationship with mycorrhizal fungi (Chapter 4) were each analyzed in respect to burn status, number of years since the last burn, and a suite of local environmental and topographic variables. Key findings include: 1) oak seedling regeneration is mostly determined by percent slope and amount of midstory basal area; 2) prescribed burns favor midstory oak over maple if the site has been burned within the last four years; and 3) arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) fungi density and diversity are differentially affected by a single prescribed fire.

In Chapter 2, I assessed how burn, topographic, and stand structural variables affected oak seedling density. Studies monitoring long-term effects of repeat prescribed surface fires are fairly rare, especially in the CHR, but these studies generally supply evidence that multiple burns benefit oak reproduction (Huddle and Pallardy 1996, Stratton 2007). The main goal of my study was to develop more details on the relationship between prescribed fire, existing stand characteristics, and competitive oak regeneration in the CHR. Unsurprisingly, mid- and overstory basal area, percent slope, number of years since the last burn, and total number of burns all affected oak seedling

density. However, percent slope and midstory basal area were two of the most important predictors of oak density. Slope greatly influences fire behavior, even during less intense prescribed burns (Pyne et al. 1996), so it is not entirely unexpected for percent slope to indirectly impact seedling density through directly impacting local burn conditions. The amount of light seedlings receive, partially controlled by mid- and overstory basal area, helps determine suitable habitat for most species, but especially shade intolerant oak (Burns and Honkala 1990).

The main conclusion of Chapter 2 was that repeat prescribed fires may condition a site for natural regeneration of oak species, but if an established midstory already exists or fire has not been used on the landscape for almost a decade, multiple fires will likely have a negligible effect on oak regeneration unless the site is burned repeatedly ($\geq$ 3 times) with high frequency ($\leq$ 4 years between burns). There is significant local variation during a single prescribed fire (Boerner 1982), so a more surefire approach may be to mechanically or chemically target mid- and overstory stems. Midstory removal can be successful when utilized to boost oak seedling regeneration, so supplementing the less-intense surface fires with stem removal may prove to be most beneficial.

Chapter 3 explored the relationship between prescribed surface fire, existing stand structure and characteristics, and midstory structure in the CHR. I previously demonstrated that the midstory basal area was a significant predictor of oak regeneration (Chapter 2), so investigating the controls of midstory basal area and structure is an important step to successfully recruiting oak seedlings to maturity. The stand structure common in the CHR, with an oak overstory but maple midstory, is thought to be novel and many managers are unsure about the best way to ensure maple do not overwhelm the canopy (Nowacki and Abrams 2008). My results indicated that overstory stand structure variables were often more important than burn variables when predicting midstory basal area and composition, but repeat fires do favor oak and hickory over more mesophytic species. However, if fire is kept off the landscape for more than four years, direct mechanical or chemical treatments of the midstory and/or overstory may also be needed to reduce maple and beech.

Midstory basal area and composition is influenced by stand structure more than the number of prescribed burns or number of years since the last burn, so promoting oak regeneration with the use of prescribed surface fire alone will likely be unsuccessful, especially if fire has been excluded from the landscape for several years. Scarification, midstory removal, or overstory thinning may need to be used in concert with prescribed fire to better enable oak to successfully reach the midstory and beyond.

Finally, the goal of Chapter 4 was to estimate the relationship between prescribed fire, mycorrhizal hypogeous fruiting body (truffle) abundance and community structure, and a small mammal dispersal agent in the CHR by extracting fungal spores from eastern chipmunk (*Tamias striatus*) scat. Mycorrhizae can affect forest composition and biodiversity (van der Heijden et al. 1998) and, at a high enough population, fungal generalists like the eastern chipmunk can become the most important spore dispersal agents (Stephens and Rowe 2020), so this is a necessary relationship to maintain in any mature forest. Eastern chipmunk density in the forest matrix at my study site was lower than in opening or edge habitats (Nelson et al. 2019), so the forest matrix itself may not be the best place to measure the dispersal importance of eastern chipmunks as they are likely moving between opening-edge-matrix habitats while they forage for fungi. The taxa with the highest density in scat samples were EM, but AM taxa had the highest incidence of occurrence. Additionally, EM taxa richness was negatively affected by burning while AM taxa richness was not significantly impacted by any burn or environmental variable measured, but overall fungal spore composition in scat was impacted by both burn variables and the local environment.

The main conclusion of Chapter 4 is that prescribed surface fire differentially affects the consumption and dispersal of AM and EM fungal taxa. However, more research is needed to explore this relationship. My study was exploratory, designed to get a first glimpse at the truffle community that eastern chipmunks are consuming in the CHR. Some truffle taxa may have been favored over others due to preferences of the chipmunks, or perhaps they foraged more in recently burned areas due to fewer physical barriers being present. To draw stronger conclusions, the mycorrhizal community in the soil must be genetically analyzed to fully understand all truffle taxa that may have been present and consumed during time of sampling. Also, we must better understand the truffle preferences and selection process of eastern chipmunks in order to make any conclusions about whether their foraging is altered by burning.

Prescribed surface fire has many direct and indirect impacts in the oak-hickory forests of the CHR, and these studies emphasize that topographic and stand structural variables often mitigate the direct effects of burning. It is therefore important to create unique prescriptions that fit each management area instead of using a one-size-fits-all approach, as a more general approach may actually encourage maple recruitment and release to the overstory. Additionally, mycorrhizal fungi are a necessary part of oak ecosystems and impacts of prescribed fire on these fungal communities should not be ignored if oak regeneration is a management goal. Smaller, patchy fires

121

that create a more heterogeneous post-burn environment should be prioritized early during the reintroduction of surface fire to an oak forest ecosystem, rather than a "scorched-earth" approach, as the former creates more opportunities for oak and their symbionts to thrive.

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APPENDIX A. CHAPTER 2 SUPPLEMENTARY MATERIAL
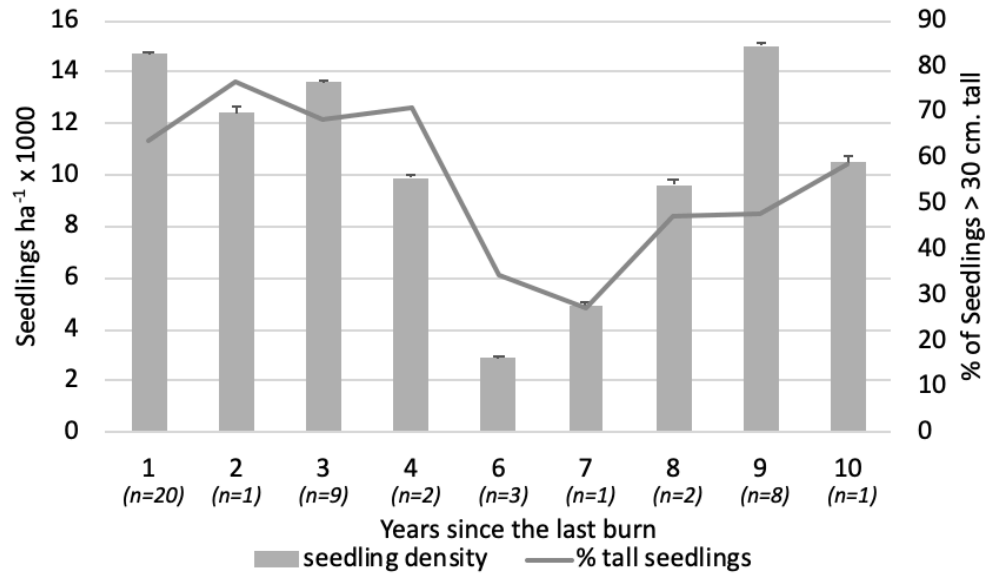
Figure A-1 Seedling density (seedlings ha^{-1} x 1000) and percentage of total seedlings taller than 30 cm displayed by the number of years since the last burn. Stand sample sizes for each year category are shown in italics below the x-axis, and error bars are displaying the standard error of the mean.
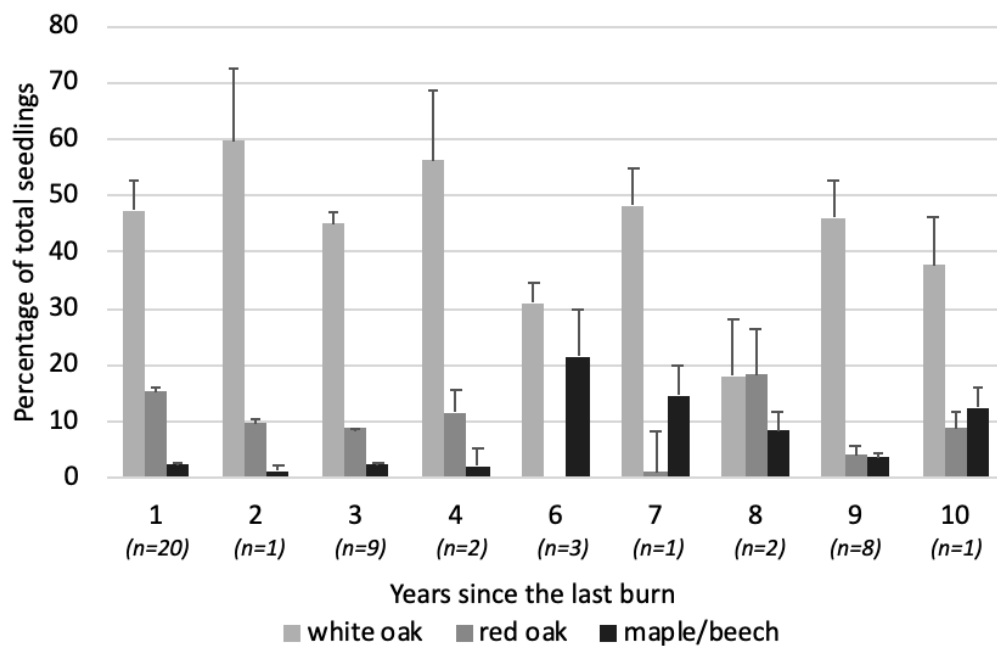
Figure A-2 Percentage of total seedlings that belong to either the white oak group, red oak group, or maple/beech group displayed by the number of years since the last burn. Stand sample sizes for each year are shown in italics below the x-axis, and error bars are displaying the standard error of the mean.

Table A-1 Range of observed values for various stand structural and environmental variables. SLOPE is percent slope and all BA variables have units m^2 ha^{-1}.

Variable	Minimum	Median	Maximum
Site index	42	63	97
Trees per acre	32	64	91
Slope	0	12	43
Midstory BA	0.3	3.6	8.0
Overstory BA	16.8	21.3	27.5
Oak BA	0.1	0.4	3.5
Mesic BA	0.0	1.2	5.7

Table A-2 Summary statistics categorized by time since the last burn. Stands = number of stands that have been burned that number of years ago; seedlings ha^{-1} = the number of seedlings per hectare for that year count ± SEM; % tall = percentage of total seedlings that are above 30 cm in height; % white oak = percentage of total seedlings that belong to the white oak species group; % red oak = percentage of total seedlings that belong to the red oak species group; % maple/beech = percentage of total seedlings that are either sugar maple, red maple, or American beech.

Years since last burn	1	2	3	4	6	7	8	9	10
Stands	20	1	9	2	3	1	2	8	1
Seedlings ha^{-1}	14,700 ± 30	12,400 ± 117	13,600 ± 45	9,860 ± 73	2,890 ± 38	4,890 ± 70	9,830 ± 89	15,100 ± 50	10,500 ± 124
% tall	63.7	76.6	68.2	71.0	34.2	27.0	47.1	47.8	58.6
% white oak	47.3	59.7	45.1	56.3	31.0	48.3	18.0	46.2	37.7
% red oak	15.3	9.7	8.8	11.7	0.0	1.1	18.3	4.0	8.9
% maple/beech	2.4	1.3	2.3	2.2	21.5	14.6	8.3	3.7	12.6

APPENDIX B. CHAPTER 3 SUPPLEMENTARY MATERIAL

Table B-1 Range of observed values, per stand, for various structural and environmental variables. All BA variables have units m^2 ha^{-1}. Shade tolerance status can be found in Table 1. Oak BA includes all oak species found on sites (listed in Table 1), and mesic BA includes common mesophilic species (sugar maple, red maple, American beech).

Variable	Definition	Minimum	Median	Maximum
Site index	site index	42	63	97
Trees per acre	trees per acre	32	64	91
Slope	percent slope	0	12	43
OverstoryBA	overstory basal area	16.8	21.3	27.5
Midstory BA	all midstory basal area	0.3	3.6	8.0
Oak BA	oak species basal area	0.1	0.4	3.5
Mesic BA	mesic species basal area	0.0	1.2	5.7
Tolerant BA	shade tolerant species basal area	0.0	1.5	5.7
Intolerant BA	shade intolerant species basal area	0.1	1.1	4.1

Table B-2 Summary statistics categorized by time since the last burn. Stands = number of stands that have been burned that many times; basal area = mean total midstory BA (m^2 ha^{-1}) ± SE for a stand; % shade tolerant = percentage of total midstory BA that is tolerant BA; % shade intolerant = percentage of total midstory BA that is intolerant BA.

Years since last burn	1	2	3	4	6	7	8	9	10
Stands	20	1	9	2	3	1	2	8	1
Basal area (m^2 ha^{-1})	1.54 ± 0.119	2.2 ± 0.384	2.48 ± 0.140	2.42 ± 0.335	2.88 ± 0.235	2.6 ± 0.368	1.84 ± 0.336	2.19 ± 0.174	3.17 ± 0.331
% shade tolerant	49.9	51.0	44.9	49.8	53.5	63.7	48.8	51.6	83.3
% shade intolerant	50.1	49.0	55.1	50.2	46.5	36.3	51.2	48.4	16.7

Figure B-1 Average midstory BA per stand displayed by the number of years since the last burn. Stand sample sizes for each year are shown in italics below each bar, and error bars are displaying the standard error of the mean.

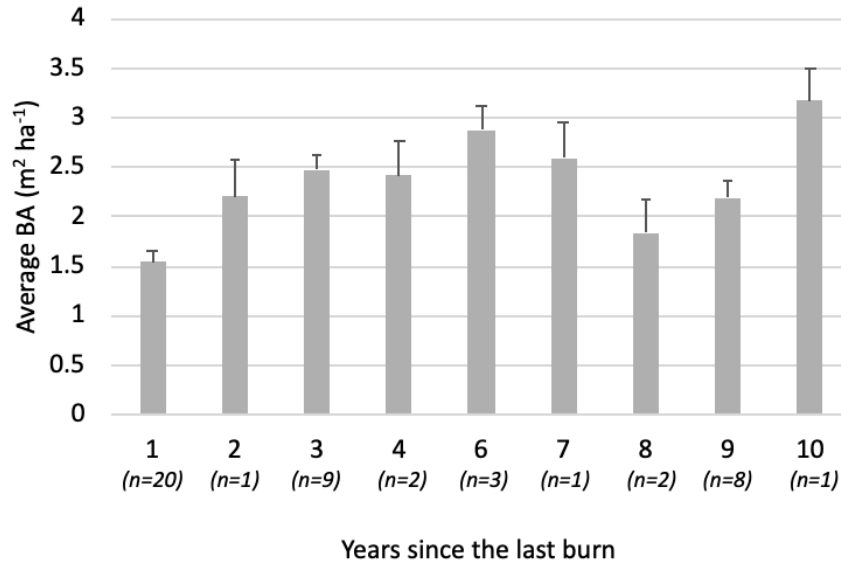Figure B-2 Avergae midstory BA per stand for both shade tolerant and intolerant species displayed by the number of years since the last burn. Stand sample sizes for each year are shown in italics below each bar, and error bars are displaying the standard error of the mean.
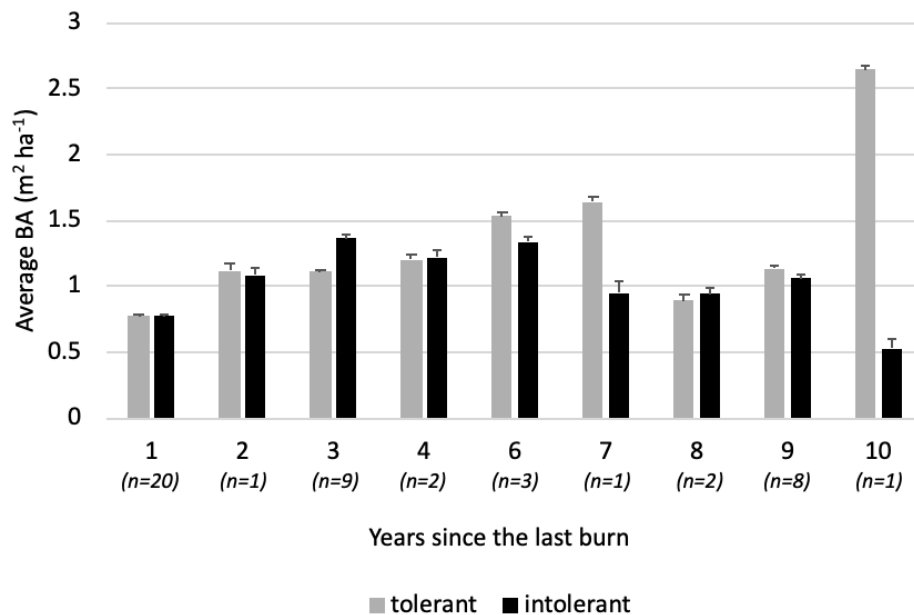
APPENDIX C. SURVEY OF FUNGAL FRUITING BODIES IN THE HARDWOOD ECOSYSTEM EXPERIMENT

C.1   Methods

C.1.1   Study Area

This study took place 23 July 2019 to 7 August 2019 at the Hardwood Ecosystem Experiment in southern Indiana. Study area details can be found in Chapter 4.

C.1.2   Plot Design

Three different management regimes were surveyed: two stands in a control unit with no known prescribed burns, two stands in a unit burned two years prior to sampling, and two stands in a stand burned three years prior. We used the already established small mammal trapping grid (described in Urban and Swihart 2013) to select 20 points in each stand for a total of 40 points in each unit. All 120 plots were circular, had an area of 4 m^2, and were placed at least 25 m away from each other. Mesic (north- and east-facing) and xeric (south- and west-facing) slopes were approximately equally represented as topography can impact both soil and climactic conditions (Claridge et al. 2000).

C.1.3   Fungal Sampling

After Claridge et al. (2000), percent slope, aspect, fire disturbance history, dominant woody plant species (by approximate percent cover) present on or adjacent to the plot, pH and organic litter depth were measured at plot center for each plot. Soil pH was measured using the Field Scout SoilStik pH meter (Spectrum Technologies, Inc., Aurora, IL). After the initial measurements were taken, the litter layer was carefully removed and sifted through all while looking for fungal fruiting bodies. Upon the discovery of a fruiting body, we took a picture of the specimen in its environment before we disturbed it during the removal process. The specimen, and any attached hyphae, was then carefully removed from the ground and we recorded the length and width, color, approximate shape, and any noticeable odor before placing it in a paper bag. The samples were then stored in a 4ºC refrigerator until they could be dried in an oven at 60ºC for 24 hours.

After the litter layer and any epigeous fruiting bodies were removed, 4-tined garden hoes and hand trowels were used to dig to a depth of 15 cm. As the soil shifted, special attention was paid to locate hypogeous fruiting bodies. If a hypogeous fruiting body was found, the same processes as described above for epigeous individuals was followed. After the drying process, the specimens were cut to reveal a "slice" of the interior. After sampling was complete, the displaced soil and leaf litter were replaced as best as possible.

C.1.4   Fungal Identification

Based on the qualitative measurements taken for each individual, fruiting bodies were tentatively assigned to genus using available literature (Baroni 2017, Castellano and Stephens 2017, Lincoff 1981). More care was taken to confirm these assignments after the fruiting bodies were dried. When available, individuals were assigned an environmental function of either parasitic, saprophytic, or mycorrhizal based on available information.

C.2   Results and Discussion

We collected a total of 78 fruiting bodies, 14 were hypogeous and the remainder were epigeous. Sixty-five of these individuals were confidently identified to an environmental function of either parasitic, saprophytic, mycorrhizal, or mycorrhizal and saprophytic. Mycorrhizal individuals were the most common (n = 36, 60% of all samples) and the mycorrhizal and saprophytic category contained only one individual (1.54% of all samples; Fig. C1). Mycorrhizal fungi were expected to be the most prevalent environmental function category as the entire Fagaceae family, which includes oak species, usually forms obligate symbioses with ectomycorrhizal fungi (Smith and Read 1997). The HEE overstory is still oak-hickory dominated, so the forest floor and soil are likely mycorrhizal fungi dominated as well.

The average pH was 4.97 ± 0.17 (mean $\pm$ SE) and all plots contained acidic or neutral soils (range: 2.85-7.24). Soil pH has been found to predict hypogeous fruiting body presence and García-Montero et al. show that some species prefer weakly acidic, neutral, or weakly basic soil matrices (2007). While this study incorporates both epigeous and hypogeous fruiting bodies, it should be noted that none of our soils were basic. This may have influenced which species were present, and another Central Hardwood Region forest with a similar oak-hickory structure but with

more basic soil may yield different species than we found at the HEE. Depth of litter present at each plot had a much smaller range of 1-7 cm, with the average plot containing 3.2 ± 0.23 cm litter. The slope ranged from 4-56% with a mean of $30.6 \pm 1.24\%$. The two most common woody plant species present at or near plots with found fruiting bodies were sugar maple (n = 15, 23.1% of all samples) and white oak (n = 11, 16.9% of all samples; Fig. C2).

Both the unburned control plots and plots burned more than 2 years prior contained 15 fruiting bodies assigned to environmental function and plots burned 2 years prior contained 35 fruiting bodies (Fig. C3). Fruiting bodies belonging to parasitic individuals were only found on burned plots, and mycorrhizal and saprophytic fruiting bodies were only found on control plots. Prescribed fire may cause vegetation to suffer injuries, leaving open wounds that can lead to scarring or an easy entry point for parasitic fungi and other diseases, leading parasitic fruiting bodies to only be found on burned plots. Parasitic fungi were not overly common in these plots, however, so it is impossible to tell if their presence is due to prescribed fire or another environmental factor. Mycorrhizal fruiting bodies were the most common on all plots and saprophytic fungi were second most common. The ratio of environmental function groups was relatively similar across all plots.

C.3   Conclusion

While this project was not meant to be an exhaustive search of all fruiting bodies in the HEE, it provides an example of what may, on average, be found during the middle of the summer by a semi-trained eye. It is important to note the potential for invasive wild boars to use fungi as a food supply. Pigs are notorious truffle hunters, so the knowledge of where a forest has a high fungal density may help predict boar foraging habits. Lastly, the fungi we found skew heavily toward mycorrhizal fungi. This bias is due to the fact that this project took place in a mature hardwood forest, where most trees have mycorrhizal associations. Fungi sprouting in grasslands or other ecosystems may be more diverse in their ecological functions; however, mushrooms are usually associated with forests so a mature forest is most often a good place to search for fungal fruiting bodies.

C.4   References

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C.5   Figures, Tables, and Images

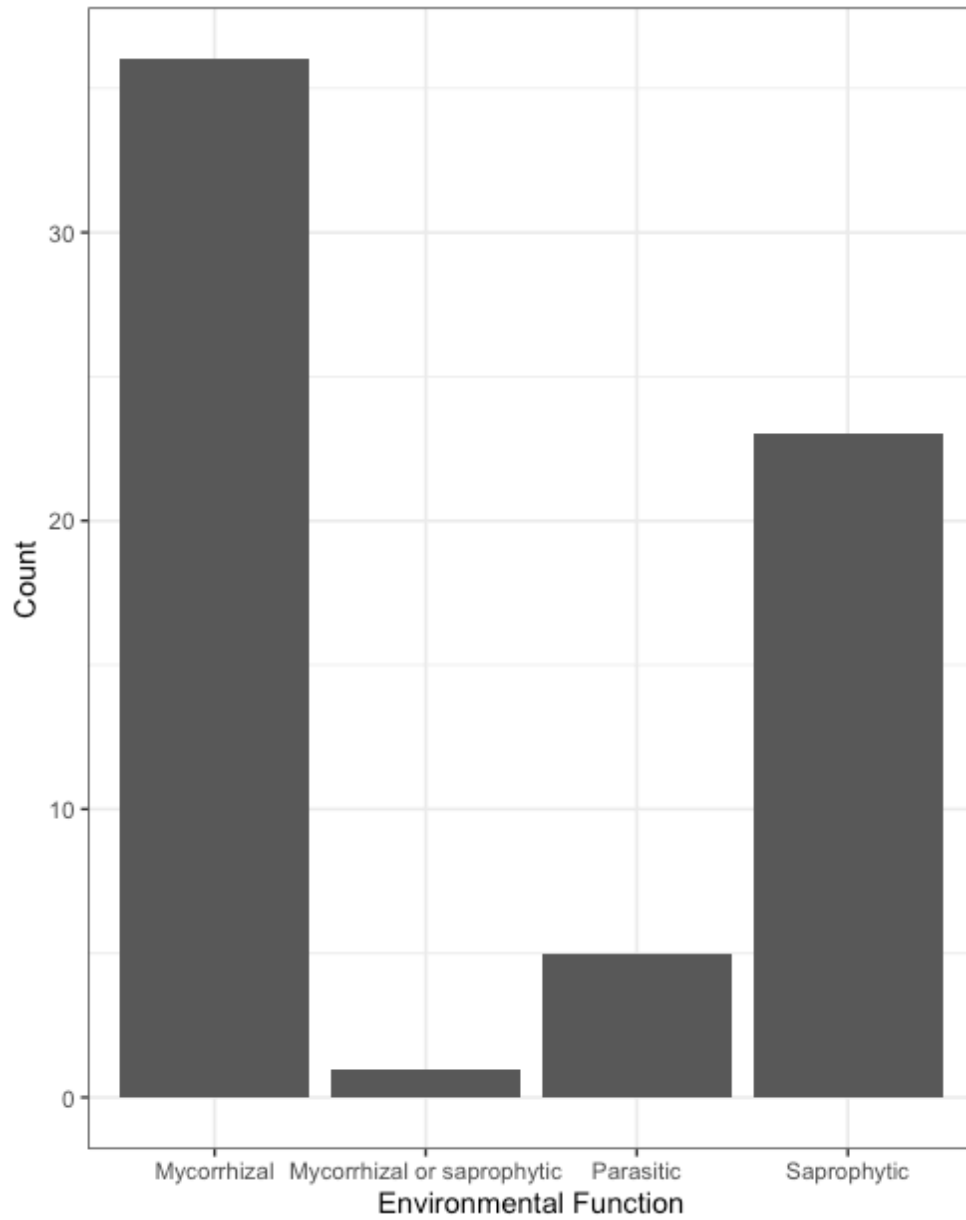Figure C-1 Count of total hypogeous and epigeous fruiting bodies (n = 65) in each environmental function assignment found at the Hardwood Ecosystem Experiment in 2019.
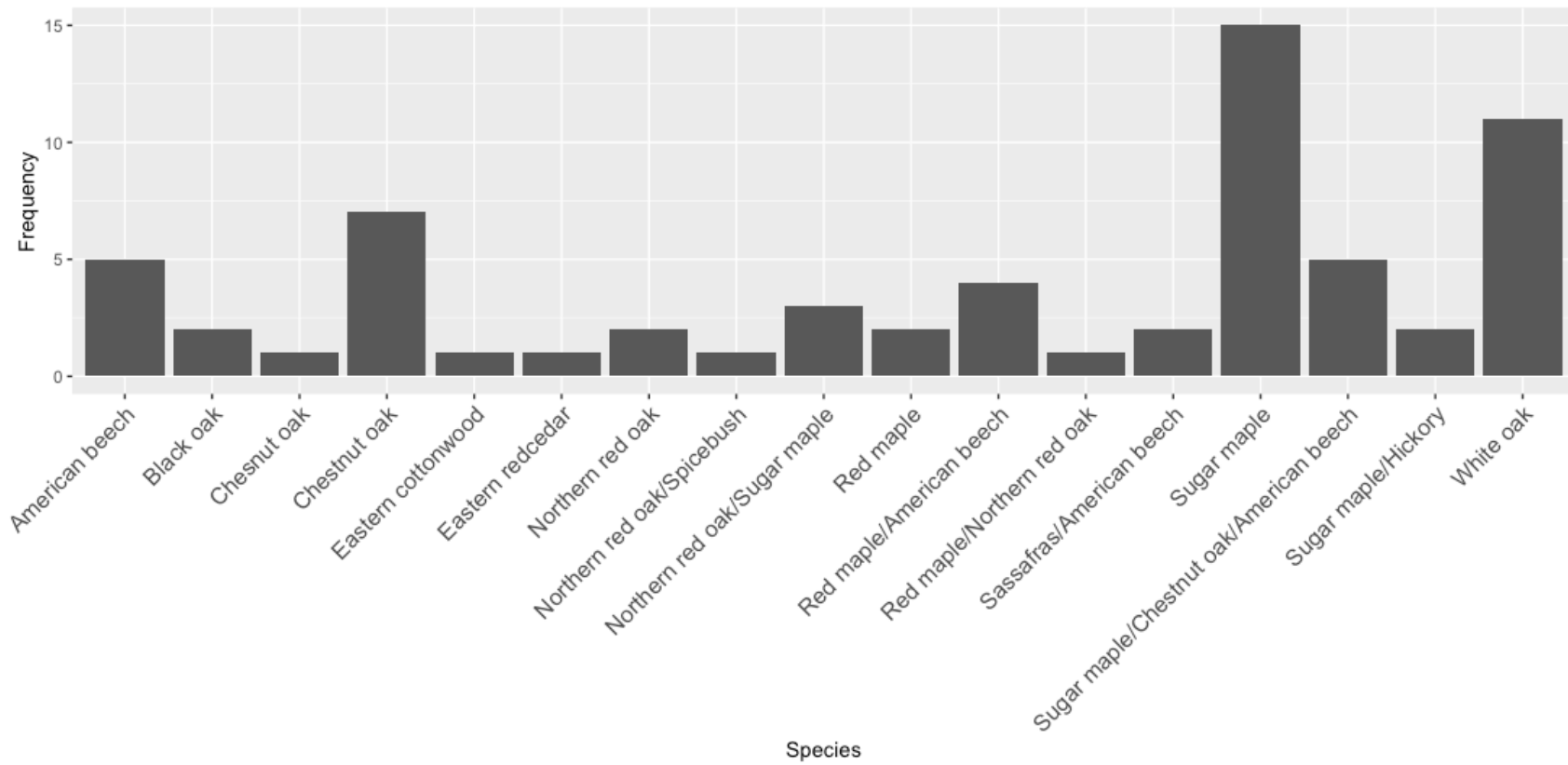
Figure C-2 Frequency of each dominant plant species group only on plots where fruiting bodies were found.
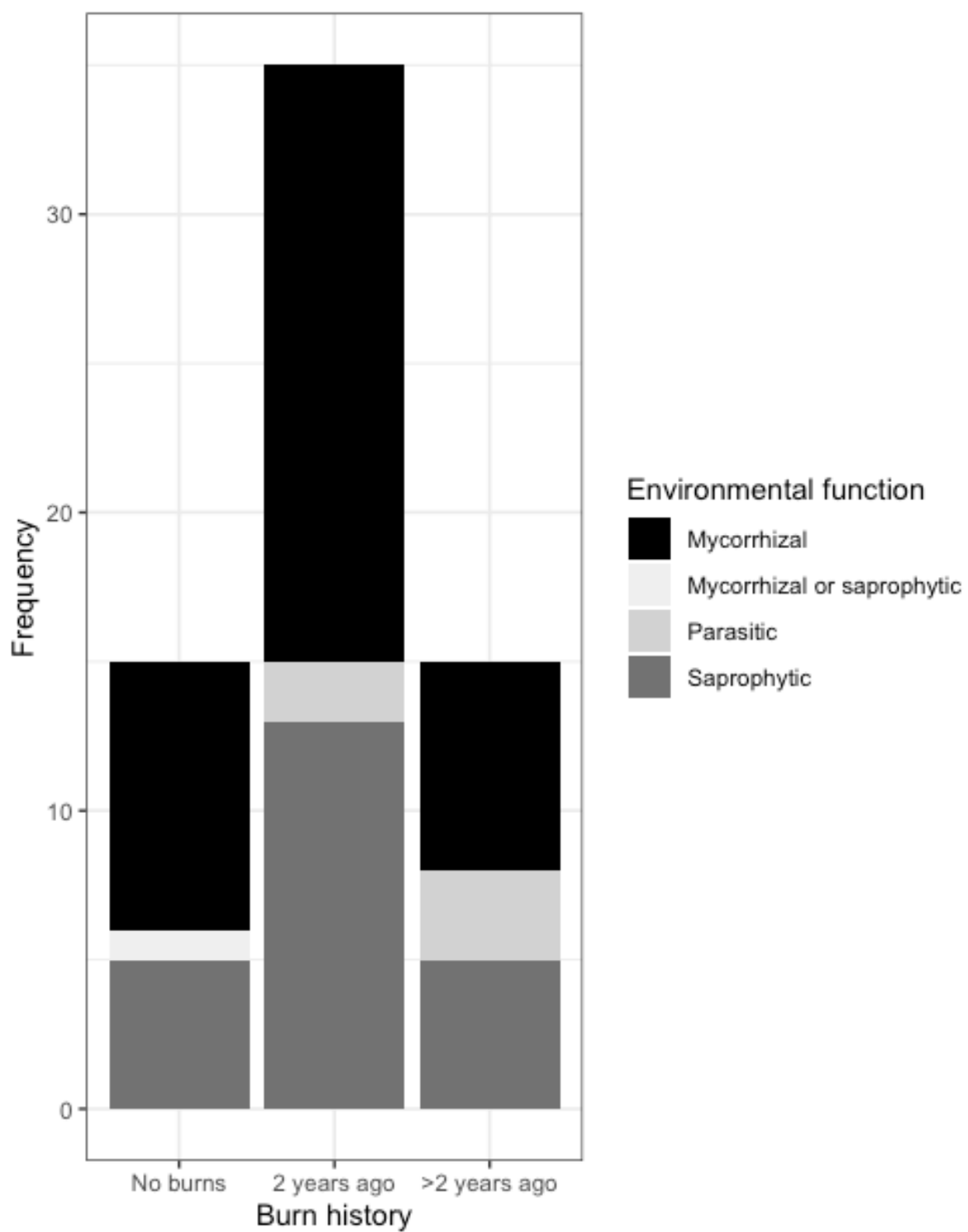
Figure C-3 Stacked bar chart displaying count of fruiting bodies separated by environmental function for each burn class.

Table C-1 Table displaying data for all fruiting bodies assigned to an environmental function. Stand IDs 3-05 and 3-16 were burned more than 2 years prior, stand IDs 6-06 and 6-10 were burned 2 years prior, and stand IDs 4-01 and 4-04 are unburned controls.

Stand ID	Plot	Slope (%)	Aspect (º)	Litter (cm)	pH	Dominant woody plant species	Taxa	Environmental function
3-05	F8	12	312	1	3.2	red maple/northern red oak	Asterophora	Parasitic
3-05	E7	45	290	2	3.8	red maple/American beech	Pluteus	Saprophytic
3-05	E7	45	290	2	3.8	red maple/American beech	Lactarius	Mycorrhizal
3-05	E7	45	290	2	3.8	red maple/American beech	Elaphomyces	Mycorrhizal
3-05	E7	45	290	2	3.8	red maple/American beech	Tricholoma	Mycorrhizal
3-05	F6	14	310	5	2.9	American beech	Amanita	Mycorrhizal
3-05	H6	23	321	4	3.3	white oak	Pholiota	Saprophytic
3-05	H6	23	321	4	3.3	white oak	Elaphomyces	Mycorrhizal
3-05	H6	23	321	4	3.3	white oak	Russula	Mycorrhizal
3-05	J2	46	316	1	3.8	sugar maple	Elaphomyces	Mycorrhizal
3-16	E9	7	86	1	5.7	Eastern redcedar	Agaricus	Saprophytic
3-16	E13	11	94	3	7.1	sassafras/American beech	Asterophora	Parasitic
3-16	E13	11	94	3	7.1	sassafras/American beech	Asterophora	Parasitic
3-16	F10	24	78	1	7.2	American beech	Entoloma	Saprophytic
3-16	H12	39	6	3	4.0	northern red oak/spicebush	Pluteus	Saptophytic
4-01	A8	34	40	2	6.6	sugar maple	Xylaria	Saprophytic

4-01	A8	34	40	2	6.6	sugar maple	Xylaria	Saprophytic
4-01	A1	33	74	2	6.6	sugar maple	Elaphomyces	Mycorrhizal
4-01	A3	24	115	4	4.7	American beech	Boletus	Mycorrhizal
4-01	B6	38	30	4	5.6	sugar maple	Laccaria	Mycorrhizal
4-01	B6	38	30	4	5.6	sugar maple	Laccaria	Mycorrhizal
4-01	D4	40	54	2	5.3	northern red oak	Clavulina	Mycorrhizal
4-01	D4	40	54	2	5.3	northern red oak	Elaphomyces	Mycorrhizal
4-04	D5	48	359	3	3.7	American beech	Boletellus	Mycorrhizal and saprophytic
4-04	D3	53	7	7	3.4	American beech	Excidia	Saprophytic
4-04	A5	24	332	2	4.1	eastern cottonwood	Lactarius	Mycorrhizal
4-04	B9	27	334	4	3.6	black oak	Cantharellus	Mycorrhizal
4-04	B9	27	334	4	3.6	black oak	Xylaria	Saprophytic
4-04	B8	26	344	3	3.4	chestnut oak	Boletus	Mycorrhizal
4-04	B8	26	344	3	3.4	chestnut oak	Xylaria	Saprophytic
6-06	D5	34	45	5	5.1	northern red oak/sugar maple	Tylopius	Mycorrhizal
6-06	D5	34	45	5	5.1	northern red oak/sugar maple	Clavulina	Mycorrhizal
6-06	D5	34	45	5	5.1	northern red oak/sugar maple	Clavulina	Mycorrhizal
6-06	D3	39	74	2	6.2	sugar maple/hickory	Xylaria	Saprophytic

6-06	D3	39	74	2	6.2	sugar maple/hickory	Xylaria	Saprophytic
6-06	E7	38	86	7	5.7	sugar maple	Russula	Mycorrhizal
6-06	E7	38	86	7	5.7	sugar maple	Clavulina	Mycorrhizal
6-06	E7	38	86	7	5.7	sugar maple	Clavulina	Mycorrhizal
6-06	E7	38	86	7	5.7	sugar maple	Clavulina	Mycorrhizal
6-06	E7	38	86	7	5.7	sugar maple	Clavulina	Mycorrhizal
6-06	E7	38	86	7	5.7	sugar maple	Xylaria	Saprophytic
6-06	G9	22	21	4	6.4	white oak	Xylaria	Saprophytic
6-06	G9	22	21	4	6.4	white oak	Xylaria	Saprophytic
6-06	G9	22	21	4	6.4	white oak	Xylaria	Saprophytic
6-06	G9	22	21	4	6.4	white oak	Xylaria	Saprophytic
6-06	G9	22	21	4	6.4	white oak	Xylaria	Saprophytic
6-06	G9	22	21	4	6.4	white oak	Xylaria	Saprophytic
6-06	G9	22	21	4	6.4	white oak	Xylaria	Saprophytic
6-06	H10	21	23	5	5.7	white oak	Xylaria	Saprophytic
6-06	E10	31	70	6	4.4	chestnut oak	Russula	Mycorrhizal
6-06	C2	33	72	2	5.7	sugar maple	Xylaria	Saprophytic
6-06	C2	33	72	2	5.7	sugar maple	Xylaria	Saprophytic
6-06	C2	33	72	2	5.7	sugar maple	Tricholoma	Mycorrhizal

6-06	C4	43	58	2	5.5	red maple	Clavulina	Mycorrhizal
6-10	G8	27	262	2	2.9	chestnut oak	Elaphomyces	Mycorrhizal
6-10	G8	27	262	2	2.9	chestnut oak	Elaphomyces	Mycorrhizal
6-10	G8	27	262	2	2.9	chestnut oak	Elaphomyces	Mycorrhizal
6-10	E5	22	386	4	3.5	chestnut oak	Elaphomyces	Mycorrhizal
6-10	G13	37	313	1	3.0	sugar maple/chestnut oak/American beech	Elaphomyces	Mycorrhizal
6-10	G13	37	313	1	3.0	sugar maple/chestnut oak/American beech	Elaphomyces	Mycorrhizal
6-10	G13	37	313	1	3.0	sugar maple/chestnut oak/American beech	Elaphomyces	Mycorrhizal
6-10	G13	37	313	1	3.0	sugar maple/chestnut oak/American beech	Elaphomyces	Mycorrhizal
6-10	G13	37	313	1	3.0	sugar maple/chestnut oak/American beech	Elaphomyces	Mycorrhizal
6-10	B5	45	280	1	4.8	red maple	Asterophora	Parasitic
6-10	C6	35	264	1	3.8	chestnut oak	Asterophora	Parasitic

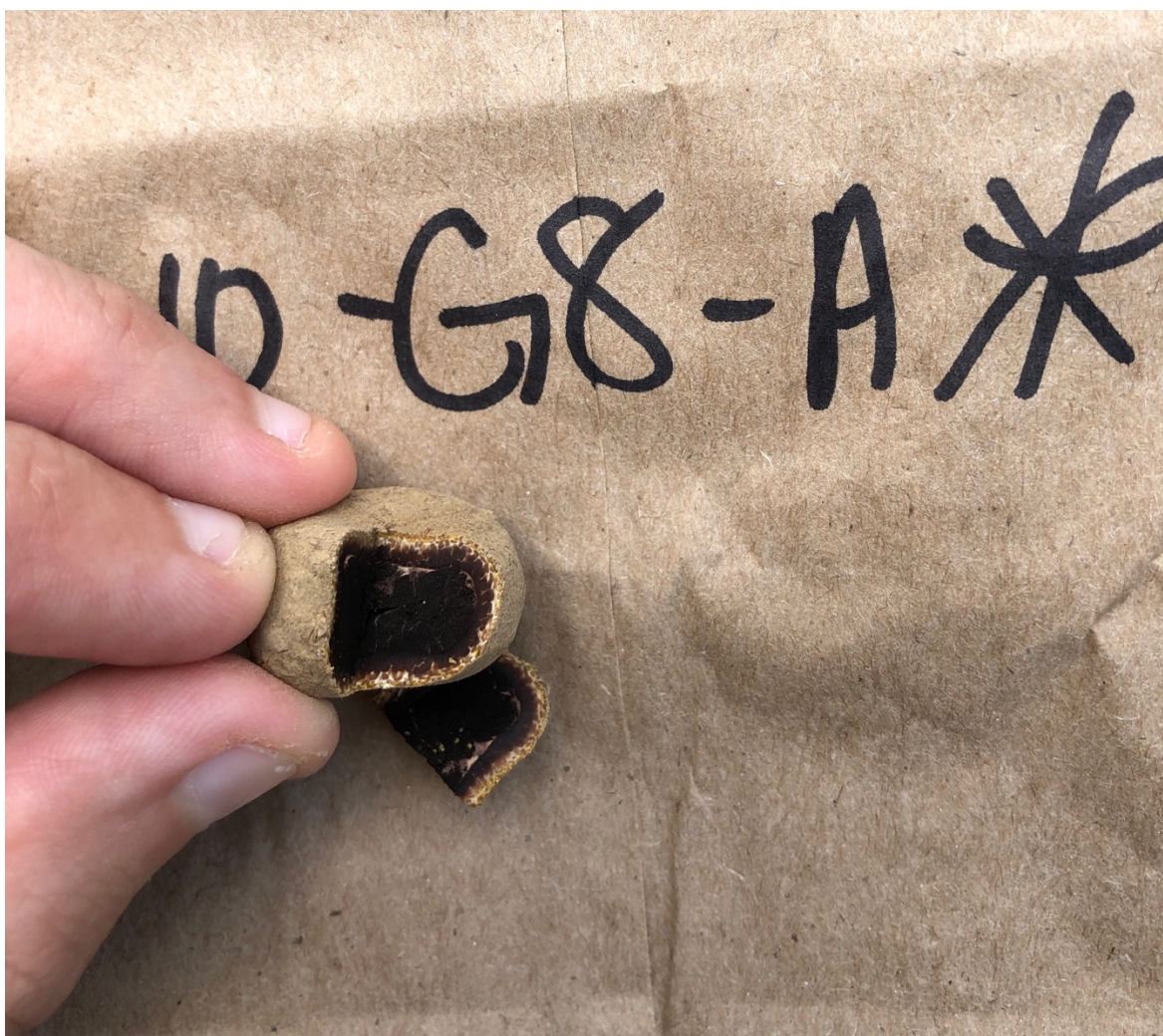Image C-1 *Elaphomyces* spp. after brushing off soil.

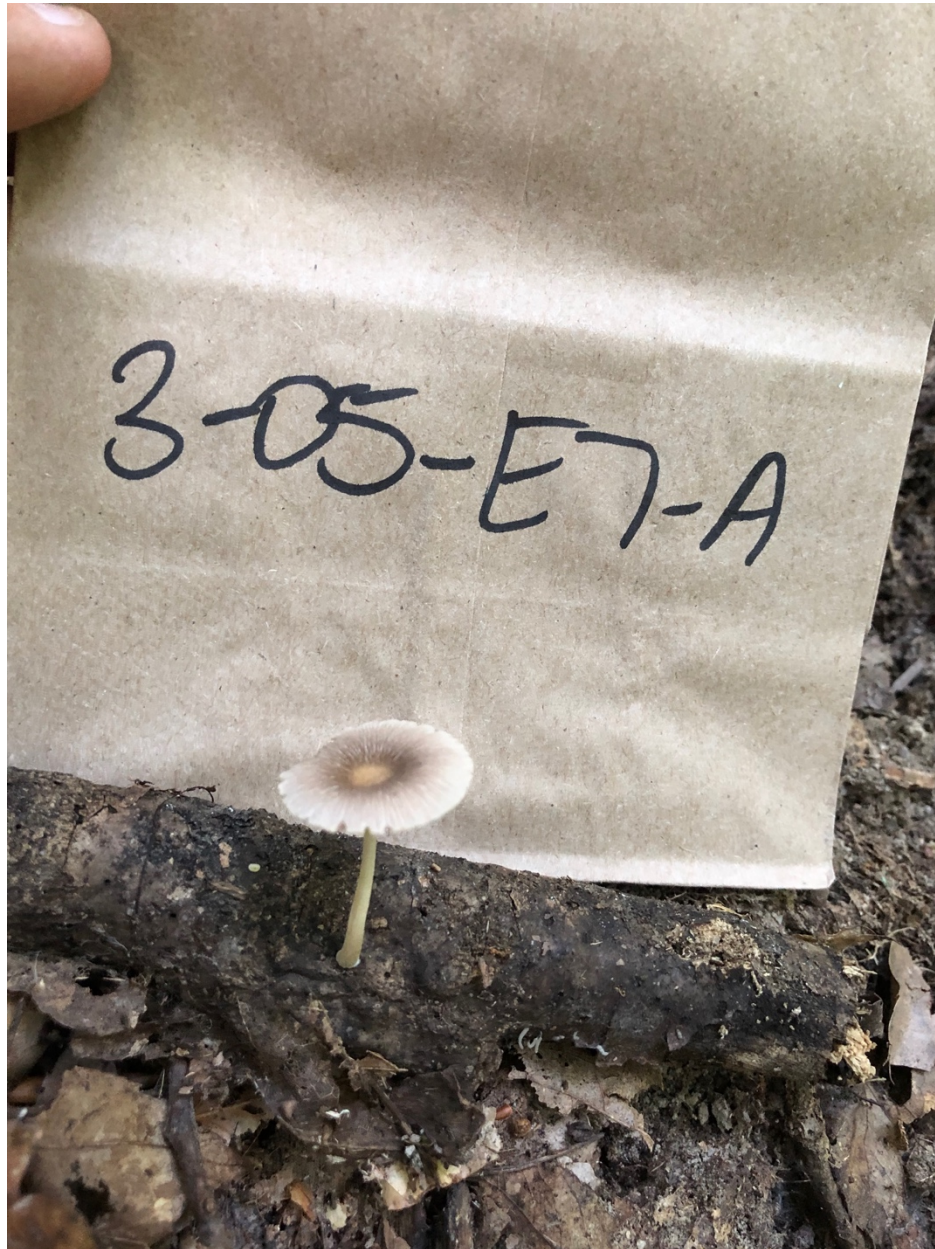Image C-2 *Elaphomyces* spp. with a "slice" removed to display spore structure.

Image C-3 Example of a field picture. Specimen is still located where we found it, on a piece of decaying wood, with the prepared storage bag in the background labelled with the unique fungal ID.