THE ROLE OF THE SOIL SEED BANK IN SOUTHERN APPALACHIAN WILDFIRE RESPONSE

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By

Kelder C. Monar

Director: Dr. Beverly Collins
Associate Professor of Biology
Biology Department

Committee Members: Dr. Kathy Gould Mathews, Biology
Dr. Diane Styers, Geosciences and Natural Resources

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ABSTRACT

THE ROLE OF THE SOIL SEED BANK IN SOUTHERN APPALACHIAN WILDFIRE RESPONSE

Kelder C. Monar, MS.
Western Carolina University (April 2018)
Director: Dr. Beverly Collins

Wildfire disturbance is likely to increase in Southern Appalachian forests due to climate change and anthropogenic land-use changes. The soil seedbank can be a source of plant community response after this disturbance. Fire can affect seeds and seed germination both positively and negatively, yet little is known about seedbank response to fire in this region. A seedbank study was conducted in the greenhouse from May 23, 2017 to January 9, 2018 using litter and mineral soil samples from burned (B) and unburned (U) plots in three mid-elevation sites to determine seedbank contribution to first-season plant community response following wildfires that swept through the region in fall, 2016. In addition, extant vegetation, woody and herbaceous seedlings, and environmental variables including canopy openness, litter cover, soil moisture, bare ground cover, temperature and tree and shrub mortality were surveyed in the field over the 2017 growing season. Seedbank-generated plant abundance and species richness were compared between B and U plots, and with field seedling and field tree communities. Seedbank abundance and richness were lower in B samples from the litter layer, but not the soil layer, suggesting that fire killed seeds near the surface but that seeds underground were insulated. Density and richness of field seedlings were higher in B plots than U plots, suggesting that post-fire environmental differences, not changes to the seedbank itself, control forest response to wildfire from the seedbank. High light, low litter, and fluctuating temperature in B plots favored germination. Species that responded most strongly in the field were generalist disturbance-adapted species such as black locust (*Robinia pseudoacacia*), not species specifically adapted to fire. Some species, such as black locust and birches (*Betula spp.*), were more common in the seedbank than in extant vegetation. Recruitment from the seedbank after periodic disturbance may allow these species to persist at these sites. Overall, the seedbank can contribute to first-year post-fire vegetation response, and has the potential to shift species composition in Southern Appalachian forests.

CHAPTER 1: INTRODUCTION

In fall 2016, at the end of a growing season that was 2°C warmer than average (at 685m; USDAc) and much drier than average (24.61cm deficit at 685m elevation, 39.10cm deficit at 1364m; USDAa, USDAb), several wildfires burned in the Southern Appalachian Mountains. The extent of the fires was unprecedented in recent decades. More than half (55%) of the acreage burned during fall in the Southern Appalachians since 1970 occurred in 2016 (Norman 2016). Although these fires and the conditions that enabled them were record-setting, they may become more common in the future. Analyses for the Southern Forest Futures Project indicate the higher temperatures and more variable precipitation that are likely to accompany climate change could increase severity of wildfire events (Stanturf and Goodrick 2013). Southern Appalachian fall wildfires are disproportionately human-caused (Norman 2016), and many are set intentionally (Freedom du Lac et al. 2016). Human population density is predicted to increase at the urban-wildland interface in this region (Stanturf and Goodrick 2013), providing more sources of ignition.

Fire has been removed from most Southern Appalachian forests since the mid-1900s (Flatley et al., 2013), though it was set or occurred naturally for thousands of years beforehand (Fesenmyer and Christensen, 2010). Fire can play an important ecological role in forests, such as enhancing diversity in herbaceous layer vegetation (Elliott et al., 2009; Hagan et al., 2015). One mechanism for this increase in diversity is recruitment from the soil seedbank. However, understanding of soil seedbanks of southern Appalachian forests is limited, and few data are available on the effect of fire on these seedbanks. This research examined the effects of fall wildfire, including high severity wildfire, on first-season soil seedbank response in the southern

Appalachians. Goals were to add to the limited body of knowledge on soil seedbanks in Southern Appalachian mixed hardwood forests, to explore the effects of wildfire on recruitment from the seedbank, and evaluate potential post-wildfire community shifts due to recruitment from the seedbank. Specific research questions were:

1. Are there quantitative differences between burned and unburned areas' soil seedbanks, litter seedbanks, and seedling recruitments in the field?
2. Are there qualitative differences between burned and unburned areas' soil seedbanks, litter seedbanks, and seedling recruitments in the field?
3. Are there qualitative differences among the seedbank, seedling, and standing vegetation communities?

CHAPTER 2: LITERATURE REVIEW

Fire as an Ecological Disturbance

Disturbance is a feature of eastern North American forests. Although the scale and intensity vary greatly, from death of a single tree to a wildfire that consumes thousands of acres, a common theme is ecological succession due to the removal of strong competitors. Disturbance promotes plant functional trait diversity in forest communities dominated by slow growing, heavy-seeded trees (Wilfahrt et al., 2014), allowing species with a “ruderal” ecological strategy to establish due to their short life-cycle and persistent seeds able to germinate rapidly in favorable conditions (Grime, 1977).

Post-disturbance, early-successional ecosystems have high species diversity, especially in the herbaceous layer (Swanson et al., 2011). In the absence of disturbance, early successional forests lose their early-successional characteristics. In lightly-disturbed areas, most understory species return to their former abundances within a few years (Aikens et al., 2007). Even after a severe disturbance, such as a clear-cut, the herbaceous component declines dramatically within 30 years (Elliott et al., 1997). Because the shift in successional state provided by any one disturbance event is temporary, reoccurring disturbances are needed to maintain landscape diversity.

Fire is the one of the most common types of disturbance in Piedmont and Coastal Plain southeastern forests (Stanturf et al., 2002). In the Southern Appalachians, the role of forest fire as a disturbance agent has changed over time. Charcoal evidence suggests fire has been common across topographic positions in this region for thousands of years (Fesenmyer and Christensen, 2010). Native American use of fire, beginning around 3000 years ago and accelerating 1000 years ago, helped shape the current forest communities in this region (Delcourt and Delcourt, 1998; Fesenmyer and Christensen, 2010). Modern fire management in the Southern Appalachians began around 1930; prior to this, some xeric forest types, such a pine-oak, burned approximately every 2-5 years, with area-wide fires occurring approximately every 6-16 years (Aldrich et al., 2010; Flatley et al., 2013). Occasionally, severe crown fires in these xeric forest types would reduce canopy coverage and basal area by 85% (Barden and Woods, 1976).

The reduction of fire disturbance in the modern era has had several consequences for Southern Appalachian forests. One has been a decline of forest community differentiation along the topographic moisture gradient as shade-tolerant, fire-intolerant tree species have established in drier topographic positions such as ridges and south-facing slopes (Flatley et al., 2015). This phenomenon, termed “mesophication,” has resulted in a decline in stand-level species richness (Nowacki and Abrams, 2008). Although the trend of mesophication is region-wide, topographic position still influences fire frequency at smaller scales, with south-facing slopes and ridges burning most frequently (Flatley et al., 2011). These patterns, however, are climate-dependent. For instance, dry years allow fires to spread into more mesic areas and reduce differentiation in fire likelihood based on topography (Flatley et al., 2011), though topographic positions can still affect fire severity (Swank et al., 1999).

Ecological effects of a fire depend on the fire’s characteristics, especially fire severity. In Great Smoky Mountains National Park, fire severity was important for predicting the magnitude of the change in plant community composition post-fire (Schwartz et al., 2016). A frequent change associated with fire is a decrease in the shrub layer (Hagan et al., 2015; Swank et al., 1999). Fire, especially high severity fire, in contrast can promote species richness in the herbaceous layer in some forest types (Hagan et al., 2015; Jenkins et al., 2011). Effects of high severity fire on herbaceous and understory diversity can extend 10 years post-fire (Elliott et al., 2009). Low severity fire has relatively little effect on species composition (Keyser et al., 2012; Swank et al., 1999). Increases in species richness associated with a fire tend to be localized, at scales as small as <1m^2, and limited by the potential immigrant species pool in the surrounding forest (Hagan et al., 2015; Reilly et al., 2006).

Soil Seed Banks and Fire

The soil seed bank includes all naturally occurring viable seeds stored in the soil or on the soil surface independent of the mother plant (Csontos, 2007). Via the "storage effect," seed banks can allow multiple communities to coexist on a site over time when species' germination requirements and environmental conditions vary (Warner and Chesson, 1985). A short-term, transient seed bank allows plants to respond to annual variation, whereas a persistent seed bank allows plants to respond to stochastic events that disturb established aboveground vegetation (Thompson and Grime, 1979). Thus, seed banks can dampen the effects of both predictable (e.g., annual) environmental variation and stochastic events such as disturbances (Templeton and Levin, 1979). The soil seed bank can be more diverse than the aboveground vegetation in the same area (Vandvik et al., 2016), and can resemble past rather than current aboveground vegetation in highly variable communities such as wetlands (Faist et al., 2013), serving as a reservoir of biodiversity. In most plant communities, seed density is concentrated near the soil surface (Csontos, 2007), potentially exposed to fire.

Fire can directly affect seeds, both positively and negatively. For instance, elevated temperatures can have mixed effects for seeds. In a lab study, ten minutes of dry heat has been shown to aid in breaking dormancy for some eastern North American species, including *Galactia regularis* and *Lupinus perennis* (80°C) and *Rhus copallinum* (90°C); however, at 110°C almost all seeds of these species were killed (Bolin, 2009). In a field study in which fire temperature ranged from <79 to <371°C, *Quercus rubra* and *Q. alba* acorns showed high mortality above 204°C, and universal mortality above 260°C (Greenberg et al., 2012). A seed's shape can affect its fire tolerance. In species otherwise unadapted to fire, compact seeds showed greater fire tolerance than elongated seeds with higher surface area (Ruprecht et al., 2015). In addition to these physical effects, the chemical legacy of fire can affect seeds. Although smoke is a well-studied germination cue in Mediterranean and prairie ecosystems (Enright and Kintrup, 2001; Moreira et al., 2010; Ren and Bai, 2016; Roche et al., 1997), the research on the effect of smoke on temperate forest seeds is limited. In a study of cool temperate secondary forest in Japan, smoke inhibited germination of more species (8) than it facilitated (3) (Tsuyuzaki and Miyoshi, 2009). In Norwegian heathland, smoke, and to a lesser degree ash, served as a germination cue for graminoids, forbs, and ericaceous shrubs (Bargmann et al., 2014). The effect of smoke can depend on life-history traits. Species of the Hungarian flora that showed positive responses to smoke were disturbance-adapted (Mojzes et al., 2015).

Recruitment strategies of individual species can determine plant community response to fire. A trade-off exists between two broad strategies: resistance (resprouting) and resilience (reproducing from seed) (Miller and Chesson, 2009). In disturbance-prone communities in temperate Australian forests, resprouting species are poorly represented in the seed bank compared to those species killed by fire (Clarke and Dorji, 2008). In a Canadian boreal forest, regeneration strategy has been observed to affect patterns of establishment depending on fire severity (Wang and Kemball, 2005), with stronger representation of resistant species at low severities and resilient species at high severities (Lee, 2004).

Southern Appalachian forests were thought to have depauperate seedbanks (Dan Pittillo, personal communication) like those of temperate forests outside the region (Matlack and Good, 1990; Roberts and Vankat, 1991). However, robust seedbanks have been found in Southern Appalachian forests (Dobberpuhl, 1980, cited in Farmer, 1997), with seed densities of 471 per m^2 reported (Keyser et al., 2012) and six woody species reported at densities of >10 seeds/m^2 in mixed hardwood forests (Ris Lambers et al., 2005). Although annual mortality is high for seeds of three common Southern Appalachians trees, *Acer rubrum*, *Liriodendron tulipifera*, and *Betula spp.*, seeds ≥2 years old are a substantial component of germination-ready seeds (Ris Lambers et al., 2005). Short-term seedbanking can also contribute to recruitment the following year, for instance in *A. rubrum* (Lambers and Clark, 2005). *Betula alleghaniensis* has been shown to have large seasonal changes in seedbank density in the litter layer, but the soil seedbank was more constant, with input from the litter balancing removal (mortality, germination) (Houle and Payette, 1990). Similarity between the seedbank and aboveground vegetation is generally low (Keyser et al., 2012).

CHAPTER 3: METHODS

Field Site Descriptions

This study was conducted in 20 burned (B) and 11 unburned (U) 7m-radius plots in three mid-elevation sites within the Nantahala National Forest in Macon and Jackson Counties of western North Carolina (Fig. 1). The three sites, Cliffside (C), Dicks Creek (D), and Wine Spring (W), experienced wildfire in fall, 2016. Sites and plot locations were chosen to capture a range of fire severity. Because of the unplanned nature of these wildfires, U plots are used to infer pre-fire conditions.

Cliffside (N 35.0817°, W 83.2392°)

Seven B and 4 U plots were at mid-elevation (1049-1186m, median 1110m), on relatively steep slopes (22.6-60.0%, median 34.9%), with a generally southeastern aspect. Fire severity ranged from low to high. The site grades from acidic cove forest, typic subtype, to chestnut oak forest, dry heath subtype (Schafale 2012), and shows signs of past human modification, such as presence of a non-native *Euonymus sp.* and possibly planted white pine (*Pinus strobus*). The largest tree in plots at the site is a white pine at 74cm DBH; the largest hardwood tree was a scarlet oak (*Quercus coccinea*) 56cm DBH. The absence of very large trees suggests disturbance at the site in the past, and that forests at Cliffside are second-growth. Common tree and shrub species at the site include *Pinus strobus, Rhododendron maximum, Acer rubrum, Kalmia latifolia,* and *Liriodendron tulipifera*.

Dicks Creek (N 35.4002°, W 83.2505°)

Nine B and 3 U plots were at mid-elevation (955-1144m, median 1048m), on very steep slopes (46.3-84.4%, median 61.6%), with a generally northwestern aspect, though three B plots had a southern aspect. Fire severity was generally high. Much of the site can be characterized as chestnut oak forest, mesic subtype, with some areas of acidic cove forest (Schafale 2012). The largest tree in plots at the site is a chestnut oak (*Quercus montana*) at 82cm DBH. This tree was atypical, and on an 80% slope; the next-largest trees at Dicks Creek were eastern hemlocks (*Tsuga canadensis*) at 61cm and 58cm DBH, a sourwood (*Oxydendrum arboreum*) at 56cm DBH and northern red oak (*Quercus rubra*) at 53cm DBH. The scarcity of very large trees at the site, except on inaccessible very steep slopes, as well as the present of several soil roads, suggests past logging disturbance at Dicks Creek, and that current forests are second growth. Common tree and shrub species at the site include *Quercus montana, Acer rubrum, Rhododendron maximum, Oxydendrum arboreum*, and *Quercus rubra.*

Wine Spring (N 35.1692°, W 83.6080°)

Four B and 4 U plots were at mid-elevation (1287-1428m, median 1366m), on relatively steep slopes (28.0-61.5%, median 46.6%), with a generally northern aspect. Fire severity was generally low. Much of the site can be characterized as northern hardwood forest, rich subtype (Schafale 2012). The largest trees in plots at the site are a black cherry (*Prunus serotina*) at 68cm DBH, a sugar maple (*Acer saccharum*) at 65cm DBH, and a northern red oak at 61cm DBH. The absence of very large trees on the site as well as the present of several soil roads, suggests past logging disturbance at Wine Spring, and that current forests are second growth. Common tree species at the site include *Prunus serotina, Acer rubrum, Quercus rubra, Magnolia acuminata,* and *Betula spp.* (*B. lenta* and *B. alleghaniensis*).

Field Methods

At each plot, vegetation was quantified in each of five structural categories: Trees, Shrubs, Vines, Herbs, and Seedlings. Field vegetation data were collected between May 25 and

July 3, 2017, with a second trip between September 16 and October 12, 2017, to confirm species identification. Species were identified with the aid of the *Audubon Society Field Guide to North American Trees: Eastern Region*, *Newcomb's Wildflower Guide*, and *Weakley's Flora of the Southern and Mid-Atlantic States*. Some plants were assigned a numerical code if it was not possible to identify them in the field.

Trees

All woody vegetation >5cm DBH (diameter at breast height, 1.4m) was sampled within the plot. Measures included species, DBH, scorch height, and a determination of whether the aboveground biomass was alive, had been killed by the fire, or had been killed prior to the fire. Scorch height was measured through 2.5m above ground, then visually estimated between 2.5 and 5m. Scorch heights above 5m were assigned the height 5.01m. Stems that split below 1.4m height were counted as separate individuals.

Shrubs and Vines

Woody vegetation <5cm DBH and >0.5m height was classified as a shrub. Plots were divided into quarters and percent cover of each living shrub species was estimated using Daubenmire cover classes. This category includes stump sprouts for otherwise dead trees. Percent dead cover was also estimated for two species of ericaceous shrubs: *Kalmia latifolia* and *Rhododendron maximum*.

Climbing woody vegetation <5cm DBH and >0.5m height was classified as a vine. The shrub procedure was also used for this category. Vines occurred in a small number of plots, and thus were added to shrubs for analysis.

Ground layer

Herbaceous vascular plants and woody plants <0.5m height were grouped as ground layer vegetation. Cover was estimated for each species in four $1m^2$ subplots per plot using Daubenmire cover classes. Subplot photos were taken of at the time of data collection. Subplots were located 2m from plot center at NE, SE, SW and NW bearings.

Seedlings

Seedling abundance by species was also collected in the subplots. Seedling status was determined primarily by presence of cotyledons. In the absence of cotyledons, root development, leaf morphology, and resemblance to nearby conspecifics with cotyledons was used as a guide.

Environmental Variables

Environmental variables were measured in plots or subplots. Canopy photos were taken with a Canon digital camera with fish-eye lens and analyzed using Gap Light Analyzer software to calculate percent canopy openness. Volumetric water content was measured with a FieldScout 350™ TDR. Measurements for the plot's center and within each subplot were averaged to produce a plot value. The percentages of rock, litter, and bare ground cover were estimated for the entire plot at the time of vegetation sampling using Daubenmire cover classes. Elevation, slope, and aspect were calculated for plot centers using a Digital Elevation Model in ArcGIS 10.5.1 (ESRI 2011). A covered iButton,™ placed 10cm above ground level at plot center, recorded temperature hourly between July 29 and September 9, 2017.

Greenhouse Methods

The litter and soil seedbanks were assessed using the emergence method, in which a sample is watered and emerged seedlings are identified and counted (Espeland et al., 2010). Between April 14 and May 18, leaf litter from 400 cm^2 of the surface (20 x 20 cm square) and 785.5 mL of soil (a cylinder 10cm deep with 5cm radius) were collected from eight sample points per plot, yielding variable amounts of litter and approximately 6.3L of soil per plot. Large debris was removed from litter samples and the remaining litter was combined with 5L of mycorrhizae-enriched sterile potting mix (Premier Horticulture Pro-Mix BX) and placed in a standard greenhouse tray with drainage holes. Soil samples were put through a 0.95cm sieve and combined with 2L of potting mix. Identifiable seeds larger than the sieve size (e.g. acorns) were added back to the trays manually. Three control trays contained 5L of potting mix only.

Litter and soil samples were exposed to conditions favorable to germination for 225 days in the greenhouse. Trays were arranged haphazardly on a greenhouse bench and watered from overhead misters for six minutes daily. Greenhouse low temperature was 15°C; greenhouse high temperatures were generally ambient on cloudy days and higher than ambient on sunny days. In some cases temperatures as high as 43°C were observed. Trays were shifted weekly according to a predetermined rotation. Tray photos were taken weekly. After 94 days, all plants were removed from trays. Identifiable plants were discarded; type specimens of unidentifiable plants were potted separately. Soil was air dried for 14 days. Dried soil was mixed thoroughly in a separate container and then returned to tray. Trays were again placed on a watering rotation with water reduced to four minutes daily. All additional emerging plants were removed 117 days after the samples were returned to the tray.

Type specimens of unidentifiable plants were potted separately in pots containing potting mix and 15.6 mL/L all-purpose organic fertilizer (Ecoscraps). Plants were watered daily and allowed to grow until identification was possible or the plant died. Taxonomy follows *Weakley's Flora of the Southern and Mid-Atlantic States* where possible. Experienced botanists including Dan Pittillo (WCU) and Jason Love (UGA) were consulted when making species identifications. In some cases, generalization to genus or higher classification was necessary when morphological characteristics could not be evaluated. Photographs and pressings taken during the study allowed identifications to be confirmed.

Data Analysis

The 20 burned (B) and 11 unburned (U) plots were treated as experimental units in an unbalanced randomized complete block design. Differences between B and U plots were tested for the litter seedbank, soil seedbank, and post-fire seedling responses in the greenhouse. Community differences between (1) B and U plots, (2) the combined (B+U) seedbank and field responses, and (3) the seedbank community and extant plant community were also tested. Analysis of the extant plant community focused on the tree (and large shrub) community due to potential ecological effects of community differences, abundance of tree seedlings, and relative ease of identification. As such, DBH values for tree species were summed per plot, shrub+vine and ground layer species cover classes were converted to species plot averages, and seedling species counts were summed per plot. For some comparisons, emergent abundances were converted to density/m^2.

Field and Greenhouse Seedling Abundance

Abundance and species richness (number of species) were calculated for emergents in each tray in the greenhouse and field plots. Differences in mean per-tray or per-plot abundance and species richness were compared between burned and unburned plots using a linear mixed-model with site as a random effect and fire (burned, unburned) as a fixed effect. The ‘lmerTest’ package in R was used (Kuznetsova et al. 2016; R Core team 2016). Separate analyses were run for (1) field seedlings, (2) litter seedbank samples in the greenhouse, and (3) soil seedbank samples in the greenhouse. The alpha used to determine significance was 0.01. Some data were log-transformed to improve normality, and a maximum of 1 “zero” value per analysis was replaced with 1 to allow log-transformation.

Community Analyses of Seedlings and Seedbank

Multi-response permutation procedure (MRPP) in PC Ord v.5 (MJM software) was used to test for differences in germinant species composition between (1) burned and unburned field plots, (2) litter seedbank samples from burned and unburned plots, (3) soil seedbank samples from burned and unburned plots, (4) litter and soil seedbank samples, (5) the standing plot trees and tree species in greenhouse samples, and (6) the germinant community in the field and greenhouse. MRPP is a non-parametric procedure for detecting the difference between previously classified groups based on a distance function; the null hypothesis is that observations are independent of membership in a group (Cai 2006; Mielke 1984). Sørensen (Bray-Curtis) distances were calculated from abundance-weighted data. In some cases, rows containing all zero values were removed from the analysis. Several minor unidentifiable species were removed from the analyses. In some cases (MRPP 5) tree species were generalized to match the less taxonomically precise germinant data. In analysis MRPP 5, pre-fire community data were used (including fire-killed trees). When comparing among communities from different source data, abundance was converted to a percentage of total source abundance. The alpha used to determine significance was 0.05.

Indicator species analyses were performed in PC Ord v.5 to determine species that indicate burned vs. unburned samples (MRPP hypotheses 1, 2, 3), vegetation layer (MRPP hypothesis 4), or community (MRPP hypothesis 5, 6). The alpha used to determine significance was 0.05.

Environmental Measures

Environmental data were used to provide context for differences observed in species abundance data, but were not used as an explanatory variable in models. Environmental data were examined at the plot level. Scorch heights from all trees in the plot were averaged. Shrub mortality was calculated by dividing fire-killed ericaceous shrub cover by total ericaceous shrub cover per plot. Burned plots without ericaceous shrub cover were assigned a mortality of zero; these six plots were lightly burned (for comparison, they averaged 0.06 tree mortality), so this seems a reasonable assumption. Tree mortality was calculated by dividing summed fire-killed tree DBH by total tree DBH per plot. Average minimum, maximum and mean temperatures were calculated per plot from iButton data in R (R Core Team 2016).

CHAPTER 4: RESULTS

Environmental Differences

Differences between burned and unburned plots varied among the three sites (Table 1). In general, compared to U plots, B plots had greater canopy openness, less ground layer cover, less surface litter, more bare ground, similar soil water content, higher maximum temperatures, similar mean temperatures, and similar low temperatures. Burned plots also had higher scorch heights, and greater shrub and tree mortality; unburned plot values for these variables were necessarily zero. Increased post-fire air temperature is most evident in daily maximum temperature. Average minimum and mean temperatures varied less than 1°C between treatments at each site; in contrast, daily maximum temperatures were at least 1°C greater in B plots at all three sites.

Characterization of the Seedbank

In general, the seed bank from samples in the greenhouse was both diverse and abundant, with approximately 70 taxa and 1610 individuals. Most taxa were uncommon; only 11 occurred in more than 10% of samples. The most common taxon in both litter and soil seedbanks was *Betula spp*., which occurred in 61.2% of samples and totaled 432 individuals.

Emergents were unevenly distributed among samples. In general, emergent density was much greater in the soil layer than in litter layer. Litter layer seedbank density ranged from 0 to 253.1 emergents/m^2 (median 31.3), whereas soil layer seedbank density ranged from 0 to 2275.9 emergents/m^2 (median 302.4). Litter taxa richness ranged from 0 to 11 (3), whereas soil taxa richness ranged from 0 to 18 (6). Burned litter samples had significantly (p=0.0001) lower abundance (mean 8.2) and lower richness (p=0.002) (2.6) than U litter samples (31.5 and 4.7, respectively). Burned soil samples were not significantly different in abundance (29.2; p=0.10) or richness (6.3; p=0.61) from U soil samples (47.0 and 7.3, respectively).

In the field, 3709 seedlings were observed: 3529 from 47 identifiable taxa and 180 unidentified individuals. Most taxa were uncommon, with only 12 occurring in more than 20% of samples. The most common taxon was *Betula spp*., which occurred in 62% of samples and totaled 889 individuals. Seedlings were unevenly distributed among plots. Density ranged from 0.3 to 147 seedlings/m^2 (median 16.8), whereas richness ranged from 1 to 17 (8). Burned plots contained significantly (p= 0.0001) more seedlings (165.1) than U plots (37.1). Burned plot species richness (11.3) was significantly (p=6.11e-05) greater than U plot species richness (5.7).

Analysis of greenhouse emergent communities by MRPP indicated significant differences in abundance-weighted species composition between (1) litter seedbanks from B and U plots (p = 0.0001), (2) soil seedbanks from B and U plots (p = 0.002), and (3) litter seedbanks and soil seedbanks (p=0.00004) (Table 2). For field seedlings, the general pattern was greater abundance and richness in B compared to U plots. Four taxa were significant indicators of B plots: *Liriodendron tulipifera, Oxydendrum arboreum, Robinia pseudoacacia*, and *Rubus spp*; *Acer pensylvanicum* was the only significant indicator of U plots in the field. The reverse pattern, greater abundance and richness in U plots, was shown by the litter seedbank, and to a lesser extent by the soil seedbank. Three taxa, *Betula spp., K. latifolia-R. maximum* complex (grouped because of morphological uncertainties during identification), and *Oxydendrum arboreum* indicated U litter samples, and three taxa, *Betula* spp., *Rubus* spp., and *Erechtites hieraciifolius* indicated U soil samples; no species indicated B litter or B soil samples. Only one taxon indicated the litter seedbank: *Acer rubrum*; in contrast, many taxa indicated the soil seedbank, including *Ageratina altissima, Dichanthelium spp., Hydrangea arborescens, Lobelia* *spp., R. pseudoacacia*, *Rubus spp., Viola spp.* and two unknown species, which were assigned codes #004 and #020.

The tree community of combined soil and litter seedbank samples differed from the standing tree community in the field ($p<0.0001$) (Table 3). Many species present in the standing tree community were absent from the seedbank. Conversely, only two species were absent from the standing tree community but present in the seed bank: native Black locust (*Robinia pseudoacacia*) and non-native Princess tree (*Paulownia tomentosa*). Indicator Species Analysis revealed that birch (*Betula spp.*) and black locust were indicative of the seedbank community, whereas *Acer pensylvanicum*, *Acer rubrum, Carya glabra, Quercus montana, Quercus rubra*, and *Tsuga canadensis* were indicative of the standing tree community ($p<0.05$).

Field and greenhouse communities differed ($p=0.003$), although there was broad overlap between the two (Table 3). Seventeen taxa were present in the greenhouse but absent from the field; 20 taxa were present in the field but absent from the greenhouse; and 28 taxa were present in both. *Acer pensylvanicum, Acer rubrum, R. pseudoacacia*, and *Viola spp*. indicated field samples, whereas *Ageratina altissima, Conyza canadensis, Hydrangea sp., Kalmia latifolia-Rhododendron maximum,* and *Lobelia spp.* were indicative of greenhouse samples.

CHAPTER 5: DISCUSSION

Broadly speaking, the environmental shifts toward higher light levels, more exposed bare ground, and higher temperatures in burned plots were conducive to seed germination. Shrub and tree mortality resulted in greater canopy openness in burned compared to unburned plots at Cliffside (C) and Dick's Creek (D); the lack of greater openness in B plots at Wine Spring (W) may reflect low severity fire at that site, or low pre-fire shrub cover. Higher light levels have been tied to greater germination in many northern temperate forest species, particularly in small-seeded species such as *Juncus effusus*, *Hypericum perforatum* and *Urtica dioica* (Jankowska-Błaszczuk and Grubb, 2006), *Digitalis purpurea* (Vranckx and Vandelook, 2012) and *Hydrangea paniculata* and *Betula platyphylla* var. *japonica* (Xia et al., 2016).

Litter cover was lower in B plots at all three sites, as was herbaceous cover at C and D, and there was a concomitant increase in exposed bare ground at all three sites. Substrate limitation, i.e., lack of exposed bare ground, has been identified as a major barrier to seed germination in northern temperate forests (Caspersen and Saprunoff, 2005). For example, in an Ohio forest, both litter removal and a low-temperature burn significantly facilitated germination of *Erechtites hieraciifolius* and *Liriodendron tulipifera* (Glasgow and Matlack, 2007), two common species in at least one site in the present study. The higher daily air temperatures in burned plots may also have stimulated germination; daily temperature fluctuation penetrates further into the ground than light cues, and can serve as a germination cue for medium- and large-seeded species such as *Phellodendron amurense, Alnus japonica, A. hirsuta, Magnolia obovata* and *M. kobus* (Xia et al., 2016).

Although moisture availability can affect community structure through an establishment filter (Vandelook et al., 2012), moisture was similar between B and U plots at all sites and likely played little role in germination patterns. Growing season precipitation in 2017 was greater than average (by 10.46cm at 685m elevation, by 30.92cm at 1364m; USDAa, USDAb), and it is possible that a drier year post-fire would have affected seedbank response through moisture limitation. Conversely, the dry conditions before the fire may have helped protect the soil seed bank. Soil moisture at the time of a fire can affect seed survival; dry seeds from several temperate herbaceous plant families display greater fire tolerance than moist seeds (Ruprecht et al., 2016).

Despite the favorable conditions for germination, comparison of field seedling density with germination from seedbank samples in the greenhouse suggests that only a minority of seeds germinated under field conditions. Seedbank limitation does not appear to constrain forest response to wildfire. The disparity between the seedbank and expressed seedling community could be the result of shading from litter or herbaceous vegetation, seed predation, or other factors in the field that suppressed germination.

Differences between field and greenhouse expressions of the seedbank could also be due to species-specific factors. Of those species with higher germination in the field, *Acer rubrum* drops its seeds from late spring to early summer (Tirmenstein 1991); its greater presence in the field may have been due to seed fall between the soil sampling and field sampling dates. *Acer pensylvanicum* germination in the greenhouse may have been suppressed by elevated greenhouse temperatures; optimum germination temperature for this species is 5°C (Baskin and Baskin 1998). Myrmecochorous groups such as *Viola* may have had reduced germination in the greenhouse due to an absence of mutualists. There is indirect evidence that eliasome removal improves seed germination by reducing pathogen-induced seed mortality in *Viola pedata*

(Gehring et al., 2013). *Robinia pseudoacacia* may have been aided by animal ingestion in the field; ingestion has been shown to increase germination by breaking dormancy (Baskin and Baskin 1998). Of those species with higher germination in the greenhouse, *Rhododendron maximum* may have been inhibited by lack of moss in the field (Dan Pittillo, personal communication). Response of *Ageratina altissima*, a disturbance-dependent forb (Redwood et al., 2016) may have been depressed in the field because it was found primarily in samples from W, the site that saw no post-fire increase in canopy openness. Finally, it is possible that some weedy plants (*Conyza canadensis, Lobelia spp*., unknown species (#020)) that were absent from the field may have entered the greenhouse as contaminants, though they were absent from control trays.

Overall, this study provides evidence for the important role played by the soil seedbank in post-disturbance forest response. Litter seedbank density was consistently and significantly lower in B samples at each site. Considering litter cover was also lower in B plots at each site, it is reasonable to conclude that a large portion of the litter seedbank was consumed by fire. *Acer rubrum*, the only significant species indicator of litter samples, drops its fruit in the spring, sufficiently early that seeds can germinate in the year that they fall (Tirmenstein 1991). In contrast, the soil may provide a more persistent or consistent post-fire seedbank. Not only was the seed density several times greater in the soil than litter seedbank even in unburned samples, but also the richness was greater in soil samples, despite a smaller area sampled per plot. Persistent seedbanks have been demonstrated in the families containing the soil seedbank indicator taxa (Baskin and Baskin 1998).

This study provides evidence for potential forest turnover after fire (Table 3). Some species, such as *Acer pensylvanicum, A. rubrum, Carya glabra, Quercus montana, Q. rubra*, and

Tsuga canadensis, were un- or under represented in the seedbank compared to their presence in the extant forest community. These seedbank-absent species can be sorted into groups: *A. pensylvanicum* and *A. rubrum*, which were observed to produce seedlings in the field and which sampling methods may have under-represented in the greenhouse; and *C. glabra, Q. montana, Q. rubra* and *T. canadensis,* for which no seedlings were observed. Previous studies in the region also found the species of the second group were absent (Ris Lambers et al., 2005; Dobberpuhl 1980, cited in Farmer 1997) or rare (Keyser et al., 2012) in Southern Appalachian seedbanks.

Species ecophysiology may contribute to *C. glabra, Q. montana, Q. rubra*, and *T. canadensis* absence from the seedbank. The large-seeded *Carya* and *Quercus spp.* do not form seedbanks, but germinate quickly to form a seedling bank in the understory (Dobberpuhl 1980, cited in Farmer 1997). Acorn mortality during fire is high, especially at high temperatures (Greenberg et al., 2012), further depressing oak representation in the seedbank. Root sprouts were observed among fire-killed oak and hickories, suggesting a resistant rather than resilient fire-survival strategy. *Tsuga canadensis* seed rain has been shown to decrease after simulated attack by the hemlock woolly adelgid (Farnsworth et al., 2012), a non-native sucking insect present in the study area; 84% of *T. canadensis* encountered in the present study had died prior to the wildfire.

In contrast to these seedbank-absent species, a few taxa were overrepresented in the seedbank relative to the standing vegetation, including *Betula spp., Robinia pseudoacacia*, and *Paulownia tomentosa*. Although *Betula* spp. was present in standing vegetation in only 32% of plots, birches were present in 71% of seedbank samples. *Betula* distribution in seedbank samples is depressed by inclusion of burned samples, which was significantly lower than unburned. Although apparently not fire-adapted, *B. alleghaniensis* is adapted for disturbance (Gaucher et al., 2005). The canopy and litter disturbance associated with the 2016 wildfires may allow *Betula* to increase at the study sites beyond current levels.

Robinia pseudoacacia was absent from the extant standing vegetation, but was nevertheless present in the seedbank of 19% of plots. Black locust seeds can remain in the soil seedbank for long periods (Stone 2009). In one study, over 15% remained viable after 39 years (Toole and Brown, 1946). Unlike other species so far discussed, black locust requires physical dormancy-breaking, which can be met by ingestion, force, or fire (Baskin and Baskin 1998). Because *R. pseudoacacia* is a fast-growing, short-lived nitrogen fixer, it can facilitate other species after disturbance (Boring and Swank, 1984) and may become temporarily dominant after disturbance (Stone 2009).

Paulownia tomentosa is the only non-native invasive species identified from the seedbank. It was present in five seedbank samples; it was also present as a seedling in three plots and was observed in several plots outside the sampling area, but was absent from the standing tree community. Anecdotally, this species was only observed in the field in plots that experienced high fire severity as measured by scorch height, litter consumption and shrub mortality; though *Paulownia* was present in the seed bank in at least one low severity plot. This species has a high light requirement for germination, and a relatively high optimum germination temperature of 30°C (Baskin and Baskin 1998); these requirements are more similar to conditions after high severity fires. In addition, smoke stimulates light-triggered germination (Todorovi et al., 2010). *Paulownia* has been documented invading other areas of the southern Appalachians after wildfire, such as Linville Gorge (Dumas et al., 2007; Hagan et al., 2015). However, long-term persistence is primarily on ridges that experience high fire severity (Kuppinger et al., 2010). *Paulownia* is difficult to eradicate once established (Innes 2009).

Conclusions

Results of this research show that the seedbank can contribute to the post-fire plant community. Both soil and litter layers of the seedbank are diverse and well-stocked, though density of viable seeds and species richness are greater in the soil layer. Additionally, the litter layer is more vulnerable to fire; burned areas at a site had lower density and richness than unburned, while burned-unburned differences were not significant for the soil layer. However, seed density and species richness in the seedbank does not appear to control first-year seedling response to wildfire in the field. In the field, seedling density and richness were greater in burned areas. Environmental conditions in burned areas were more favorable to seed germination than in unburned areas, suggesting that seeds in burned areas responded to the indirect effects of fire rather than direct effects of fire on seeds. Species that responded most strongly to fire in the field were generalist disturbance-adapted species, not specialist fire-adapted species. This implies that except for the reduction in the litter seedbank, from a seed's perspective fire functioned similarly to other disturbances, such windthrow or logging, at the sites studied.

In the studied forests, which do not appear to have experienced major disturbance in recent decades, the seedbank does serve as a reservoir of biodiversity when compared to standing trees. For instance, black locust exists in at least two of these sites in the seedbank but not in the extant vegetation. Its presence as a field seedling post-fire, as well as its seed physiology, suggests that over long timescales black locust is maintained at these sites by seedbank response to periodic disturbance. A storage effect from the seedbank seems probable for this tree. Another ecologically consequential contribution of the seedbank to forest response is *Paulownia*, a non-native invasive tree previously absent from the extant vegetation. Managers should be prepared to address *Paulownia* invasion after wildfire in these forests.

Caveats and Limitations

This study demonstrates that the seedbank can contribute to post-wildfire forest response; however, the seedbank is not the only source of forest response to disturbance. This study does not attempt to characterize response from resprouting roots, crowns, or below-ground storage organs. It is likely that these also play an important role in post-disturbance response at the study sites. In particular, tree species that were absent from the seedbank, such as oaks and hickories, may employ a resistance (resprouting) strategy.

Even considering seeds alone, the seedbank is not a full representation of all seed sources. Seed rain from plants both within and outside a plot can contribute to post-disturbance response. It may be that post-fire seed input to plots was contained on the soil surface in the litter layer. If post-fire seed rain were a major input to the litter seedbank, it might be expected to affect burned and unburned plots equally. The much lower burned litter seed density observed in this research suggests that seed rain was relatively minor. However, other than sampling the litter and soil separately, this study does not explicitly address seed rain.

Plant community or plot placement could have influenced the outcome of this research. Sites were selected to capture a range of fire severity, keep travel time reasonable, and avoid duplicating efforts of other researchers. One consequence of these choices is unequal representation of plant communities among the sites: forest communities at Cliffside and Dicks Creek are more similar to each other than to Wine Spring. Thus, trends specific to Wine Spring may not be reflected in this analysis of all plots. Furthermore, the limited number of plots per treatment at each site, necessitated by the small research crew, could have allowed a plot to be sited in a location that does not reflect typical site characteristics. As a final caveat, this study focused on first-year post-fire effects, which are not likely to capture the long-term effects. For instance, field seedling density of 17 or more individuals per square meter will be unsustainable as the seedlings mature. Continued monitoring will be needed to determine long-term effects of the fires. In addition, future research that includes more plots within each site and combines results from the sites studies here with ongoing research across a larger range of fire severities and forest communities can provide more comprehensive and broader data for evaluating variation in fire responses within Southern Appalachian forests.

CHAPTER 6: LITERATURE CITED

Aikens, M.L., Ellum, D., McKenna, J.J., Kelty, M.J., Ashton, M.S., 2007. The effects of disturbance intensity on temporal and spatial patterns of herb colonization in a southern New England mixed-oak forest. For. Ecol. Manag. 252, 144–158.

Aldrich, S.R., Lafon, C.W., Grissino-Mayer, H.D., DeWeese, G.G., Hoss, J.A., 2010. Three centuries of fire in montane pine-oak stands on a temperate forest landscape. Appl. Veg. Sci. 13, 36–46.

Barden, L.S., Woods, F.W., 1976. Effects of Fire on Pine and Pine-Hardwood Forests in the Southern Appalachians. For. Sci. 22, 399–403.

Bargmann, T., Måren, I.E., Vandvik, V., 2014. Life after fire: smoke and ash as germination cues in ericads, herbs and graminoids of northern heathlands. Appl. Veg. Sci. 17, 670–679.

Baskin C. C. and J. M. Baskin. 1998. Seeds. Ecology, biogeography, and evolution of dormancy and germination. 666 pp. San Diego: Academic Press.

Bolin, J.F., 2009. Heat Shock Germination Responses of Three Eastern North American Temperate Species. Castanea 74, 160–167.

Boring, L. R., Swank, W. T., 1984. The Role of Black Locust (Robina Pseudo-acacia) in Forest Succession. J. Ecol. 72, 749.

Cai, L. 2006. Multi-response permutation procedure as an alternative to the analysis of variance: An SPSS implementation. Behavior Research Methods. 38: 51.

Caspersen, J.P., Saprunoff, M., 2005. Seedling recruitment in a northern temperate forest: the relative importance of supply and establishment limitation. Can. J. For. Res. 35, 978–989.

Clarke, P.J., Dorji, K., 2008. Are Trade-Offs in Plant Resprouting Manifested in Community Seed Banks? Ecology 89, 1850–1858.

Csontos, P., 2007. Seed banks: ecological definitions and sampling considerations. Community Ecol. 8, 75–85.

Delcourt, P.A., Delcourt, H.R., 1998. The influence of prehistoric human-set fires on oak-chestnut forests in the Southern Appalachians. Castanea 63, 337–345.

Dobberpuhl, J. E. 1980. Seed Banks of Forest Soils in East Tennessee. M Sc. thesis. University of Tennessee, Knoxville.

Dumas, S., Neufeld, H.S., Fisk, M.C., 2007. Fire in a Thermic Oak-Pine Forest in Linville Gorge Wilderness Area, North Carolina: Importance of the Shrub Layer to Ecosystem Response. Castanea 72, 92–104.

Elliott, K., Vose, James, Hendrick, R., 2009. Long-term effects of high intensity prescribed fire on vegetation dynamics in the Wine Spring Creek Watershed, Western North Carolina, USA. Fire Ecol. 5266-85 5, 66–85.

Elliott, K.J., Boring, L.R., Swank, W.T., 1997. Successional changes in plant species diversity and composition after clearcutting a Southern Appalachian watershed. For. Ecol. Manag. 92, 67–85.

Enright, N.J., Kintrup, A., 2001. Effects of smoke, heat and charred wood on the germination of dormant soil-stored seeds from a Eucalyptus baxteri heathy-woodland in Victoria, SE Australia. Austral Ecol. 26, 132–141.

Espeland, E.K., Perkins, L.B., Leger, E.A., 2010. Comparison of Seed Bank Estimation Techniques Using Six Weed Species in Two Soil Types. Rangel. Ecol. Manag. 63, 243–

247.

ESRI. 2011. ArcGIS Desktop: Release 10. Redlands, CA: Environmental Systems Research Institute.

Faist, A.M., Ferrenberg, S., Collinge, S.K., 2013. Banking on the past: seed banks as a reservoir for rare and native species in restored vernal pools. AoB Plants 5.

Farmer, J. E. 1997. Seed Ecophysiology of Temperate and Boreal Zone Forest Trees. St. Lucie Press Delray Beach FL. 253 pp.

Farnsworth, E.J., Barker Plotkin, A.A.., Ellison, A.M.., 2012. The relative contributions of seed bank, seed rain, and understory vegetation dynamics to the reorganization of Tsuga canadensis forests after loss due to logging or simulated attack by Adelges tsugae. Can. J. For. Res. 42, 2090–2105.

Fesenmyer, K.A., Christensen, N.L., Jr, 2010. Reconstructing Holocene fire history in a southern Appalachian forest using soil charcoal. Ecology 91, 662–670.

Freedom du Lac, J., S. Larimer and L. Bever. 2016. ‘Two juveniles charged with arson in Tennessee wildfires that left 14 people dead,’ Washington Post. Available at https://www.washingtonpost.com/news/post-nation/wp/2016/12/07/two-juveniles-charged-with-arson-in-deadly-east-tennessee-wildfires/?utm_term=.7cbc0942ff30. Accessed 21 March 2018.

Flatley, W.T., Lafon, C.W., Grissino-Mayer, H.D., 2011. Climatic and topographic controls on patterns of fire in the southern and central Appalachian Mountains, USA. Landsc. Ecol. 26, 195–209.

Flatley, W.T., Lafon, C.W., Grissino-Mayer, H.D., Laforest, L.B., 2013. Fire history, related to climate and land use in three southern Appalachian landscapes in the eastern United States. Ecol. Appl. 23, 1250–1266.

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

Gaucher, C., Gougeon, S., Mauffette, Y., Messier, C., 2005. Seasonal variation in biomass and carbohydrate partitioning of understory sugar maple (Acer saccharum) and yellow birch (Betula alleghaniensis) seedlings. Tree Physiol. 25, 93–100.

Gehring, J.L., Cusac, T., Shaw, C., Timian, A., 2013. Seed germination of Viola pedata, a key larval host of a rare butterfly. Native Plants J. Univ. Wis. Press 14, 205–212.

Glasgow, L.S., Matlack, G.R., 2007. Prescribed burning and understory composition in a temperate deciduous forest, Ohio, USA. For. Ecol. Manag. 238, 54–64.

Greenberg, C.H., Keyser, T.L., Zarnoch, S.J., Connor, K., Simon, D.M.., Warburton, G.S.., 2012. Acorn viability following prescribed fire in upland hardwood forests. For. Ecol. Manag. 275, 79–86.

Grime, J.P., 1977. Evidence for the Existence of Three Primary Strategies in Plants and Its Relevance to Ecological and Evolutionary Theory. Am. Nat. 111, 1169–1194.

Hagan, D.L., Waldrop, T.A., Reilly, M., Shearman, T.M., 2015. Impacts of repeated wildfire on long-unburned plant communities of the southern Appalachian Mountains. Int. J. Wildland Fire 24, 911–920.

Houle, G., Payette, S., 1990. Seed dynamics of Betula alleghaniensis in a deciduous forest of north-eastern North America. J. Ecol. 78, 677–690.

Innes, Robin J. 2009. Paulownia tomentosa. In: Fire Effects Information System, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire

Sciences Laboratory (Producer). Available: https://www.fs.fed.us/database/feis/plants/tree/pautom/all.html [2018, March 21].

Jankowska-Błaszczuk, M., Grubb, P.J., 2006. Changing perspectives on the role of the soil seed bank in northern temperate deciduous forests and in tropical lowland rain forests: parallels and contrasts. Perspect. Plant Ecol. Evol. Syst. 8, 3–21.

Jenkins, M.A., Klein, R.N., McDaniel, V.L., 2011. Yellow pine regeneration as a function of fire severity and post-burn stand structure in the southern Appalachian Mountains. For. Ecol. Manag. 262, 681–691.

Keyser, T.L., Roof, T., Adams, J.L., Simon, D., Warburton, G., 2012. Effects of Prescribed Fire on the Buried Seed Bank in Mixed-Hardwood Forests of the Southern Appalachian Mountains. Southeast. Nat. 11, 669–688.

Kuppinger, D.M., Jenkins, M.A., White, P.S., 2010. Predicting the post-fire establishment and persistence of an invasive tree species across a complex landscape. Biol. Invasions 12, 3473–3484.

Kuznetsova, A., P. B. Brockhoff and R. H. Bojesen Christensen (2016). lmerTest: Tests in Linear Mixed Effects Models. R package version 2.0-33. https://CRAN.R-project.org/package=lmerTest

Lambers, J.H.R., Clark, J.S., 2005. The benefits of seed banking for red maple (Acer rubrum): maximizing seedling recruitment. Can. J. For. Res. 35, 806–813.

Lee, P., 2004. The impact of burn intensity from wildfires on seed and vegetative banks, and emergent understory in aspen-dominated boreal forests. Can. J. Bot. 82, 1468–1480.

Matlack, G.R., Good, R.E., 1990. Spatial Heterogeneity in the Soil Seed Bank of a Mature Coastal Plain Forest. Bull. Torrey Bot. Club 117, 143–152.

McCune B, Mefford MJ. 2006. PC-ORD 5.0. Multivariate analysis of ecological data. Gleneden Beach, Oregon, USA.

Mielke, P. W. 1984. Meteorological applications of permutation techniques based on distance functions. In P. R. Krishnaiah & P. K. Sen (Eds.), Handbook of statistics: Vol. 4. Nonparametric methods (pp. 813–830). Amsterdam: North-Holland.

Miller, A.D., Chesson, P., 2009. Coexistence in Disturbance-Prone Communities: How a Resistance-Resilience Trade-Off Generates Coexistence via the Storage Effect. Am. Nat. 173, E30–E43.

Mojzes, A., Csontos, P., Kalapos, T., 2015. Is the positive response of seed germination to plant-derived smoke associated with plant traits? Acta Oecologica 65–66, 24–31.

Moreira, B., Tormo, J., Estrelles, E., Pausas, J.G., 2010. Disentangling the role of heat and smoke as germination cues in Mediterranean Basin flora. Ann. Bot. 105, 627–635.

Norman, S. P. 2016. Presentation to WCU Biology Seminar on 10 November 2017. Western Carolina University, Cullowhee, NC.

Nowacki, G.J., Abrams, M.D., 2008. The Demise of Fire and “Mesophication” of Forests in the Eastern United States. BioScience 58, 123–138.

R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Redwood, M.E., Matlack, G.R., Huebner, C.D., 2016. Seed longevity and dormancy state in a disturbance-dependent forest herb, Ageratina altissima. Seed Sci. Res. 26, 148–152.

Reilly, M.J., Wimberly, M.C., Newell, C.L., 2006. Wildfire effects on plant species richness at multiple spatial scales in forest communities of the southern Appalachians. J. Ecol. 94, 118–130.

Ren, L., Bai, Y., 2016. Smoke originating from different plants has various effects on germination and seedling growth of species in Fescue Prairie. Botany 94, 1141–1150.

Ris Lambers, J.H., Clark, J.S., Lavine, M., 2005. Implications of Seed Banking for Recruitment of Southern Appalachian Woody Species. Ecology 86, 85–95.

Roberts, T.L., Vankat, J.L., 1991. Floristics of a Chronosequence Corresponding to Old Field-Deciduous Forest Succession in Southwestern Ohio. II. Seed Banks. Bull. Torrey Bot. Club 118, 377–384.

Roche, S., Koch, J.M., Dixon, K.W., 1997. Smoke Enhanced Seed Germination for Mine Rehabilitation in the Southwest of Western Australia. Restor. Ecol. 5, 191–203.

Ruprecht, E., Fenesi, A., Fodor, E.I., Kuhn, T., Tökölyi, J., 2015. Shape determines fire tolerance of seeds in temperate grasslands that are not prone to fire. Perspect. Plant Ecol. Evol. Syst. 17, 397–404.

Ruprecht, E., Lukács, K., Domokos, P., Kuhn, T., Fenesi, A., 2016. Hydration status influences seed fire tolerance in temperate European herbaceous species. Plant Biol. Stuttg. Ger. 18, 295–300.

Schafale, M. P. 2012. Guide to the Natural Communities of North Carolina Fourth Approximation. North Carolina Natural Heritage Program, North Carolina Department of Natural and Cultural Resources. Raleigh, NC.

Schwartz, N.B., Urban, D.L., White, P.S., Moody, A., Klein, R.N., 2016. Vegetation dynamics vary across topographic and fire severity gradients following prescribed burning in Great Smoky Mountains National Park. For. Ecol. Manag. 365, 1–11.

Stanturf, J.A., Wade, D.D., Waldrop, T.A., Kennard, D.K., Achtemeier, G.L., 2002. Fire in southern forest landscapes. Wear DM Greis J Ed. 2002 South. For. Resour. Assess. P 607-630 Chapter 25 Gen Tech Rep SRS-53 Asheville NC US Dept Agric For. Serv. South. Res. Stn.

Stanturf, J.A. and S. L. Goodrick. 2013. The Southern Forest Futures Project: technical report. Wear DM Greis J Ed. 2013 South. For. Futures Proj. P 509-542 Chapter 17 Gen Tech Rep SRS-GTR-178. Asheville NC US Dept Agric For. Serv. South. Res. Stn.

Stone, Katharine R. 2009. Robinia pseudoacacia. In: Fire Effects Information System, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). Available: https://www.fs.fed.us/database/feis/plants/tree/robpse/all.html [2018, March 21].

Swank, W.T., Vose, J.M., Elliott, K.J., Hendrick, R.L., Major, A.E., 1999. Vegetation dynamics after a prescribed fire in the southern Appalachians. For. Ecol. Manag. 114, 199.

Swanson, M.E., Franklin, J.F., Beschta, R.L., Crisafulli, C.M., DellaSala, D.A., Hutto, R.L., Lindenmayer, D.B., Swanson, F.J., 2011. The forgotten stage of forest succession: early-successional ecosystems on forest sites. Front. Ecol. Environ. 9, 117–125.

Templeton, A.R., Levin, D.A., 1979. Evolutionary Consequences of Seed Pools. Am. Nat. 114, 232–249.

Thompson, K., Grime, J. p., 1979. Seasonal Variation in the Seed Banks of Herbaceous Species in Ten Contrasting Habitats. J. Ecol. 67, 893.

Tirmenstein, D. A. 1991. Acer rubrum. In: Fire Effects Information System, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). Available: https://www.fs.fed.us/database/feis/plants/tree/acerub/all.html [2018, March 21].

Todorovi, S., BoŽI, D., 2, Simonovi, A., Filipovi, B., DragiĆevi, M., Giba, Z., GrubiŠi, D.,

2010. Interaction of fire-related cues in seed germination of the potentially invasive species Paulownia tomentosa Steud. Plant Species Biol. 25, 193–202.

Toole, E.H., Brown, E., 1946. Final results of the Duvel buried seed experiment. J. Agric. Res. 72, 201–10.

Tsuyuzaki, S., Miyoshi, C., 2009. Effects of smoke, heat, darkness and cold stratification on seed germination of 40 species in a cool temperate zone in northern Japan. Plant Biol. Stuttg. Ger. 11, 369–378.

USDA, USFS Southern Research Station. 2018. Coweeta Hydrologic Laboratory: Low Elevation Precipitation (Main Office) [Excel Document]. Available from www.srs.fs.usda.gov/coweeta/tools-and-data/

USDA, USFS Southern Research Station. 2018. Coweeta Hydrologic Laboratory: High Elevation Precipitation (Mooney Gap) [Excel Document]. Available from www.srs.fs.usda.gov/coweeta/tools-and-data/

USDA, USFS Southern Research Station. 2018. Coweeta Hydrologic Laboratory: Average Air Temperature (Climate Station 01) [Excel Document]. Available from www.srs.fs.usda.gov/coweeta/tools-and-data/

Vandelook, Filip, Verdú, M., Honnay, O., 2012. The role of seed traits in determining the phylogenetic structure of temperate plant communities. Ann. Bot. 110, 629–636.

Vandvik, V., Klanderud, K., Meineri, E., Måren, I.E., Töpper, J., 2016. Seed banks are biodiversity reservoirs: species-area relationships above versus below ground. Oikos 125, 218–228.

Vranckx, G., Vandelook, F., 2012. A season- and gap-detection mechanism regulates seed germination of two temperate forest pioneers. Plant Biol. Stuttg. Ger. 14, 481–490.

Wang, G.G., Kemball, K.J., 2005. Effects of fire severity on early development of understory vegetation. Can. J. For. Res. 35, 254–262.

Warner, R.R., Chesson, P.L., 1985. Coexistence Mediated by Recruitment Fluctuations: A Field Guide to the Storage Effect. Am. Nat. 125, 769–787.

Wilfahrt, P.A., Collins, B., White, P.S., 2014. Shifts in functional traits among tree communities across succession in eastern deciduous forests. For. Ecol. Manag. 324, 179–185.

Xia, Q., Ando, M., Seiwa, K., 2016. Interaction of seed size with light quality and temperature regimes as germination cues in 10 temperate pioneer tree species. Funct. Ecol. 30, 866–874.

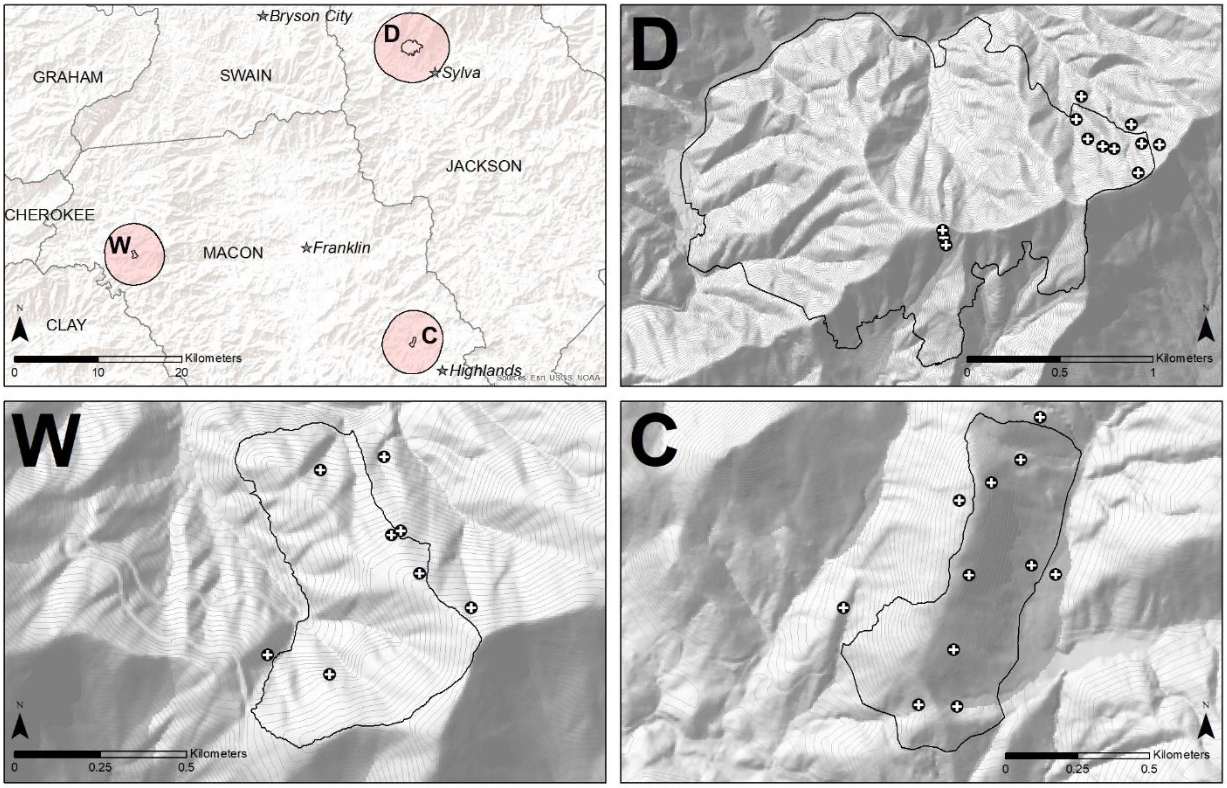

Fig. 1. Location of 3 study sites in Jackson and Macon counties in western North Carolina; distribution of plots inside and outside the fire lines at Dicks Creek (D), Cliffside (C) and Wine Spring (W) sites.

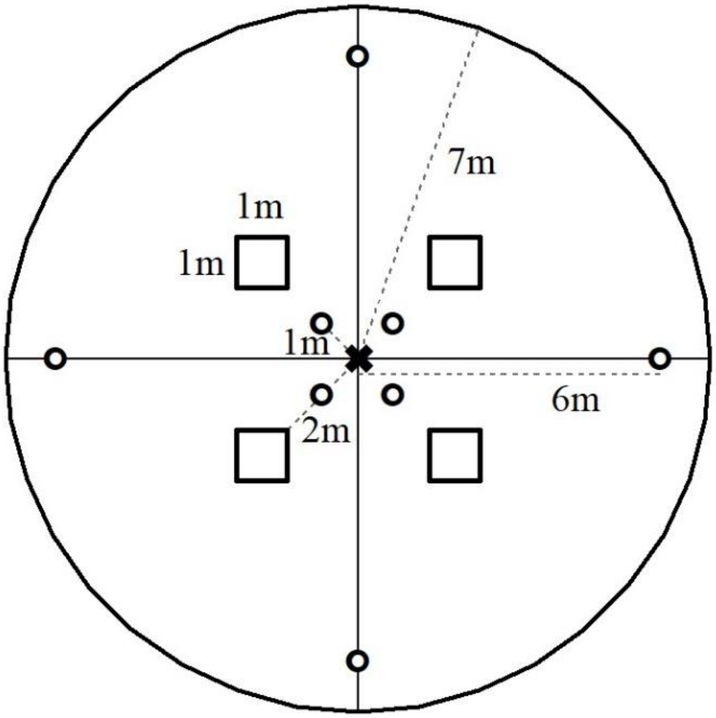

Figure 2. Sampling plot layout. Includes 7m radius plot (circle), plot center (cross), soil/litter sample points (small circles), and ground layer/seedlings subplots (squares).

Table 1. Means and standard deviation of environmental response variables in site-treatment combinations. * indicate variables that differed significantly between burned and unburned treatments with site as random effect.

	Cliffside		Dicks Creek		Wine Spring	
Response	B	U	B	U	B	U
Canopy openness (%)*	**20.5 (±8.6)**	**13.3 (±1.4)**	**31.9 (±16.1)**	**10.3 (±2.2)**	**13.8 (±1.0)**	**13.7 (±0.9)**
Soil water content (%)	24.4 (±3.4)	24.6 (±2.5)	24.6 (±5.7)	21.5 (±1.1)	36.3 (±5.5)	35.8 (±6.1)
Litter cover (%)*	**61.8 (±19.4)**	**97.5 (±0.0)**	**25.7 (±17.9)**	**97.5 (±0.0)**	**41.9 (±13.8)**	**94.4 (±6.3)**
Bare cover (%)*	**35.5 (±22.0)**	**1.1 (±1.6)**	**71.9 (±17.6)**	**1.5 (±1.8)**	**55.6 (±13.8)**	**3.3 (±3.0)**
Herbaceous cover (%)	10.9 (±9.8)	25.5 (±31.8)	6.2 (±8.4)	16.3 (±8.8)	53.3 (±16.4)	42.2 (±15.0)
Scorch height (cm)	43.3 (±26.2)	.	298.3 (±148.8)	.	36.0 (±8.3)	.
Shub mortality (%)	38.9 (±45.4)	.	86.1 (±33.2)	.	0.0 (±0.0)	.
Tree mortality (%)	17.3 (±21.8)	.	25.2 (±27.3)	.	6.7 (±2.4)	.
Mean min. temp. (°C)	14.7 (±0.6)	14.5 (±0.8)	15.8 (±0.4)	16.2 (±0.0)	14.7 (±0.1)	14.3 (±0.2)
Mean mean temp. (°C)	18.6 (±0.9)	17.8 (±0.8)	19.8 (±1.4)	19.0 (±0.1)	17.0 (±0.0)	16.4 (±0.2)
Mean max. temp. (°C)*	**25.0 (±3.5)**	**22.5 (±1.7)**	**28.5 (±5.5)**	**23.6 (±1.5)**	**20.6 (±0.0)**	**19.4 (±0.2)**

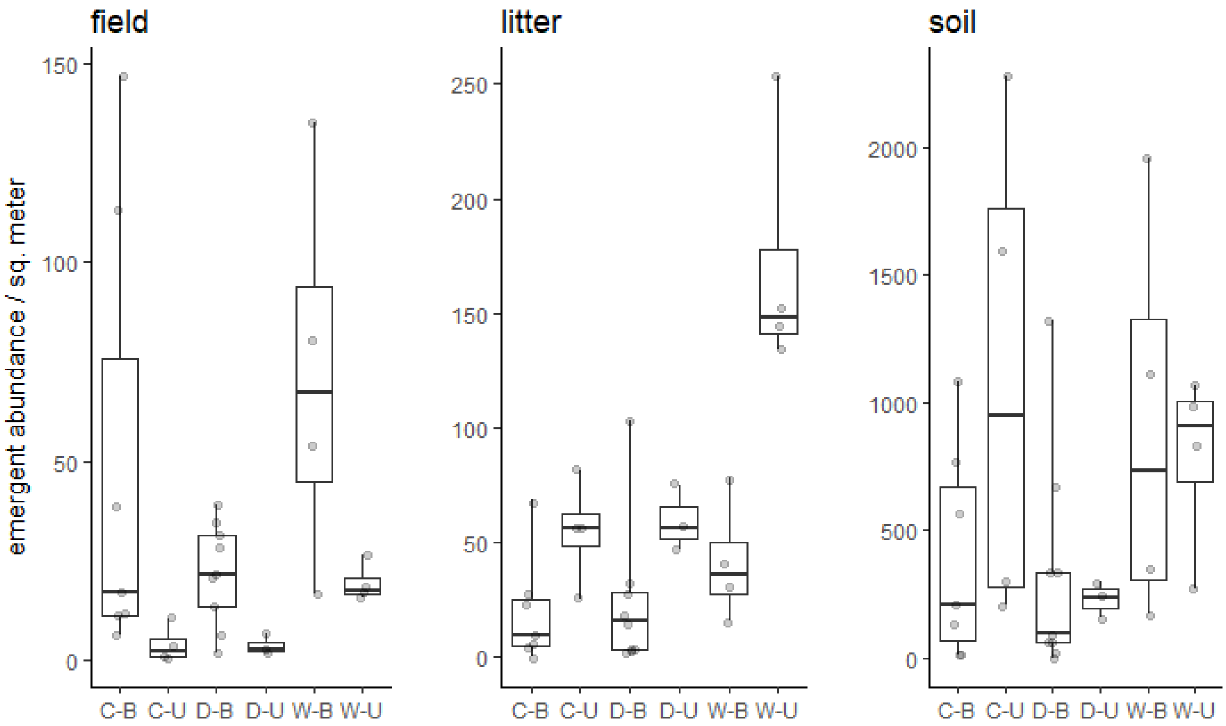

Fig. 3. Density per m^2 for seedlings in the field and for viable seeds in greenhouse litter and soil samples.

Table 2. Field, litter seedbank and soil seedbank responses by treatment, showing the proportion of samples in which a taxon was present. * denotes a taxon that significantly indicated either burned (B) or unburned (U) samples within each source, as tested by abundance-based Indicator Species Analysis. Only indicator taxa, taxa present in at least 6 samples from at least one source, and other taxa mentioned in the narrative are shown.

	Field		Litter		Soil	
Taxon	B	U	B	U	B	U
unknown (#004)	0.25	0.09	0.10	0.09	0.45	0.27
unknown (#020)	0.00	0.00	0.00	0.00	0.25	0.09
Acer pensylvanicum	0.10	**0.45***	0.00	0.00	0.00	0.00
Acer rubrum	0.75	0.73	0.30	0.27	0.05	0.00
Ageratina altissima	0.10	0.00	0.00	0.09	0.20	0.27
Betula spp.	0.65	0.55	0.45	**0.91***	0.40	**1.00***
Conyza canadensis	0.00	0.00	0.30	0.18	0.05	0.09
Dichanthelium spp.	0.35	0.00	0.00	0.00	0.20	0.27
Erechtites hieraciifolius	0.20	0.00	0.05	0.09	0.00	**0.36***
Halesia tetraptera	0.20	0.18	0.00	0.09	0.15	0.09
Hydrangea arborescens	0.15	0.00	0.00	0.00	0.30	0.18
K. latifolia - R. maximum	0.00	0.00	0.00	**0.27***	0.25	0.27
Liriodendron tulipifera	**0.55***	0.09	0.20	0.36	0.10	0.27
Oxydendrum arboreum	**0.55***	0.27	0.15	**0.64***	0.35	0.45
Robinia pseudoacacia	**0.70***	0.09	0.00	0.00	0.10	0.36
Rubus spp.	**0.45***	0.09	0.00	0.09	0.20	**0.55***
Viola spp.	0.35	0.18	0.00	0.00	0.20	0.18
Vitis spp.	0.45	0.09	0.05	0.00	0.15	0.18

#004 and #020 are unknown taxa. #004 appears to be an herbaceous perennial or biennial, possibly *Lysimachia ciliata*. #020 is also herbaceous, possibly *Pseudognaphium obtusifolium*.

Table 3. Proportion of samples from each source (litter vs. soil seedbanks; field seedlings vs. seedbank; extant trees vs. seedbank trees) in which a taxon is present. * denotes taxa that significantly indicate a community, as tested by abundance-based Indicator Species Analysis. Non-tree taxa are indicated by “-“ in tree-based analysis. Only indicator taxa, taxa present in at least 6 samples (0.19) from at least one source, and other taxa mentioned in the text are shown.

	Greenhouse		Seedlings		Trees	
Taxon	Litter	Soil	Field	Greenhouse	Extant	Greenhouse
unknown (#004)	0.10	**0.39***	0.19	**0.42***	-	-
unknown (#020)	0.00	**0.19***	0.00	**0.19***	-	-
Acer pensylvanicum	0.00	0.00	**0.23***	0.00	**0.19***	0.00
Acer rubrum	**0.29***	0.03	**0.74***	0.29	**0.58***	0.29
Ageratina altissima	0.03	**0.23***	0.06	**0.23***	-	-
Betula spp.	0.61	0.61	0.61	0.71	0.32	**0.71***
Carya glabra	0.00	0.00	0.00	0.00	**0.19***	0.00
Conyza canadensis	0.26	0.06	0.00	**0.29***	-	-
Dichanthelium spp.	0.00	**0.23***	0.23	0.23	-	-
Erechtites hieraciifolius	0.06	0.13	0.13	0.19	-	-
Halesia tetraptera	0.03	0.13	0.19	0.16	0.16	0.06
Hydrangea arborescens	0.00	**0.26***	0.10	**0.26***	-	-
K. latifolia / R. maximum	0.10	0.26	0.00	**0.26***	0.35	0.26
Liriodendron tulipifera	0.26	0.16	0.39	0.29	0.19	0.29
Lobelia spp.	0.00	**0.16***	0.00	**0.16***	-	-
Oxydendrum arboreum	0.32	**0.39***	0.45	0.55	0.39	0.55
Paulownia tomentosa	0.10	0.06	0.10	0.13	0.00	0.13
Quercus montana	0.00	0.00	0.00	0.00	**0.35***	0.00
Quercus rubra	0.00	0.00	0.00	0.00	**0.42***	0.00
Robinia pseudoacacia	0.00	**0.19***	**0.48***	0.19	0.00	**0.19***
Rubus spp.	0.03	**0.32***	0.32	0.32	-	-
Solidago spp.	0.13	0.06	0.06	0.19	-	-
Tsuga canadensis	0.00	0.00	0.00	0.00	**0.19***	0.00
Viola spp.	0.00	**0.19***	**0.42***	0.19	-	-
Vitis spp.	0.03	0.16	0.32	0.16	-	-

#004 and #020 are unknown taxa. #004 appears to be an herbaceous perennial or biennial, possibly *Lysimachia ciliata*. #020 is also herbaceous, possibly *Pseudognaphium obtusifolium*.