Multiple Disturbances, Multiple Legacies: Fire, Canopy Gaps, and Deer Jointly Change the Forest Seed Bank

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Abstract

The manipulation of pre-colonial disturbances in U.S. forests can play a critical role in determining ecological composition, structure, and function. However, our understanding of how concurrent disturbances influence non-tree species is extremely limited in forests. To this end, we used a long-term, multi-disturbance experiment in an oak dominated forest in West Virginia, U.S.A. that factorially manipulated understory fire, deer fencing, and canopy gaps. Thirteen years after disturbance initiation, we sampled and germinated the seed bank from each disturbance treatment. We found long-term seed banks differed only in plots with understory fire, with effects contingent on canopy gaps and deer fencing. Fire combined with canopy gaps caused a 205% increase in seed abundance. Combined fire, deer fencing, and canopy gaps led to the lowest diversity of all treatments and the dominance of the shrub *Rubus* in the seed bank, reflecting the continued legacy of extant plants that grew immediately after disturbance. Lastly, in plots with multiple reintroduced disturbances, seed communities were distinct from extant understory species at all time points, highlighting how the seed bank is an important reservoir of biodiversity. Each reintroduced disturbance combination left a unique legacy in the seed bank that will likely influence future forest reorganization following disturbance, adding to our understanding of how disturbances influence forest succession and organization. Our study highlights the many unexpected ways that multiple disturbances can change an understudied, but influential, component of the forest for well over a decade.

Significance Statement

In forests throughout the United States, pre-colonial disturbances, such as understory fire, canopy gaps, and deer populations, are being manipulated in hopes of restoring historic biodiversity. Each disturbance (or lack thereof) can lead to unique plant communities, but we do not understand how these combined disturbances change plant diversity and the resulting soil seed bank. Changes in the soil seed bank, which depends on the plants that survive post-disturbance, can strongly influence biodiversity and forest development. By using an experiment that manipulates combined understory fire, canopy gaps, and deer exclusion, we found that multiple seed bank communities arise depending on the disturbance combination. These altered seed banks will likely have long-term effects on future biodiversity and development.

Main Text

Introduction

Early and mid-twentieth century management practices in eastern U.S. forests have led to radical deviations from historic disturbance regimes and subsequent forest ecosystems (1–3). This scenario is particularly acute in Appalachian hardwood forests, which have lost oak (*Quercus* spp.) tree regeneration and are transitioning to wetter, maple-dominated (*Acer* spp.) systems (4). This transition from oak to maple forests was initiated by the forced removal of Indigenous peoples and their use of cultural burning as a management tool (5). This was followed by mass forest liquidation and slash wildfires in the late 19th and early 20th century (6). Negative perceptions of these wildfires led to a century of state-sanctioned fire exclusion and suppression that favored maple growth and wetter understories (7, 8). As a result, Appalachian forests became dominated by even-aged stands with few large (20 to 25-m + diameter) canopy gaps and infrequent understory fires (4, 9, 10). In some areas the fire return interval is now over 10,000 years, as opposed to the historic 1-to-2-decade fire return interval common under Indigenous stewardship (6). Meanwhile, white-tailed deer (*Odocoileus virginianus*) populations have increased dramatically above historical baselines (above 4 and 8 deer/km^2) in most of the eastern United States, driving ecological change depending on their population density (11, 12). To reverse the long-tailed negative effects of historic management and sustain oak-dominated plant communities, forest managers are reintroducing disturbances like prescribed burns, canopy gap creation through tree harvesting, and lowering deer densities through hunting or fencing off vulnerable areas (10, 13).

Reintroducing multiple disturbances can be a powerful tool in efforts to restore and change ecological communities (14–17). For instance, combined understory fire and canopy gap creation can lead to greater oak regeneration and reduced maple dominance, while these individual disturbances alone are less effective (18–20). In this example, the surviving oak trees represent a post-disturbance legacy, which is broadly characterized as the adaptations, individuals, and biomass that remain on the landscape following a disturbance (21). Disturbance legacies can be material (e.g., wood and nutrient pools) and informational (e.g., species' adaptive responses and genetic material), although the categories are not mutually exclusive (22). Each disturbance that occurs in a given area modifies the legacy community of the previous disturbance, and in certain cases, the disturbance combination and timing may lead to unique communities depending on how the disturbances in question interact (23). Thus, in eastern U.S. forests, the disturbance legacies of combined understory fire, canopy gap creation, and deer browsing may have a particularly influential role in determining how forests reorganize and develop into the future when compared to the legacies of these disturbances individually (24–26).

To this end, the soil seed bank represents an important, but understudied, entity that may be strongly influenced by the reintroduced disturbances in the eastern U.S. and may influence future disturbance regimes (27). Seed banking is a reproductive adaptation that allows plants to persist belowground as dormant seeds, wherein the soil serves as a buffer from aboveground disturbances (28). When a disturbance creates suitable conditions for certain seed species, they can germinate. The forest seed bank is a reservoir of biodiversity, holding many herbaceous and woody species like *Viola* spp*.,* *Carex* spp*.,* *Rubus* spp., *Acer* spp*., Prunus* spp*.,* and *Betula* spp. (29–31). The seed bank is also a latent source of genetic diversity, making the seed bank both a material and information legacy. Germinated plants that survive the disturbance eventually mature and release seeds, reestablishing the seed banking process that allows for plant communities to reorganize with future disturbance, thereby setting another legacy depending on the seeds that are returned to the soil (32, 33). Hypothetically, more disturbance will lead to a seed bank that is more similar to aboveground vegetation, as the herbaceous layer is homogenized and a few ruderal species survive and reproduce (27). These changes in the seed bank with disturbance can have long-lasting ecological ramifications. For example, rampant timber harvesting and slash wildfires in the United States during the late 19th and early 20th centuries likely allowed the shrub *Rubus* to spread and saturate forest seed banks with its long-lived seeds, creating a century-old legacy of heavy *Rubus* regeneration following overstory disturbance throughout the eastern U.S. (34, 35). *Rubus* can then survive as a recalcitrant understory for decades (36–38).

Prescribed burns, canopy gap creation, and deer browsing each provide a unique and important opportunity for new vegetation to grow from the seed bank and for the seed bank to change (27, 39). Prescribed fires clear plant material, catalyzing seed germination with increased light, heat, smoke, and nutrients (40). In fire-prone ecosystems, Pausas and Lamont (2022) found that ≈42% of seed banking species are adapted to germinate with heat or smoke (41). Canopy gaps increase understory resources like light, soil temperature, and soil moisture, which are critical for seeds to germinate (42, 43). Both fire and canopy gaps result in a temporary depletion of seeds in the seed bank as plants germinate, but over time, newly established vegetation will grow, reproduce, and replenish the seed bank (44, 45). This replenishment process may be disrupted by white-tailed deer, as chronic over-browsing can constrain seed set, reduce plant abundance, and lower long-term understory plant diversity by shifting composition to browse tolerant species (46–48). These direct consumptive effects may, thus, indirectly reduce the abundance and diversity of seed banking species (49, 50) However, in regions where deer populations are low and similar to historic estimates, deer browsing has been shown to increase understory diversity by reducing competitive ruderal species, which could then lead to a more diverse seed bank (51).

Despite the increasing prevalence and co-occurrence of these reintroduced disturbances in the eastern U.S., our understanding of how individual and combined understory fires, canopy gaps, and white-tailed deer change long-term forest seed banks is minimal. This highlights a significant gap in our understanding of post-disturbance legacies, as seed banks are critical for maintaining forest biodiversity in light of disturbance. Therefore, the primary question guiding our research is: **Do multiple reintroduced disturbances cause more substantial long-term changes in the seed bank than each respective individual disturbance?** To test this question we used a unique, multi-disturbance forest experiment that factorially manipulated understory fire via controlled burn, canopy gap creation via girdling and herbicide injection, and deer density via fenced exclosures (Fig. 1). Thirteen years after the experiment's initiation, we sampled the seed bank in each disturbance combination treatment and tested how seed composition varied by disturbance treatment and in comparison to extant vegetation at multiple time points.

We expected low-intensity fire to be the predominant driver of increased seed abundance and diversity, as the Appalachian Mountains are a historically burned ecosystem and many plant species are likely favored by fire (**H1**). Similarly, we expected canopy gaps to lead to a modest increase in seed bank diversity and density, mirroring the increased aboveground plant diversity with gaps by Royo et al. (2010) (51) (**H2**). We hypothesized that fire combined with canopy gaps would cause the greatest increases in seed bank abundance and diversity, leading to concomitant changes in seed community composition (**H3**). Based on studies showing negative impacts of deer herbivory on aboveground plant growth and reproduction, we expected deer to have a negative effect on seed bank abundance and diversity, particularly when combined with fire (**H4**). Lastly, when comparing the seed bank to extant vegetation, we expected the seed bank community to be most similar to extant vegetation in highly disturbed plots, as many seed banking species are favored by disturbance and may have been able to saturate the seed bank (**H5**).

Results

A total of 3,642 seeds germinated in our trials (across all trays there was an average of 309 germinants / m^2 and 12,331 germinants / m^3), representing at least 59 different taxa, with 38 forb species, 8 shrub species, 7 graminoid species, 5 tree species, and 1 vine species (Table 1). *Rubus* spp. accounted for 28% of total seeds, followed by

Betula spp. (9%), *Ageratina altissima* (6%), *Aralia spinosa* (5%), and *Robinia pseudoacacia* (5%). Among all germinating taxa, 9 species were non-native, accounting for 16% of all taxa (Table 1). However, non-native species' proportional abundance among all seeds was low, with non-native species representing 1% of total seeds germinated. The most common non-native species was *Stellaria media*, while one individual of *Rosa multiflora*, a non-native invasive species of concern in West Virginia (WV DNR), was found in the entire seed bank survey in an unburned plot with fencing and no canopy gap overhead.

Seed Abundance:

The effects of fire on total and life form-specific seed abundance varied depending on whether there was a canopy gap overhead or deer fencing. Fire under a closed canopy led to a 63% increase in mean seed abundance in comparison to unburned plots under a closed canopy ($z = 2.5$, $p = 0.07$), but when fire and canopy gaps were combined, there was a 205% increase in seed abundance in comparison to unburned plots with a closed canopy ($z = 6.8$, $p < 0.001$; Fig. 2; Table 2). This increase in seed abundance was driven by a 478% increase in forb seed abundance with fire and canopy gaps in comparison to unburned plots with a closed canopy ($z = 6.7$, $p < 0.001$; Fig. 2; Table 3). In addition, canopy gaps in burned areas decreased vine seed abundance by 93% in comparison to unburned plots with a canopy gap overhead ($z = -4.6$, $p < 0.001$), whereas canopy gaps in unburned plots had no influence on vine seed abundance. Lastly, fire alone decreased tree seed abundance by 33% in comparison to unburned plots ($\chi^2 = 5.3$, $df = 1$, $p = 0.02$).

The combination of fire and deer fencing had a strong influence on life-form specific seed abundance as well. Burned and fenced plots lead to 750% greater shrub seed abundance in comparison to unburned plots that are unfenced ($z = 6.1$, $p < 0.001$; Fig. 2). Fire and fencing had an interactive effect on graminoid abundance ($\chi^2 = 3.2$, $p = 0.07$), wherein fencing in burned plots decreased graminoid seed abundance by 62% in comparison to burned plots that were unfenced ($z = -2.2$, $p = 0.03$). There was a three-way interaction among fire, canopy gaps, and deer fencing on *Rubus* abundance, the most common germinant in our trials ($\chi^2 = 3.0$, df =1, $p = 0.08$). Burning only increased *Rubus* seed abundance with a canopy gap or fencing present. Fire, fencing, and canopy gaps together caused a 1432% increase in *Rubus* seed abundance in comparison to unburned plots with no fencing and a closed canopy ($z = 6.1$, $p < 0.0001$).

Seed Diversity:

We found that fire increased average seed species density from an average of 57.2 ± 2.7 species/m^2 to 68.8 ± 3.3 species/m^2 in comparison to unburned plots ($\chi^2 = 6.4$, df =1, $p = 0.01$; Table 2). Fencing decreased average seed bank Shannon diversity by 8% in comparison to unfenced plots ($F_{1, 58.1} = 9.1$, $p = 0.004$). Fire and no fencing (or presence of deer) increased forb seed species density to 39.2 ± 4.1 species / m^2 in comparison to 18.7 ± 3.1 forb species / m^2 in unburned plots with fencing ($z = - 4.2$, $p < 0.001$). Fire increased average forb seed Shannon diversity by 45% in comparison to unburned plots ($F_{1, 56.1} = 7.9$, $p = 0.003$). In contrast, fire led to a 40% decrease in average shrub seed Shannon diversity in comparison to unburned plots ($F_{1, 58.1} = 9.5$, $p = 0.007$).

However, the results from our linear models differed from rarefied and extrapolated Hill richness and diversity. When extrapolating seed species richness across all treatments in the iNEXT package, Hill richness was highly variable and there were no substantial differences between treatments (Fig. 3; Table S1). In contrast, Hill

Shannon diversity varied substantially depending on whether the plot had been burned. We found that fire with deer fencing and a canopy gap led to the lowest Hill Shannon diversity of all the treatments (6.7 species; 95% CI [6.0 - 7.5]; Fig. 3; Table S1). Fire with fencing and no canopy gap led to the second lowest Hill Shannon diversity of all treatments (9.1 species; 95% CI [7.9 - 10.3]; Fig. 2). In contrast, burned areas with a canopy gap and without fencing had the highest Hill Shannon diversity (18.1 species; 95% CI [16.4-19.7]; Fig. 3). Unburned plots with no fencing or gap overhead had roughly the same Hill Shannon diversity as the most disturbed plots (17.9 species; 95% CI [15.3 - 20.4]; Fig. 3), although there were no major differences in Hill Shannon diversity between any of the unburned treatments.

Seed Community Composition:

Despite having similar Hill Shannon diversities, burned plots with a canopy gap overhead and no fencing had very different seed species compositions in comparison to unburned plots with no fencing and no canopy gap ($F = 3.4$, $p = 0.004$; Fig. 4; Table S2). Further, burning led to seed communities that were significantly different from nearly all unburned plot seed communities ($F = 2.5$, $p < 0.001$; Fig. 4; Table S2). Burned plots with no gap overhead and no fencing had the most similar seed bank communities to most of the unburned treatments (Table S2).

Within burned treatments, plots with a canopy gap and no fencing had significantly different communities than all other burned treatments (Fig. 4; Table S2). Burned plots with no canopy gap and no fencing had different seed communities than burned plots with fencing and a canopy gap overhead ($F = 4.8$, $p = 0.006$). In contrast, burned plots with fencing and no canopy gap overhead had the most variable seed community, which overlapped with two other treatment’s seed communities (burned plots with fencing and a canopy gap; burned plots with no fencing and no canopy gap (Fig. 4; Table S2). The presence or absence of canopy gaps and fencing in unburned plots had no effect on seed bank communities and there were no differences in seed community amongst any of the unburned plots (F = 0.99, p = 0.5; Fig. 3; Table S2).

In addition, burned areas with canopy gaps and fencing had several indicator species, including *Rubus* (p = 0.003), *Phytolacca americana* (p = 0.02), *Sambucus* (p = 0.02), and *Aralia spinosa* (p = 0.05). In contrast, burned areas with canopy gaps and no deer fencing had *Ageratina altissma* (p = 0.001), *Carex* spp. (p=0.005), *Verbena urticifolia* (p = 0.03), *Viola pubescens* (p = 0.02), and *Solanum carolinense* (p = 0.1) as primary indicator species. *Viola rotundifolia* (p = 0.06) was the primary indicator for burned and fenced plots with no gaps. There were no indicator species for burned plots with no canopy gap and no fencing, whereas the indicator for unburned plots with a gap overhead and no fencing was *Sassafrass albidum* (p = 0.07).

When comparing 2013 seed communities to extant plant communities sampled in disturbance treatments over time (2000 [pre-treatment], 2001, 2002, 2006, 2013), burned plots with a canopy gap overhead and no fencing had extant plant and seed bank communities that differed at every measured time point (F = 19, p < 0.001; Fig. 5; Table S3). By 2013, burned plots with a canopy gap and no fencing maintained a unique seed bank in comparison to the extant understory, with 75% of the seed species not being found in the extant understory.

Discussion

Using a unique, long-term experiment that manipulated several historically important disturbances, we provide evidence of how disturbance interactions cause lasting imprints on the seed bank community. Specifically, interactions among understory fire, canopy gap creation, and continuous deer browsing left distinct disturbance legacies in the seed bank community over 13 years (Fig. 6). Despite the frequency and importance of these disturbances in forests broadly, this study is the first, to our knowledge, to test how these interacting disturbances can change the seed bank. This work on seed bank legacies provides new applied and theoretical insight towards how biodiversity and forest communities are maintained and develop in light of multiple disturbances. Further, these altered seed banks represent a critical reservoir of biodiversity that will influence community structure and reorganization following subsequent disturbances (32, 52).

Disturbance Effects on Seed Banks

Individual Disturbances

In partial agreement with our first hypothesis, only burning increased average seed bank species density, likely driven by a post-fire increase in the proportion of forb species. These increases in species density in the seed bank after a single fire were relatively small and similar to the changes in aboveground forb species density following prescribed fire found by Hutchinson et al. (2005) and Keyser et al. (2012) (53, 54). However, these results contrast Huebner et al. (2023) which found that fire prone regions in the Monongahela National Forest didn't increase seed bank richness, but rather, frequent historic burns increased seed bank Shannon diversity (55). These results also contrast with Shi et al.'s (2022) global meta-analysis of relationships between fire and seed bank diversity, which found either null or negative influences of fire on seed species richness (56). These differences in results are likely due to different sampling intensities and our sites only having a single fire that occurred in recent memory.

Canopy Gaps & Fencing

In contrast to our second hypothesis, we found no strong effects of combined canopy gaps and fencing in unburned areas on seed abundance, species density, richness, or diversity. This contrasts with the strong interaction these disturbances had on seed bank species occurrence probability found by Shinoda and Aksaka (2020) (45). That canopy gaps and deer exclusion, or lack thereof, had no influence on seed banks except in the presence of fire indicates the importance of burning in shaping fire-dependent plant communities. However, if deer densities were higher in our study site, it is possible that there would have been an interaction between fencing and canopy gaps in unburned plots, as several studies have found that intense browsing under an open canopy can create a growth bottleneck and altered plant communities (57–59).

Fire & Canopy Gaps

In agreement with our third hypothesis, we found that the combination of a prescribed fire and canopy gaps strongly increased seed abundance in comparison to plots that were only burned or only had a canopy gap overhead. In this scenario, fires cleared existing biomass, created microsite heterogeneity, and stimulated seed germination through heat and smoke, which allowed for a pulse of plant growth from the seed bank. These plants then grew into an environment with light levels that were approximately 260% higher than that of control plots (51). The abundant resources and associated heterogeneity within burned plots with a canopy gap then allowed for plants to invest in seed production within the growing season, thereby replenishing the seed bank (60–62).

Burning and canopy gaps also led to an increase in forb seed abundance and species density, similar to Hyatt (1999), who found forbs increased substantially with open canopies (63). Increased forb seed abundance parallels the increase in forb cover with fire and canopy gaps found by Royo et al. (2010) in this same experiment, which suggests that the forbs that initially germinated following these disturbances reached sexual maturity and established a long-term presence in the seed bank (51). It is also possible that combined fire and canopy gaps may be a way to increase forb growth and seed bank persistence, which is often desired within fire dependent systems (17, 64).

Fire & Fencing

In contrast to our fourth hypothesis, we found that fire and fencing decreased seed bank average Shannon diversity and Hill Shannon diversity due to a parallel increase in shrub seed abundance, while other seed life forms remained at low relative abundances. These shrubs were likely able to grow prolifically from the seed bank or as advanced regeneration after fire and could invest in reproduction without stress or death from deer herbivory (62). As a result, long-lived shrub seeds accumulated in the seed bank and made the relative proportions of seed life forms highly uneven, thereby decreasing average Shannon diversity and Hill Shannon diversity. These results provide nuance to the finding that high deer densities reduce seed abundance (49, 50, 65, 66), as we found that low and moderate deer browse in burned areas seemingly maintains higher seed biodiversity by creating a more heterogeneous environment that allows for more even relative abundances of seed banking species.

Fire, Canopy Gaps, & Fencing

We found that the combination of fire, canopy gaps, and low deer browsing pressure led to the most unique seed communities and the highest seed bank Hill Shannon diversity, similar to results found by Royo et al. (2010) within this same experiment (51). In contrast, fire, canopy gaps, and deer fencing led to the lowest Hill Shannon diversity, likely due to these treatment's particularly strong combined influence on *Rubus* seed abundance. We saw the highest *Rubus* seed abundance in areas that were burned and had both fencing and a canopy gap. Burning likely cleared most aboveground vegetation other than pole and mature trees, while canopy gaps provided abundant sunlight, leading to *Rubus* dominance in the understory. This *Rubus* dominated understory was then unmoderated by deer browsing and its highly palatable fruits dropped into the seed bank, thereby saturating the soil with *Rubus* seeds (37, 67, 68). *Rubus* seeds can survive belowground for decades and readily germinate with disturbance, making it very likely that this seed legacy will become apparent in the understory following the next fire or canopy gap creating event.

Shrubs are known to heavily compete with herbaceous species such as forbs and graminoids (69). The increase in Rubus seeds inside of fencing may explain why we see a concurrent decrease in graminoid and forb seed abundance, as dense and fast-growing Rubus would shade out shade intolerant herbaceous species before they could reproduce and drop seed. That we might expect entirely different seed legacies depending on the presence or absence of herbivores in a burned environment with a canopy gap provides critical insight for land managers battling woody encroachment and trying to maintain biodiversity in formerly fire prone ecosystems. The influence of herbivory on encroaching shrubs in the presence of burning and tree harvesting has been demonstrated in savanna ecosystems (70), but few have tested how these combined disturbance influence the subsequent seed bank. Our results suggest that the reintroduction of herbivores in open and burned ecosystems would reduce shrubs both aboveground and belowground.

Fire-Driven Plant Communities

Fire combined with canopy gaps and fencing treatments led to unique seed bank communities in comparison to unburned areas with fencing or a canopy gap treatment (Fig. 3). These results support Grubb (1988) who found that fire and seed communities are coupled and that different disturbance events lead to different long-term seed banks (71). In our study, fire was the strongest driver of community change, with added community differentiation when deer and canopy gap treatments were applied (Fig. 6). This result highlights the importance of fire in shaping plant diversity in formerly fire-prone systems, while added canopy gaps or deer exclusion can lead to substantial variation in seed bank community trajectories.

Several studies theorize that aboveground and belowground plant communities should homogenize and converge with increasing disturbance due to there being greater ruderal species survival (72, 73). In our study, seed communities in burned plots with a canopy gap and no fencing were substantially different from all extant communities at each time point (Table S3). This result does not support our fifth hypothesis and suggests that the seed bank consistently maintains a unique species assemblage and likely changes over time due to many seeds having relatively short longevity *in situ* (30, 74). Nevertheless, even in the plots with the most reintroduced disturbances, 75% of all species in the seed bank in burned plots with a canopy gap and no fencing were not found in the extant understory in 2013, highlighting how the seed bank maintains an important reservoir of biodiversity over time and in light of multiple disturbance.

Additionally, extant plant communities were compositionally variable in comparison to the seed bank, indicating that we likely under-sampled the seed bank and there is greater diversity to be found within seed banks than our sampling intensity indicates (73). Thus, we believe the forest seed bank represents an important reservoir of native plant biodiversity, especially considering that only 16% of taxa were non-native to North America and these taxa represented only 1% of total germinants.

Ruderal Species, Ecological Memory, and Future Considerations

The novelty of this seed bank study highlights our limited understanding of how multiple disturbances influence forest succession, reorganization, and future legacies. Research on how forests respond to multiple disturbances is often limited to studying how trees respond to the combined influences of high intensity disturbances, such as wildfires and stand-leveling windstorms (22). Often, many other layers of a forest are forgotten in relation to multiple disturbances, such as the understory or the seed bank (75–77). Further, studies on multiple disturbances tend to be theoretical or observational rather than experimental or do not adequately interpret results within the theoretical assumptions of disturbance ecology (2, 78). It is critical that we consider disturbances in tandem, especially since disturbances like understory fire and mid-sized canopy gaps were the most common pre-colonial forest disturbances throughout the eastern U.S. and are being broadly reintroduced (5, 79), while white-tailed deer are far above historic densities and are now a dominant contemporary disturbance with many indirect effects (16, 80).

In our experiment, reintroducing disturbances favored many ruderal species in the seed bank. Although many of these seed banking species would be considered “weeds” by land managers, ruderal species can play an important role in ecosystems

(81, 82). For instance, *Rubus*, the most important indicator species in our germination experiment and the only species to have a significant response to combined fire, fencing and canopy gaps, has been shown to reduce forest floor temperatures, hide tree seedlings from deer, and fix nutrients as biomass, which prevents nitrogen from leaching post-disturbance (37, 68). These ruderal species are eventually shaded-out by growing trees, so their survival strategy is to create dense seed banks that buffer them from year-to-year variability and can germinate after the next vegetation-clearing disturbance (83, 84).

There could also be generalizable patterns in how seed banks respond to certain combinations of disturbance. For instance, based on our results, we expect fire, canopy gaps, and herbivore exclusion to create a long-lasting *Rubus* seed legacy, especially considering that shrub seeds are often more persistent than extant understory plants (72). A shrub seed bank would then continually recur following infrequent understory fire and canopy gap creation in the absence of herbivores, thereby cementing a feedback loop of disturbance and the ecological memory of a shrub-dominated system in the seed bank (22). This ecological memory likely cannot be broken except through burning more frequently or low intensity deer browsing (4, 85). These disturbance legacies and ecological memories in the seed bank make sampling the forest seed bank a valuable exercise to determine which combination of disturbances to apply in a restoration or management setting, particularly as the climate changes and many seed banking species are favored by warmer conditions (86, 87).

The high numbers of disturbance-dependent species in the seed bank may also shed light on why many studies find a ‘seed bank bias,’ wherein forest seed bank communities have low compositional similarity to those of aboveground plants (88, 89).

Our study appears to represents what Larson and Suding (2022) define as a "parallel tracking" seed bank bias, wherein diversity increases in both the extant understory and seed bank across a gradient of increasing disturbance frequency despite there being compositional dissimilarity between both communities (89). The seed communities in our study are likely different from the extant understory because seeds of formerly living plants have slowly accumulated in the soil over 13 years and the ecosystem has not been burned or harvested recently. Compounding this seed bank bias, many studies under-sample the seed bank (73). These factors may then contribute to the assumption that seed banks are not a particularly important source of biodiversity or component of forest development (88, 89); however, our study provides evidence to the contrary.

Future studies that evaluate the influence of multiple disturbances on forest seed banks should make several considerations. First and foremost, our study highlights why researchers should think beyond how trees respond to multiple disturbances (77, 90). These fast-growing seed bank species can heavily influence forest succession and it can take decades for the effects of multiple reintroduced disturbances to become fully apparent amongst tree communities, making the understory and seed bank an excellent study system in a multi-disturbance scenario. Further, seed bank studies should measure several germinant functional traits rather than just species richness or diversity. This is one of the primary gaps in our understanding of seed communities and the traits of species may be important in determining seed bank legacies following multiple disturbances (89). Future work should also evaluate how seed banks vary with specific disturbance intensities, frequencies, and severities rather than just evaluating how seeds respond to disturbance binaries (78). From this, we may be able to better detect nonlinear responses and improve research integration into meta-analyses (91). For instance, ongoing restoration of the fire regime in our study may lead to even more substantial changes in the seed bank (55). Nevertheless, by taking an experimental and long-term approach to measuring how multiple disturbance influence the seed bank, we take a considerable step in our limited understanding how multiple drivers of change influence the forest seed bank, succession, and biodiversity.

Materials and Methods

Study Site

To test how the forest seed bank responds to multiple reintroduced disturbances, we experimentally manipulated prescribed fire, deer presence, and canopy gap creation in four replicate Appalachian hardwood stands in central West Virginia, USA (Fig. 1). We established this experiment in 2000 in the Western Allegheny Mountain ecological subsection using two stands in the Monongahela National Forest (39º06’ N, 79º43’ W) and two stands in the Fernow Experimental Forest (39º01’ N, 79º42’ W). Each stand was 60 to 90 years old and between 670 to 800 m in elevation. All stands were dominated by oak (*Quercus rubra* L., *Q. alba* L., & *Q. montana* L.) with associated maple (*Acer saccharum* Marsh., *A. rubrum* L.), cherry (*Prunus serotina* Ehrh.), beech (*Fagus grandifolia* Ehrh.) and birch (*Betula* spp.) (51) The herbaceous layer in this forest is highly diverse, with up to 461 plant species recorded (92). Mean annual max temperature is 15.1 °C, mean annual min temperature is 3 ºC, with an average of 122 cm of precipitation (93). For further details about the pre-existing manipulative experiment and aboveground plant sampling, please see Royo et al. (2010) (51).

Disturbance Treatments

Our experimental design was a split-plot factorial (Fig. 1), with each stand split in half and randomly assigned a burn treatment (burned or unburned). In each burned and unburned half stand, we established eight treatment plots (20 x 20 m, 400 m^2) for a total of 64 plots with either fencing (no deer), canopy gap, fencing + canopy gap, or no gap and no fence. Treatment plots were 20 m from one another, stand edges, and burn lines to avoid nonindependence and edge effects.

In May and June of 2000, we established 2-m high fencing around treatment plots to prevent deer entry. Deer densities in this location are between 4 and 7 deer/km^2, which are slightly higher than historical estimates, but low relative to most eastern forests (Horsley et al. 2003). We created all canopy gaps in June 2000 by girdling multiple canopy-dominant trees. By summer 2001, all trees in canopy gaps were standing dead, fallen, or near-dead. Canopy gaps were mid-sized (284 m^2 ± 16 SE) (94). We lit each understory fires between April 27 and May 1, 2001. Fire temperatures on the mineral soil surface were 245 ± 15.4 °C, while temperatures at 1 m from the ground were 91.9 ± 1.7 °C (51). Fires took place during the historic peak spring fire season (6, 13), during understory bud-break but prior to canopy bud-break. Historically, fire return intervals in this region are between 25 and 30 years (6, 95). Considering that there was a minimum of 20 m of space between burned and unburned regions, we assume that drifting smoke did not lead to a substantial germination event within unburned plots. Fire was only used once at each site.

Seed Sampling & Germination

We placed five permanent 1-m^2 sampling quadrats within each treatment plot. Within these quadrats, we measured forb, graminoid, shrub, and vine species abundances in 2000 (pre-treatment), 2001 (post-treatment), 2002, 2006, and 2013. One fire and canopy gap treatment plot could not be found in 2013, thus n = 63. We sampled soil in early June 2013 and germinated seeds using a standard seedling emergence methodology described below (96). Following the recommendations of Plue and Hermy (2012) (97), we sampled ca. 3% of the quadrat surface area to represent seed bank abundance and diversity. We collected seed bank samples at each corner of the five quadrats using a 5-cm long section of a 10-cm PVC pipe ($78.5\ cm^2 \times 4 = 314\ cm^2$ soil sampled per quadrat). All 20 soil cores per treatment plot were then pooled, mixed, and subsampled for use in emergence trials. Three subsamples were taken from each of the 63 treatment plot's pooled soils and placed in separate $625\ cm^2$ square trays in a greenhouse ($625\ cm^2 \times 3 = 1875\ cm^2$ soil per plot), with 2.5 cm of subsampled soil placed on top of 2 cm of sterile sand in each tray. We watered all 189 trays (63 treatment plots x 3 subsamples) daily and occasionally rotated the trays to minimize any greenhouse positional effects (e.g., light, temperature). All germinants were identified to species or genera depending on life form, counted, and removed from the tray. After 5 months, we subjected trays to a 90-day, 5° C cold stratification period, after which they were returned to the greenhouse for another germination phase.

Statistical Analysis

For operational purposes we define seed species density as the total number of species found across the three trays representing a single treatment plot. Seed species density is different from seed richness because it is not rarefied and does not represent an asymptotic estimate (98). Similarly, seed abundance is defined as the total number of germinants found across the three trays per plot.

We conducted analyses using R software (R v4.3.1). We tested differences in average species density and abundance using a generalized linear mixed effects model

(GLMM) with either Poisson or quasi-Poisson distributions in the ‘glmmTMB’ package (99). We calculated average seed Shannon diversity within the ‘vegan’ package based on species abundances per plot (100) and modeled responses using a linear mixed effects model in the ‘lme4’ package (101). The use of average Shannon diversity and species density in our linear models allowed us to more easily explore two-way interactions between treatments. We also calculated Hill richness and Hill Shannon diversity based on total seed abundance using the ‘iNEXT’ package, which rarefies and extrapolates diversity metrics (102). Hill diversity metrics provide a host of benefits, such as an asymptotic estimate for diversity comparisons and providing additional clarity regarding our diversity inferences. To explore life form-specific seed diversity metrics, we subset the data according to life form (forb, graminoid, shrub, tree, vine) and modeled life form abundance, species density, and Shannon diversity response to treatments. Graminoid Shannon diversity was modeled using a GLMM with a Tweedie distribution due to the data being zero-inflated. Since *Rubus* is a critical species occupying the seed bank, particularly in light of disturbances, we tested how total *Rubus* seed abundance varied with disturbance treatments using a GLMM due to this species being extremely common in the seed bank and highly influential in successional dynamics. We constructed different models with individual disturbances and their interactions as fixed effects to determine best fit (Fire, Fence, Gap, Fire x Fence, Fire x Gap, Gap x Fence, Fire x Gap x Fence). We compared models using AICc (103). We report the results from models with the lowest AICc. For our random effects, treatment plot was nested within experimental site. If models failed to converge, we only used experimental site as a random effect. All model assumptions were tested using the ‘DHARMa’ package (104), while post-hoc tests were done with the ‘emmeans’ package using a Bonferroni correction (105).

We tested differences in community composition between treatments using seed species abundance data and the ‘adonis2’ PERMANOVA function, while checking community dispersion with the ‘betadisper’ function in order to meet the assumptions of PERMANOVA (100). Data was not transformed. We visualized differences among communities using 3-dimensional NMDS to keep stress below 0.20. We identified indicator species using vegan’s ‘multipatt’ function. When comparing extant plants and the seed bank at various time points, certain species were concatenated by genera as they could not be identified to species immediately after germination in 2013 (*Carex*, *Galium*, *Rubus*, *Solidago*, & *Viola*). We removed species that did not occur in at least 3 plots for multivariate analysis. When comparing the seed bank and annual plant cover, all data were converted to presence-absence format prior to analysis. Similar to Plue et al. (2021) (106) we used Raup-Crick similarity within PERMANOVA to test for community differences between 2013 seeds and extant plants at each time point.

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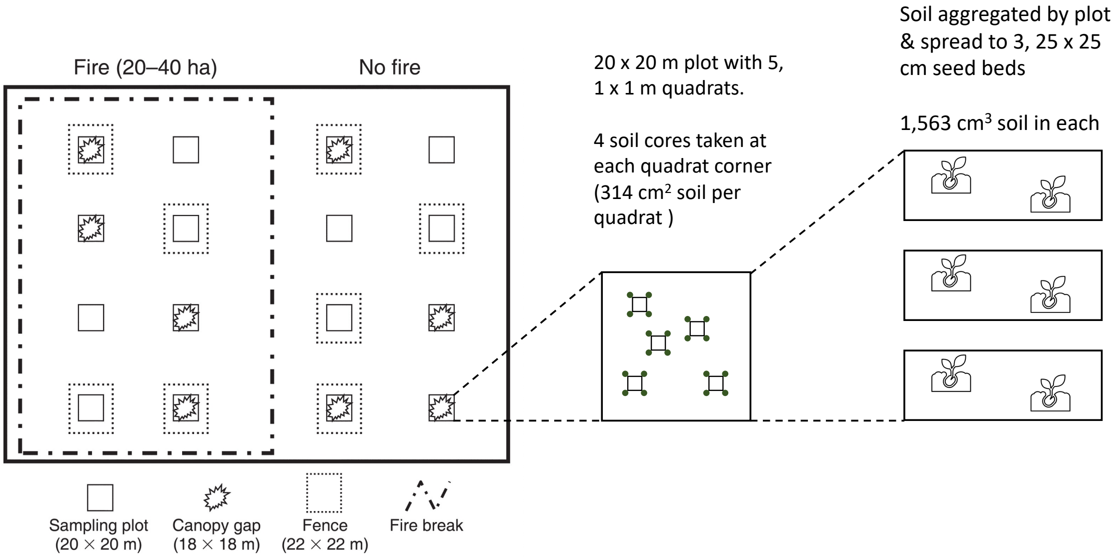


Figure 1. Diagram showing the organization of our replicated, factorial multi-disturbance study. Four stands were split into burned and unburned halves. Within each half, various fencing and canopy gap treatments were applied in 20x20 m plots. With each of these plots, soil was collected and aggregated. This soil was then split into 3 seed beds and placed in a greenhouse for germination trials. Figure adapted from Thomas Van Gundy et al. 2014

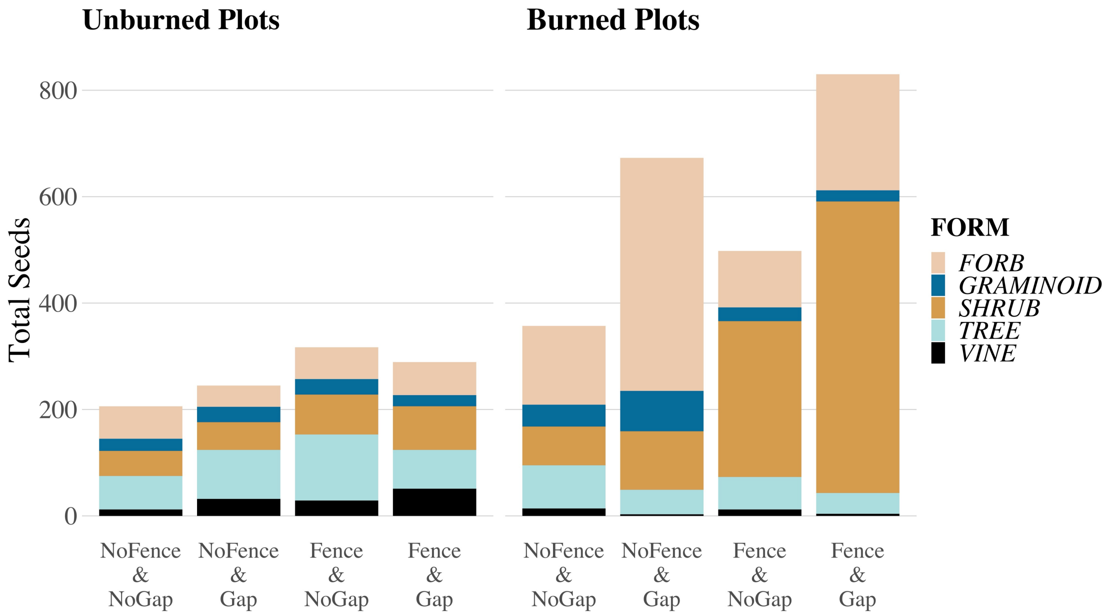


Figure 2. Bar charts that represent the relative proportion of seeds for each plant life form in disturbance treatments.

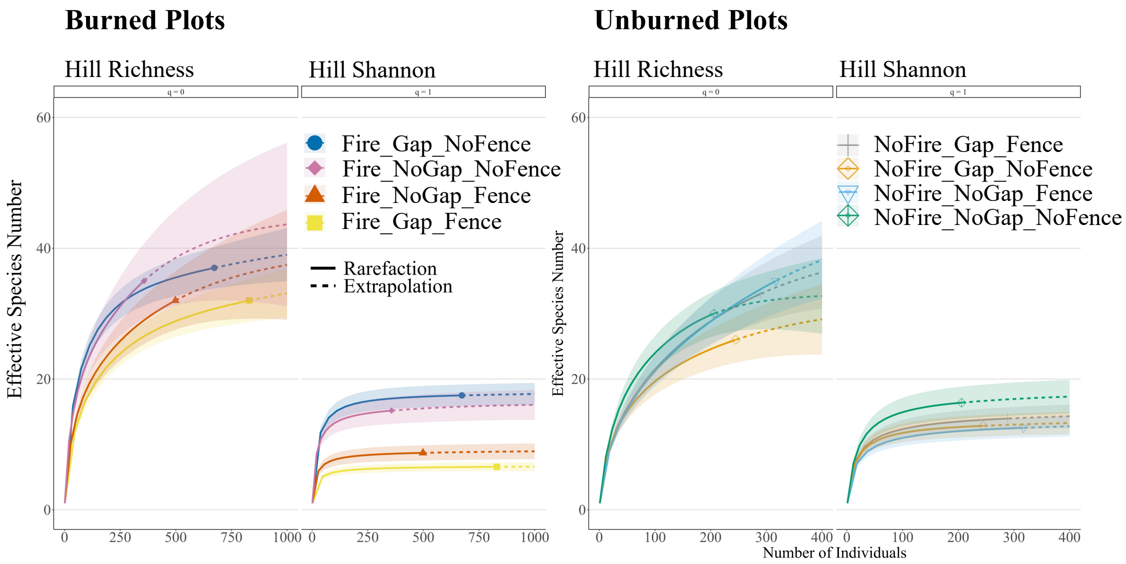


Figure 3. Seed bank Hill richness and Hill Shannon diversity by treatment. Shaded regions represent 95% confidence intervals. Rarefaction and extrapolation were calculated following Chao et al. (2014) (107).

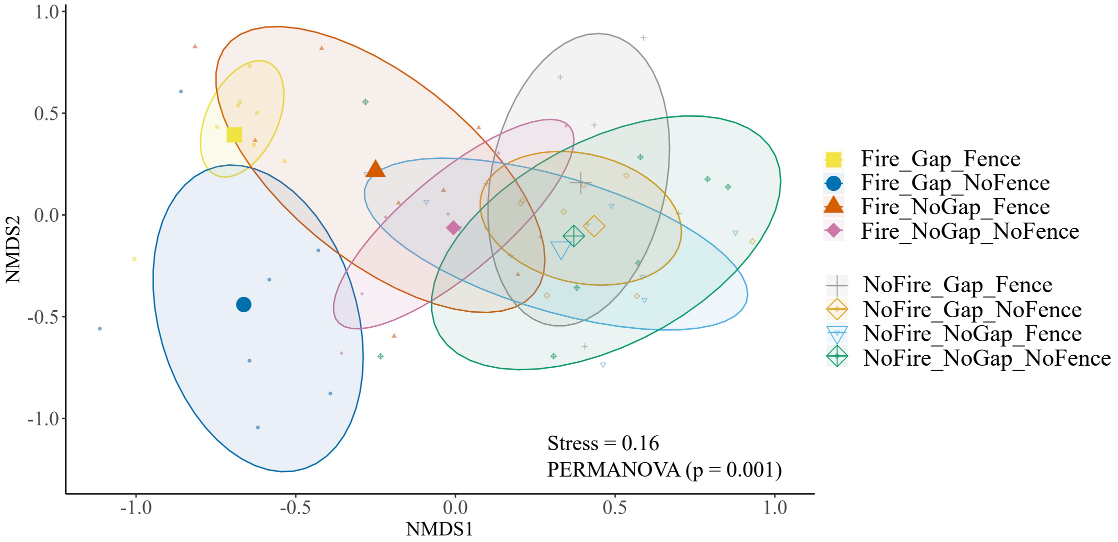


Figure 4. NMDS showing seed community differences amongst burned and unburned disturbance treatments.

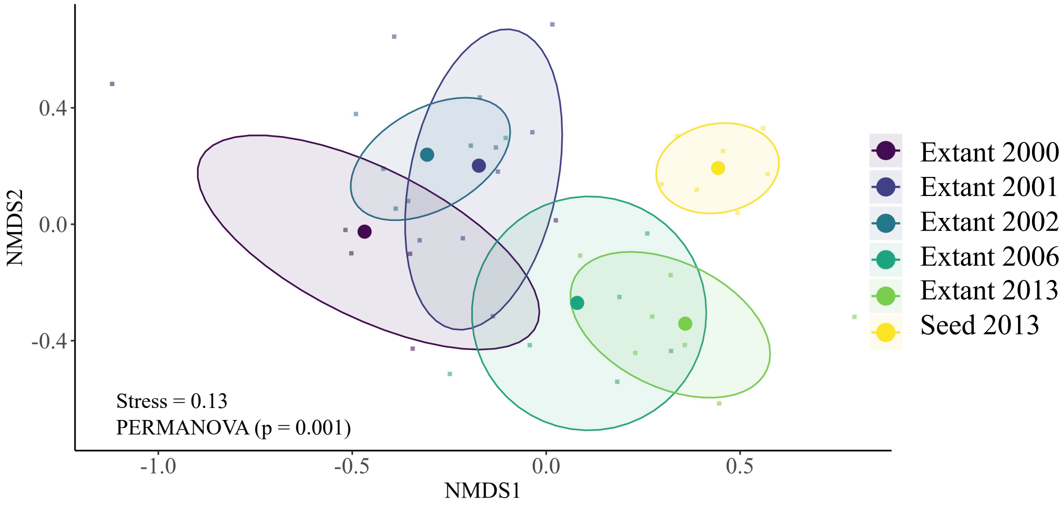


Figure 5. NMDS showing differences within burned plots with a canopy gap and no fencing amongst seed communities in 2013 (yellow) and extant plant communities sampled in each treatment in 2000 (pre-treatment), 2001, 2002, 2006, and 2013.

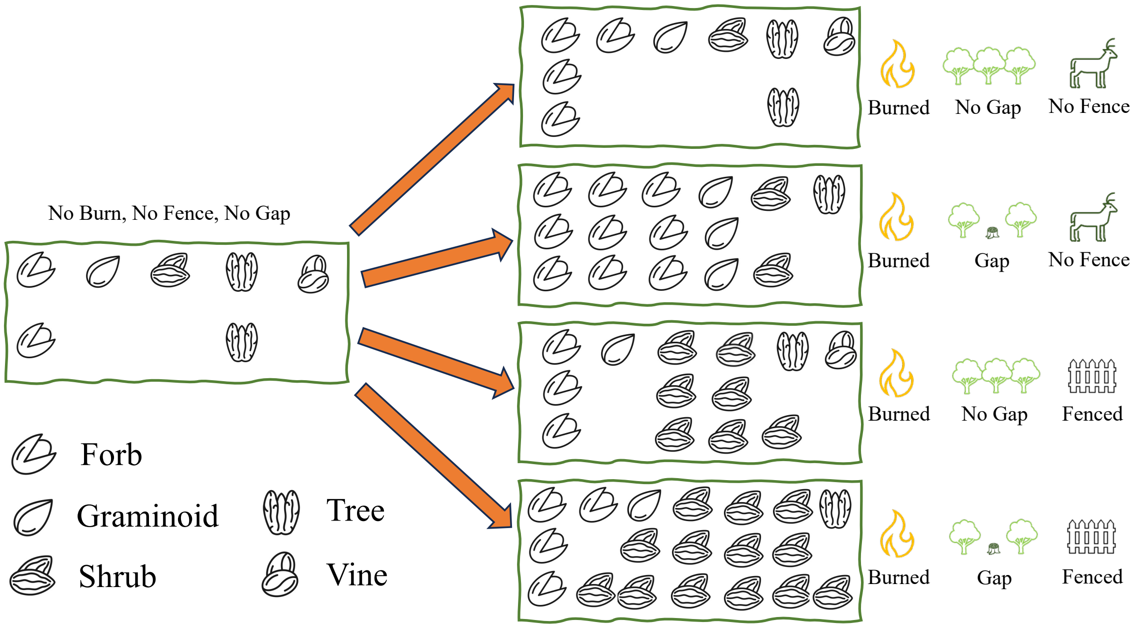


Figure 6. Schematic representing disturbance pathways to different seed bank communities found in our results. Burning with a closed canopy and no fencing led to a slight increase in forb seed abundance, burning with no fencing and a canopy gap led to a substantial increase in forb and graminoid abundance. In contrast, burning and fencing led to a substantial increase in shrub seed abundance, particularly with a canopy gap overhead.

Table 1. All seed bank survey species, their corresponding germination totals, and whether the species is native to North America.

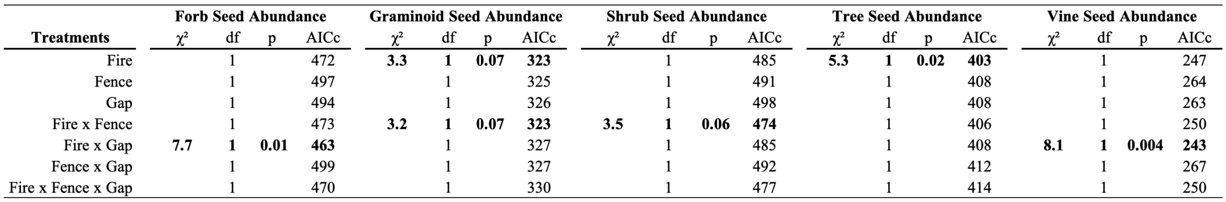
Species	Total Germinants	Native to North America?
Forbs		
Ageratina altissima	216	Yes
Phytolacca americana	133	Yes
Erechtites hieraciifolius	111	Yes
Viola blanda	111	Yes
Dalibarda repens	68	Yes
Saxifraga spp.	51	Yes
Pilea pumila	50	Yes
Viola macloskeyi	44	Yes
Viola spp.	42	Yes
Viola canadensis	39	Yes
Potentilla canadensis	26	Yes
Stellaria media	25	No
Verbena urticifolia	24	Yes
Viola rotundifolia	23	Yes
Galium spp.	23	Yes
Packera aurea	20	Yes
Viola sororia	18	Yes
Veronica officinalis	17	No
Verbascum thapsus	17	No
Eurybia divaricata	16	Yes
Viola pubescens	11	Yes
Eupatorium serotinum	10	Yes
Hypericum pseudomaculatum	6	Yes
Mitchella repens	6	Yes
Prenanthes trifoliata	3	Yes
Apocynum spp.	2	Yes
Boehmeria cylindrica	2	Yes
Gaultheria procumbens	2	Yes
Juncus effusus	2	Yes
Plantago lanceolata	2	No

Polygonum persicaria	2	No
Solanum carolinense	2	Yes
Trillium spp.	2	Yes
Lobelia spicata	1	Yes
Medeola virginiana	1	Yes
Solanum nigrum	1	No
Uvularia sessilifolia	1	No
Graminoids		
Carex spp.	178	Yes
Dichanthelium clandestinum	45	Yes
Luzula multiflora	25	Yes
Sisyrinchium spp.	9	Yes
Agrostis perennans	2	Yes
Andropogon spp.	1	Yes
Shrubs		
Rubus spp.	1035	Yes
Aralia spinosa	199	Yes
Sassafras albidum	17	Yes
Sambucus spp.	16	Yes
Berberis thunbergii	6	No
Rhus typhina	6	Yes
Rosa multiflora	1	No
Trees		
Betula spp.	337	Yes
Robinia pseudoacacia	199	Yes
Liriodendron tulipifera	26	Yes
Acer rubrum	15	Yes
Acer pensylvanicum	2	Yes
Vines		
Vitis aestivalis	157	Yes

Table 2. Seed species density, average Shannon diversity (H'), and abundance response to individual disturbance and their combinations. Each variable corresponds to a single model.

	Seed Species Density				**Seed H'**				**Seed Abundance**			
Treatments	χ^2	df	p	AICc	F	df	p	AICc	χ^2	df	p	AICc
Fire	**6.4**	**1**	**0.01**	**329**		1, 58		-10		1		603
Fence		1		334	**9.1**	**1, 58**	**0.004**	**-18**		1		621
Gap		1		334		1, 58		-10		1		615
Fire x Fence		1		332		1, 56		-9		1		605
Fire x Gap		1		332		1, 56		1	**4.9**	**1**	**0.03**	**591**
Fence x Gap		1		337		1, 56		-8		1		618
Fire x Fence x Gap		1		339		1, 52		10.6		1		597

Table 3. Forb, graminoid, shrub, tree, and vine seed abundance response to individual and combined disturbance treatments. Each variable corresponds to a single model.



	Forb Seed Abundance				Graminoid Seed Abundance				Shrub Seed Abundance				Tree Seed Abundance				Vine Seed Abundance			
Treatments	χ^2	df	p	AICc	χ^2	df	p	AICc	χ^2	df	p	AICc	χ^2	df	p	AICc	χ^2	df	p	AICc
Fire		1		472	**3.3**	**1**	**0.07**	**323**		1		485	**5.3**	**1**	**0.02**	**403**		1		247
Fence		1		497		1		325		1		491		1		408		1		264
Gap		1		494		1		326		1		498		1		408		1		263
Fire x Fence		1		473	**3.2**	**1**	**0.07**	**323**	**3.5**	**1**	**0.06**	**474**		1		406		1		250
Fire x Gap	**7.7**	**1**	**0.01**	**463**		1		327		1		485		1		408	**8.1**	**1**	**0.004**	**243**
Fence x Gap		1		499		1		327		1		492		1		412		1		267
Fire x Fence x Gap		1		470		1		330		1		477		1		414		1		250