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Comparison of seed bank composition over a gradient of pyrophilic vegetation[1]

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Abstract. Seed bank compositions provide a record of past disturbances, the legacies of which may last thousands of years. Our goal was to determine if sites defined historically by fire-related (pyrophilic) tree species have seed bank compositions different from sites defined historically by pyrophobic tree species. We selected three 80+ year-old forested sites within the Monongahela National Forest, West Virginia, to represent three pyrophilic zones based on percent-pyrophilic-witness tree cover: Low (0–10%), Medium (40–50%), and High (80–90%). Soil plugs were collected to evaluate the seed bank and determine soil nutrients and charcoal abundance; cover of all understory plants was also estimated. The results, supported by the charcoal analyses, show significant differences between extant and seed bank vegetation, pyrophilic zones, and pyrophilic zones within each vegetation lifeform (extant *vs*. seed bank). The High zone seed bank had the greatest species richness, the most pyrophilic species, and differed the most from its extant vegetation, but was the least dense of all zones and a pyrophobic species ranked among its top five indicator species. These findings indicate mesophication and a depletion of pyrophilic species in the seed bank. The High site appears to be reaching a potential threshold where passive recovery to fire-adapted conditions may be unlikely.

Key words: charcoal, deciduous forest, fire, indicator species, multivariate, understory

Soil seed banks have been studied for decades as a source of relic, specialist species for restoration of native vegetation. Relic species' seed abundance and richness in seed banks vary with habitat as well as existing and historic management (van der Valk and Verhoeven 1988; Brown 1998; Valkó *et al*. 2011; Weerasinghe *et al*. 2019; Abella *et al*. 2020). Time since disturbance or cessation of disturbance regimes also affects seed bank composition and subsequent usefulness in restoration (van der Valk *et al*. 1992; Brown 1998; Laughlin 2003; Overdyck and Clarkson 2012). Seed banks of deciduous forest soils often differ in species composition from the existing understory and overstory vegetation, with compositions resembling the early-successional old fields that preceded the second-growth forest. The latter is due in part to changes in life-history types associated with successional stage, revealing greater seed predation, decreasing seed longevity, and lower seed abundance for seed banks as forests mature (Roberts and Vankat 1991; Bossuyt and Hermy 2000; Bossuyt *et al*. 2002; Plue *et al*. 2010a). Although old-growth and mature forests tend to have fewer seeds in their seed banks, some of the seeds may be of late-successional species (Leckie *et al*. 2000). Nonetheless, re-establishment of ancient forest (*i.e.,* intact forests that established *ca*. 1770 or earlier) species may take over a century despite being adjacent to ancient forests. This is due in part to the lack of abundant seed in the soil (Bossuyt and Hermy 2000).

Though mature forest seed bank compositions are often dominated by similar weedy early-successional species, they may still provide a record of past disturbances, the legacies of which could last for as long as 1,600 years since human occupation (Plue *et al*. 2008; Plue *et al*. 2010b). For example, Gallo-Roman sites in northern France have a more homogenized seed bank composition than sites showing no history of Gallo-Roman occupation, likely due to evident alterations in the soil with increased phosphorus and pH levels (Plue *et al*. 2008). Indeed, the fact that many seed banks of young forests are dominated by early-successional species may reflect a history of arable land use instead of forest age. For instance, disturbed tropical forests in Panama with no agricultural history, distant from any agricultural land use, show no difference in seed bank species density or composition across

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forest age (Dalling and Denslow 1998). Similarly, a lack of historic or current cultivation may explain a depauperate seed bank in a southern Appalachian oak forest (Schiffman and Johnson 1992). Nonetheless, agricultural systems with frequent tillage and cover crops reduce the germinable seed bank, so the importance of agricultural history on seed bank composition likely depends on the agricultural system (Mirsky *et al*. 2010) as well as the presence of any forest seed sources. Similarity between seed bank and extant species composition is lower in Mediterranean oak forests grazed by ruminants and *Sus scrofa* L. (wild boar) than in ungrazed forests, suggesting a similar impact as with crop cultivation, but in contrast, diversity is lower for both seed bank and extant species composition in grazed forests than ungrazed forests (Chaideftou *et al*. 2008). Similarly, seed banks of young deciduous forests in Maryland, USA, decrease in density and richness with increasing *Odocoileus virginianus* Zimm. (Eastern white-tailed deer) browse intensity (Beauchamp *et al*. 2013). Conversely, site seed bank species diversity increases in sites with greater fire frequency in heathy woodlands of southeast Australia but burning more than 5% of the area per year reduces combined above-ground and seed bank diversity (Chick *et al*. 2018; Chick *et al*. 2019). Seed banks found primarily in the duff and litter layer, rather than in mineral soil, are vulnerable to fires (Schiffman and Johnson 1992). Nonetheless, a lack of fire often correlates with a decrease in extant understory vegetation diversity (30–70% lower in southeast Australia; Chick *et al*. 2019) and may lead to the formation of alternative species assemblies, transitioning from pyrophilic (vegetation assemblages favored by fire) to pyrophobic (fire-sensitive vegetation assemblages) stable states (Wright *et al*. 2019). Pyrophilic forests often require frequent fire to maintain their species composition or system integrity (Stephens *et al*. 2018).

In eastern forests of the US, this shift from pyrophilic to pyrophobic vegetation is also called mesophication, a positive feedback cycle in which the removal of fire has resulted in a landscape that is increasingly fireproof and less amenable to either the restoration of fire regimes or the maintenance of historically pyrophilic, often *Pinus* or *Quercus*-dominated, forests (Nowacki and Abrams 2008; Caldwell *et al*. 2016; Varner *et al*. 2016; Palus *et al*. 2018; Alexander *et al*. 2021; Hanberry 2021). The impacts of mesophication on soil seed banks in US eastern forests are under-investigated, with a few studies that focus on the return of fire to the landscape to restore dominant tree species (Brose *et al*. 2008; Iverson *et al*. 2008). In addition to the loss of tree species that dominate pyrophilic communities, fire suppression and the resulting mesophication are threats to several understory species in eastern forests, including a relatively common sedge, *Carex digitalis* Willd. (slender woodland sedge; Romand-Monnier 2014), *Chamerion angustifolium* (L.) Holub (fire weed; Foster 1985), *Iliamna corei* Sherff (Peter's mountain mallow; Hoss *et al*. 2008), and *Xerophyllum asphodeloides* (L.) Nutt. (turkeybeard; Bourg *et al*. 2015). Graminoid species, such as *Dichanthelium commutatum* (Schult.) Gould and *Carex* L. spp., have also been documented as fire-adapted (Hutchinson *et al*. 2005); however, lists of fire-adapted herbaceous species in eastern forests could not be located by these authors in the literature, though the Fire Effects Information System (FEIS) is a good source of fire-adapted plant species that is being updated continuously, albeit currently dominated by Western U.S. species. (FEIS 2022).

Plant species responding to fire can be classified as evaders, endurers, and/or resisters. Evaders are defined as species with long-lived propagules stored in the soil; endurers are defined as resprouters; resisters are defined as species that survive low-intensity fire due to certain adaptive characteristics. Plant species are also classified as increasers, maintainers, or decreasers. Increasers are likely to increase in abundance after a burn; maintainers are likely to maintain their current population size after a burn; decreasers are likely to decrease in abundance after a burn (Rowe 1983; Harrod and Reichard 2001). Here, we define fire-adapted or pyrophilic species as most likely to maintain or increase their populations on sites that experience short to intermediate fire intervals and not those that may just increase temporarily (*e.g.,* one growing season) after a single burn. More specifically, fire-adapted or pyrophilic species are maintainers or increasers that do so by either evading, enduring, and/or resisting multiple fires, some of which may be severe, over short or intermediate fire intervals.

Several studies have evaluated the effects of fire on seed banks and extant understory vegetation by comparing pre- and post-burn seed bank compositions. In a study that focused on the effects of two burns, spaced two to three years apart, on tree and shrub species composition, both the pre- and post-prescribed burn seed banks were found to be dominated by the pyrophobic species *Betula lenta* L. (black birch), *Liriodendron tulipifera* L. (yellow-poplar), *Rubus* L. spp. (blackberry), and *Vitis* L. spp. (grapevine; Thomas-Van Gundy and Nowacki 2013). However, *B. lenta*, *L. tulipifera*, and *Vitis* spp. abundance decreased after the burns (Schuler *et al*. 2010). Density of *Robinia pseudoacacia* L. (black locust), a pyrophilic species (Thomas-Van Gundy and Nowacki 2013), a minor component of the seed bank, increased after the prescribed burns (Schuler *et al*. 2010). In a Virginia study, a sparse seed bank (less than 1 seedling m^{-2}) may have resulted from frequent fire, but the seed bank's sparse herbaceous species composition consisted of more forest herb species similar to the extant vegetation than ruderal or early successional species (Shiffman and Johnson 1992). The latter suggest that these frequent fires may be helping to maintain the existing vegetation and not just acting as a disturbance to be colonized by ruderals. Knox and Clarke (2012) found that pyrophilic species were maintained in a long-lived seed bank responding to rare, severe fire events. Similarly, in a study that compared burned and unburned plots after a single West Virginia wildfire with areas of higher severity, burned plots were found to have higher species richness and diversity than unburned plots, but the analyses also included vegetative structures (rhizomes, corms, bulbs) in addition to seeds (Nowlin 1993). While over 80% of the seed bank species were found concomitantly in forest extant vegetation, some seed bank species typically associated with dry, fire-prone habitats and/or poor soils were found only in the burned sites, especially the more severely burned areas. These species included *Cunila origanoides* (L.) Britton (wild oregano), *Danthonia spicata* (L.) Beauv. (poverty grass), *Polygala sanguinea* L. (blood milkwort), and *Potentilla simplex* Michx. (old-field cinquefoil) (Nowlin 1993).

In contrast, Elliott and Vose (2005) showed that a single burn did not promote regeneration of pyrophilic species, including *Pinus echinata* Mill. (shortleaf pine), *Andropogon gyans* Ashe. (bluestem), or *Schizachyrium scoparium* (Michx.) Nash. (little bluestem). Another study in North Carolina forests showed little difference between pre-fire and post-fire seed bank compositions after a single burn, with both containing ruderal and forest species (Keyser *et al*. 2012). Similarly, overall species diversity, and the density of graminoid species, increased in the seed bank in an Arkansas study after a single prescribed fire, but with no evident increase in pyrophilic species (Schuler and Liechty 2008).

These varied responses to fire may be explained by legacy effects of previous disturbances including fire and cultivation. They may also be due in part to differences in climate and physiography that define the underlying vegetation. Pyrophilic species are often adapted to drier conditions or strongly associated with drier habitats that are prone to fires. Thus, pyrophilic species could be fire-adapted (fitness improves with fire) or just fire-related (found in fire-prone sites; Fulé 2008, Lamont and He 2017). However, drier sites in eastern US forests are still subject to mesophication (Caldwell *et al*. 2016; Palus *et al*. 2018; Alexander *et al*. 2021), though it may occur at a slower rate or be less likely to occur in some more xeric forests found in the western part of the central hardwood region (Olson *et al*. 2014).

Because short-term pre- and post-burn studies vary greatly and do not reliably predict how fire might impact seed banks and their potential as seed sources for restoration and the maintenance of fire-resistant communities over the long term, a different approach is needed. Instead of comparing pre- and post-fire short-term impacts, our goal was to evaluate seed banks over a historic pyrophilic vegetation gradient defined by witness trees. Witness trees (also called bearing trees) were described historically to mark property boundaries. Though these land ownership records could suffer from surveyor bias, they are the best data available to determine European settlement-era forest vegetation. Though the witness trees, most of which are now dead, provide important information about historic vegetation, they may not differentiate between vegetation dependent on fire *vs*. vegetation defined by climate and physiography (*i.e*., dry sites that are fire-prone), but seed bank composition may help make this distinction. We hypothesized that (1) seed bank compositions located in zones with a higher percentage of historic pyrophilic witness tree species would also have more pyrophilic seed bank species than sites with a lower percentage of pyrophilic witness tree species, and that (2) because of fire suppression and the presence of long-lived seeds, differences between extant understory vegetation and seed bank compositions would be greatest within sites with the highest percentage of pyrophilic witness trees. The latter comparison of seed bank and extant vegetation may help differentiate the effects of fire and mesophication vs climate and physiography. This research will help measure the degree of mesophication in eastern US forests and define the potential for restoration of fire-adapted and fire-related species and their communities. Eastern US forests once defined by fire but now showing signs of mesophication in their extant vegetation are unlikely to be restored after reintroducing fire if their current seed banks have also converted to pyrophobic species.

Materials and Methods. Pyrophilic Zones. The pyrophilic gradient sampled in this study is derived from an analysis of witness trees from the first deeds associated with the area that is now the Monongahela National Forest (Thomas-Van Gundy and Strager 2012). Witness trees are those trees cited in deeds to indicate corners and represent an inadvertent ecological data source describing mainly overstory trees at the time of the land survey. Approximately 22,000 witness trees from deeds between 1752 and 1899 were used to develop the geospatial layer with each corner representing a point; one to six trees could be found at each corner. The 49 species of witness trees were categorized into two classes, pyrophiles and pyrophobes. Species with traits (*e.g.*, thick bark, serotinous cones, sprouting) that can persist under frequent and low- to moderate-severity fires were categorized as pyrophiles, and those with fire-sensitive and mesophytic traits (*e.g.*, thin bark, leaves with high water content) were categorized as pyrophobes. A pyrophilic percentage was determined for each corner by dividing the number of pyrophilic individuals by the total number of individuals of both pyrophiles and pyrophobes and multiplying by 100. Ordinary kriging was used to develop a continuous pyrophilic value between all points (Thomas-Van Gundy and Nowacki 2013). The area described below is ideal for this study because the interpolated pyrophilic percentage transitions acutely over a relatively short distance from the Tygart Valley River (historically a pyrophilic overstory) to the shoulders of Cheat Mountain (historically a pyrophobic overstory; Fig. 1).

Site Description. Our study site is located in the Monongahela National Forest, Greenbrier Ranger District, in Randolph County and the Allegheny Plateau Province, near the Tygart Valley River and Huttonsville, West Virginia (WV) (Fig. 1). Three sites were selected to represent three distinct pyrophilic zones: Low (0–10% pyrophilic witness trees; 38.6785500, –79.8939200, elevation 1,128 m), Medium (40–50% pyrophilic witness trees; 38.6730956, –79.9193730, elevation 975 m), and High (80–90% pyrophilic witness trees; 38.6555005, –79.9673850, elevation 792 m). Each site was approximately 0.2 ha in size. Soil types are Buchanan Ernst stony soil at the Low site, Meckesville stony silt loam at the Medium site, and Highsplint Berks Association moist, extremely stony soil at the High site (Web Soil Survey 2021). Annual precipitation is approximately 160 cm for the area. All three sites were located on south-facing slopes with 20–25% inclination and were forested with trees at least 80 years of age. The potential natural vegetation (as documented by the witness tree data) differs at the three sites spanning the inferred pyrophilic gradient from *Picea rubens* Sarg. (red spruce) on the Low site, *Quercus montana* Willd.-*Acer saccharum* Marsh.-*Tilia americana* L. (chestnut oak-sugar maple-basswood) on the Medium site, and *Q. montana*-*Q. rubra* L.-*Q. alba* L. (chestnut oak-northern red oak-white oak) on the High site. The sites are located along an approximately 7-km transect moving from east to west across the study area. The valleys along this transect may have been cultivated in the late 1700s, but there is no documented agricultural land use at the three sites for at least 80 years.

After the first railroads were built in West Virginia in 1853, timber removal denuded most of the slopes in what is now the Monongahela National Forest. These timber operations were often followed by large fires for about 25 years in the late 1800s. Consequently, it is likely our sites experienced one or more fires in the late 1800s and early 1900s. Forest reestablishment began with the designation of the Monongahela National Forest in 1915 (McKim 1970).

Sampling Design. In early June 2016, two parallel 50-m transects on south-facing slopes, one from just below the ridgetop and the other about midway down the slope, each at least 10 m apart (Fig. 2), were selected within each of the three pyrophilic zones (Low, Medium, and High as

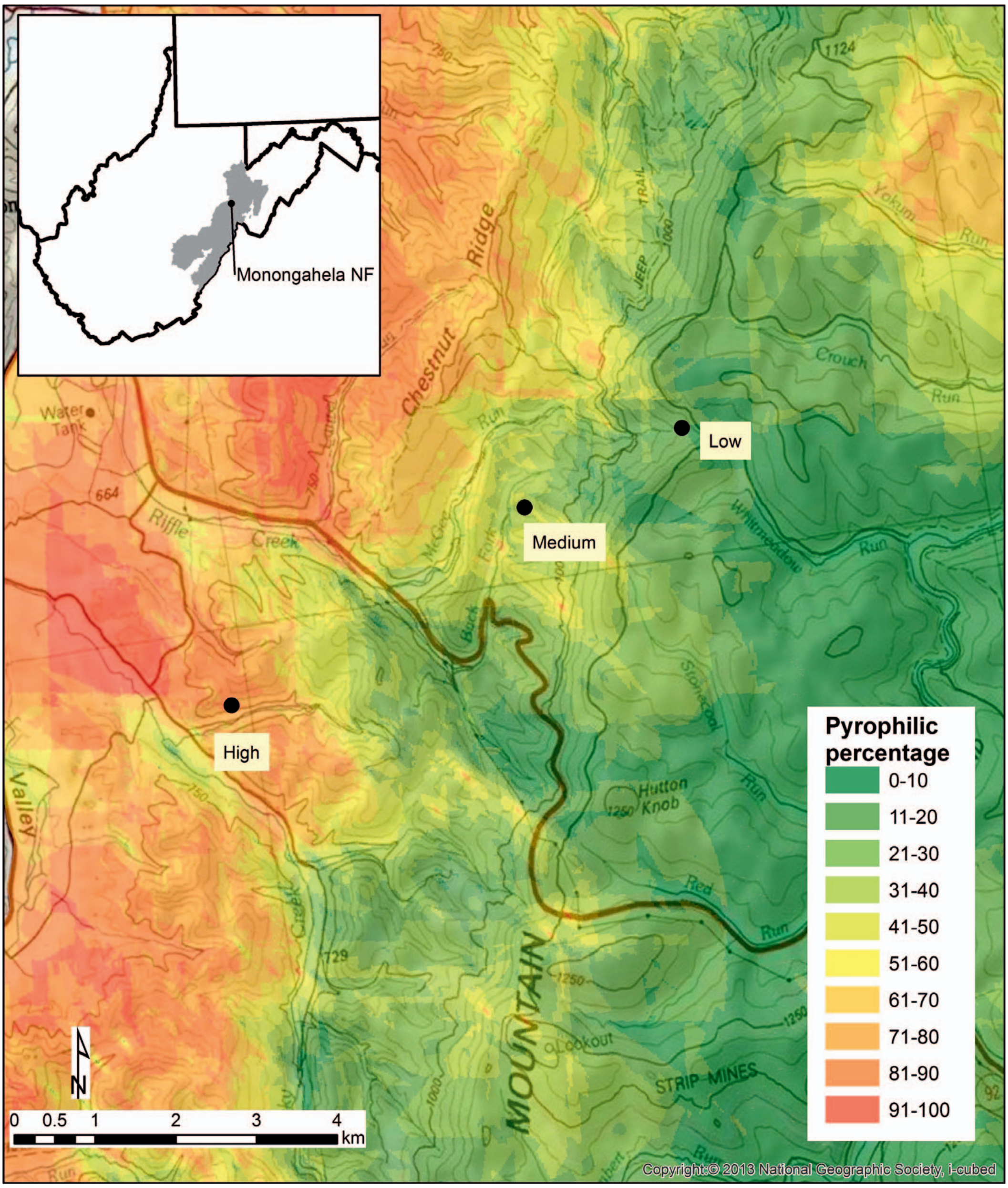

FIG. 1. Study area showing the pyrophilic zones and the three site locations.

defined above). Along each transect, five circular 1-m² plots were placed at 10-m intervals. Within each 1-m² plot, herbs, shrubs, and vines were identified and their abundance (ocular estimate of percent cover at 1 m above ground) recorded. All tree seedlings under 1 m tall were identified and their abundance and density recorded. Herbs and tree seedlings were only included if rooted in the plot, but the cover of shrubs and vines was estimated if hanging in the plot as well as rooted in the plot with no height limit. Sites were also walked for the presence of any plant species not found in the 1-m² plots.

Soil samples for the seed bank analyses were also collected in June 2016 and were located just outside the 1-m² circular plots in each cardinal direction (Fig. 2), resulting in a total of 20 samples per transect, 40 samples per pyrophilic zone, and

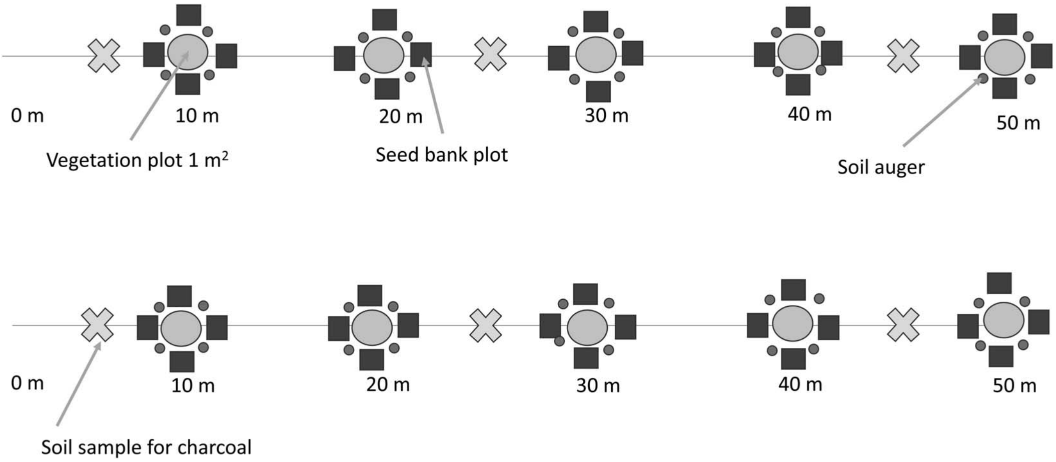

Fig. 2. Schematic drawing of the lay out of the two transects, vegetation plot, seed bank samples, soil chemistry samples, and soil charcoal samples at each of the three sites.

120 total samples. Each soil sample was 15 × 15 cm and 10 cm deep. The loose litter layer was removed prior to collection in the field, but the duff (humus) and all soil horizons within this 10-cm depth were included. Soil samples were placed in a walk-in refrigerator at 4 °C for 5 months of cold storage. With this design, approximately 9% of each 1-m^2 circular plot ([4 × 15 cm × 15 cm]/10,000 = 0.09 m^2) was sampled, well over the suggested minimum of 3% for seed bank samples, and all soil samples were collected at least 50 cm apart (Plue and Hermy 2012). The soil sample depth of 10 cm is deeper than most seed bank samples of 5–6 cm depth and was used to potentially capture rarer or longer-lived species (Leckie *et al*. 2000).

Starting Nov 21, 2016, the seed bank samples collected at the N and S cardinal directions from each plot were mixed, as were the samples collected at the E and W cardinal directions. Roots, tubers, corms, bulbs, and rocks were removed from all mixed samples. The combinations resulted in 4,500 cm^3 of soil for both the N + S and E + W mixtures per plot. The soil mixtures were placed in 103 × 46 × 6 cm flats on top of 4-cm potting soil mix and distributed uniformly across the flats to a depth of approximately 1 cm. This process produced 60 mixed soil samples. The 60 flats were placed in a greenhouse with temperature ranging between 25 °C and 30 °C (day) and 15 °C and 20 °C (night) with standardized day lengths (13 hours 6:30 am–7:30 pm) provided by high-pressure sodium lighting to mimic early spring conditions and promote germination. After 3 months, controlled day length was extended to 14 hours (6:00 am–8:00 pm) to promote germination of later season species.

Flats were rotated weekly and watered daily as needed. Fertilizer was added after two weeks and then each week. Germinating species were marked with colored toothpicks, identified, and tallied every two weeks. Tallies were completed until there were no new germinating individuals. Individuals with confirmed identifications were removed from the flats at each tally period, with some pressed as voucher specimens. Some plants were transplanted to enable them to flower and fruit for more certain identification. The seed bank was monitored for a total of 5 months. For this study, we use the term "vegetation lifeforms" when comparing the vegetation grown from the seed bank *vs*. the extant understory vegetation.

Soil samples for soil chemistry analyses were taken at the same time as the vegetation surveys (June 2016). Soil chemistry (pH, % organic material, total N, total C, Ca, K, Mg, P, Al, Fe, Mn, Zn, Na, acidity, and cation exchange capacity) was evaluated using four mixed 1-cm diameter and 10-cm depth auger samples per 1-m^2 plot collected from just outside the plot boundary at the northeastern, southwestern, northwestern, and southeastern directions (Fig. 2). Soil samples were analyzed at the Maine Agriculture and Forest Experiment Station Analytical Laboratories and Maine Soil Testing Service, Orono, ME. Soil pH was measured in distilled water. Organic matter was measured by percent lost on ignition at 550 °C. Total nitrogen and carbon were measured as percentages by combustion analysis at 1350 °C. Exchangeable acidity was extracted in potassium chloride and measured by titration. Exchangeable cations (Ca, K, Mg, P, Al, Fe, Mn, and Zn) were extracted in ammonium chloride and measured by ICP-OES with units of mg/kg. Effective cation exchange capacity (ECEC) was calculated by the summation of milliequivalent levels of Ca, K, Mg, Na, and acidity with units of milliequivalents per 100 g of soil. ECEC is a measure of available cations and an estimate of soil fertility. We used the Ca:Al ratio to estimate potential system stress per Cronan and Grigal (1995), who estimated there to be a 50% chance of a plant-stress growth response if the ratio is $\leq$ 1.0, a 75% chance if the ratio is $\leq$ 0.5, and a 100% if the ratio is $\leq$ 0.2. We also included the C:N ratio in our analyses. The C:N ratio may be used as an approximate indicator of organic matter quality (Ostrowska and Porębska 2015), fertility, and primary productivity (Watt and Palmer 2012).

Soil samples for charcoal analysis were collected in October 2018 using a tubiform root auger to extract contiguous 10-cm intervals to the depth of refusal (typically parent material rock) or 1 m, following methods described by Horn and Underwood (2014). Six cores were collected at each site along the previously described 50-m transects at the midpoints between 0 and 10 m, 20 and 30 m, and 40 and 50 m on both transects at each site (Fig. 2). The average depth of the cores was 22 cm. Each 10-cm soil core increment was soaked overnight in 0.75 L of water to disaggregate the soil. Samples were then wet-sieved using a 20.5-cm diameter sieve with a mesh size of 2 mm. Macroscopic charcoal was removed under magnification from the material remaining on the sieve, rinsed with distilled water, and transferred to a glass vial for drying at 100 °C for 36 hours. Once dry, the charcoal from each core section was weighed to determine an aggregate mass. Twenty individual charcoal fragments were randomly selected from across all sites for taxonomic identification and radiocarbon dating, yielding four samples from the Low and High sites and 12 samples from the Medium site. Randomly selected charcoal fragments were cut transversely with a razor blade to analyze wood morphology and identify the species of tree that burned to produce the charcoal. Charcoal fragments were pretreated using an acid-alkali-acid protocol to remove allochthonous sources of carbon and then dated by the Radiocarbon Collaborative of the Northern Institute of Applied Climate Science (Supplemental Table S1). Radiocarbon years were converted to calibrated ages (Bronk Ramsey 2009) using the OxCal Version 4.4 radiocarbon calibration program (OxCal 2020) and the calibration curve datasets of Reimer *et al.* (2020) and Hua *et al.* (2021), for one modern (post-1950) date. We used the weighted mean of the calibration probability distribution to assign a single calendar year to each dated sample (Telford *et al.* 2004).

Statistical Analyses. The goal of this study was to evaluate a single site within each pyrophilic zone intensively in terms of its seed bank and understory vegetation; to do so, we treated the sample plots within each as replicates. Separately, we averaged vegetation plots, seed-bank samples, and soil samples of the same distance point along both transects, resulting in five replicates per site. We averaged the corresponding plots for the two transects because of their similarity and to reduce the potential for overfitting our models. For the charcoal samples, depth values were averaged, and the resulting six samples were treated as replicates.

Species richness, diversity (Shannon index), evenness, and total density/cover per plot of both the seed bank and extant vegetation were determined and compared across the three pyrophilic classes using ANOVA; all means were compared using the Tukey's multiple-comparison test (Proc GLM; SAS v. 9.4). All variables except Ca, K, Na, and Ca:Al, which were log-transformed, met normality assumptions. Soil charcoal abundances were analyzed using a generalized linear model with a gamma distribution and log link function (Proc GenMod; SAS v. 9.4). Soil chemistry variables were compared across pyrophilic class using ANOVA (Proc GLM; SAS v. 9.4) noting any trends within pyrophilic class. Species compositions of both seed bank and extant vegetation were evaluated using a nonmetric multidimensional scaling (NMS) ordination using a Sørensen distance measure. Nonmetric multidimensional scaling diagrams were compared using groups for vegetation lifeform, pyrophilic zones, and the two categories combined. Significant differences in composition between the three pyrophilic classes were evaluated using a permutation-based MANOVA (perMANOVA, using 4999 permutations) and multi-response permutation (MRPP; PCORD v.7), also based on a Sørensen distance measure. MRPPs and perMANOVAs were run for the

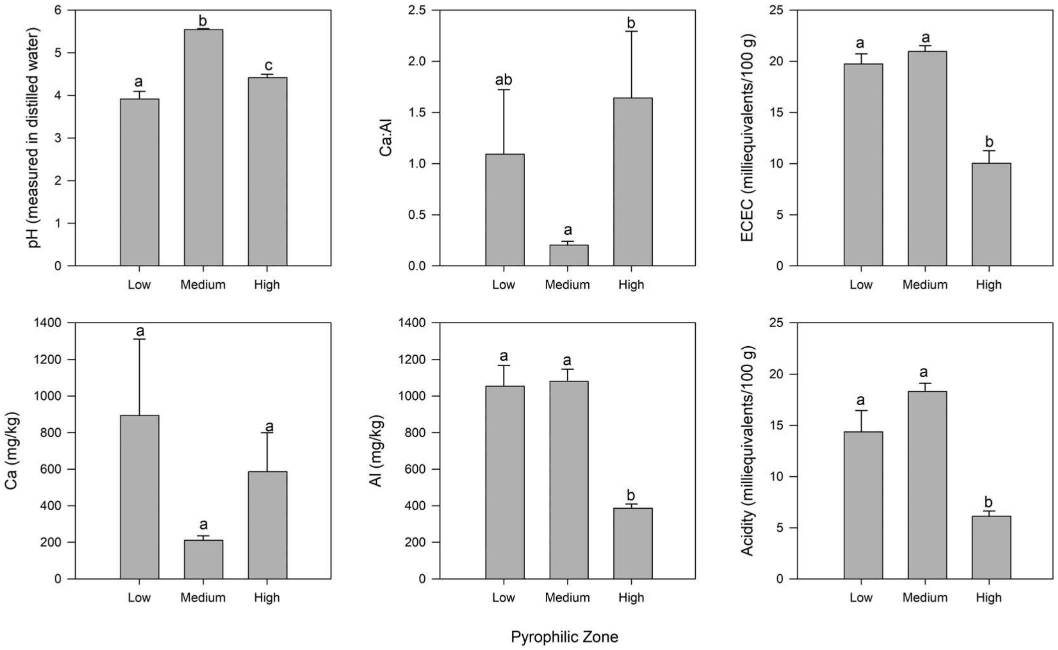

Fig. 3. Comparison, using a one-way ANOVA, of soil pH (F = 53.64, $P = 0.0001$), Ca:Al (F = 4.06; $P = 0.0451$), Ca (mg/kg; F = 2.26, $P = 0.15$), Al (mg/kg; F = 26.10, $P = 0.0001$), ECEC (milliequivalents/100 g soil; F = 38.33, $P = 0.00001$), and acidity (milliequivalents/100g soil; F = 22.12, $P = 0.0001$) across the three pyrophilic zones. Different letters within each graph are significantly different at $P < 0.05$.

vegetation lifeforms and pyrophilic zones separately and combined. After doing a log10 transformation on the distance measures, an ANOVA was used to compare extant vegetation and seed bank composition dissimilarities by pyrophilic zone using the Sørensen distance measures of the NMS.

Species indicator analyses were also conducted following Dufrěne and Legendre (1997), which defines a perfect indicator of group as both always being present within the group and not being present in other groups. Both proportional abundance and frequency of each species relative to all species in a particular group were included in the indicator species calculations. Indicator values were tested for statistical significance using Monte Carlo randomization (4999 permutations; PCORD, v. 7; McCune and Grace 2002).

Results. Soil Chemistry. All three sites exhibited acidic soils, but the Medium site was significantly less acidic than both the Low and High sites, and the Low site was the most acidic (Fig. 3). The Ca:Al ratio for the Medium site was < 0.5, indicating stressed growth conditions, whereas that of the Low site was just > 1 and the High site was > 1.5. Variation was great, but the Medium and High sites differed significantly (Fig. 3). The effective cation exchange capacity was lower for the High site, despite the higher Ca:Al ratio, due to lower values of exchangeable acidity associated with very low Al values. The Low site, like the High site, had high levels of Ca but also had high levels of Al (Fig. 3). The Medium site had significantly more organic matter and higher C:N ratios than both the Low and High sites; the High site had significantly higher C:N ratios than the Low site, which does not appear to be due to organic matter (Fig. 4). Unlike, Ca and Al, the C and N amounts changed proportionally by site (*i.e.*, sites with relatively high C values also had high N values; Fig. 4). The Medium site had the highest levels of measured soil nutrients, with significantly higher levels of K, P, Zn, and Na than both the Low and High sites and significantly higher levels of Fe than the High site, all of which agree with the higher available cations at the Medium site (Table 1; Figs. 4 and 5).

Soil Charcoal. Soil charcoal mass was most abundant in the Medium site and consistently highest in the upper 10 cm of soil for all three

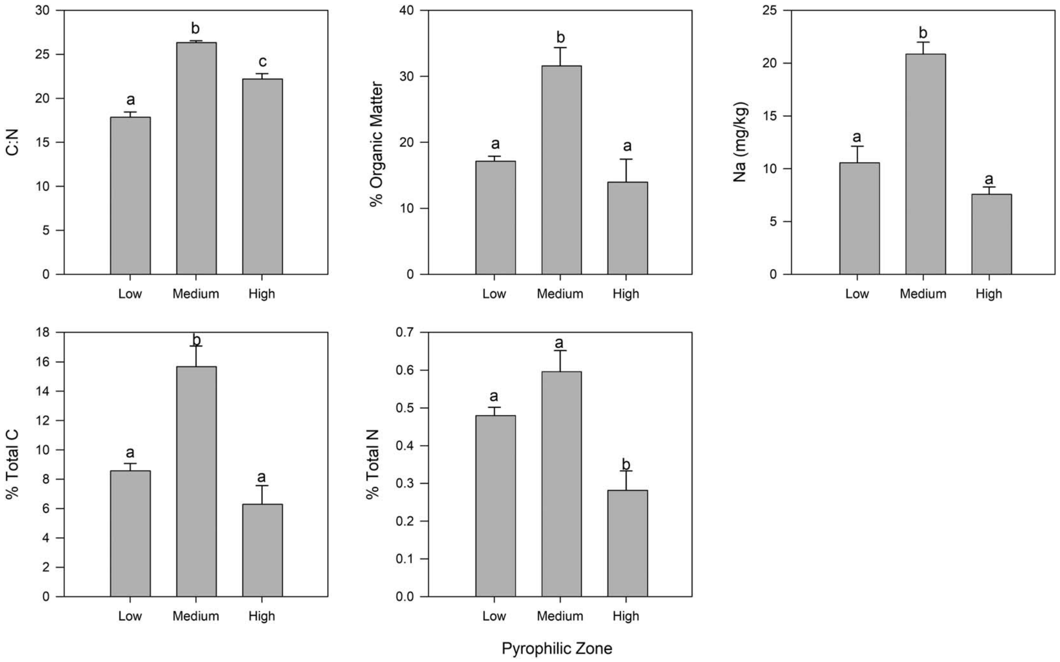

FIG. 4. Comparison, using a one-way ANOVA, of soil Na (mg/kg; F = 27.90; $P = 0.0001$), % Total C (F = 18.74, $P = 0.0002$), % Total N (F = 12.16, $P = 0.0013$), percent organic matter (F = 12.91, $P = 0.001$), and C:N (F = 68.79, $P = 0.0001$) across three pyrophilic zones. Sites with different letters within each graph are significantly at $P \leq 0.05$.

pyrophilic zones (Fig. 6). Sites and depths differed significantly ($X^2 = 12.6$, $P = 0.0018$ and $X^2 = 12.2$ and $P = 0.0067$, respectively) but there were no significant interactions ($X^2 = 4.44$ and $P = 0.35$). The Low site had the oldest charcoal overall with three dates from the late Woodland Period (900–1650 AD). Low site charcoal was primarily *Acer rubrum* L. The Medium and High sites had charcoal more similar in age from the 17th and/or 18th centuries, with the High site having somewhat older charcoal. Medium site charcoal was dominated by *A. rubrum* and *Castanea dentata* (Marshall) Borkh. High site charcoal was all *C. dentata* (Supplemental Table S1).

EXTANT VEGETATION AND SEED BANK RICHNESS, DIVERSITY, AND EVENNESS. There was a total of 61 different extant vascular plant species in all sites, with 24 species in the Low site, 10 in the Medium site, and 37 in the High site. There were an additional 19 species not in the plots (offplot) in the Low site, 8 in the Medium site, and 34 in the

Table 1. Multiresponse permutation procedure (MRPP) results by vegetation lifeform and pyrophilic zone as groups using a Sørensen distance measure. The T-statistic describes the separation of the defined groups, the more negative the T, the greater the separation. A = the chance-corrected within group agreement (within group homogeneity). A = 1 when all members in a group are identical; A = 0 when heterogeneity within a group equals what is expected by chance; A < 0 means that there is less agreement within the groups than expected by chance; A > 0 but less than 1 indicate within-group agreement differs significantly from that expected by chance from the within-group agreement of the other groups. Pairwise comparisons within pyrophilic zone group are also presented.

Group	Group size	T-value	Chance corrected within group agreement—A	*P*-value
Vegetation lifeform	15	−16.81	0.22	0.00000003
Pyrophilic zone	10	−3.41	0.064	0.0084
Low *vs*. Medium	5	−2.67	0.070	0.026
Low *vs*. High	5	−2.87	0.050	0.020
Medium *vs*. High	5	−1.40	0.030	0.090

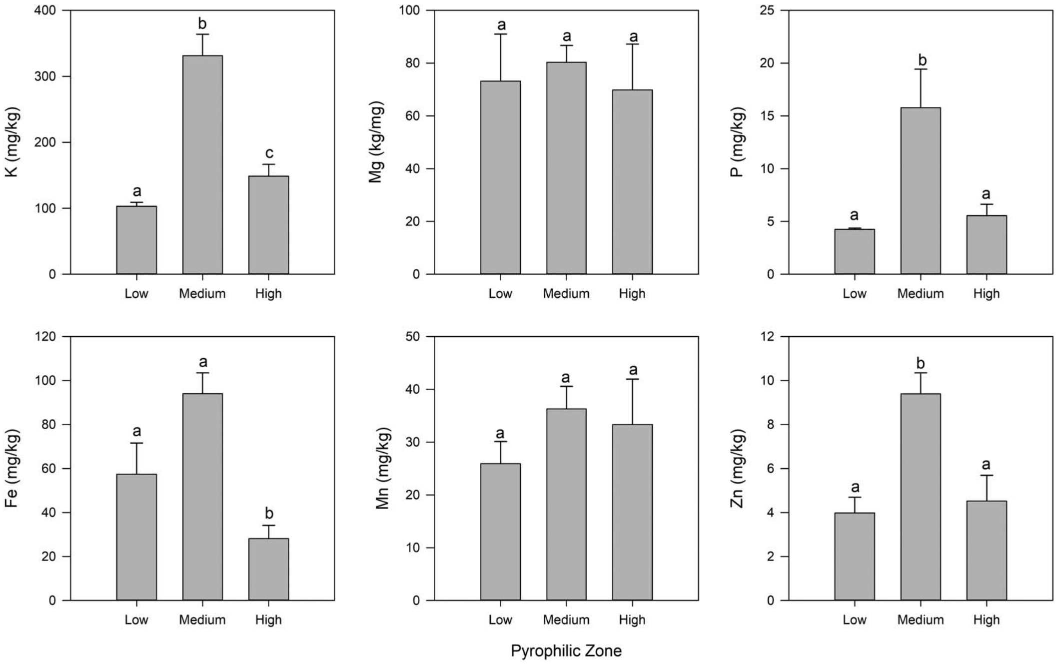

FIG. 5. Comparison, using a one-way ANOVA, of soil K (mg/kg; $F = 91.80$, $P = 0.0001$), Mg (mg/kg; $F = 0.18$, $P = 0.87$), P (mg/kg; $F = 8.3$, $P = 0.0055$), Fe (mg/kg; $F = 9.99$, $P = 0.0028$), Mn (mg/kg; $F = 0.78$, $P = 0.48$) and Zn (mg/kg; $F = 9.56$, $P = 0.0033$) across the three pyrophilic zones. Sites with different letters within each graph are significantly different at $P \leq 0.05$.

High site. There were 56 different species in the seed bank, with 33 species in the Low site, 31 in the Medium site, and 44 in the High site. Though there was a trend for higher extant vegetation richness, diversity, and evenness for the High site, the differences among pyrophilic zone were not significant (Fig. 7). Similarly, seed bank richness was slightly higher for the High site (with no statistical significance), and seed bank diversity and evenness for the High site were significantly greater than for both the Low and Medium sites (Fig. 7). Total extant vegetation cover did not differ significantly across the pyrophilic zones, but total seed bank density of the High site was significantly lower than at both the Low and Medium sites (Fig. 8), signifying more species, but none was abundant in number in the seed bank High site.

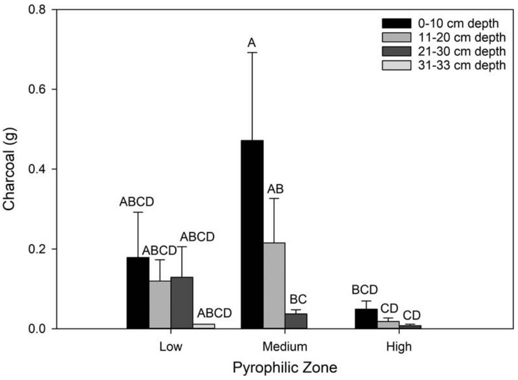

FIG. 6. Soil charcoal abundance for each pyrophilic zone and by depth. Analysis used a generalized linear model with a gamma distribution and log link function. Charcoal amounts with different letters were significantly different at $P \leq 0.05$.

SPECIES COMPOSITION COMPARISONS. We used NMS to compare the species compositions of the seed bank and extant vegetation within each pyrophilic zone. The vegetation lifeform (extant vegetation or seed bank) had the strongest separation (Fig. 9), but the pyrophilic vegetation category also showed clear separation (Fig. 10). The NMS ordination was run using a Sørensen distance measure, 250 random and real data runs, and a random number seed; it showed a 3-dimensional configuration as the most reliable, with an 8.78 final stress value and 0.00 final instability value after 89 iterations. Stress values

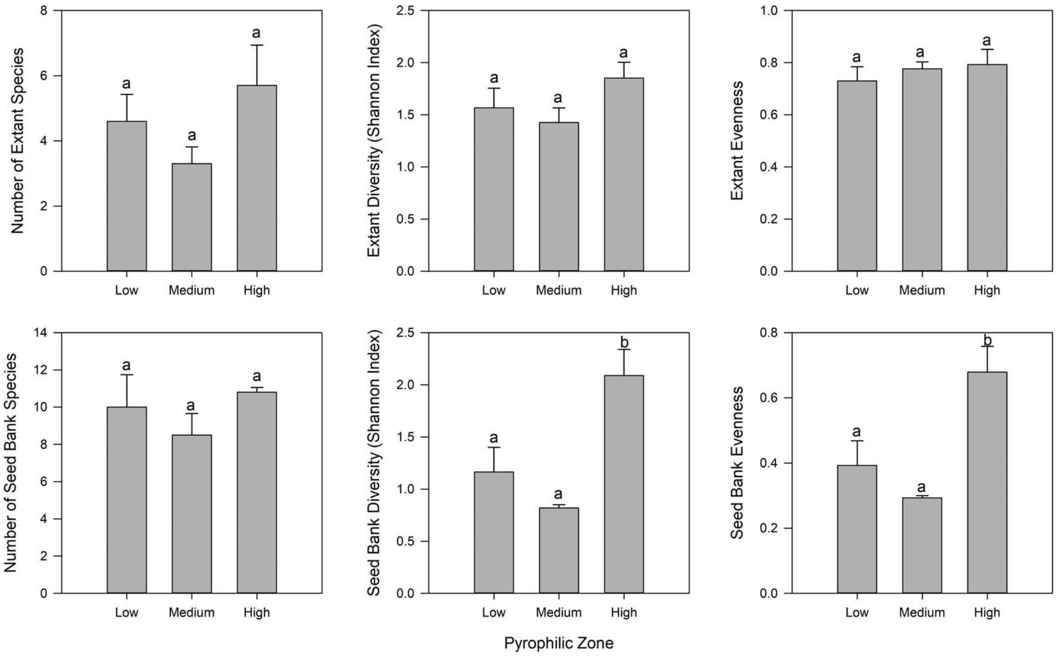

FIG. 7. Comparison, using a one-way ANOVA, of extant and seed bank richness (F = 0.92, $P = 0.42$), diversity (Shannon index; F = 10.78, $P = 0.0021$) and evenness (F = 10.03, $P = 0.0027$) across the three pyrophilic zones. Sites with different letters within each graph are significantly different at $P \leq 0.05$.

below 10 are considered good with no real risk of drawing false inferences (McCune and Grace 2002; Peck 2016). The best separation of the vegetation lifeforms was axis 1, whereas axes 2 and 3 better separated the pyrophilic zones.

The multi-response permutation procedure (MRPP) comparing vegetation lifeform (with all pyrophilic zones combined) showed that the extant vegetation and seed bank compositions differed significantly from what would be expected at

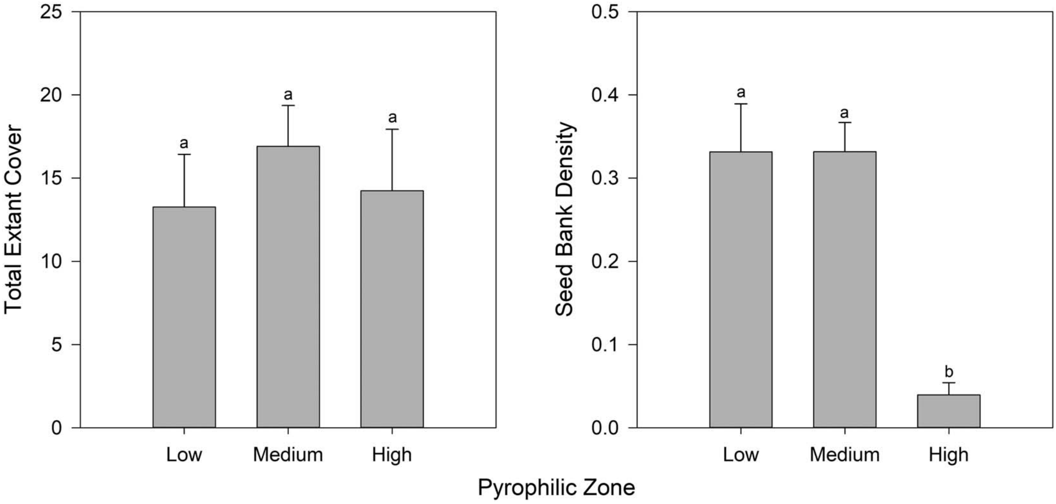

FIG. 8. Comparison, using a one-way ANOVA, of extant vegetation total cover (F = 36, $P = 0.7048$) and seed bank total density (F = 17.82, $P = 0.0003$) across the three pyrophilic zones. Sites with different letters within each graph are significantly different at $P \leq 0.05$.

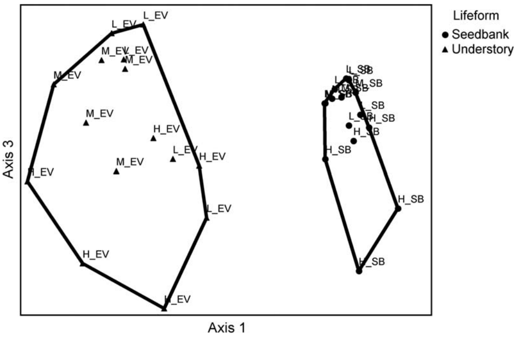

Fig. 9. Nonmetric multidimensional scaling ordination showing the vegetation lifeform (extant vegetation (EV) and seed bank (SB)) with convex hulls (the smallest convex polygon that encloses all points in the same set; a convex polygon is one that has no corners that bend inward).

random. Likewise, the compositions associated with the three pyrophilic zones (with both vegetation lifeforms combined) also differed significantly than what would be expected by chance, though the difference was weaker than that found comparing the vegetation lifeforms, and the Medium and High sites did not differ significantly (Table 1). The results of a two-way factorial perMANOVA agreed with the MRPP results but also manifested the impacts of the vegetation lifeform, showing that the Medium and High zones differed significantly from each other if the effects of the vegetation lifeform and any interactions were removed (partitioned out; Table 2). Vegetation lifeform explained most of the variance (49.6%), while the pyrophilic zone explained 13.2%.

To evaluate the degree of separation between extant vegetation and seed bank compositions

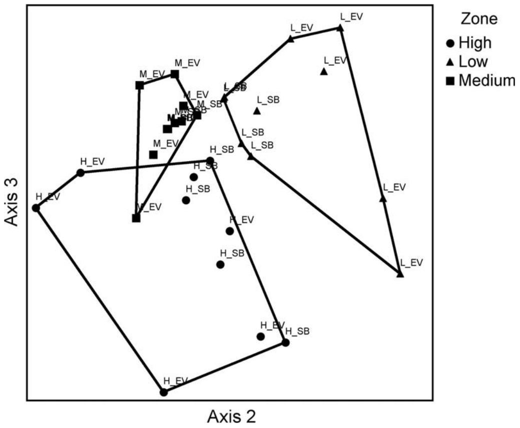

Fig. 10. Nonmetric multidimensional scaling ordination showing the pyrophilic zones (L = Low, M = Medium, and H = High) with convex hulls (the smallest convex polygon that encloses all points in the same set; a convex polygon is one that has no corners that bend inward); EV =extant vegetation and SB = seed bank.

within each pyrophilic zone, we analyzed a combined vegetation lifeform and pyrophilic zone variable using MRPP and perMANOVA (PCORD v. 7). Our NMS ordination using the combined vegetation lifeform and pyrophilic zone as the group reveals that the Medium site was the most homogeneous and the High site was the most heterogeneous for both extant vegetation and seed bank compositions (Fig. 11). The pairwise comparison of High site and extant vegetation *vs*. High site and seed bank had the lowest chance-corrected within-group agreement (A), showing the greatest within-group heterogeneity when comparing extant vegetation with seed bank compositions across each pyrophilic zone. However, the Medium site

Table 2. The results of a perManova using a two-way factorial design with vegetation lifeform as fixed factor; 4,999 randomizations; Sørensen distance measure. The subtable shows the results of the multiple comparisons with the pyrophilic zone group with the fixed factor (vegetation lifeform) and interaction effects partitioned out or included.

Group	d.f.	SS	MS	F	*P*-value
Vegetation lifeform	1	3.69	3.69	21.70	0.00020
Pyrophilic zone	2	1.61	0.80	4.73	0.00020
Interaction	2	0.86	0.43	2.52	0.011
Residual	24	4.08	0.17		

Pyrophilic zone—vegetation lifeform and interaction partitioned out	t	*P*-value	Pyrophilic zone—vegetation lifeform and interaction not partitioned out	t	*P*-value
Low *vs*. Medium	2.82	0.00040	Low *vs*. Medium	1.83	0.023
Low *vs*. High	2.07	0.0018	Low *vs*. High	1.62	0.020
Medium *vs*. High	1.74	0.018	Medium *vs*. High	1.28	0.13

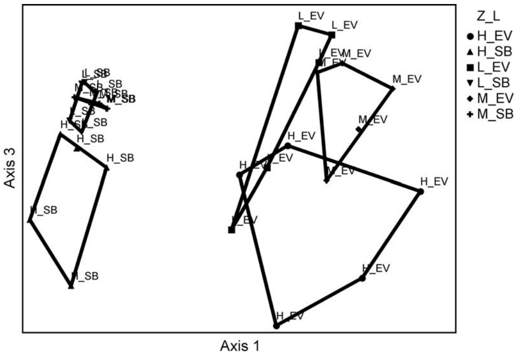

FIG. 11. Nonmetric multidimensional scaling ordination showing the combined (Z + T or pyrophilic zone + vegetation lifeform) pyrophilic zones (L = Low, M = Medium, and H = High) and vegetation lifeforms (EV = extant and SB = seed bank) with convex hulls (the smallest convex polygon that encloses all points in the same set; a convex polygon is one that has no corners that bend inward); EV = extant vegetation and SB = seed bank (SB).

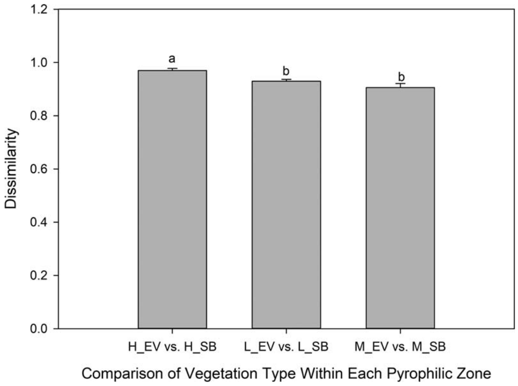

FIG. 12. Comparison of extant vegetation (EV) and seed bank (SB) composition dissimilarity values within each pyrophilic zone (L = Low, M = Medium, and H = High). Dissimilarity was determined from Sørensen distance measures and compared using a one-way ANOVA. Comparisons with different letters are significantly different at $P < 0.05$.

had the highest A or the greatest within-group homogeneity when comparing extant vegetation with seed bank compositions (Table 3). A one-way perMANOVA using a combined variable showed significant differences in the pair-wise comparisons (Table 4). A direct comparison of extant vegetation composition with seed bank composition within each pyrophilic zone showed that the High site had significantly greater dissimilarity between compositions of the extant vegetation and the seed bank than both the Medium and Low sites, with Low site dissimilarity trending to be higher than that of the Medium site (Fig. 12).

Indicator species analyses comparing vegetation lifeform showed that the extant vegetation composition had very few indicator species compared to the seed bank composition, and only one of these (*Vaccinium pallidum* Aiton) was a fire-adapted/fire-related species (Arthur *et al*. 1998; FEIS 2022). The seed bank composition also had only one species, *R. pseudoacacia*, that was fire-adapted (Table 5). A comparison of indicator species among the three pyrophilic zones but within each vegetation lifeform revealed more differences in fire-adapted indicator species. Within the extant vegetation, *Gaultheria procumbens* L. (fire-related; Ducey *et al*. 1996, FEIS 2022) and *Q. montana* (fire-adapted/fire-related; Hoss *et al*. 2008, FEIS 2022) were the only species significant at *P*-value < 0.05, and they both were indicators of the Medium zone. *Melampyrum lineare* var. *latifolium* W. P. C. Barton and *Q. rubra*, both fire-adapted/fire-related species (Reed 1984; Crow

Table 3. Multiresponse permutation procedure results comparing combined vegetation lifeform and seed bank groups using a Sørensen distance measure. The T-statistic describes the separation of the defined groups, the more negative the T, the greater the separation. A = the chance-corrected within group agreement (within group homogeneity). A = 1 when all members in a group are identical; A = 0 when heterogeneity within a group equals what is expected by chance; A < 0 means that there is less agreement within the groups than expected by chance; A > 0 but less than 1 indicate within-group agreement differs significantly from that expected by chance from the within-group agreement of the other groups. Pairwise comparisons within the combined group are also presented. L, M, and H = Low, Medium, and High pyrophilic zones, respectively; EV = extant vegetation, and SB = seed bank.

Group	Group size	T-value	Chance corrected within-group agreement—A	*P*-value
Combined vegetation lifeform and pyrophilic zone	30	−11.38	0.36	0.00000
L-EV *vs*. L-SB	5	−5.34	0.34	0.0016
M-EV *vs*. M-SB	5	−5.76	0.47	0.0017
H-EV *vs*. H-SB	5	−5.20	0.18	0.0016

Table 4. The results of a one-way perManova using 4,999 randomizations and a Sørensen distance measure. The subtable shows the results of the pairwise comparisons across vegetation lifeform within each pyrophilic zone. L, M, and H = Low, Medium, and High pyrophilic zones, respectively; EV = extant vegetation, and SB = seed bank.

Group	d.f.	SS	MS	F	*P*-value
Combined vegetation lifeform and pyrophilic zone	5	6.15	1.23	7.24	0.00020
Residual	24	4.08	0.17		
Pairwise comparisons	t	*P*-value			
L-EV *vs*. L-SB	3.14	0.0064			
M-EV *vs*. M-SB	4.40	0.0080			
H-EV *vs*. H-SB	2.21	0.0078			

1988; Gibson 1993; Varner *et al*. 2016), were indicator species of the High zone but only marginally significant (*Q. rubra* less significant than *M. lineare* var. *latifolium*; Table 6). In the seed bank, two fire-adapted/fire-related species were significant indicators of the High zone, including *Hieracium paniculatum* L. (Silberhorn 1966; Keyser *et al*. 2012), *Dichanthelium latifolium* (L.) Harvill (Catling and Brownell 1999; Slaughter and Paskus 2015), and three fire-adapted species were marginally significant indicators of the High zone, including *Dichanthelium dichotomum* (L.) Gould (Taft and Solecki 2002; Glitzenstein *et al*. 2012), *Poa sylvestris* A. Gray (Taft 2003; McClain and Ebinger 2007), and *Sphenopholus obtusata* (Michx.) Scribn. (Hadley 1970; Jenkins and Parker 2000; Klips 2003; Taft 2003). However, *Liriodendron tulipifera* L., a pyrophobic species (Thomas-Van Gundy and Nowacki 2013; FEIS 2022), was also a significant indicator in the High zone (Table 7). The Medium zone showed *Kalmia latifolia* L. (Elliott *et al*. 2013; FEIS 2022) and *Fragaria virginiana* Duchesne (Klips 2003; Wilk *et al*. 2009), both fire-adapted/fire-related (Table 7). No fire-adapted/fire-related species were indicators of the Low zone in either the extant vegetation or the seed bank (Tables 6 and 7).

The complete species list for the sites (Supplemental Table S2) shows that there were six exotic species found, five of which were only in the seed banks and five of which only occurred in the High site. Also, 10 fire-adapted/fire-related species that were present only in the High site but too uncommon in the extant vegetation (eight) and the seed bank (three) to be an indicator species (*i.e*., occurring only offplot in the extant vegetation), included *Antennaria plataginifolia* (L.) Richardson (extant vegetation; Tester 1989; Copeland *et al*. 2002; Ebinger *et al*. 2013), *Carex wildenovii* Schkuhr ex Willd. (seed bank; Allard and Leonard 1962; Hutchinson *et al*. 2005), *Carya glabra* (Mill.) Sweet (extant vegetation; FEIS 2022), *Covallaria majalis* var. *montana* (Raf.) H. E. Ahles (extant vegetation; Ruch *et al*. 2010; Gierczyk *et al*. 2017), *Dichanthelium boscii* (Poir.) Gould & C.A. Clark (both; Taft and Solecki 2002; *Dichanthelium linearifolium* (Scribn.) Gould/*depauperatum* (Muhl.) Gould (seed bank; Taft and Solecki 2002; McClain and Ebinger 2007; Burton *et al*. 2011), *Hieracium venosum* L. (extant vegetation; Homoya 1994; Cipollini *et al*. 2012;

Table 5. Indicator species for each vegetation lifeform (extant and seed bank). Only species with relative importance values > 40 are shown.

Species	Vegetation lifeform	Relative importance value (> 40)	*P*-value	Fire-adapted/ fire-related
Amelanchier arborea (F. Michx.) Fernald	Extant	53.3	0.0020	
Smilax rotundifolia L.	Extant	46.7	0.0066	
Vaccinium pallidum Aiton	Extant	46.7	0.0066	X
Dennstaedtia punctilobula (Michx.) T. Moore	Seed Bank	100.0	0.0002	
Carex laxiflora Lam.	Seed Bank	66.7	0.0002	
Rubus pensylvanicus Poir.	Seed Bank	66.7	0.0002	
Liriodendron tulipifera L.	Seed Bank	60.0	0.0010	
Robinia pseudoacacia L.	Seed Bank	60.0	0.0010	X
Carex swanii (Fernald) Mack.	Seed Bank	46.7	0.0076	

Table 6. Indicator species for each pyrophilic type (Low, Medium, and High) in the extant vegetation. Only species with importance values 60 or greater are included.

Species	Pyrophilic zone	Relative importance value (> 60)	*P*-value	Fire-adapted/ fire-related
Melampyrum lineare var. *latifolium* W.P.C. Barton	High	60.0	0.0612	X
Quercus rubra L.	High	53.2	0.1096	X
Dryopteris intermedia (Muhl. ex Willd.) A. Gray	Low	100.0	0.0010	
Betula sp. L.	Low	80.0	0.0098	
Prunus serotina L. f.	Low	80.0	0.0138	
Arisaema triphyllum (L.) Schott	Low	60.0	0.0612	
Viola rotundifolia Michx.	Low	60.0	0.0612	
Gaultheria procumbens L.	Medium	100.0	0.0010	X
Quercus montana Willd.	Medium	76.3	0.0108	X
Acer rubrum L.	Medium	64.6	0.0120	

Townsend and Ludwig 2020), *Houstonia longifolia* Gaertn. (extant vegetation; Schiffman and Johnson 1992; Davis *et al*. 2002; Li and Waller 2015)*, Quercus velutina* Lam. (extant vegetation; McClain and Ebinger 2007; Burton *et al*. 2011)*,* and *Rosa carolina* L. (extant vegetation; Taft and Solecki 2002; McClain and Ebinger 2007). There were a few fire-adapted/fire-related species found as uncommon species in both the Medium and High sites, namely *Andropogon virginicus* L (seed bank; Hughes *et al*. 1991; D'Antonio *et al*. 2000; Cipollini *et al*. 2012) and *Vaccinium stamineum* L (both; Ford 1995; Arthur *et al*. 1998; Kreher *et al*. 2000) and one uncommon species, *Gaylussacia baccata* (Wangenh.) K. Koch (extant vegetation; Matlack *et al*. 1993; Arthur *et al*. 1998; Elliott *et al*. 1999) found only in the Medium site (Supplemental Table S2).

Discussion. Hypothesis 1: Seed bank compositions located in zones with a higher percentage of historic pyrophilic witness tree species will also have more pyrophilic seed-bank species than sites with a lower percentage of pyrophilic witness tree species. Our results show that the High pyrophilic site seed bank composition differed significantly from the Low site and also the Medium site, but only from the latter if the effects of vegetation lifeform (seed bank *vs*. extant vegetation) were also removed. The High site had more fire-adapted/fire-related seed bank species than both the Low and Medium pyrophilic sites. However, the Medium site, in addition to the pyrophilic witness tree gradient, may exhibit more xeric site characteristics (understory with abundant *Kalmia latifolia*, a mor humus (partially decomposed plant material, often small roots) or duff

Table 7. Indicator species for each pyrophilic zone in the seed bank. Only species with importance values $\geq$ 60 are included.

Species	Pyrophilic zone	Relative importance value ($\geq$ 60)	*P*-value	Fire-adapted/ fire-related
Hieracium paniculatum L.	High	100.0	0.0012	X
Dichanthelium latifolium (L.) Harvill	High	80.0	0.0120	X
Liriodendron tulipifera L.	High	71.5	0.0372	
Dichanthelium dichotomum (L.) Gould	High	60.0	0.0644	X
Poa sylvestris A. Gray	High	60.0	0.0666	X
Sphenopholus obtusata (Michx.) Scribn.	High	60.0	0.0644	X
Sambucus nigra ssp. *canadensis* (L.) R. Bolli	Low	100.0	0.0016	
Viola rotundifolia Michx.	Low	99.0	0.0024	
Viola blanda Willd.	Low	97.0	0.0036	
Carex swanii (Fernald) Mack.	Low	96.3	0.0062	
Rubus pensylvanicus Poir.	Low	86.2	0.0060	
Carex laxiflora Lam.	Low	66.5	0.0678	
Betula sp. L.	Low	64.3	0.0016	
Kalmia latifolia L.	Medium	79.5	0.0020	X
Lysimachia quadrifolia L.	Medium	77.2	0.0114	
Juncus tenuis Willd.	Medium	60.4	0.0628	
Fragaria virginiana Duchesne	Medium	60.0	0.0634	X

layer, and relatively abundant *Q. montana* in the overstory) than the High site (Braun 1940; Niering 1953). Despite this, the High site manifested more fire-adapted/fire-related indicator species than the Medium site; though the High site had the lowest significant densities, it had the highest significant richness values of all three zones.

Compared to other 80-year or older deciduous forest seed banks in eastern North American forests, our sites were relatively depauperate in terms of both richness and density, with an average richness of 6.88 and density of 82.46 m^{-2}. For instance, one Tucker County, West Virginia, study (Huebner, unpublished) had 52 species and density of 1,569 m^{-2}. A Canadian seed bank study of an old-growth forest had 49 species and density of 1,218 m^{-2} (Leckie *et al*. 2000). Landenberger and McGraw (2004) found 44 species and density of 2,579 m^{-2} in West Virginia mixed-mesophytic forests, and Ashton *et al*. (1998) documented an average of 17 species and a density of 1,257 m^{-2} across three land types. The driest of the land types in the Ashton *et al*. (1998) study had 16 species and a density of 659 m^{-2}. Huebner (unpublished) found 28 species and a density of 1,445 m^{-2} in a northern hardwood forest in Pennsylvania, and Royo and Ristau (2013) found 15 species and an approximate density of 1,000 m^{-2} in another Pennsylvania northern hardwood forest. While these seed bank analyses clearly had high richness and density values compared to our sites, Schiffman and Johnson (1992) showed an average density of only 0.43 m^{-2} for an Appalachian oak forest in Virginia, with forest herbs having greater densities than even the ruderals. Schiffman and Johnson (1992) reasoned that the sparse seed banks were due to no historic cultivation and past low-intensity burns with no outside source of ruderal species propagules. Our High site having the lowest density values (7.22 m^{-2}) of the three pyrophilic zones may support the hypothesis of past low-intensity burns being the cause of low seed bank densities proposed by Schiffman and Johnson (1992). This is further supported by our High site having the highest and significantly different diversity and evenness values, showing a lack of dominant species, the cover of which relatively frequent burns may have helped reduce.

However, deer pressure may also play a role in the sparse seed banks. Increased deer browse was strongly associated with decreased seed bank abundance in a temperate deciduous forest in Maryland (Beauchamp *et al*. 2013) and recently fallowed agricultural fields in New York (DiTommaso *et al*. 2014). Deer may affect not only seed abundance but also seed bank composition, with higher proportions of plants that survive deer browse and still produce seeds than plants that do not survive the browse and/or produce seeds (Tamura 2019). Grazing in general has been noted to act antagonistically with fire in terms of species richness. Davies *et al*. (2013) found that ungrazed (by livestock) seed bank samples treated with simulated smoke and heat treatments had significantly higher species richness, including several fire-adapted species, than grazed samples. Though there is at least one species (*Ageratina altissima* var. *altissima* (L.) King & H. Rob.; white snakeroot) which deer avoid, most of the species found in our sites' seed banks and extant vegetation are browsed by deer, suggesting deer pressure has not played a prominent, recent role in defining the seed banks at these sites. Nonetheless, the lack of oak saplings (personal observation), as is common to many forested areas in West Virginia, supports an underlying, ubiquitous deer problem that may be exacerbated in more xeric sites (Regula *et al*. 2015).

The greater number of fire-adapted/fire-related species found in the seed bank of the High site supports our hypothesis, but the low densities of these species are cause for concern in terms of their longevity and ability to prevent future mesophication of the High zone site. None of the fire-adapted species in our sites are rare or threatened species, also suggesting that at one time these species may have been abundant and any threatened species that may have been present have since been extirpated from the site. Several plant communities with frequent fires are noted for their rare species in part because these communities (*e.g*., barrens, glades, balds, prairie pockets) are rare as well (Campbell *et al*. 1991; Latham 2003). In a Kentucky study in Appalachian pine-oak barrens, rare plants were associated with disturbed habitats, such as roadsides, but notably also were linked to recent fires. Indeed, similar disturbed habitats without any burning did not share the same rare species (Campbell *et al*. 1991). Though our study did not contain any of the same rare species as this Kentucky study, *Melampyrum lineare* var. *latifolium* (cow-wheat) is a shared relatively common indicator species in the extant vegetation and *Lespedeza* Michx. was a shared genus between the

Kentucky study and our sites (*L. capitata* Michx., bushclover, in Kentucky [Campbell *et al.* 1991] and *L. hirta* (L.) Hornem, hairy bushclover, found only in the High site). However, *L. hirta* was too rare to be defined as an indicator species in our study area. While all the fire-adapted species found in our sites share traits that would enable them to survive xeric conditions, *L. hirta* may be an example of a species where its physical dormancy due to the hard seed coat (typical of many Fabaceae) provides evidence of historical fire at the High site.

Though we added horticultural-grade charcoal to our seed bank samples, we did not simulate fire by adding smoke or heat. Several studies show that treating seed bank soil samples with smoke and/or heat may stimulate germination of some fire-adapted species, with liquid smoke being most effective. The effect is mostly to increase the density of species that may be found without the added liquid smoke or heat (Enright and Kintrup 2001; Chou *et al.* 2012; Nelson *et al.* 2012; Davies et al. 2013). Liquid smoke may also inhibit germination of other species (Chou *et al.* 2012; Nelson *et al.* 2012). Latham (2003) also noted that in many northeastern US rare communities, many of the rare species benefit only indirectly from fire that is critical for maintaining other more dominant shrub species. Nonetheless, the number of uncommon fire-adapted/fire-related species found in the extant vegetation of the High site support the need for future research to include applying liquid smoke derived from litter collected from each of our sites.

HYPOTHESIS 2: BECAUSE OF FIRE SUPPRESSION AND THE PRESENCE OF LONG-LIVED SEEDS, DIFFERENCES BETWEEN EXTANT UNDERSTORY VEGETATION AND SEED BANK COMPOSITIONS WOULD BE GREATEST WITHIN SITES WITH THE HIGHEST PERCENTAGE OF PYROPHILIC WITNESS TREES. The site with the highest percentage of pyrophilic witness trees (High site) did show the greatest difference between the extant understory vegetation and the seed bank composition. However, the Low and Medium sites' extant vegetation and seed bank compositions did not differ significantly, indicating this pattern may not follow the pyrophilic gradient completely. Instead, the greater similarity between the Low and Medium sites may be driven by comparatively higher fire suppression and/or stress levels than found in the High site. Fire suppression at the Medium site is supported by its relatively deep duff layer due to abundant *K. latifolia*, which propagates best in duff (Lorente *et al.* 2012). Although very dry duff conditions can lead to highly destructive hot burns that remove all the duff (Waldrop *et al.* 2010), fire ignition can be more difficult with deep duff layers (Varner *et al.* 2007; Pipkin 2012). Further, low ratios of Ca to K and Mg at the Medium site are indicative of xeric sites (Klinka and Lowe 1976), soil weatherization, and lack of fire (McKee 1991). The very low Ca values with corresponding high levels of Al or a low Ca:Al value, in contrast, support a stressed environment compared to the Low and High sites (Klinka and Lowe 1976). Such stressed conditions may further reduce the likelihood of early successional species in the seed bank of the Medium site compared to the Low site, resulting in greater similarity between the Medium site's extant vegetation and seed bank compositions. Given that the indicator species associated with the Medium site were also examples of xeric/stressed species and no fires have been documented at the site for at least 80 years, the presence of these species in the seed bank may not be due to fire. The Low site's high Ca values explain a lack of difference in the stress level, as defined by the Ca:Al, between the Low and High sites (Klinka and Lowe 1976; Farr *et al.* 2009; Elliott *et al.* 2013).

The dissimilarity between vegetation lifeform (extant vegetation and seed bank) was much higher than between pyrophilic zone, but the species compositional differences between extant vegetation and seed bank composition differed for each zone. The Low and Medium sites had seed bank compositions defined more by mesic and xeric forest species, respectively, with a few open-area species (*e.g.*, *Lysimachia quadrifolia* L., four-flowered loosestrife), especially in the Medium site. Sharing at least some extant species and being relatively depauperate makes the Low and Medium seed banks similar to the seed banks described by Leckie *et al.* (2000) and Schiffman and Johnson (1992) with possible seed bank depletion caused by deer (Beauchamp *et al.* 2013). In contrast, the High site's seed bank was defined primarily by pyrophilic seed bank species and shared few species with the extant vegetation. Moreover, the densities of the High site pyrophilic seed bank species were lower than the densities of the seed bank species found in the Low and Medium sites. Many of the uncommon fire adapted/fire-related species remaining in the High site's extant understory may be being maintained vegetatively but with decreasing abundances. The High site contained more pyrophobic species in the extant vegetation (nine +) or and seed bank (eight +) than pyrophilic species (Supplemental Table S2), in addition to the indicator seed bank species *L. tulipifera*. Thus, the High site is exhibiting mesophication with decreasing potential to recover even if fire were to be reintroduced. It is also concerning that the High site had the highest number of exotic species in both the extant and seed bank vegetation, albeit they were still at low density. The High site was geographically closest to private land, though the Medium and Low sites were closer to roads, both of which are known sources of exotic plant propagules (Mortensen *et al*. 2009; Gavier-Pizarro *et al*. 2010; Huebner 2021).

We expected there to be greater charcoal abundance in the High pyrophilic zone. However, the Low zone had the most charcoal, with some dates from the Woodland Period, which may indicate this location was burned by indigenous people at a time when plant husbandry and domestication were increasing in parts of the Appalachian Plateau with probable use of fire to clear areas for cultivation (Delcourt *et al*. 1998; Lafon *et al*. 2017). Our results, which are limited by a small number of samples, do not necessarily indicate that the High and Medium zones do not also share a similar Woodland Period history. Instead, comparatively recent fires may have been much more frequent in these zones than in the Low zone, increasing charcoal amounts from those dates such that a small random sample made selection of older dates less probable. In the late 1600s, the first Europeans began exploring West Virginia, and in the mid- to late 1700s the first European settlements were established in Randolph County, West Virginia. The earlier fires at the High zone may be linked to Native American land use and weather (lightning). The fires at the Medium and High zones site may additionally have been associated with European settlements, though none are documented specifically in this location at that time (Bosworth 1916). Tree species represented by the randomly selected charcoal fragments align with our hypotheses regarding species distributions across the pyrophilic gradient. Low site charcoal was dominated by the mesophyte *A. rubrum*, the Medium site had a mix of *A. rubrum* and *C. dentata*, a fire-adapted species, and High site samples were all *C. dentata*. Although *C. dentata* is no longer a dominant species in these forests, its presence in this charcoal record is a reminder of its once broad distribution throughout eastern North American forests.

Conclusions. The High site had the greatest number of pyrophilic species in both the extant vegetation and the seed bank composition as well as the greatest diversity of the three zones. This greater diversity was, in part, due to having a mixture of pyrophilic and pyrophobic species. The High site also had exceptionally low seed bank densities, especially of the pyrophilic species. In addition, the extant vegetation and seed bank composition differed the most from each other at the High site compared to the Low and Medium sites, reflecting a transition from fire-adapted species to mesophytic species. Our results are based on one site per pyrophilic zone but reveal a vulnerability of fire-prone vegetation in West Virginia to mesophication and a decreasing propensity to restoration of fire-adapted plant communities even with the reintroduction of fire. The degree of mesophication is also influenced by site conditions, with stressed xeric sites being more likely to show an increase in dominance of stress-tolerant species that may also be fire-adapted. Implications for management include increasing efforts of reintroducing fire to once fire-prone sites, monitoring natural species re-establishments from the seed bank in response to increased fire frequencies, and, if such responses are weak, seeding or planting pyrophilic species known historically to be present at the fire-prone sites. Soil charcoal results provide direct evidence of past fire on these forests, dating to at least the Woodland Period for the Low site. Further soil charcoal investigations with more radiocarbon dates could provide a more comprehensive and robust fire and forest composition history for these forests. Other sites will need to be sampled with similar intensity to confirm that vulnerability of fire-prone vegetation to mesophication is a common phenomenon in eastern temperate deciduous forests.

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Supplemental Table S1. Charcoal age results for samples with identifiable species/genera using ^{14}C dating. Analyses were conducted by the National Institute of Applied Climate Science. (NIACS). CADN = *Castanea dentata* and ACRU = *Acer rubrum*.

Lab Number	Site, Transect, Core, and Depth	Sample ID	Δ^{14}C (‰)	Uncalibrated ^{14}C age (^{14}C yr BP)	Calibrated age range (cal yr BP) ± 2σ	Rel. area under prob. curve	Weighted mean calibration age (cal yr BP)
256130	Pyro 1, Lower Transect, 50 m, 0–10 cm deep	CADN	−142.0	1160 ± 20	1178–1161	0.124	1071
					1127–1049	0.509	
					1034–976	0.321	
256113	Pyro 1, Lower Transect, 50 m, 20–28 cm deep	ACRU	−143.8	1180 ± 20	1178–1056	0.917	1113
					1021–1006	0.038	
256126	Pyro 1, Upper Transect, 25 m, 20–28 cm deep	Acer sp.	−293.6	2725 ± 20	2861–2766	0.954	2814
256125	Pyro 1, Upper Transect, 50 m, 0–10 cm deep	ACRU	−95.3	735 ± 20	689–653	0.954	671
256122	Pyro 2, Lower Transect, 0 m, 20–24 cm deep	ACRU	−33.2	200 ± 20	295–265	0.254	211
					217–145	0.592	
256123	Pyro 2, Lower Transect, 0 m, 20–24 cm deep	CADN	−28.6	165 ± 20	284–254	0.171	196
					226–166	0.401	
					156–137	0.099	
					112–72	0.072	
256127	Pyro 2, Lower Transect, 25 m, 0–10 cm deep	ACRU	−32.1	195 ± 15	290–266	0.236	208
					215–146	0.611	
256128	Pyro 2, Lower Transect, 25 m, 0–10 cm deep	CADN	−14.8	50 ± 20	253–227	0.265	121
					138–115	0.264	
					71–39	0.34	
					−1 to −3	0.015	
					−4 to −5	0.07	
256112	Pyro 2, Lower Transect, 25 m, 10–20 cm deep	CADN	−21.3	105 ± 20	259–222	0.251	126
					141–30	0.704	
256117	Pyro 2, Lower Transect, 50 m, 0–10 cm deep	ACRU	−19.5	90 ± 20	256–224	0.268	129
					139–33	0.686	
256118	Pyro 2, Lower Transect, 50 m, 0–10 cm deep	ACRU	−21.0	100 ± 20	258–223	0.257	127
					140–31	0.697	
256119	Pyro 2, Lower Transect, 50 m, 0–10 cm deep	CADN	−34.8	215 ± 20	305–266	0.372	219
					214–195	0.077	
					190–147	0.468	
256114	Pyro 2, Lower Transect, 50 m, 10–20 cm deep	ACRU	−17.5	75 ± 20	255–225	0.287	131
					139–88	0.314	
					83–33	0.354	
256121	Pyro 2, Upper Transect, 0 m, 0–10 cm deep	ACRU	−20.6	100 ± 20	258–223	0.257	127
					140–31	0.697	
256124	Pyro 2, Upper Transect, 50 m, 0–10 cm deep	ACRU	−16.8	65 ± 15	253–226	0.298	134
					138–115	0.288	
					67–40	0.369	
256131	Pyro 2, Upper Transect, 50 m, 20–27 cm deep	CADN	−109.5	865 ± 20	793–724	0.954	758
256129	Pyro 3, Lower Transect, 0 m, 0–10 cm deep	CADN	−26.3	145 ± 20	280–240	0.152	165
					232–170	0.235	
					153–129	0.101	
					119–56	0.269	
256115	Pyro 3, Lower Transect, 0 m, 10–20 cm deep	CADN	−77.0	575 ± 20	634–589	0.636	591
					563–535	0.318	
256116	Pyro 3, Lower Transect, 0 m, 10–20 cm deep	CADN	−36.7	230 ± 20	310–274	0.534	238
					208–199	0.016	
					185–150	0.404	
256120	Pyro 3, Upper Transect, 25 m, 0–10 cm deep	CADN	−21.4	105 ± 20	259–222	0.251	126
					141–30	0.704	

Supplemental Table S2. Species found in the extant understory vegetation (EV) and/or the seed bank (SB) by pyrophilic zone (L = Low, M = Medium, H = High. P = present only in the offplot walk through. Numbers represent the frequency of occurrence on both transects (1 = occurring in every plot). * = exotic. Determination of fire-adapted/fire-related (marked with an X) is based on having at least one published source indicating an increase in abundance or stable abundance that is not just transitory post-burn (only a temporary increase via germination immediately after the burn).

		Low		Medium		High		Fire-Adapted/ Fire-Related
Species	Common Name	EV	SB	EV	SB	EV	SB	
Tree/shrub/woody vine								
Acer pennsylvanicum L.	Striped maple	0	0	0	0	0.2	0	
Acer rubrum L.	Red maple	1	0.6	1	0	0.8	0.2	
Acer saccharum Marsh.	Sugar maple	0.2	0	0	0	0	0	
Amelanchier arborea (F. Michx.) Fernald	Common serviceberry	0	0	0.8	0	0.8	0	
Aralia spinosa L.	Devil's walking stick	0	0	0	0.8	0	0.2	
Aristolochia macrophylla Lam.	Dutchman's pipe	0	0	0	0	P	0	
Betula L. sp.	Birch	0.8	1	0	1	0	0.8	
Buddleja davidii Franch. *	Butterfly bush	0	0	0	0	0	0.4	
Carpinus caroliniana Walter	Muscle wood	0	0	0	0	P	0	
Carya glabra (Mill.) Sweet	Pignut hickory	0	0	0	0	0.2	0	X
Carya tomentosa (Lam. ex Poir.) Nutt.	Mockernut hickory	0	0	0	0	P	0	
Cornus florida L.	Flowering dogwood	0	0	0	0	P	0	
Fagus grandifolia Ehrh.	American beech	0.2	0	0	0	P	0	
Fraxinus americana L.	White ash	P	0	0	0	0.2	0	
Gaultheria procumbens L.	Teaberry	0	0	1	0	0	0	X
Gaylussacia baccata (Wangenh.) K. Koch	Black huckelberry	0	0	0.2	0	0	0	X
Hamamelis virginiana L.	Witchhazel	0	0	0	0	0.2	0	
Ilex montana Torr. & A. Gray ex A. Gray	Mountain holly	0	0	P	0	0	0	
Kalmia latifolia L.	Mountain laurel	0	0	0.8	1	0.4	0.6	X
Liriodendron tulipifera L.	Tulip poplar	0	0	0	1	0	0.8	
Magnolia fraseri Walter	Umbrella tree	P	0	0	0	0	0	
Nyssa sylvatica Marshall	Black gum	0	0	0.2	0	0	0	
Parthenocissus quinquefolia (L.) Planch.	Virginia Creeper	0	0	0	0	0.2	0	
Picea rubens Sarg.	Red spruce	0.4	0	0	0	0	0	
Pinus strobus L.	White pine	0	0	0	0	P	0	
Prunus pensylvanica L. f.	Pin cherry	0	0.6	0	0	0	0	
Prunus serotina Ehrh.	Black cherry	0.8	0	0	0	0	0	
Quercus alba L.	White oak	0	0	0	0	0.2	0	X
Quercus montana Willd.	Chestnut oak	0	0	0.8	0	0.2	0	X
Quercus rubra L.	Northern red oak	0	0	0.4	0	0.6	0	X
Quercus velutina Lam.	Black oak	0	0	0	0	0.2	0	X
Rhododendron calendulaceum (Michx.) Torr.	Flame azalea	0	0	0	0	P	0	
Robinia pseudoacacia L.	Black locust	0	0.2	0	0.8	P	0.8	X
Rosa carolina L.	Pasture rose	0	0	0	0	P	0	X
Rubus allegheniensis Porter	Common blackberry	0	0	0	0.6	0	0.2	
Rubus L. sp.	Blackberry	P	1	0	0.8	0	0.2	
Rubus pensylvanicus Poir.	Pennsylvania blackberry	0	1	0	0.6	0	0	
Sambucus nigra ssp. *canadensis* (L.) R. Bolli	Common elderberry	0	1	0	0	0	0	
Sassafras albidum (Nutt.) Nees	Sassafras	P	0	P	0.6	0.2	0.2	
Smilax glauca Walter	Saw brier	0	0	0	0	0.4	0	
Smilax rotundifolia L.	Common greenbrier	0	0	0.8	0	0.6	0	
Tilia americana L.	American basswood	P	0	0	0	0	0	
Tsuga canadensis (L.) Carrière	Eastern hemlock	0	0	0	0	P	0	
Vaccinium pallidum Aiton	Late low blueberry	0	0	0.6	0	0.8	0	X
Vaccinium stamineum L.	Deerberry	0	0	0	0.2	0.2	0.2	X
Viburnum acerifolium L.	Maple-leaf viburnum	0	0	0	0	0.4	0	
Vitis L. sp.	Grape	0	0.4	0	0.4	0	0.4	

Supplemental Table S2. Continued.

Species	Common Name	Low		Medium		High		Fire-Adapted/ Fire-Related
		EV	SB	EV	SB	EV	SB	
Forb								
Actaea racemosa L.	Black cohosh	P	0	0	0	0	0	
Ageratina altissima var. *altissima* (L.) King & H. Rob.	White snakeroot	0.2	0.4	0	0	P	0.6	
Antennaria plantaginifolia (L.) Richardson	Plantainleaf pussytoes	0	0	0	0	0.2	0	X
Arisaema triphyllum (L.) Schott	Jack in the pulpit	0.6	0	0	0	0	0	
Conopholis americana (L.) Wallr.	Cancer root	0	0	0	0	P	0	
Convallaria majalis var. *montana* (Raf.) H.E. Ahles	American lily-of-the valley	0	0	0	0	0.2	0	X
Cypripedium acaule Aiton	Pink lady's slipper	0	0	P	0	0	0	
Desmodium nudiflorum (L.) DC.	Naked-flowered tick trefoil	0	0	0	0	P	0	
Dioscorea villosa L.	Wild yam	0	0	0	0	0.2	0	
Dryopteris intermedia (Muhl. ex Willd.) A. Gray	Intermediate wood fern	1	0	0	0	0	0	
Erechtites hieraciifolius (L.) Raf. ex DC.	Fire weed	0	0	0	0.2	0	0.4	
Erigeron annuus (L.) Pers.	Daisy fleabane	0	0.6	0	0.2	0	0.4	
Eurybia divaricata (L.) G.L. Nesom	White woodland aster	0.2	0	0	0	P	0	
Eutrochium purpureum (L.) E.E. Lamont	Joe pye weed	0.2	0	0	0	0	0	
Fragaria virginiana Duchesne	Virginia strawberry	0	0	0	0.6	0	0	X
Galium aparine L.	Cleavers	P	0	0	0	0	0	
Galium circaezans Michx.	Wild licorice	0	0.2	0	0	0.4	0	
Galium lanceolatum Torr.	Lanceleaf wild licorice	0	0	0	0	P	0	
Galium triflorum Michx.	Sweetscented bedstraw	0.2	0.2	0	0	P	0	
Goodyera pubescens (Willd.) R. Br.	Downy rattlesnake plantain	0	0	0	0	0.2	0	
Helianthus L. sp.	Sunflower	0	0	0	0	P	0	
Hieracium L. sp. *	Hawkweed	0	0	0	0	0	1	X
Hieracium paniculatum L.	Panicled hawkweed	0	0	0	0	P	1	X
Hieracium venosum L.	Rattlesnake weed	0	0	0	0	0.2	0	X
Houstonia longifolia Gaertn.	Long-leaved summer bluets	0	0	0	0	P	0	X
Hypericum L. sp.	St. John's wort	0	0.2	0	0	0	0.2	
Hypericum punctatum Lam.	Dotted St. John's wort	P	0	0	0	0	0	
Impatiens L. sp.	Jewelweed	P	0.2	0	0	0	0	
Laportea canadensis (L.) Wedd.	Wood nettle	P	0	0	0	0	0	
Lespedeza hirta (L.) Hornem.	Hairy bushclover	0	0	0	0	0	0.2	X
Leucanthemum vulgare Lam. *	Ox-eye daisy	0	0	0	0	0	0.4	
Lobelia inflata L.	Indian tobacco	0	0	0	0.2	0	0	
Lysimachia quadrifolia L.	Four-flowered loosestrife	0	0.2	P	0.8	0	0	
Maianthemum canadense Desf.	Canada lily	P	0	0	0	0	0	
Maianthemum racemosum ssp. *racemosum* (L.) Link	False Solomon's seal	P	0	0	0	0	0	
Medeola virginiana L.	Cucumber root	P	0	0	0	0	0	
Melampyrum lineare var. *latifolium* W.P.C. Barton	Cow-wheat	0	0	0	0	0.6	0	X
Mitchella repens L.	Partridge berry	0.4	0	0	0	0	0	
Monotropa uniflora L.	Indian pipe	0	0	0	0	P	0	
Phytolacca americana L.	Pokeweed	0	0.2	0	0.6	0	0.4	
Plantathera orbiculata (Pursh) Lindl.	Round-leaved orchid	P	0	0	0	0	0	
Polygonatum biflorum (Walter) Elliott	Solomon seal	P	0	0	0	P	0	
Polygonatum pubescens (Willd.) Pursh	Downy Solomon seal	0.2	0	0	0	0	0	
Potentilla simplex Michx. /*canadensis* L.	Old field/running cinquefoil	0	0	0	0.6	0.2	0.6	
Prenanthes L. sp.	White lettuce	0.2	0	0	0	0.2	0	
Prosartes laguninosa (Michx.) D. Don	Yellow mandarin	0	0	0	0	P	0	
Ranunculus hispidus Michx.	Hispid buttercup	0	0.2	0	0	0	0	

Supplemental Table S2. Continued.

		Low		Medium		High		Fire-Adapted/ Fire-Related
Species	Common Name	EV	SB	EV	SB	EV	SB	
Rumex acetosella L. *	Sheep sorrel	0	0.2	0	0	0	0	
Rumex crispus L. *	Curly dock	P	0	0	0	0	0.2	
Solidago arguta Aiton	Cutleaf goldenrod	0	0	0	0	P	0	
Solidago caesia L.	Blue-stem goldenrod	0	0	0	0	P	0	
Solidago canadensis L.	Canada goldenrod	0	0	0	0	0	0.2	
Solidago curtisii Torr. & A. Gray	Curtis' goldenrod	0.2	0.2	0	0	0	0	
Solidago rugosa Mill.	Wrinkled-leaf goldenrod	0	0.4	0	0	0	0.4	
Symphyotrichum lateriflorum var. *lateriflorum* (L.) A. Love & D. Love	Calico aster	0	0.4	0	0	0	0.4	
Symphyotrichum undulatum (L.) G.L. Nesom	Wavy-leaf aster	0	0	0	0	0.2	0	
Veronica officinalis L.*	Common speedwell	0	0.2	0	0.2	0	0.4	
Viola blanda Willd.	Sweet white violet	0.4	1	0	0	0	0.2	
Viola hastata Michx.	Halberd-leaf yellow violet	0	0	0	0.2	0	0	
Viola palmata L.	Palmate-leaf violet	0	0	0	0	0.2	0	
Viola rotundifolia Michx.	Roundleaf violet	0.6	1	0	0.2	0	0	
Viola sororia Willd./*palmata* L.	Downy wood violet/palmate-leaf violet	0.4	0.6	0	0.2	0.2	0.2	
Grass/rush/sedge								
Agrostis perennans (Walter) Tuck.	Autumn bent grass	0	0	0	0.2	0	0.6	
Andropogon virginicus L.	Broomsedge	0	0	0	0.2	0	0.2	X
Carex communis L.H. Bailey	Colonial sedge	0	0.4	0	0.4	0	0	
Carex debilis Michx.	Weak sedge	P	0.4	0	0.2	0	0.2	
Carex digitalis Willd.	Finger sedge	0	0.4	0	0	0.2	0	X
Carex L. sp.	Sedge	0	0.4	0	0	0	0.4	
Carex laxiflora Lam.	Loose-flowered sedge	0	1	0	0.6	P	0.4	
Carex normalis Mack.	Right-angle sedge	0	0.2	0	0.2	0	0.2	
Carex pensylvanica Lam.	Pennsylvania sedge	0	0.2	0	0	0	0.2	
Carex straminea Willd. ex Schkuhr	Straw-colored sedge	0	0.8	0	0.2	0	0.2	
Carex swanii (Fernald) Mack.	Swan's sedge	0	1	0	0.4	0	0	
Carex willdenovii Schkuhr ex Willd.	Wildenow's sedge	0	0	0	0	0	0.4	
Danthonia compressa Austin	Poverty grass	0	0.2	0	0	0	0.4	
Dichanthelium boscii (Poir.) Gould & C.A. Clark	Bosc's panic grass	0	0	0	0	0.2	0.2	X
Dichanthelium commutatum (Schult.) Gould	Variable panic grass	0	0	0	0	0.2	0.4	X
Dichanthelium dichotomum (L.) Gould	Bushy panic grass	0	0	0	0	0.2	0.6	X
Dichanthelium latifolium (L.) Harvill	Broad-leaved panic grass	0	0	0	0	0	0.8	X
Dichanthelium linearifolium (Scribn.) Gould/*depauperatum* (Muhl.) Gould	Linear-leaved panic grass/ starved panic grass	0	0	0	0	0	0.2	X
Festuca subverticillata (Pers.) E.B. Alexeev	Nodding fescue	P	0	0	0	P	0	
Glyceria R. Br. sp.	Mannagrass	0	0.2	0	0	0	0	
Glyceria striata (Lam.) Hitchc.	Fowl mannagrass	P	0	0	0	0	0	
Juncus effusus L.	Common rush	0	0.2	0	0.6	0	0.4	
Juncus tenuis Willd.	Path rush	0	0	0	0.8	0	0.4	
Melampyrum lineare var. *latifolium* W.P.C. Barton	Cow-wheat	0	0	0	0	0.6	0	X
Panicum L. sp.	Panic grass	0	0	0	0	0	0.8	X
Poa alsodes A. Gray	Woodland bluegrass	0.2	0.4	P	0	0	0.2	
Poa sylvestris A. Gray	Sylvan bluegrass	0	0	0	0	0.2	0.6	X
Scirpus L. sp.	Bulrush	0	0.6	0	0.4	0	0	
Sphenopholis obtusata (Michx.) Scribn.	Prairie wedgegrass	0	0	0	0	0.2	0.6	X

Supplemental Table S2. Continued.

Species	Common Name	Low		Medium		High		Fire-Adapted/ Fire-Related
		EV	SB	EV	SB	EV	SB	
Fern								
Asplenium platyneuron (L.) Britton, Sterns & Poggenb.	Ebony spleenwort	0	0	0	0	P	0	
Dennstaedtia punctilobula (Michx.) T. Moore	Hayscented fern	0	1	0	1	P	1	
Dryopteris marginalis (L.) A. Gray	Marginal wood fern	0	0	0	0	0.2	0	
Polystichum acrostichoides (Michx.) Schott	Christmas fern	P	0	0	0	0.2	0	
Thelypteris noveboracensis (L.) Nieuwl.	New York fern	0.4	0	0	0	0	0	