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Assessing the Effects of Prescribed Fires on Mycorrhizal Fungi in Upland Mixed Oak-Pine Forests at DuPont State Recreational Forest, NC

Ari Puentes
Department of Biology
The University of North Carolina at Asheville
One University Heights
Asheville, North Carolina 28804 USA

Faculty Advisors: Dr. Jonathan Horton and Dr. Graham Reynolds

Abstract

Fungi are often overlooked when planning forest management practices such as prescribed fires, but fungal communities are incredibly important to ecosystem health. Almost all trees have been found to rely on mutualistic fungal symbionts, known as mycorrhizal fungi. This study investigated the effects of prescribed fire on mycorrhizal fungi in Dupont State Recreational Forest in Western North Carolina. Prescribed burn units in mixed oak-pine communities that differed only in the time since they were last burned (unburned, 1, 2, and 13 years) were surveyed monthly from June to September 2022. Surveys consisted of photographing, counting, collecting specimens, and taking notes on all mycorrhizal fungi found within the three 0.1 ha^2 research plots per burn unit. Field collection was done by citizen scientists from the Asheville Mushroom Club and UNC Asheville student volunteers. The collected specimens were brought back to the lab for identification and detailed descriptions before they were preserved. Shannon-Weaver diversity index, species richness, and community composition were calculated and compared between burn units. There were no statistically significant differences in any of the diversity indices between the burn units, though the recently burned areas had higher diversity and higher richness than the unburned ones. There were different

communities present in the recently burned and unburned areas indicating that certain species react more to fire than others. These preliminary data encourage further research so that forest management methods can take the mycorrhizal fungal communities into account. Specimens with DNA barcodes were deposited into the newly created UNC Asheville Fungarium. This information was digitized on the UNC Asheville Fungarium Mycology Collection Portal (MyCoPortal) and uploaded to iNaturalist. The results of this project provide many resources for citizen scientists, students, and researchers to utilize for continued mycological research.

1. Introduction

Fungi are an extremely diverse group of organisms, with global diversity estimates ranging from 2.2[1] to 12 million[2] species, the vast majority of which are undescribed. Many fungi dwell in the soil, within plant roots and tissues, and form complex relationships with other organisms that prevent the ability to culture them in a lab, which are some reasons why describing species can be very challenging. Due to the great diversity within the fungal kingdom, fungi can play many important roles within an ecosystem, some of which are still not well understood. Some examples include forming mutualistic symbioses with plants, acting as decomposers, and nutrient cycling.[3] One of these mutualistic symbioses is the mycorrhizal relationship that almost all plants have been found to rely on. Mycorrhizal relationships can be especially important for seedling establishment, nutrient uptake, and carbon transfer to plants.[4] In addition to supporting the plant communities through mycorrhizal relationships, fungi also play a huge role in decomposition of litter and woody debris, which contributes largely to the productivity of an ecosystem.

Despite the important role that fungi play in ecosystems and their tremendous diversity, fungal communities are largely ignored in forest management practices and conservation. In the United States, only two species of lichenized fungi, *Cladonia perforata* and *Cetradonia linearis,* are protected under the Endangered Species Act.[5] *Cetradonia linearis* is endemic to the Southern Appalachians, making it the only federally protected fungal species in North Carolina. On a state level, there are currently 22 species of lichenized fungi listed as having conservation concern by the Natural Heritage Program (NHP).[5] Considering the diversity of fungi and the number of wildlife and plant species that are protected, this seems like a gross underrepresentation. Fungal ecology and succession need to be considered when managing for forest ecosystem health, because almost all plants rely on their fungal symbionts for survival.

In the Southern Appalachian Mountains, it is thought that indigenous peoples have used fires to influence the vegetation for 4000 to 10,000 years.[6,7] The area of this study was traditionally inhabited and managed by the Cherokee people, and in the early 1800's many settlers had adopted the Cherokee land tending practices which utilized prescribed fire, specifically for maintaining open hunting grounds.[8] Fire suppression

began in the late 1890's due to the destructive effects of wildfire on irresponsibly logged forests.[7] The decision to suppress all forest fires did not consider forest ecology nor the indigenous history of fire use, but resulted in many people adopting the idea that fire is harmful to forests, which is still prevalent today.[9] In more recent years, with support from ecological research, there have been efforts to reintroduce fire to ecosystems through prescribed fires. Some of the benefits of prescribed fire include wildfire hazard reduction, control of vegetation, habitat enhancement for certain wildlife, and reduced risk of insect and disease outbreak.[9] The application of prescribed fires depends largely on the desired management goals. In DuPont State Recreational Forest (DSRF), fire has been used as a management tool for over 13 years. The primary goal of the prescribed fires in DSRF is to reduce certain shrub cover such as *Rhododendron spp.* and Mountain Laurel (*Kalmia latifolia* L.), and to reintroduce fire into the ecosystem to promote oak (*Quercus spp.)*, pitch pine (*Pinus rigida* Mill.*)*, and chestnut (*Castanea spp*.) regeneration.

This study aimed to better understand fungal succession ensuing prescribed fire by assessing mycorrhizal fungal community composition across a chronosequence of burned areas in DSRF. Understanding how fungi respond to prescribed fire can be useful to forest managers because fungal succession following fires likely drives the effects on plant community composition.[10] These data on fungal succession -- how long it takes for fungal communities to return to a preburned composition -- can be used to inform forest managers about the most desirable prescribed fire time interval. For example, a previous study done in boreal forests found higher fungal diversity in recently burned plots than in plots that were burned less recently.[11] Another study conducted in loblolly pine stands in the southern piedmont of Georgia, USA, found that diversity did not change after burns, but the importance of certain species changed over time.[12] To date, there have been no studies examining the effects of prescribed fire on fungal communities in Southern Appalachian forests. The amount of time that fungal communities remain altered by burns has been found to be between ten[12] and 15 years.[13] This recovery time is likely dependent on many factors, such as fire intensity, soil type, forest type, and average rainfall. Because of this variability, it is important to research the effects of prescribed fire in different forest types. Different geographic regions and forest types have different fungal communities, so research results from one region such as the Pacific Northwest cannot be assumed to apply to another region such as the Southern Appalachian Mountains. Data from specific regions should be used to inform fungal conservation and management efforts in that particular region.

Community science (also known as citizen science) is when people who are not professional scientists voluntarily collect data for scientific research.[14] Community science has been a method for gathering data for centuries, and was even the most frequent source of scientific data prior to the professionalization of science in the 19th century.[15] In recent years, this method of research has become more widely-accepted

and utilized by the scientific community, especially in areas of research that cover a broad geographic area.[16] Community science not only benefits the scientists using the data, but makes science more accessible to people by allowing them to gain experience firsthand, rather than having to read scientific papers that are often not easily digestible to those who are not professionals.[17] Community scientists from the Asheville Mushroom Club were an important component of this project, as their participation helped to expand our data collecting capabilities. The Asheville Mushroom Club (AMC) was formed in 1983 and is a North American Mycological Association (NAMA) affiliated club that is mostly comprised of amateur mushroom enthusiasts.[18] A working relationship between the AMC and UNC Asheville was formed so that the community scientists from the AMC could continue to contribute to the UNC Asheville Fungarium.

A fungarium is a collection of dried fungal specimens and is a valuable resource for many reasons. Fungarium specimens can be used to inform many areas of research including phenology, ecology, conservation biology, and taxonomy/systematics.[19] These collections allow researchers to update distribution information on certain species, find previously unknown plant pathogens, and extract and sequence DNA, which can be used to support or update systematic relationships or for various other purposes. Fungaria exist worldwide, the largest one being the Fungarium at Kew Gardens in the United Kingdom. The Kew fungarium has over 1.25 million dried specimens, some dating back to the early 18th century.[20] If properly maintained, a fungarium can preserve specimens for an indefinite amount of time. There was not a fungarium located in Western North Carolina prior to this study, the closest one being over 200 miles away at NC State University or Duke University. Because this area is a hotspot for fungal biodiversity, having a local fungarium seemed necessary, which is why the UNC Asheville Fungarium was created.

2. Methods

2.1. Study sites

All field work took place in DuPont State Recreational Forest (DSRF) which is located in Henderson and Transylvania counties, in the Blue Ridge Mountains of Western North Carolina. DSRF is approximately 12,500 acres and has been a state forest since 1995, when the state initially bought 7,640 acres from the DuPont Corporation. There is an area commonly known as the “doughnut hole” that is just under 500 acres, which is where the DuPont plant was located from 1956 to 2002.[21] The plant produced silicone chips then developed x-ray films, which left the soil and groundwater very polluted. DuPont donated this area to the state over the course of many years. Remediation efforts have been in place, but the area is still closed to the public. No research plots were located within this area. DSRF is primarily dominated by Montane Oak-Hickory forest followed by Acidic Cove forest, the latter being where this study took place.[22]

There are many areas in DSRF that were previous eastern white pine (*Pinus strobus* L.) plantations, which the forest managers are trying to return to a more natural forest type that includes other native pine species that are in decline such as pitch pine (*Pinus rigida*) and shortleaf pine (*Pinus echinata* Mill.). In addition to increasing the diversity of pines, forest managers are also trying to reintroduce hardwoods through various management methods, including different silvicultural techniques and prescribed fire. DSRF has been using prescribed fires for over 13 years to reintroduce fire into the ecosystem after a long period of fire suppression.

2.2 Experimental Design for Sample Plots

A list of every location in DSRF that had been burned was acquired from the management forester in March 2022. From these, the burn units that had only been burned once and had the same forest canopy type (white pine-hardwood) and soil type (sandy loam) were chosen. Three burn units were chosen that only differed in length of time since they were burned (1, 2, and 13 years) as well as an unburned control site with the same canopy and soil types (Figure 1).

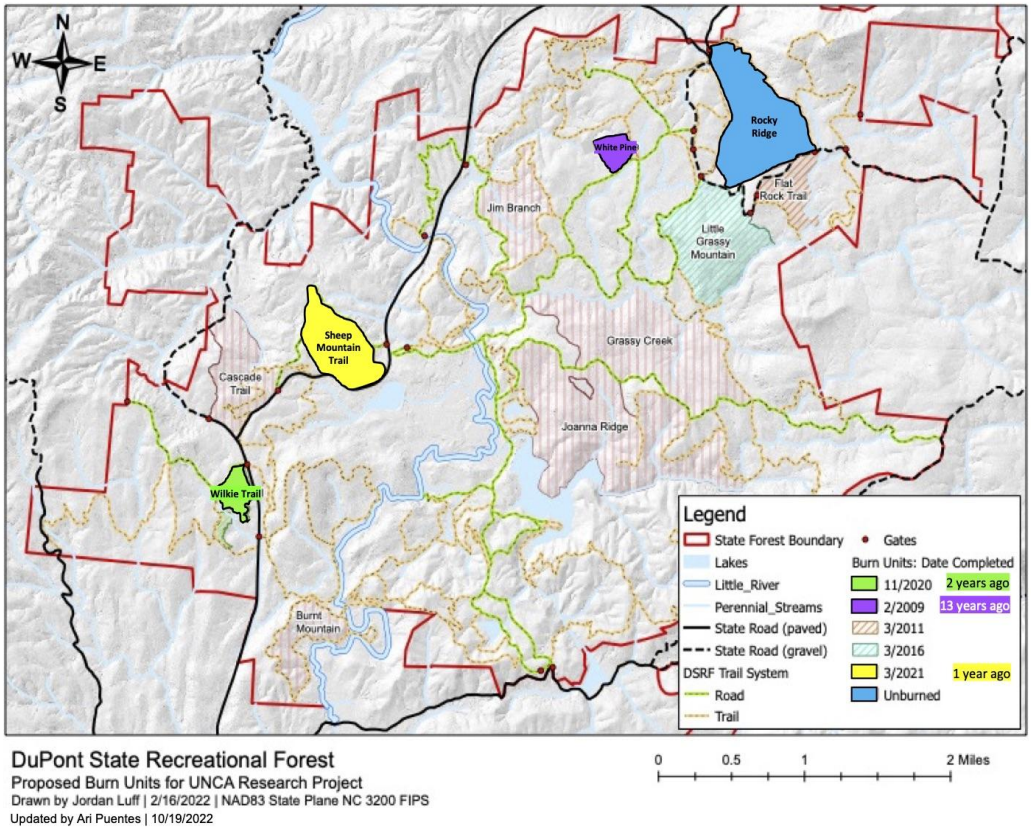

Figure 1. Map showing location of burn units in DSRF.

Within the burn units and unburned control site, permanent, square 0.1 ha plots (n = 3 in each unit, N = 12 total plots) were established in April and May 2022. To determine where to put the plots, the burn units were manually surveyed and locations were chosen carefully to ensure they had similar slope aspects, comparable vegetation types, and did not overlap with other management practices (e.g., harvest sites, new plantations, etc.). The burn units were surveyed monthly between June and September 2022 to assess the seasonal production of macrofungi. Due to unforeseen circumstances, not all burn units were sampled each month, and collection had to be scaled back to only include mycorrhizal fungi (Table 1). All burn units were sampled within two weeks to avoid variation due to timing and weather as much as possible.

Table 1. Sampling efforts for each month.

Sampling Month	Burn Units Sampled	Fungi Type(s)
June 2022	1-year, 2-year	All macrofungi
July 2022	1-year, 2-year, 13-year, control	All macrofungi
August 2022	1-year, 2-year, control	Mycorrhizal
September 2022	1-year, control	Mycorrhizal

Monthly collection was done by student volunteers and citizen scientists from the Asheville Mushroom Club. Upon arrival to each plot, tapes were run to establish the collection boundary and to split each 0.1 ha plot into quarters. Five volumetric soil moisture measurements (VSM) were taken in the four corners and center of every plot with a Time Domain Reflectometer (Hydrosense II Soil Moisture Meter, Campbell Scientific, Logan UT). These soil moisture percentages were averaged and recorded as a possible variable explaining differences in fruiting body production between plots due to soil moisture. During each collection, all visible fungi greater than 5 mm in diameter[23] were photographed, identified if possible, and the number of fruiting bodies was recorded. For polypores and other fungi too prolific to count the individual fruiting bodies, the number of trees/sticks the species was present on was recorded instead. These counts were used to quantify species abundance in each plot. Representative samples of all taxa were collected in the field and notes about habitat and substrate were recorded on data slips for each specimen. All collected fungi were put into separate wax paper bags with their respective data slips and brought back to the lab at UNC Asheville. Spore prints were obtained whenever possible by leaving the specimens on top of paper or microscope slides overnight (Figure 2), and subsequent microscopy was done to confirm identification. After identification, all specimens were dried in a dehydrator at 35°C for 48 hours and stored in sealed plastic bags in the UNC Asheville Fungarium. Specimens that were too small to put in the dehydrator were instead put into sealed plastic bags with desiccant packets to dry.

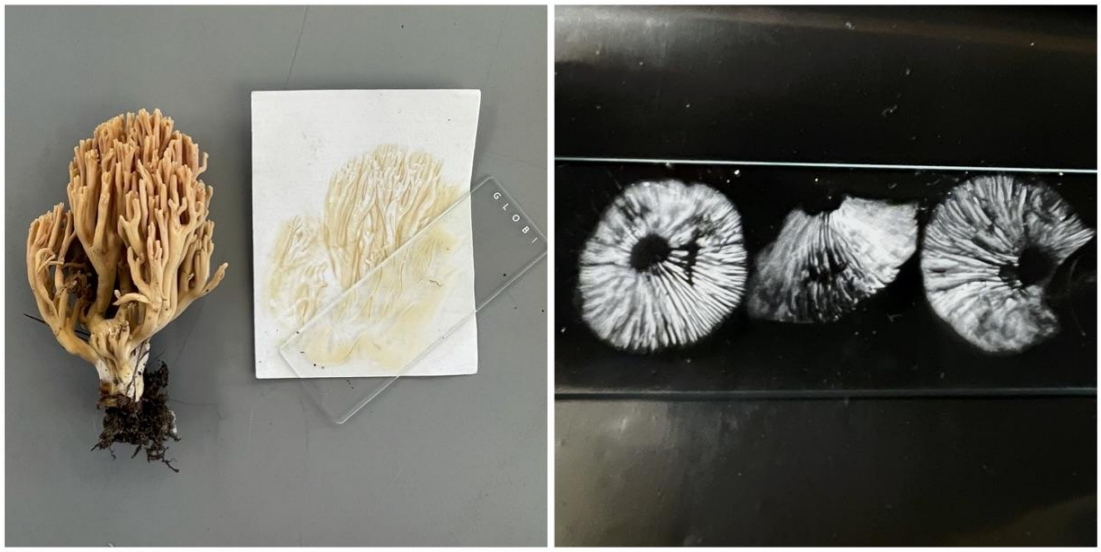

Figure 2. Spore prints from two species of fungi. Left photo shows spore print from *Ramaria conjunctipes* on paper and microscope slide. Right photo shows spore print from three specimens of *Lactarius camphoratus* on a microscope slide.

2.3 Statistical Analyses

Several calculations were done in October 2022 to compare fungal community composition and diversity between the burn units. Because saprobic fungi were only sampled during June and July, all indices and calculations were done for saprobes and mycorrhizal fungi separately. The month of June was omitted from analyses of mycorrhizal community comparisons because very few mycorrhizal species were found, and because the control unit was not sampled that month. To calculate diversity, Shannon-Wiener species diversity index (H')[24] was calculated:

$$\text{Species diversity } (H') = - \ P_i \log P_i$$

P_i indicates species evenness, or the count of one species divided by the total count of all species. Mycorrhizal species diversity was calculated for each burn unit by month across a three-month (July-September) sample period. The one-year and control units were the only units sampled consistently across the sample period. Saprobic species diversity was calculated for the one-year and two-year burn units during June and July. These were the only burn units that were sampled for saprobic fungi during multiple months. This diversity index is one of the most widely used because it is sensitive to evenness and sample size, so it will allow for our results to be compared to many other studies.[25] Coefficient of community (CC)[26] was calculated:

$$\text{Coefficient of community } (CC) = 2c/(a+b)$$

Where a = total number of species present in the first community, b = total number of species present in the second community, and c = number of species present in both communities.[25] This equation yields a value between 0 (no species common to both communities) and 1 (all species are found in both communities). Coefficient of community (CC) was used to analyze the differences in community composition between the burn units and the control.[26] Because not all the burn units were sampled each month (Table 1), the 1-year burn unit and the control were the only units in which the compiled mycorrhizal communities could be compared over a three-month sample period (July-September). Species richness (n), or the total number of species recorded in a burn unit was also recorded and analyzed for each burn unit by month. Because data did not meet the assumptions of normality, they were compared among burn units each month using Kruskal-Wallis non-parametric analysis of variance (ANOVA) using SAS v9.4.[27]

2.4 Fungarium Establishment

The UNC Asheville Fungarium was established in June 2022 with the specimens from the first month of field collection at DSRF. It is housed in the Plant Physiology lab in Zeis Hall, room 334. All subsequent specimens collected at DSRF were curated by me and AMC volunteers, then deposited into the UNC Asheville Fungarium. Some specimens from AMC forays were donated and deposited into the fungarium as well. For each fungarium specimen, the metadata from field data slips was recorded (location, date collected, substrate) as well as the name of the collector, the name of the determiner (person who identified the species), detailed descriptions of physical characteristics, and details about microscopic features. These data were recorded on paper in the lab to make things more convenient for volunteers. But to make this information available to as many people as possible, the data was also digitized. To digitize the data, the UNC Asheville Fungarium was registered on the Mycology Collections data Portal (MyCoPortal) which is an online database used worldwide by a plethora of fungal curators,[28] in September 2022.

2.5 Molecular Work

It is important for fungarium specimens to have publicly-accessed DNA sequences available as part of the specimen record, as well as for identification. Fungal DNA was extracted from dried tissue of fungarium specimens using Qiagen DNeasy Plant kits. The tissue from each specimen was ground with a mortar and pestle prior to extraction. Polymerase chain reaction (PCR) was used to amplify the nuclear ribosomal DNA (rDNA) from fungal-specific ITS regions, ITS1F and ITS4R.[29,30] Each PCR reaction contained a mix of 12.5 uL GoTaq Green Master Mix, 2.5 uL sterilized distilled water, 2.5 uL ITS1F and ITS4R primers, and 5uL extracted DNA. Standard PCR parameters

were followed consisting of 35 cycles of: 3 minutes at 95°C, 30 seconds at 55°C, 1 minute at 72°C, 30 seconds at 95°C, and 10 minutes at 72°C. PCR products were verified by gel electrophoresis on agarose gels. The resulting amplified rDNA was sent to the NC State Genomic Sciences lab for Sanger sequencing. 52 samples were sent for forward and reverse sequencing in November 2022. Once the sequences were received, contiguous sequences of F and R reads (contigs) were formed when applicable and they were manually cleaned up using Geneious v. 10. The cleaned up sequences were BLASTed against records in GenBank and species matches were confirmed if sequences had greater than 98% similarity. Another 48 sequences are to be sent to NC State for sequencing in May 2023, then all cleaned up sequences will be uploaded to GenBank and linked to their respective MyCoPortal entries.

2.6 Community Science

Communication with the AMC pertaining to this project began in January 2022, which is when a relationship was formed with the AMC board for this collaboration. In order to introduce the project and begin to recruit volunteers from the Asheville Mushroom Club, Dr. Jonathan Horton gave a talk at the club's monthly meeting on April 21, 2022, titled "Investigating the Effects of Prescribed Fires on Fungal Diversity." This talk gave background information on prescribed fires, why there is interest in its effects on fungal diversity, the project design and goals, and announced the creation of the fungarium and the collaboration between UNC Asheville and the AMC. I created a short informational presentation that went into more detail about the project and what the volunteer opportunities (field collection, lab work/curation) would require in order to train and prepare potential volunteers. This video was attached to a digital sign-up sheet which was sent out by the AMC through the monthly newsletter each month from June-September. Students from Dr. Camila Figueiras' NEMA lab and former students who took mycology at UNC Asheville were also contacted each month to be made aware of volunteer opportunities.

In the field, all volunteers were walked through the collection process and protocol. Depending on the number of volunteers, they were split into small groups of 3-4 people and assigned roles (photographer, field slip data collector, plot data collector, seeker). In the lab, all volunteers were shown the lab protocol and given many informational handouts and field guides to aid in the identification and curation processes. As an incentive to volunteer and a way to show our appreciation, all AMC volunteers were offered the opportunity to attend a fungal microscopy workshop hosted by Dr. Jonathan Horton and myself. Because our volunteers were doing us a great favor by helping us collect data, we tried to focus on helping them to develop useful skills such as field data collection, different field sampling techniques, fungal identification, and fungal microscopy.

The Fungal Diversity Survey (FunDiS) is a national effort to document fungal diversity using community science, particularly through iNaturalist.[31] iNaturalist is a widely utilized community science platform that can be accessed through a website or an app. FunDiS has an iNaturalist project titled “FunDiS - Fungal Biodiversity Survey” which serves as their primary database. I created an iNaturalist project titled “Macrofungi of DuPont State Recreational Forest” and all photos taken in the field are being uploaded to this project. My iNaturalist project is set up so that all fungal observations (not including lichens per the FunDiS guidelines) found in DSRF will automatically be added to the project once a user joins. The observations from my project will be contributed to the FunDiS project.

3. Results

3.1 Species Richness and Diversity

An estimated total of 222 unique taxa were collected over the four-month sampling period. Of these taxa, 146 were assigned a species name, 55 were identified to genus, and 21 were not able to be identified to genus level. Obtaining DNA barcodes from specimens will allow for more precise identification. Species richness (n) for each burn unit was calculated by averaging the total number of taxa found within the burn unit during each sampling period month (Table 2).

Shannon-Weiner diversity index (H’) was used to quantify diversity in the burn units. These diversity results were then compared using ANOVA. There were no significant differences found among any of the burn units, so these data were omitted. Coefficient of community (CC) was calculated to compare similarity between the 1-year burn unit and the control. For total mycorrhizal community from June to September 2022, CC = 0.365. There were no patterns found among the monthly CC results.

Table 2. Monthly comparison of diversity indices for mycorrhizal fungi. Mean (± SE) species richness (n), mean (± SE) species diversity (H’), coefficient of community (CC) comparing the burn units to the control. CC ranges from 0 – no similarity to 1 – completely similar. There were no statistically significant differences among these indices for any of the burn units.

Month	Burn Unit	n	H’	CC
July	1-year	3.67 ± 0.88	1.10 ± 0.23	0.154
	2-year	1.67 ± 0.67	0.35 ± 0.35	0
	13-year	1.67 ± 0.67	0.35 ± 0.35	0.25
	control	1.33 ± 0.67	0.44 ± 0.22	--

August	1-year	14 ± 3.5	2.02 ± 0.34	0.298
	2-year	11.67 ± 3.67	1.73 ± 0.42	0.25
	control	8 ± 0.58	1.67 ± 0.21	
September	1-year	19.67 ± 4.33	1.86 ± 0.34	0.342
	control	14.33 ± 3.28	1.83 ± 0.41	--

Although there were no statistically significant differences among any of the burn units, there was higher diversity (H') and species richness (n) observed in the 1-year burn unit each month. Additionally, comparing the total mycorrhizal species composition of the 1-year burn unit and the control over the entire sample period showed that there were different species present in the burn units, and that the species that were present in both units occurred in different abundances (Figure 3).

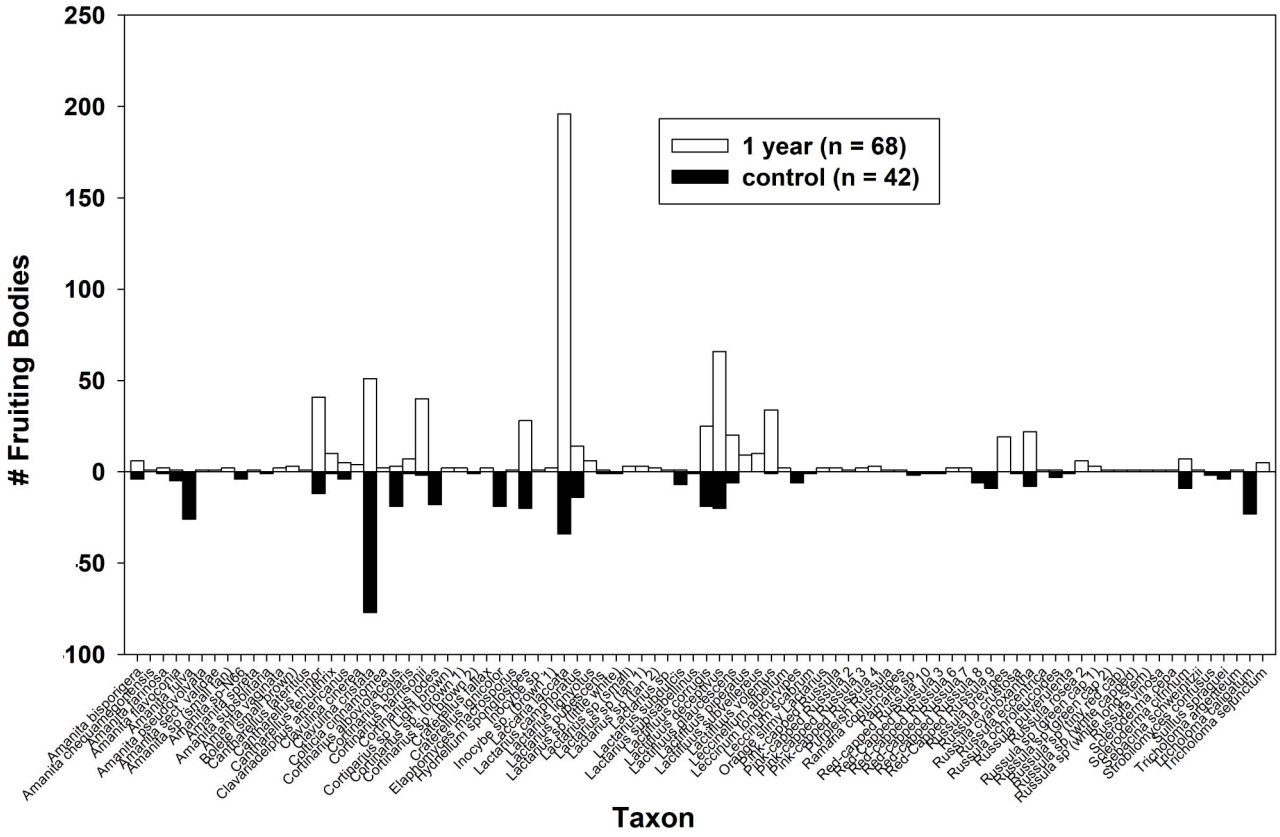

Figure 3. Comparison of total mycorrhizal species composition (Jun-Sep 2022) between the 1-year burn unit and unburned control.

Saprobic fungi sampled in June and July from the 1-year and 2-year burns units were compared using the same diversity indices as mycorrhizal fungi. Alternatively, coefficient of community (CC) was used to compare the communities in the 1-year and 2-year units, not the control. The saprobic communities were more similar to each other in June than in July, but not significantly (Table 3).

Table 3. Monthly comparison of diversity indices for saprobic fungi. Mean (± SE) species richness (n), mean (± SE) species diversity (H'), coefficient of community (CC) comparing the 1-year and 2-year burn units. CC ranges from 0 – no similarity to 1 – completely similar. There were no statistically significant differences among these indices for any of the burn units.

Month	Burn Unit	n	H'	CC
June 2022	1-year	10.0 ± 1.16	2.13 ± 0.09	0.316
	2-year	8.67 ± 3.84	1.67 ± 0.44	
July 2022	1-year	8.67 ± 1.86	1.90 ± 0.31	0.25
	2-year	13.0 ± 1.0	2.31 ± 0.22	

Fruiting body production is heavily influenced by rainfall, additionally the slope aspect and vegetation cover of the plots affected the amount of moisture in the soil. Precipitation data was obtained from the Guion Farm Remote Automatic Weather Station (RAWS) located in DSRF (Table 4). Low rainfall in June likely explains the lack of macrofungi found that month, which is why it was omitted from mycorrhizal calculations.

Table 4. Monthly precipitation measurements at DuPont State Recreational Forest.

Month	Average Daily Precipitation (in)	Total Accumulated Precipitation (in)
June 2022	0.049	1.47
July 2022	0.288	8.94
August 2022	0.169	5.24
September 2022	0.322	9.34

3.2 Fungarium Data

193 specimens were deposited into the UNC Asheville Fungarium between June and September 2022. Each fungarium specimen contained detailed descriptions and pictures of macro- and microscopic features (Figure 4), a spore print if obtained, notes about habitat, and the names of the people who collected and identified them. The pictures and descriptions for all fungarium specimens are in the process of being uploaded to the UNC Asheville Mycology Collections Portal (MyCoPortal) collection profile, which can be accessed here: https://www.mycoportal.org/portal/collections/misc/collprofiles.php?collid=158

Figure 4. Macro- and microscopic characteristics of *Amanita cinereoconia* var. cinereoconia. A) Underside shot showing the gills, gill attachment, and partial veil remnants. B) Stipe texture and ornamentation. C) Cap details. D) Spore-bearing structures (basidia) at 400X magnification (pointer is located directly between the two visible basidia).

3.3 Molecular Results

Of the 52 samples sequenced, 33 contigs were successfully assembled and cleaned up. Of these 33 sequences, 28 had a specific match in GenBank. Out of the sequences that had matches, two specimens in the genus *Amanita*, had the fungus *Vanrija pseudolonga* amplified during PCR instead of the target taxon and returned a 100% match to *V. pseudolonga* in GenBank.

3.4 Community Science Results

There are approximately 230 observations to be uploaded to my iNaturalist project (Fig. 4), which will then be contributed to the larger FunDiS Biodiversity Database Project. From the Asheville Mushroom Club (AMC) there were 16 volunteers who contributed to data collection as well as 12 UNC Asheville students and alumni. For the AMC volunteers, in-person recruitment and networking were more effective than online efforts whereas the opposite was true for UNC Asheville students. Eight AMC members attended the fungal microscopy workshop held on October 6, 2022. Many of the AMC volunteers expressed interest or inquired about contributing specimens to the UNC Asheville fungarium.

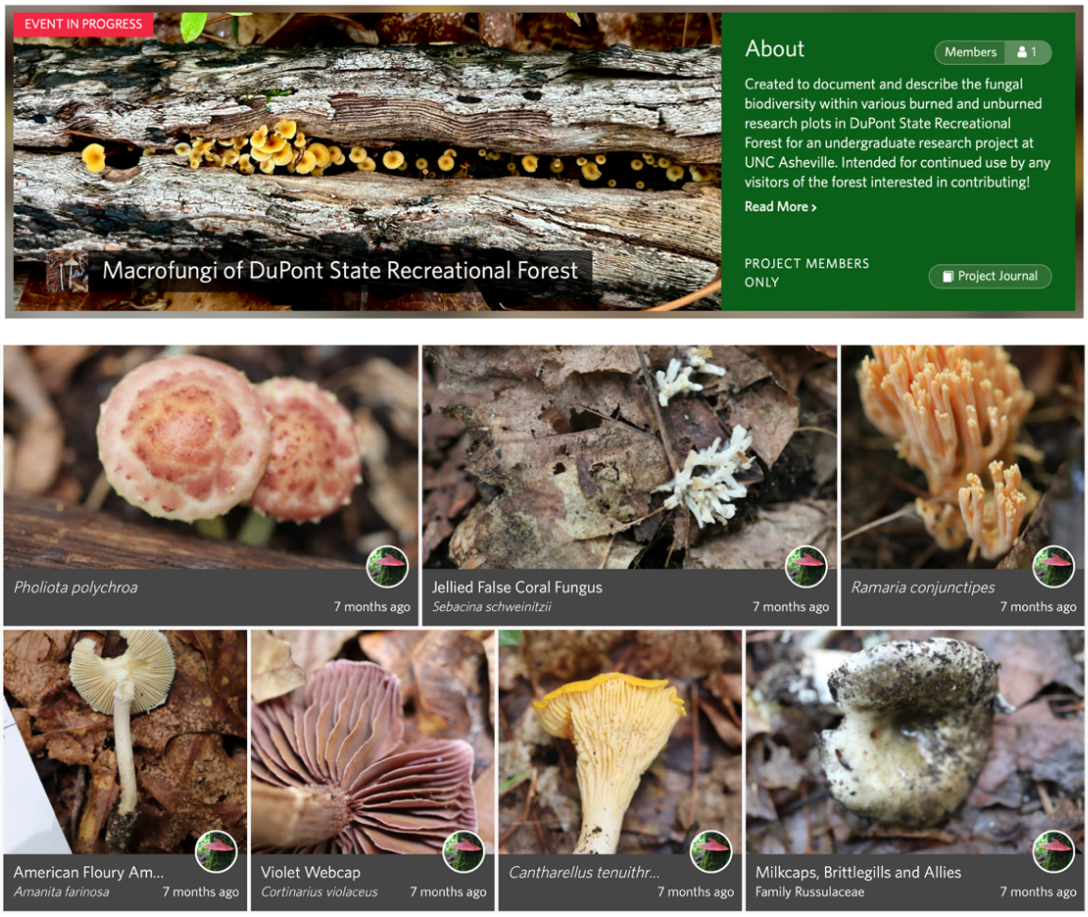

Figure 4. Pictures of my iNaturalist project "Macrofungi of DuPont State Forest." Top picture shows the project banner and description, bottom photo shows seven project observations.

4. Discussion

In my study, it was found that neither the mycorrhizal species richness (number of species present) or diversity (H') were statistically significantly different among any of the burn units or controls. This lack of statistical significance is likely due to our small sample size (n = 3). There were consistently higher richness (n) and diversity (H') values in the 1-year burn unit than any other unit for the entire sampling period (Table 2). Although the mycorrhizal communities were not significantly affected in these areas that have only been burned once, it is not indicative that mycorrhizal communities are unaffected by prescribed fires, because fire interval (the amount of time between prescribed burns) is another very important factor to consider.

Fire interval is particularly important to consider in DSRF because prescribed burns are used primarily for the purpose of reducing shrub cover, which usually takes repeated burns to achieve. Making sure that these burns aren't done too frequently is important for maintaining the fungal and plant health of the forest. A study done in Georgia that looked at the effects of prescribed fire on soil fungal communities in loblolly pine forests found that frequent and infrequent burns had different effects on the fungal communities, and that frequent prescribed fires could damage the mycorrhizal community.[32] This study concludes that although frequent fires are required to change forest composition, this could damage the mycorrhizal community, which could negatively affect the health of the remaining plant community.[32] More research needs to be done on the effects of fire intervals on the fungal communities in this area to better inform forest managers on the most effective and responsible amount of time to wait between prescribed burns. There are burn units located within DSRF that have been burned more than once which could be sampled to assess these affects.

Different types of fungi were found in areas that had recently been burned versus those that had never been burned. The CC result of the 1-year burn unit and the unburned control being 0.365 means that roughly 1/3 of the total species found in were common to both communities. In comparison, a study done in ponderosa pine stands in Oregon found that mycorrhizal species richness was not reduced significantly after prescribed fires, but that different species were abundant before and after.[33] I found that mycorrhizal species richness was not statistically significantly higher, although there was consistently higher richness values in the 1-year burn unit. I do not have data on the fungal communities from before the burns were done, but many of my research plots are located in places that are scheduled to be burned again in the future, so my

results can be used as the preliminary data to see how the mycorrhizal fungal communities in the burn units that we sampled might differ before and after a burn.

Although some similar studies have been conducted in various locations around the world, none used the same sampling methods that I did, and none occurred in the Southern Appalachian Mountains. It must be taken into consideration that fungal sampling methodologies are all limited in their own ways. In my study, only aboveground macrofungi were assessed, ignoring the majority of fungal diversity, which dwell underground.[34] Aboveground macrofungi fruiting body production is heavily influenced by rainfall and other unpredictable conditions, so the diversity documented in this study is far from comprehensive. In the months that there was little precipitation, the macrofungal richness that was recorded was likely a bad representation of the actual fungal diversity present in the soil.

To gain a better understanding of the effects of prescribed fires on mycorrhizal fungal communities, soil samples and/or root tips should be collected and sequenced using next generation sequencing. Additionally, more long-term studies are needed and would benefit from more replications. In my study, initially, research plots were set up in five burn units (18 plots total, including the control plots) in DSRF that had last been burned one, two, six, 11, and 13 years ago so that a more complete chronosequence could be assessed. All of the research plots that were set up could be found again in the future with GPS coordinates. All are marked with flags and metal tags on trees -- these plots will be used for further research at UNC Asheville and are available for use by other researchers as well. Due to lack of volunteers, time, and other unforeseen challenges, my project had to be scaled back significantly, leaving us with only the one-year and control units being sampled consistently throughout the sampling period. Therefore, the chronosequence is incomplete but can be used as preliminary data for further research.

The sampling methods that I used required many experienced individuals and community scientists who needed to be trained. In addition to requiring many people and training, gathering the amount of data needed to properly identify specimens, taking photos in the field, and organizing these data is a very time-consuming process. It is recommended that future studies utilizing similar methods to mine collaborate with as many people as possible to increase data collection capabilities, and that the study is conducted over a longer time period. Utilizing community science platforms such as iNaturalist during forays is a great way to have all the data collected in one place. Because time and volunteers were often scarce and volunteers already had many daunting tasks, I did not train volunteers on how to use iNaturalist, rather I had them upload their photos to a shared folder, then uploaded them to iNaturalist myself. I recommend that community science volunteers who will be taking photos in the field are trained on how to use iNaturalist prior to collection, so that the photos can be uploaded from them directly. This platform is also useful because it makes the data widely available to anybody who would like to use it. Data uploaded to iNaturalist can be added

to other projects for analyses and can be distributed with ease because they are available to anybody with internet access. Species lists can also be easily generated from iNaturalist, which is of great interest to many forest managers.

The specimens collected during this project were the first to be deposited into the UNC Asheville Fungarium. Although this resource is newly created, it will continue to be added to and if maintained properly, can last for hundreds of years. The closest fungarium to UNC Asheville is over 200 miles away, which is why establishing this resource in this area was so important. The members of the AMC and UNC Asheville students will continue to add to this resource giving it the potential to be a great source of information on local fungal biodiversity. Having genetic information tied to the fungarium specimens is also very useful for confirming identification and for subsequent research reference. Even if molecular data is not extracted from specimens at the time that they are deposited into the fungarium, as long as they are dried and stored properly, DNA can be extracted from them after an indefinite time period. MyCoPortal is an excellent database because so many fungaria utilize it around the world, so our information is available in the place where it is most likely to be accessed and used by other researchers.

5. Conclusion

Looking at how forest management methods such as prescribed fires affect the mycorrhizal fungal communities is important for overall forest health, gaining a better understanding of forest ecology, and for informing fungal conservation efforts. Without looking into how fungi are affected by certain practices, an entire kingdom and essential component of the ecosystem is being overlooked. We have much to learn about mycorrhizal interactions and other plant-fungal interactions, the best way to accomplish this is through continued research and efforts to conserve and protect fungi. Since most fungi dwell in the soil, protecting fungi will also protect and promote healthy soil, in addition to plant health. More research needs to be done on fungal ecology in this region, and resulting data needs to be used to inform conservation efforts. We must protect fungi to be able to understand them more, and by understanding more about fungi, we will be able to better understand the complex ecology of the forest and beyond.

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