Multiple Disturbances and the Turbulent Forest

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Mary and Jack Powers,

Loving grandparents

Abstract

I have always been drawn to change, whether I liked it or not. As a kid, I was obsessed with dinosaurs and their extinction, all while having to move constantly as a Navy brat. As an adult, I am devoted to understanding how and why systems change, likely inspired by the unending change of my childhood. However, as I matured, I realized that change is almost never spurred by a single event, but rather a multitude of shocks to the system. This dissertation focuses on the complexity of multiple ecological disturbances and highlights their importance in the world. Using several multi-disturbance experiments, I explore a wide variety of disturbance interactions in the temperate deciduous forest. The first chapter of this dissertation focuses on how deer and canopy gaps influence invasive earthworms, shedding new light on how aboveground events can change belowground communities. The second chapter builds upon the first and tests how combined deer and canopy gaps influence understory regeneration over 15 years, with some reference to invasive earthworms. Lastly, the third chapter explores how combined fire, deer, and canopy gaps change the seed bank over 13 years. Each of the aforementioned disturbances are common and influential in eastern forests, although they are not often studied together. In each chapter we find that community responses vary depending on the disturbances in question. This dissertation is meant to highlight how little we know about the many ways in which multiple disturbances change ecosystems and how critical it is that we start to study these complex drivers of change, particularly as the climate warms and disturbances become more frequent on the landscape.

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Introduction

Humans are particularly good at thinking about one thing at a time. Thus, we gravitate towards binaries, as they simplify and reduce complexity. Ecologists are no different: The experimental underpinnings of our field come from a binary perspective. Did x increase y? Did an organism respond to an experimental treatment or didn't it? How does an unburned forest compare to a burned forest? And although this is a valid and informative way to approach the natural world, it can force us into a trap, wherein we ignore the complexity and nuance that actually occurs on the landscape.

The field of disturbance ecology is particularly influenced by the binary perspective. We compare areas that are disturbed and undisturbed, using these categorical variables to differentiate how communities respond. Often, we do not consider any other disturbances that may be co-occurring on the landscape and whether these disturbances interact. For instance, fire and deer can have an interactive effect on ecosystems (Although generally not at the exact same time). Rather, the legacies of disturbance interact and can lead to drastically altered community outcomes. A fire can rip through a stand (disturbance), leading to a pulse of vegetation (legacy), which attracts large herbivores (disturbance), and these herbivores could consume so many plants they leave behind an altered community (legacy). Hypothetically, after this fire, the plants could regenerate with such strength that the effects of herbivores are negated. Or, the sheer amount of herbivores eating plants could drastically change which plant species can survive post-fire, leading to a different ecological trajectory. Point being, each of these disturbances have different intensities and durations, all of which mediate the resulting legacy and the degree of interactive effects. The nuance and complexity in these scenarios seems endless.

However, despite these complexities, multiple-disturbance ecology is making headway on the many ways in which disturbances might interact. This dissertation uses several large experiments throughout the eastern United States to untangle several of the most important drivers of change in temperate forests. Namely, I investigate combinations of treefall and resulting canopy gap creation, low intensity fire, deer herbivory, and earthworm invasion. Each of these disturbances are highly influential in forest development and they all interact in some way on the landscape. Thus, each of these disturbance combinations could lead to new and different community outcomes than their individual counterparts alone. And although we cannot test all the ways in which these disturbances interact, this dissertation provides an important platform for thinking about multiple disturbance within a relatively nascent field and highlights their many management implications.

One of the main themes of this work is that different disturbance combinations can lead to different community outcomes. In Chapter 1, we found that deer and canopy gaps can lead to dramatic changes in earthworm invasion across two separate experiments in northern Wisconsin. We generally found the highest earthworm populations in areas with deer and closed canopies overhead. In Chapter 2, we used the same experiment as in Chapter 1 to test how deer, canopy gaps, and ruderal vegetation (*Rubus* and *C. pensylvanica*) influence woody plant regeneration and community composition over 15 years. Sapling density was substantially higher in areas with deer fencing and a gap overhead (which was also the least favorable location for invasive earthworms in Chapter 1) and reduced the dominance of ruderal shrubs and sedge. Lastly, in Chapter 3, we used an experiment in West Virginia to test how understory fire, canopy gaps, and deer exclosures can change the forest seed bank after 13 years. We found that fire combined with gaps and fencing led to radically different seed bank communities than any individual disturbance.

Although this work highlights the many unique and unexpected ways in which multiple disturbances can influence a landscape, multi-disturbance ecology is in its infancy and will be critically important as disturbances become more frequent with a changing climate. Thus, I have several recommendations for ecologists that are considering an experiment that tests for the effects of multiple disturbances. First and foremost, record and publish each disturbance's intensity, duration, and spatial scale as these quantitative variables are likely playing a substantial role in mediating how a community responds to a disturbance. Fires burn at different intensities, deer populations and browse intensity vary, and winds blow at different speeds. Each of these factors can lead to different ecological outcomes, especially when disturbances interact on the landscape. In addition, when these disturbance metrics are measured and quantified, it allows for more direct comparisons between disturbance events in different ecosystems.

In addition, it is critical that we consider and test how multiple disturbances influence multiple strata of an ecosystem. In forests, we tend to focus on how trees respond to disturbance. However, the understory and midstory layer of shrubs and herbs are arguably more vulnerable to disturbance and just as important for forest development and function. Soil micro and macro-faunal communities represent another highly vulnerable and influential component of ecosystems that likely change depending on the combination of disturbances in question. However, each of these ecological groups have been largely forgotten when testing the effects of multiple disturbances.

Lastly, our field should start to give more consideration to the long-term effects of low and moderate intensity disturbances. In addition to humans preferring simple binaries, we also have relatively short attention spans. Thus, much of our contemporary multi-disturbance theory is constructed from only a few years of data using disturbance events that are high intensity and disrupt a large spatial extent. Although these large, infrequent disturbances are highly influential, it does not negate that low and moderate intensity disturbances are far more frequent and as influential in ecosystem development. Yet, we do not have a good understanding of how these low and moderate intensity disturbance interactions can influence ecosystems in the long term. This point is particularly important as we consider how many disturbances are expected to become more frequent with a changing climate.

Chapter 1:

Linked disturbance in the temperate forest: Earthworms, deer, and canopy gaps[1]

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Summary

Despite the large body of theory concerning multiple disturbances, there have been relatively few attempts to test the theoretical assumptions of how and if disturbances interact. Of particular importance is whether disturbance events are linked, as this can influence the probability and intensity of ecological change. Disturbances are linked when one disturbance event increases or decreases the likelihood or extent of another. To this end, we used two long-term, multi-disturbance experiments in northern Wisconsin to determine whether earthworm invasion is linked to canopy gap creation and white-tailed deer browsing. These three disturbances are common and influential within North American temperate forests, making any interactions among them particularly important to understand. We expected both deer and canopy gaps to favor invasive earthworms, particularly species that live close to or on the soil surface. However, we found only partial support of our hypotheses, as both deer exclosures and canopy gaps decreased earthworms in each experiment. Further, earthworm density increased the most over time in areas far from the gap center and in areas with deer present. Deer exclosures primarily decreased *Aporrectodea* and *Lumbricus* species, while gaps decreased *Dendrobaena* and *Lumbricus* species. Our findings show that earthworm invasion is linked to deer presence and gap creating disturbances, which provides new insight towards multiple disturbance theory, aboveground-belowground dynamics, and temperate forest management.

1.1: Introduction

Our understanding of terrestrial disturbances is largely derived from researching individual phenomena (Turner 2010). Although single disturbance events can significantly impact ecosystems, their intensity may be dependent on the presence of other disturbances on the landscape (Foster et al. 2016; Johnstone et al. 2016). Disturbance events can be linked and increase or decrease the likelihood, intensity, or spatial extent of another (Buma 2015), such as blowdowns increasing wildfire intensity (Cannon et al. 2017). These linked disturbances can then compound, allowing an ecosystem little time for recovery between events and potentially sending it on a new developmental trajectory (Paine et al. 1998; Burton et al. 2020). As climate change increases disturbance frequency and duration, these interactions are expected to become more prevalent and influential in socio-ecological systems (Dale et al. 2001; Frelich 2002).

However, despite a well-established theoretical understanding of multiple disturbances and their potential consequences, there has been insufficient effort devoted to testing disturbance interactions relative to the effects of individual events (Graham et al. 2021). To this end, North American temperate forests are a relevant system to investigate disturbance interactions, given their ecological and functional importance (Landuyt et al. 2019). Three influential temperate forest disturbances are canopy gaps from treefall, white-tailed deer (*Odocoileus virginianus*) browsing, and European earthworm invasions (Frelich 2002; Bohlen et al. 2004b; Hanberry & Faison 2023). Each disturbance has unique and relatively well-understood individual effects on vegetation, but their long-term influence on one another is largely unknown, leaving a gap in our understanding of how common disturbances interact (Côté et al. 2004; Bohlen et al. 2004b; Muscolo et al. 2014).

Canopy gaps can be created by windstorms, diseases, or tree harvest and often spur plant growth by increasing light availability in the understory. Gaps can also alter understory microenvironments, favoring certain plant species depending on location within or around the gap (Kern et al. 2013; VanderMolen et al. 2021). Canopy gap induced understory plant growth can then attract deer and increase browse pressure (Forrester et al. 2014). Over time, high browsing can transform the understory community of herbs and saplings, eventually leading to changes in succession and forest structure (Royo & Carson 2006; Royo & Carson 2022). Deer also indirectly change forest soil properties (Rooney & Waller 2001; Sabo et al. 2017), which can affect soil-dwelling fauna like earthworms.

Earthworms have long been recognized for their ability to transform soils (Darwin 1881). When introduced to formerly glaciated and earthworm-free landscapes in the Midwest U.S. earthworms function as a disturbance, causing significant losses in native plant biodiversity through soil mixing, consumption of seeds and litter, and alteration of soil chemical characteristics (Hale et al. 2006; Frelich et al. 2019). Earthworm impacts depend on the genera in question, as certain species occupy different soil niche spaces and can serve as an indicator of how an ecosystem is changing (Bottinelli et al. 2020). *Dendrobaena* species occupy the litter layer and do not impact soil or plant species (Hale et al. 2005b). *Aporrectodea* species occupy the first 15 cm of soil, are sensitive to soil chemical alterations, and can cause long-lasting changes in soil microbial communities (Bart et al. 2019), while *Lumbricus* species occupy multiple soil niche-spaces and can change soil faunal communities, nutrient levels, and seed communities (Cassin & Kotanen 2016).

There is limited and variable evidence regarding linkages among canopy gaps, deer, and invasive earthworms, despite each of their respective influences on the forest. Some studies link increased earthworm populations to deer presence, but this phenomenon has only been found in a handful of locations in the eastern United States (Davalos et al. 2015c; Cope & Burns 2019; Mahon & Crist 2019). Some hypothesize that deer create favorable soil environments for earthworms through increased soil heterogeneity from trampling or greater nutrient availability from deer waste (Davalos et al. 2015c; Cope & Burns 2019). Others have found no relationship between deer and earthworms (Shelton et al. 2014; Dobson & Blossey 2015). Thus, no conclusions can be made regarding this disturbance link's potential mechanisms or generalizability. Research on connections between canopy gaps and invasive earthworms is even more limited. Nachtergale et al. (2002) found that single-tree gaps decrease earthworm biomass through post-harvest soil disturbance, whereas Ganault et al. (2021) found that more open canopies may favor earthworms through abiotic factors.

To investigate whether influential temperate disturbances are linked, we tested how earthworm invasion is influenced by canopy gaps and deer in two long-term experiments in northern Wisconsin, USA. These experiments are unique in their factorial manipulation of dozens of large (>20-m) canopy gaps and deer exclosures over a decade and across a wide spatial scale. Since earthworms were already established at each experimental site, we could assess earthworm community variation across individual and combined gap creation and deer exclusion treatments. One site was surveyed for earthworms prior to experimental treatments, providing a baseline for us to test if earthworm density varied with disturbance treatments over 13 years. We hypothesized that increased earthworm biomass and density would be linked with deer presence and canopy gaps (H1 & H2) and that both disturbances would synergistically increase the biomass and density of all earthworm species (H3). In this scenario, increased vegetative biomass under a canopy gap would increase the number of deer, which would then concomitantly increase earthworm populations through more favorable soil environments. Deer and canopy gaps likely affect the upper layers of soil, therefore we also expected earthworm species that reside on or near the surface to be particularly influenced by these disturbances.

1.2: Methods

Site Description

This study used two long-term experiments in Wisconsin, USA. The first experiment is the Managed Old Growth Silviculture Study (MOSS), which was established in 2007 and has three, ≈200 ha replicate sites located in the Northern Highlands American Legion State Forest (NH), the Flambeau River State Forest (FL), and the Chequamegon-Nicolet National Forest’s Argonne Experimental Forest (AR) (Fig. 1.1). The second experiment, ‘The Flambeau Experiment’ (FE), was established in 2006 and is in the northern portion of the Flambeau River State Forest (Fig. 1.1). Stands at each site are representative of a maturing Great Lakes northern hardwood forest and are dominated by even-aged, second-growth sugar maple (*Acer saccharum*) with subdominant assemblages of intermediate shade-tolerant species such as white ash

(Fraxinus americana), yellow birch (*Betula alleghaniensis*), and red oak (*Quercus rubra*) (Forrester et al. 2014; Fassnacht & Steele 2016). Soils differed among sites, with both Flambeau locations having silt loam over sandy loam soil, AR having sandy loam with high stone proportion, and NH having sandy loam soil over stratified outwash sand (Fassnacht et al. 2013). All sites in this study were separated by a maximum of 140 km across roughly the same latitude, with mean temperatures of ≈4.2° C and mean precipitation of ≈88.6 cm (Climate Normals 2006–2020; NOAA NCEI - Station USC00475516).

Managed Old Growth Silviculture Study (MOSS) – The MOSS experiment has several harvest-created canopy gap treatments across the three replicate sites that were established in the winter of 2007 and 2008 (for further detail regarding establishment see Fassnacht et al. 2013). We used the "Large Gaps" treatment, which consists of 16, 24-m diameter canopy gaps within a 48-ha stand at each site. One canopy gap could not be found at AR, leading to a total gap count of 47 across the three sites. Each gap contains a deer exclosure in one of four locations along a north-south gradient: the north transitional edge, the northern gap, the center gap, or the south transitional edge (Fig. 1.1). All exclosures were established in summer 2007. Each fence is 5.25 x 5.25m wide and 1.5m tall, except for the north gap locations, which are longer (3.75 x 7.5m) to capture potential plant variation in this location of the canopy opening. Hereafter we also refer to exclosure treatments as "fenced" or "unfenced," with the latter signifying deer presence.

We sampled earthworms in August and September of 2019 in MOSS. In sites with a gap overhead, we placed one circular earthworm plot (0.07 m^2) in the center of exclosures and a paired earthworm plot 4.5 m away from the western or eastern edge of fencing (n = 47; Fig. 1.1). We sampled inside and outside of 19 fences without a gap overhead across the three site's controls (AR [n=4]; FL [n = 4]; NH [n=11]). Prior to sampling, all litter in a plot was collected and searched for earthworms. Soil moisture and temperature were measured adjacent to the plot. We then poured a liquid mustard solution on the soil (40 g of powdered mustard to 3.8 L of water) and collected all earthworms that rose to the surface over 15 minutes (Hale et al. 2006). All earthworms associated with a single gap and exclosure were collected on the same day. Each earthworm that emerged from the soil was placed in 70% isopropyl alcohol for preservation. Isopropyl alcohol was replaced every three weeks for two months until earthworms were preserved. One earthworm sample within the control sites was lost due to the test tube cracking. Each earthworm was identified to species or genus and measured lengthwise. These lengths were input into Hale et al. (2004)'s allometric equations to estimate ash-free dry biomass (afdb).

The Flambeau Experiment – The Flambeau Experiment (FE) is another long-term study that tests how forest structure influences fine-scale vegetation and soil processes (Forrester et al. 2012). The FE has 35, 80 x 80 m plots with seven fully replicated treatments. We used the canopy gap (n =5) and fenced canopy gap (n = 5) treatment's 22-m diameter gaps, which is similar to the gap sizes in the MOSS experiment (Fig. 1.1). Gaps were established in January 2007. Each gap is surrounded by an uncut transitional edge that is the same radius as the gap itself (11-m) and an additional 5-m wide buffer area, which we used as a control. The fenced canopy gap plots are surrounded by an 80 x 80 m deer exclosure (2.1-m tall), which were established in fall 2007.

We sampled earthworms prior to gap and fencing treatments in late spring 2006, then sampled earthworms again 13 years after treatment in the fall of 2019. Earthworms were sampled along a north-south transect across each 22-m canopy gap, from the north buffer to the south transitional edge (Fig. 1.1; n = 5 at each gap location). The 2019 earthworm sampling locations were approximately where earthworms were sampled in 2006. The only difference between sampling periods is that the 2019 north gap location was 1.5 m north of the 2006 north gap location. Earthworms were extracted, measured, and identified using the same methodologies as the MOSS experiment. Weather conditions between spring 2006 and fall 2019 were also consistent, with neither period experiencing drought, which reduces earthworm biomass (US Drought Monitor; Hale et al. 2006).

Statistical Methods

Across both MOSS and FE, earthworm biomass and density were used as the primary response variables in our study. Similar to Hale et al. (2005b) and Davalos et al. (2015c), species were binned according to the most common genera - *Dendrobaena*, *Aporrectodea*, and *Lumbricus*. Earthworms were grouped according to genera rather than functional group due to the current debate concerning this categorization framework (Chang et al. 2016; Bottinelli et al. 2020). Several *Dendrodrilus rubidus* specimens were found and binned with *Dendrobaena*, as both species are very similar in size and feeding patterns (Hale et al. 2005b). The genus *Octolasion* was present at some sites, but we did not have the statistical power to test if this genus varied with disturbance treatments. We also tested whether earthworm communities in 2019 varied with treatment using the ‘adonis2’ and ‘betadisp’ functions in the vegan package (Oksanen et al. 2022).

In MOSS, total earthworm biomass, total *Lumbricus* biomass, and total *Dendrobaena* biomass were either cube or square root transformed to meet the assumptions of a linear mixed effects model in the ‘lme4’ package (Bates et al. 2015). Our fixed effects were deer exclosures, gap presence, and their interaction. Our random effects structure for all MOSS models was location within a gap nested within gap number nested within site. Soil moisture and soil temperature were initially incorporated into models, but each of these variables had little influence on earthworm biomass and density. *Aporrectodea* biomass was analyzed using generalized linear mixed effects models (GLMM) in the ‘glmmTMB’ R package with a tweedie error distribution, which allows for zero-inflated, continuous data (glmmTMB; Brooks et al. 2017). Total and genera-specific earthworm densities were also tested using a GLMM with either a Poisson or negative binomial error distribution using the same model structure as above.

For FE, we scaled 2006 and 2019 earthworm densities to a 1-m^2 area. The differences in earthworm density between 2006 and 2019 were then tested using GLMMs with year, gap location, and their interaction as fixed effects, or with year, fencing, and their interaction as fixed effects. Our random effects were gap location nested within plot number. Earthworm metrics from 2019 were tested as a function of deer exclosure, location within a gap, and their interaction, with plot number as a random effect. Significance within mixed effects models was tested with Type III ANOVA with a Kenward-Roger degrees of freedom adjustment and significance within generalized linear mixed effects models was tested using a Type III Wald Chi-Square test. All corresponding model assumptions were met and tested using the ‘DHARMa’ package (Hartig 2017). The ‘emmeans’ package was then used for pairwise analysis with a

Bonferroni adjustment (Lenth et al. 2022). We report proportional differences between treatments based on untransformed means, but median values also showed similar patterns with disturbance treatment. We also report data to meet several minimum descriptive standards set by Buma (2021) to simplify integration into disturbance ecology related meta-analyses.

1.3: Results

For this experiment we collected, measured, and identified nearly 2000 European earthworms. Population biomass and density varied by site, with the FE having the greatest earthworm biomass and density, followed by AR, then NH, and finally FL (Table 1.1). Species densities varied among sites, but all species aggregate biomass rankings were consistent among sites. *Lumbricus* had the highest biomass, followed by *Aporrectodea*, and then *Dendrobaena*. However, earthworm communities did not vary with disturbance treatments in either experiment. Between MOSS and FE, we found that deer and canopy gaps both influence earthworm density and biomass. Deer presence led to increased earthworm biomass and density, whereas canopy gaps led to decreased earthworm biomass and density.

Managed Old Growth Silviculture Study (MOSS)

Within MOSS, mean earthworm biomass was 21% lower in the fenced treatments ($F_{1,63}$ = 5.5, p = 0.02; Fig. 1.2), while mean earthworm biomass was 62% lower under a canopy gap than under a closed canopy ($F_{1,61.3}$ = 9.4, p = 0.003; Fig. 1.2). Earthworm density had a similar trend with mean density being 17% lower inside of fencing (χ^2 = 4.6, df = 1, p = 0.03) and 8% lower under canopy gaps (χ^2 = 0.33, df =1, p = 0.56). No significant interactions were found between fencing and canopy gap presence, nor did earthworm biomass and density vary by location within the gap.

Each earthworm genus followed a similar response pattern to fencing and gap treatments, although certain genera were more strongly influenced by disturbance treatments than others (Fig. 1.3). In fenced treatments, *Aporrectodea* mean biomass and density were 25% and 8% lower respectively ($\chi^2 = 4.2$, df = 1, $p = 0.04$; $\chi^2 = 4.1$, df = 1, $p = 0.04$), but canopy gaps had little influence on *Aporrectodea* biomass ($\chi^2 = 0.13$, df =1, $p = 0.71$). Fencing and canopy gaps had an interactive effect on *Aporrectodea* density ($\chi^2 = 3.7$, df =1, $p = 0.05$); Mean earthworm density was lower inside of fencing under a closed canopy (t = -2.0, df = 122, $p = 0.04$). Deer also influenced *Lumbricus* species, with fenced treatments leading to a 23% and 16% decrease in mean *Lumbricus* biomass and density respectively ($F_{1,63} = 6.0$, $p = 0.02$; $\chi^2 = 2.1$, df = 1, $p = 0.15$). Canopy gaps decreased mean *Lumbricus* biomass and density by 77% and 25% respectively ($F_{1,61.6} = 7.9$, $p = 0.006$; $\chi^2 = 4.8$, $p = 0.03$). Finally, deer fencing had no impact on *Dendrobaena* biomass or density, but gaps decreased mean *Dendrobaena* biomass by 33% ($F_{1,61.2} = 4.8$, $p = 0.03$).

Flambeau Experiment

At FE, there was a significant interaction between the fencing treatment and year of sampling ($\chi^2 = 26$, df =1, p < 0.0001); Therefore, the change in earthworm density in the fenced versus unfenced areas in 2019 from prior to initiation of fencing (in 2006) were different. Mean earthworm density strongly increased outside of fencing by 69% from pre-treatment conditions ($t = -16.4$, df = 88, $p < 0.0001$), while increasing by only

9% inside fencing (t = -7.79, df = 88, $p < 0.0001$; Fig. 1.4a). During this 13-year period, mean earthworm density increased the most under a closed canopy in the north buffer location (+62%; t = -14.6, df = 66, $p < 0.0001$), followed by the north transitional edge (+37%, t = -9.9, df = 64, $p < 0.0001$), then the south transitional edge location (+24%, t = -6.8, df = 64%, $p < 0.0001$), but stayed the same in the north gap location (t = -0.6, df = 64, $p = 0.55$; Fig. 1.4b; Table S1.1). In 2019, mean earthworm density and biomass were 22% and 24% lower in fenced treatments than unfenced treatments respectively (χ^2 = 10.6, df = 1, $p = 0.001$; $F_{1,8} = 1.9$, $p = 0.21$). In 2019 we also found an interaction between fencing and gap location (χ^2 = 11.5, df = 4, $p = 0.02$), where mean earthworm density was 57% lower in the center of fenced gaps than the center of unfenced gaps (t = -3.3, df = 39, $p = 0.002$; Table S1.2). In 2019, deer and canopy gaps in FE influenced earthworm species' density more than biomass. Mean *Aporrectodea* and *Lumbricus* density decreased by 30% and 23% respectively in fenced treatments (χ^2 = 4.0, df = 1, $p = 0.05$; χ^2 = 9.3, df = 1, $p = 0.002$). There was an interactive effect between gap location and exclosure on mean *Lumbricus* density (χ^2 = 15.0, df = 4, $p = 0.005$), which was lowest in the fenced center of a canopy gap compared to other gap locations (t = -3.0, df = 39, $p = 0.004$). *Lumbricus* mean density decreased by 65% respectively from the center of unfenced to fenced gaps. *Dendrobaena* did not respond to disturbance treatments.

1.4: Discussion

Our results support the idea that common and influential disturbance events can be linked to one another, shedding new light on disturbance dynamics in temperate forests. Using northern Wisconsin's forests as a study system, we found that fencing out white-tailed deer and creating canopy gaps decreased earthworm biomass and density (Fig. 1.5).

These patterns were maintained across two separate experiments that differed in spatial scales, with FE exclosures being 230 times larger than MOSS exclosures. The consistency of our results highlights the potential ubiquity of earthworm invasion being linked to deer and canopy gaps in temperate deciduous forest landscapes. Given each disturbance's power to shape the function, composition, and structure of a stand, the links we found have important implications for contemporary forest ecology and management.

H1: Deer increase invasive earthworms

Although most research concerning ungulate herbivores relates to their strong influence on vegetation (Côté et al. 2004), increasing focus is being placed on indirect effects of ungulates on invertebrate communities (Bernes et al. 2018) and soil properties (Ohira et al. 2022). Our finding that deer can increase earthworm populations aligns with several studies throughout the eastern United States (Davalos et al. 2015c; Cope & Burns 2019; Mahon & Crist 2019) and provides a deeper understanding of the relationship between deer and invasive earthworms. In particular, we found that ambient deer populations lead to greater earthworm densities over time (in comparison to fenced treatments) and that certain earthworm genera are more likely to be influenced by deer.

Our finding that earthworm densities increased the most over time outside of fencing and increased slightly inside of fencing differs from both Davalos et al. (2015c) and Mahon and Crist (2019), who found that earthworm density generally decreases inside of fencing with time. These differences among studies could be due to the timing of earthworm invasion (i.e. how long after initial invasion that earthworms were sampled). In addition, Davalos et al. (2015c) found lower *Aporrectodea* density with fencing treatments but were unsure of whether this was due to confounding effects. Our study supports this result and points to several potential mechanisms that have not been adequately tested when evaluating deer and earthworm linkages. Since Aporrectodea are particularly influenced by deer in our study and receive most of their nutrients from the soil, the mechanism driving increased earthworm biomass and density with deer presence could be due to altered soil properties. One potential mechanism is that deer are indirectly increasing belowground microbial biomass as plants reallocate nutrients to their roots to compensate for losses in foliar tissue (Bardgett & Wardle 2003; A'Bear et al. 2014). Increased soil microbial biomass would then favor a microbe-feeding group like *Aporrectodea*. The second potential mechanism is that deer reduce the density and diversity of plants aboveground, which then reduces soil nutrient uptake outside of exclosures, thereby favoring increased earthworm biomass and density (Bardgett & Wardle 2003). The third and fourth potential mechanisms are that deer are directly increasing earthworm populations by transporting earthworm cocoons with their hooves or by increasing soil nitrogen through fecal and urinary inputs, although deer-mediated increases in nitrogen would likely be heterogeneous and operate at scales of 1-20 m (Murray et al. 2013). Since direct aboveground and belowground relationships between trophic levels are rare, the first two indirect mechanisms seem most likely (Bardgett & Wardle 2003).

The management and ecological implications of deer increasing invasive earthworms may be substantial, as deer and earthworms both function as a disturbance and an ecological engineer (Fisichelli et al. 2013; Hanberry & Faison 2023). Individually, overabundant deer and earthworms reduce forest biodiversity and structure (Frelich et al.

2019; Reed et al. 2022). However, these linked disturbances may have a compounding effect on ecosystem nutrients and vegetation, leading to understory dominance of ruderal species and altered successional trajectories (Powers & Nagel 2009). In a recent study showing how combined deer and earthworms can change ecosystems, Mahon et al. 2020 found that deer increased earthworm populations, which then increased leaf litter decomposition rates and changed soil nutrient dynamics. However, more research is needed on the combined effects of earthworms and deer, considering their many indirect effects on ecosystems (Rooney & Waller 2003; Frelich et al. 2019).

H2: Canopy gaps decrease invasive earthworms

Our initial hypothesis that gaps increase invasive earthworms was not supported, as canopy gaps decreased earthworm biomass and density. This is likely due to closed canopies having nutrient-rich tree leaf litter, which serves as shelter and food for earthworms (Mathieu et al. 2010). Further, our finding that *Lumbricus* and *Dendrobaena* population biomass increased under a closed canopy points to leaf litter quality as a driving force in determining earthworm populations (Suárez et al. 2006). Additionally, a lack of recent soil disturbances in the closed canopy treatments may favor earthworms (Nachtergale et al. 2002).

Earthworm densities also differed among specific gap locations. Over time, earthworm densities increased the most in locations further from the gap center and the least underneath an open canopy. There are several potential reasons gap location may cause earthworm densities to vary. Canopy gap edges may have more deer browsing pressure and preferable abiotic soil conditions, which would then increase the number of earthworms (Burton et al. 2021). It is also possible that differences in earthworm density are caused by earthworms slowly migrating outwards from gap centers and into the surrounding forest, as Hale et al. (2005) showed that earthworms can move several meters per year in the right conditions. However, since the total number of earthworm cocoons is far greater than the total number of earthworms at any given time (Butt 1992; Fernández et al. 2010), it is more likely that environmental conditions under a canopy gap are influencing the rates of mortality and recruitment of hatchlings rather than leading to mass-migration of earthworms. Reductions in hatchling success would then lead to differential earthworm biomass and density in certain areas over time. In addition, Aporrectodea species have been shown to burrow downwards with adverse environmental conditions (Perreault & Whalen 2006; Nuutinen & Butt 2009), which would likely reduce their movement laterally into the forest. Therefore, we believe that these gap and fence treatments are primarily influencing the population growth of earthworms rather than their dispersal. Assuming that canopy gaps decrease earthworm populations, our results suggest that efforts to increase canopy complexity towards old-growth structure may have benefits that extend beyond increased plant biodiversity and could slow earthworm invasion. Nevertheless, how canopy gaps and increasing canopy complexity effects earthworm migration and reproduction requires more research.

H3: Connections among canopy gaps, deer, and invasive earthworms

Earthworm biomass and density are likely a product of net interactions between canopy gaps and deer, wherein gaps decrease earthworms and deer increase earthworms (Fig. 1.5). Our interpretation of results is benefitted by considering these disturbances through a “press-pulse” lens. Many studies on disturbance linkages are focused on combinations of high-severity, short-duration pulse events, such as fire and blowdowns (Cannon et al. 2017, Jentsch and White 2019). Although these pulse-pulse disturbance interactions can quickly and dramatically change landscapes, their influence can hinge on the severity of existing press disturbances (Bergstrom et al. 2021). A press disturbance, such as species invasion or deer browse, operates continuously on a system from year to year and is not temporally confined (Lake 2000). Our work suggests that press disturbances can be linked with other press or pulse disturbances, which can have particularly long-lasting effects on ecosystem structure and function (Ratajczak et al. 2017).

Our study shows that the intensity and extent of one press (earthworm invasion) can be linked to another press disturbance (deer herbivory). This implies that disturbances can also potentially be "unlinked" and indirectly controlled to maintain ecological structure and function. Similar to targeting climate-mediated positive feedback loops, by identifying and breaking the links between influential disturbances we may be able to slow ecosystem degradation. This strategy may be particularly important when trying to control influential press disturbances that seem impossible to manage, such as invasive earthworms. In temperate forests, deer can be a dominant press disturbance that are seemingly linked to the extent and intensity of other disturbances across a wide spatial scale. By reducing deer populations or fencing out deer from target areas, by proxy we may be able to better control "unmanageable" disturbances such as invasive earthworms over the long term. In places where deer management is culturally sensitive or not preferred (Cambronne 2013), canopy gap creation through group selection harvesting may be a more feasible way to take advantage of disturbance links and slow earthworm invasion. Thus, by identifying and targeting a suite of linked press and pulse disturbances, we may be able to make or break links to control certain undesirable disturbances, save management time and effort, and discover new connections within disturbance ecology.

Future Research & Conclusions

Our work provides evidence of links between canopy gaps, deer, and invasive earthworms, but future research should explicitly address the mechanisms behind these disturbance links, such as changes in soil nutrients, microbial biomass, or litter quality. By identifying how aboveground disturbances are linked to belowground disturbance we will gain a deeper understanding of ecosystem function, biodiversity, and restoration (A'Bear et al. 2014). Researchers should also evaluate if deer and canopy gaps can influence other important soil-dwelling fauna, such as *Amynthas* species, a group of earthworms that are likely to be a highly damaging wave of disturbance that many temperate forests will face (Chang et al. 2021). Lastly, future work should test whether deer concentrate at gap edges and if this microsite preference facilitates an increase in invasive earthworms.

Our work highlights the importance of linked disturbances and our limited knowledge regarding how a belowground disturbance may be connected to aboveground disturbances. Deer, canopy gaps, and invasive earthworms are likely to become more frequent with a changing climate, which would make their linkages more important to understand in order to conserve soil communities and ecosystem processes (Park et al. 2014; Fisichelli & Miller 2018). Therefore, our understanding of disturbance theory will be incomplete without greater effort to understand disturbance’s linked nature and its potential influence on forest ecology and management. To this end, reduced deer populations and strategic tree harvesting could be a means to take advantage of disturbance links and slow earthworm invasion.

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1.5: Tables

Table 1.1: Genera-specific mean earthworm density and biomass by experimental site.

	Mean Earthworm Density (# / m^2)				**Mean Earthworm Ash-Free Dry Biomass (g / m^2)**			
Site	***Aporrectodea***	***Dendrobaena***	***Lumbricus***	**Total**	***Aporrectodea***	***Dendrobaena***	***Lumbricus***	**Total**
AR (MOSS)	47.0	85.7	80.1	212.8	2.1	0.4	3.2	5.7
FL (MOSS)	8.6	25.7	27.5	61.8	0.1	0.1	1.1	1.3
NH (MOSS)	29.7	51.1	44.2	125.0	0.7	0.2	1.9	2.7
The FE	50.9	29.7	112.0	192.9	2.0	0.2	3.9	6.1

Abbreviations: AR = Argonne Experimental Forest; FL = Flambeau River State Forest; NH = Northern Highlands American Legion State Forest; MOSS = Managed Old Growth Silviculture Study; The FE = The Flambeau Experiment

1.6: Figures

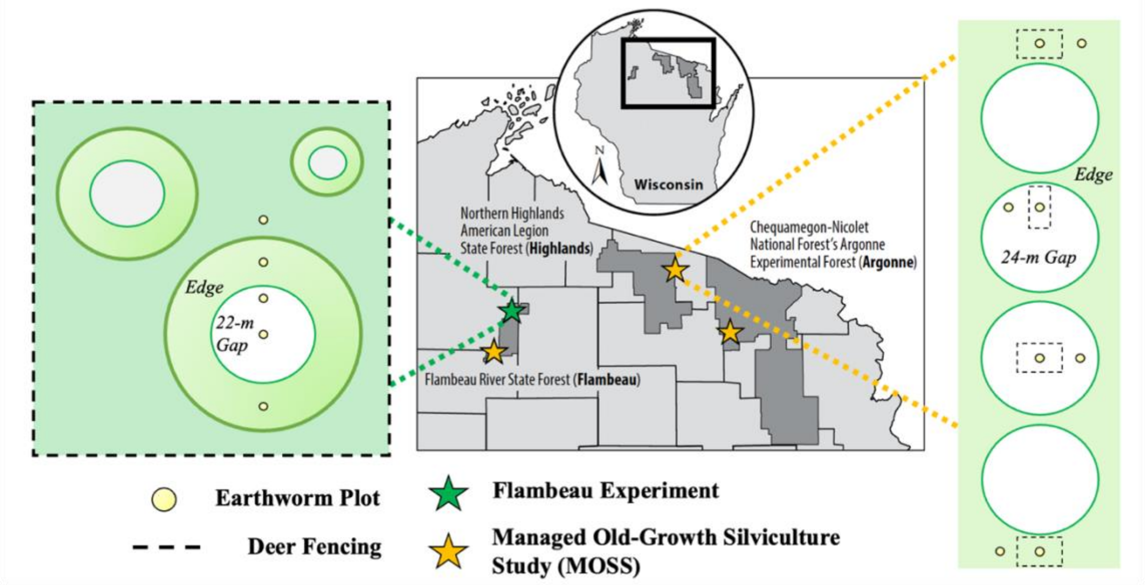

Fig. 1.1: Map of N. Wisconsin, USA with depictions of canopy gap and earthworm sampling design. Gold stars represent the MOSS experiment, where earthworms were sampled inside and outside of small fences underneath a canopy gap (n=47). The green star represents the Flambeau Experiment, where earthworms were sampled along a north-south transect across a fenced (n=5) or unfenced gap (n=5) in both 2006 and 2019. Sampling points along this transect extended from the north buffer (25-m from gap center), to the north transitional edge (16-m from gap center), to the north gap (7-m from gap center), to the gap center, to the south transitional edge (16-m from gap center). Map adapted from Fassnacht et al. 2013.

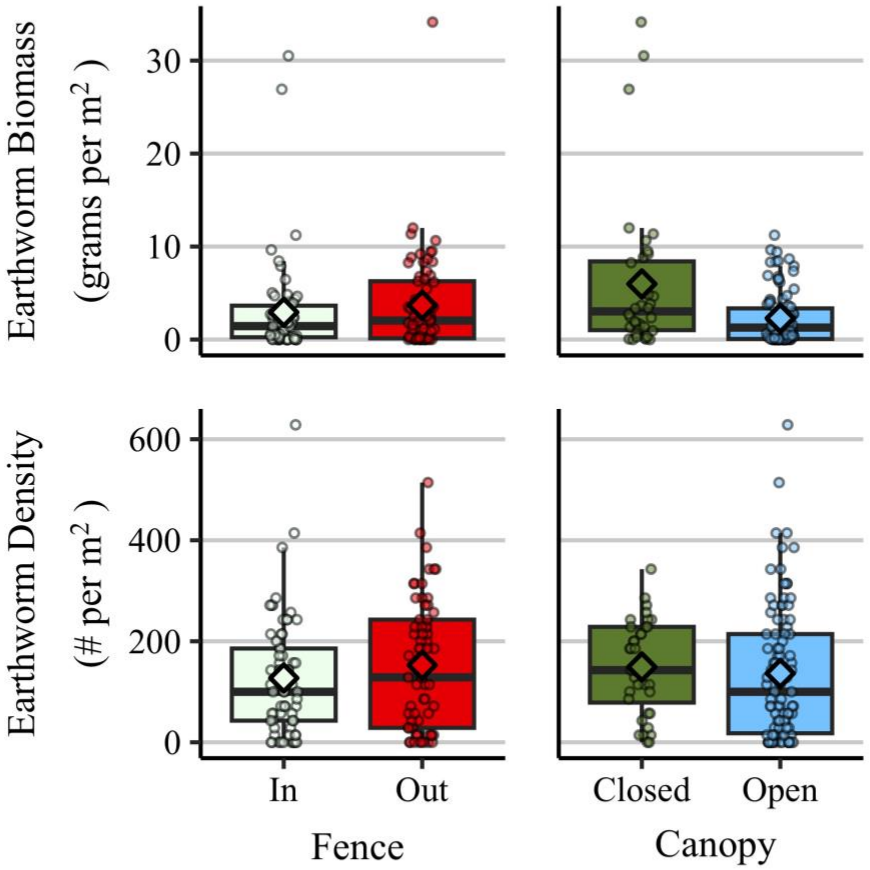

Fig. 1.2: Mean earthworm biomass and density response to fencing and canopy gaps. Diamonds are means, while bold horizontal lines are medians.

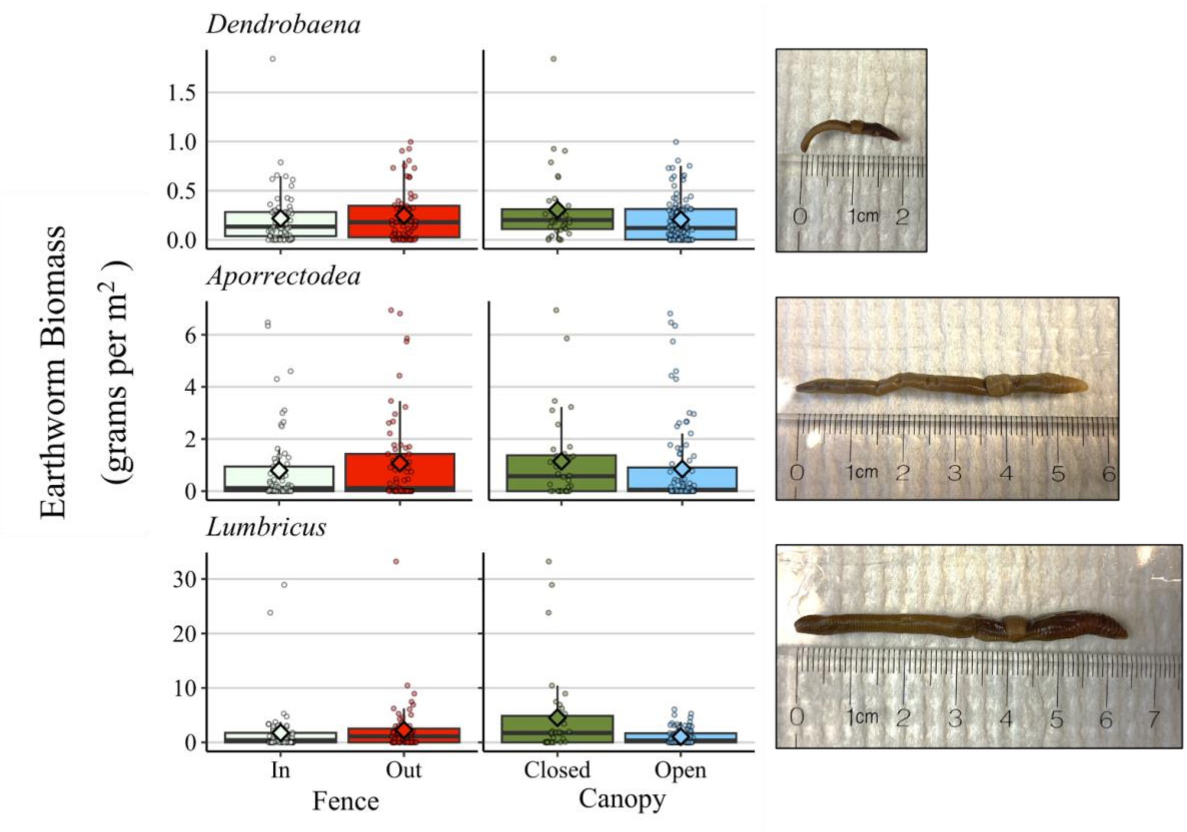

Fig. 1.3: Earthworm genera response to deer fencing and gap creation. *Aporrectodea* and *Lumbricus* biomass decreased inside of fencing, while *Lumbricus* biomass decreased with gap creation. All genera follow a pattern of lower biomass inside of fencing and decreased biomass under a canopy gap. Photos by Samuel Reed.

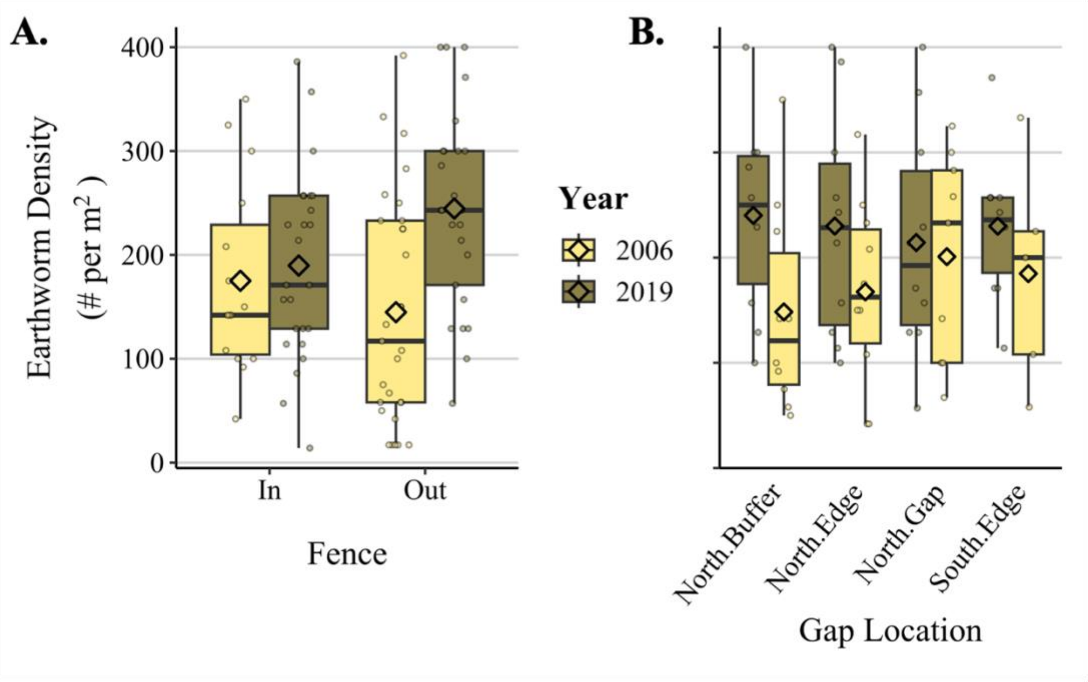

Fig. 1.4: A) From pre-treatment (2006) to 13 years post-treatment (2019), earthworm density increased by 69% in unfenced treatments, while earthworms only increased by 9% in fenced treatments. **B)** Earthworm density also increased the most over 13 years in the north buffer location (+62%).

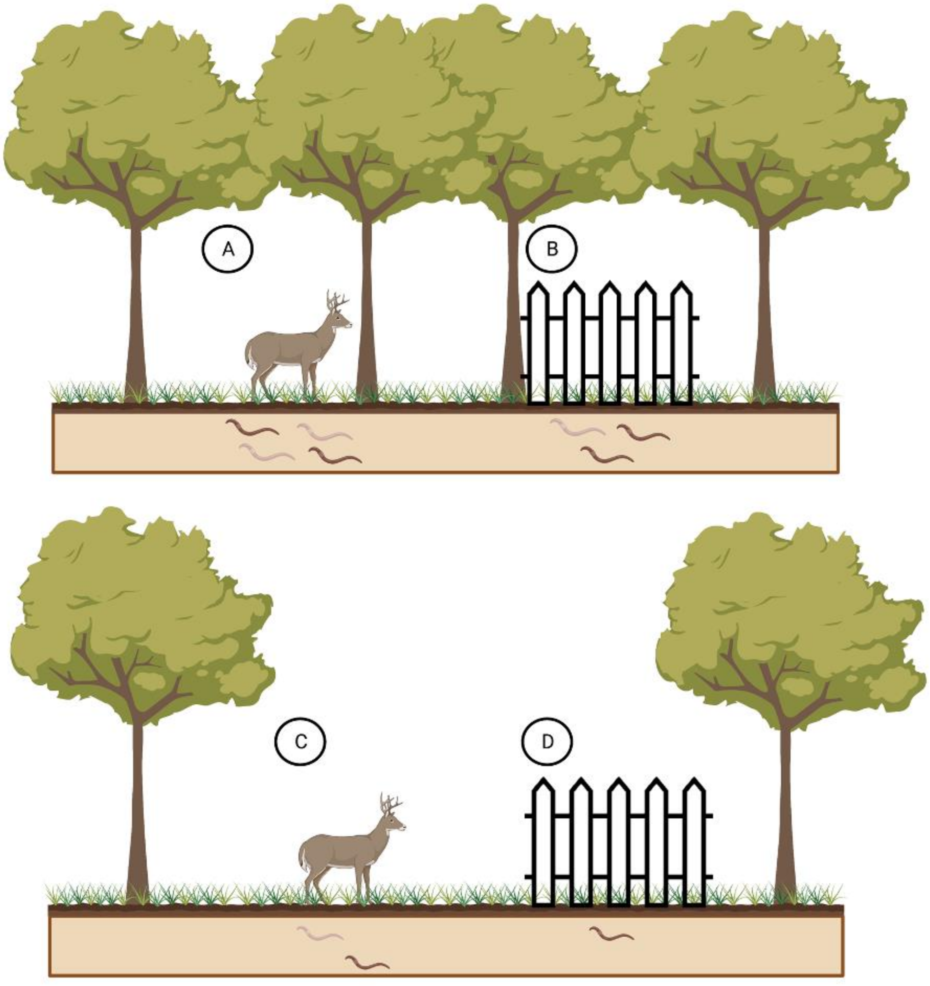

Fig. 1.5: Diagram showing how earthworms are linked to disturbance treatments in our study: **A)** Deer presence under a closed canopy leads to the highest earthworm biomass and density **B)** Fencing out deer decreases earthworm biomass and density; **C)** Gap creation substantially decreases earthworm biomass and density; **D)** Deer presence and canopy gaps cause the lowest earthworm biomass and density. Created with BioRender.

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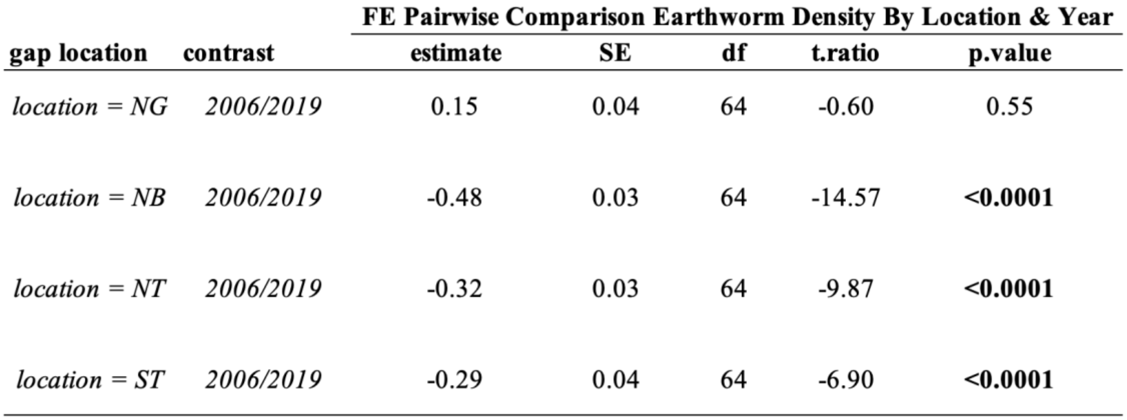
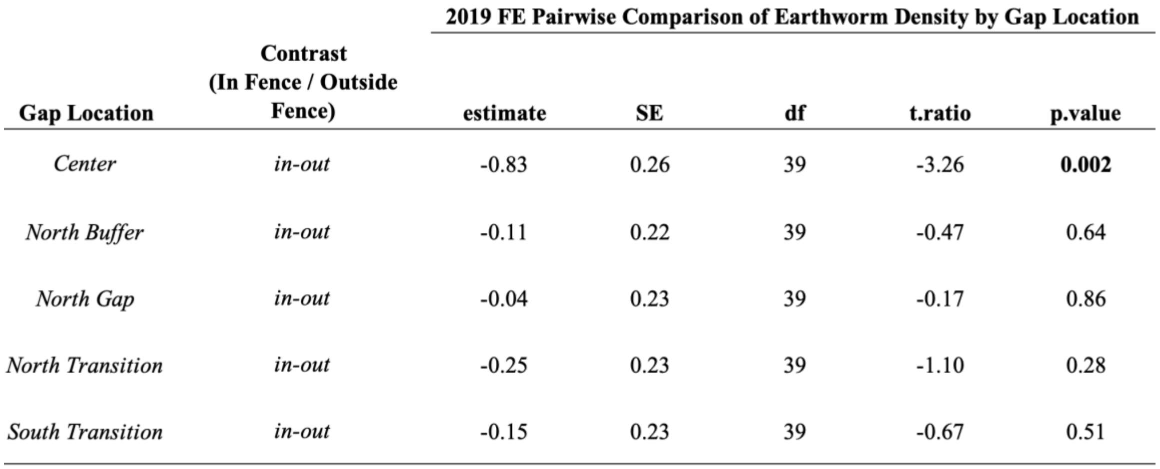
1.8: Supplementary Information: Further supplemental information provided in Reed et al. (2023). Citation above.

Table S1.1: Pairwise comparison of earthworm density by gap location between years 2006 and 2019



		FE Pairwise Comparison Earthworm Density By Location & Year				
gap location	**contrast**	**estimate**	**SE**	**df**	**t.ratio**	**p.value**
location = NG	*2006/2019*	0.15	0.04	64	-0.60	0.55
location = NB	*2006/2019*	-0.48	0.03	64	-14.57	**<0.0001**
location = NT	*2006/2019*	-0.32	0.03	64	-9.87	**<0.0001**
location = ST	*2006/2019*	-0.29	0.04	64	-6.90	**<0.0001**

Table S1.2: Pairwise comparison of earthworm density by gap location and fencing treatments in 2019



		2019 FE Pairwise Comparison of Earthworm Density by Gap Location				
Gap Location	**Contrast (In Fence / Outside Fence)**	**estimate**	**SE**	**df**	**t.ratio**	**p.value**
Center	*in-out*	-0.83	0.26	39	-3.26	**0.002**
North Buffer	*in-out*	-0.11	0.22	39	-0.47	0.64
North Gap	*in-out*	-0.04	0.23	39	-0.17	0.86
North Transition	*in-out*	-0.25	0.23	39	-1.10	0.28
South Transition	*in-out*	-0.15	0.23	39	-0.67	0.51

Chapter 2:

Long term effects of deer exclosures, canopy gaps, and ruderal species on woody seedling and sapling regeneration

Summary

Northern hardwood forests are influenced by a wide variety of disturbances, many of which interact and can change vegetation. Two of the most common disturbances are canopy gap creating events and deer browsing. Canopy gaps allow light into the understory, leading to a flush of plant growth, while deer consume plants and can dramatically change biodiversity. Both canopy gaps and deer may lead to the proliferation of ruderal shrubs and herbs such as *Rubus* spp. and *C. pensylvanica*, both of which are hypothesized to slow forest succession. Yet, it is unclear how the combination of these two disturbances and ruderal species can change regeneration of woody plant density, species richness, diversity, and species composition. To address this knowledge gap, we used a multi-disturbance experiment in northern Wisconsin's hardwood forests that manipulates combined deer and canopy gap treatments and examined 15-year responses to these manipulations. We found evidence of a tree-height bottleneck, wherein neither gaps nor fencing influenced tree seedling (<10 cm height or <2 cm DBH) density, while canopy gaps primarily increased tree sapling density (Between 2 cm and 12 cm DBH) inside of fencing. This disturbance response pattern was particularly strong for sugar maple, the dominant overstory species. Alternatively, gaps and fencing led to a decrease in *Rubus* density over time. *Rubus* density was also negatively correlated with tree sapling density, indicating that advanced regeneration and a healthy sapling layer are critical to reduce *Rubus* density. Our findings provide insight towards the complex ways in which gaps and deer influence trees and ruderal species, highlighting how multiple disturbances can lead to multiple regenerative outcomes.

2.1: Introduction

Ecologists and land managers have long focused on testing the effects of individual disturbances in temperate forests. However, disturbances do not occur independently of one another on the landscape and certain combinations of forest disturbances are more influential than their individual counterparts (Abrams et al. 1985; Graham et al. 2021; Yantes et al. 2023). In northern temperate forests, regenerating and maturing tree communities are susceptible to multiple disturbances and can change rapidly in response (Frelich 2002). If given enough time, the tree species that survive disturbance and understory competition can eventually come to occupy the overstory (Reed et al. 2022). Thus, it is critical that we understand how tree and woody plant regeneration respond to multiple disturbances over long time scales and how this can influence successional development and forest management (Fahey et al. 2018).

Canopy gaps and deer are some of the most common and influential disturbances in temperate forests. Gaps can be created through tree harvesting, pathogens, or windstorms, with mid-sized gaps (20-30 m in diameter) being the most commonly found gap size in temperate forests (Hanson & Lorimer 2007). Generally, plant dynamics within a canopy gap are mediated by light availability, with ruderal and early-successional species quickly capitalizing on this resource within larger canopy gaps (20+ m diameter; Muscolo et al. 2014). Eventually, these ruderal species are expected to be overtopped by shade-tolerant trees that were present as advanced regeneration and for successional development to continue (Widen et al. 2018). Some researchers have theorized that ruderal species like *Rubus* can be beneficial for regrowing trees, despite their persistent labeling as weeds (Donoso & Nyland 2006). Shrubs and herbs may provide more desirable abiotic conditions for trees by reducing harmful U.V., increasing shade, or by retaining nutrients within a gap (Donoso & Nyland, 2006; Montgomery et al. 2010).

Some hypothesize that canopy gaps can increase understory plant diversity depending on gap size, however this effect is likely mediated by deer browsing (Kern et al. 2012; Burton et al. 2021). White-tailed deer (*Odocoileus virginianus*) are generally present at historically high populations in temperate forests, which places consumptive pressure on preferred plant species (Cote et al. 2004). This voracious plant consumption by deer can reduce diversity and has a wide variety of indirect effects, such as altered soil characteristics and canopy structure (Rooney & Waller 2003; Reed et al. 2022; Reed et al. 2023) or acting as a brake on climate change adaptation (Fisichelli et al. 2012).

Both deer and canopy gaps have well understood individual effects on tree regeneration, but their combined influence on plants is complex and varied, particularly over longer time periods (Donoso & Nyland 2006; Kern et al. 2017). Some have found that understory shrubs, herbs, and ferns can be a threat to tree regeneration and cause arrested succession, particularly with overabundant deer populations (Thrippleton et al. 2016; Royo and Carson 2022). Kern et al. (2012) found that shrubs like *Rubus* can shade-out and slow tree growth within a canopy gap over the course of a decade. This tree-shrub competition is then exacerbated with deer browsing, as seedlings and saplings are unable to grow above the shade of ruderal species (Kern et al. 2012). Others have found that shrubs can protect and hide growing seedlings from herbivores, but once these saplings are in view of deer, they are heavily browsed. Thus, shade-tolerant seedlings in a canopy gap may not be influenced by deer, as they are hidden from view beneath a shrubby midstory (Walters et al. 2016; Jones et al. 2023). This could create a tree-height bottleneck, where deer-preferred species cannot grow above a certain height, even with increased light resources (Walters et al. 2020; VanderMolen et al. 2021; VanderMolen & Webster 2021). In addition, deer may indirectly slow or stop tree growth, as Penn sedge (*Carex pensylvanica*), a fast-spreading sedge species that is theorized to be favored by deer and gaps, has been shown to slow tree regeneration (Powers & Nagel 2009).

To test how different combinations of deer, canopy gaps, and ruderal species influence the woody understory, we used a long-term, multi-disturbance experiment in northern Wisconsin, U.S.A, that manipulates canopy gaps and deer fencing. Woody plant density and herbaceous cover have been measured within this experiment for 15 years, allowing us to tease apart how combinations of canopy gaps and deer can change tree, herb, and shrub regeneration over time. Considering that most studies testing the combined effects of deer and canopy gaps on tree regeneration are short term and do not consider the effects of non-tree species, the structure of this experiment is unique (Roberts 2004; Spicer et al. 2020).

To this end, we hypothesized that canopy gaps would increase woody seedling and sapling density and diversity over time due to there being increased resources for shade tolerant plants in the understory (**H1**). However, we expect combined canopy gaps and deer fencing to increase sapling density and diversity over time, while fencing under a gap will not influence seedlings, as ruderal species hide seedlings from deer regardless of fencing (**H2**). In addition, we expect the highest *Rubus* and Penn sedge density to be under a gap and outside of fencing, as deer will give shrubs and sedge a competitive advantage by browsing regrowing saplings (**H3**). Lastly, we hypothesize that these differing woody plant responses to canopy gaps and deer will lead to distinct communities over time, particularly among species under a gap and outside of fencing (**H4**).

2.2: Methods

Site Description:

To test how woody species respond to deer fencing and canopy gap treatments, we used the Managed Old Growth Silviculture Study (MOSS). MOSS was established in 2007 and is comprised of three ≈200 ha replicate sites located in Wisconsin's Northern Highlands American Legion State Forest (NH), Flambeau River State Forest (FL), and Chequamegon-Nicolet National Forest's Argonne Experimental Forest (AR). Stands at each site are maturing Great Lakes northern hardwood forests and dominated by second-growth sugar maple (*Acer saccharum*). Subdominant trees in each stand include white ash (*Fraxinus americana*), red oak (*Quercus rubra*), and yellow birch *(Betula alleghaniensis*) (Fassnacht & Steele 2016). Soils vary among sites, with the NH having sandy loam over stratified outwash sand, FL having silt loam over sandy loam soil, and then AR having sandy loam with a high stone proportion (Fassnacht et al. 2013). All sites occur at roughly the same latitude and are within 140 km of one another.

Experimental Design:

MOSS has several canopy gap-creation treatments at each site that were established in winter 2007 and 2008. We used the "Large Gaps" treatment, which consists of 36, 24-m diameter gaps at each site that had understories that were either uncleaned, cleaned (removal of all species >2cm DBH), or cleaned and scarified. We omitted gaps that had their understories cleaned or scarified and cleaned following harvest as this management treatment can lead to substantially different tree composition and density (Reuling et al. 2019). Thus, we have an uncleaned gap total of 36. Among these canopy gaps, 24 contain a small deer exclosure that is in one of four locations along a north-south gradient, from the north transitional edge, to the north gap, to the center gap, to the south transitional edge (Fig. 2.1). Most exclosures are 5.25 x 5.25 m wide and 1.5 m tall, while fencing in the north gap location is 3.75 x 7.5 m in order to capture plant variation in this region of the gap. There were also 33 deer fences that were established underneath closed canopies across the sites. All fencing was established in 2007. We refer to these treatments as “fenced” or “unfenced,” with the latter signifying ambient deer presence.

We measured woody and herbaceous plants in 2007, 2010, 2017, and 2022 in MOSS’s regeneration quadrats and denote these times as t0 (pre-treatment), t3, t10, and t15 in figures. Underneath each canopy gap, there are 3 to 4 regeneration quadrats that capture the variety of light environments underneath a gap. One regeneration quadrat is within each deer exclosure. Regeneration quadrats are 5 x 5 m and contain 4, 1 x 1 m subplots at each edge, along with a 1.5 m radius circular plot in the center. Seedlings of all woody species (<2 cm DBH or <10 cm height) were identified and counted within each quadrat’s 1x1 m subplot and the 1.5 m radius circular plot (Table 2.1). We include both shrub and tree species in woody seedling density. Tree saplings (2 – 12 cm DBH) were identified and counted in the entire 5 x 5 m regeneration quadrat (Table 2.2). *Rubus* density was also measured within each circular plot starting in t3. In addition, cover classes for six native and non-native invasive herbaceous species were measured each year. For the purposes of this study, we only focus on Penn sedge cover as it is particularly influential as an understory species (Powers and Nagel 2009).

Statistical Analysis

We scaled woody seedling and sapling density up to the hectare basis for our analysis and used canopy gaps as our unit of replication. To analyze how seedling and sapling variables responded to disturbance treatments, we used generalized linear mixed effects hurdle models with quasi-Poisson or negative binomial distributions in the 'glmmTMB' package (Brooks et al. 2017). For seedling and sapling species richness and diversity, we used glmm's with Poisson and Tweedie distributions, respectively. Fencing, gap presence, year since treatment, and their interaction were our primary fixed effects, while gap number nested within site was our primary random effect. We also tested how fencing, canopy gaps, and their interaction influenced variables that were averaged across post-treatment years. To test how tree seedling and sapling density varied with *Rubus* density and *C. pensylvanica* cover we used both linear mixed effects models in the 'lme4' package and glmm's with negative binomial distributions in the 'glmmTMB' package. *C. pensylvanica* cover was tested using the average of the cover class midpoints, which converted the ordinal cover classes into a continuous variable. All model assumptions were tested using the 'DHARMa' package and post hoc comparisons were done in the 'emmeans' package (Hartig 2017; Lenth et al. 2022).

2.3: Results

Woody Seedlings & Saplings

Woody seedlings and saplings had different responses to canopy gaps and deer fencing. Year and canopy gap presence had an interactive effect on woody seedling density (χ^2 = 9.6, df =3, *p* = 0.02; Fig. 2.2; Table S2.1). In year 3, canopy gaps increased woody seedling density by 130% in comparison to pre-treatment plots at year 0 (z = -4.9, p = 0.0001). By year 10 and 15 post-treatment, woody seedling density had decreased substantially and there was no difference in woody seedling density between canopy gaps and pre-treatment plots. Similarly, woody seedling richness also increased over time following canopy gap creation (χ^2 = 7.1, df = 3, p < 0.07; Table S2.1), being 3.3 ± 0.3 species at year 0, then 4.6 ± 0.3 species at year 3 under a gap, 6.6 ± 0.4 species at year 10 under a gap, and 5.8 ± 0.3 species at 15 under a gap. Canopy gaps also increased woody seedling diversity over time, which was lowest at pre-treatment (0.61 ± 0.07) and highest at t10 (0.98 ± 0.07) (z = -3.4, p = 0.0001). Combined canopy gaps and fencing reduced average woody seedling density, richness, and diversity by 23%, 40%, and 34% respectively in comparison to outside of fencing and under a canopy gap (z = -2.4, p = 0.02; z = -4.4, p < 0.0001; z = -3.1, p < 0.002; Table S2.1). Fencing under a closed canopy did not influence woody seedling density, richness, or diversity.

Gap creation decreased mean sapling density by 33% from pre-treatment to three years post treatment (z = 2.9, p = 0.0003; Table S2.2). With time, canopy gaps increased sapling density above pre-treatment levels by 114% at year 10 and 145% at year 15 (z = -4.1, p = 0.0003; z = -5.5, p < 0.0001). Fencing under a canopy gap increased sapling density above pre-treatment levels by 174% in year 10 and 188% in year 15 (z = -3.3, p = 0.005; z = -3.8, p = 0.0008; Fig. 2.3). On average, fencing under a canopy gap increased sapling density by 82% in comparison to areas with no fencing under a canopy gap and by 205% in comparison to areas with no fencing and under a closed canopy (z = 3.1, p = 0.01; z = 4.5, p = 0.0001; Table S2.2). Sapling density did not vary between inside and outside of fencing under a closed canopy.

Fencing and time since treatment had a significant interactive effect on sapling species richness and diversity ($\chi^2 = 13$, df = 3, $p < 0.003$; $\chi^2 = 11.6$, df = 3, $p < 0.009$; Table S2.2). Prior to gap creation, sapling species richness was 1.6 ± 0.2 and did not change 3 years after gap creation, but by years 10 and 15 following gap creation, sapling species richness had increased to 2.6 ± 0.2 species and 3.0 ± 0.2 species respectively (z = -3.6, p = 0.002; z = -4.2, p = 0.0001). There was no difference in sapling species richness between years 10 and 15 underneath a gap. Gap creation also increased sapling diversity over time, causing a 215% increase in diversity from pre-treatment to 15 years post-treatment ($\chi^2 = 11.6$, df = 3, $p < 0.009$). On average, gap creation increased sapling richness and diversity by 90% and 71% respectively above unharvested controls ($\chi^2 = 8.4$, df = 1, $p < 0.004$; $\chi^2 = 8.6$, df = 1, $p < 0.003$; Table S2.2). However, fencing did not influence sapling species richness or diversity.

Tree and Species-Specific Responses to Treatments

Time was the primary variable that influenced tree seedling density, with tree seedling density decreasing by 23% from year 0 to year 3, decreasing 20% from year 3 to year 10, and then increasing by 68% from year 10 to year 15 ($\chi^2 = 6.3$, df = 3, p = 0.1; Table S2.3). Tree sapling density was strongly influenced by year since treatment, fencing, and canopy gaps, being the highest inside of fencing and under a gap at year 15 (2139 ± 444 saplings / ha) and being the lowest outside of fencing and under a gap at year

3 (363 ± 68.7 saplings / ha). On average canopy gaps and fencing increased tree sapling density by 82% in comparison to areas with no fencing under a canopy gap and by 205% in comparison to areas with no fencing and under a closed canopy ($z = 2.9$, $p = 0.02$; $z = 3.9$, $p = 0.0006$; Table S2.3). There was no difference in sapling density in fencing that was under a closed canopy.

Sugar maple was the most abundant seedling and sapling species in the experiment (Table 2.1; Table 2.2). Sugar maple seedlings initially decreased by 60% with gap creation from t0 to t3 ($z = 5.1$, $p = 0.0001$), but then increased by 136% from t3 to t15 ($z = 3.0$, $p = 0.01$; Table S2.4). Fencing did not influence sugar maple seedlings. In contrast, there was an interaction between fencing and canopy gap presence on sugar maple sapling density ($\chi^2 = 5.6$, df = 1, $p = 0.02$), with average sugar maple sapling density being 167% higher inside of fencing under a gap than outside of fencing under a gap ($z = 3.8$, $p = 0.0001$; Fig 2.4). There were no differences in sugar maple sapling density under a closed canopy with deer fencing.

Ironwood (*O. virginia*) was the sixth most common tree seedling and second most common tree sapling. Prior to treatment, ironwood seedling density was 67% higher in areas where fencing and gaps would be established in comparison to areas that would have a gap and no fencing ($z = 2.5$, $p = 0.01$; Table S2.4). With fencing and canopy gap treatments, ironwood seedling density dropped by 56% from pre-treatment to year 3, by 25% from year 3 to year 10, and by 5% from year 10 to year 15. By year 15, there was no difference in ironwood seedlings between inside and outside of fencing. Ironwood sapling density followed a slightly different pattern than ironwood seedlings, being 7%

lower in areas where fencing and gaps would be established in comparison to areas to where there would be a canopy gap and no fencing ($z = 2$, $p = 0.04$). However, by year 3, ironwood sapling density was 157% greater inside of fencing and under a gap in comparison to outside of fencing and under a gap ($z = 3.4$, $p = 0.0006$). By year 10 and 15, there was only a 31% and 14% respective difference in ironwood sapling density between inside and outside of fencing under a canopy gap ($z = 2.5$, $p = 0.01$; $z = 2.2$, $p = 0.03$). There was no difference in ironwood seedling and sapling density under a closed canopy.

There was a significant three-way interaction between year, fencing, and canopy gap treatment on *Rubus* density ($\chi^2 = 32.1$, df =3, $p < 0.0001$; Table S2.5). Canopy gaps increased Rubus density substantially by year 3 in comparison to pre-treatment conditions and the unharvested matrix ($z = -17.2$, $p < 0.0001$; $z = 17.6$, $p < 0.0001$). There was no difference in Rubus density under a gap between years 3 and 10. By year 15 post-treatment, Rubus density under a canopy gap had fallen substantially in comparison to year 10 ($z = 6.6$, $p = 0.0001$; Fig. 2.5). In particular, by year 15 fencing under a canopy gap had decreased Rubus density by 74% in comparison to outside of fencing under a canopy gap ($z = -6.3$, $p < 0.0001$). *Rubus* density did not correlate with tree seedling density underneath a canopy gap, but *Rubus* density was negatively correlated with tree sapling density underneath a canopy gap ($\chi^2 = 9.9$, $p = 0.002$; Fig. 2.6).

C. pensylvanica cover changed over time, peaking in year 10 and decreasing 66% from year 10 to year 15 ($\chi^2 = 14.8$, df = 3, $p = 0.0006$). Year and fencing had an interactive effect on *C. pensylvanica* ($\chi^2 = 10.4$, df = 3, $p = 0.02$) wherein sedge cover was no different inside and outside of fencing in year 3, but significantly higher outside of fencing in years 10 and 15 ($z = -2.6$, $p = 0.01$; $z = -3.3$, $p = 0.001$). *C. pensylvanica* cover was not correlated with tree seedling density or tree sapling density.

Community Responses To Treatment

Prior to the disturbance treatments, there were no differences among woody plant communities ($F = 0.73$, $p = 0.8$; Fig. 2.7a; Table S2.6). However, with combined canopy gaps and fencing, we saw substantial changes in woody plant communities, while fencing under a closed canopy did not influence community composition. In year 3, 10, and 15 post-treatment, woody plant communities in fenced areas with a gap and unfenced areas with a gap were significantly different from one another and all communities under a closed canopy ($F = 9.1$, $p < 0.001$; $F = 9.2$, $p < 0.0001$; $F = 4.0$, $p = 0.002$; Fig. 2.7; Table S2.7; Table S2.8; Table S2.9). Woody plant communities outside of fencing and under a gap had the least variation and tended to be the most different from communities outside of fencing and under a closed canopy in years 3, 10, and 15 ($F = 21.2$, $p < 0.001$; $F = 21.8$, $p < 0.001$; $F = 8.6$, $p < 0.001$; Fig. 2.7). By year 15, woody plant communities inside of fencing and under a gap had returned to being similar to closed canopy communities regardless of fencing, while woody plant communities outside of fencing and under a gap remained significantly different from fenced and unfenced communities under a closed canopy ($F = 7.4$, $p < 0.001$; $F = 8.6$, $p < 0.001$; Fig. 2.7d; Table S2.9).

2.4: Discussion

Using a unique, long-term disturbance experiment in northern Wisconsin, we found strong combined influences of canopy gaps and deer on the forest understory over fifteen years. Woody seedling density, richness, and diversity were highest underneath a canopy gap and outside of deer fencing. In contrast, woody sapling density increased substantially inside of fencing and under a canopy gap, highlighting this height class's sensitivity to disturbances. In addition, species-specific and community-wide responses to fencing and gaps may have long-lasting implications for forests, as these individuals are the most likely to occupy the canopy and play a significant role in reducing the dominance of ruderal species (Frelich 2002; Donoso & Nyland 2006). The changes in *Rubus* density in response to disturbance treatments over time provides new insight towards how ruderal species are influenced by disturbance and may influence forest development in the Lake States. Each of these results highlights the complexity of multiple disturbances in silvics and how these phenomena can lead to different management outcomes depending on the combination of disturbance in question (Kern et al. 2017).

Seedlings

Woody seedlings were strongly influenced by canopy gaps and the time since gap creation. Initially, gaps increased seedling density in comparison to the unharvested control, likely because increased light resources allowed plants to germinate from the seed bank (Muscolo et al. 2014). Eventually, woody seedling density decreased over time as tree seedlings entered the next height class and competitive exclusion occurred

(Frelich 2002). The decrease in woody seedling density, species richness, and diversity inside of fencing under canopy gaps is likely due to increased sugar maple sapling density shading-out woody seedlings. This heavy shade produced by a canopy of saplings would then reduce shade-intolerant woody species and reduce overall species richness (Donoso and Nyland 2006). It's likely that deer did not influence seedling density under canopy gaps because shrubs outside of fencing hid seedlings from view, making it harder for deer to browse these individuals (Walters et al. 2016). This mutualism between shrubs and seedlings is likely why certain studies do not find an effect of deer on seedlings underneath a canopy gap (Jones et al. 2023).

Neither deer nor canopy gaps influenced tree seedling density, diversity, or richness in our experiment. In addition, *Rubus* density and *C. pensylvanica* cover were not influential covariates in determining tree seedling density even though several studies have found that *Rubus* and *C. pensylvanica* can have negative effects on tree seedlings (Kern et al. 2012; Randall & Walters 2019). Our finding that ruderal species did not influence tree seedling density may be due to our experiment having a greater density of shade-tolerant understory species that can survive under a dense midstory of saplings, *Rubus*, or *C. pensylvanica*.

C. pensylvanica cover decreased with fencing, which supports the hypothesis that deer can increase the cover of this species (Powers and Nagel 2009; Fisichelli et al. 2013). Increased *C. pensylvanica* cover outside of fencing could also be attributed to a compounding effect of deer and invasive earthworms, seeing as Reed et al. (2023) found that deer increased earthworm biomass and density at MOSS. Deer could be eating plants that grow above the sedge layer and increasing invasive earthworms, which would then reduce soil health and nutrients, thereby providing a more favorable environment for *C. pensylvanica*. These effects would likely be magnified in moderately sandy soils like those found at our site (Randall & Walter 2019). *C. pensylvanica* did not respond to canopy gap creation, despite Powers & Nagel (2009) finding that increased light can benefit this species. Our results highlight the complex nature of plant competition following disturbance and how ruderal species influence on woody species is likely a symptom of adverse environmental conditions, such as deer or invasive earthworms.

Saplings

In contrast to seedlings, woody saplings were highly responsive to the disturbance treatments in MOSS. Total woody sapling, tree sapling, and sugar maple sapling density were all highest in areas with fencing and a gap overhead, indicating that mid-sized canopy openings and reduced deer browsing had the greatest combined benefit for tree regeneration in these forests. These results agree with several other studies in the eastern United States and highlight the tree height bottleneck within gap-based management practices (Kern et al. 2012; Walters 2020; Henry et al. 2021). It is likely that deer are attracted to canopy gaps because these saplings are easy to see, readily available, and have high foliar nitrogen content (Naidu & DeLucia 1997; Kuijper et al. 2009; Zaret et al. 2023). This combined effect of deer and canopy gaps then leads to shorter trees that cannot escape the browse line (Forrester et al. 2014). Notably, in our study, we can see how differences in sapling density between inside and outside of fencing under a canopy gap are only widening with time.

Sapling richness and diversity were also highest underneath canopy gaps and peaked at 10 years post-treatment but were not influence by deer fencing. Increasing richness and diversity in large gaps is to be expected as a range of shade and mid tolerant woody species are released (Kern et al. 2014). However, fencing having no influence on richness and diversity contrasts with several studies that have found that deer generally reduce species richness and diversity within a gap (Kern et al. 2012; Nuttle et al. 2013; Sabo et al. 2019). This lack of difference in sapling species richness and diversity with fencing is likely due to there being advanced regeneration of unpalatable species that was already present inside of fencing.

Advanced regeneration is of particular importance when considering gap-based regeneration (Kern et al. 2013; Widen et al. 2018). In our study, advanced regeneration likely caused differences in sapling density, diversity, and richness between treatments prior to canopy gap and fencing establishment. For instance, increased ironwood seedling density in areas where fencing and gaps would be established led to high ironwood sapling density following canopy gap and fencing establishment (Knapp et al. 2021). However, as time passed, the difference in ironwood seedling and sapling density between inside and outside of fencing decreased, likely due to this species being favored outside of fencing due to its unpalatability to deer (VanderMolen & Webster 2021). If we had only measured ironwood seedling and sapling density in year 3 post-treatment, we might have come to an entirely different conclusion regarding the effects of deer and canopy gaps on tree saplings. Thus, considering advanced regeneration in disturbance studies is critical to untangle which effects are due to disturbances over time and which are due to a priori environmental factors.

Further, a lack of advanced regeneration may contribute to tree regeneration failures and competitive exclusion by *Rubus* and *C. pensylvanica* in the understory (Miller & McGill 2019). The relatively high sapling density prior to treatment in our study explains why *Rubus* decreased substantially over time with fencing, as it was shaded out by maturing trees that were not browsed by deer (Donoso & Nyland 2006). This interpretation is further supported by the negative correlation between sapling density and *Rubus* density, which suggests that increased sapling density can help to reduce *Rubus* dominance in the understory. Thus, by reducing deer we can increase sapling density and concomitantly decrease the dominance of Rubus within canopy gaps.

Community Change

Our multivariate analysis highlights how the combination of deer and canopy gaps can lead to long-term changes in the understory community, as both disturbances led to unique woody plant communities over time. However, by year 15, woody communities under a gap and inside of fencing had returned to being similar to communities that were under a closed canopy, regardless of fencing. In contrast, woody communities under a gap and outside of fencing did not return to being similar to closed canopy communities in year 15. These results may be indicative of combined deer and canopy gaps casing arrested succession in the understory, as the community is static and not developing towards the closed canopy state (Royo and Carson 2006). This stasis within the understory community with deer and gaps could have important ramifications, as it may be indicative of reduced ecological resilience and increase the likelihood that the forest eventually shifts to a different stable state (Johnstone et al. 2016).

Conclusions and Implications:

Our results paint a complicated, but important, story regarding how deer and canopy gaps influence vegetative dynamics. Generally, mid-sized canopy gaps and reduced deer browsing pressure in areas with high advanced regeneration led to the greatest sapling density over time, particularly for species such as sugar maple. This advanced regeneration then shades out ruderal species such as *Rubus* and self-reinforces tree dominance within a gap without deer, as predicted by Donoso & Nyland (2006). When deer are present, gap-based regeneration changes, with ruderal species remaining abundant and saplings struggling to grow above the browse line. This dominance of ruderal species and abundant herbivores then leads to arrested succession and slowed forest development (Royo et al. 2006). However, in this scenario, it is possible that slow growing and unpalatable species such as ironwood could eventually shade out ruderal species and give shade-tolerant sugar maple a better environment to grow. Despite ironwood generally being considered an undesirable species for forest managers, it may be worthwhile to leave as advanced regeneration to reduce *Rubus* dominance in a canopy gap.

This study also highlights the importance of considering how different combinations of disturbance can lead to multiple plant regenerative outcomes. Deer and canopy gaps are two of the most common drivers of change in temperate forests and had an interactive effect on resulting vegetation, but we are still missing some key information regarding this interaction. Namely, how do gaps and deer change forest regeneration across multiple decades and how might this known interaction change with different gap sizes and deer population densities? Our study tests these disturbances within a binary framework, such as deer or no deer, when in reality canopy gaps and deer browsing occur at different intensities and spatial scales across a landscape (Callan et al. 2013). Combinations of different browsing intensities and different sizes of canopy gaps may lead to radically different outcomes. Further, whether mid-sized canopy gaps and reduced deer populations lead to landscape-scale improvements in forest regeneration and structure over time scales longer than three decades is also virtually unknown. Thus, despite the strengths of our experimental approach, such as highly replicated multi-disturbance treatments and having collected data on multiple forest strata for 15 years, there are still many open questions regarding how multiple disturbances are changing temperate forests.

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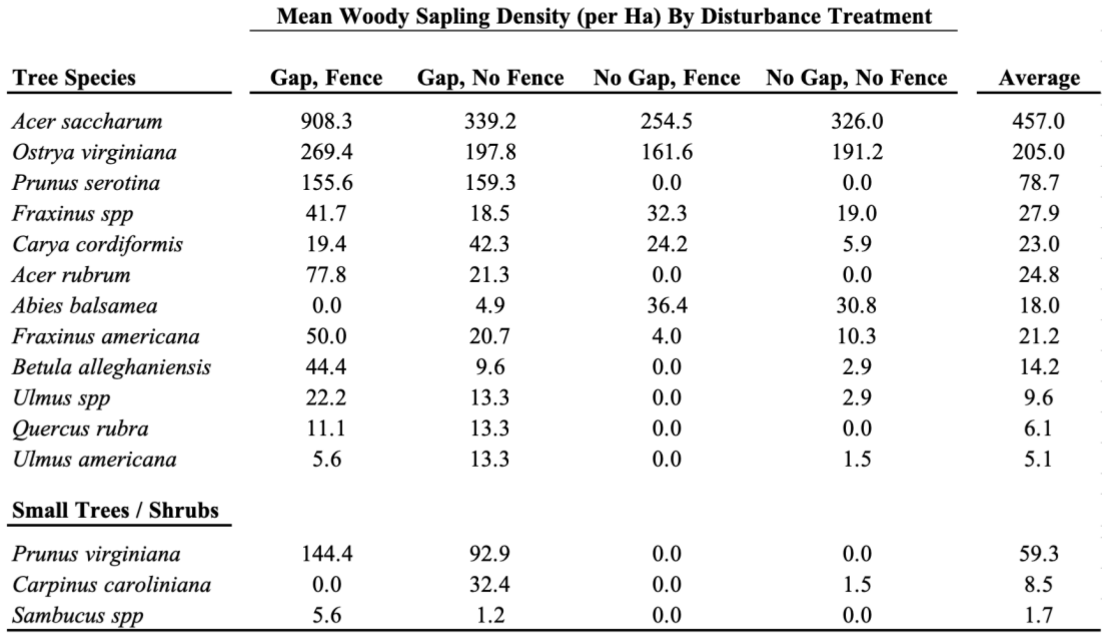
2.5: Tables

Table 2.1: Mean woody species seedling richness by treatment and growth form.

	Mean Seedling Density (per Ha) By Disturbance Treatment				
Tree Species	**Gap, Fence**	**Gap, No Fence**	**No Gap, Fence**	**No Gap, No Fence**	**Average**
Acer saccharum	23948	28114	26104	25908	26018
Fraxinus spp	3056	2434	1920	2293	2425
Prunus serotina	5432	1665	116	313	1882
Acer rubrum	1477	1721	388	101	922
Fraxinus americana	358	624	688	885	639
Ostrya virginiana	428	1064	279	323	523
Quercus rubra	488	241	330	547	402
Carya cordiformis	298	306	400	262	317
Ulmus spp	123	764	166	209	316
Fraxinus nigra	189	228	155	313	221
Tilia americana	112	355	29	125	155
Ulmus americana	67	279	98	110	138
Abies balsamea	0	56	127	77	65
Tsuga canadensis	0	0	100	99	50
Betula alleghaniensis	0	138	0	1	35
Prunus pensylvanica	0	2	47	13	16
Populus tremuloides	0	20	0	21	10
Small Trees / Shrubs					
Rubus spp	32778	52588	202	678	21561
Prunus virginiana	3133	2878	2144	236	2098
Ribes cynosbati	2368	2496	576	698	1535
Ribes spp	421	522	29	253	306
Acer spicatum	0	4	893	0	224
Parthenocissus quinquefolia	0	480	29	225	183
Corylus spp	284	420	20	4	182
Sambucus racemosa	495	140	0	62	174
Dirca palustris	84	55	254	74	117
Carpinus caroliniana	0	180	14	102	74
Lonicera canadensis	22	5	143	70	60
Sambucus spp	52	22	30	36	35
Cornus alternifolia	6	13	0	93	28
Rubus hispidus	0	75	29	5	27
Diervilla lonicera	0	9	0	87	24
Cornus spp	0	7	0	37	11
Amelanchier spp	11	28	0	3	11

Table 2.2: Mean sapling density per ha by disturbance treatment and growth form.



	Mean Woody Sapling Density (per Ha) By Disturbance Treatment				
Tree Species	**Gap, Fence**	**Gap, No Fence**	**No Gap, Fence**	**No Gap, No Fence**	**Average**
Acer saccharum	908.3	339.2	254.5	326.0	457.0
Ostrya virginiana	269.4	197.8	161.6	191.2	205.0
Prunus serotina	155.6	159.3	0.0	0.0	78.7
Fraxinus spp	41.7	18.5	32.3	19.0	27.9
Carya cordiformis	19.4	42.3	24.2	5.9	23.0
Acer rubrum	77.8	21.3	0.0	0.0	24.8
Abies balsamea	0.0	4.9	36.4	30.8	18.0
Fraxinus americana	50.0	20.7	4.0	10.3	21.2
Betula alleghaniensis	44.4	9.6	0.0	2.9	14.2
Ulmus spp	22.2	13.3	0.0	2.9	9.6
Quercus rubra	11.1	13.3	0.0	0.0	6.1
Ulmus americana	5.6	13.3	0.0	1.5	5.1
Small Trees / Shrubs					
Prunus virginiana	144.4	92.9	0.0	0.0	59.3
Carpinus caroliniana	0.0	32.4	0.0	1.5	8.5
Sambucus spp	5.6	1.2	0.0	0.0	1.7

2.6: Figures

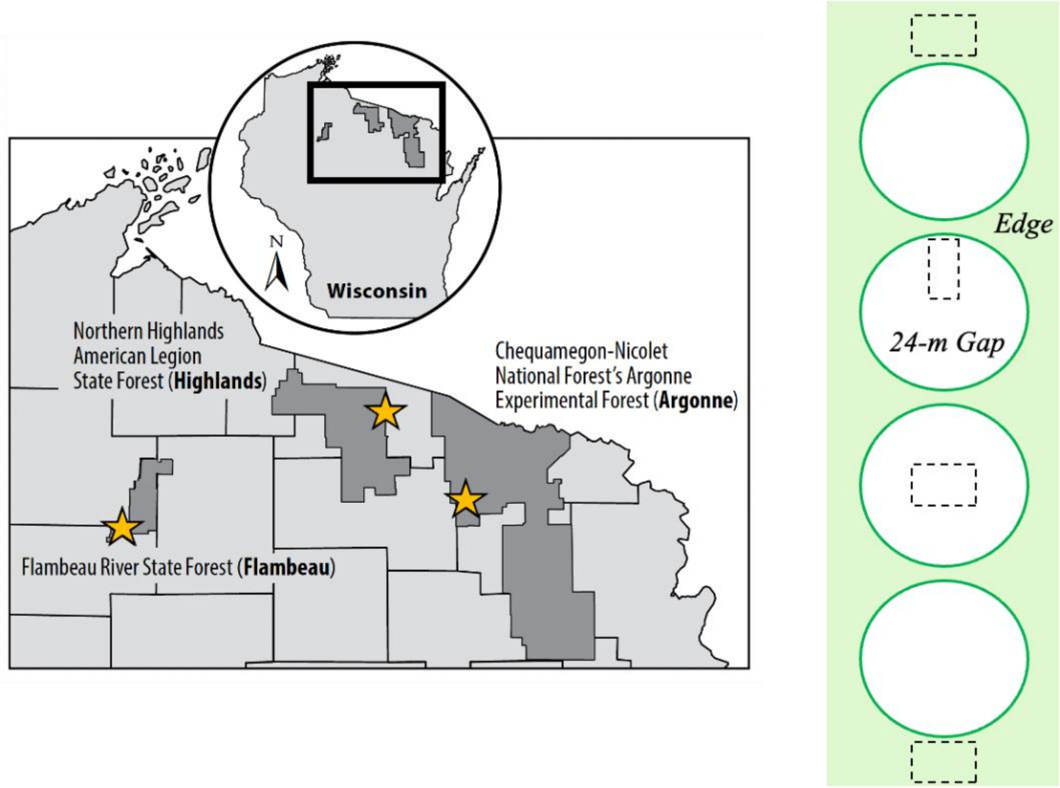


Fig. 2.1: Map illustrating the location of each Managed Old Growth Silviculture Study (MOSS) site, along with accompanying canopy gap and deer fencing organization.

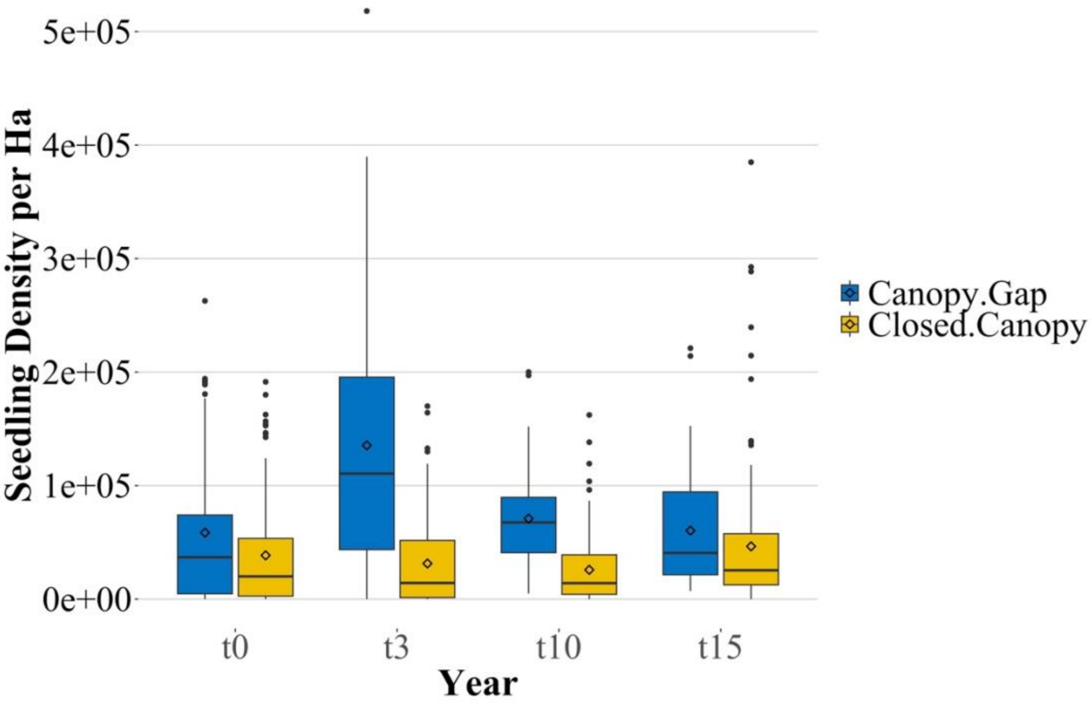


Fig. 2.2: Woody seedling density response to gap creation and fencing treatment combinations over time. Diamonds represent means while bold lines represent medians.

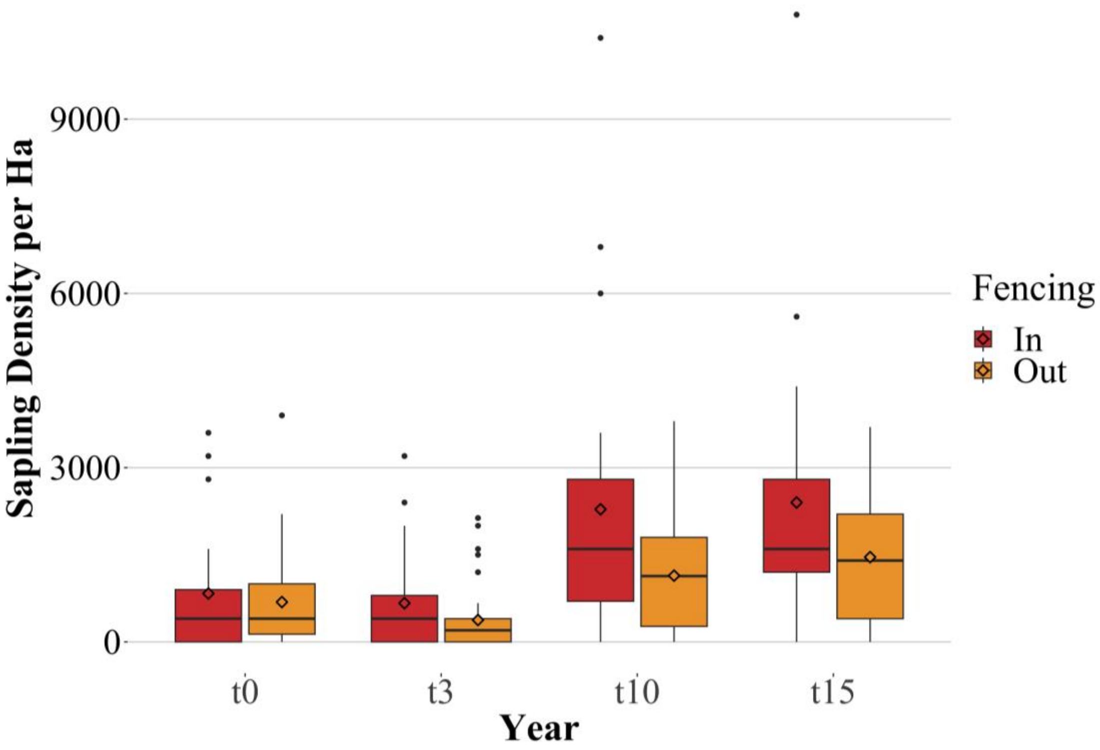


Fig. 2.3: Woody sapling density response to combined canopy gaps and deer fencing over time. All data for this figure is from areas where canopy gaps were established.

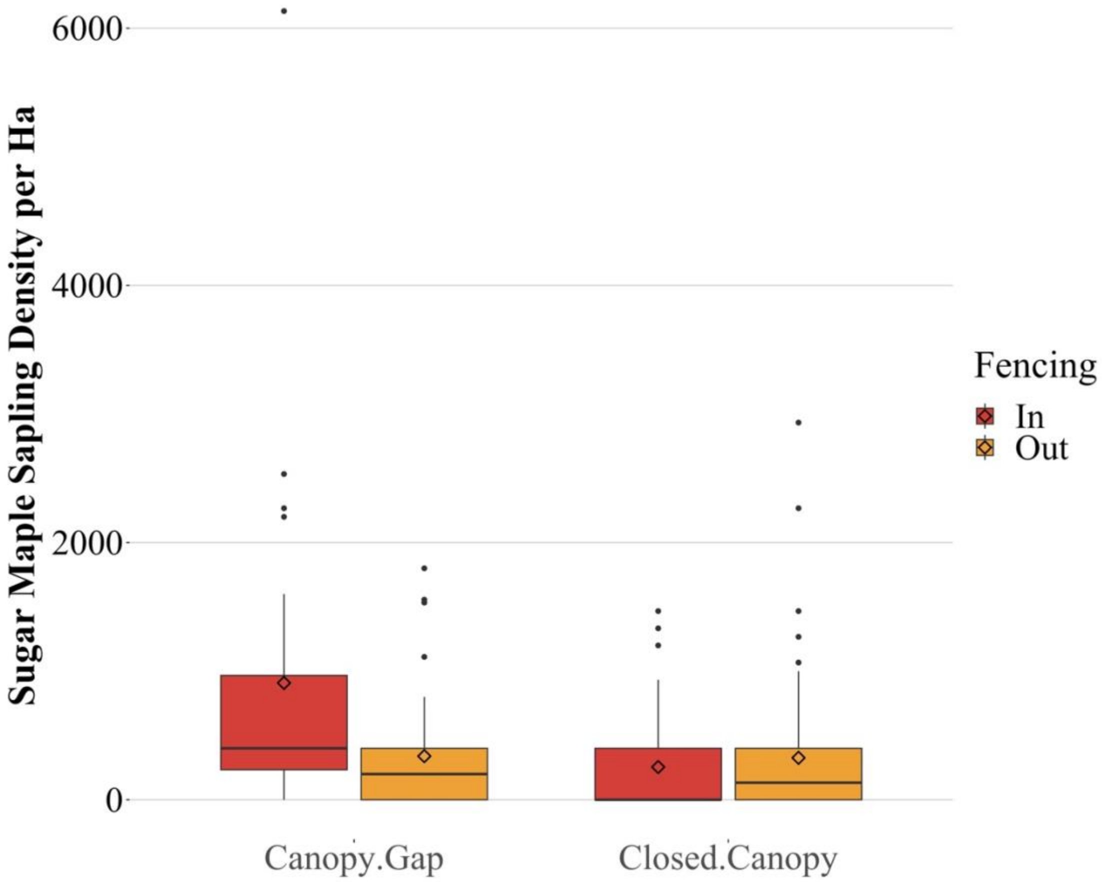


Fig. 2.4: Average sugar maple sapling density response to fencing and canopy gap treatments.

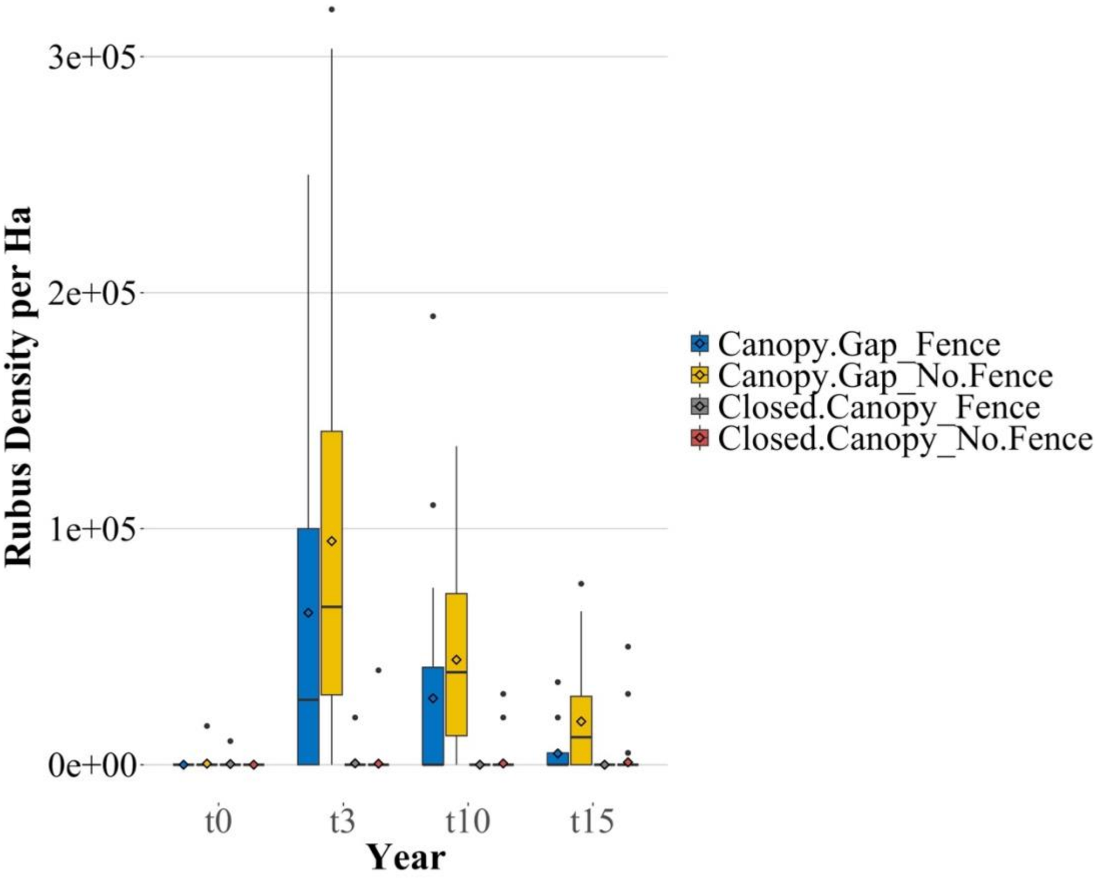


Fig. 2.5: *Rubus* response to combined gap and fencing treatments by year.

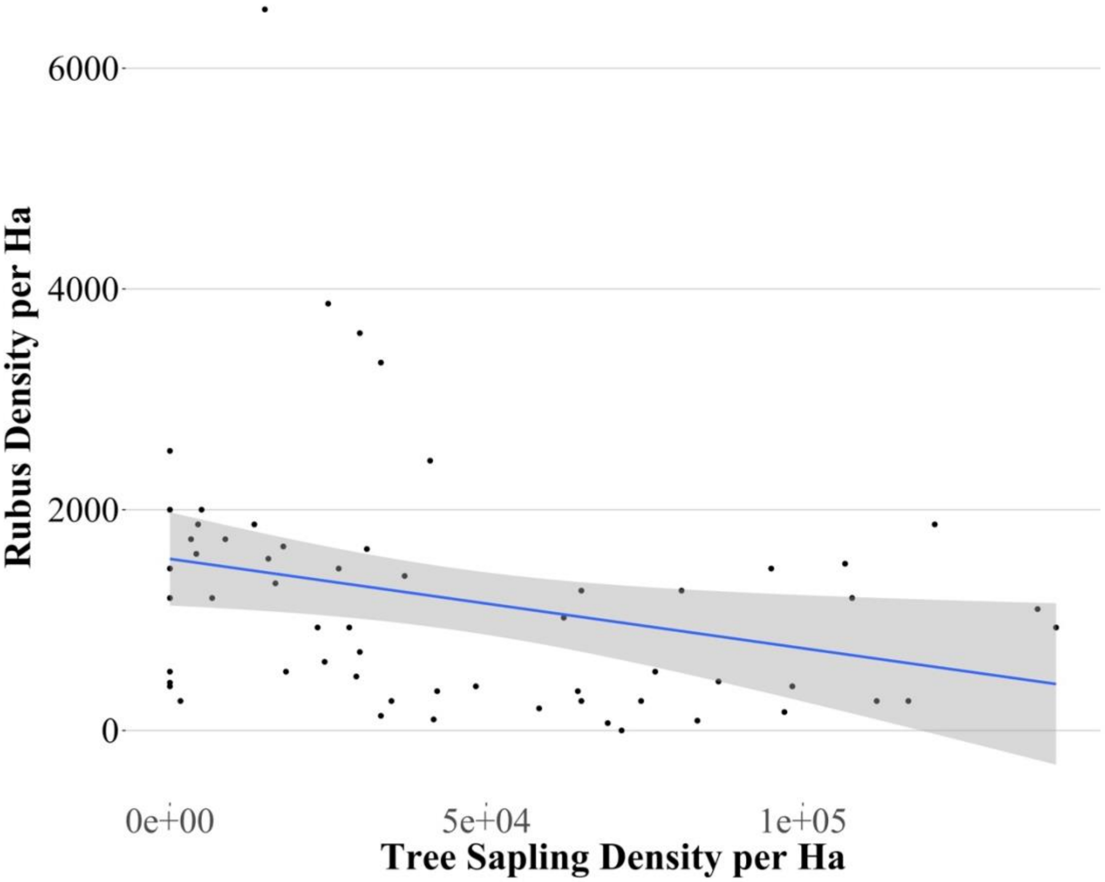


Fig. 2.6: Relationship between *Rubus* density per ha and tree sapling density per ha.

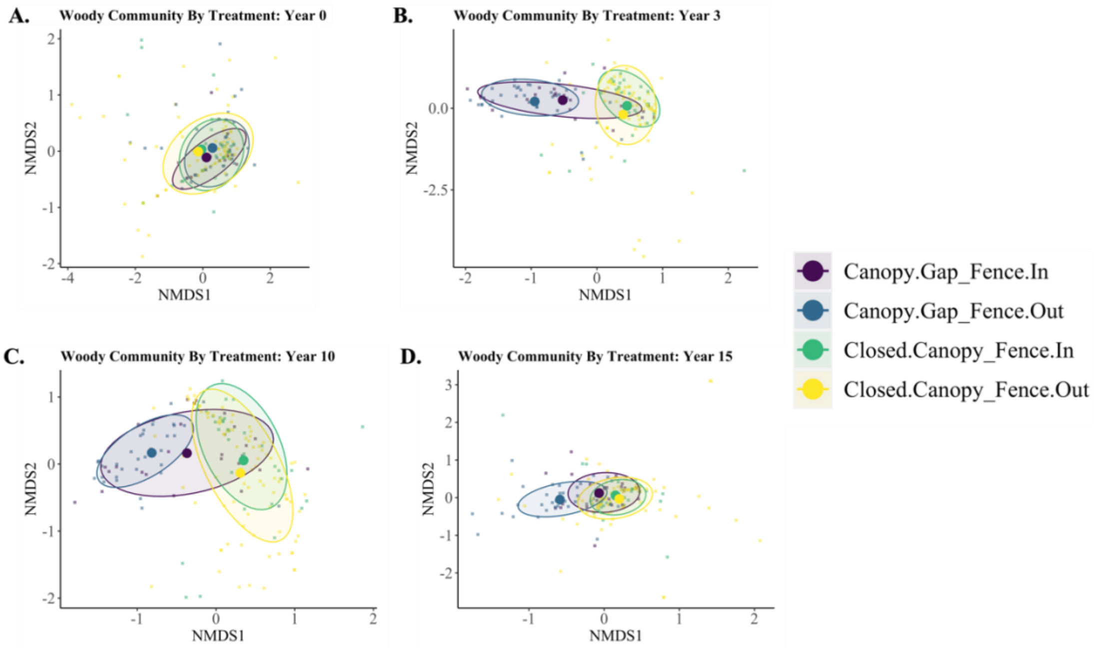


Fig. 2.7: NMDS showing woody community response to gap and fence treatments at pre-treatment (Year 0), year 3 post-treatment, year 10 post-treatment, and year 15 post-treatment.

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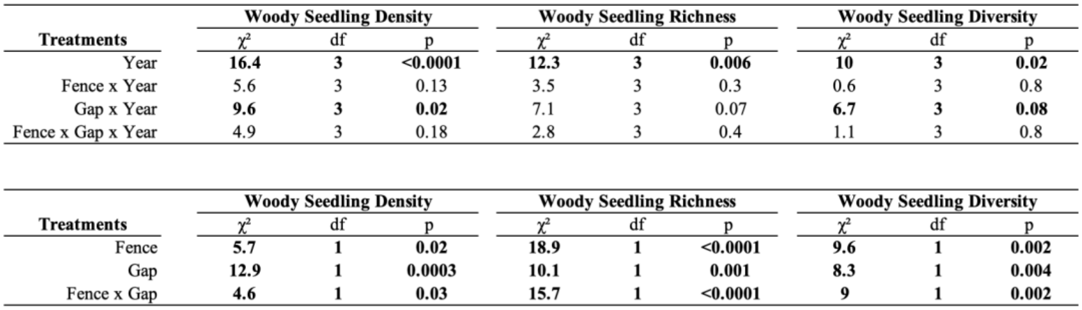
2.8: Supplementary Information

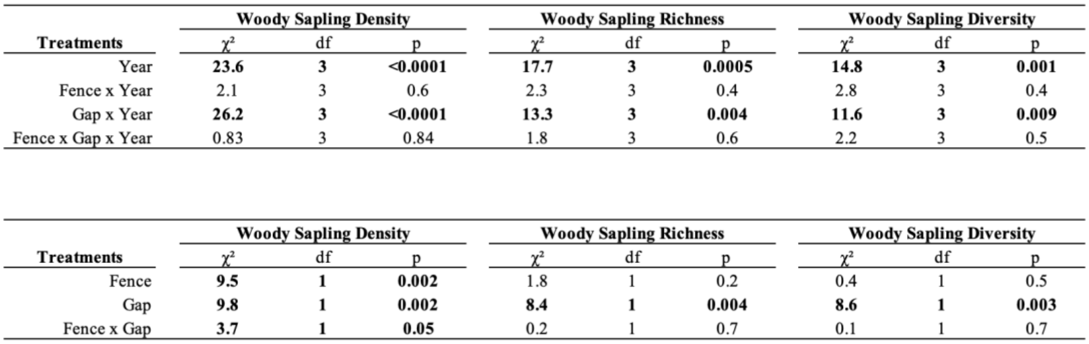
Table S2.1: Woody seedling density, richness, and diversity responses to year, fencing, and canopy gap treatments. Individual disturbance effects tested with variables averaged across years 3, 10, and 15.



	Woody Seedling Density			Woody Seedling Richness			Woody Seedling Diversity		
Treatments	χ^2	df	p	χ^2	df	p	χ^2	df	p
Year	**16.4**	**3**	**<0.0001**	**12.3**	**3**	**0.006**	**10**	**3**	**0.02**
Fence x Year	5.6	3	0.13	3.5	3	0.3	0.6	3	0.8
Gap x Year	**9.6**	**3**	**0.02**	7.1	3	0.07	**6.7**	**3**	**0.08**
Fence x Gap x Year	4.9	3	0.18	2.8	3	0.4	1.1	3	0.8

	Woody Seedling Density			Woody Seedling Richness			Woody Seedling Diversity		
Treatments	χ^2	df	p	χ^2	df	p	χ^2	df	p
Fence	**5.7**	**1**	**0.02**	**18.9**	**1**	**<0.0001**	**9.6**	**1**	**0.002**
Gap	**12.9**	**1**	**0.0003**	**10.1**	**1**	**0.001**	**8.3**	**1**	**0.004**
Fence x Gap	**4.6**	**1**	**0.03**	**15.7**	**1**	**<0.0001**	**9**	**1**	**0.002**

Table S2.2: Woody sapling density, richness, and diversity responses to year, fencing, and canopy gap treatments. Individual disturbance effects tested with variables averaged across years 3, 10, and 15.



	Woody Sapling Density			Woody Sapling Richness			Woody Sapling Diversity		
Treatments	χ^2	df	p	χ^2	df	p	χ^2	df	p
Year	**23.6**	**3**	**<0.0001**	**17.7**	**3**	**0.0005**	**14.8**	**3**	**0.001**
Fence x Year	2.1	3	0.6	2.3	3	0.4	2.8	3	0.4
Gap x Year	**26.2**	**3**	**<0.0001**	**13.3**	**3**	**0.004**	**11.6**	**3**	**0.009**
Fence x Gap x Year	0.83	3	0.84	1.8	3	0.6	2.2	3	0.5

	Woody Sapling Density			Woody Sapling Richness			Woody Sapling Diversity		
Treatments	χ^2	df	p	χ^2	df	p	χ^2	df	p
Fence	**9.5**	**1**	**0.002**	1.8	1	0.2	0.4	1	0.5
Gap	**9.8**	**1**	**0.002**	**8.4**	**1**	**0.004**	**8.6**	**1**	**0.003**
Fence x Gap	**3.7**	**1**	**0.05**	0.2	1	0.7	0.1	1	0.7

Table S2.3: Tree seedling and sapling density responses to year, fencing, and canopy gap treatments. Individual disturbance effects tested with variables averaged across years 3, 10, and 15.

	Tree Seedling Density			Tree Sapling Density		
Treatments	χ^2	df	p	χ^2	df	p
Year	6.3	3	0.1	**21.9**	**3**	**<0.0001**
Fence x Year	4.2	3	0.2	6.4	3	0.1
Gap x Year	0.7	3	0.9	**28.5**	**3**	**<0.0001**
Fence x Gap x Year	1.4	3	0.7	4	3	0.3

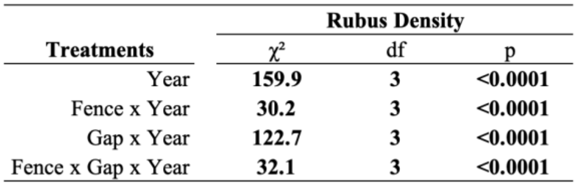
	Tree Seedling Density			Tree Sapling Density		
Treatments	χ^2	df	p	χ^2	df	p
Fence	0.2	1	0.7	**8.3**	**1**	**0.004**
Gap	0.3	1	0.6	**10.8**	**1**	**0.001**
Fence x Gap	0.12	1	0.7	**5**	**1**	**0.03**

Table S2.4: Sugar maple and ironwood seedling and sapling responses to year, fencing, and canopy gap treatments. Individual disturbance effects tested with variables averaged across years 3, 10, and 15.

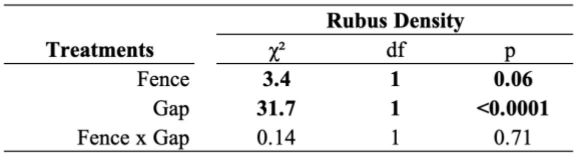
	Sugar Maple Seedling Density			Ironwood Seedling Density			Sugar Maple Sapling Density			Ironwood Sapling Density		
Treatments	χ^2	df	p	χ^2	df	p	χ^2	df	p	χ^2	df	p
Year	**17.4**	**3**	**0.0006**	**45.8**	**3**	**<0.0001**	2.9	3	0.4	0.39	3	0.9
Fence x Year	2.5	3	0.5	**8.8**	**3**	**0.03**	3.3	3	0.4	4.2	3	0.2
Gap x Year	**7.1**	**3**	**0.07**	**12.6**	**3**	**0.005**	2.3	3	0.5	2.4	3	0.5
Fence x Gap x Year	1.9	3	0.6	6.8	3	0.07	1.1	3	0.8	2.9	3	0.4

	Sugar Maple Seedling Density			Ironwood Seedling Density			Sugar Maple Sapling Density			Ironwood Sapling Density		
Treatments	χ^2	df	p	χ^2	df	p	χ^2	df	p	χ^2	df	p
Fence	1.1	1	0.3	3.3	1	0.07	**14.8**	**1**	**0.0001**	**7.7**	**1**	**0.005**
Gap	0.02	1	0.9	0.1	1	0.7	**3.3**	**1**	**0.07**	0.3	1	0.6
Fence x Gap	2.7	1	0.1	0.5	1	0.5	**5.6**	**1**	**0.02**	**5.2**	**1**	**0.02**

Table S2.5: Rubus density responses to year, canopy gap, and fencing treatments. Individual disturbance effects tested with variables averaged across years 3, 10, and 15.

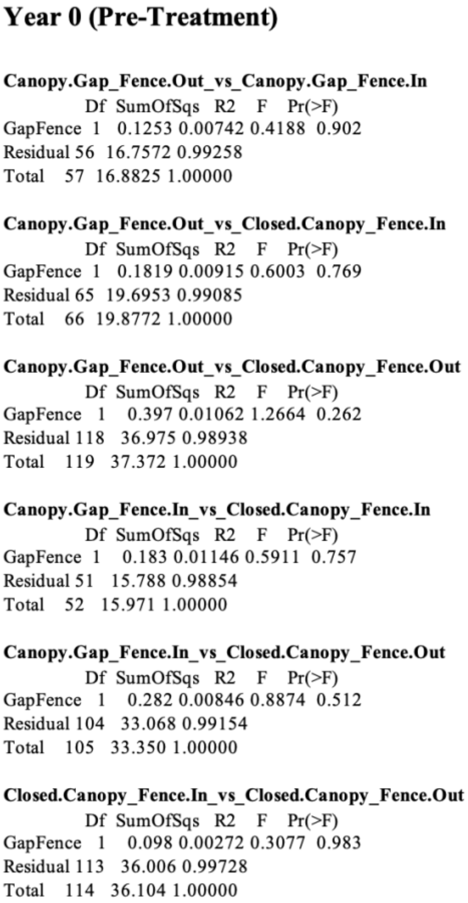


Treatments	Rubus Density		
	χ^2	df	p
Year	**159.9**	**3**	**<0.0001**
Fence x Year	**30.2**	**3**	**<0.0001**
Gap x Year	**122.7**	**3**	**<0.0001**
Fence x Gap x Year	**32.1**	**3**	**<0.0001**



Treatments	Rubus Density		
	χ^2	df	p
Fence	**3.4**	**1**	**0.06**
Gap	**31.7**	**1**	**<0.0001**
Fence x Gap	0.14	1	0.71

Table S2.6: Pairwise PERMANOVA comparison of pre-treatment communities.



Year 0 (Pre-Treatment)

Canopy.Gap_Fence.Out_vs_Canopy.Gap_Fence.In

```
             Df  SumOfSqs   R2     F    Pr(>F)
GapFence  1    0.1253 0.00742 0.4188  0.902
Residual 56  16.7572 0.99258
Total    57  16.8825 1.00000
```

Canopy.Gap_Fence.Out_vs_Closed.Canopy_Fence.In

```
             Df  SumOfSqs   R2     F    Pr(>F)
GapFence  1    0.1819 0.00915 0.6003  0.769
Residual 65  19.6953 0.99085
Total    66  19.8772 1.00000
```

Canopy.Gap_Fence.Out_vs_Closed.Canopy_Fence.Out

```
             Df  SumOfSqs   R2     F    Pr(>F)
GapFence   1     0.397 0.01062 1.2664  0.262
Residual 118   36.975 0.98938
Total    119   37.372 1.00000
```

Canopy.Gap_Fence.In_vs_Closed.Canopy_Fence.In

```
             Df  SumOfSqs   R2     F    Pr(>F)
GapFence  1     0.183 0.01146 0.5911  0.757
Residual 51    15.788 0.98854
Total    52    15.971 1.00000
```

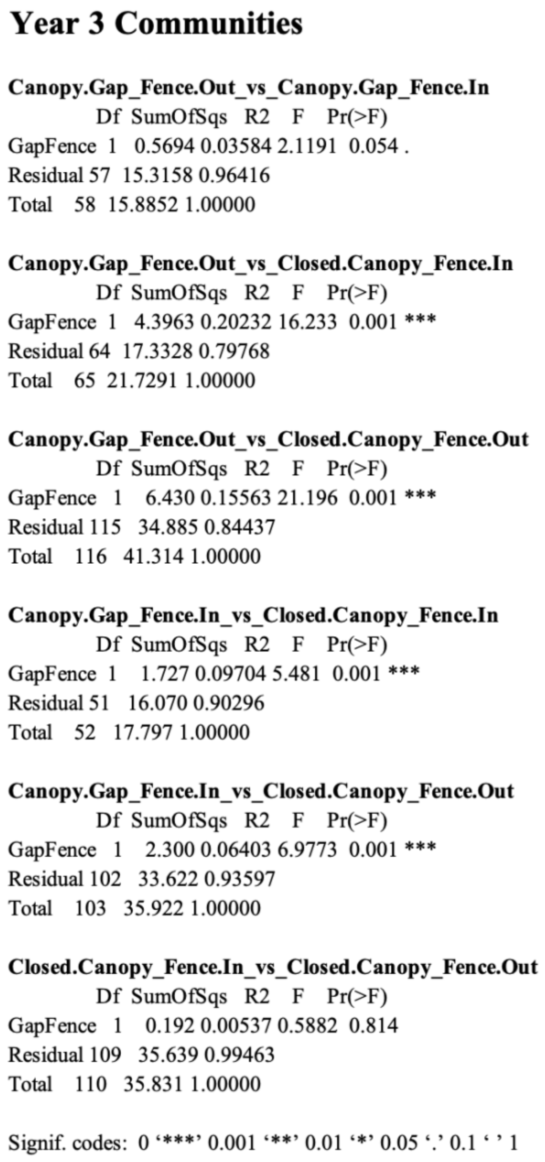
Canopy.Gap_Fence.In_vs_Closed.Canopy_Fence.Out

```
             Df  SumOfSqs   R2     F    Pr(>F)
GapFence   1     0.282 0.00846 0.8874  0.512
Residual 104   33.068 0.99154
Total    105   33.350 1.00000
```

Closed.Canopy_Fence.In_vs_Closed.Canopy_Fence.Out

```
             Df  SumOfSqs   R2     F    Pr(>F)
GapFence   1     0.098 0.00272 0.3077  0.983
Residual 113   36.006 0.99728
Total    114   36.104 1.00000
```

Table S2.7: Pairwise PERMANOVA comparison of communities at 3 years post-treatment.



Year 3 Communities

Canopy.Gap_Fence.Out_vs_Canopy.Gap_Fence.In
```
          Df SumOfSqs   R2    F    Pr(>F)
GapFence  1   0.5694 0.03584 2.1191  0.054 .
Residual 57  15.3158 0.96416
Total    58  15.8852 1.00000
```

Canopy.Gap_Fence.Out_vs_Closed.Canopy_Fence.In
```
          Df SumOfSqs   R2    F    Pr(>F)
GapFence  1   4.3963 0.20232 16.233  0.001 ***
Residual 64  17.3328 0.79768
Total    65  21.7291 1.00000
```

Canopy.Gap_Fence.Out_vs_Closed.Canopy_Fence.Out
```
          Df SumOfSqs   R2    F    Pr(>F)
GapFence   1    6.430 0.15563 21.196  0.001 ***
Residual 115   34.885 0.84437
Total    116   41.314 1.00000
```

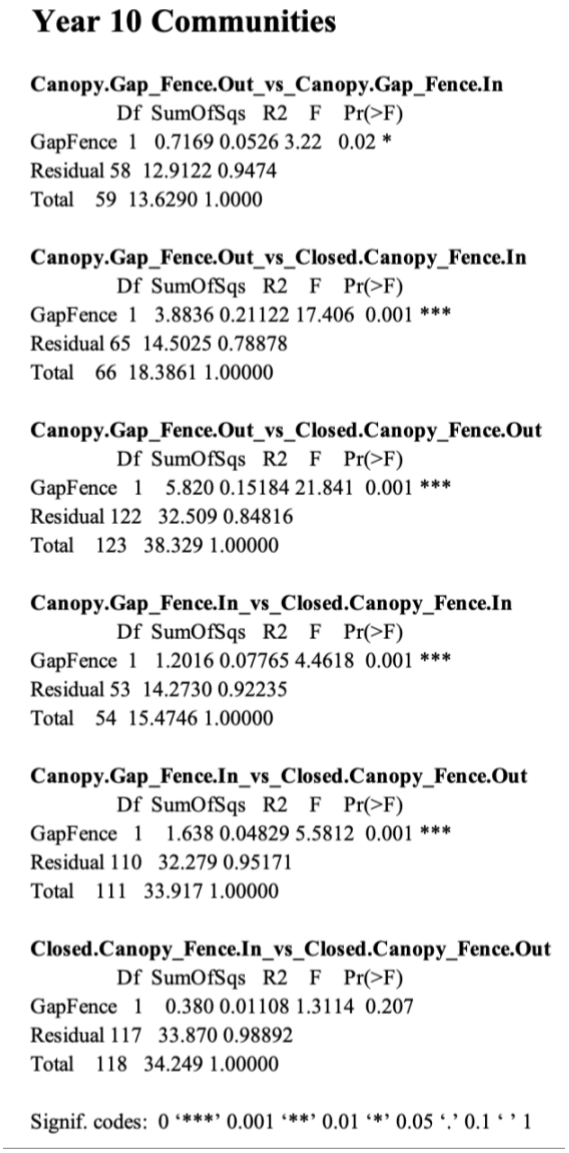
Canopy.Gap_Fence.In_vs_Closed.Canopy_Fence.In
```
          Df SumOfSqs   R2    F    Pr(>F)
GapFence  1    1.727 0.09704 5.481  0.001 ***
Residual 51   16.070 0.90296
Total    52   17.797 1.00000
```

Canopy.Gap_Fence.In_vs_Closed.Canopy_Fence.Out
```
          Df SumOfSqs   R2    F    Pr(>F)
GapFence   1    2.300 0.06403 6.9773  0.001 ***
Residual 102   33.622 0.93597
Total    103   35.922 1.00000
```

Closed.Canopy_Fence.In_vs_Closed.Canopy_Fence.Out
```
          Df SumOfSqs   R2    F    Pr(>F)
GapFence   1    0.192 0.00537 0.5882  0.814
Residual 109   35.639 0.99463
Total    110   35.831 1.00000

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Table S2.8: Pairwise PERMANOVA comparison of communities at 10 years post-treatment.



Year 10 Communities

Canopy.Gap_Fence.Out_vs_Canopy.Gap_Fence.In

```
          Df SumOfSqs  R2    F    Pr(>F)
GapFence  1   0.7169 0.0526 3.22   0.02 *
Residual 58  12.9122 0.9474
Total    59  13.6290 1.0000
```

Canopy.Gap_Fence.Out_vs_Closed.Canopy_Fence.In

```
          Df SumOfSqs  R2    F    Pr(>F)
GapFence  1   3.8836 0.21122 17.406  0.001 ***
Residual 65  14.5025 0.78878
Total    66  18.3861 1.00000
```

Canopy.Gap_Fence.Out_vs_Closed.Canopy_Fence.Out

```
          Df SumOfSqs  R2    F    Pr(>F)
GapFence  1    5.820 0.15184 21.841  0.001 ***
Residual 122   32.509 0.84816
Total    123   38.329 1.00000
```

Canopy.Gap_Fence.In_vs_Closed.Canopy_Fence.In

```
          Df SumOfSqs  R2    F    Pr(>F)
GapFence  1   1.2016 0.07765 4.4618  0.001 ***
Residual 53  14.2730 0.92235
Total    54  15.4746 1.00000
```

Canopy.Gap_Fence.In_vs_Closed.Canopy_Fence.Out

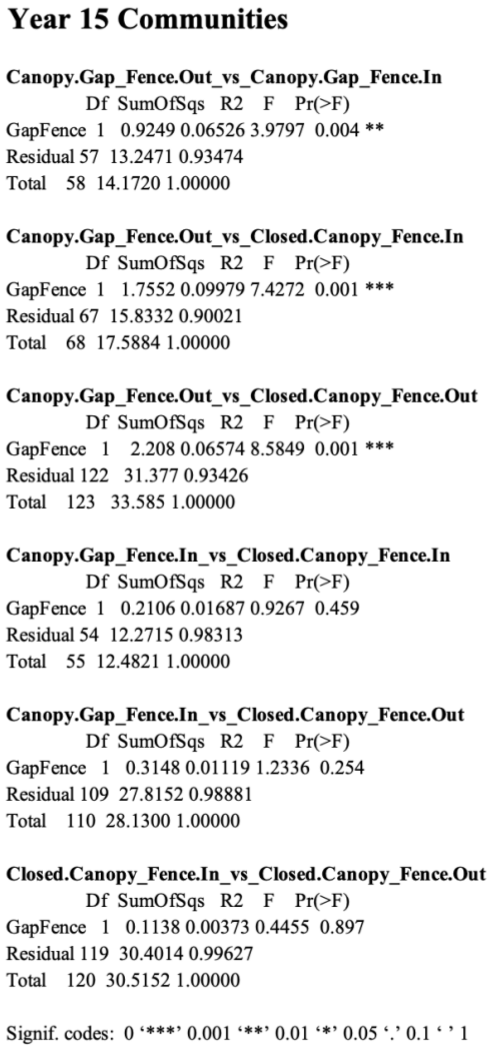
```
          Df SumOfSqs  R2    F    Pr(>F)
GapFence  1    1.638 0.04829 5.5812  0.001 ***
Residual 110   32.279 0.95171
Total    111   33.917 1.00000
```

Closed.Canopy_Fence.In_vs_Closed.Canopy_Fence.Out

```
          Df SumOfSqs  R2    F    Pr(>F)
GapFence  1    0.380 0.01108 1.3114  0.207
Residual 117   33.870 0.98892
Total    118   34.249 1.00000

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Table S2.9: Pairwise PERMANOVA comparison of communities at 15 years post-treatment.



```
Year 15 Communities

Canopy.Gap_Fence.Out_vs_Canopy.Gap_Fence.In
            Df  SumOfSqs   R2    F    Pr(>F)
GapFence  1   0.9249 0.06526 3.9797  0.004 **
Residual 57  13.2471 0.93474
Total    58  14.1720 1.00000

Canopy.Gap_Fence.Out_vs_Closed.Canopy_Fence.In
            Df  SumOfSqs   R2    F    Pr(>F)
GapFence  1   1.7552 0.09979 7.4272  0.001 ***
Residual 67  15.8332 0.90021
Total    68  17.5884 1.00000

Canopy.Gap_Fence.Out_vs_Closed.Canopy_Fence.Out
            Df  SumOfSqs   R2    F    Pr(>F)
GapFence   1    2.208 0.06574 8.5849  0.001 ***
Residual 122   31.377 0.93426
Total    123   33.585 1.00000

Canopy.Gap_Fence.In_vs_Closed.Canopy_Fence.In
            Df  SumOfSqs   R2    F    Pr(>F)
GapFence  1   0.2106 0.01687 0.9267  0.459
Residual 54  12.2715 0.98313
Total    55  12.4821 1.00000

Canopy.Gap_Fence.In_vs_Closed.Canopy_Fence.Out
            Df  SumOfSqs   R2    F    Pr(>F)
GapFence   1   0.3148 0.01119 1.2336  0.254
Residual 109  27.8152 0.98881
Total    110  28.1300 1.00000

Closed.Canopy_Fence.In_vs_Closed.Canopy_Fence.Out
            Df  SumOfSqs   R2    F    Pr(>F)
GapFence   1   0.1138 0.00373 0.4455  0.897
Residual 119  30.4014 0.99627
Total    120  30.5152 1.00000

Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Chapter 3:

Multiple Disturbances, Multiple Legacies:
Combined Fire, Canopy Gaps, & Deer Change the Forest Seed Bank

Authors: Reed S.P., Royo A.A., Carson W. A., Olmsted C.F., Frelich L.E., Reich P.B.

Summary:

Whether multiple disturbances can lead to multiple legacies in forest ecosystems is a question that has primarily been tested using tree seedlings and saplings. However, there are many other strata of the forest that may be even more sensitive to disturbance and show strong legacies depending on the combination of disturbance. Forest seed banks represent a highly undervalued, but optimal study system to investigate how multiple disturbances can change vegetative legacies. Seed banks are an important reservoir of biodiversity, are sensitive to disturbance, and can play a critical role in forest development. They are also likely influenced by the restoration of pre-colonial disturbances such as understory fire, mid-sized canopy gaps, and low deer populations. However, whether these combinations of disturbance can lead to changes in the seed bank is undetermined. To this end, we used a long-term, multi-disturbance experiment in West Virginia, U.S.A. that factorially manipulates understory fire, deer fencing, and canopy gaps. Thirteen years after disturbance initiation, we sampled and germinated the seed bank from each disturbance treatment. Over a decade since treatments were first applied, we found that seed banks differed depending on the combination of fire, gaps, and deer fencing. Seed abundance showed a synergistic increase with combined fire and gaps, while seed species richness increased with burning. Fire and deer decreased seed Shannon diversity and combined fire, deer fencing, and canopy gaps led to dominance of the seed bank by *Rubus*, likely reflecting the legacy of extant plants that grew immediately after disturbance. Lastly, seed communities were always distinct from extant understory species in the most disturbed plots but were particularly similar to understory species from later years of sampling. Each of these findings represent a different seed

bank legacy depending on the combination of disturbance, adding to our limited understanding of how disturbance influences forest seed bank dynamics. Our study highlights the many indirect and unexpected ways that multiple disturbances can change an understudied, but influential, component of the forest for well over a decade.

3.1: Introduction

As disturbances become more frequent and severe with a changing climate and increased human activity, there is increasing likelihood of disturbance interactions (Dale et al. 2000, Turner 2010). Multiple, interacting disturbances can lead to vastly different ecological communities than individual disturbances (Abrams et al. 1985, Buma 2015; Graham et al. 2021). These communities represent a disturbance legacy (Cuddington 2011), which is broadly defined as the adaptations, individuals, and materials that persist on the landscape following a disturbance. 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 (Johnstone et al. 2016). Disturbance legacies can have important ramifications for long-term ecological composition and structure (Cuddington 2011; Seidl et al. 2014). For instance, windstorms knock over trees, leaving behind a legacy of woody fuel, which could then lead to more intense wildfires (Anoszko et al. 2022). However, our understanding of how disturbance legacies change with multiple disturbances is limited, despite their importance for ecological succession and development (Seidl & Turner 2022).

In temperate forest ecosystems, the soil seed bank often represents a particularly important, but understudied, disturbance legacy. Seed banking is a reproductive adaptation that allows plants to persist belowground as ungerminated seeds, wherein the soil serves as a buffer from aboveground disturbances (Baskin & Baskin 2022). 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. (Ashton et al. 1998, Hille Ris Lambers et al. 2005, Royo and Ristau 2013). When a disturbance creates optimal conditions for certain seed species, they can germinate, making the seed bank both a material and information legacy. These plants eventually release seeds and reestablish 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 (Seidl & Turner 2022). Thus, disturbances shape vegetation, which indirectly changes the resulting seed bank and either reinforces or creates a new legacy, as these seeds are likely to germinate following future disturbances. These changes in the seed bank can then have serious ecological ramifications. For example, rampant timber harvesting and slash wildfires in the United States during the late 19th and early 20th century 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 with tree harvesting throughout the eastern U.S. (Dunn et al. 1983, Peterson & Carson 1996).

Now, due to past forest mismanagement and altered disturbance regimes, eastern U.S. temperate forests are radically different from pre-colonial forests (Leckie et al. 2000; Webster et al. 2018; Vander Yacht et al. 2020). This scenario is particularly acute in Appalachian hardwood forests, which have lost regenerating oak species and are transitioning to wetter, maple-dominated systems (Nowacki & Abrams 2008). This transition from oak to maple species was largely caused by the forced removal of indigenous peoples and cultural burning (Abrams et al. 2021), which allowed for industrial forest liquidation in the late 19th and early 20th century, followed by slash wildfires, and then a century of fire exclusion (Lafon et al. 2017). As a result, Appalachian forests became dominated by even-aged stands with few large (20 to 25-m +

diameter) canopy gaps and very few understory fires (Clebsch & Busing 1989; Nowacki and Abrams 2008; Raymond et al. 2009). Within the Monongahela National Forest, our area of interest, the fire return interval is now over 10,000 years as opposed to the historic 1-to-2-decade fire return interval with cultural burning (Lafon et al 2017). Meanwhile, white-tailed deer (*Odocoileus virginianus*) populations have increased dramatically above historical baselines (between 4 and 8 deer/km^2) in most of the eastern United States, driving ecological change depending on their population density (Horsley et al. 2003; Reed et al. 2022). To reverse the legacies of historic mismanagement and sustain oak-dominated plant communities, forest managers are reintroducing pre-colonial disturbances like prescribed burns, tree harvesting to emulate old growth characteristics, and deer population control through fencing or hunting (Raymond et al. 2009; Nuttle et al. 2013).

Disturbances like prescribed burns, tree harvesting, and deer browsing each provide a unique and important opportunity for new vegetation to grow and for the seed bank to change (Ma et al. 2021; Gioria et al. 2022; Fig. 3.1). Prescribed fires clear plant material, catalyzing seed germination with increased light, heat, smoke, and nutrients (Keeley & Fotheringham 2000). In fire-prone ecosystems, Pausas and Lamont (2022) found that ≈42% of seed banking species are adapted to germinate with heat or smoke. Canopy gaps increase understory resources like light, soil temperature, and soil moisture, which are critical for seeds to germinate (Pakeman & Small 2005; Muscolo et al. 2014). Both gaps and fire result in a temporary reduction in the seed bank as plants germinate, but with enough time, newly established vegetation will grow, reproduce, and replenish the seed bank (Auld & Denham 2006; Shinoda & Aksaka 2020). White-tailed deer overpopulation can lead to low understory plant diversity and density, which may reduce the abundance and diversity of seed banking species (Beauchamp et al. 2013; Tamura 2019). Deer browsing can also stress plants, leading to resource reallocation and reduced seed production, which could then reduce seed bank density (Brody & Irwin 2012). Other studies have found that moderate deer populations have no effect on the seed bank's abundance or diversity (Levine 2012). While in regions where deer populations are low and similar to historic estimates, deer have been shown to increase understory diversity by reducing competitive ruderal species, which could then lead to a more diverse seed bank (Royo et al. 2010).

Despite the increasing prevalence and co-occurrence of these reintroduced pre-colonial disturbances in the eastern U.S., our understanding of how individual and combined fires, canopy gaps, and white-tailed deer change long-term forest seed banks is unclear. 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 historic 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 fire via controlled burns, canopy gap creation via girdling and herbicide injection, and deer density via fenced exclosures (Fig. 3.2). 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 *in situ* vegetation at different time points.

We expected 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 and the low deer density in our study site to lead to a modest increase seed bank diversity and density, mirroring the increased aboveground plant diversity with gaps and deer found by Royo et al. (2010) (**H2**). We hypothesized that fire combined with either canopy gaps or low intensity deer browsing would cause the greatest increases in seed bank abundance, diversity, and community composition (**H3**). The restoration of pre-colonial disturbances should favor a more diverse and abundant seed bank since many of the plant species in our area of interest are adapted to these drivers of change. Lastly, we expected the seed bank community to be most similar to extant vegetation that was sampled closer to when the seed bank was surveyed due to the known transience of many seeds (**H4**).

3.2: Methods

Study Site

To test how the forest seed bank responds to multiple disturbances, we experimentally manipulated prescribed fire, deer presence, and canopy gap creation in four replicate Appalachian hardwood stands in central West Virginia, USA (Fig. 3.2). This experiment was established in 2000, 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, between 670 to 810 m in elevation, and in predominantly upland locations. 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.) (Royo et al. 2010). The herbaceous layer in this forest is highly diverse, with up to 461 plant species recorded (Coxe et al. 2006). Mean annual temperature is 9°C with an average of 145 cm of precipitation. For further details about the pre-existing manipulative experiment, please see Royo et al. (2010).

Disturbance Treatments

Our experimental design was a split-plot factorial (Fig. 3.2), with each stand being split in half and randomly assigned a burn treatment (burned or unburned). In each burned and unburned half stand, eight treatment plots were established (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). All canopy gaps were created in June 2000 by girdling multiple canopy-dominant trees. By summer 2001, all trees were standing dead, fallen, or near-dead. Gaps were mid-sized (284 m^2 ± 16 SE) and meant to simulate those found in old-growth forests (Collins and Carson 2003). Each respective understory fire was then lit 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 (Royo et al. 2010). Fires took place during the historic peak spring fire season (Nuttle et al. 2013), during understory bud-break but prior to canopy bud-break. Historically, fire return intervals in this region are between 25 and 30 years (Collins & Carson 2003; Guyette et al. 2012). Considering that there was a minimum of 20 m of space between burned and unburned regions, we assume that smoke did not lead to a substantial germination event within unburned plots.

Seed Sampling & Germination

Five permanent 1-m^2 sampling quadrats were randomly placed 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 gap treatment plot could not be found in 2013, thus n = 63. In early June 2013, soils were sampled and seeds were germinated using a standard seedling emergence methodology (Brown 1992). Following the recommendations of Plue and Hermy (2012), we sampled ca. 3% of the quadrat surface area to represent seed bank abundance and diversity. Seed bank samples were collected at each corner of the five quadrats using a 5-cm long section of a 10-cm PVC pipe (78.5 cm^2 x 4 = 314 cm^2 soil sampled per quadrat). All 20 soil cores per treatment plot were 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 x 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. All 189 trays (63 treatment plots x 3 subsamples) were then watered daily and occasionally rotated in order to standardize growing light. All germinants were identified to species or genera depending on life form, counted, and removed from the tray. After 5 months, all trays were subjected to a 90-day, 5º C cold stratification period, after which trays were returned to the greenhouse for another germination phase. Although our unit of measure was germinants, from this point on we use either “seeds” or “seed bank” to describe our results, as the latter is a proxy for the former.

Statistical Analysis

For operational purposes we define seed species richness as the total number of species found across the three trays representing a single treatment plot. Similarly, abundance is defined as the total number of germinants found across the three trays. Differences in richness and abundance were tested using a generalized linear mixed effects model (GLMM) with either Poisson or quasi-Poisson distributions in the glmmTMB package (Brooks et al. 2017). Seed Shannon diversity was calculated within the vegan package based on species abundances per plot (Oksanen et al. 2022) and primarily tested using a linear mixed effects model in the lme4 package (Lenth et al. 2022). Graminoid seed Shannon diversity was tested using a GLMM with a Tweedie distribution. 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). All models were then compared using AICc and BIC (Burnham and Anderson 2004). We report the results from models with the lowest AICc and BIC. For our random effects, treatment plot was nested within experimental site. If models failed to converge, we just used experimental site as a random effect. All model assumptions were tested using the ‘DHARMa’ package (Hartig 2017), while post-hoc tests were done with the ‘emmeans’ package using a Bonferroni correction (Lenth et al. 2022).

Differences in community composition between treatments and between years were tested using the ‘adonis2’ PERMANOVA function, while community dispersion was checked with the ‘betadisper’ function in order to meet the assumptions of PERMANOVA (Oksanen et al. 2022). Differences between communities were visualized in a 3-dimensional NMDS to keep stress below 0.20. Indicator species were evaluated with vegan’s ‘multipatt’ function. When comparing above and belowground communities 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*). Species that did not occur in at least 3 plots were removed from multivariate analysis. All yearly plant abundance data were converted to presence-absence format prior to analysis. Similar to Plue et al. (2021) we used Raup-Crick similarity within PERMANOVA to test for community differences between germinants and extant plants at each time point.

3.3: Results

A total of 3,642 seedlings germinated in our trials (average of 310 germinants / m^2), representing at least 59 different taxa, with 38 forb species, 8 shrub species, 7 graminoid species, 5 tree species, and 1 species of vine (Table 3.1). *Rubus* spp. accounted for 28% of total germinants, followed by *Betula* spp. (9%), *Ageratina altissima* (6%), *Aralia Spinosa* (5%), and *Robinia psuedoacacia* (5%) as the next most common species to germinate. Among all of the germinating seed taxa, 9 species were non-native, accounting for 16% of all taxa. Non-native species’ proportional abundance among all germinants was low, with non-natives 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, was found in the entire seed bank survey.

Individual Disturbance

Disturbance effects on the seed bank were generally greatest when multiple disturbances were combined. However, in several instances, models with a single disturbance were best suited to describe seed variables. We found that fire increased seed richness from an average of 10.7 ± 0.5 species to 12.9 ± 0.62 species ($\chi^2 = 6.4$, df =1, $p = 0.01$; Table 3.2), while fencing and gaps had no influence on seed species richness. Certain seed life forms responded strongly to individual disturbances as well. Fire increased forb seed richness from 3.6 ± 0.4 species to 6.4 ± 0.5 species ($\chi^2 = 19.9$, df = 1, $p < 0.0001$). Similarly, burning increased forb seed Shannon diversity by 45% ($F_{1, 58} = 7.9$, $p = 0.003$). In contrast, fire led to a 40% decrease in shrub seed diversity ($F_{1, 58.1} = 9.5$, $p = 0.007$). Fire also decreased tree seed abundance by 33% ($\chi^2 = 6.4$, df = 1, $p = 0.02$; Table 3.3).

Canopy Gaps & Fencing:

Canopy gaps and fencing alone did not influence seed abundance, diversity, or species richness. Plots with both canopy gaps and fencing led to a different plant community than areas without either gaps or fencing ($F = 2.7$, $p = 0.0004$; Fig. 3.6c). Fencing and canopy gap treatments alone did not cause differences in the plant community when compared to one another or to areas without gaps or fencing (Fig. 3.6c).

Fire & Gaps:

The effects of fire on seed abundance were magnified with a gap overhead. Fire under a closed canopy led to a 63% increase in mean seed abundance in comparison to unburned plots under a closed canopy, while canopy gaps in unburned areas led to a 19% increase in mean seed abundance in comparison to closed canopies in unburned plots. When fire and gaps were combined there was a 205% increase in seed abundance in comparison to unburned plots with no canopy gap (t = 6.8, $p < 0.0001$; Fig. 3.3). Fire and gaps also had an interactive effect on forb seed abundance ($\chi^2 = 7.7$, df = 1, p = 0.006), wherein burned plots with gaps increased forb seed abundance by 175% in comparison to burned plots with a closed canopy (z = 3.5, $p < 0.001$; Fig. 3.4b). Gaps in burned areas decreased vine seed abundance by 75% in comparison to burned plots with no gap overhead (z = -2.7, $p < 0.006$), while unburned gaps increased did not influence vine seed abundance in comparison to unburned areas with no canopy gaps.

The combination of fire with a canopy gap led to highly distinct communities in comparison to unburned plots with closed canopies and unburned plots with a canopy gap (F = 2.2, p = 0.001; Fig. 3.6b). Canopy gaps in unburned plots did not influence seed communities. When comparing seed communities to extant plants in burned plots with a canopy gap, the seed community was significantly different from the extant plant community at every measured time point (2000, 2001, 2002, 2006, 2013; Table S3.2; Fig. 3.7a). There were no indicator species for combined fire and gap treatments, whereas the indicator for unburned plots with a gap overhead was *Sassafrass albidum* (p = 0.07).

Fire & Fencing:

There was a weak interaction between fencing and fire on seed bank Shannon diversity ($F_{1, 56.1} = 2.7$, $p = 0.1$), wherein fencing and burning led to 13% lower seed diversity than unfenced and burned plots ($t = -3.3$, df = 56.2, $p = 0.002$; Fig. 3.5). Fire and fencing had an interactive effect on shrub seed abundance ($\chi^2 = 3.5$, df =1, $p = 0.06$), with burned and fenced plots leading to 750% greater shrub seed abundance in comparison to unburned plots without a fence ($z = 6$, $p < 0.0001$; Fig. 3.4a). Fencing in burned plots decreased graminoid seed abundance by 62% in comparison to burned plots without fencing ($z = -2.2$, $p = 0.03$), while there was no difference in graminoid abundance between fencing treatments in unburned plots.

Seed communities in burned plots with fencing were highly distinct from unburned plots with and without fencing ($F = 6.8$, df =1, $p < 0.001$; Fig. 3.6a). Burned plots with and without fencing treatments also formed distinct communities from one another ($F = 3.3$, df = 1, $p < 0.004$). Fencing in unburned plots did not significantly influence seed communities. The seed community in the fire and fenced treatments was significantly different from the extant plant community at every measured time point (2000, 2001, 2002, 2006, 2013; Table S3.1; Fig. 3.7b). *Viola rotundifolia* ($p = 0.06$) was the primary indicator for burned and fenced plots.

Fire, Gaps, & Fencing:

There were no significant three-way interactions between fire, gaps, and fencing on total seed bank abundance, richness, or diversity. However, these disturbances had a slight three-way interaction on *Rubus* abundance, the most common seed germinant in our trials ($\chi^2 = 3.0$, df =1, $p = 0.08$). Burning only increased *Rubus* seed abundance with a gap or fencing present, with fire, fencing, and canopy gaps causing a 1432% increase in *Rubus* seed abundance in comparison to unburned plots with no fencing and no canopy gaps (z = 6.1, p < 0.0001). In addition, burned areas with gaps and fencing had several indicator species, including *Rubus* (p = 0.005), *Phytolacca americana* (p = 0.02), *Sambucus* (p = 0.02), and *Aralia spinosa* (p = 0.06). In contrast, burned areas with gaps and deer had *Ageratina altissma* (p = 0.001), *Carex* spp. (p=0.007), and *Verbena urticifolia* (p = 0.03) as primary indicator species.

3.4: Discussion

Using a unique, long-term experiment that manipulates several historically important disturbances, we provide evidence of how disturbance interactions cause lasting imprints on the seed bank community. Specifically, interactions between understory fire, canopy gap creation, and continuous low-intensity deer browsing left distinct disturbance legacies in the seed bank community over 13 years. Despite the frequency and importance of these disturbances, this study is the first to our knowledge to test how these interacting disturbances can change the forest seed bank. These seed bank legacies provide new theoretical insight towards how biodiversity and forest communities are maintained in light of multiple disturbances. Further, these altered seed banks represent a critical reservoir of biodiversity that will influence community structure and composition following subsequent disturbances (Gioria & Pysek 2016).

Disturbance Effects on Seed Banks

Individual Disturbances

Only burning increased long term seed bank richness, likely driven by an increase in the proportion of forb species with fire. These increases in seed species richness after fire were relatively small and are similar to the changes in aboveground forb species richness following prescribed fire found by Hutchinson et al. (2005) and Keyser et al. (2012). However, these results contrast 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 richness. Fire also decreased tree seed abundance, similar to the findings of Schuler et al. (2010). Reduced tree seed abundance is likely due to a temporal delay between tree seed germination following disturbance and the age at which tree species are able to flower and reproduce. Trees growing post-fire would invest in growth rather than reproduction, highlighting how there may be a delay in how long it takes for a seed bank to represent certain tree species, such as *Betula* spp. (Royo and Ristau 2013).

Canopy Gaps & Fencing

We found no strong effects of combined canopy gaps and fencing on seed abundance, richness, or diversity, which contrasts the strong interaction these disturbances had on seed bank species occurrence probability found by Shinoda and Aksaka (2020). However, we did find that combined gaps and fencing led to different plant communities in comparison to sites with closed canopies and no fencing, while canopy gaps and fencing alone did not influence the seed community (Fig. 3.6c). This interactive effect between gaps and fencing on the seed community points to how deer can play an influential role moderating plant growth underneath a canopy gap and indirectly shift seed community composition (VanderMolen & Webster 2021).

Fire & Gaps

We found that the combination of prescribed burns and canopy gaps dramatically increased seed abundance in comparison to plots that were only burned or had a canopy gap overhead. In this scenario, fires cleared existing biomass 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 (Royo et al. 2010). The abundant resources within burned plots with a canopy gap then allowed for plants to invest in seed production, thereby replenishing the seed bank.

Burning and canopy gaps also led to an increase in forb seed abundance, similar to Hyatt (1999), while vine seed abundance decreased with these disturbances. Increased forb seed abundance parallels the increase in forb cover with fire and gaps found by Royo et al. (2010) in these plots, suggesting that the forbs that initially germinated following these disturbances can have a long-term presence in the seedbank. It is 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 (Lettow et al. 2014; Yantes et al. 2023).

Fire & Fencing

Deer exclusion and fire decreased seed 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 after fire and could invest in reproduction without stress or death from herbivory. As a result, shrub’s long-lived seeds accumulated in the seed bank and made the relative proportions of seed life forms highly uneven, thereby decreasing Shannon diversity. These results provide nuance to the finding that high deer densities can reduce seed abundance, as low and moderate deer browse seemingly maintains higher seed biodiversity by creating a more heterogeneous environment that allows for more even relative abundances of seed banking species (Tamura 2019; Beauchamp et al. 2013; DiTommaso et al. 2014; Levine et al. 2012).

Fire, Canopy Gaps, & Fencing

The combined influence of burning, thinning, and herbivory on shrubs has been demonstrated in savanna ecosystems (Knapp et al. 1999; Yantes et al. 2023), but few have tested whether these aboveground vegetation legacies are then reflected in the seed bank. In our study, we found that fire, canopy gaps, and fencing had a 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, similar to Royo et al. (2010). This outcome is likely exacerbated by *Rubus* increasing with canopy gap creation and having fruit that is highly palatable to deer (Mladenoff 1990; Donoso & Nyland 2006; Widen et al. 2018). *Rubus* seeds are able to survive belowground for decades, making it very likely this legacy in the seed bank will become apparent in the understory following the next fire or gap. Further, this increase in *Rubus* seeds inside of fencing may be why we see a concomitant decrease in graminoids inside of fencing, as these two life forms are known to heavily compete (Van Auken 2009).

Fire-Driven Plant Communities

Fire combined with gaps or fencing led to unique seed bank communities in comparison to areas with only fencing or a canopy gap, as evidenced by multivariate analysis and NMDS. 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. In our study, fire was the strongest driver of community change, with added community differentiation when deer and canopy gap treatments were applied. This result highlights the importance of fire in shaping plant diversity in formerly fire-prone systems and how 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 (Plue et al. 2017; Plue et al. 2021). In our study, seed communities were most similar to extant plant community data in 2006 and 2013. This result is likely due to many seeds having relatively short longevity *in situ* (Hille Ris Lambers et al. 2005; Probert et al. 2009), making it more likely that the seed bank community would be more representative of extant plant communities closer to sampling. However, even in the most disturbed plots, seed bank communities never converged with extant plant communities at any time point, highlighting how the seed bank maintains an important reservoir of biodiversity. Notably, extant plant communities were more dispersed and variable in comparison to the seed bank, indicating that we likely under-sampled the seed bank and there is far greater diversity to be found within seed banks than our sampling intensity indicates (Plue et al. 2021). However, our seed bank survey represents 13 years of seed accumulation and death after the disturbance treatments, which would maintain a different community than the vegetation occurring aboveground. Thus, we believe the seed bank represents an important component of forest biodiversity, especially considering that only 16% of species were non-native to North America and 1% of total germinants were non-native.

Ruderal Species, Ecological Memory, and Future Considerations

Research on how forests respond to multiple disturbances is often limited to studying how trees respond to the combined influence of high intensity disturbances, such a wildfires and stand-leveling windstorms (Johnstone et al. 2016). Often, many other layers of a forest are forgotten in light of multiple disturbances, such as the understory or the seed bank (Roberts & Gilliam 2003; Roberts 2004; Spicer et al. 2020). Further, many studies on multiple disturbance are conceptual rather than experimental or do not adequately interpret results within the theoretical assumptions of disturbance ecology (Foster et al. 2016; Webster et al. 2018). It is critical that we consider these disturbances in tandem, since understory fire and mid-sized canopy gaps were the most common pre-colonial forest disturbances throughout the eastern U.S. and are being broadly restored (Hanson & Lorimer 2007; Abrams et al. 2021), while white-tailed deer are far above historic densities and are now a dominant contemporary disturbance with many indirect effects (Rooney & Waller 2003; Reed et al. 2023). The novelty of our study highlights our limited understanding of these disturbance combinations and their potential influence on forest succession, development, and disturbance legacies.

In our experiment, restoring pre-colonial 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 play an important role in ecosystems (Richmond et al. 2005; Palacio et al. 2016). 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 (Donoso & Nyland 2006; Widen et al. 2018). These ruderal species are eventually shaded-out by growing trees, so they create dense seed banks that buffer them from year-to-year variability and can germinate after the next vegetation-clearing disturbance (DeMalach et al. 2021). Although some studies have found that ruderal species like *Rubus* can lead to arrested succession, this effect is likely due to overpopulation of deer giving a competitive advantage to shrubs over trees (Kern et al. 2012; Widen et al. 2018).

Our results fundamentally differ from most gap-deer-*Rubus* studies because of the prescribed fire and relatively low deer population density in our region of study. Burning likely cleared most aboveground vegetation, while gaps provided abundant sunlight, leading to *Rubus* dominance in the understory. This *Rubus* dominance was then moderated and reduced by deer browsing. These shrub reductions with historic disturbances are then reflected as a legacy in the seed bank. Alternatively, outside of fencing, we see greater graminoid seed abundance, which are species that provide little forage value to wildlife and have been shown to increase with deer presence (Rooney 2009). Thus, differing seed banks with fencing and fire could eventually influence trophic interactions, as there are fewer seeds that produce nutritious soft-mass outside of fencing.

The high numbers of disturbance-dependent species in the seed bank may shed light on why many studies find a seed bank bias, wherein forest seed banks have low similarity to aboveground plants (Hopfensperger 2007, Larson & Suding 2022). These seed communities are different from extant plants because the ecosystem has not been disturbed recently. Compounding this issue, many studies under-sample the seed bank (Plue et al. 2021). These factors may also contribute to the assumption that seed banks are not a particularly important source of biodiversity or component of forest development, which our study provides evidence otherwise.

There could also be generalizable patterns in how seed banks respond to certain combinations of disturbance. For instance, based on our results, we’d expect fire and herbivore exclusion to create a long-lasting *Rubus* seed legacy, especially considering that shrub seeds are often less transient than extant understory plants (Plue et al. 2017). A shrub seed bank would then continually reoccur following occasional fire and gap creation, thereby cementing the ecological memory of a shrub-dominated system following disturbance (Johnstone et al. 2016). Repeated burns and canopy gap creating events would continually favor fast-growing shrubs that drop seeds at extremely high densities, creating a feedback loop of disturbance and shrub dominance. This ecological memory likely cannot be broken except through the manipulation of more intense or targeted disturbances, such as low intensity deer browsing (Nowacki & Abrams 2008; Jogiste 2017). These disturbance legacies and ecological memories in the seed bank make sampling a forest seed bank a valuable exercise to determine which combination of disturbances to apply in a restoration or management setting, particularly as the climate warms and many seed banking species are favored by warmer conditions (Abella 2022; Auffret et al. 2023).

Future studies that evaluate the influence of multiple disturbances on forest seed banks should make several considerations. First and foremost, researchers should think beyond how trees respond to multiple disturbances (Roberts & Gilliam 2003; Gilliam 2007; Spicer et al. 2020). It can take decades for the effects of multiple disturbances on a stand to become fully apparent, 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 (Larson & Suding, 2022). Future work should also evaluate how seed banks vary with multiple disturbance rather than just evaluating how seeds respond to disturbance binaries (Foster et al. 2016). By taking a continuous approach to measuring multiple disturbance we may be able to better detect nonlinear responses and improve research integration into meta-analyses (Buma 2021).

We found that the forest seed bank primarily varied depending on the disturbance combinations in question. These altered seed banks are an important reservoir of biodiversity and represent likely regenerative outcomes following subsequent disturbances, further justifying the seed bank’s importance in forest ecosystems. Our work also takes a first step towards understanding whether there are common patterns in forest seed bank responses to multiple disturbances and how the reintroduction of pre-colonial disturbances will likely leave important legacies that extend far beyond the tree line.

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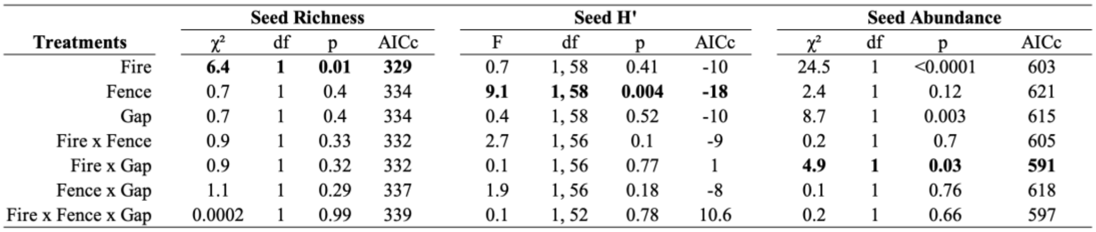
3.5: Tables

Table 3.1: 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
Erichtites hieraciifolius	111	Yes
Viola blanda	111	Yes
Dalibarda repens	68	Yes
Saxifraga	51	Yes
Pilea pumea	50	Yes
Viola macloskeyi	44	Yes
Violet 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 psuedomaculatum	6	Yes
Mitchella repens	6	Yes
Prenanthes trifoliata	3	Yes
Apocynum	2	Yes
Boehmeria cylindrica	2	Yes
Gaulitheria procumbens	2	Yes
Juncus effusus	2	Yes

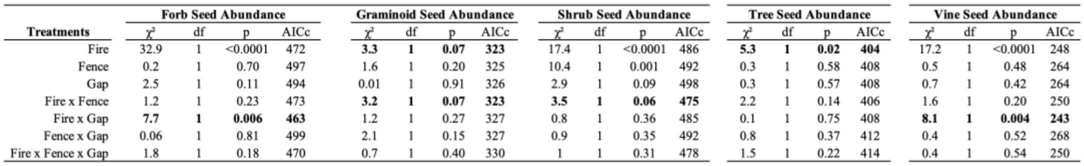
Plantago lanceolate	2	No
Polygonum persicaria	2	No
Solanum carolinense	2	Yes
Trillium spp.	2	Yes
Lobelia spicata	1	Yes
Medeola virginiana	1	Yes
Solanum nigrum	1	No
Uvularia sessifolia	1	No
Graminoids		
Carex spp.	178	Yes
Dichanthelium clandestinum	45	Yes
Luzula multiflora	25	Yes
Sisyrinchium	9	Yes
Agrostis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

Table 3.2: Seed richness, diversity (H') and abundance response to individual disturbance and their combinations. Each variable corresponds to a single model.



Treatments	Seed Richness				Seed H'				Seed Abundance			
	χ^2	df	p	AICc	F	df	p	AICc	χ^2	df	p	AICc
Fire	**6.4**	**1**	**0.01**	**329**	0.7	1, 58	0.41	-10	24.5	1	<0.0001	603
Fence	0.7	1	0.4	334	**9.1**	**1, 58**	**0.004**	**-18**	2.4	1	0.12	621
Gap	0.7	1	0.4	334	0.4	1, 58	0.52	-10	8.7	1	0.003	615
Fire x Fence	0.9	1	0.33	332	2.7	1, 56	0.1	-9	0.2	1	0.7	605
Fire x Gap	0.9	1	0.32	332	0.1	1, 56	0.77	1	**4.9**	**1**	**0.03**	**591**
Fence x Gap	1.1	1	0.29	337	1.9	1, 56	0.18	-8	0.1	1	0.76	618
Fire x Fence x Gap	0.0002	1	0.99	339	0.1	1, 52	0.78	10.6	0.2	1	0.66	597

Table 3.3: Forb, graminoid, shrub, tree, and vine seed abundance response to individual and combined disturbance treatments. Each variable corresponds to a single model.



Treatments	Forb Seed Abundance				Graminoid Seed Abundance				Shrub Seed Abundance				Tree Seed Abundance				Vine Seed Abundance			
	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	32.9	1	<0.0001	472	**3.3**	**1**	**0.07**	**323**	17.4	1	<0.0001	486	**5.3**	**1**	**0.02**	**404**	17.2	1	<0.0001	248
Fence	0.2	1	0.70	497	1.6	1	0.20	325	10.4	1	0.001	492	0.3	1	0.58	408	0.5	1	0.48	264
Gap	2.5	1	0.11	494	0.01	1	0.91	326	2.9	1	0.09	498	0.3	1	0.57	408	0.7	1	0.42	264
Fire x Fence	1.2	1	0.23	473	**3.2**	**1**	**0.07**	**323**	**3.5**	**1**	**0.06**	**475**	2.2	1	0.14	406	1.6	1	0.20	250
Fire x Gap	**7.7**	**1**	**0.006**	**463**	1.2	1	0.27	327	0.8	1	0.36	485	0.1	1	0.75	408	**8.1**	**1**	**0.004**	**243**
Fence x Gap	0.06	1	0.81	499	2.1	1	0.15	327	0.9	1	0.35	492	0.8	1	0.37	412	0.4	1	0.52	268
Fire x Fence x Gap	1.8	1	0.18	470	0.7	1	0.40	330	1	1	0.31	478	1.5	1	0.22	414	0.4	1	0.54	250

3.6: Figures

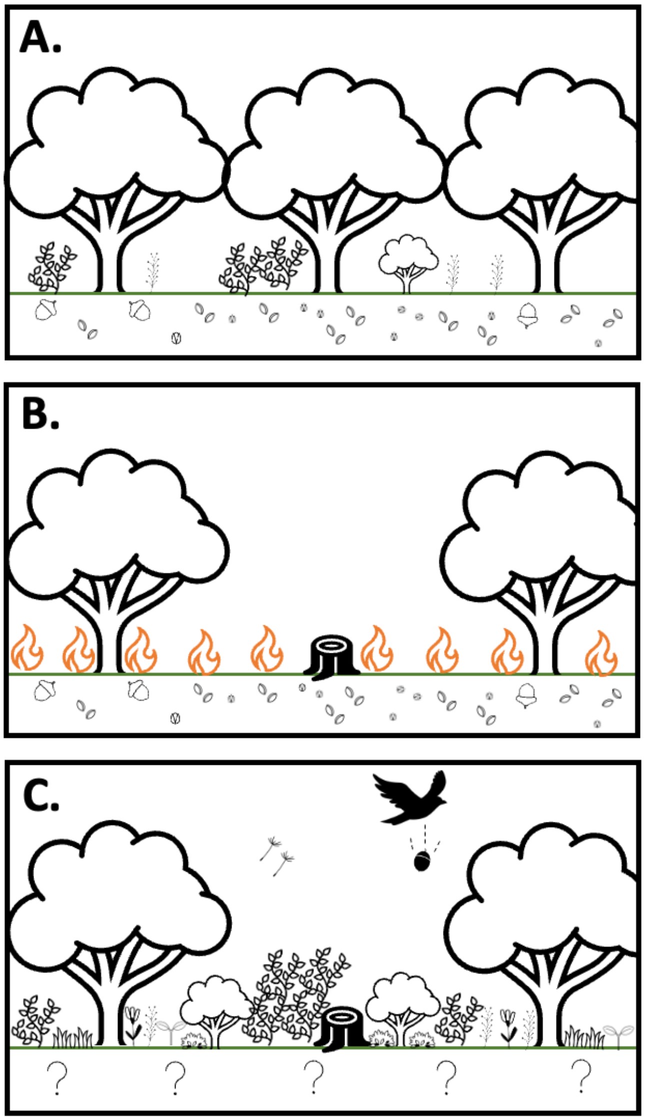


Fig. 3.1: Conceptual diagram illustrating how aboveground vegetation can respond to multiple disturbances such as fire and treefall gaps. One major unknown in forest ecology is how the seed bank responds after multiple disturbances.

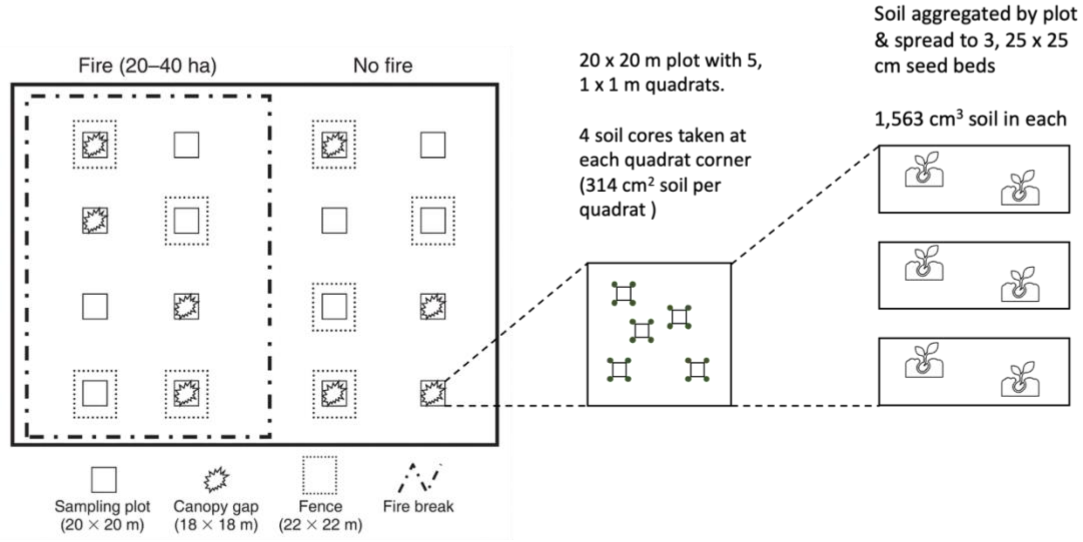


Fig. 3.2: Diagram showing the organization of our replicated, factorial multi-disturbance study. Four stands were split into burned and unburned halves. Within each half, various fencing and gap treatments were applied in 20x20 m plots. With each of these plots, soil was collected and aggregated. This 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.

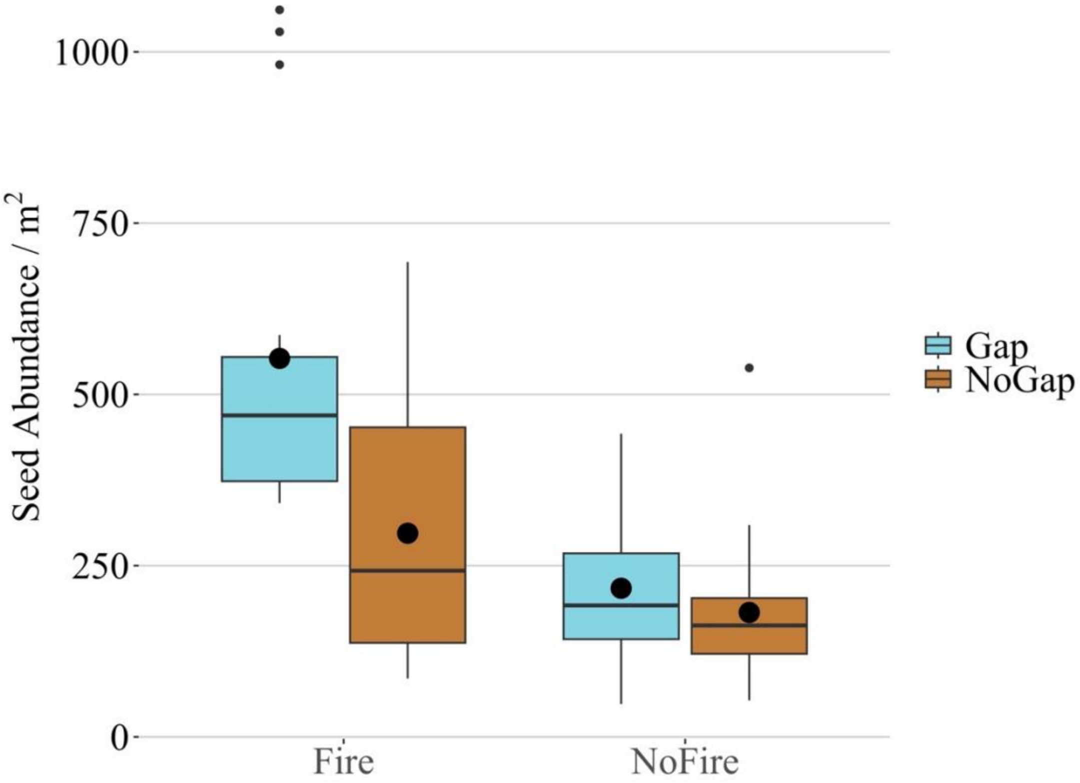


Fig. 3.3: Seed abundance response to combined gap and fire treatments. Black circles represent means whereas bold lines represent medians.

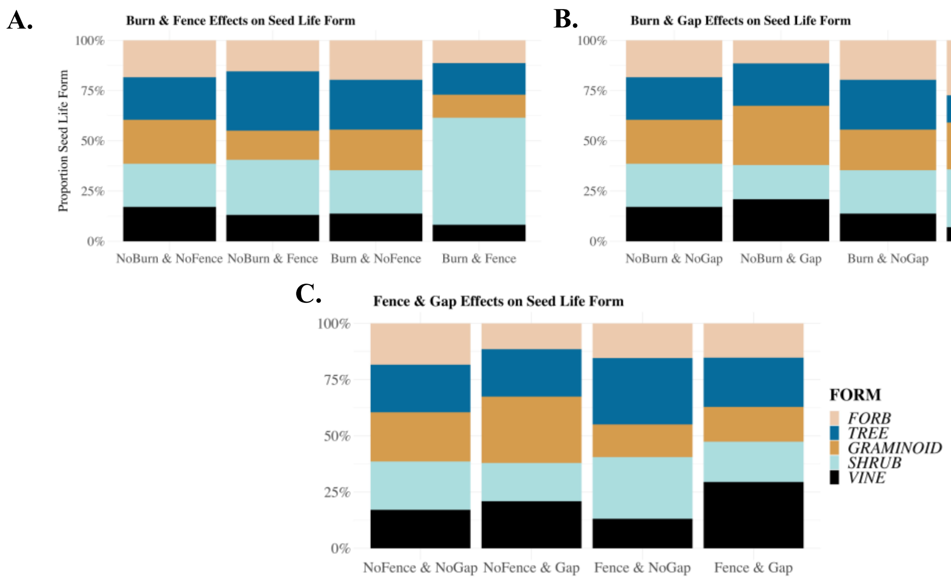


Fig. 3.4: Bar charts that represent the relative mean proportion of each germinant life form in unpooled disturbance treatments.

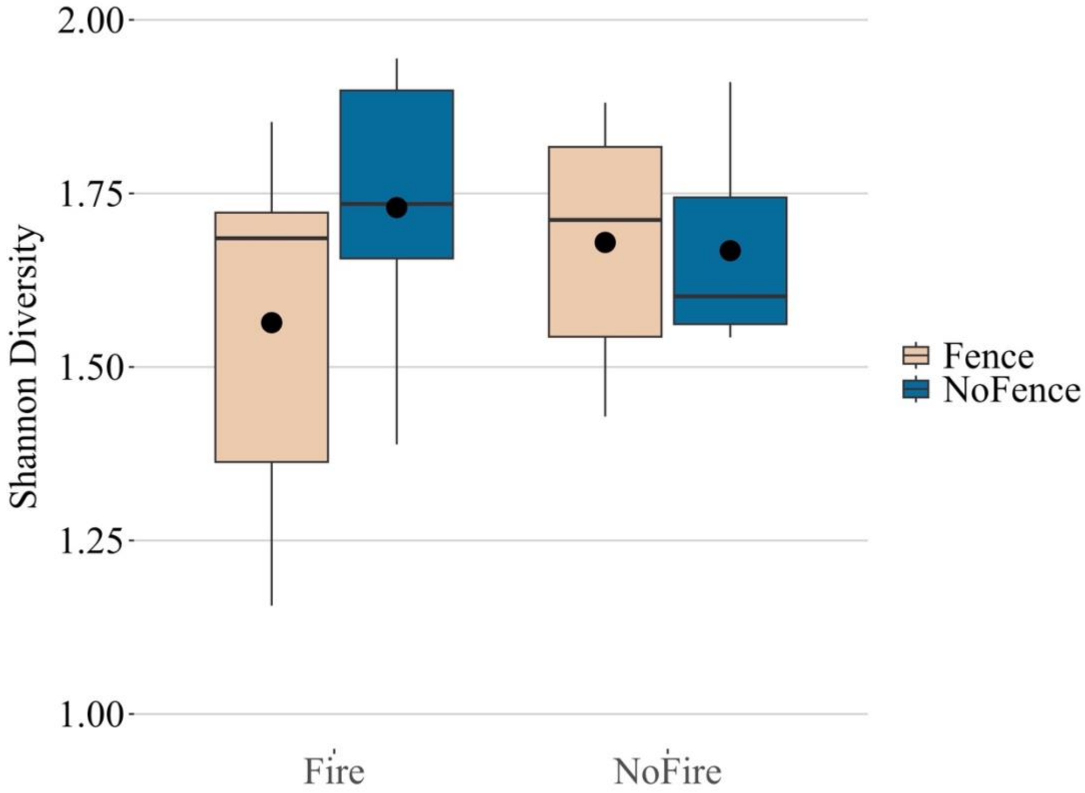


Fig. 3.5: Seed Shannon diversity response to deer fencing and prescribed fire.

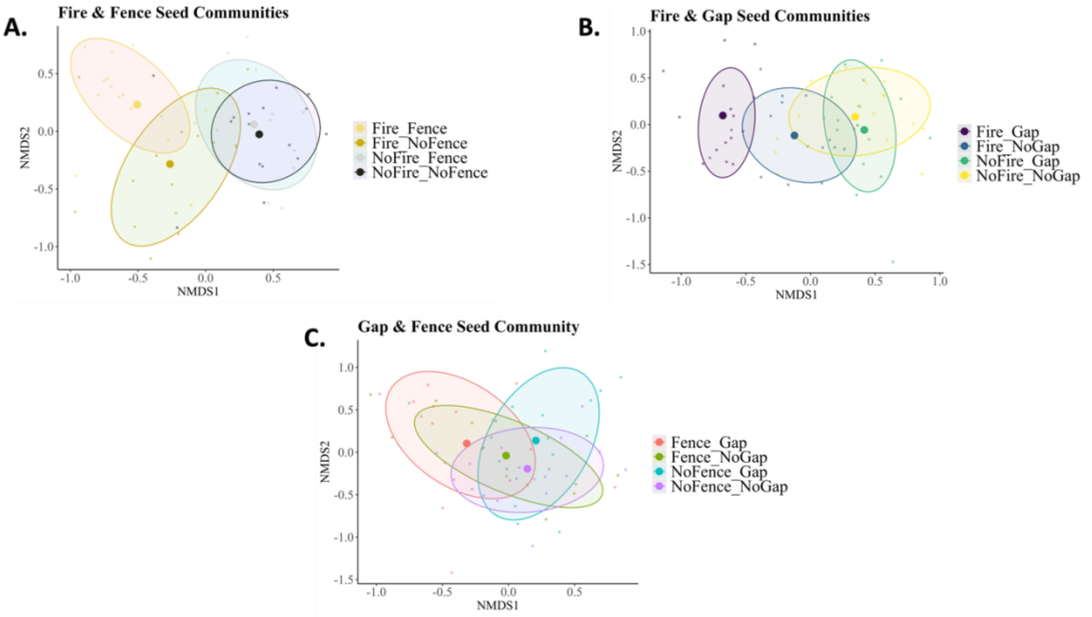


Fig. 3.6: NMDS showing seed community differences amongst disturbance treatments. Understory fire causes the most distinct shifts in community with additional variation from canopy gaps (**A.**) and deer fencing (**B.**). There are slight differences in communities with gaps and deer fencing, although not as pronounced as with fire.

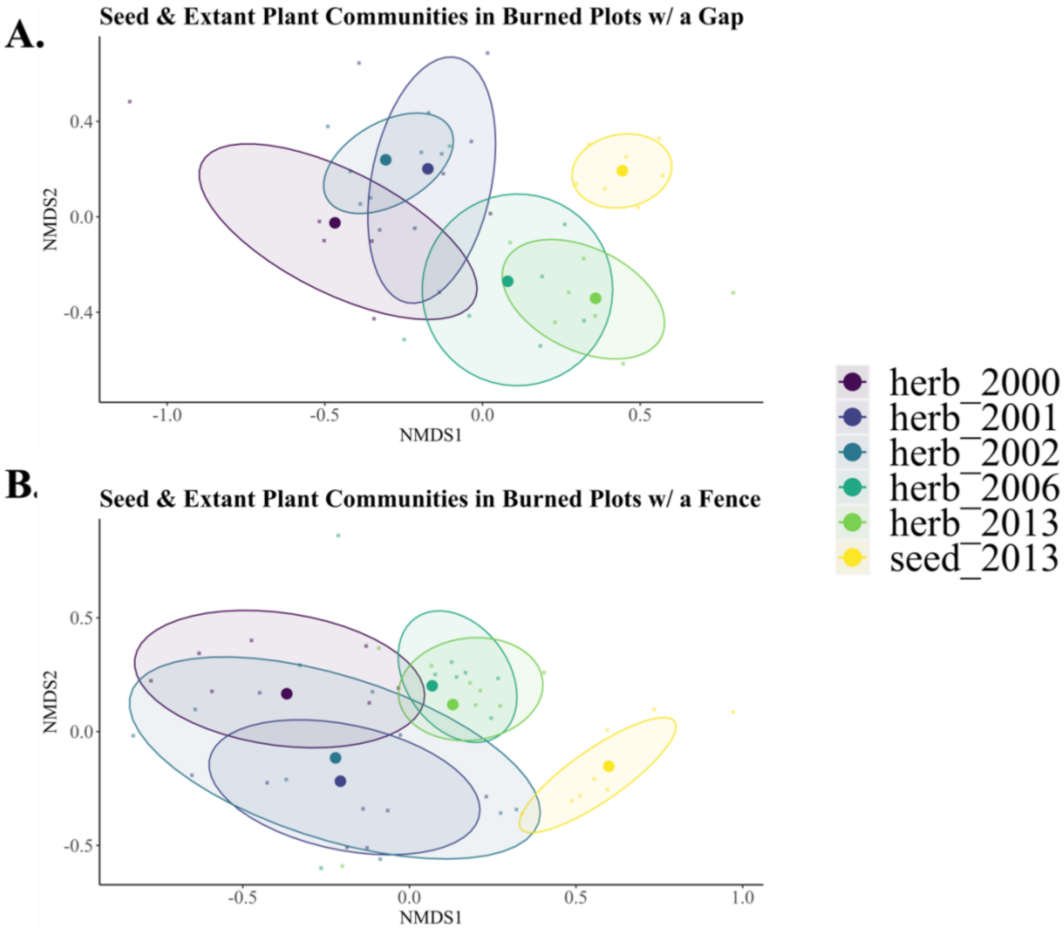


Fig. 3.7: NMDS showing differences amongst seed communities in 2013 and extant plant communities sampled in each treatment in 2000, 2001, 2002, 2006, and 2013. **A.** Represents plant communities from above and belowground in plots that were burned and had a canopy gap overhead. **B.** Represents plant communities from above and belowground that were burned and had a canopy gap overhead.

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3.8: Supplementary Information

Table S3.1: PERMANOVA pairwise comparison using Raup-Crick dissimilarity between seed and herbaceous layer communities between years in plots that had a fence and were burned.

Seed_2013_vs_Herb_2013

```
          Df SumOfSqs      R2      F Pr(>F)
YearLayer  1  0.34938 0.48184 13.019  0.003 **
Residual  14  0.37572 0.51816
Total     15  0.72510 1.00000
```

Seed_2013_vs_Herb_2006

```
          Df SumOfSqs      R2      F Pr(>F)
YearLayer  1  0.60928 0.46955 12.393  0.002 **
Residual  14  0.68829 0.53045
Total     15  1.29757 1.00000
```

Seed_2013_vs_Herb_2002

```
          Df SumOfSqs      R2      F Pr(>F)
YearLayer  1   1.3922 0.47829 12.835  0.001 ***
Residual  14   1.5185 0.52171
Total     15   2.9107 1.00000
```

Seed_2013_vs_Herb_2001

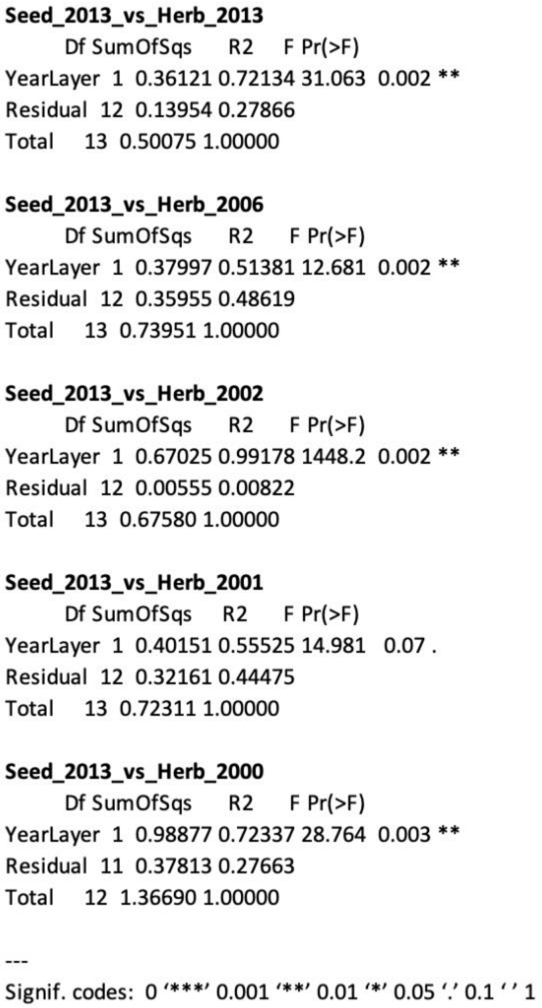
```
          Df SumOfSqs     R2      F Pr(>F)
YearLayer  1  1.39722 0.59101 20.231  0.001 ***
Residual  14  0.96691 0.40899
Total     15  2.36412 1.00000
```

Seed_2013_vs_Herb_2000

```
          Df SumOfSqs      R2      F Pr(>F)
YearLayer  1  2.07439 0.69244 31.52  0.001 ***
Residual  14  0.92138 0.30756
Total     15  2.99577 1.00000
```

```
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Table S3.2: PERMANOVA pairwise comparison using Raup-Crick dissimilarity between seed and herbaceous layer communities between years in plots that had a gap and were burned.



```
Seed_2013_vs_Herb_2013
          Df SumOfSqs      R2      F Pr(>F)
YearLayer  1  0.36121 0.72134 31.063  0.002 **
Residual  12  0.13954 0.27866
Total     13  0.50075 1.00000

Seed_2013_vs_Herb_2006
          Df SumOfSqs      R2      F Pr(>F)
YearLayer  1  0.37997 0.51381 12.681  0.002 **
Residual  12  0.35955 0.48619
Total     13  0.73951 1.00000

Seed_2013_vs_Herb_2002
          Df SumOfSqs      R2      F Pr(>F)
YearLayer  1  0.67025 0.99178 1448.2  0.002 **
Residual  12  0.00555 0.00822
Total     13  0.67580 1.00000

Seed_2013_vs_Herb_2001
          Df SumOfSqs      R2      F Pr(>F)
YearLayer  1  0.40151 0.55525 14.981   0.07 .
Residual  12  0.32161 0.44475
Total     13  0.72311 1.00000

Seed_2013_vs_Herb_2000
          Df SumOfSqs      R2      F Pr(>F)
YearLayer  1  0.98877 0.72337 28.764  0.003 **
Residual  11  0.37813 0.27663
Total     12  1.36690 1.00000

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```