Fall 2023

EFFECTS OF PERENNIAL FORAGE COMPOSITION AND HARVEST INTENSITY ON THE WEED SEEDBANK COMMUNITY

Lilly E. Hartman
University of New Hampshire

Recommended Citation

Hartman, Lilly E., "EFFECTS OF PERENNIAL FORAGE COMPOSITION AND HARVEST INTENSITY ON THE WEED SEEDBANK COMMUNITY" (2023). *Master's Theses and Capstones*. 1760.
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EFFECTS OF PERENNIAL FORAGE COMPOSITION AND HARVEST INTENSITY ON THE WEED SEEDBANK COMMUNITY

BY

LILLY HARTMAN

BA Biology, Earlham College, 2020

THESIS

Submitted to the University of New Hampshire
in Partial Fulfillment of
the Requirements for the Degree of

Master of Science
in
Agricultural Science
September, 2023

This thesis has been examined and approved in partial fulfillment of the requirements for the degree of Master of Science in Agricultural Science by:

Thesis Co-Director, David A. Mortensen, Professor Emeritus, Agriculture, Nutrition, and Food Systems

Thesis Co-Director, Richard G. Smith, Professor, Natural Resources and the Environment

Rebecca G. Sideman, Professor, Agriculture, Nutrition, and Food Systems

Jessica Ernakovich, Associate Professor, Natural Resources and the Environment

On 20 July, 2023

Original approval signatures are on file with the University of New Hampshire Graduate School.

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ACKNOWLEDGMENTS

This work was conducted on the ancestral home of the Pennacook, Abenaki, and Wabanaki people who have stewarded these lands and waters for generations and continue to do so. As researchers at a Land Grant institution, we benefit from the seizure and re-sale of indigenous lands enabled by the Morrill Act of 1862, or the Land-Grant College Act. The U.S. gave 144,073 acres of stolen land to UNH to be resold to settlers at an 11-fold increase from what was paid to tribal nations (https://www.landgrabu.org/universities).

Immense thanks to my advisors, Dave Mortensen and Rich Smith, for your thoughtfulness, encouragement, and love of science. Thanks to my committee members, Becky Sideman and Jessica Ernakovich, for your guidance and for the perspective you bring to our discussions. Infinite thanks to Nick Warren for being incredibly present for and aiding in every component of this thesis work. Thanks to the rest of the UNH Agroecology lab for your feedback on research development, interpretation, and communication, and for your good company: Natalie Lounsbury, Palash Mandal, Isaac Ativor, and Shyloh Favreau. Thank you to Luke Hydock and Amber Kittle for emergence experiment care and maintenance, and to Julia Hobbie, Robert Pease, Ben Nikitas, Ben Fehr, Elizabeth Cain, Liza DeGenring, Catherine Coverdale, and Eliza Balch for technical assistance. Thank you to Anissa Poleatewich and Laura Gabel-Hartman for comments on previous drafts, and to Iago Hale for comments on statistical analysis.

Thank you to my family and community for your tremendous support. Thank you to my family for encouraging my interests always. Thank you to my partner, Eliza, for your unwavering support. I am grateful to the dear friends who have made the last few years a joy. To

all of my past and present mentors, thank you for your encouragement which has led me where I am today.

Funding for this research was provided by the USDA NIFA OREI Program (Project No. 2020-51300-32196).

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ABSTRACT

EFFECTS OF PERENNIAL FORAGE COMPOSITION AND HARVEST INTENSITY ON THE WEED SEEDBANK COMMUNITY

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Most arable weeds arise from a resident soil seedbank and are typically controlled with tillage and herbicides, each of which pose sustainability challenges to growers and consumers. However, agronomic management practices that reduce weed seed input to the seedbank and that accelerate mortality of seeds already in the soil could reduce the need for more conventional weed control. Previous research and farmer practice has demonstrated that rotation of annual crops with several years of perennial forage can reduce weed abundance and the need for chemical weed control. In perennial forage systems, crop species and harvest intensity may have important effects on seedbank composition due to the multiple stress and mortality factors these systems impose on weeds, including facilitation of strong crop-weed competitive interactions, periodic defoliation of crop (and weed) canopies, and potentially crop species-specific associations with soil faunal and microbial communities that cause seed damage and mortality. Unfortunately, we know little about the influence of agronomic practices such as forage crop species selection or harvest frequency and harvest height on weed population dynamics or the specific mechanisms that regulate weed seed survival in the soil in perennial forage systems. We used a three-year factorial field experiment established in 2018 that included four mixtures of perennial forage legumes, each grown with orchardgrass, *Dactylis glomerata* L., two harvest height treatments (5 and 10 cm residual forage height), and two harvest frequency treatments (three and five harvests per year) to investigate how perennial forage composition and management act as community assembly filters on the composition and abundance of the weed seedbank. The composition and abundance of the weed seedbank was quantified at the end of the third year of the study using the direct germination method in a heated greenhouse. While overall seedbank density did not differ across treatments, seedbank community composition was influenced by the interactive effects of both harvest height and harvest frequency. More intensely harvested plots (shorter stubble remaining) favored weed seed bank communities more heavily dominated by weed species with specific functional traits: mat-forming weeds that set seed close to the soil surface were favored in short stubble harvest regimes, possibly due to their ability to grow and reproduce while evading defoliation. Conversely, the harvest regime in less intensely harvested plots enabled a more generalist weed community. In order to determine whether weed seed decay mechanisms might vary across the treatments, we conducted a weed seed burial experiment with seeds of a common weed, velvetleaf (*Abutilon theophrasti* Medik.). Seeds were buried in each experimental unit to simulate seed shed and incorporation in the soil and were extracted in the following spring. Seed death over the burial period was not different in more intensely harvested plots compared to less intensely harvested plots. This suggests that when considering how forage canopy management practices mediate seed mortality, seed decay may be of lesser importance compared to other mechanisms of seedbank decline such as fatal germination and seed predation. An indicator plant material was buried to gain a secondary measurement of cellulose decay in the system without the complex effects of seed dormancy and viability. In red clover and white clover biculture plots, cellulose decay was greater in more frequent harvest treatments when compared to less frequent. Lastly, both the decay of velvetleaf seeds and of indicator cellulose were significant predictors of seedbank community composition, demonstrating that microbial activity acts as a biological filter on weed seedbank community assembly.

CHAPTER ONE:
Perennial forage and the weed seedbank

Agricultural weeds pose a significant challenge to farmers by competing with crop plants for limited resources. Weed infestations arise from a resident seed and vegetative propagule bank. Weed seeds are deposited into the seed bank when mature weeds shed their seeds. Seeds exit the seedbank when they emerge successfully or when they die due to abiotic extremes, fatal germination, decay by cellulolytic microbes, seed predation by insects and small mammals, or other mortality processes (Dalling et al. 2011, Davis 2007, Fawcett and Slife 1978, Pakeman et al. 2012).

Booth and Swanton (2002) and Smith and Mortensen (2017) apply ecological community assembly theory to agricultural weed communities and discuss a hierarcy of assembly filters. Like other ecological communities, weed communities assemble from the pool of geographically available species. A series of “environmental filters” determine what species can establish and persist in the habitat. Some weed species then pass through “management filters,” being the anthropogenic disturbances which are characteristic of agricultural systems, thereby evading weed control. Furthermore, species interactions that affect the establishment and persistence of weeds, or “biotic filters,” act on the realized weed community. What then persists is a realized weed community where some portion of the geographically available species are expressed, based on their traits that enabled them to pass through environmental, management, and biotic filters, and persist in the ecological community.

The majority of current weed control practices, or community assembly filters at the management level, target weeds at their seedling stage using tillage, herbicides, or both (Buhler et al. 1997). Each of these approaches comes with their own sustainability challenges. Frequent tillage, or the mechanical turning or mixing of the soil, has been demonstrated to adversely affect soil health, carbon storage, and water quality (Lehmann et al. 2020, Mohler et al. 2018, Ogle et al. 2019). Pesticides, including herbicides, pose threats to human health and the environment (Köhler and Triebskorn 2013, Lechinovski et al. 2022, Rani et al. 2021, Rohr and McCoy 2010, Rose et al. 2016, Tang et al. 2021), and their widespread and frequent use has led to the evolution of herbicide-resistant weeds (Baucom 2019, Harper 1957). Due to these challenges, the adoption of weed management strategies that reduce dependency on conventional approaches to weed control is critical (Liebman et al. 1997, Mortensen et al. 2012). Furthermore, the pursuit of nonchemical approaches to manage weeds in reduced-tillage production systems offers dual benefits: first, it can promote wider adoption of reduced tillage practices by providing effective weed management strategies that reduce reliance on herbicides, and second, it aids in reducing herbicide usage in production systems already employing reduced tillage, reducing the selection pressure for herbicide-resistant weeds (Brainard et al. 2013).

Practices that reduce weed seed input and accelerate weed seed death in the soil have shown a greater effect on emerged weed density than practices that target only seedlings (Gonzalez-Andujar and Fernandez-Quintanilla 1991, Jordan et al. 1995). Further, targeting weeds at multiple life stages has been shown to bolster weed management outcomes compared to management at one life stage only (Liebman et al. 1997, Westerman et al. 2005). The challenge of weed management can be reduced by incorporating seedbank-focused and multi-faceted approaches.

Crop rotation is a millennia-old innovation by Indigenous agriculturists (Magcale-Macandog and Ocampo 2005, Sands et al. 2023), wherein crops with different ecological niches are grown sequentially in the same field. Diversification via crop rotation has been shown to increase yield (Smith et al. 2008), increase profitability, reduce need for synthetic agrichemical inputs (Davis et al. 2012), and suppress weeds (Picasso et al. 2008, Wang et al. 2008). Integrated crop and livestock systems, a form of crop rotation, were historically common but lost popularity in the mid 20th century when novel synthetic fertilizers replaced on-farm nutrient recycling. Reintegrating crop and livestock systems has gained traction more recently as a means to minimize the use of fossil fuels, chemical fertilizers, and environmental contaminants (Naylor et al. 2005). Rotation to perennial forage, namely pastures and hayfields, has the potential to be used as a weed management tool in low-input and reduced tillage systems. When compared to annually tilled systems, integration of perennial forages can better suppress annual weeds (Teasdale et al. 2004), increase overall profitability, provide favorable habitat for beneficial arthropods (Schipanski et al. 2017) and seed predators (Meiss et al. 2010), increase beneficial microbial biomass and diversity (Schlautman et al. 2021), and shift the composition of weed communities (Entz et al. 2002) due to the diversification of the community assembly filters acting on the realized weed community.

An improved ecological understanding of the environmental variables that regulate seedbank density and composition is paramount if we are to reduce the environmental footprint of industrial agriculture (Forcella et al. 1993). The following chapters describe two field experiments in which we investigated how three years of perennial forage management influenced the composition and abundance of the soil weed seedbank (Chapter 2) and the fate of seeds of a common annual weed, velvetleaf, buried in the soil (Chapter 3).

CHAPTER TWO:
Effects of perennial forage composition and harvest intensity on the germinable weed seedbank community

INTRODUCTION

Previous theoretical and empirical research suggests that perennial forage cropping systems (pastures and hayfields) may enhance biologically-based mechanisms of weed suppression relative to conventional annual row cropping systems. For example, Davis et al. (2012) demonstrated that by integrating two years of a perennial forage into a corn-soybean rotation, herbicide inputs could be six to ten times lower without heightened weed abundance relative to a conventional corn-soy rotation. Schipanski et al. (2017) showed that systems rotated to perennial forage for two years maintained equal profitability to purely annual systems while displaying greater weed suppression. Rotation to perennial cover can be used as a weed management tool in low-input and reduced tillage systems because it enables the manager to drive the seedbank density of summer annual weeds down before rotating back to a summer annual crop, suppressing problematic populations of weeds by varying management filters on weed and weed seedbank community assembly.

Several biological mechanisms contribute to the high levels of weed suppression observed in perennial cropping systems (Nikolić et al. 2020, Ilnicki and Enache 1992). Perennial forage systems are characterized by periodic livestock grazing or mowing, wherein aboveground plant material is harvested (Gilmullina et al. 2020). Weed suppression occurs at multiple life stages. Weeds experience season-long resource competition from crops (e.g. Bradshaw and Lanini 1995), and weed seed input is less likely because the periodic removal of biomass reduces weed fitness (Donald 2006). The lack of soil disturbance results in weed seeds remaining on the soil surface, susceptible to seed predators and allelochemicals (Liebman and Davis 2001), and seed predators tend to be more abundant in perennial cover (Meiss et al. 2010). Canopy cover can reduce weed seed germination cues (Sias et al. 2021). Further, seeds are more likely to decompose for multiple reasons: frequent defoliation results in root exudates which stimulate microbial activity (Hamilton et al. 2008), and reduced soil disturbance results in development of a more abundant decomposer community (Helgason et al. 2009, Schlautman et al. 2021). Lastly, legumes alter rates of soil C mineralization (Li et al. 2020), potentially increasing seed decay (Mohler et al. 2012, 2018), and some forage crops produce allelochemicals harmful to weeds and weed seeds (Farooq et al. 2020).

The concept of “weed suppressive soils” has a long history in the weed management and especially the weed seed bank literature (Davis et al. 2006, Gallandt et al. 2004, Kennedy and Kremer 1996, Kremer 1993, Kremer and Li 2003). Under this framework, cropping systems can be managed to enhance populations of naturally occurring microbial and macrofaunal communities that decompose and predate weed seeds thereby reducing weed seed bank abundance (Gallandt et al. 2005, Kremer 1993, Mohler et al. 2018). Despite evidence that perennial forage crops can foster improved weed suppression through a variety of biological mechanisms, it remains unknown the degree to which forage management practices such as harvest intensity and species selection may act as management filters on the weed seedbank community and mediate these effects.

The objective of this study was to investigate how perennial crop community composition and harvest intensity affects weed seedbank community composition and abundance. We used a factorial experiment in a field of grass and legume mixtures which had been established three years prior. The experiment included four perennial mixtures of legumes and *Dactylis glomerata* L., two harvest stubble height treatments at 5 and 10 cm, and two harvest frequency treatments at three and five events per year. The seedbank was sampled after 3.75 years of field treatments in 2022 and quantified and identified to species or genus using the direct emergence method (Thompson and Grime 1979). We hypothesized that treatments with more intense harvest and with lower proportions of legumes to grasses would have higher germinable weed seedbank abundance and lower richness and diversity because these plots would have the least favorable habitats for seed predators and decomposers. We also hypothesized that seedbank community composition would be influenced both by forage species identity and by harvest intensity. Lastly, we hypothesized that seedbank abundance would be correlated to aboveground weed biomass and crop biomass due to differences in seed input effects.

MATERIALS AND METHODS

Study site

We conducted this research on ancestral Pennacook, Abenaki, and Wabanaki land. A three-year field experiment was conducted at the University of New Hampshire Kingman Research Farm in Madbury, New Hampshire (43.17°N, -70.94°W). Soils at the site are Charlton fine sandy loams with 3 to 8% slopes (Soil Survey Staff 2022). Prior to the establishment of the experiment, the site was in mixed production of cover crops and strawberry for eight years, and cucurbits prior to 2010.

Experimental field design

The experiment was established on 8 August 2018 and included four perennial forage legume-grass mixtures harvested either three or five times per season and cut to either 5 or 10 cm residual forage height. Harvest frequency, harvest height, and mixture composition treatments were arranged in a 2 by 2 by 4 factorial in a randomized complete block design with a split plot. Whole-plot factors were each combination of harvest frequency and cutting height. Mixture treatments were applied to subplots. All mixtures included orchardgrass (*Dactylis glomerata* L.) and one or more perennial forage legume species. Plots were harvested by mowing and removal of biomass with an RCI 36A small plot research harvester (RCI Engineering LLC, Mayville, WI). Harvest height was either 5 or 10 cm residual forage height after cutting. Harvest frequency included either three or five harvest events per growing season. Plots harvested five times per season (hereafter ‘5x’) were harvested on an approximate 30-day interval, while plots harvested three times per season (hereafter ‘3x’) were timed to optimize stand quality and productivity: the first harvest occurred when orchardgrass was at the swollen boot stage, and the second and third harvests coincided with pre-to early-bloom legume stages. The plots were harvested together in late May or early June, after which the 3x harvests continued until September and the 5x harvests continued until October. Subplots were four levels of mixture composition (described below) and were 2.29 m by 6.10 m. (Fig. 2.1). Total seeding rate was 1,076 pure live seeds per square meter. Pure live seed proportions of the grass and legumes were:

1. 30% orchard grass (or OG), 70% alfalfa (*Medicago sativa* L.);

2. 30% orchard grass, 70% red clover (*Trifolium pratense* L.);
3. 30% orchard grass, 70% white clover (*Trifolium repens* L.);
4. 30% orchard grass, 17.5% red clover, 17.5% bird's foot trefoil (*Lotus corniculatus* L.), 17.5% alfalfa, 17.5% white clover.

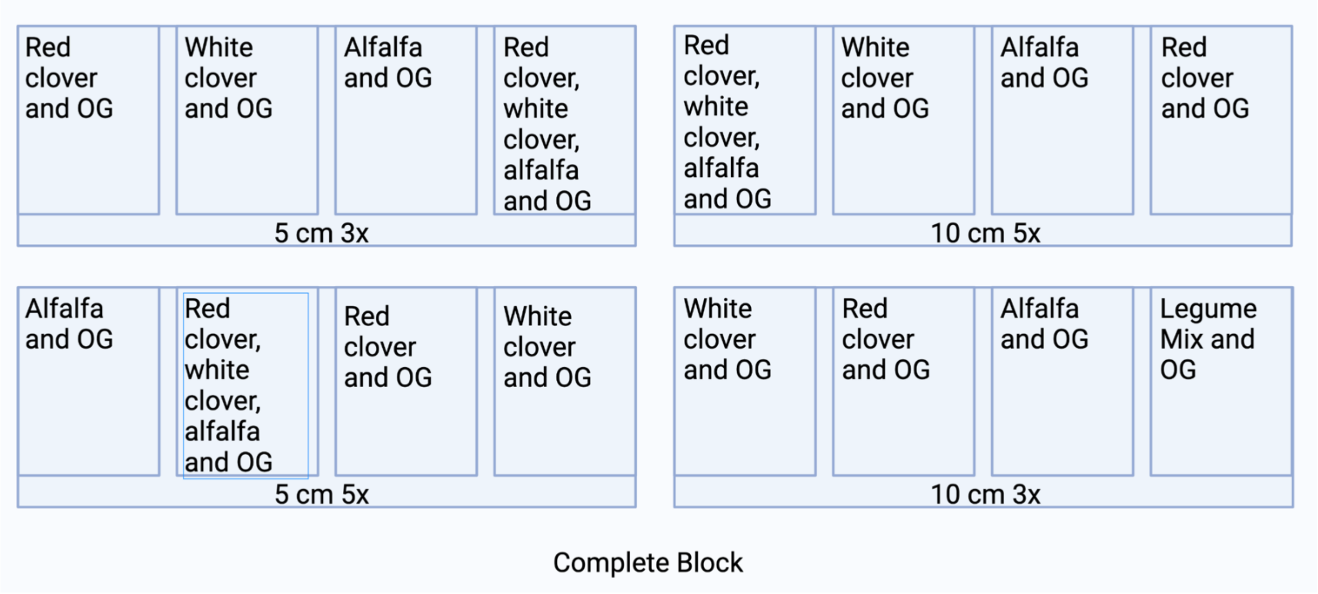

Figure 2.1. Illustration of experimental field design portraying a single block. Experiment was replicated in five blocks with one replication per block-treatment combination. Four harvest treatments were imposed on main plots. Subplots were seeded with one of four mixture treatments.

Aboveground biomass and environmental variable data collection

Before each harvest event, crop and weed biomass was sampled from one 0.25 m^2 quadrat placed in the center of each subplot. All harvested biomass was sorted to species, oven-dried, and weighed. Total weed biomass, total crop biomass, total legume biomass, total orchard grass biomass, and ratio of orchardgrass biomass to legume biomass were calculated across years as kg ha^{-1}. Soil volumetric water content was measured 27 September 2022.

Seedbank emergence assay

The soil weed seed bank was sampled in each subplot in May 2022, 3.75 years after the treatments were established, and weed seed density and species composition were assessed using the emergence method (Gross and Renner 1989) where a soil sample is treated to break weed seed dormancy, and germinated seeds are identified and counted at their seedling stage so that seedling count is a surrogate for seedbank density (Fig. 2.2). Twelve 2.6 by 10 cm soil cores were collected per subplot and mixed into one composite sample per plot. Samples were spread on 25.4 cm by 50.8 cm greenhouse flats filled with a 2.5 cm deep weed seed free growing medium of peat moss, vermiculite, and perlite (PRO-MIX BX, Premier Tech Growers and Consumers, Quakertown, PA, USA). Flats were watered with tap water daily to encourage seed germination and seedling emergence. Weed seedlings were censused weekly at which time seedings were counted and identified to species or genus. Once identified, seedlings were removed. The census was conducted for 7 weeks. Soils in the trays were then allowed to dry completely for one week, then lightly disturbed and re-watered, and emerged seedlings were censused for an additional 6 weeks.

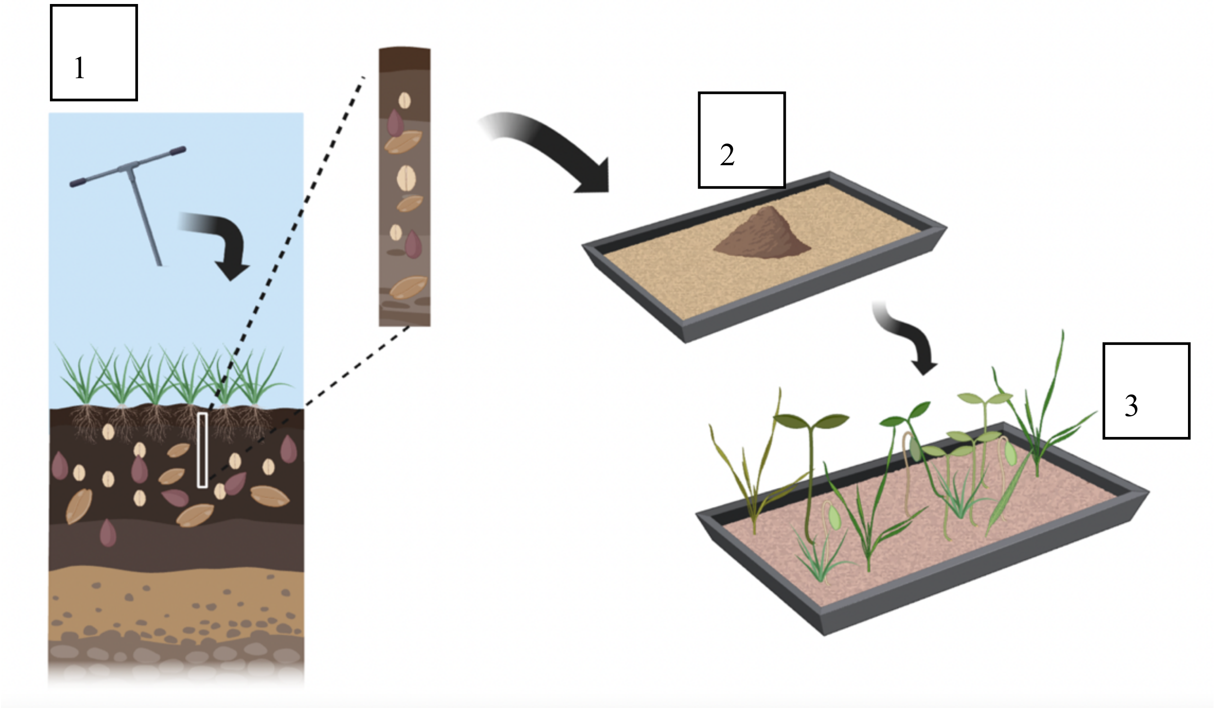

Figure 2.2. Workflow used to collect soil samples from the study site to evaluate treatment effects on seedbank emergence. Twelve samples per subplot were collected May 2022 (1) and homogenized. Composite samples were spread over media (2). Seedlings were allowed to emerge for 14 weeks total and were identified and removed weekly (3). Figure created in Biorender.

Seedbank density was calculated as emerged weed seedlings m^{-2}, considering two-dimensional field surface area sampled as is standard in the literature (e.g., Sanderson et al. 2014, Smith and Gross 2006). Any crop species present in the seedbank were excluded from weed seedbank calculations when crop species aligned with mixture treatment. Weed seedbank community diversity was described with species or genus richness and Shannon's Diversity Index (H) (Shannon 1948). Seedlings were further classified in the data into monocot or dicot, and into annual, biennial, or perennial based on descriptions from Haines et al. (2011), Rhoads et al. (2007), and Uva et al. (1997). Lastly, weed species that were present in the germinable seedbank but not in the aboveground weed community sampled during 2021 were considered "legacy seeds." All legacy seeds were present in the aboveground weed community in 2019-2020. Germinable legacy seed abundance was summed to create a total legacy seeds response variable.

Statistical analysis

Weed seedbank density data were tested for normality with the Shapiro-Wilks test and for homogeneity of variance with Levene's Test. We used a factorial analysis of variance (ANOVA) to test for differences in weed seedbank density, species richness, and H. The model included mixture composition, harvest frequency, and cutting height as fixed effects, with all possible two-way and three-way interactions considered, and with block as a random effect. The three-way interaction between block and the two main plot factors were specified as the error term in the model (Appendix C). Where a significant treatment effect was detected, means were separated using Tukey's Honestly Significant Difference test at $\alpha = 0.05$. Linear regression was used to assess the effects of previous harvest crop and weed biomass on germinable weed seedbank density.

Several multivariate analyses were used to identify and characterize differences in community composition attributable to our treatments. Prior to analyses, species absent in 95% of trays were removed from the dataset (McCune and Grace 2002), leaving 29 remaining species. Abundance data were relativized by row totals to minimize the influence of extreme values. A Bray-Curtis distance matrix was calculated using the relativized abundance data. To visualize weed seedbank communities we performed an ordination with non-metric multidimensional scaling (NMDS) using 250 runs. We used permutational multivariate analysis of variance

(PERMANOVA; Anderson 2001) to test for treatment effects on the weed seedbank community. Indicator species analysis (ISA; Dufrêne and Pierre Legendre 1997) was used to identify species associated with treatments using data that were square-root transformed rather than relativized in order to normalize the skewed distribution but let differences in total abundance remain. All statistical analyses were carried out in R v. 4.1.2 (R Core Team 2022).

RESULTS AND DISCUSSION

Effects of harvest management and mixture composition on seedbank abundance and diversity

A total of 5,547 seedlings representing 38 species or genera emerged from the seedbank samples over the course of the study. Mean weed seedbank density was 10,823 ± 712 seeds m^{-2}, somewhat less abundant than the average seedbank sampled by Smith et al. (2018) in their study of 77 organic farms and their seedbanks in our region. Seedbank communities were dominated by slender rush (*Juncus tenuis* Willd.), garden yellowrocket (*Barbarea vulgaris* W.T. Aiton), redroot pigweed (*Amaranthus retroflexus* L.), carpetweed (*Mollugo verticillata* L.), common purslane (*Portulaca oleracea* L.), common plantain (*Plantago major* L.), speedwell (*Veronica* spp. L.), and common lambsquarters (*Chenopodium album* L.). A complete list of weed species in the germinable seedbank can be found in Appendix B. Total weed seedbank density did not differ across any of the treatments (ANOVA: $p > 0.05$).

Diversity (H) did not differ among harvest treatments or mixture treatments (ANOVA: $p > 0.05$). Mean richness across the experiment was 13.32 ± 0.32, and richness was significantly higher (p=0.01) in alfalfa plots across harvest treatments (mean richness = 14.4) compared to red clover plots across harvest treatments (mean richness = 11.6; Fig. 2.3).

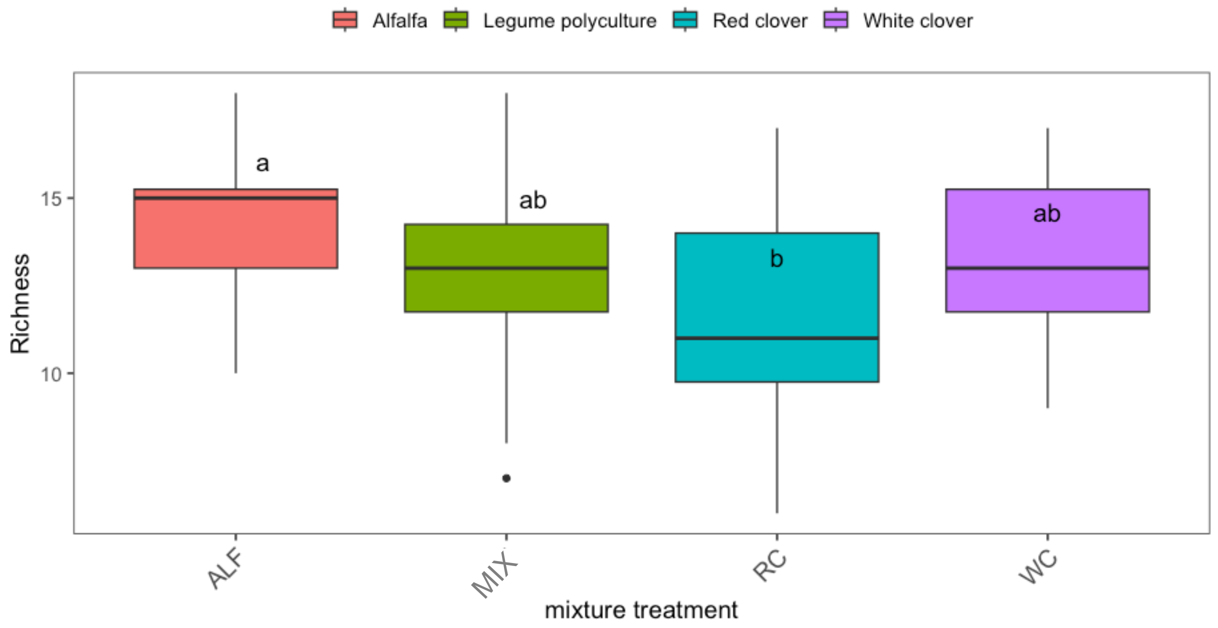

Fig. 2.3. Richness of weed seedbank taxa (species or genus resolution) across mixture treatments. Mixture treatments are ALF, alfalfa-orchardgrass; RC, red clover-orchardgrass; WC, white clover-orchardgrass; and MIX, alfalfa-red clover-white clover-bird's foot trefoil-orchardgrass. Bold lines are median and boxes are interquartile range. Boxes sharing the same letter are not significantly different (Tukey HSD test, p<0.05).

Mixture treatments appeared to differentially influence taxa richness. The mechanism of treatment differences in richness is unresolved, but a major functional difference in alfalfa and red clover plots was relative legume abundance. Alfalfa plots had very little legume biomass compared to red clover plots and were much more dominated by orchardgrass. We hypothesize that this could have resulted in differential abundance of open niches for weeds to exploit.

Effects of harvest management and mixture composition on seedbank community composition

Seedbank community composition and abundance was not affected by mixture treatment (PERMANOVA: $p>0.05$) but did differ based on the harvest treatments. Specifically, there was an interaction between harvest height and harvest frequency (PERMANOVA: $p=0.001$). Pairwise comparisons indicated the 3x5cm harvest treatment was different from the three other harvest groups (Fig. 2.4). This agrees with previous findings that grazing and mowing management history affect the community composition of the weed seedbank (Sanderson et al. 2014, Sanou et al. 2018). None of the three other treatment groups were different from one another.

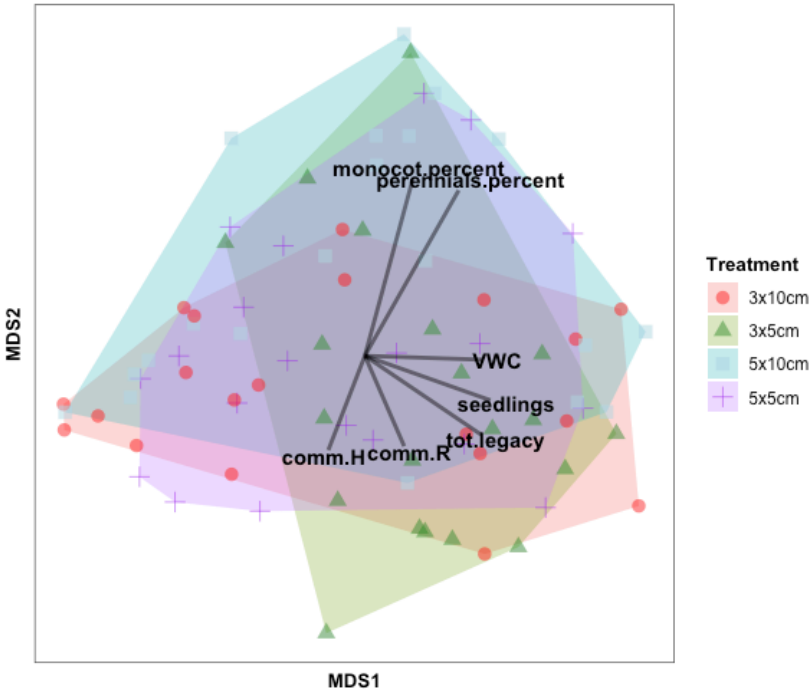

Fig. 2.4. NMDS ordination of species response in the weed seedbank using relative abundance data. Points represent experimental plots and are color coded with harvest treatment. Minimum stress of the best solution was 0.134 with three dimensions included. Biplots are environmental variables (VWC, volumetric water content) and community summary variables (clockwise: monocot.percent, percent of the germinable seedbank that was monocots; perennial.percent, percent of the germinable seedbank that was perennials; seedlings, total seedbank abundance; tot.legacy, abundance of legacy seeds in the seedbank; comm.R, germinable seedbank richness; comm.H, seedbank diversity using Shannon's Diversity Index) that were significantly correlated with ordination axes. Biplot vector lengths are scaled to correlation coefficient.

Ordination axis 1 was correlated with variation in total abundance, total legacy seeds, and VWC, while axis 2 was associated with richness, H, percent monocots, and percent perennials represented in the weed seedbank.

Five weeds were detected in the soil seedbank that were not detected in aboveground biomass in 2021 (most recent harvest year): *A. retroflexus, C. album, Persicaria maculosa, P. oleracea, and Veronica* spp. These were all considered "legacy species" under the assumption that their seeds are in the seedbank due to seed inputs prior to the experiment. *P. oleracea* and

Veronica spp. are included even though they are mat-forming species that could have evaded biomass sampling at 5 and 10 cm, because they were detected in aboveground weed biomass in previous years but not in 2021.

Indicator species were detected in the seedbanks of the 5x5cm and 3x5cm treatments. *M. verticillata* (p=0.008, IV=59.3) and *C. vulgatum* (p=0.099, IV=63.6) were associated with the 5x5cm treatments. *P. oleracea* (p=0.051, IV=58.6) and *Silene alba* (Mill.) E.H.L. Krause (p=0.047, IV=62.7) were both associated with the 3x5cm treatments. *M. verticillata, C. vulgatum*, and *P. oleracea* are mat-forming and set seed close to the soil surface, whereas *S. alba* at its maturity stands from 30-100cm tall (Uva et al. 1997). Viewed within a community assembly framework (e.g., Booth and Swanton 2002), these results suggest that harvest intensity may act as a relatively weak filter on species traits related to stature, favoring species in the community that set seed close to the ground, thereby evading harvest. Conversely, in less intensely harvested plots the defoliation regime enabled a more generalist (in terms of growth form and positioning of reproductive structures) weed community. No significant indicator species were detected for any mixture treatments (ISA: p>0.05), suggesting that despite significant differences in taxa richness among mixture treatments, legume species-specific factors may not be meaningful community filters under the time scales encompassed in this study.

Relationships between weed seed bank density and weed biomass from previous harvests

We assessed the relationship between germinable weed seed bank density and the weed biomass in the harvested plant community compiled over the three years of the study. Regression analysis indicated that seedbank densities were positively related with the biomass of weeds in the harvested plant material. Seedbank density was higher where more weed biomass was harvested across years (R^2= 0.09, p=0.006) (Fig. 2.6). We also assessed this relationship within each of the four harvest treatments.

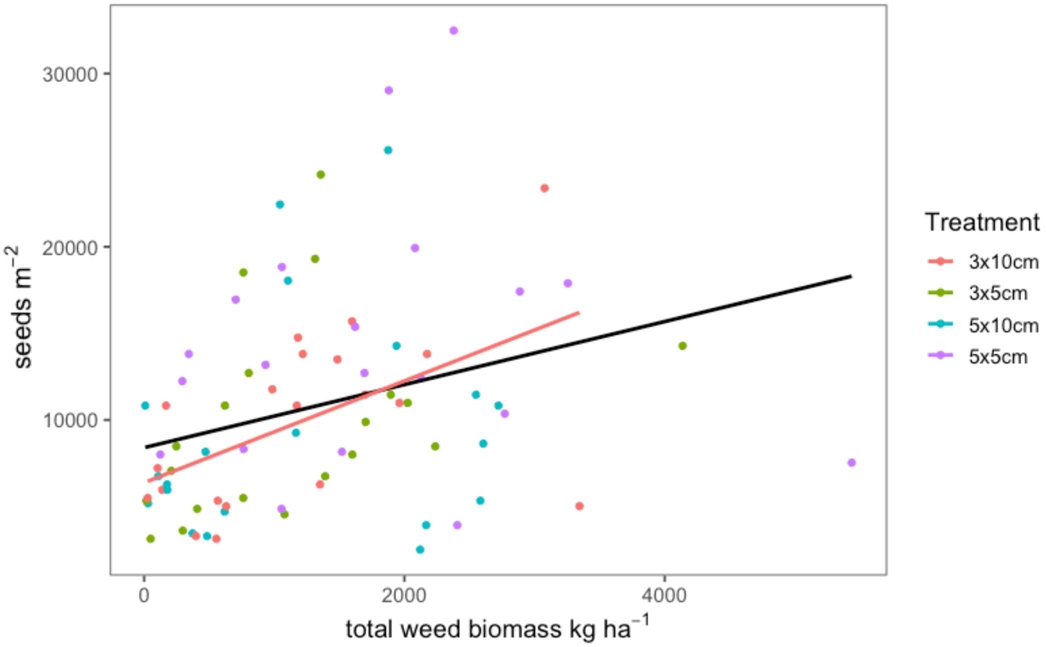

Fig. 2.5. Linear regression of germinable seedbank density by total weed biomass. Each of the four harvest treatments is shown in color. While the overall relationship (black) was significant (R^2= 0.09, p=0.006), the relationship was driven by the 3x10cm (red) treatment group (R^2= 0.25, p=0.01).

The relationship was driven by the 3x10cm harvest treatment group (R^2= 0.25, p=0.01), while the other three treatment groups with more intense harvest did not show a significant relationship between overall weed biomass and germinable seedbank density. This could be because weeds did not have the chance to shed seed as readily as they did in less intensely managed plots. Further work is needed to investigate the role of weedy plant height in seed input in a harvested field. Of the ten most abundant species in the seedbank in this study, five shed seed at ~30 cm or lower (Uva et al. 1997). Short weeds could make seeds more readily than tall weeds when defoliation is occurring at 5 cm or 10 cm. This result provides further evidence that the aboveground plant community drives seedbank community assembly, and reiterates what farmers already know: a weedier field produces a weedier seedbank.

Seedbank abundance relationship to orchard grass and legume crop biomass

Weed seedbank abundance was higher where more orchard grass biomass was harvested across years ($R^2=0.065$, $p=0.02$) and where less legume biomass was harvested in the year 2019 ($R^2= 0.08$, $p=0.011$), though it is worth highlighting that these correlations are relatively weak. Further, the above listed relationships likely are not causal but point toward other drivers of seedbank dynamics. The orchard grass biomass pattern follows the weed biomass by seedbank abundance trend (similar R^2 across all four treatments), indicating that this relationship could have more to do with the productivity on a plot level than with a crop/seedbank relationship. The legume biomass relationship could be because in the establishment year, plots with less established and successful legume biomass would have been less weed suppressive.

Density of the 2022 weed seedbank was unrelated to overall crop biomass harvested, which was unexpected based on previous evidence that dense perennial cover is more weed suppressive and provides more favorable habitat for decomposers and seed predators (Sias et al. 2021). Density of the 2022 weed seedbank was unrelated to overall legume biomass harvested, or to the ratio of orchard grass to legume biomass harvested, a coarse proxy for crop C:N.

Our study shows that perennial weeds are more associated with the seedbank of more intensely harvested treatments, while less intense harvest results in more legacy seeds. Previous work has shown that reduced tillage systems often increase perennial weed presence (Blackshaw et al. 1994, Entz et al. 2002, Peigné et al. 2007), and our work is consistent with evidence that weed communities shift to grasses against broadleaves when in a perennial grass dominated system (Duchene et al. 2023). Our treatments may be differentially favoring a shift to a perennial weed dominated seedbank with repeated mowing because removal of aboveground biomass reduces annual weed fitness disproportionately.

Because we are not seeing overall differences in abundance, either differential input and seed death are occurring that offset eachother's effects, or else seedbank processes among treatments are equivalent. Because legacy seed abundance and total seedbank abundance are both correlated with NMDS axis 1, we hypothesize that the weed seedbank in this system overall is being reduced over time. Further investigation is required to explore how specific seedbank processes relate to the observed treatment variations. Our current understanding of weed seedbank processes hampers the development of targeted seedbank management strategies, but investigating the role of field management on seedbank processes including specific mechanisms of seed death and seed input and their relative importance could aid in developing seedbank management strategies that consider optimal weed seed fates.

CONCLUSIONS

This work provides further evidence that cropping system management acts as a filter on weed community assembly (Menalled et al. 2001, Smith and Mortensen 2017). We showed that in a perennial forage system, the frequency and height of harvest impact the composition of the germinable seed bank. However, these factors seem to be relatively weak filters on the community assembly of the weed seed bank.

We showed that certain weed species are more likely to thrive under more frequent and shorter harvest, and these species share a similar characteristic of low stature. Managers grappling with problematic mat-forming weeds and rotating into perennial forage for weed suppression may be wise to opt for a less intense harvest height and schedule, whereas managers aiming to reduce abundance of problematic tall annuals such as *C. album* and *A. retroflexus* may wish to employ a more aggressive forage harvest approach.

We sought to determine the degree to which we can manipulate harvest management and the composition of forage species to enhance belowground weed suppression in perennial forage systems, whether integrated into annual row crop rotations or otherwise. Based on the findings of this study, the potential for such manipulation to produce biologically meaningful outcomes may be somewhat limited. The treatments tested in this study did not differentially affect seedbank abundance in perennial forage systems. Further exploration in this area is needed to identify contexts where such an approach would be worthwhile to growers.

CHAPTER THREE:
Harvest intensity and composition of a perennial forage affect decay of indicator plant material and the fates of viable velvetleaf (*Abutilon theophrasti*) seeds

INTRODUCTION

Perennial forage cropping systems, namely hayfields and pastures, may enhance biological mechanisms of weed suppression and reduce input needs relative to conventional annual cropping systems (Davis et al. 2012, Schipanski et al. 2017, Smith and Mortensen 2017). In perennial forage systems, plant material is regularly removed from the field by grazing animals or by mowing. Rotation from annual crops to perennial cover can be used as a weed management tool in low-input and reduced tillage systems. Despite evidence that perennial forage crops can foster improved weed suppression through various biological mechanisms, it is unknown the degree to which forage management practices such as harvest intensity and species selection may mediate these effects.

Developing soils that promote microbial decay of weed seeds death could aid significantly in sustainable weed management (Chee-Sanford et al. 2006, Davis 2007, Gallandt et al. 2004, Müller-Stöver et al. 2016). Under this framework, cropping systems can be managed to enhance microbial and macrofaunal communities that decompose and predate weed seeds, reducing weed seed bank abundance (Gallandt et al. 2005, Kremer 1993, Mohler et al. 2018). Weed seed decay has been shown to be influenced by management history and subsequent variation in the microbial decomposer community (Davis et al. 2006) and related parameters such as seed burial depth (Benvenuti et al. 2001), soil temperature (Davis et al. 2005), soil moisture (e.g. Mickelson and Grey 2006, Schafer and Kotanen 2003), and soil nutrient content for some species of weeds (Davis 2007).

Recent literature has called for an improved understanding of the relative importance of weed seed fates (e.g., predation, fatal germination, and decay) and their underlying mechanisms (Geddes 2021). Much of the existing research on weed seed viability loss has not separated losses by mechanisms of seed death. The few studies that have attempted to quantify the relative importance of different seed fates have found variable results. For example, Gallandt et al. (2004) investigated fates of the problematic weed wild oat (*Avena fatua* L.) and found that minor field seedbank decline was due to microbial decay when compared to fatal germination. In contrast, Davis et al. (2006) found that seedbank decline of both giant foxtail (*Setaria faberi* Herrm.) and velvetleaf (*Abutilon theophrasti* Medik.) were linked to field management history and microbial community composition. Lastly, factors such as increased nitrate availability (such as after incorporation and decomposition of legume crop residue or after addition of nitrate fertilizer) can promote seed germination in some weed species (Fawcett and Slife 1978). Effects promoting germination can therefore increase fatal germination can make it difficult to distinguish fatal germination from other fates such as seed decay or predation that may also lead to reduced weed emergence. Resolving the underlying mechanisms of seed mortality and quantifying their relative contribitions are crucial steps toward clarifying unknowns in the field of sustainable weed management.

While previous research has demonstrated that perennial forage crops can lead to substantial reductions in weed abundance and the need for chemical control practices when integrated into annual crop rotations (Davis et al. 2012), little is known about the mechanisms underpinning these responses or the degree to which forage crop management decisions may contribute to the relative weed suppressiveness of these systems. Especially unclear is whether forage crop management may influence the degree to which weed seeds decompose in the soil. Considering our findings that perennial crop harvest intensity affects seedbank community composition (Chapter 2), our objective was to explore the mechanisms underlying this difference by quantifying seedbank decay. We investigated how perennial forage crop mixture composition and harvest intensity treatments influenced the overwinter survival and viability of seeds of a common annual weed species, *A. theophrasti*. We also examined forage management treatment effects on soil organic matter decay more broadly with cellulose cards that were buried at the same time as the velvetleaf seeds.

We expected greater seed and cellulose decay in less intensely harvested (more frequent harvests and shorter stubble) treatments because of the associations between more dense plant cover and soil temperature, moisture, and microbial decay. We also expected greater seed decay in mixture treatments with greater relative aboveground legume abundance because of the previously observed link between N availability and *A. theophrasti* decay (Davis 2007) and because of belowground differences in N and C:N previously observed within this field experiment (Teixeira et al. 2023). We hypothesized there would be a positive relationship between *A. theophrasti* seed viability loss and cellulose mass loss, due to previous literature demonstrating a strong relationship between decay of native plant material and experimentally buried cellulose (e.g. Kurka 1999). By examining the effect of perennial forage management on weed seed decay, we aimed to provide insight into the potential of managing perennial forages to create weed-suppressive soils.

MATERIALS AND METHODS

Study site

We conducted this research on ancestral Pennacook, Abenaki, and Wabanaki land. A field experiment was conducted at the University of New Hampshire Kingman Research Farm in Madbury, New Hampshire (43.17°N, -70.94°W). Soils at the site are Charlton fine sandy loams with 3 to 8% slopes (Soil Survey Staff 2022). Prior to the establishment of the experiment, the site was in mixed production of cover crops and small fruit for eight years and conventional cucurbits prior to 2010.

Experimental field design

The experiment was established on 8 August 2018 and involved four perennial legume-grass mixtures harvested either three or five times per year and cut to either 5 or 10 cm residual forage height. Harvest frequency, harvest height, and mixture composition treatments were arranged in a 2 by 2 by 4 factorial in a randomized complete block design with a split plot. Whole-plot factors were each combination of harvest frequency and cutting height. Mixture treatments were applied to subplots. All mixtures included orchardgrass (*Dactylis glomerata* L.) and one or more perennial forage legume species. Plots were harvested by mowing and removal of biomass with an RCI 36A small plot research harvester (RCI Engineering LLC, Mayville, WI). Plots harvested five times per season were harvested on an approximate 30-day interval, while plots harvested three times per season were timed to optimize stand quality and productivity: the first harvest occurred when orchardgrass was at the swollen boot stage, and the second and third harvests occurred at pre-to early-bloom stages of legume species. The plots were initially harvested together each year in late May or early June, after which the 3x/year harvests continued until September and the 5x/year harvests continued until October. Subplots were four levels of mixture composition (described below) and were 2.29 m by 6.10 m. (Fig. 2.1). Total seeding rate was 1,076 pure live seeds per square meter. Pure live seed proportions of the grass and legumes were:

1. 30% orchard grass, 70% alfalfa (*Medicago sativa* L.);
2. 30% orchard grass, 70% red clover (*Trifolium pratense* L.);
3. 30% orchard grass, 70% white clover (*Trifolium repens* L.);
4. 30% orchard grass, 17.5% red clover, 17.5% bird's foot trefoil (*Lotus corniculatus* L.), 17.5% alfalfa, 17.5% white clover.

Abutilon theophrasti seed burial

Viable *A. theophrasti* seeds and cellulose cards were buried in experimental plots on 3 and 4 November 2022. *Abutilon theophrasti* was chosen because it is an annual weed that is common in the region, it has been used in previous research investigating weed seed decay (e.g. Davis 2007, Davis et al. 2008, Schutte et al. 2008, Schutte et al. 2010), and its seeds are easily identifiable. While *A. theophrasti* is not a problematic weed in perennial forages, it was chosen for its utility as a model weed seed rather than for its relevance to the system. Further, *A. theophrasti* relies on physical seed protection for persistence in the soil and is therefore vulnerable to physical damage to the seed coat (Davis et al. 2008).

Seeds from a single well-mixed and viable lot were sterilized with a solution of 1% NaClO and 1% Tween surfactant in DI water (Van Mourik et al. 2005), rinsed with DI water, and fan-dried before burial. Two subsamples of 50 seeds each were buried per subplot in nylon mesh bags to exclude seed input and macrofaunal seed predation while maintaining access by the soil microbial community. Bags were buried in each subplot between plants, laid flat at 5 cm depth with soil layers maintained. Germination was predominantly discouraged by burying seeds in the fall when soil temperatures had dropped below *A. theophrasti*'s preferred germination range and by burying at the maximum of *A. theophrasti*'s preferred germination depth. Burial at 5 cm also maximizes relevance of this study to reduced tillage systems where the seedbank is closer to the soil surface.

Three indicator bags intended to monitor potential late-season germination were buried outside of the experimental units at the same time seed bags were buried in the experimental plots. These bags were recovered on 16 November and indicated that 3, 4, and 3 seeds had germinated over the 12 to 13-day period since they were buried. Indicator bags were then reburied. Additionally, five control replications of 50 seeds each were stored in mesh bags at 4.2° C for the duration of the burial experiment. Indicator bags were monitored in April 2023 so as to ensure the experimental seed bags were recovered from the field prior to the onset of seed germination. Seed bags were recovered on 14 April 2023. Viability of seeds recovered from the bags was tested with medium forceps pressure ("crush test," e.g. Davis 2007, Warnes and Andersen 1984). Crush tests and tetrazolium redox indicator tests are the primary viability measures for unimbibed seeds, each carrying potential for some nonviable seeds to be classified as viable. For velvetleaf seed lots with a "narrow range of viability" as defined by Sawma and Mohler (2002), the results of the two methods are not statistically different. Seeds that were brittle, easily crushed, or not intact were classified as nonviable. Intact (unimbibed and firm) seeds were classified as viable and “firm.” Individuals with emerged radicals and imbibed individuals which presented a radical upon light pressure were classified as viable and “germinated.” Categorized seeds of the two subsample bags in each subplot were averaged and considered as one observation per category per subplot. Viability remaining was calculated as (firm seeds plus germinated seeds) divided by 50 intial viable seeds.

Cellulose card burial

Cellulose card decomposition is an indicator of decomposition activity; soils with greater microbial cellulolytic decomposer activity will also have greater decomposition of plant-based materials (e.g. Concheri et al. 2018). Previous work has shown a significant positive correlation between mass loss of cellulose strips or cards, local cellulose litter, and basal respiration of the local soil (e.g. Kurka 1999).

Cellulose cards (2.6 cm^2) were weighed, sterilized, rinsed, and dried before burial. Two cards were buried per subplot in the same nylon bags as the seeds mentioned above but separated by a seam in the bags to create independent compartments shared by experimental units. Cards were recovered on April 14, 2023, washed with water, oven-dried, and weighed. Proportion mass remaining was calculated as remaining mass divided by initial mass, averaged across subsamples. All remaining mass averages were nonzero values and the data were usable for analysis.

Soil moisture sampling

Volumetric water content of soil (%) in each experimental unit was measured 27 September 2022 with a Field Scout TDR probe (Spectrum Technologies Inc., Aurora, IL) in order to provide supplementary snapshot data on an important environmental variable. This was immediately prior to the last 5x cut of the season and before seed and card burial, targeting a time in the growing season when greater aboveground biomass variations may enhance the likelihood of detecting a wide range of VWC representation.

Statistical Analysis

Seed viability and card mass data were tested for normality with Shapiro-Wilks test and for homogeneity of variance with Levene’s Test. We used a factorial analysis of variance (ANOVA) to test for differences in weed seedbank density, species richness, and H. The model included mixture composition, harvest frequency, and cutting height as fixed effects, with all possible two-way and three-way interactions considered, and with block as a random effect. The three-way interaction between block and the two main plot factors were specified as the error term in the model (Appendix C). Where a significant difference was detected, means separation was conducted using Tukey’s Honestly Significant Difference where $\alpha = 0.05$. Linear regression was used to assess the relationship between seed viability loss and card mass loss and between each of those measures and soil moisture. Proportion data were arcsine transformed when necessary to manage a right-skewed distribution. All statistical analyses were carried out in R v. 4.1.2 (R Core Team 2022).

RESULTS AND DISCUSSION

Effect of harvest intensity on velvetleaf seed decay, germination, and viability

Across the experiment, 61.2% of seeds were still firm and 3.8% were germinated for a combined 65% of individuals still "viable," leaving 35% of the buried seeds presumed dead (by difference). In the refrigerated control group, 99.6% of seeds were firm, 0.4% decayed, and none were germinated. Percent viable, percent dead, and percent firm were not different among harvest treatments or mixture treatments (ANOVA: $p>0.05$, arcsine transformation applied to manage a right-skewed distribution).

However, there was a harvest height/harvest frequency interaction effect on germinated seeds (ANOVA: $p=0.04$). Germinated seeds were more common in 3x5cm plots than in the other 3 treatment combinations by about 125% (Figure 3.1).

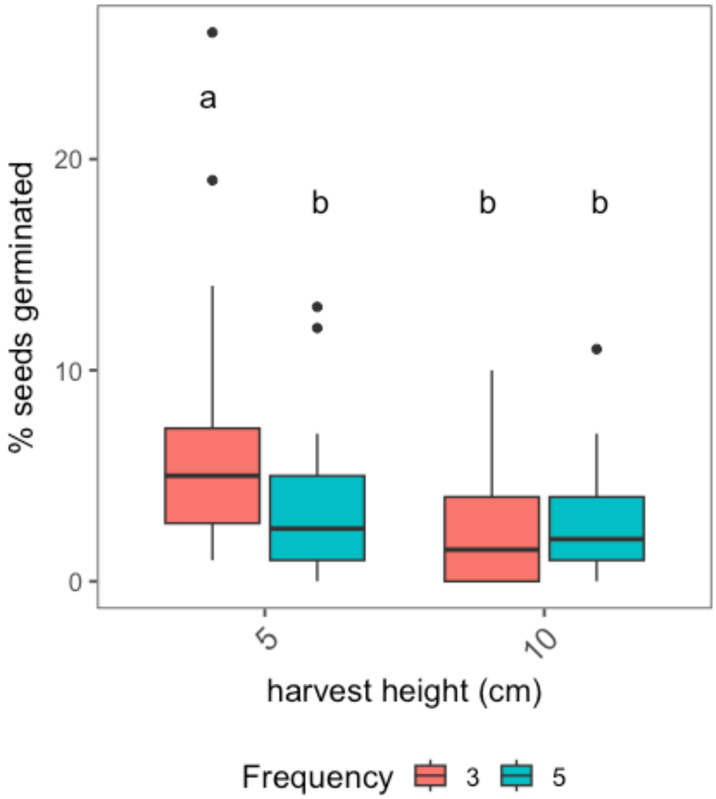

Fig. 3.1. Percent germinated seeds by harvest treatment. Bold lines are median and boxes are interquartile range. Bars with different letters are significantly different (Tukey HSD test $p<0.05$).

These results suggest that forage harvest practices may influence the germination rates of some weed species; however, the mechanism driving these treatment differences is unclear. There could have been treatment differences in soil temperature, plant residue, and light penetration which were not measured in this work. Teixeira et al. (2023) investigated several soil chemical and biological parameters in these experimental plots, focusing on soil organic carbon, nitrogen, and extracellular enzymes, and found that harvest height did not affect any of these variables, but greater harvest frequency was associated with greater permanganate oxidizable carbon, particulate organic carbon, mineral- associated organic carbon, and greater activity of β-glucosidase and cellobiohydrolase.

A possible limitation of our study was that buried *A. theophrasti* seeds included both germinable and dormant seeds. Seed mortality experiments allow for more precise measurement of decay when only persistent seeds (capable of remaining ungerminated in a dormant state) are buried. Nondestructive testing methods for viable seeds with physical seed dormancy, such as *A. theophrasti*, allow for exclusion of easily germinable seeds in burial (Schutte et al. 2010). Fatal germination may contribute to variability in our viability loss data because of buried seeds that were easily germinable (not dormant). While outcome of this study may have been marginally more precise had persistence testing been employed before burial, the very few seeds that could have germinated and died before bag recovery are not meaningfully affecting our results: we used linear regression to assess the relationship between germinated seeds and viability loss and found none, so our treatment differences in viability loss are not attributed to fatal germination.

Harvest frequency and mixture influenced cellulose decay

Cellulose decay was affected by the interaction of harvest frequency and mixture treatment (ANOVA: $p=0.034$, Fig. 3.2) but not by harvest height (ANOVA: $p>0.05$). In red clover and white clover subplots, cellulose card mass was 43% and 86% greater in plots cut 5x per year compared to those cut 3x.

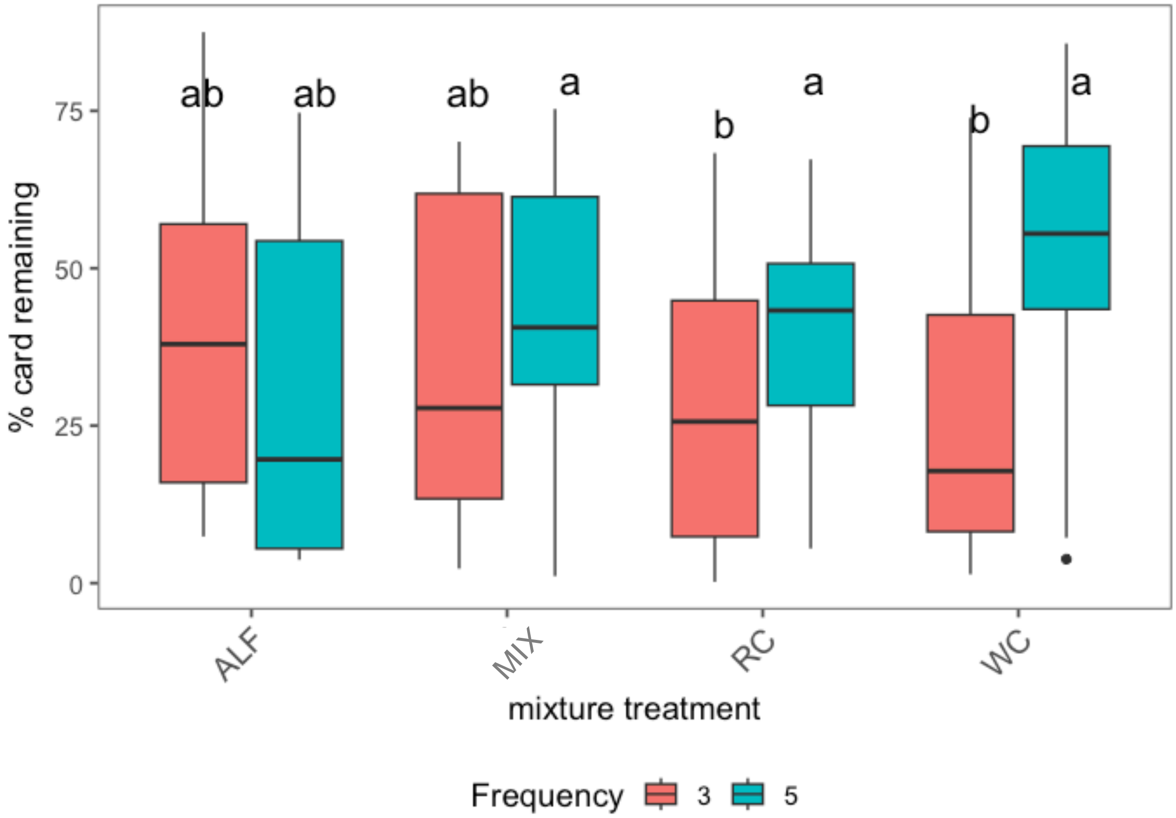

Fig. 3.2. Percent of buried cellulose card mass remaining by harvest frequency and mixture treatment. Mixture treatments are ALF, alfalfa-orchardgrass; RC, red clover-orchardgrass; WC, white clover-orchardgrass; and MIX, alfalfa-red clover-white clover-bird's foot trefoil-orchardgrass. Plots harvested more frequently had more card mass remaining at the end of the burial period when in RC and WC subplots ($p=0.034$). Bold lines are median and boxes are interquartile range. Bars with different letters are significantly different (Tukey HSD, $p<0.05$).

Our hypothesis that cellulose decomposition would be greater in less frequent and higher harvest was partially supported. This could have been due to microenvironment differences resulting from more biomass cover. In this experimental system, soil organic C pools and C-degrading enzyme activity were affected by defoliation frequency (Teixeira et al. 2023), where β-glucosidase and cellobiohydrolase activity, considered indicators of microbial activity, were higher in 5x/year soil. Teixeira et al. hypothesize that forages in 5x/year plots stimulated microbial activity by exuding more C into the soil and resulting in a larger TOC pool. Hence, it is unexpected that cellulose card mass remaining would be greatest in 5x/year plots.

Cellulose mass loss, as demonstrated in previous studies that link greater card decay with native plant material decay, is a coarse measurement of microbial activity, and its scope of inference has been criticized (Howard 1988), though it has continued to be used in peer reviewed publications over decades (Kurka 1999). However, this work brings the method further into question, as patterns in cellulose mass loss did not align with the more direct measurement, being cellobiohydrolase activity (Teixeira et al. 2023).

Velvetleaf seed viability loss was unrelated to cellulose mass loss

The average burial bag had 63.2 ± 0.03% card mass loss. Our hypothesis that seed decay and card mass loss would be correlated was not supported by regression analysis. These findings suggest that the decay of cellulose cards and the loss of *A. theophrasti* seed viability are unrelated and are mediated by different environmental parameters. Cellulose card burial is often used as a proxy for cellulolytic decomposer activity. These findings suggest that cellulose decay may a less appropriate supporting variable when investigating decay of viable seeds, which have more complicated death and decay processes when compared to other cellulose-based detritus.

Mixture treatment did not influence velvetleaf seed decay

Velvetleaf seed decay and remaining viability were not different among mixture treatments. This was unexpected due to previous evidence that *A. theophrasti* decay is associated with lower soil C:N (Davis 2007), and the findings by Teixeira et al. (2023) that in the red clover/orchardgrass and the alfalfa/orchardgrass subplots studied here, mixture composition affected hot water extractable C, N, and C:N. It is possible that the differences in soil chemistry and biology among mixture treatments were not great enough to see a difference in *A. theophrasti* decay. Because all aboveground forage biomass was removed at harvest in this system, the soil chemical effects of legume decomposition on or in the soil were dramatically reduced. Therefore, it is possible that the effects of different crop species production may have been minimized in this study.

Soil volumetric water content was correlated to cellulose card mass loss

Volumetric water content from fall 2022 was weakly correlated with cellulose card mass remaining (R^2=0.06, p=0.02). This weak relationship suggests that while there was treatment variation in soil moisture, this correlation had minimal effects on the outcome of the study. VWC was significantly greater in 5x and 5 cm plots than 3x and 10 cm plots. VWC was not correlated with viability loss of velvetleaf seeds, which was unexpected due to moisture-limitation in microbial decomposers (Schafer and Kotanen 2003, Mickelson and Grey 2006). A limitation of our study was that soil VWC was measured only on one day as a supporting environmental variable. Our findings could differ had VWC been routinely sampled, and/or soil water retention characteristics been measured (e.g. water holding capacity). Future work is needed to effectively pursue these relationships.

CONCLUSIONS

Our results suggest that management of a perennial forage system does not influence decomposition of *A. theophrasti*, at least not in the time scale studied here or to an extent that may be biologically meaningful for agroecosystem managers. Manipulating harvest height, frequency, and mixture treatment did not affect seed death of *A. theophrasti*, though the percent of seeds that germinated was relatively greater in the 3x10cm treatment when compared to the other three harvest regimes. Further research is needed to understand the mechanisms of seed death in these systems and the potential impacts of a wider range of harvest regimes on weed seed fates.

No differences were observed in seed viability loss among different forage crop mixture treatments. Choice of forage crop composition may not have a direct influence on seed viability loss, or the range of mixture treatments in this study may not have been sufficient to capture potential variations in decay processes. Findings could differ in systems where biomass is not removed from the field after harvest, or where animals are grazing and returning fertility.

We observe that the decay of cellulose material in the soil was influenced by the interaction of forage harvest frequency and forage legume mixture. Greater harvest frequency (5x) was associated with more remaining card mass compared to less frequent harvest (3x) when in plots with red clover/orchardgrass and white clover/orchardgrass bicultures. Cellulose decay was also associated with greater soil volumetric water content. Card decay is a rough proxy for cellulolytic decomposer activity, but our hypothesis that card mass loss would be related to weed seed viability loss was not supported. Using cellulose decay as a proxy for overall decomposition activity has limitations and should be interpreted with caution. This study demonstrates that the mass loss of cellulose cards may be an inappropriate supporting measurement for *A. theophrasti* seed decay in the field.

While promoting microbial decay of weed seeds could be a promising approach in sustainable weed management, focusing management decisions on decay may not be an effective primary strategy for seedbank remediation in perennial forage systems like the one studied here. Further investigation is needed to unravel the mechanisms underlying seed mortality and to target development of weed-suppressive soils in perennial forage and rotation systems.

CHAPTER FOUR:
Conclusions

Research that focuses on finding nonchemical methods to manage weeds in reduced-tillage production systems has the potential to achieve two important goals: it can both increase the adoption of reduced tillage practices by providing effective weed management strategies that do not rely heavily on herbicides, and it can help minimize the use of herbicides in production systems that already employ reduced tillage, thus reducing the selection pressure for herbicide resistance in weeds (Brainard et al. 2013).

A guiding hypothesis of this work was that perennial forage management could be manipulated to improve the weed suppression potential via seedbank processes. Rotation to perennial cover is used as a weed management tool in low-input and no-till systems, which tends to reduce weed pressure in the following crop and shift weed community composition (Entz et al. 2002). Integrating both annual and perennial crops into organic systems has been shown to reduce annual weed pressure. We saw effects of forage management on seedbank community composition and differences in experimentally buried seed germination and cellulose decay. Overall seedbank abundance did not differ among experimental treatments tested here. This leads us to believe that cultural intervention on the weed seedbank with targeted management (harvest or species selection) practices is not quite enough to meaningfully drive the weed seedbank situation. With our *A. theophrasti* burial study, we also found that producers may manage fields for greater harvest intensity without a tradeoff on seedbank decay.

Interestingly, the seed viability and cellulose card data from chapter 3 are significantly correlated with NMDS axis 1 from chapter 2 (Fig. 4.1), indicating that microbial decay could be a predictor of seedbank community composition.

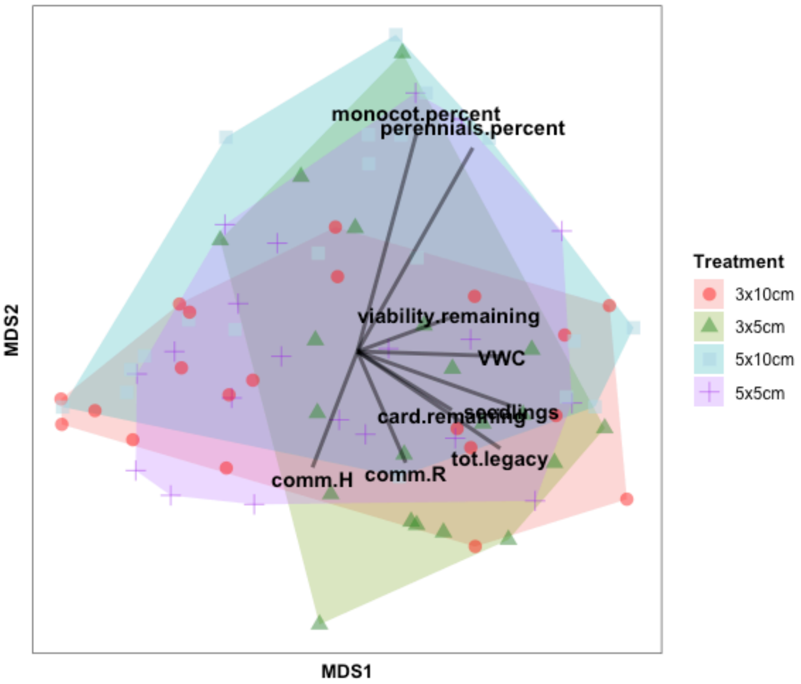

Figure 4.1. NMDS ordination of species response in the weed seedbank using relative abundance data (chapter 2) where chapter 3 results are included in environmental biplot. Points represent experimental plots and are color coded with harvest treatment. Minimum stress of the best solution was 0.134 with three dimensions included. Biplots are environmental variables (VWC, volumetric water content), community summary variables (clockwise: monocot.percent, percent of the germinable seedbank that was monocots; perennial.percent, percent of the germinable seedbank that was perennials; seedlings, total seedbank abundance; tot.legacy, abundance of legacy seeds in the seedbank; comm.R, germinable seedbank richness; comm.H, seedbank diversity using Shannon's Diversity Index), and chapter 3 findings (viability.remaining, percent buried seeds that were still living after burial; card.remaining, percent card mass remaining after burial) that were significantly correlated with ordination axes. Biplot vector lengths are scaled to correlation coefficient.

Our community composition findings could guide management decisions for those grappling with a very specific weed community. We found that some species sharing similar traits favored intense management practices. Mat-forming weed species seemed to be selected for in more intensely harvested treatments. Were a grower rotating a field into perennial forage as a weed management strategy for a problematic weed community dominated by a mat-forming species, it may behoove them to harvest less frequently or leaving a shorter stubble.

Interestingly, the factor with the greatest effect throughout our work was the block in our experimental field. In other words, the overriding effect for germinable weed seedbank abundance and community was the position in the field landscape. This agrees with previous work demonstrating that edaphic factors may drive seedbank community composition moreso than management (Lowry et al. 2021). If the strongest filter on the weed seedbank community is location in the field, that suggests that the legacy effect of the seedbank matters much more than management decisions. However, it is essential to recognize the impact of management decisions on the expressed aboveground weed community, a critical consideration for growers. To achieve effective weed suppression, our community composition findings can guide targeted management practices tailored to specific weed communities.

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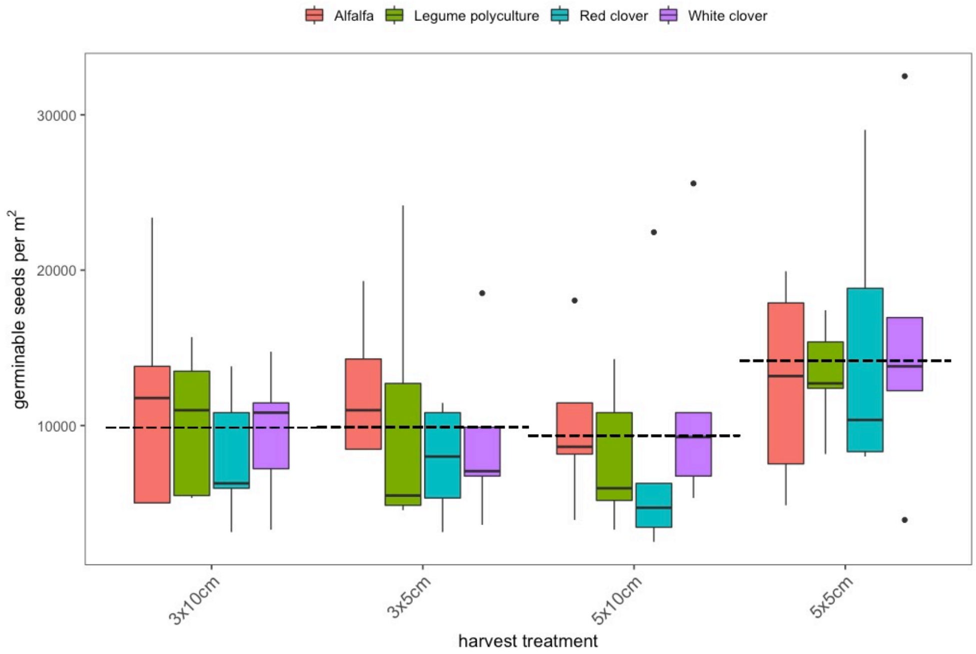
APPENDIX A:
Germinable seedbank abundance across treatments – Chapter TWO



Germinable seedbank abundance was not significantly different among mixture treatments nor harvest treatments. Dashed lines indicate harvest group means. Bold lines are treatment combination median and boxes are interquartile range.

APPENDIX B:
Weeds represented in the germinable seedbank—Chapter TWO

Germinable seed ID	Class	Family	Life Cycle	Seedlings*
Juncus tenuis (Slender rush)	Monocot	Juncaceae	Perennial	947
Barbarea vulgaris (Yellow rocket)	Dicot	Brassicaceae	Biennial	815
Amaranthus retroflexus (Red-root pigweed)	Dicot	Amaranthaceae	Annual	795
Mollugo verticillata (Carpetweed)	Dicot	Molluginaceae	Annual	584
Portulaca oleracea (Purslane)	Dicot	Portulaceae	Annual	414
Plantago major (Broadleaf plantain)	Dicot	Plantaginaceae	Perennial	330
Veronica spp. (Speedwells)	Dicot	Plantaginaceae	Annual, perennial	305
Chenopodium album (Common lambsquarters)	Dicot	Chenopodiaceae	Annual	210
Oxalis spp. (Woodsorrel)	Dicot	Oxalidaceae	Perennial	191
Lamium amplexicaule (Henbit deadnettle)	Dicot	Lamiaceae	Annual	123
Taraxacum officinale (Common dandelion)	Dicot	Asteraceae	Perennial	121
Capsella bursa-pastoris (Shepherd's purse)	Dicot	Brassicaceae	Annual	83
Erigeron spp. (Horseweeds and fleabanes)	Dicot	Asteraceae	Annual	77
Stellaria media (Common chickweed)	Dicot	Caryophyllaceae	Annual	61
Digitaria sanguinalis (Large crabgrass)	Monocot	Poaceae	Annual	58
Silene latifolia (White campion)	Dicot	Caryophyllaceae	Annual or short-lived perennial	51
Persicaria maculosa (Lady's thumb)	Dicot	Polygonaceae	Annual	50
Sonchus spp. (Sowthistle spp.)	Dicot	Asteraceae	Annual, perennial	46
Eragrostis (Lovegrass)	Monocot	Poaceae	Annual, perennial	39
Acalypha (Virginia copperleaf)	Dicot	Euphorbiaceae	Annual	39
Poa spp. (Bluegrass)	Monocot	Poaceae	Annual, perennial	35
Digitaria ischaemum (Smooth crabgrass)	Monocot	Poaceae	Annual	28
Equisetum (Horsetail)	Gymnosperm	Equisetaceae	Perennial	20
Cerastium fontanum (Mouseear chickweed)	Dicot	Caryophyllaceae	Perennial	20
Senecio vulgaris (Common groundsel)	Dicot	Asteraceae	Annual	16
Lepidium campestre (Field pepperweed)	Dicot	Brassicaceae	Annual	12
Potentilla spp. (Cinquefoil spp.)	Dicot	Rosaceae	Perennial	10
Ambrosia artemisiifolia (Common ragweed)	Dicot	Asteraceae	Annual	9
Spergula arvensis (Corn spurry)	Dicot	Caryophyllaceae	Annual	6
Matricaria discoidea (Pineappleweed)	Dicot	Asteraceae	Annual	5

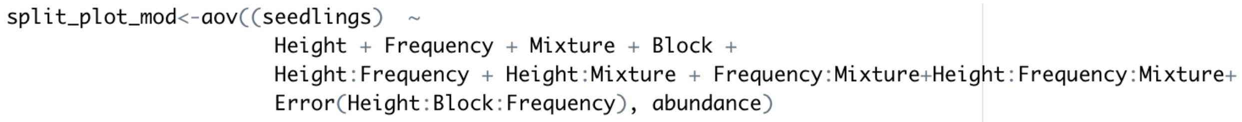
Rumex acetosella (Red sorrel)	Dicot	Polygonaceae	Perennial	4
Bidens frondosa (Devils beggarticks)	Dicot	Asteraceae	Annual	3
Galinsoga quadriradiata (Hairy galinsoga)	Dicot	Asteraceae	Annual	2
Gnaphalium uliginosum (Low cudweed)	Dicot	Asteraceae	Annual	2
Chenopodium Glaucum (Oakleaf goosefoot)	Dicot	Chenopodiaceae	Annual	2
Cyperus esculentus (Yellow nutsedge)	Monocot	Cyperaceae	Perennial	2
Solidago spp. (Goldenrod spp.)	Dicot	Asteraceae	Perennial	1
Euphorbia maculata (Spotted spurge)	Dicot	Euphorbiaceae	Annual	1

*cumulative seedlings in study across treatments

APPENDIX C:
Statistical model for factorial ANOVA with a split plot – Chapters TWO and THREE

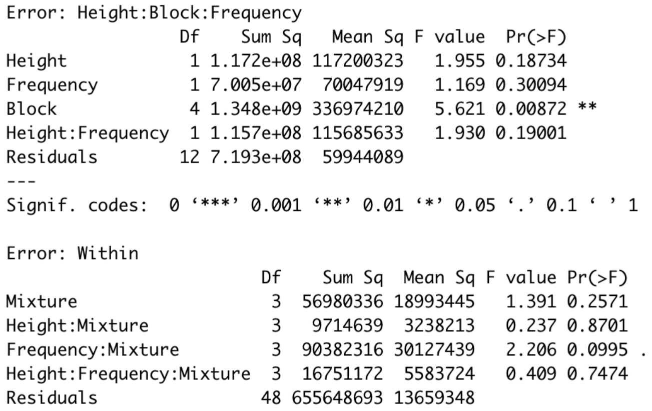
The statistical model for the factorial ANOVA with a split plot is demonstrated below with the response variable of seedling density as an example.

The error term for the main plot is the Block:Height:Frequency interaction. The error term for Mixture (subplot) and the Height:Frequency:Mixture interaction is the residual error.



```
split_plot_mod<-aov((seedlings)  ~
                        Height + Frequency + Mixture + Block +
                        Height:Frequency + Height:Mixture + Frequency:Mixture+Height:Frequency:Mixture+
                        Error(Height:Block:Frequency), abundance)
```

The resulting R output includes an ANOVA table for the main plot (top) and the subplot (bottom).



```
Error: Height:Block:Frequency
                 Df    Sum Sq   Mean Sq F value  Pr(>F)   
Height            1 1.172e+08 117200323   1.955 0.18734   
Frequency         1 7.005e+07  70047919   1.169 0.30094   
Block             4 1.348e+09 336974210   5.621 0.00872 **
Height:Frequency  1 1.157e+08 115685633   1.930 0.19001   
Residuals        12 7.193e+08  59944089                   
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Error: Within
                         Df    Sum Sq  Mean Sq F value Pr(>F)  
Mixture                   3  56980336 18993445   1.391 0.2571  
Height:Mixture            3   9714639  3238213   0.237 0.8701  
Frequency:Mixture         3  90382316 30127439   2.206 0.0995 .
Height:Frequency:Mixture  3  16751172  5583724   0.409 0.7474  
Residuals                48 655648693 13659348                 
```