THE INVESTIGATION OF THE SOIL SEEDBANK IN A LONG-TERM MOWING AND FERTILIZATION EXPERIMENT

By
Skyler DeWall
May, 2024

Director of Thesis: Dr. Carol Goodwillie
Major Department: Biology

ABSTRACT

Seedbanks, the assemblage of dormant seeds found in soils, play a vital role in plant community dynamics and can aid in environmental restoration efforts. Studies have shown that fire suppression, anthropogenic nutrient addition, and human-altered hydrology can all have negative effects on aboveground vegetation, such as a decrease in biodiversity. However, little is known about the effects of these factors on seedbanks. Here I investigated how fertilization, drainage, and disturbance (mowing) have affected the seedbank community in a long-term experimental study of a coastal plain wetland. After 20 years of mowing, unmowed plots are dominated by trees with a limited understory, while the mowed plots are dominated by herbaceous perennial species. Long-term fertilization and drainage have also altered the species composition of the aboveground plant community, with these treatments causing a decrease in species richness. We collected soil samples from each plot at two depths and used the seedling emergence method to characterize the soil seedbank community in a growth room. Long-term fertilization could alter seedling emergence either through its effect on the aboveground community and the seeds that enter the seedbank or through effects on germination. To explore this, I conducted an additional growth room experiment comparing seedling emergence with or without the addition of nutrients to soils of each treatment plot. I then conducted multiple univariate and multivariate analyses to investigate the effects of both the long-term treatments and the nutrient addition experiment on diversity, density and composition of the seedbank and aboveground vegetation.

A total of 2,509 seedlings emerged over the course of the experiment, including 20 families and 59 species. I found that fertilization, drainage, and mowing increased both the total number of seedlings and the number of species that emerged. The multivariate analysis revealed that species composition was also altered by the three treatments. The addition of nutrients increased both the number and diversity of the seedlings that emerged. I calculated a Sorenson similarity index to compare the belowground seedbank community to aboveground vegetation both at the start of the experiment and in the year that soil was collected. Greater similarity was found between the plots that were mowed in the year 2022. While the aboveground plant community demonstrated a decrease and loss of species because of the introduced treatments, the seedbank did not show this pattern. This could be due to the seeds not experiencing the competition the aboveground plant communities face and to a time lag in changes in the belowground environment. The seedbank had species from before the introduction of the treatments and also had species introduced from the new treatments. This time lag demonstrated between changes in the seedbank and aboveground vegetation in this experiment suggests that the seedbank can be valuable tool for conservation efforts. This data set is important because seedbanks are highly understudied, and they are vital to the future and potential restoration of plant communities.

The Investigation of the Soil Seedbank in a Long-term Mowing and Fertilization Experiment

A Thesis

Presented to the Faculty of the Department of Biology

East Carolina University

In Partial Fulfilment of the Requirements for the Degree

Master of Science in Biology

By

Skyler DeWall

May, 2024

Director of Thesis: Carol Goodwillie, Ph.D.
Thesis Committee Members:
Grace Chen, Ph.D.
Heather Vance-Chalcraft, Ph.D.
Ariane Peralta, Ph.D.

ACKNOWLEDGEMENTS

I would like to first thank my parents and grandparents for their continual support in my education and for their motivation to obtain degrees in the sciences. This project and research would not have been possible without Dr. Carol Goodwillie, the director of my thesis. I thank Dr. Goodwillie for inspiring my love and passions for botany. I would also like to thank my committee members, Dr. Peralta, Dr. Vance-Chalcraft, and Dr. Chen, for their contributions to my thesis. I extend gratitude to the undergraduates Alyssa Price and Peyton Suellen for their help throughout the soil collection, growth room upkeep, and data collection. I thank the East Carolina Vegetation Sampling classes, that have collected the aboveground plant data over the course of the past 20 years. I appreciate the East Carolina Biology Department, as well as the ECU Grounds Crew for maintaining the West Research Campus. I thank Anthony Molton, Maddison Salois, Paul Salib, and Carter Stancil for help in collection and watering processes. Lastly, I would like to thank Dr. Michael Larsen, and Dr. Chris Havran for preparing me with the research skills and plant knowledge for this graduate degree.

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CHAPTER I: INTRODUCTION

The suppression of natural fires and the addition of anthropogenic nutrients has changed the natural vegetation of North Carolina and led to a decrease in biodiversity of plant communities (Goodwillie et al., 2020; Krupa, 2003; Opdekamp et al., 2012). Plant diversity supports animal biodiversity and aids in creating healthy ecosystems that have vigor, organization, and resilience (Levins,1998). Biodiversity also provides crucial benefits to humans in the form of food, material, medicine, cleaning of water, protection from natural disasters, and can have cultural and religious significance (Haines-Young & Potschin, 2010). Seedbanks--seeds that lie dormant beneath the soil awaiting favorable factors for germination--may provide a means for plant communities to return to their former composition after natural disturbances and soil conditions are restored. Even if the biodiversity in aboveground vegetation is low, the plant community can be preserved underground, waiting for germination to occur (Vandvik et al., 2026).

Human activities can both increase and decrease the frequency of disturbance. Ecological disturbance is defined as an event that destroys biomass and therefore alters the ecosystem (Lear et al., 2020). Natural fire has historically been an important source of disturbance in the coastal plain of the southeastern United States (Spencer et al., 2017). Before European settlement in 1740, the longleaf pine communities that dominated the southeast are estimated to have experienced wildfires every three to five years (Langley, 2000). Native Americans would also start fires in this region for a variety of benefits to their community, including hunting, collecting, and supporting pioneer species growth (Fowler & Konopik, 2007). However, due to cultural shifts, humans no longer promote or allow for natural fires to disturb the natural vegetation, therefore changing the vegetative landscape. Certain plant species are dependent on fire disturbance, as it clears out competitors for light and restores nutrients to the soil (Luna & Moreno, 2000; Ames et al., 2015). Therefore, without wildfires, many pioneer species that require disturbance are not able to thrive.

Anthropogenic nutrient enrichment through activities such as fertilizer use, runoff, and industrial processes, have also impacted plant communities worldwide. Rainwater can contain nutrients, such as nitrates, that are being added to ecosystems by emission of fossil fuels and other industrial processes (Galloway et al., 2008). Studies have shown that there is a negative linkage between the increased availability of nutrients and plant biodiversity (Krupa, 2003; Phoenix et al., 2006). Fertilizer is predicted to reward fast growing and tall plants that then outcompete shorter or slower growing species for light and other resources (Goldberg & Miller, 1990). Studies have shown that elevated levels of nutrients added to plant communities anthropogenically can cause irreversible effects to the ecosystem, including declines in species richness (Gough et al., 2000; Isbell et al., 2013).

Anthropogenic effects on hydrology can also lead to alteration in plant community composition (Wilcox, 1995). Humans alter natural hydrology by establishing drainage ditches and channelizing streams (Rambaud et al., 2009). Ditching causes soil to drain, which affects the plant community's composition (Lu et al., 2009; Vincent et al., 2013). An example of this change is the loss of wetland specialists to newly drained areas.

The potential for altered communities to recover from anthropogenic impacts may depend on the composition of the seedbank. Seedbanks are seeds located on top of and within soils that can potentially germinate when the optimal environmental factors are present (Roberts, 1981). Seeds can remain dormant, yet still be able to germinate for many years (Quick, 1971). Dormancy is when viable seeds are unable to germinate because of unfavorable conditions (Koorneef et al., 2002). Different species have distinct dormancy-breaking requirements such as a cold stratification, oxygen levels, wetness, levels of light, pH, and soil texture (Baskin and Baskin, 1998). The longevity of seed dormancy varies among species. Plants with long-lived seedbanks tend to be opportunistic, pioneer species; in this life history strategy, plants create many seeds, disperse them widely, and wait for the disturbance events to occur to germinate (Gutterman, 1997). Plant community dynamics are strongly influenced by the bank of seeds buried underground. In a disturbance such as a fire, treefall, or heavy rainfall, what replaces this aboveground vegetation depends on the seeds available to germinate in the seedbank (Hyatt & Casper, 2001).

Adding nutrients to the soil seedbank has been shown to negatively affect its diversity and density (Basto et al., 2015). Studies have demonstrated that the addition of nitrogen can negatively modify germination rates and plant establishment (Ochoa-Hueso & Manrique, 2010). It has also been found that anthropogenic addition of nitrogen and phosphorus indirectly changed the composition of the persistent seedbank because of alterations to soil pH and aboveground vegetation (Zhang et al., 2019). These effects of nutrient addition could limit how well a soil seedbank can restore native plant communities.

During the process of succession, seedbanks can retain the history of the plant community (Kalamees & Zobel, 1997; Sanou et al., 2018). After a disturbance, either natural or anthropogenic, the trajectory of succession depends on the viable seeds in the seedbank. Most seeds found in these recently disturbed habitats are early successional species, such as annuals (Skoglund, 1992). Therefore, the first plants to grow after disturbances are these small, fast growing, and highly dispersed species. Later in succession, the bigger, slower growing plant species, such as trees, will begin to dominate. Soil seedbank composition becomes more different from that of aboveground vegetation the longer the site is left undisturbed (Saatkamp et al., 2018), resulting in a time lag between succession of above and belowground communities (Dalling & Brown, 2009; Zhang et al., 2019). This time lag may be due to the slow maturity of the late successional species, and the persistence of seeds of pioneer species. In suppressing disturbance, such as wildfire, humans have dramatically altered some naturally disturbed plant communities. Therefore, if natural disturbances are reintroduced, seedbanks may have the potential to restore native plant communities (Bossuyt & Honnay, 2008).

A long-term fertilization and mowing (a disturbance that mimics fire by removing biomass) experiment in a North Carolina wetland plant community provided an opportunity to study the effects of these treatments on the seedbank. During the experiment, fertilizer and mowing have decreased diversity and changed the composition of the aboveground vegetation (Goodwillie et al., 2020). I investigated whether after 20 years of treatments creating substantial changes in the aboveground vegetation, the seedbank communities retain similar composition across treatments or have diverged. I used a growth room experiment and the seedling emergence method to investigate the effects of the long-term treatments on the seedbank. Long-term fertilization could alter seedling emergence either through its effect on the aboveground community and thus the seeds that enter the seedbank or through direct effects on germination. To explore this, I conducted an additional growth room experiment comparing seedling emergence with or without the addition of nutrients to soils of each treatment plot. I then conducted multiple univariate and multivariate analyses to investigate the effects of both the long-term treatments and the nutrient addition experiment on diversity, density, and composition of the seedbank and aboveground vegetation.

This study addressed the following research questions: 1) Does seedbank composition differ according to soil depth? 2) How does fertilization, mowing, and drainage affect the diversity, density, and composition of the seedbank? 3) Does fertilization affect the rate of seed germination? Does it affect all species similarly? 4) What is the similarity of the seedbank to current and past aboveground communities? Do the treatments affect the extent of similarity?

CHAPTER II: MATERIALS AND METHODS

Study site

This long-term experiment was established in 2002 at the West Research Campus of East Carolina University. It is in North Carolina's coastal plain and has elevations of 22-25 m. More than half of the land at this site is classified as a jurisdictional wetland because of the lack of drainage caused by the flat topography. The soil is acidic and poorly drained; these characteristics are vital for the successful growth of wetland native plant species. The plant communities present have been characterized as wet pine flatwood, pine savanna, and mixed hardwoods (Chester, 2004). Species present include plants that are fire-adapted, which suggests that in the past this land underwent frequent fires (Chester, 2004). Before the experiment was initiated, the land was managed with prescribed burns and frequent mowing to maintain an open landscape.

Design of long-term experiment

The long-term experiment examines the effects of fertilizer and mowing and their interaction on the plant community. Four treatment plots are replicated on eight 20 m × 30 m blocks. The four plots include 1) no mowing, no fertilizer 2) no mowing, fertilizer, 3) mowing, no fertilizer 4) mowing and fertilizer. Mowing is applied once annually, and fertilizer is applied three times annually to the long-term treatment plots. There is a long-term drainage ditch alongside the plots. This has created a gradient where the four plots that are near to it have drained soil while the four plots farther away from the ditch have wetter soil. Aboveground plant community composition data are collected annually in three randomly and permanently located 1 m^2 quadrats per treatment plot. In each quadrat, the stem count and percent cover of each species present is recorded.

After 20 years, plant communities in mowed and unmowed plots have diverged. The unmowed plots are dominated by woody species such as sweetgum, blackgum, and red maple trees. Meanwhile, the mowed plots contain herbaceous plants such as *Eupatorium* and *Solidago* species. The drained versus the undrained plots also differ in plant species. For instance, the wetter, undrained plots have higher amounts of *Juncus*, sedges, and other wetland specialist species. The drained plots do not have many of these wetland species, and instead favor species that are adapted to drier soils such as winged sumac (Goodwillie et al., 2020).

Design of seedbank study

Soil collection: Soil for the seedbank study was collected in mid-January 2023 using a core sampler (3.1 cm diameter). At each of the 32 plots (4 treatments × 8 blocks), 22 soil samples were collected. To obtain the volume of soil needed for the growth room experiment, seven samples were collected near each of the three vegetation sampling quadrats, and one more core was collected at a haphazardly chosen location in the plot. The soil collected in each core was split into two depths: surface-6 cm and >6-12 cm. Shallow and deep soil samples were placed into separate Ziploc bags and pooled together with the other soil samples of the same depth and block. To avoid cross-contamination, the first sample taken in each plot was discarded before soil collection began.

Effects of treatments on seedbank communities: The soil samples were stored in the refrigerator for three days and then air dried at room temperature for one day and processed to remove plant debris and stones. Plastic pots (10.2 × 10.2 cm) were filled with 450 mL of greenhouse potting mix (Sungro Professional Growing Mix). A layer of 160 mL of field collected soil was then placed on top. From each plot, five pots were filled with soil from the shallow layer (0-6 cm) and five with soil from the deep layer (>6-12 cm), for a total of 320 pots (8 blocks × 4 treatments × 2 depths × 5 replicates). These pots were randomly arranged in 20 trays. The trays were relocated periodically within the greenhouse room so that they were equally exposed to variation in artificial and natural light throughout the experimental period. The seedling emergence method (Heerdt et al., 1996) was used to quantify and characterize the seedbank. I allowed a seedling to grow until it could be distinguished as a specific seedling type, and then it was counted, recorded, and removed to allow for other seedlings to germinate. For every seedling type that emerged, one or two individuals were transplanted to a separate pot to allow for growth of structures such as flowers, needed for species identification.

Effect of nutrient addition on seed germination: To test the direct effects of nutrient addition on seed germination, I compared the emergence of seedlings in soils with and without fertilizer added during the growth room experiment (hereafter, “nutrients added vs no-nutrients added”). Liquid fertilizer (Miracle Gro All Purpose Plant Food 24-8-16 NPK) was applied to soils of each plot at a concentration of 0.23 g/mL of N every two weeks in 30 mL quantities. This concentration and volume were selected because it is comparable to the rate of nutrient application to fertilized plots in the long-term experiment at the West Research Campus. The nutrition addition treatments were replicated on three pots per plot using only soils from the shallow collection. Methods for planting and care followed the protocol for the main experiment above. These additional 96 pots (8 blocks × 4 treatments × 3 replicates) were included in the randomized array of pots in the experiment above. The seedling emergence data for the nutrient added pots were compared with the no-nutrients added shallow soil pots for each block and treatment in the experiment above. We compared the three replicates of the nutrient addition to three randomly selected replicates of the no-nutrient addition pots.

Analyses

Effects of treatments on seedbank density and diversity: To test experimental effects of the long-term treatments on the seedbank, I conducted multiple ANOVAs (analysis of variance) for each dependent variable using only data for the no-nutrient added pots (see above). The independent variables included in the model were depth, long-term fertilization, mowing, ditch proximity, and block. Depth, fertilizer, mowing, and ditch proximity were treated as fixed variables, while the block was included as a random variable nested within ditch proximity. The dependent variables included species richness and seed density (number of seedlings emerging). All analyses in this study were conducted using SPSS version 29.0.1.0 (171).

Effects of treatments on seedbank composition: I conducted NMDS (non-metric multidimensional scaling) and PERMANOVA (permutational multivariate analysis of variance) to explore the effects of treatments on the species composition of the seedbanks, using the data from no-nutrients added pots. The NMDS allowed us to visualize patterns in seedbank composition, while the PERMANOVA tested for significance of treatment effects on the multivariate response variables. I combined data from the five replicates for each plot and soil depth for the NMDS analysis. For NMDS analysis, The PROXSCAL function was selected because it includes improvements to minimize the raw stress. For both NMDS and PERMANOVA, proximities for the distance matrix were calculated using Euclidean distance. Alternative algorithms to calculate distance were explored, and results showed no qualitative differences.

Effects of nutrient addition on seed germination: To explore the results of the nutrient addition study, I conducted separate ANOVAs. The independent variables included in the model were: long-term fertilization, mowing, ditch proximity, nutrient addition, and block. Long-term fertilizer, mowing, ditch proximity, and nutrient addition were treated as fixed variables, while the block location was included as a random variable nested within ditch proximity. The dependent variables include species richness and seed density (number of seedlings emerging). I also inspected the data to see if individual species were positively or negatively affected by the nutrient addition treatment. I looked for trends in the most common seedling species and conducted ANOVAs as needed.

Effects of treatments on aboveground vegetation: I conducted the analyses of the aboveground vegetation data using the same ANOVA models. Importance values, which combine data on the number of stems in a quadrat with percent cover values, were used for analysis of aboveground vegetation. I also conducted NMDS analysis on this aboveground vegetation data to visualize the plant community patterns. To consider temporal changes in the aboveground vegetation, analyses were applied to both data from 2022, the year in which soil samples were collected, and 2004, the first year that data was collected.

Comparison of aboveground vegetation and seedbank communities: The species composition of aboveground vegetation in both 2004 and 2022 were compared to the seedbank communities (from 2023) for each plot. Because most of the seedling emergence I observed was from shallow soils, I used this depth only to represent the seedbank community. Similarity of soil seedbank and aboveground plant communities was quantified using Sorensen's Index (Sørensen, 1948), which has been widely used in seedbank/aboveground vegetation comparisons (Hopfensperger, 2007):

Coefficient of Community = 2c / (a + b + 2c)

where a = the number of species in the aboveground community, b = the number of species in the seedbank community, and c is the number of species shared by both communities. Replicate data for each plot were pooled for this analysis. I conducted ANOVAs using similarity values as the dependent variable to explore the effects of mowing, fertilization, and drainage. A separate analysis was conducted for the 2004 and 2022 vegetation sampling year on the similarity of aboveground and seedbank communities.

CHAPTER III: RESULTS

We stopped collecting data on seedling emergence on June 15th. We chose this date because we observed a sharp decrease in the rate of seedling emergence, and seedlings were beginning to emerge from airborne seeds that had dispersed from a *Gamochaeta purpurea* plant that had matured in the growth room. A total of 2,509 seedlings of 59 species, from over 20 different families, emerged from the seedbanks. The species included 25 graminoids, 31 forbs, and 3 woody species (Appendix 1). Of the 59 total species documented, 48 of those species were identified completely, with the aid of two experts consulted (A. Krings of the NC State University herbarium and B. Sorrie of the NC Botanical Garden). Eleven seedling types were not identified to species, but three have been identified to genus, seven to family, and only one species is completely unknown (Appendix 1). Of the 416 pots, 54 did not have any seedlings emerge throughout the entire experiment, with 46 of these from the deep soils. Out of the eight pots with no seedlings that were from the shallow soils, five were from the control blocks (no mowing and no fertilizer).

Effects of treatments on seedbank density and diversity: In all the analyses of variation, the block nested within ditch was not a significant factor, nor were its interactions significant with other variables, so it was removed from the model. In the seedbank data, two dependent variables, species richness and total seedlings, had similar findings (Tables 1 and 2). Depth of soil significantly affected both variables. Pots with shallow soil, on average, had approximately four times the number of seedlings and more than double the number of species present. Long-term fertilization and mowing significantly increased both the number of seedlings and species richness of pots (Tables 1 and 2). Pots with soil from fertilized plots had nearly double the number of seedlings emerging and higher species richness compared to those with soil of unfertilized plots. Similarly, soils from mowed plots had more seedlings emerging and higher species richness than that of unmowed plots. Proximity to the ditch did not have a significant effect on the total number of seedlings that emerged per pot, and while it did not significantly affect species richness, there was a slight trend toward more species emerging from drained soils in plots adjacent to the ditch. The interactions between depth and fertilization and between depth and mowing were significant for both response variables (Tables 1 and 2). Both treatments (mowing and fertilization) had a greater positive effect on seed density and diversity in shallow than in deep soils. Fertilization and mowing interacted significantly in their effect on both dependent variables, with fertilization having a greater effect in mowed than in unmowed plots. The interaction between depth, fertilizer and mowing was also significant, which largely reflects the extremely low overall seedling number in deep soils.

Effects of treatments on seedbank composition: The NMDS analysis revealed variation in species composition among treatments (Figures 1, 2, 3, and 4) Analysis of shallow soils revealed effects of mowing on the extent of variation among plots (Figures 3 and 4). The unmowed plots were tightly clustered, indicating that they were similar to each other in species composition. In contrast, the mowed plots were separated not only from the unmowed plots, but also from each other, suggesting that disturbance by mowing increases beta diversity. The NMDS analysis of deep seedbanks demonstrated a similar pattern with respect to drainage as seen in shallow soils (Figures 1 and 2). The wet and the dry plots were also separated along axis 1. However, mowed and unmowed plots were not strongly separated. This suggests the deep seedbank is only showing consequences of the drainage ditch, in place long before the introduction of the mowing and fertilization experiment 20 years ago. The PERMANOVA results supported the same pattern found in the NMDS (Tables 3 and 4). The PERMANOVA demonstrated a significant effect of all treatments on species composition in the shallow seedbank, but the analysis found a significant effect of only drainage in the deep seedbank.

Effect of nutrient addition on seed germination: In the experiment that focused on the effects of nutrient addition on seed germination, the results showed that the nutrients applied in the growth room increased species richness and the number of seedlings significantly (Tables 5 and 6). Pots that had nutrients added during the seedling growth period had 32% higher species richness and 37 % higher seedling emergence than control pots. To investigate whether certain species were affected differently than others by nutrient addition, we conducted ANOVAs on individual species data for the most abundant species, using the same model as in the overall analysis (*Lobelia nuttallii, Dichanthelium dichotomum, Eupatorium capillifolium, Dichanthelium scoparium, and Chasmanthium laxum*). In all of them, the trend was toward an increase in density and species richness with nutrient addition, however no species was significantly affected by nutrient addition. These results suggest that the nutrient addition increased germination rates of individual species only slightly but led to an overall significant effect on seedling emergence. Another interesting observation, though not tested with quantitative data, is that seedlings in pots that were given this addition of nutrients seemed to emerge faster than those in control nutrient pots.

Effects of treatments on aboveground plant community diversity: In the analysis of variation, the block nested within ditch was not a significant factor, nor were its interactions significant with other variables, so it was removed from the model. At the beginning of the long-term experiment in 2004, drainage significantly altered the species richness of the aboveground plant community (Table 7). Plots that were drained by the ditch had 26% lower mean species richness than plots away from the drainage ditch. Surprisingly, just one year into the experiment, mowed plots showed significantly higher species richness. It was unexpected for the treatment to alter the aboveground plant community in such a short period of time. Fertilization and interaction effects were not significant. In 2022, drainage also had a significant negative effect on species richness (Table 8). However, the magnitude of the effect was not as large as at the beginning of the long-term experiment with only 13% lower species richness in plots drained by the ditch. Mowing continued to have a significant effect, with species richness in mowed plots more than double that unmowed plots. The effect of the fertilizer treatment also became significant by 2022, with the addition of fertilization causing a 11% decrease in species richness. The interactions between treatments also became significant in 2022, with fertilization and drainage having a greater positive effect on species richness in mowed plots.

Effects of treatments on aboveground vegetation composition: The NMDS analysis also revealed variation among treatments in aboveground vegetation composition (Figures 5, 6, 7, and 8). The analysis for 2004 showed clustering of plots along axis 1 depending on whether they were close to or away from the drainage ditch (Figure 6). The mowing and the fertilization treatment effects had not altered the composition as they had just been introduced (Table 5). In contrast, the NMDS analysis for 2022 showed a clustering of plots along axis 1 depending on whether they were mowed or unmowed (figure 8). When observing the effects of fertilization, it seems the plots first cluster dependent on the mowing treatment, then subdivide that cluster in fertilized versus unfertilized subclusters (Figure 7). This result suggests that the mowing treatment had the highest effect on composition, followed by the fertilizer. The NMDS plot for 2022 did not have an observable pattern of clustering associated with drainage. This introduces the idea that the new treatments might have overpowered the effect that drainage once had on aboveground vegetation composition.

Comparison of aboveground vegetation and seedbank communities: To investigate the similarity between the species composition of the seedbank in 2022 and aboveground vegetation of both 2004 and 2022, we used Sorenson's index of similarity. Overall, this experiment had similarity index values lower than expected based on results of similar studies (Hopfensperger, 2007) ranging from 0 to 0.333. The similarity of the current seedbank to the aboveground plant community in 2004 was not affected by the treatments of mowing and fertilizer, besides a slight increase in similarity between the aboveground vegetation and seedbanks in the wetter plots (Table 9). When comparing the seedbank composition to the 2022 aboveground vegetation, fertilized plots had only a slightly but significantly higher similarity (Table 10). However, there was a substantial and significant effect of mowing on similarity between the aboveground vegetation and the seedbank. The species composition of aboveground vegetation in plots that are mowed was more like their seedbanks, with similarity indices ranging from 0.136 to 0.333, compared to those that are unmowed, which ranged in similarity from 0 to 0.200. This could demonstrate a time lag between successional changes in the seedbank and the aboveground plant community, where the seedbank holds herbaceous species that were previously abundant, instead of the woody species that now occupy the aboveground vegetation of the unmowed plots.

Table 1: Seedbank Analysis of Variance (ANOVA) of seed density (number of seedlings emerging per pot)

Source	Type III Sum of Squares	df	F	P-Value
Depth	2,832.200	1	173.811	<0.001
Fertilizer	616.050	1	37.807	<0.001
Mowing	781.250	1	47.945	<0.001
Drainage	19.013	1	1.167	0.281
Depth*Fert	409.513	1	25.132	<0.001
Depth*Mow	632.812	1	38.835	<0.001
Depth*Drain	2.450	1	0.150	0.698
Fert*Mow	94.613	1	5.806	0.017
Fert*Drain	76.050	1	4.667	0.032
Mow*Drain	4.050	1	0.249	0.618
Depth*Fert*Mow	125.000	1	7.671	0.006
Depth*Fert*Drain	46.513	1	2.854	0.092
Depth*Mow*Drain	49.613	1	3.045	0.082
Fert*Mow*Drain	12.013	1	0.737	0.391
Depth*Fert*Mow* Drain	1.250	1	0.077	0.782
Error	4,953.600	304.00	16.295	
Total	17,706.000	320.00		

Table 2: Seedbank Analysis of Variance (ANOVA) of species richness (number of species emerging per pot)

Source	Type III Sum of Squares	df	F	P-Value
Depth	548.628	1	206.965	<0.001
Fertilizer	63.903	1	24.107	<0.001
Mowing	89.253	1	33.670	<0.001
Drainage	8.128	1	3.066	0.081
Depth*Fert	37.128	1	14.006	<0.001
Depth*Mow	41.328	1	15.591	<0.001
Depth*Drain	0.003	1	0.001	0.973
Fert*Mow	0.703	1	0.265	0.607
Fert*Drain	5.778	1	2.180	0.141
Mow*Drain	0.903	1	0.341	0.560
Depth*Fert*Mow	7.503	1	2.830	0.094
Depth*Fert*Drain	1.378	1	0.520	0.471
Depth*Mow*Drain	1.953	1	0.737	0.391
Fert*Mow*Drain	2.628	1	0.991	0.320
Depth*Fert*Mow* Drain				
Error	805.850	304.00		
Total	1,615.222	320.00		

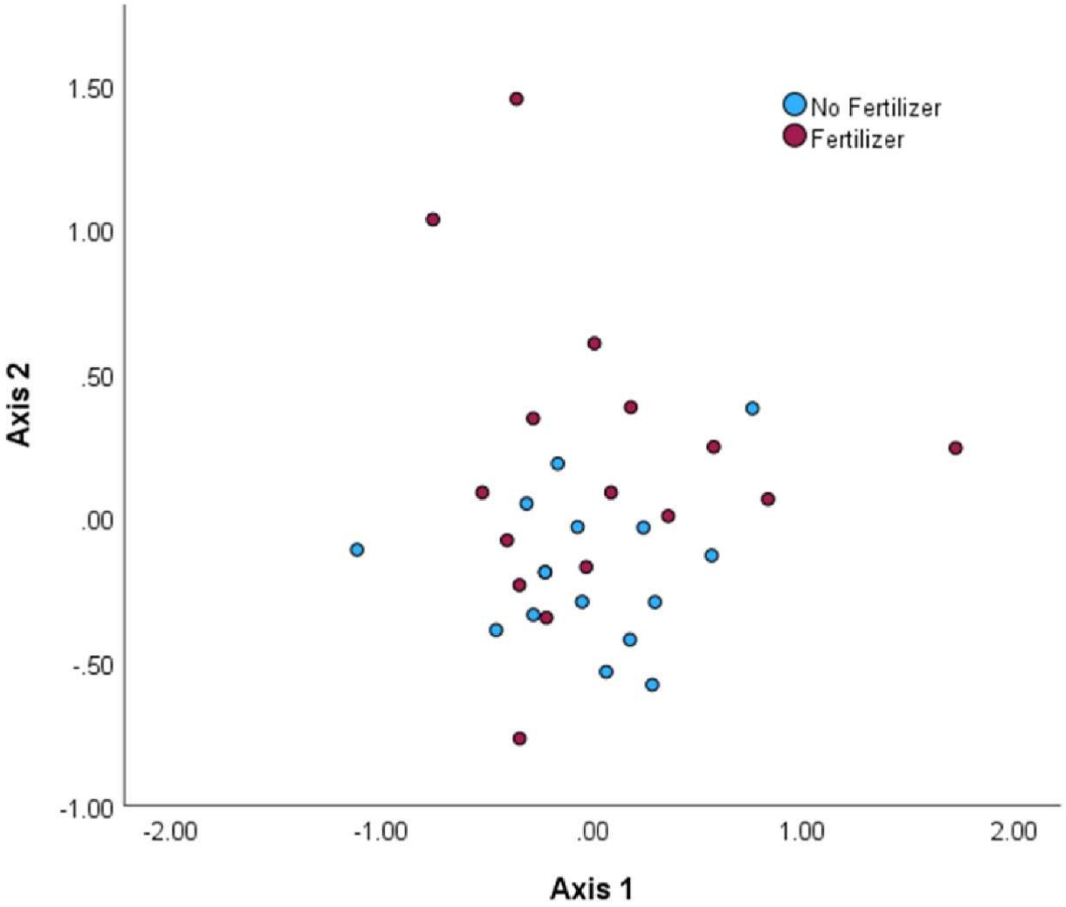

Figure 1: NMDS analysis of the deep seedbank showing effects of fertilizer. Data from five pots were combined for each plot. Colors indicate plots with and without the fertilizer treatment.

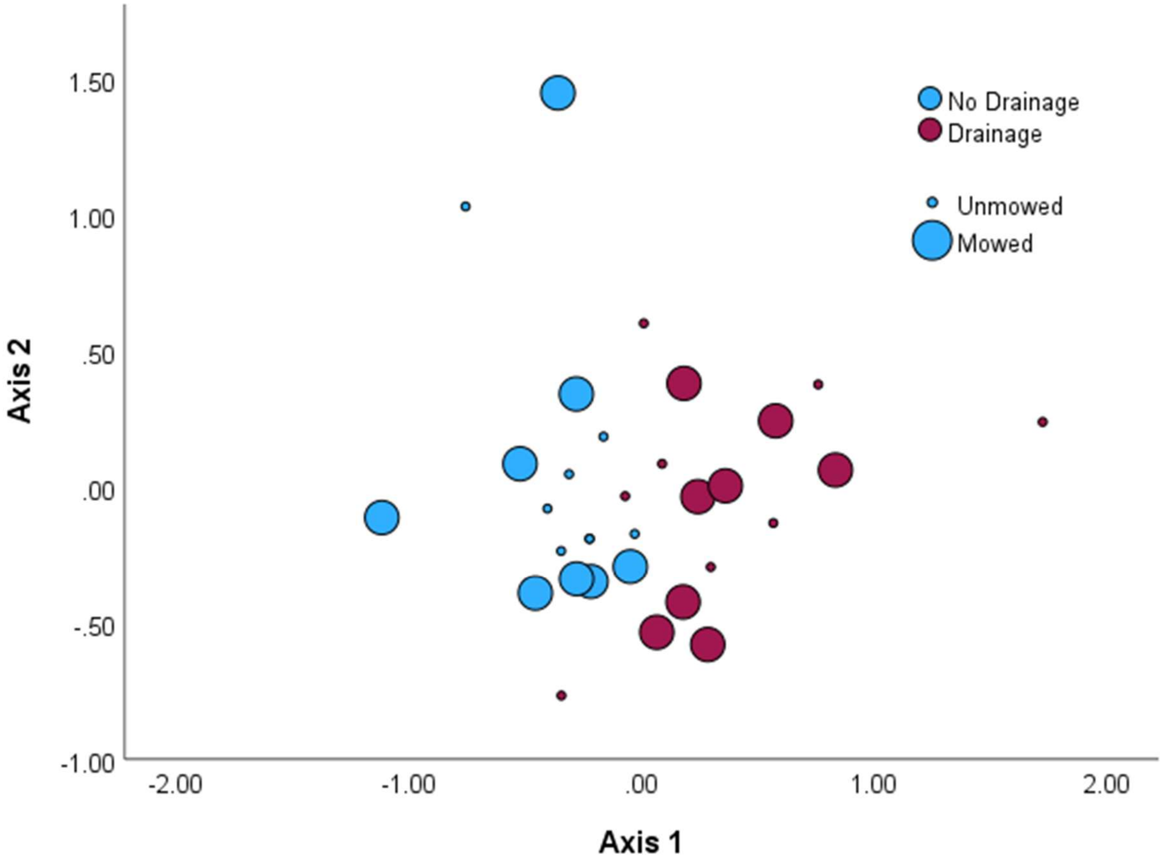

Figure 2: NMDS analysis of the deep seedbank showing the effects of ditch drainage and mowing. Data from five pots were combined for each plot. Colors indicate plot proximity to a drainage ditch ("dry" plots are adjacent to ditch, "wet" plots are away from ditch). Size indicates whether plots were mowed (large) or unmowed (small).

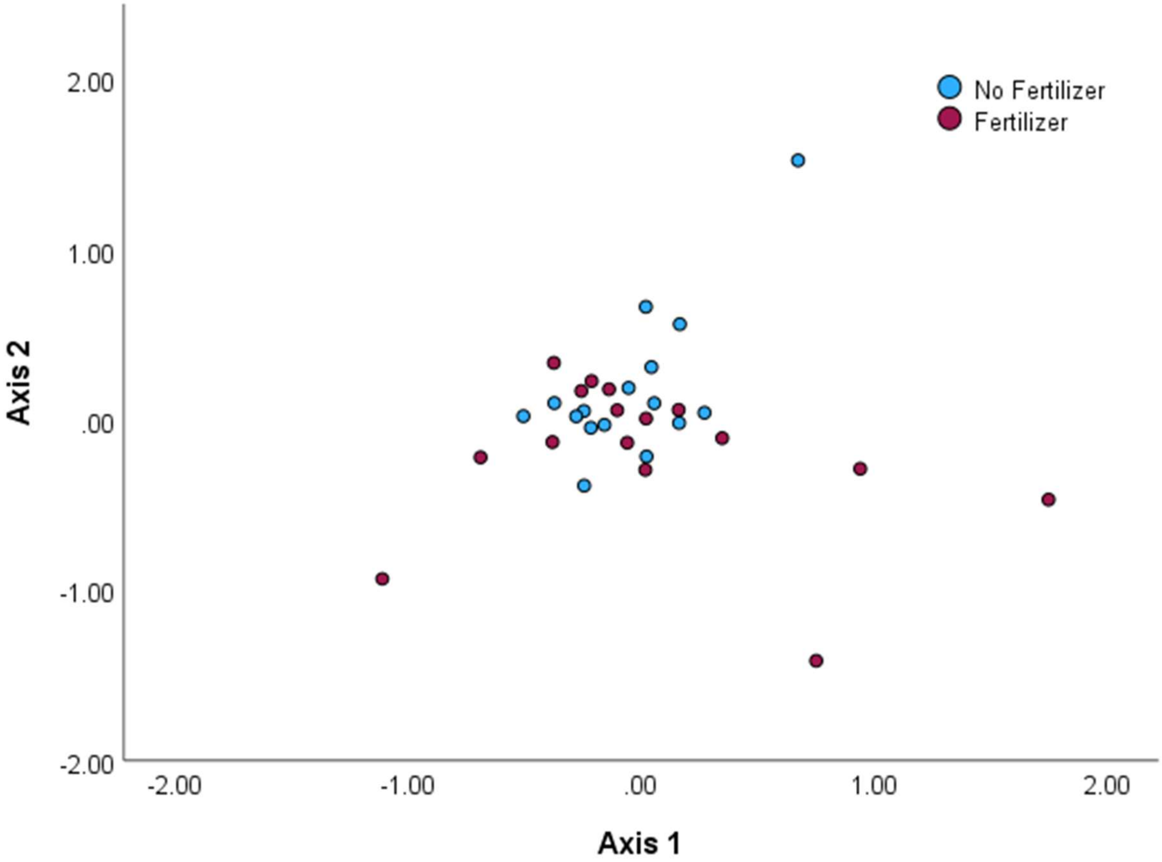

Figure 3: NMDS analysis of the shallow seedbank showing effects of fertilizer. Data from five pots were combined for each plot. Colors indicate plots with and without the fertilizer treatment.

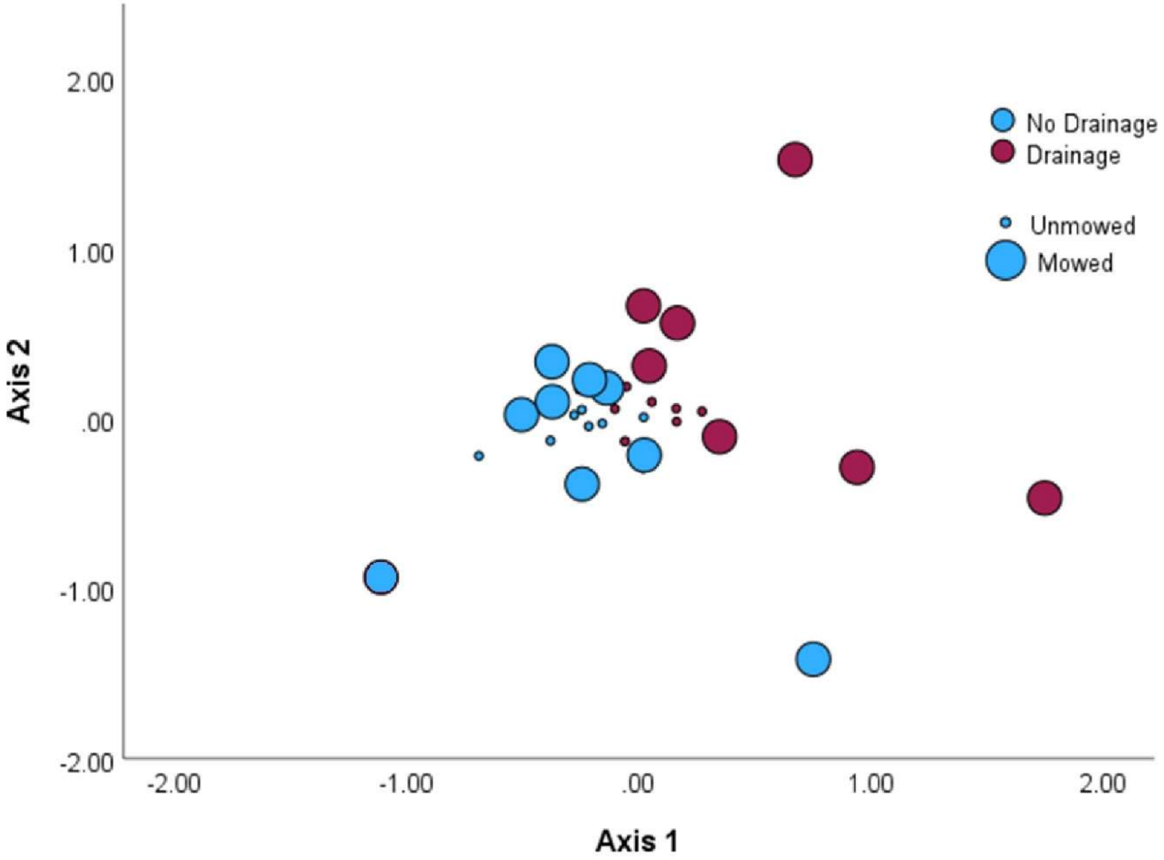

Figure 4: NMDS analysis of the shallow seedbank showing the effects of ditch drainage and mowing. Data from five pots were combined for each plot. Colors indicate plot proximity to a drainage ditch (“dry” plots are adjacent to ditch, “wet” plots are away from ditch). Size indicates whether plots were mowed (large) or unmowed (small).

Table 3: PERMANOVA analysis of the shallow seedbank

Source	Type III Sum of Squares	df	Pseudo-F	P(Perm)
Mowing	998.000	1	3.5351	0.003
Fertilizer	1165.000	1	4.1266	0.001
Drainage	1433.100	1	5.0764	0.002
Fert*Mow	562.000	1	1.9907	0.077
Mow*Drain	365.370	1	1.2942	0.261
Fert*Drain	884.870	1	3.1344	0.005
Mow*Fert*Drain	500.380	1	1.7724	0.104
Residual	6775.500	24		
Total	12684.000	31		

Table 4: PERMANOVA analysis of the deep seedbank

Source	Type III Sum of Squares	df	Pseudo-F	P(Perm)
Mowing	10.312	1	0.57225	0.803
Fertilizer	30.813	1	1.7098	0.101
Drainage	93.688	1	5.1988	0.001
Fert*Mow	19.812	1	1.0994	0.354
Mow*Drain	14.687	1	0.81503	0.582
Fert*Drain	26.937	1	1.4945	0.168
Mow*Fert*Drain	15.188	1	0.84277	0.560
Res	432.500	24		
Total	643.940	31		

Table 5: Seedbank Analysis of Variance (ANOVA) of seed density (number of seedlings emerging per pot) of the Nutrient Addition experiment.

Source	Type III Sum of Squares	df	F	P-Value
Fertilizer	1645.021	1	49.790	<0.001
Mowing	2537.521	1	76.803	<0.001
Nutrient Addition	385.333	1	11.663	<0.001
Fert*Mow	295.333	1	8.929	0.003
Fert*NA	18.750	1	0.568	0.452
Mow*NA	126.750	1	3.836	0.052
Fert*Mow*NA	5.333	1	0.161	0.688
Error	6079.250	184		
Total	26898.000	192		

Table 6: Seedbank Analysis of Variance (ANOVA) of species richness (number of species emerging per pot) of the Nutrient Addition experiment.

Source	Type III Sum of Squares	df	F	P-Value
Fertilizer	141.797	1	29.876	<0.001
Mowing	218.880	1	46.117	<0.001
Nutrient Addition	64.172	1	13.521	<0.001
Fert*Mow	11.505	1	2.424	0.121
Fert*NA	1.172	1	0.247	0.621
Mow*NA	24.797	1	5.225	0.023
Fert*Mow*NA	0.130	1	0.027	0.869
Error	873.292	184		
Total	5287.000	192		

Table 7: Analysis of Variance (ANOVA) of species richness (number of species emerging per pot) of the aboveground vegetation in 2004.

Source	Type III Sum of Squares	df	F	P-Value
Fertilizer	0.167	1	0.019	0.890
Mowing	84.375	1	9.755	0.002
Drainage	759.375	1	87.793	<0.001
Fert*Mow	0.375	1	0.043	0.836
Fert*Drain	51.042	1	5.901	0.017
Mow*Drain	2.667	1	0.308	0.58
Fert*Mow* Drain	2.667	1	0.308	0.58
Error	761.167	1		
Total	35562.000	1		

Table 8: Analysis of Variance (ANOVA) of species richness (number of species emerging per pot) of the aboveground vegetation in 2022.

Source	Type III Sum of Squares	df	F	P-Value
Fertilizer	49.594	1	6.165	0.015
Mowing	1953.010	1	242.776	<0.001
Drainage	78.844	1	9.801	0.002
Fert*Mow	75.260	1	9.356	0.003
Fert*Drain	12.760	1	1.586	0.211
Mow*Drain	58.594	1	7.284	0.008
Fert*Mow* Drain	3.760	1	0.467	0.496
Error	707.917	1		
Total	18065.000	1		

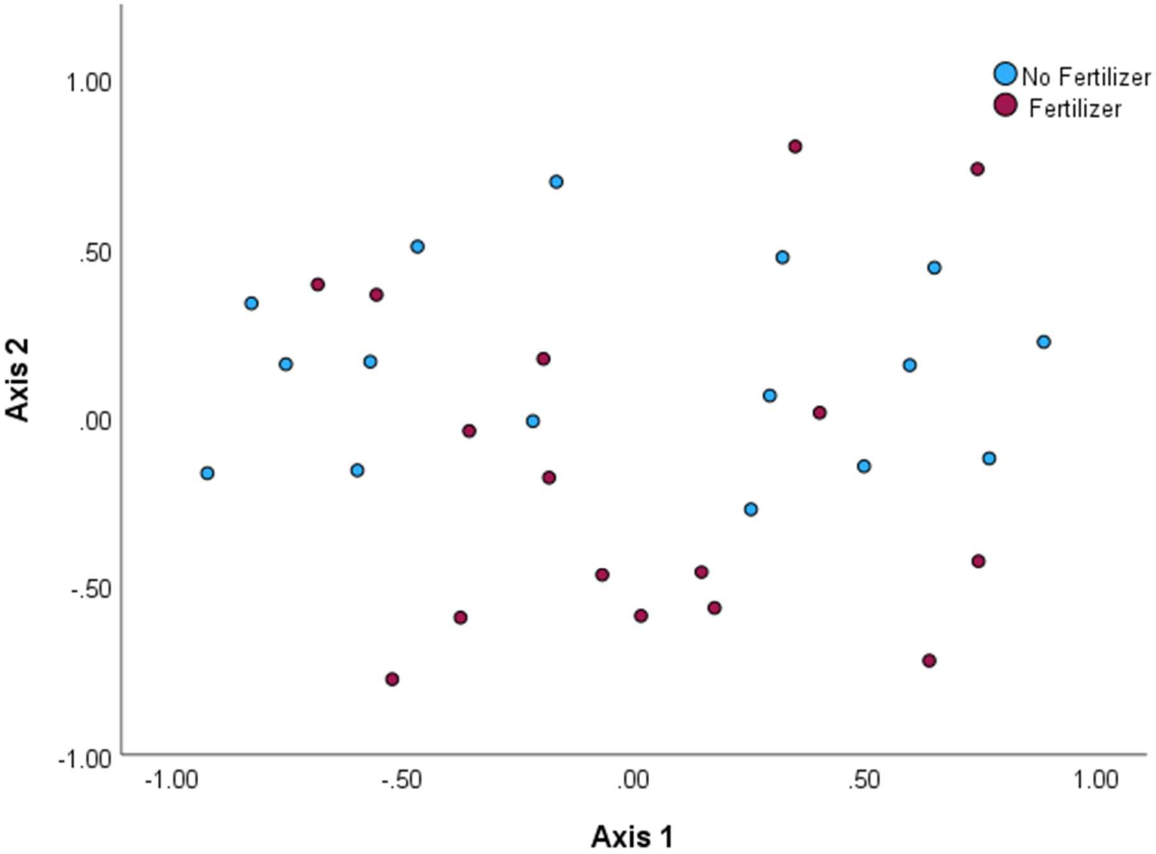

Figure 5: NMDS analysis of the 2004 aboveground vegetation showing effects of fertilizer. Colors indicate plots with and without the fertilizer treatment.

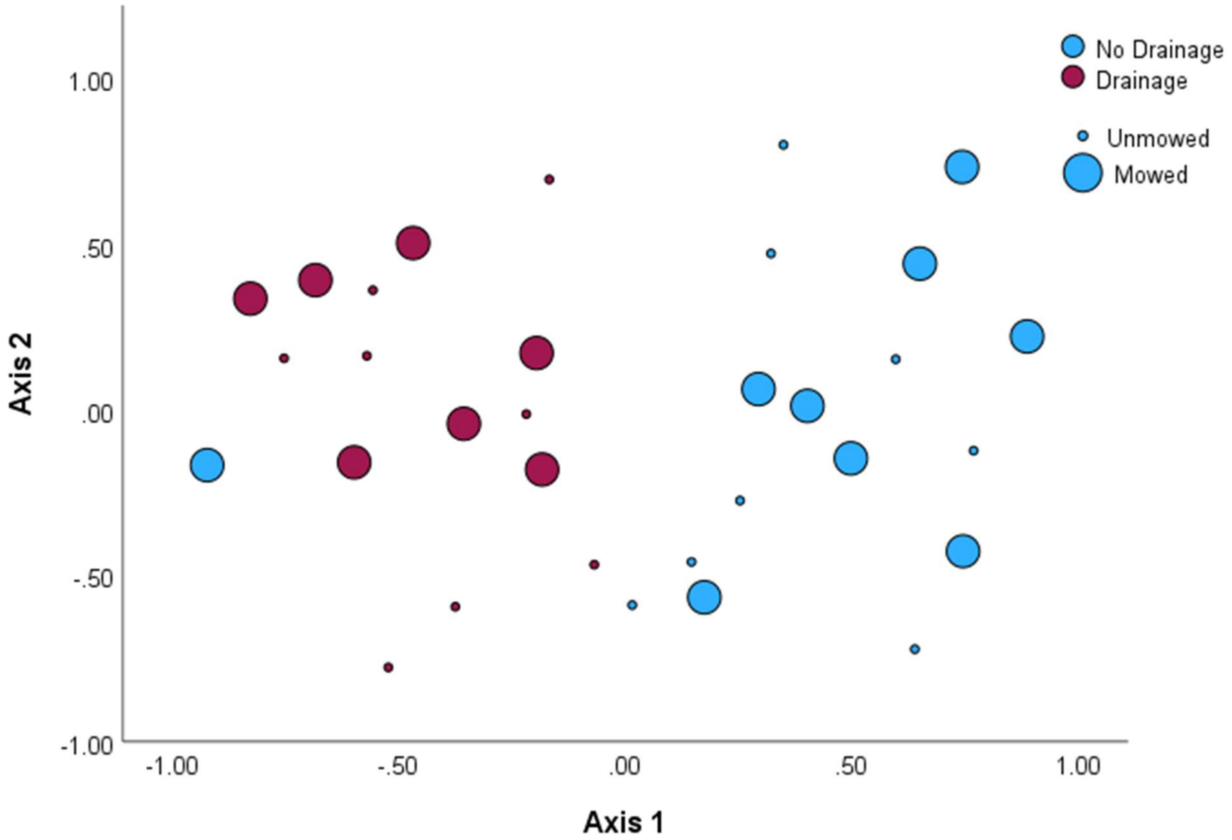

Figure 6: NMDS analysis of the 2004 aboveground vegetation showing the effects of ditch drainage and mowing. Colors indicate plot proximity to a drainage ditch ("dry" plots are adjacent to ditch, "wet" plots are away from ditch). Size indicates whether plots were mowed (large) or unmowed (small).

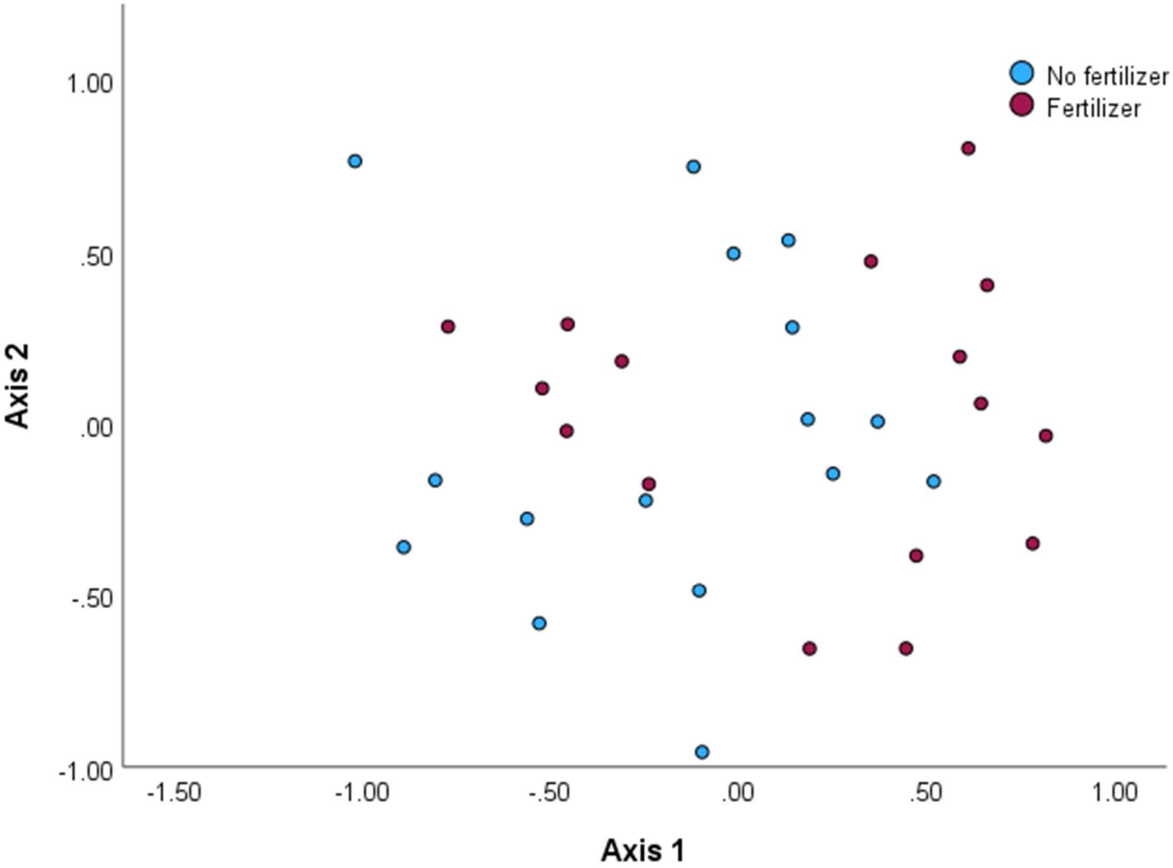

Figure 7: NMDS analysis of the 2022 aboveground vegetation showing effects of fertilizer. Colors indicate plots with and without the fertilizer treatment.

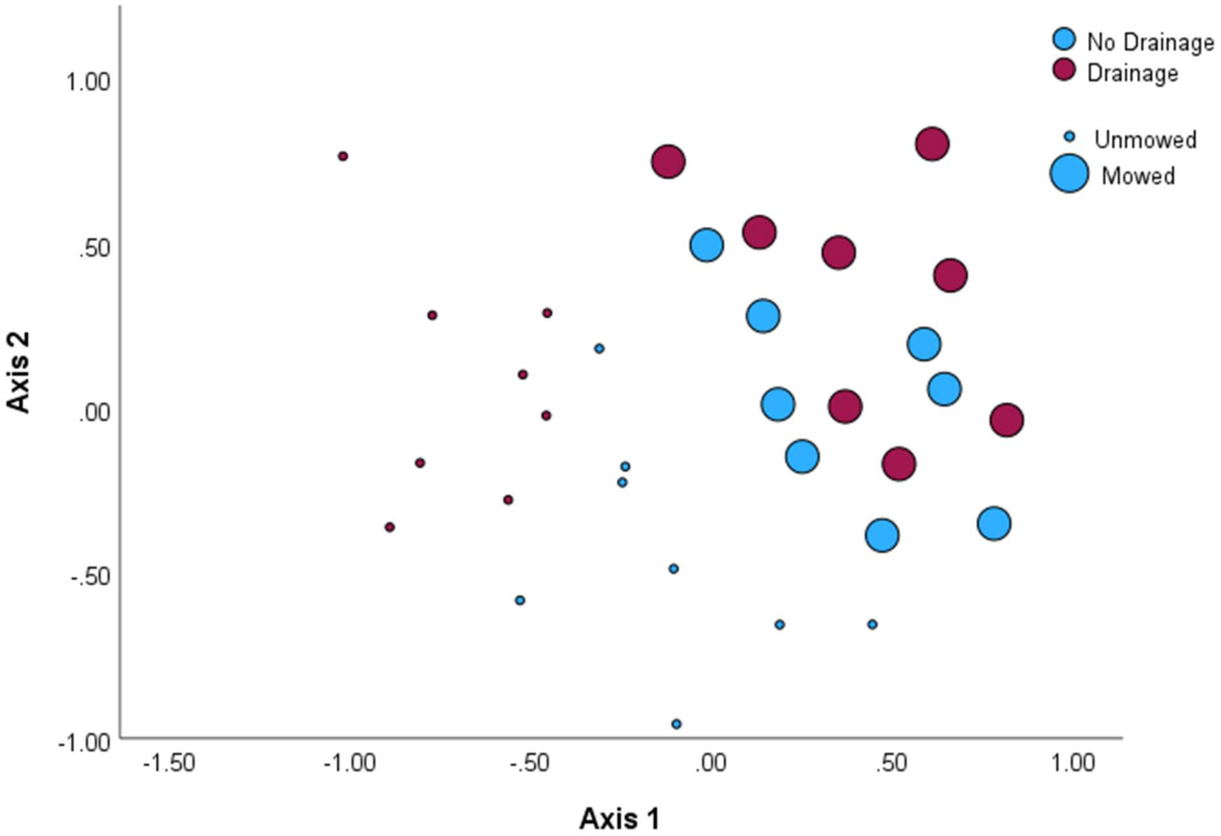

Figure 8: NMDS analysis of the 2022 aboveground vegetation showing the effects of ditch drainage and mowing. Colors indicate plot proximity to a drainage ditch ("dry" plots are adjacent to the ditch, "wet" plots are away from the ditch). Size indicates whether plots were mowed (large) or unmowed (small).

Table 9: Analysis of Variance (ANOVA) of Sorenson similarity index between 2004 aboveground vegetation and shallow seedbank.

Source	Type III Sum of Squares	df	F	P-Value
Mowed	0.001	1	0.548	0.466
Fertilizer	0.001	1	0.877	0.358
Drainage	0.007	1	4.429	0.046
Fert*Mow	0.001	1	0.748	0.396
Mow*Drain	0.001	1	0.0459	0.504
Fert*Drain	0.001	1	0.346	0.562
Mow*Fert* Drain	6.05E-05	1	0.038	0.846
Error	0.038	24		
Total	1.453	32		

Table 10: Analysis of Variance (ANOVA) of Sorensen similarity index between 2022 aboveground vegetation and shallow seedbank.

Source	Type III Sum of Squares	df	F	P-Value
Mowed	0.122	1	45.211	<0.001
Fertilizer	0.014	1	5.188	0.032
Drainage	0.000	1	0.000	1.000
Fert*Mow	0.002	1	0.810	0.377
Mow*Drain	0.004	1	1.609	0.217
Fert*Drain	0.003	1	1.281	0.269
Mow*Fert*Drain	0.004	1	1.441	0.242
Error	0.065	24		
Total	1.397	32		

Table 11: Mean values (and standard deviation) of seed density (number of seedlings emerging per pot) for each treatment group and seedbank depth.

	Ditch +	Ditch -	Fertilizer +	Fertilizer -	Mowing +	Mowing -
Shallow Seedbank	8.00 (5.951)	7.34 (7.461)	10.19 (7.723)	5.15 (4.337)	10.64 (7.561)	4.7 (4.036)
Deep Seedbank	1.88 (1.453)	1.56 (2.359)	1.98 (2.062)	1.46 (1.828)	1.88 (1.878)	1.56 (2.037)

Table 12: Mean values of species richness (number of species emerging per pot) for each treatment group and seedbank depth.

	Ditch +	Ditch -	Fertilizer +	Fertilizer -	Mowing +	Mowing -
Shallow Seedbank	4.14 (1.998)	3.81 (2.521)	4.76 (2.285)	3.19 (1.982)	4.86 (2.299	3.09 (1.877)
Deep Seedbank	1.51 (0.981)	1.20 (1.444)	1.46 (1.043)	1.25 (1.410)	1.52 (1.321)	1.19 (1.137)
Aboveground 2004	15.98 (3.077)	21.60 (3.120)	18.75 (4.174)	18.83 (4.234)	19.78 (4.051)	17.85 (4.141)
Aboveground 2022	11.65 (3.077)	13.46 (6.562)	11.83 (4.516)	13.27 (6.411)	17.06 (4.051)	17.85 (4.141)

CHAPTER IV: DISCUSSION

After 20 years of long-term treatments, this study shows that the drainage ditch, (established before the installation of the experiment) mowing and fertilizer treatments have affected the composition and density of the soil seedbank significantly. All three factors have had a positive effect on both the species diversity and the density of the seedbank, sometimes in contrast to the effects on the aboveground plant community and caused the species composition of the seedbanks to diverge. We found overall low similarity between the aboveground and seedbank plant communities, and the patterns of similarity observed indicate that the seedbank has changed more slowly than the aboveground plant community.

Analyses of the seedbank in the long-term mowing and fertilization experiment suggest that changes in the composition of soil seedbank show a time lag relative to the aboveground vegetation. Twenty years of mowing and fertilization have affected the composition of the seedbank, as seen in the significance of both factors in the PERMANOVA analysis. However, the rate of successional change belowground has been slower than aboveground. The aboveground vegetation in the mowed plots contain diverse herbaceous species, resembling communities present before the start of the experiment, while the unmowed plots have undergone rapid succession to a community dominated by woody species. In the unmowed plots woody species make up almost half of the aboveground composition (43%) as measured in importance values, while in the mowed plots, woody species account for only 13% of the aboveground vegetation. In contrast, the seeds that germinated from soils of both mowed and unmowed plots were almost entirely from herbaceous species, with woody species limited to one seedling of *Liquidambar styraciflua*, *Rubus argutus*, and eight *Rhus copallinum.* In the unmowed plots, the soil seedbank contains rapidly growing ruderal species that no longer are found in the aboveground community. Other studies have described seedbanks as time-capsules because of this ability to harbor species that are no longer found in the aboveground community (Nadkami & Haber, 2009). Supporting this idea, the seedbank in mowed plots was significantly more like the corresponding aboveground vegetation present in 2022, in which vegetation has remained relatively unchanged (mean similarity = 0.254)), compared to the unmowed plots where the plant community has undergone succession (mean similarity = 0.131). In contrast, similarity of soil seedbanks in 2023 to aboveground vegetation in 2004 did not differ in mowed and unmowed plots, as aboveground vegetation had not yet diverged at the start of the experiment (mean of 0.215 compared to 0.204).

In this experiment, approximately five times more seedlings emerged from the shallow (0-6 cm) compared to the deep (6-12 cm) soils collected. It is a common result that the density of seedbanks decreases as depth increases (Savadogo et al., 2017; Hu et at., 2019). Seed rain occurs at the surface of the soils, and through time, the seeds enter the deeper soil layers. Through this time, seeds with high persistence can survive at these deeper layers (Espinar et al., 2005). Therefore, in most cases, the seeds in the deeper soils are the oldest seeds in the seedbank, and there is a significant decrease in the number of seeds found there (Thompson et al., 1997).

Comparisons of deep and shallow soils provide more insight into the time course of succession in the seedbank. This study demonstrated that the long-term treatments had a greater effect on both the density and species richness of the shallow seedbank, compared to the deeper seedbank, as indicated by significant interactions between depth and treatments in the analysis of variance. For instance, species richness was 57% higher in mowed than in unmowed plots in the shallow seedbank but was only 28% higher with mowing in the deep seedbank. Moreover, in shallow seedbanks, mowing appeared to increase diversity among plots as well. In NMDS plots, unmowed plots were tightly clustered, while mowed plots were scattered. Similarly, the fertilizer treatment caused a 49% increase in species richness in the shallow seedbank, and only a 17% increase in the deep seedbank. Seeds found in the deeper seedbank are older and more persistent compared to the shallow seedbank (Thompson et al., 1997). Therefore, the deeper soil retains the imprint of an earlier aboveground community. Another important piece of evidence for a time lag between the deep and shallow seedbank are the patterns in plant composition demonstrated by the NMDS and PERMANOVA analysis of both soil layers. The NMDS analysis of the deep seedbank revealed a strong influence of the ditch drainage (established before the beginning of the experiment) but little effect of mowing and fertilizer, while the shallow seedbank indicated differences in composition associated with both drainage and mowing. The PERMANOVA also showed supporting results with ditch drainage being the only factor to significantly affect plant composition in the deep seedbank, while the fertilizer and mowing treatments were both significant in the shallow seedbank.

One reason there is contrast between the types of species found between the deep and shallow seedbank is that different species have different survival strategies (Grimes, 1977). Seeds of ruderal species are expected to persist in these deeper, older layers because they have greater longevity than seeds of other woody and grassland species. Ruderal species that are dependent on disturbance can survive by distributing many small seeds with high longevity, allowing them to persist until the next disturbance event (Grimes, 1977; Bekker et al., 1998). Therefore, we can expect to find these species in the deeper seedbank. Woody species also tend to have lower dispersal capacity compared to the ruderal species that produce many more seeds with higher dispersal rate (Dölle & Schmidt, 2009). Once the seeds are distributed to the soil, their size and shape also contribute to the seed's ability to move through the soil layers (Dias, 2022; Toth et al., 2022). Many studies disregard deep soil seedbanks because they generally have low seed densities. However, our study and others that have included the deep seedbank, have found distinct patterns of composition between the shallow and deep soils. Therefore, the deep soils should continue to be investigated for insights into seedbank dynamics and conservation efforts (Toth et al., 2022).

The long-term fertilization treatment has led to a loss of approximately 10% of species richness in the aboveground plant community, consistent with other studies that show a loss of diversity in plant communities with nutrient enrichment (Goodwillie et al., 2020; Manrique, 2010; Tillman, 1987). Loss of aboveground vegetation can occur with the addition of fertilizer because certain species can dominate their access to light sources, which can lead to competitive exclusion (Goldberg and Miller, 1990). The addition of nutrients can also cause certain species to increase in biomass, which in turn depletes other species (Oksanen, 1996). In our long-term experiment, the dominance of taller species that are better able to exploit resources in fertilized plots supports this idea (Tate, 2018). In contrast to the aboveground vegetation, however, the long-term fertilization caused more than a 40% increase of species richness in the soil seedbank. Adding nutrients such as nitrogen and phosphorus to the underground plant communities can increase seed density due to the positive effects of nutrients on reseeding processes (Iannucci, 2014). The increases in seed density could therefore lead to an increase in species richness due to a sampling effect; that is, species richness per pot might have been strongly limited by the number of seedlings that emerged. Other studies have found that fertilization has negatively altered the belowground seedbank community significantly less than the aboveground plant community (Zhang et al., 2019), supporting the overall trend that the soil seedbank changes more slowly than the vegetation and is more stable in response to alterations such as fertilization (Ma et al., 2014; Zhang et al., 2019). In our study, however, we found not just a reduced impact but an increase in diversity in the seedbank with long-term fertilization. Fertilizer may cause competitive exclusion of plants aboveground, but these species may remain in the seedbank through seed persistence. Also, the addition of fertilizer may allow new species to be introduced to the seedbank to thrive. For example, *Galium tinctorium* only appeared in the pots that came from the long-term fertilization plots. Dogfennel also appeared significantly more in the long-term fertilization plots compared to the unfertilized. The species remaining in the seedbank and the addition of the new species that thrive with fertilization could lead to higher species richness.

The nutrient addition experiment results suggest that the apparent higher seed density, as measured by the seedling emergence method, might be caused in part by a higher germination rate in response to nutrients in the soil with long-term fertilizer. The seedling number was 32% higher with the addition of nutrients. Other studies have found that the fertilizer has rewarded a few species, while negatively influencing other species (Kirkham & Kent, 1997). The aboveground plant community shows this trend with fertilization increasing the abundance of dogfennel and excluding species such as broomsedge and loblolly pine (Goodwillie et al., 2020). However, the analysis of the top five species found in our seedbank study found similar, modest positive trends in germination in all of them, although none were individually significant.

The proximity to the drainage ditch strongly affected the aboveground plant community and the seedbank composition. The NMDS analysis for both depths of the seedbank, as well as the NMDS analysis for 2004 and 2022 aboveground vegetation, conveys clustering based on proximity to the ditch (Figure 2, 4, 6, and 8). This shows that plant composition in the long-term plots is heavily dependent on adjacency to the drainage ditch. The species composition has been altered based on the disappearance or diminished numbers of wetland species in the drained plots. The proportion of wetland species found in the aboveground vegetation was 70% lower in plots that were closer to the ditch compared to those away from the ditch (Goodwillie et al., 2020). A similar pattern was found in the seedbank, where wetland species were diminished in numbers, and sometimes eliminated altogether. In the seedbank, the wetland specialist *Xyris* was found 59 times in the undrained plots, and only 5 times in the drained plots. There was also a substantial reduction in the abundance of *Juncus* species in the drained soils, with the species of *Juncus dichotomus* appearing 28 times in the undrained, and only 8 times in the drained soils. Other species appeared only in the undrained soils including *Eupatorium mohrii* and *Lobelia canbyi,* which are facultative wetland species. This finding is common in other studies: drained and undrained soils support different species, and wetland species do not thrive in the drained soils (Van der Valk., 2013).

While both the aboveground vegetation and the seedbank showed that wetland species have been reduced in abundance or eliminated from the drained plots, species richness showed opposite trends. Both in 2004 and 2022, the aboveground plant community had significantly lower species richness in plots that were near the ditch. However, even though the aboveground plant community showed a decrease in species richness in both years, the magnitude of the drainage effect has been reduced. At the beginning of the experiment in 2004, the drained plots had a 26% reduction in species richness, while in 2022 there was only a 13% decrease. It is possible that as the ditch is getting older and clogged with plants and other litter debris, it has lost its ability to drain the soil as well as it did in the past. It could also show that the newly introduced treatments of mowing and fertilizer have overpowered the ditch's effect on plant composition.

The seedbank demonstrated the opposite results of ditch proximity compared to the aboveground plant community; species richness in the overall seedbank was 13% higher in the drained soils. The observed increase in species richness in the drained seedbank conflicts with other studies. In other research, seedbanks in drained wetlands showed rapid declines in both species richness and seed density after five years of drainage (Wienhold & Valk, 1989). It is possible that in our study, with ditch drainage occurring over 20 years, the competitive generalist species might have had more time to colonize, reproduce, and integrate into the seedbank. This combined with the persistence of seeds of wetland species whose numbers may be reduced aboveground could explain the higher species richness in our study. Another possibility is that the undrained plots are not able to preserve seeds as long as the drained plots because the seeds rot in the soggy soils.

A novel finding in this study is that both the fertilization treatment and the drainage ditch led to an increase in seedling emergence. We offer a potential explanation that because seedlings belowground do not compete, seeds that have persisted from the past can coexist with seeds that have been added with the new arrival of species that flourish in dry and nutrient rich soils. Interestingly, analysis of other belowground biota in this long-term experiment showed similar trends. Soil microbial diversity also increases with the fertilization treatment (Bledsoe et al., 2020). This suggests that other abiotic and biotic interactions could be leading to the contrasting effect that fertilization and drainage cause to the aboveground vegetation and belowground seedbank.

The similarity index values comparing the seedbank with the concurrent aboveground vegetation (2022) in this study were lower than expected based on relevant studies. Hopfensperger (2007) reviewed 42 seedbank studies located in a wetland area and found that the average Sorensen similarity value was 0.47, which is far greater than even our highest similarity found, 0.33. Several factors might limit the degree of similarity including limitations in sampling and inaccuracy in the seedling emergence method. Soils cores haphazardly collected only a small fraction of the total seedbank and could have missed areas that held many more species. Similarly, vegetation sampling does not record every species present because some species never fall within the randomly sampled quadrats. The quadrats are also sampled during late summer, which could cause the recorders to miss species that have already germinated and died. As a result, soil and aboveground samples might contain different subsets of the total species present in each.

The seedling emergence method assumes that seeds present in the soil collected will germinate. However, studies have found that high seed densities decrease the chances for successful germination (Bergelson & Perry, 1989). More importantly, the growth room conditions may not have provided the factors required for germination of all species, which could have prevented some from germinating. For instance, seeds that are larger take longer to germinate than smaller seeds; therefore, small seeds are favored to germinate (Limón et al., 2016). Woody species tend to have larger seeds, which could limit them from being sampled and successfully germinating during the growth room experiment. In other studies that used the seedling emergence method, forested areas have far lower similarities between the aboveground and belowground plant communities than herbaceous communities (Hopfensperger, 2007), and while our study takes place in a jurisdictional wetland, half of the plots are forested. Our study followed the trend that forested plots had significantly lower similarity between the aboveground vegetation and the corresponding seedbank.

CHAPTER IV: CONCLUSIONS

The coastal plain area of North Carolina is subject to the addition of anthropogenic nutrients through atmospheric deposition (Willey & Kieffer, 1993). The diversity of the aboveground plant community in our study has been negatively affected by long-term fertilization. In this study, we found evidence that fertilization may affect the number and composition of seeds underground but also can increase their germination rates. As one of the first studies to test for both effects, the results were important because they suggest that nutrient addition may influence the dynamics of seedbanks in complex ways that are not yet known.

Seedbanks have the potential to aid in restoration efforts. In our experiment *Xyris ambigua* and *Lobelia canbyi* appeared in substantial amounts from the soils in our growth room experiment. However, they are extremely rare to find in the field in the long-term plots. In fact, they have not been recorded in the past 20 years by vegetation samplers. The seedbank has preserved these wetland specialist species, which has implications for other studies and conservation efforts. Other studies have found that some endangered wetland species can be restored in the aboveground plant community through soil seedbanks (Bernhardt & Ulbel, 2000). This study serves as a model of what the seedbank could look like after 20 years of anthropogenic impacts. However, although it may hold the potential to restore some species that were present earlier because it has changed more slowly than the aboveground communities, the seedbank has been altered by the treatments that have been applied for 20 years in the long-term experiment. Therefore, when applying restoration to an area like this one, the seedbank cannot be relied on entirely to reintroduce all the species before anthropogenic effects. In this area, prescribing burns, limiting nutrient addition, while also reintroducing species to the area that are no longer in the seedbank would be the best way to restore the native plant community.

Seedbanks are important components of plant community dynamics, yet we know little about them relative to our knowledge of the aboveground vegetation. Furthermore, understanding seedbanks may be essential for restoration of native plant communities. Humans have suppressed natural wildfires, added nutrients to the environment, and affected hydrology across the world. These alterations in anthropogenic disturbances have been shown to cause a range of impacts on plant communities (Goodwillie et al., 2020; Krupa, 2003; Wilcox, 1995). Therefore, it is important to know how best to begin restoration efforts to bring back natural biodiversity. The first step in restoring plant communities will be finding out if the diverse herbaceous species are still present within the seedbank. Then, by reintroducing disturbance, and limiting nutrient pollution, we will know what the plant community can reach by itself. By studying seedbanks, we can better understand what is possible for the future of a plant community. The finding in our study of persistent *Xyris* and *Lobelia* seeds, despite their loss aboveground, provides hope that the seedbank can maintain and conserve components of the past plant communities. However, the overall alteration that the treatments have caused the seedbank warns us to better protect our plant communities.

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Appendix 1. List of species identified in the seedlings that emerged in the seedbank study

Family	Genus	Species epithet and authority	Common name	Symbol	Shallow	Deep
Altingiaceae	*Liquidambar*	*styraciflua* L.	sweetgum	LIST2	1	0
Anacardiaceae	*Rhus*	*copallinum* L.	winged sumac	RHCO	7	1
Asteraceae	*Ambrosia*	*artemisiifolia* L.	annual ragweed	AMAR2	1	0
Asteraceae	*Erechtites*	*hieracifolia* L.	American burnweed	ERHI12	4	0
Asteraceae	*Eupatorium*	*capillifolium* LAM.	dogfennel	EUCA5	224	38
Asteraceae	*Eupatorium*	*rotundifolium* L.	roundleaf thoroughwort	EURO4	35	7
Asteraceae	*Eupatorium*	*semiserratum* DC.	smallflower thoroughwort	EUSE	55	6
Asteraceae	*Eupatorium*	*mohrii* Greene	Mohr's thoroughwort	EUMO4	5	2
Asteraceae	*Eupatorium*	*pilosum* Walter	rough boneset	EUPI2	1	0
Asteraceae	*Euthamia*	*caroliniana* (L.) Greene ex Porter & ritton	slender goldentop	EUCA26	23	4
Asteraceae	*Gamochaeta*	*purpurea* (L.) Cabrera	spoonleaf purple everlasting	GAPU3	16	2
Asteraceae	*Gamochaeta*	unknown	unknown	unknown	11	1
Asteraceae	*Krigia*	*virginica* (L.) Wild	Virginia dwarfdandelion	KRVI	1	0
Asteraceae	*Solidago*	*stricta* Aiton	wand goldenrod	SOST	5	0
Asteraceae	*Solidago*	*rugosa* Mill.	wrinkleleaf goldenrod	SORU2	15	1
Campanulaceae	*Lobelia*	*nuttallii* Schult.	Nuttall's lobelia	LONU	287	52
Campanulaceae	*Lobelia*	*canbyi* A. Gray	Canby's lobelia	LOCA	17	0
Cyperaceae	*Carex*	*glaucescens* Elliott	southern waxy sedge	CAGL5	4	1
Cyperaceae	*Carex*	unknown	unknown	unknown	37	0
Cyperaceae	*Cyperus*	*lupulinus* (Spreng.) Marcks	Great Plains flatsedge	CYLU2	2	1
Cyperaceae	*Cyperus*	unknown	unknown	unknown	76	5
Cyperaceae	*Killinga*	*ordorata*	fragrant spikesedge	KYOD	27	9
Cyperaceae	unknown	unknown	unknown	unknown	57	3

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Cyperaceae	unknown	unknown	unknown	unknown	1	0
Cyperaceae	unknown	unknown	unknown	unknown	12	0
Fabaceae	*Lespedeza*	*capitata* Michx.	roundhead lespedeza	LECA8	0	1
Fabaceae	*Lespedeza*	*cuneata*	Sericea lespedeza	LECU	0	2
Hypericacaceae	*Hypericum*	*multilum* (L.)	dwarf St. Johnswort	HYMU	3	0
Hypericacaceae	*Hypericum*	*crux-andreae* (L.) Crantz	St. Peterswort	HYCR3	0	1
Juncaceae	*Juncus*	*dichotomus* Elliot	forked rush	JUDI	45	2
Juncaceae	*Juncus*	*biflorus* Elliott	bog rush	JUBI	12	2
Juncaceae	*Juncus*	*canadensis* J. Gay ex Laharpe	Canadian rush	JUCA3	3	2
Lamiaceae	*Scutellaria*	*integrifolia* L.	helmet flower	SCIN2	1	0
Loganiaceae	*Gelsemium*	*sempervirens* (L.) W.T Aiton	evening trumpetflower	GESE	2	0
Melastomataceae	*Rhexia*	*mariana* L.	Maryland meadowbeauty	RHMA	44	12
Onagraceae	*Ludwigia*	*alternifolia* L.	seedbox	LUAL2	17	2
Onagraceae	*Ludwigia*	*hirtella* Raf.	spindleroot	LUHI	2	0
Oxalidaceae	*Oxalis*	*dillenii* Jacq.	slender yellow woodsorrel	OXID2	2	1
Poaceae	*Amphicarpum*	*purshii* Kunth	blue maidencane	AMPU6	1	0
Poaceae	*Aristida*	*virgata*	arrowfeather threeawn	ARPUV	3	0
Poaceae	*Chasmanthium*	*laxum* (L.) Yates	slender woodoats	CHLA6	107	7
Poaceae	*Dichanthelium*	*scoparium* (LAM.) Gould	velvet panicum	DISC3	244	28
Poaceae	*Dichanthelium*	*dichotomum* (L.) Gould var. *tenue* (Muhl.) Gould & C.A Clark	cypress panicgrass	DIDIT	45	5
Poaceae	*Dichanthelium*	*ovale (Elliot) Gould & C.A Clark* var. ovale	eggleaf rosette grass	DIOVO	15	5

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Poaceae	*Dichanthelium*	*dichotomum* (L.) Gould var dichotomum	cypress panicgrass	DIDID	97	17
Poaceae	*Muhlenbergia*	* *curtisetosa* (Scribn.) Bush (pro sp.) *[frondosa * schreberis]*	muhly	MUCU2	4	1
Poaceae	unknown	unknown	unknown	unknown	78	7
Poaceae	unknown	unknown	unknown	unknown	6	0
Poaceae	unknown	unknown	unknown	unknown	7	1
Poaceae	unknown	unknown	unknown	unknown	424	23
Poaceae	*Andropogon*	*virginicus* L.	Broomsedge	ANVI2	14	3
Primulaceae	*Lysimachia*	*nummularia* L.	creeping jenny	LYNU	1	0
Rosaceae	*Rubus*	*argutus* Link	sawtooth blackberry	RUAR2	8	3
Rubiaceae	*Galium*	*tinctorium (L.) Scop*	stiff marsh bedstraw	GATI	17	2
Scrophulariaceae	*Gratiola*	*pilosa* Michx.	shaggy hedgehyssop	GRPI	15	2
Smilacaceae	*Smilax*	*rotundifolia* L.	roundleaf greenbrier	SMRO	1	0
Smilacaceae	*Smilax*	*glauca* Walter	cat greenbrier	SMGL	1	0
Violaceae	*Viola*	*primulifolia* L. (pro sp.) [lanceolata × macloskeyi]	N/A	VIPR4	6	0
Xyridaceae	*Xyris*	*ambigua* Bey. Ex Kunth	coastal plain yellow eyed grass	XYAM	83	17
Unknown	Unknown	Unknown	Unknown	Unknown	1	0