2023

The Effects of Trailside/Non-trailside Sample Collection and Tree Communities on Biodiversity of Mushroom-forming Fungi in Public Green Spaces of New London, Connecticut

Quincy Dowling
Connecticut College, qsdowling52@gmail.com

The Effects of Trailside/Non-trailside Sample Collection and Tree Communities on Biodiversity of Mushroom-Forming Fungi in Public Green Spaces of New London, Connecticut

An honors thesis presented by
Quincy Dowling

To the department of Biology
In partial fulfillment of the requirements for Honors

Connecticut College
New London, CT

May 3, 2023

Thesis Committee:

Thesis Advisor, Assistant Professor Eric Vukicevich, PhD.
Department of Botany, Connecticut College

Reader, Assistant Professor Yan Zhuang, PhD.
Department of Statistics, Connecticut College

Reader, Senior Lecturer Kristine Hardeman, PhD.
Departments of Botany and Biology, Connecticut College

TABLE OF CONTENTS

1.0 ABSTRACT

The Kingdom Fungi holds a wealth of biodiversity and potential for better supporting both natural and human communities (Lange, 2014). Mushroom-forming fungi have potential value as sustainable and accessible sources of food in public green spaces (Rombach & Dean, 2023). The purpose of this investigation was to examine the effect of trailside disturbance and tree communities on the diversity and community composition of mushroom-forming fungi in New London, Connecticut. Six public green spaces were identified and surveyed for species richness of mushroom-forming fungi. At each site, two foraging areas of equal area were defined: one along either side of a trail, and one in an undisturbed section of forest adjacent to the trailside area. Each site and both foraging areas within each site were visited twice between the months of September and October of 2022. Temperature and precipitation data were gathered for the 72 hours surrounding each sample collection, and the number of days since September 1 at the time of sampling was recorded as a measure of seasonality. Tree diversity and the proportion of tree species that associate with ectomycorrhizal fungi (ECM) were also assessed at each site. Fungal species richness was found to be associated with sites that had higher proportions of ECM tree species. Disturbance caused by the presence of a trail in the foraging area did not have a significant impact on fungal species richness observed. Fungal community composition varied between sites and with seasonality of the sampling date. Tree diversity, temperature and precipitation levels, and sampling site did not significantly impact fungal species richness. Identifying the proportion of ECM tree species present as a predictor for fungal diversity is helpful in expanding our knowledge of fungal biodiversity and making mushroom foraging more accessible to people in urban environments.

2.0 INTRODUCTION

2.1 Global Significance of Fungal Biodiversity

Fungi play many influential ecological roles, and yet they are vastly understudied compared to the Plant and Animal kingdoms. While global fungal biodiversity is estimated to be in the millions, only 120,000 species have been described thus far (Blackwell & Vega, 2018). Much more research is required to get an accurate estimate of their true numbers. Mycology is not only relevant to understanding the essential biology of Fungi, but also to the progression of antibiotic drug development, agriculture, climate adaptation, alternative medicine, plant pathology, and more (Lange, 2014). In the face of widespread global biodiversity loss due to climate change, it is more imperative than ever to improve our understanding of fungal biodiversity so that we can protect our ecosystems from further damage and loss.

Fungi are often cast aside as the "decomposers" of the natural world, simply breaking down organic matter so that it can be taken up and turned into new life by plants. This is far from an accurate representation of the variety and diversity found within Kingdom Fungi. Fungi influence nutrient cycling as well as soil carbon storage, soil structure, nutrient availability, plant productivity and microbial community composition (Frąc et al., 2018). Fungi can have a wide variety of interactions with their environment; they can exist as microscopic or macroscopic organisms, and within each of those categories exists a multitude of lifestyles and morphologies. Mycorrhizal fungi are soil fungi that live in a mutualistic, symbiotic relationship with a plant host, providing them with phosphorus and nitrogen in exchange for carbon (van der Heijden, 2009). These symbiotic relationships have a significant impact on the health of plant hosts and even improve ecosystem productivity. Fungi also operate as both saprotrophs and opportunistic pathogens, utilizing the nutrients and potential hosts that are made available to them (Boddy &

Hiscox, 2016). Fungi inhabit a multitude of roles, and yet many species or categories of fungi are overlooked because of the challenges that come with studying these organisms. For example, species that are entirely microscopic, have ephemeral macroscopic structures, or are obligate symbionts are challenging to study in a lab setting.

Macrofungi are fungi that produce macroscopic fruiting bodies, or what we think of as mushrooms, toadstools, and the like. The fruiting body is a reproductive structure formed by a fungus to disperse its spores (Fig.1). Most of the biomass of a fungus is its mycelium, or root-like network of cells growing throughout or over the surface of its substrate. The substrate is the material from which a fungus is growing and obtaining nutrients (Molina et al., 1993; Arora, 1986). Not all fungi form macroscopic fruiting bodies; some rely on abiotic factors or soil dynamics as means of dispersing their spores to new substrates and hosts or rely on the growth of their mycelium into new territories (Boddy & Hiscox, 2016). Mushrooms often serve as an easily observable marker of the mycodiversity in an area because of their visibility and identifiability (Halme et al., 2012).

For species that form fruiting bodies, mushrooms provide a structure for spore creation and dispersal, playing an essential role in the fungus' reproductive cycle. The hymenium is the region on a mushroom on which spores are produced; typically, the hymenium is on the surface underneath the mushroom cap (Molina et al., 1993). The hymenium is often covered by a web-like structure, called a veil, during the early formation of a mushroom. Once mushroom formation is complete and the spores have reached maturity, the veil will naturally fall off or disintegrate and the spores are then dispersed both through the active release by surface tension catapults on the hymenium, or by the flow of air and water vapor beneath the hymenium which are able to dissipate the spores from the hymenium surface (Dressaire et al., 2016). The

hymenium differs in its structure and morphology between different species; two common descriptors for the hymenium type of a mushroom are those that are called “gilled” or “non-gilled” mushrooms, which refers to the pattern of indentations (or lack thereof) on the hymenium’s surface. Common types of hymenia surfaces are forked gills, pores, tubes, and teeth. Macrofungi have evolved to create fruiting bodies with unique caps, surfaces, shapes, colors, textures, smells, tastes, and toxicities (Baroni, 2017; Arora, 1986). Identifying a macrofungus based purely on observation of its fruiting body is a common practice, given that the mycelium of fungi can be difficult to see with the naked eye, let alone identify morphologically. In comparison, a mushroom can be collected and examined in the field or in the lab to help identify the species. Analyzing the spores found on a fruiting body can also provide further clues about the identity of that fungus (Molina et al., 1993).

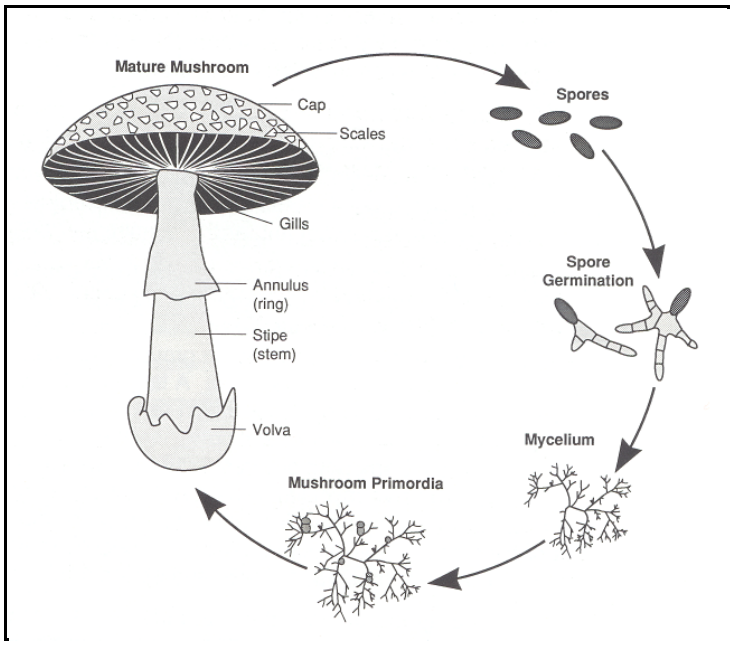


Figure 1. The lifecycle of a macrofungus. Mycelia grow outward in search of nutrients until the fungus is mature enough to form fruiting bodies. The stipe, cap, gills, volva, annulus, and scales are all features that a mushroom may or may not have, the presence and characteristics of which help to identify the mushroom’s species (Molina et al., 1993).

The importance of mushrooms also goes beyond their reproductive function. There are many edible species of mushrooms, some of which are even considered delicacies. Mushrooms provide nutritional value through their mineral, vitamin, and protein content (van Dijk et al., 2003). Edible mushrooms can be found across many genera, including *Agaricus*, *Boletus*, *Pleurotus*, *Cantharellus*, and *Auricularia* (Karwa & Rai, 2009). Popular examples are common morels (*Morchella esculenta*), and truffles (*Tuberaceae* family) (Arora, 1986; de Mattos-Shipley, 2016). Many edible species of fungi can be foraged in parks and forested areas across the world, making them an accessible food source for visitors to green spaces (Rombach & Dean, 2023; Arora, 1986). Additionally, mushrooms have had cultural significance for centuries as food, medicine and ceremonial practices (Pérez-Moreno et. al., 2021). Ancient civilizations such as those in China, various nations across Africa, and in eastern Europe are known to have utilized the medicinal properties of mushrooms. In Central and South America, indigenous groups such as the Aztecs were known to use hallucinogenic mushrooms in the *Psilocybe* genus (Subramanian, 1995). Many of those traditions persist to this day. In Chinese medicine, the Reishi or Lingzhi mushroom (*Ganoderma lucidum*) has been used for centuries to promote general health as well as being anti-inflammatory and an immunostimulant (Subramanian, 1995). *G. lucidum* is even being investigated for its ability to promote anticancer activity in the body (Monroy et al., 2021; Jin et. al., 2012). In Japan, a natural product called PSK extracted from the turkey tail fungus (*Trametes versicolor*) has been approved for use in cancer treatment (de Mattos-Shipley, 2016). Fungi are also an essential part of antibiotic drug development. In the coming decades, the need for alternative medicines and new antibiotics will continue to increase (Karwehl & Stadler, 2016). Fungi can provide possible answers for these problems. Investigating the full extent of fungal biodiversity and better understanding how mushrooms interact with their

environment will allow us to better utilize their potential for drug development, agriculture, and food accessibility and sustainability (Lange, 2014).

2.2 Mushroom Biodiversity and the Environment

The Kingdom Fungi encompasses multiple life history strategies, including saprobes, parasites, pathogens, and mycorrhizal symbionts. Saprotrophic fungi fill the ecological role of breaking down nutrients and returning them to the soil. They retrieve nutrients by secreting enzymes into their substrate; many utilize lignin-cellulose degrading enzymes to break down organic matter from plants (Boddy & Hiscox, 2016). Common examples of saprotrophic macrofungi are turkey tail (*Trametes versicolor*), chicken of the woods (*Grifola frondosa*) and oyster mushrooms (*Pleurotus* species). The growth and development of saprobes can be influenced by competition for available detritus, as well as stress and disturbance from their surroundings (Boddy & Hiscox, 2016; Elevitch, 2004). Saprotrophic fungi and other decomposers are essential components of ecosystems like forests with high rates of nutrient turnover; without them, plants would not have available nutrients to grow and support the biodiversity of other organisms.

Fungal parasites and pathogens pose serious concerns to farmers, arborists, and public health experts alike. A large portion of research within mycology, especially in fungal genetics, is dedicated to improving our understanding of fungi that could potentially become or are already pathogenic (Kogel et. al., 2006). Fungal pathogens are parasites that cause a disease in their host, such as *Batrachochytrium dendrobatidis*, which causes chytridiomycosis in frogs and other amphibians (Fisher et. al., 2009). There are many common types of fungal infections in humans, such as minor skin infections like athlete's foot, but there are also serious diseases such as aspergillosis and systemic candidosis that have high mortality rates in humans (Kavanaugh,

2005). Common forest macrofungi that are parasitic include *Armillaria* species and conks (e.g. *Heterobasidion annosum*), which can both persist as saprobes as well (Molina et al., 1993). Fungi are constantly adapting to their environment and the resources that are made available to them. While parasites and pathogens are a serious concern for both environmental and human health, these species still contribute significantly to the processes of returning nutrients to an ecosystem and to the biodiversity of the Kingdom Fungi, as well as some edible species such as honey mushrooms (*Armillaria mellea*) (Arora, 1986).

Mycorrhizae form networks of hyphae in the soil around the root tips of their host plant, essentially increasing the spread of the plant's root system and allowing for greater access to available nutrients and minerals in the soil, especially phosphorus and nitrogen. Mycorrhizal plants can obtain up to 80% of their required nitrogen and up to 90% of phosphorus from their fungal symbionts (van der Heijden, 2009). Plants tend to have multiple fungal symbionts connected to their root systems, and mycorrhizae tend to expand their networks to multiple hosts, even reallocating resources between different areas of their network, between hosts that have greater or lesser amounts of certain nutrients (van der Heijden et al., 2003; 2009). Mycorrhizal fungi play host to their own endosymbiotic bacteria and can also increase the populations of soil bacteria in their proximity; hence, their presence in the soil can change the dynamic of the whole soil microbial community (Andrade, 1997).

The four categories of mycorrhizae are arbuscular mycorrhizal fungi (AMF), ectomycorrhizal fungi (ECM), ericoid mycorrhizal fungi, and orchid mycorrhizal fungi (Brundrett & Tedersoo, 2018). Approximately 80% of all plants are associated with arbuscular mycorrhizal fungi (AMF), including a majority of food crops (Pele, 2018). AMF are characterized by their arbuscules, which are clusters of hyphae which grow inside the cells of

their host plant's roots and facilitate the direct transfer of nutrients to their host in exchange for carbon-containing molecules. Ectomycorrhizal fungi (ECM) are the only kind of mycorrhizal fungi that produce mushrooms (Molina et al., 1993). ECM do not penetrate their plant host's cells, but instead their hyphae grow in between the cells and form what is called a Hartig net. ECM belong to the phyla Basidiomycota and Ascomycota (van der Heijden, 2009). Only 2% of vascular plants (around 8,000 species) associate with ECM, but among them are many common tree species within the Birch (*Betulaceae*), Pine (*Pinaceae*) and Beech (*Fagaceae*) and dipterocarp (*Dipterocarpaceae*) families, among others, which dominate many boreal, temperate, and tropical forests (Dahlberg et al., 2001). ECM fungi and their tree hosts are often key players in ecosystem health and resilience (Usman et al., 2021); these organisms are worth studying to expand our knowledge of how to protect the biodiversity of fungi, plants, and other taxa.

Mycorrhizae can directly influence the diversity and identity of plants in their environment (Tendersoo et al., 2020). Mycorrhizal fungi, including both their hyphal networks and their fruiting bodies, can potentially be used to tackle problems such as environmental contamination. Bioremediation is the process of using a living organism to remove or degrade a harmful substance from the environment. Reforestation and bioremediation utilizing plants along with their inherent symbionts are two strategies for decreasing the concentration or spread of contaminants at a polluted site, especially in the case of heavy metals (Henke et al., 2015). Establishing a plant community, such as a forest that hosts ectomycorrhizal trees, is one such method to remove a pollutant from the environment by accumulating it into the biomass of the fungi or trees present.

White rot fungi, such as common button mushrooms (*Agaricus bitorquis)*, brown rot fungi (such as *Gloephyllum* species) and even portobellos (*Agaricus bisporus*) are commonly

studied for mycoremediation of heavy metals (Budzynska et al., 2022). One study in northern Croatia observed several species of mushrooms, including wood hedgehog mushrooms (*Hydnum repandum*) and porcinis (*Boletus edulis*), that were able to accumulate radioactive carbon from soil that had been contaminated from the nuclear meltdown at Chernobyl (Tucaković et al., 2018). Additionally, multiple species of fungi such as Turkey tail (*Trametes versicolor*) and oyster mushrooms (*Pleurotus ostreatus*) are capable of transforming organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) into non-toxic compounds (Budzynska et al., 2022). Some pathogenic fungi and xerophilic fungi including *Aspergillus glaucus* and *Botryosphaeria laricina* have been investigated as potential remediators for pesticides as well (Budzynska et al., 2022; Maqbool et al., 2016). Fungi not only support ecosystem health through nutrient cycling and their symbiosis with plants, but also through their potential to remove contaminants from their environment (Harms et al., 2011).

Quantifying mycodiversity in an area is a valuable lens through which to view the functioning of an ecosystem because fungi directly impact the chemistry of their surroundings and the soil microbiome, play important roles as plant-symbionts, and are a significant food source for animals and insects as well as humans. Understanding the impact of disturbance on mushroom diversity could help improve our overall understanding of fungal diversity in different environments and the best ways to make mushroom foraging more accessible to nearby communities. As fungi are essential for nutrient cycling and have strong benefits for plant communities, it is imperative to understand how anthropogenic forces as well as natural ones affect fungal biodiversity. In the coming decades, knowledge of fungal communities and their interrelatedness with the environment and other organisms will continue to shape how we

improve our agriculture, progress our biotechnology research, and protect our natural ecosystems from biodiversity loss.

2.3 What Influences Fungal Biodiversity?

Climate and habitat are two of the most influential factors on the ability of fungi to thrive. Each mushroom or fungus has their own preferences for substrate, temperature, and moisture level, but many are apt to thrive in damp, shaded environments (Tomao et al., 2020; Boddy et al., 2014). The presence of a consistent nutrient source is essential for sustained fungal growth in an area. Soil fungi and saprotrophic fungi tend to grow in areas with more vegetation and organic matter upon which to grow (Boddy & Hiscox, 2016). Precipitation and soil water retention are key factors in enabling fungal growth because fungi rely on soil water potential to uptake and retain water in their mycelia. Additionally, fruiting body formation depends on carbon and mineral nutrient availability, along with temperature, water availability, light, and interactions with other microbes in the soil (Moore et al., 2008; Boddy et al., 2014).

Fungal metabolism is higher at warmer temperatures, which causes the mushroom fruiting season to generally last from late spring to early fall; some species prefer the beginning of the season, while others prefer the end when temperatures begin to drop. As climate change causes elongated periods of summer in regions like Europe and North America, mushroom fruiting season has also begun to expand for some species, while shrinking for others (Boddy et al., 2014). In a global study done on soil fungi, researchers found that overall, fungal biodiversity increased with closer proximity to the equator. Diversity of most fungal groups was highest in tropical regions except for ECM species, for whom biodiversity was highest in temperate or boreal ecosystems. After climate, they concluded that edaphic factors (such as pH, mineral

composition, and soil texture) and spatial patterning (habitat zone, latitude) were the best predictors of soil fungi richness and community composition (Tedersoo et al., 2014).

For soil fungi that associate with a plant host, the distribution of host plants is one of the largest influences on the presence of those fungi. Tree communities and fungal communities are inherently linked, and so the diversity and species composition within those communities also influence each other (Frąc et. al., 2018). A higher presence of mycorrhizal fungi supports soil microbial diversity as well as plant health (Tendersoo et al., 2020; Andrade et al., 1997). Fungal populations are also significantly impacted by the plant communities around them (Frąc et al., 2018). Community composition of ECM fungi is largely determined by host plant identity and the soil conditions (Molina et al., 1993). For example, oak trees and oak dominated forests are likely to favor the presence of specific fungi in the *Amanita*, *Boletus*, and *Hygrophorus* genera, among others (Nguyen et al., 2016). Many edible mushroom species belong to these genera, making ECM tree-fungi associations critical for improving accessibility of mushroom foraging as a means of decreasing food scarcity (Arora, 1986; Rombach & Dean, 2023). Old growth forests have also been observed to support a diversity of ECM species (Tomao et al. 2020). Furthermore, AMF-dominated forests have been shown to have more saprotrophs and parasites along with greater soil fungal diversity overall compared to ECM-dominated forests (Eagar et al., 2022). This goes to show how fungal communities and their tree associations can have largescale implications on the diversity found in an ecosystem.

Disturbance is also a factor that influences mushroom growth and fungal diversity. Natural disturbance events like animal movements, storms causing trees to fall, wildfires and other natural disasters are examples of disturbance to which fungi adapt regularly. There are some species that thrive most in disturbed ecosystems, likely due to different nutrients being

made available and the pH and moisture content of the soil being changed by the disruption (Arora, 1986). In other circumstances, anthropogenic disturbance can negatively impact plant and soil communities, and in turn, fungal communities. A study done in Japan investigated whether soil microbial communities and fungal communities differed in natural versus artificial forests. They looked specifically at forests that had previously been transformed into plantations or other curated forest areas. They found that the fungal and bacterial communities in the soil were different between natural and modified forests. The diversity indexes of microbes did not change between the two, but the microbial co-occurrence network was changed (Nakayama et al., 2019). Forest management practices, which can involve the modification of tree communities, available deadwood, and other ecological factors, can also impact fungal communities. Forest management has been seen to increase the species richness of saprotrophic fungi, but current research has come to contrasting conclusions on forest management's effects on ECM fungi, depending on if ECM diversity was measured by observing sporocarps or ECM root morphotypes (Tomao et al., 2020).

Another form of disturbance that comes with proximity to human activity is compaction, which can occur during events such as logging or heavy footfall on walking or running trails. Compaction limits the air and water conductivity in soil, making it more difficult for fungi to retrieve nutrients. A study done on the effects of compaction caused by logging showed that compaction significantly reduced abundance, but increased the diversity of fungi (Hartmann et al., 2014). Parks and green spaces that are in immediate proximity to cities are exposed to additional disturbance. In a study on fungal biodiversity in urban forests of Portland, Oregon compared to Mount Hood National Park, greater diversity of mycorrhizal fungi was seen in the national forest, but overall fungal community composition was not significantly different

between the two ecosystems (Leis, 2022). Human activities in green spaces, whether rural or urban, can have unseen impacts on biodiversity.

Mushroom foraging can provide an accessible and sustainable additional source of food to people who have access to parks and other green spaces. Foraging for edible mushrooms has been gaining popularity in the US over the past decade as a way to procure additional food and connect with nature at the same time (Rombach & Dean, 2023). Improving our understanding of mushroom biodiversity in urban parks and green spaces will help better inform local and regional mushroom foragers on where they can most reliably find edible species. Trails provide convenient avenues for mushroom foragers; but whether mushrooms grow more readily along disturbed edges such as trails compared to off-trail patches of forest has not been thoroughly researched. This study investigated the impact of trailside disturbance on biodiversity of mushroom-forming fungi in public green spaces of New London, Connecticut. It was hypothesized that fungal species richness and fungal community composition would vary significantly between sampling sites, due to seasonality, and between foraging areas (trailside or in the undisturbed plot). It was predicted that tree communities present would also have an impact on the fungal communities. Foraging in the trailside sampling area was hypothesized to increase fungal diversity and affect fungal community composition due to the ecological disturbance caused by the presence of a trail and soil compaction, along with the effects of trail management practices on available deadwood and leaf litter. Seasonality was predicted to affect fungal species richness and community composition due to the variation in peak fruiting season timeframe between species of fungi. It was predicted that temperature and precipitation levels might also interact with seasonality and cause changes in fungal species richness observed.

3.0 METHODS

3.1 Experimental Design and Sampling Methods

Six public parks and reserve areas were selected in the New London area, and each was sampled for mushroom specimens twice during the months of September and October, 2023. These sites were chosen based on their proximity to Connecticut College, accessibility of these sites for the public, and knowledge of areas used by local mushroom foragers. Limiting the number of sites included in the study allowed for each site to be visited twice during the experiment. As a measure of the effects of disturbance, two separate areas of equal size were sampled at each site: one along either side of a trail, and the other in a relatively undisturbed rectangular plot area away from the trail. Both foraging areas, trailside or non-trailside, had a total area of 600 square meters. The trailside sampling area included 3 meters on either side of the trail for a stretch of 100 meters. The undisturbed plot was selected at least 10 meters away from the trail or other disturbance, and was 20 by 30 meters in area. This strategy allowed for comparison to be drawn between trailside and non-trailside sampling areas within each site, as well as across all 6 sites.

Sample collection days were within 2-4 days of a rain event so as to maximize the presence of possible fruiting bodies. During sampling, a fruiting body of each unique species observed at that site was photographed, removed from the substrate, and collected for later identification (Fig. 2). Only one specimen of each species was collected in each respective sampling area, regardless of abundance. Specimens were identified using a combination of David Arora's *Mushrooms Demystified* (Arora, 1986), as well as the online identification forum, iNaturalist, and other mushroom identification keys such as mushroomexpert.org (Kuo, 2022). Most specimens were identified to the genus level or lower.

Figure 2. Mushroom Collection Process. **Top Left:** Habitat adjacent to the foraging areas at Mamacoke Island. **Top Center & Top Right, Bottom Left & Bottom Center**: Mushroom bodies collected during the sampling process from the genera *Trametes* (top center) *Amanita* (top right), *Russula* (bottom left) and *Mycena* (bottom center). **Bottom Right**: Foraging in the undergrowth of Bates Woods Park.

3.2 Foraging Sites

The sites chosen for the study were Avery Nature Reserve (41 24’27”N 72 06’04”W), Bates Woods Park (41 21’18”N 72 07’34”W), Mamacoke Island within the Connecticut College Arboretum (41°23’29”N 72°06’01”W), Virginia Conover Nature Reserve (41°24’07”N 72°08’34”W), Stenger Farm Park (41°21’05”N 72°07’53”W), and Mitchell Woods (41°19’36”N 72°05’57”W). These sites were chosen based on their proximity to Connecticut College and the

accessibility of these sites for both students and the public **(**Fig. 3). Trailside and undisturbed sample areas within each open space were selected based on prior mushroom foraging and habitat parameters, e.g. relatively mature forest, dominance of ectomycorrhizal host trees, proximity to streams, creeks, or other wetlands, and previous foraging experience in the area. Trailside and undisturbed areas within each site were adjacent to each other.

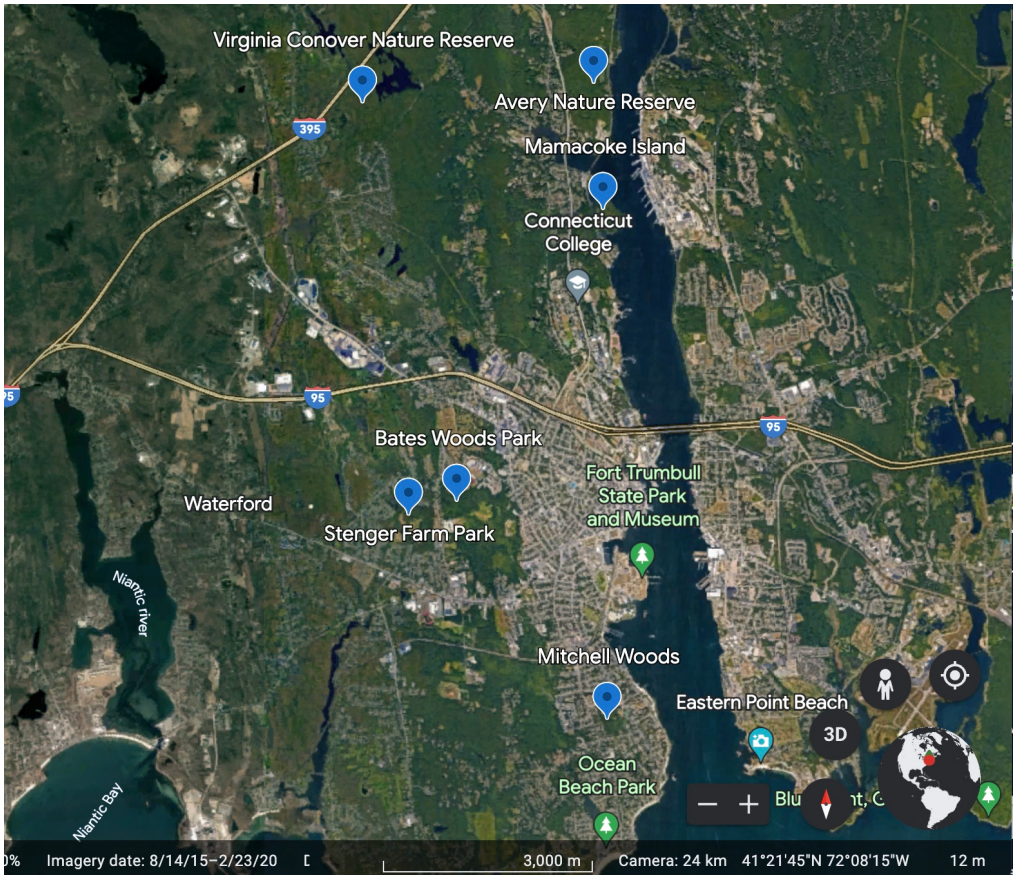


Figure 3. Map of the study sites, each with a blue pin showing their position in the greater New London and Waterford area of Connecticut (Google Earth version 7.3).

3.3 Further Data collection

After identification had been attempted for all specimens, the number of unique species (including unidentified but morphologically unique specimens) at each site and in the trailside or non-trailside area was recorded. Precipitation data for the days surrounding each mushroom

collection were gathered from the National Oceanographic and Atmospheric Administration (National Centers for Environmental Information, 2022). A representation of the tree community composition at each site was gathered by recording the observed tree species richness and abundance within a 20 m x 30 m transect along both foraging areas at each site (undisturbed plot and trailside) as well as recording the proportion of those tree species that form ectomycorrhizal (ECM) associations at each site. To include a measure of seasonality over the course of the experiment, we coded each sampling date as the number of days since September 1. This was used as an approximate measure of the change in season from late summer to early fall over the course of the experiment, as fruiting season varies between species of fungi (Boddy et al., 2014).

3.4 Statistical Analyses

The data was initially visualized to compare the richness of mushroom-forming species observed at each site and within each foraging area (trailside or undisturbed forest plot). Fungal species richness was also plotted with seasonality (number of days since September 1 at the time of sampling), precipitation, and temperature. The initial visualizations of the fungal richness data across each site and foraging area also indicated that there was a potential interaction between these two predictor variables, and we therefore added an interaction term for site and disturbance (foraging area) to our statistical analysis. Based on all of the preliminary visualizations, a four-way ANOVA was conducted to test whether fungal species richness was influenced by the predictor variables of sampling site, foraging area, and seasonality (Anderson et al., 2001).

Comparison of fungal communities between sites was conducted at the genus level. The assessment of fungal communities refers only to the variety of mushroom-forming fungal species that were found at a site; microscopic soil fungi or non-ECM fungi species were not included in this study. The fungal species richness measure in our data was reflective of the number of

distinct morphological species within each genus and was included (as opposed to presence/absence of each genus) to give weight to the potentially ecologically and culinary significance of the number of different species within each genus at a given sampling. A Constrained Analysis of Principal Coordinates (CAP) (Anderson & Willis, 2003) using Bray-Curtis dissimilarity (Bray and Curtis, 1957) was used to visualize differences in fungal communities among sites and foraging areas through an ordination plot. To examine the effects of site, disturbance (foraging area) and seasonality on fungal community structure, we conducted a three-way PERMANOVA (Anderson et al., 2001) using the 'adonis' function in the R package 'vegan' (Oksanen et al., 2016) with Bray-Curtis dissimilarity (Bray and Curtis, 1957). Indicator species analyses (De Caceres & Legendre, 2009) were also used to identify fungal genera that were found significantly more frequently at different sites and foraging areas.

Finally, Shannon diversity indices of tree diversity and the proportion of ectomycorrhizal (ECM) tree species within the tree community at a site were incorporated into a six-way ANOVA with site, disturbance, the interaction between site and disturbance, and seasonality to assess the effects of those factors on fungal species richness. All statistical analyses were conducted using the program R (R Core Team, 2021), and the code that was used is available at the following link: https://osf.io/r6kje/.

4.0 RESULTS

4.1 Effects of Sampling Site, Disturbance and Climate on Fungal Species Richness

The trailside and undisturbed plots were not significantly different in species richness (*F=0.718, P=0.41*) as determined by the six-way ANOVA on fungal species richness. Trailside sampling areas had an average of 22 unique species with a standard deviation of ± 9 and the undisturbed sampling plots had an average of 19 ± 7 unique species (Fig. 4A). Fungal species

richness was higher on the trailside at four of the six sites, except for Bates Woods Park and Stenger Farm, where fungal species richness was higher in the undisturbed plots (Fig. 4B and 4C). Mamacoke Island had the highest overall fungal species richness with of 27 unique species averaged between the two sampling occasions and foraging areas, while Mitchell Woods had the lowest overall fungal species richness with an average of 11. The highest average fungal species richness observed at any specific sampling area within a site was recorded at the Mamacoke Island undisturbed plot, with 44 unique species (Fig. 4B). There were no direct correlations between fungal richness and sampling occasion (first or second sample collection) (see Appendix 4). Climate data also indicated no direct correlation between fungal richness and temperature or amount of precipitation in the previous 72 hours before sampling (see Appendices 2 and 3).

The four-factor ANOVA comparing the effects of site, foraging area, the interaction between site and foraging area, and seasonality on the species richness of fungi indicated that there was not a significant effect of these factors on the species richness observed, except perhaps for site ($F=2.715, P=0.08$). Initial analyses found a slight negative correlation between fungal species richness observed and seasonality (see Appendix 1), but there was not a statistically significant effect of seasonality on fungal species richness ($F=0.366, P= 0.5573$).

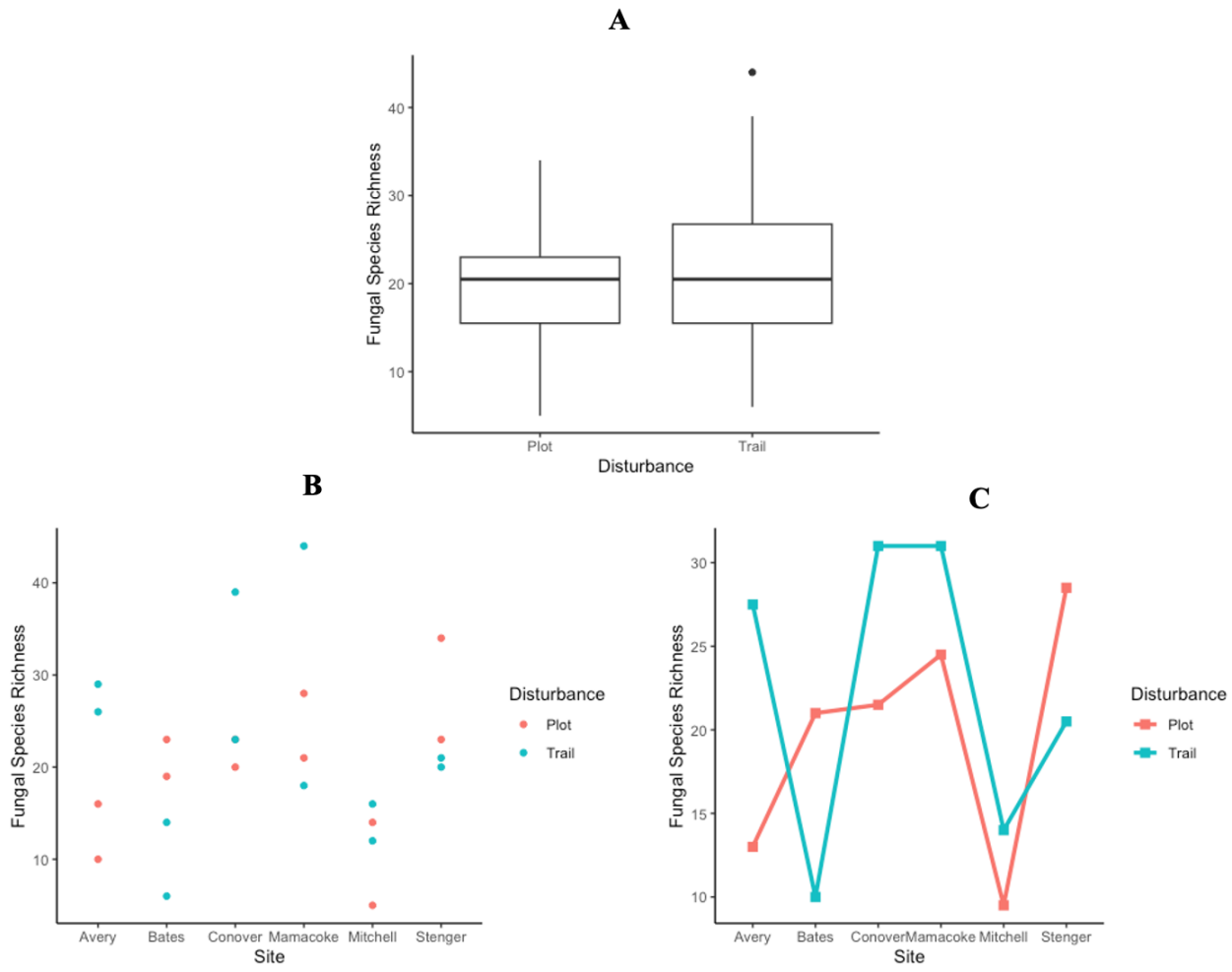


Figure 4. Effects of sampling site and disturbance within that site (trailside vs undisturbed plot) on fungal species richness. **A:** Distribution of fungal species richness observed in trailside sampling (trail) versus in undisturbed sampling plots (plot). **B:** Fungal species richness observed at each site, including sampling occasion 1 and 2 as separate points, and distinguishing between fungal species richness observed on the trailside and in the undisturbed plot. **C:** Interaction plot displaying the effects of sampling site and disturbance on fungal species richness.

4.2 Effect of Site and Disturbance on Fungal Communities

Fungal communities differed between sites and depending on seasonality ($F = 1.71$, $P = 0.004$, and $F = 2.17$, $P = 0.019$, respectively) as determined by the three-way PERMANOVA. Foraging area did not have a significant impact on fungal community composition. The CAP ordination (Fig. 5) generated ellipses representing the 95% confidence interval of each site's fungal community composition, where overlap in the ellipses indicates similarity in the fungal

communities of those sites. Mamacoke's fungal community composition showed significant similarity with that of Bates Woods, Avery, and Stenger Farm, as well as some similarity with Conover. Avery's fungal community showed similarity with the fungal communities of all other sites, but especially Bates Woods, Mamacoke and Conover. Meanwhile, Mitchell showed the least similarity with any other sites' fungal community compositions, having no overlap at all with Mamacoke or Stenger, and showing minimal similarity to the fungal community composition of Conover, Bates Woods, and Avery. The *Amanita* genus was identified as an indicator genus for Mamacoke Island ($P = 0.035$), while *Merulius* was an indicator genus for Mitchell Woods ($P = 0.011$). *Stereum* was an indicator genus for the trailside foraging area.

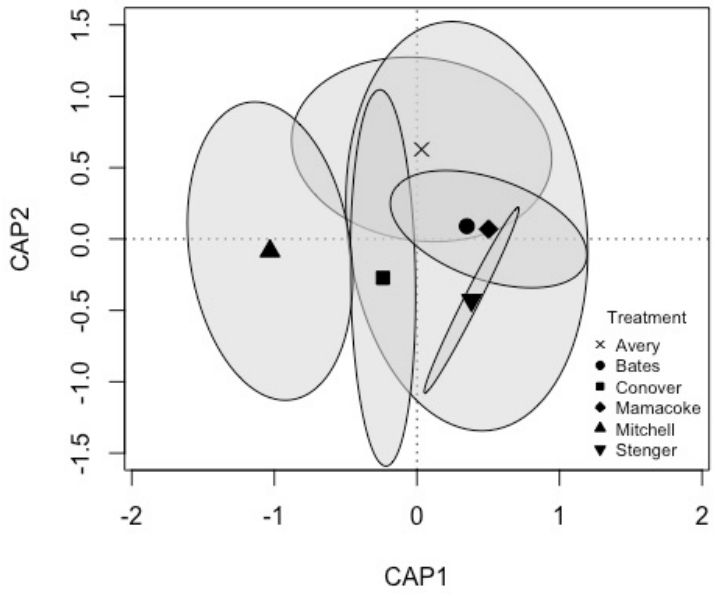


Figure 5. Constrained Analysis of Principal Coordinates (CAP) using Bray-Curtis Dissimilarity Matrix, comparing fungal community composition between different sites and displaying 95% confidence intervals as ellipses around the center symbol representing each site. Each symbol and corresponding ellipse represents the average of all four replicates, two sampling occasions and both foraging areas.

4.4 Tree communities and Fungal Communities

Tree species richness showed only slight variation across sites and between foraging areas. Conover had the highest tree species richness on average with 8 in the undisturbed plot and 9 on the trailside, while Bates Woods Park had the lowest at 5 in the plot and 6 on the trail.

Table 1. **Tree diversity and percent ectomycorrhizal tree species (ECM) at each site and foraging area within that site.**

Foraging Area	Site	Tree Species richness	Shannon Diversity Index	Percent ECM
Trailside	Avery	8	1.992	84.615
	Bates	6	1.044	28.571
	Conover	9	1.947	87.180
	Mamacoke	5	1.772	92.857
	Mitchell	7	1.826	42.857
	Stenger	8	1.954	64.000
Undisturbed Plot	Avery	8	1.992	69.231
	Bates	5	1.088	35.294
	Conover	8	1.956	73.333
	Mamacoke	7	1.304	92.308
	Mitchell	5	1.459	40.000
	Stenger	6	1.712	69.231

Initial comparison between of the proportion of ECM trees and the fungal species richness found at that site indicated a positive correlation between these two factors (Fig. 6), which was confirmed as statistically significant by the six-way ANOVA ($F = 7.04$, $P = 0.023$) which also tested site, foraging area, the interaction between site and foraging area, seasonality, and tree diversity. None of the other predictor variables had significant effects on fungal species richness.

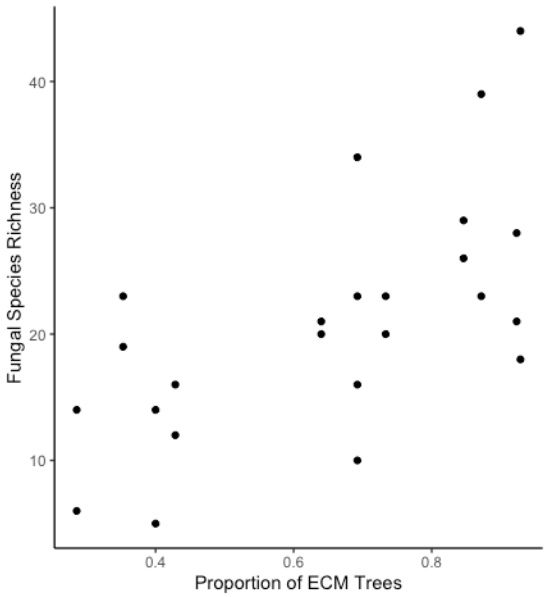


Figure 6: Proportion of Ectomycorrhizal tree species found at a site and the fungal species richness found at that site.

5.0 DISCUSSION

5.1 Fungal Species Richness Impacted by Presence of ECM-Associated Trees

The proportion of ECM-associating tree species at a given site significantly impacted species richness of mushroom-forming fungi. The three sites with the highest proportions of ECM tree species were Mamacoke, Conover and Avery. These sites had high abundance of oak trees (*Quercus*) as well as some presence of birch (*Betula*). Notably, these sites were also high in fungal species richness and in their overlap with fungal community composition of other sites. Mamacoke had high diversity of species in the *Amanita* genus, many of which are ECM fungi (Yang et al., 1999). These results agree with the results of Cavard et al. (2011), which found that ECM fungal species richness increases with higher ECM tree diversity. ECM fungi have high variation in their spatial distribution within a forest ecosystem; most species aggregate together, leading to a high density of a species in one area and a very low density of that same species in a different area (Dahlberg et al., 2001). In a study on ECM fungi in boreal forests disrupted by

wildfires, spatial variation was shown to have a greater impact on ECM fungal species composition than the impact of disturbance caused by wildfires (Jonsson et al.,2003). The chosen study area at Mamacoke Island containing a high abundance of oak (*Quercus*), hickory (*Carya*) and birch (*Betula*) trees, all of which are ectomycorrhizal, could have played a role in the *Amanita* genus being highly associated with this site. The impact of ECM trees on the broader ecosystem has become of greater interest to plant and fungal ecologists as more research supports the importance of diverse mycorrhizal communities for forest resilience. Quantifying abundance and diversity of mushrooms can be used to assess the responses of fungal communities to disturbance and change, which will continue to be relevant as climate change progresses and more ecosystems are susceptible to disruption (Stephens et al., 2017).

5.2 Fungal Species Richness Largely Uninfluenced by Disturbance, Seasonality, Tree Diversity, Precipitation or Temperature

The results indicated that site, foraging area, and seasonality at the time of sampling did not have significant effects on fungal species richness observed. As foraging area served as a measure of disturbance for this experiment, the disturbance caused by the presence or absence of a trail in the foraging area was not deemed significant. This result did not support the hypothesis that trailside foraging areas would have greater fungal diversity due to increased compaction and available deadwood; however, the genus *Stereum* was identified as an indicator for the trailside foraging areas. *Stereum* is a common saprotrophic shelf fungus found on hardwood; it follows that this genus would commonly be found along the sides of trails where there is a plethora of cleared vegetation and fallen branches upon which those fungi could grow. This is supported by the findings of Tomao et al. (2020) whose results suggested that disturbances that lead to greater availability of deadwood could promote diversity of fungi that use wood as their substrate.

Tree species diversity did not significantly impact fungal species richness across sampling sites. This contrasts the findings of Tomao et al. (2020), who found that fungal diversity was positively correlated with tree species diversity. Their study was focused on the effects of forest management; they found that high intensity forest management correlated with lower fungal species richness. Tomao et al. (2020) also concluded that low impact forms of forest management, such as more sustainable logging practices and reduction of habitat fragmentation, can help to preserve fungal diversity. These findings could lead to improved understandings of how parks, forests and preserves can be responsibly managed to benefit the biodiversity of all taxa, including the Kingdom Fungi, that may be present. Maintaining trail systems and supporting ectomycorrhizal and native trees could augment the biodiversity and health of these spaces, as well as improving their accessibility and resiliency.

Temperature and precipitation levels in the days leading up to the sample collection also had no correlation with fungal species richness. It is relevant to note that the summer before this experiment had lower rainfall than an average year (National Centers for Environmental Information, 2022). The inconsistency of mushroom fruiting patterns and the lower amounts of precipitation experienced in this area of Connecticut prior to the experiment could have affected the species richness observed. Mushroom fruiting can be triggered by a variety of factors and many mushrooms are ephemeral, only lasting for a few days if they do form. There are even species that do not form fruiting bodies annually (Boddy et al., 2014). Because of all these uncertainties, sampling mushrooms is more reflective of what fungi happen to be thriving and producing fruiting bodies in the conditions of that transient moment in time rather than all of the fungal diversity that may be present.

5.3 Differences in Fungal Community Composition based on Site and Seasonality

Fungal communities were significantly impacted by site and seasonality, while disturbance of the foraging area by the presence or absence of a trail had no significant impact. The CAP ordination showed overlap between many of the sites, such as Avery and Mamacoke, but also highlighted differences in fungal community composition between some key sites. The fungal community composition of Mitchell Woods was the least like other sites, and this site was also the lowest in fungal species richness. Mamacoke and Mitchell woods also were significantly associated with particular fungal genera, *Amanita* and *Merulius* respectively, which further emphasizes the difference between the fungal community composition of these two sites. Differences between the mycodiversity observed at each site could be due to the unique site histories and characteristics of the soil such as moisture retention and mineral composition (Tomao et al., 2020). Each site within this study had a different total area, as well as contrasting land uses in the adjacent areas. Grilli et al. (2017) found that habitat fragmentation largely negatively impacted fungal diversity, except occasionally in the case of pathogenic fungi. Mitchell Woods had the least amount of total area within that park, making its interior habitat more susceptible to the effects of fragmentation. Avery and Conover Nature Reserves had larger total areas; Mamacoke Island also had a large area, as well as being bordered by water and the Connecticut College Arboretum. The fungal diversity at these sites could have been supported by their greater land area and decreased proximity to urban development.

Seasonality is well documented as having an impact on which species of fungi undergo fruiting (Boddy et al. 2014). In our study, seasonality was shown to have a significant impact on fungal community composition, but not fungal species richness. Regarding seasonality, it is plausible that changes in niche availability occurred over the course of the experiment as

temperature and precipitation changed. Seasonal preferences of different species of fungi could explain why fungal species richness was not impacted by seasonality, unlike fungal community composition. Two additional hypotheses that attempt to explain the distribution of fungal diversity are 1) niche differentiation between species with contrasting competitive abilities, and 2) stochastic factors which enhance coexistence of species with similar competitive abilities (Dahlberg et al., 2001). As fungi have little control of the dispersal of their spores once they have left the fruiting body, it is up to factors such as wind, rain, and animals to determine how far the spores will spread and upon which substrate they will land. Successional changes, such as the regrowth of primary vegetation after a disturbance, also goes hand in hand with primary colonization of mycorrhizal fungi, therefore influencing what fungal communities can be found in an ecosystem along a successional chrono sequence (Dahlberg et al., 2001). Furthermore, the timing of mushroom morphogenesis is influenced by a combination of environmental factors such as nutrient availability, temperature, water availability, and interactions with other soil microorganisms (Moore et al., 2008). Mushroom fruiting is therefore subject to seasonal conditions as well as short term weather events, which accounts for the individual variations in fungal species richness that may have been observed between different occasions or foraging areas within a site (Halme & Kotiaho, 2012).

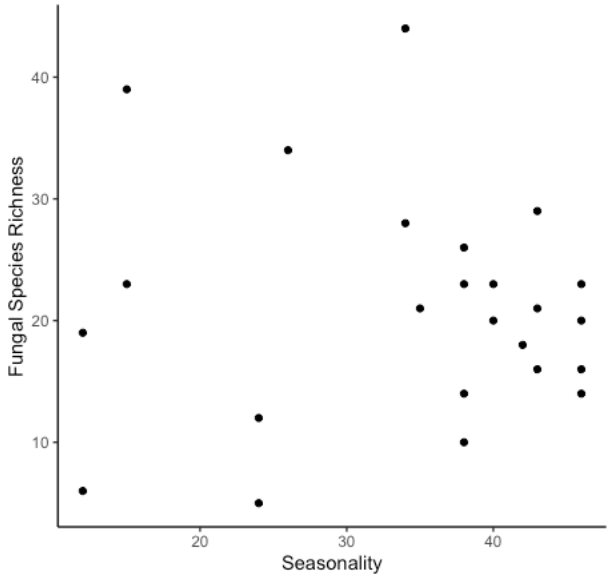
6.0 CONCLUSIONS

Fungal species richness was correlated with higher proportions of ECM trees within the tree community at a site, indicating the importance of ECM hosts in predicting fungal diversity. Fungal species richness was not significantly impacted by foraging area or tree diversity at the given site. Site and seasonality had significant impacts on fungal community composition, but not on the species richness. Niche availability likely played a role in the impact of seasonality on

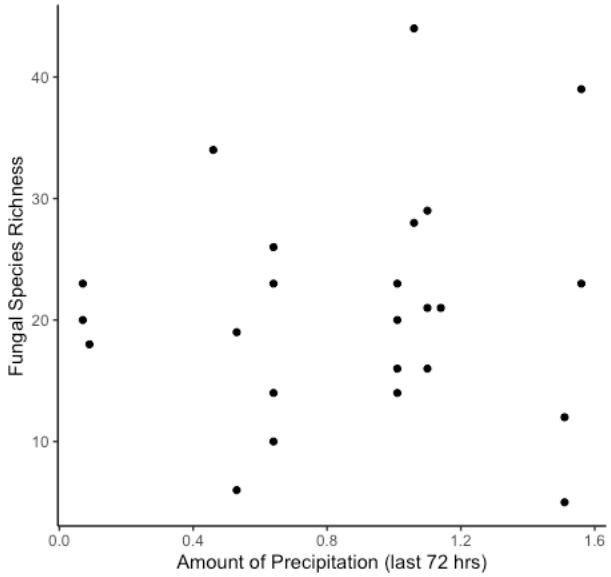
fungal species richness, as different niches can emerge over the course of changing temperatures and precipitation levels between late summer and early fall. Factors such as the tree community composition and habitat fragmentation likely affected the fungal community composition at each site. Ultimately, foraging alongside a trail versus in an undisturbed area of forest did not impact the fungal richness observed, indicating that remaining on or off a trail in a public green space will not impact the accessibility of particular species of fungi, such as those that are edible or used for medicinal purposes. Fungi in urban environments can help improve our connection to nature, decrease food insecurity, and better support biodiversity and ecosystem resilience. As biodiversity continues to be threatened globally by anthropogenic forces and climate change, it is important to recognize both the inherent value of fungal biodiversity and the ways in which we can better protect and utilize our green spaces (Martinez-Garcia et al., 2017; Lange, 2014).

7.0 APPENDICES

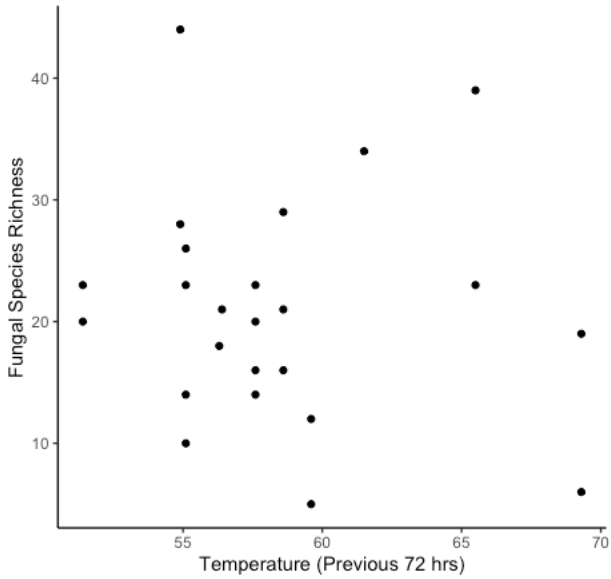
Appendix 1. Seasonality and Fungal Species Richness, where each dot represents one observation at each foraging area within each site.



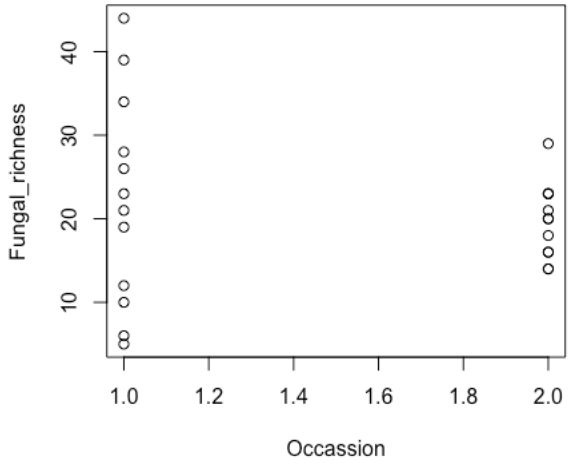
Appendix 2. Precipitation in the last 72 hours before sampling and Fungal Species Richness.



Appendix 3: Fungal Species Richness and Temperature in the 72 hours prior to sampling.



Appendix 4: Sampling occasion (1st or 2nd collection) and Fungal Species Richness



REFERENCES

Anderson, M.J. (2001). A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46. https://doi.org/10.1046/j.1442-9993.2001.01070.x.

Anderson, M.J. & Willis, T.J. (2003). Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. Ecology 84, 511--525.

Andrade, G., Mihara, K. L., Linderman, R. G., & Bethlenfalvay, G. J. (1997). Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. *Plant and Soil, 192*(1), 71-79. 10.1023/A:1004249629643

Arora, D. (1986). Mushrooms demystified: A comprehensive guide to the fleshy fungi / David Arora (2nd ed. ed.)

Blackwell, M., & Vega, F. E. (2018). Lives within lives: Hidden fungal biodiversity and the importance of conservation. *Fungal Ecology, 35*, 127-134. 10.1016/j.funeco.2018.05.011

Boddy, L., Büntgen, U., Egli, S., Gange, A. C., Heegaard, E., Kirk, P. M., Mohammad, A., & Kauserud, H. (2014). Climate variation effects on fungal fruiting. *Fungal Ecology, 10*, 20-33. 10.1016/j.funeco.2013.10.006

Boddy, L., & Hiscox, J. (2016). Fungal Ecology: Principles and Mechanisms of Colonization and Competition by Saprotrophic Fungi. Microbiology Spectrum, 4(6), 4.6.17. 10.1128/microbiolspec.FUNK-0019-2016

Bray, J. R., & Curtis, J. T. (1957). An Ordination of the Upland Forest Communities of Southern Wisconsin. *Ecological Monographs, 27*(4), 326-349. 10.2307/1942268

Brundrett, M. C., & Tedersoo, L. (2018). Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytologist, 220*(4), 1108-1115. 10.1111/nph.14976

Cavard, X., Macdonald, S. E., Bergeron, Y., & Chen, H. Y. H. (2011). Importance of mixedwoods for biodiversity conservation: Evidence for understory plants, songbirds, soil fauna, and ectomycorrhizae in northern forests. *Environmental Reviews, 19*, 142-161. 10.1139/a11-004

Dahlberg, A. (2001). Community ecology of ectomycorrhizal fungi: an advancing interdisciplinary field. *New Phytologist, 150*(3), 555-562. 10.1046/j.1469-8137.2001.00142.x

De Caceres, M., Legendre, P., 2009. Associations between species and groups of sites: indices and statistical inference. Ecology 90, 3566–3574.

de Mattos-Shipley, K. M. J., Ford, K. L., Alberti, F., Banks, A. M., Bailey, A. M., & Foster, G. D. (2016). The good, the bad and the tasty: The many roles of mushrooms. Studies in Mycology, 85(1), 125-157. 10.1016/j.simyco.2016.11.002

Dressaire, E., Yamada, L., Song, B., & Roper, M. (2016). Mushrooms use convectively created airflows to disperse their spores. *Proceedings of the National Academy of Sciences, 113*(11), 2833-2838. 10.1073/pnas.1509612113

Eagar, A. C., Mushinski, R. M., Horning, A. L., Smemo, K. A., Phillips, R. P., & Blackwood, C. B. (2022). Arbuscular Mycorrhizal Tree Communities Have Greater Soil Fungal Diversity and Relative Abundances of Saprotrophs and Pathogens than Ectomycorrhizal Tree

Communities. Applied and Environmental Microbiology, 88(1), e0178221. 10.1128/AEM.01782-21

Elevitch, C. R. (.)., Permanent Agriculture Resources, Holualoa, HI (USA) eng, & Stamets, P. (2004). The overstory book: cultivating connections with trees. Holualoa, HI (USA) PAR.

Fisher, M. C., Garner, T. W. J., & Walker, S. F. (2009). Global Emergence of Batrachochytrium dendrobatidis and Amphibian Chytridiomycosis in Space, Time, and Host. *Annual Review of Microbiology, 63*(1), 291-310. 10.1146/annurev.micro.091208.073435

Frąc, M., Hannula, S. E., Bełka, M., & Jęyczka, M. (2018). Fungal Biodiversity and Their Role in Soil Health. *Frontiers in Microbiology, 9*, 707. 10.3389/fmicb.2018.00707

Google Earth version 7.3. (8/14/15 – 2/23/20). New London and Waterford County, Connecticut. 41°21’45” N, 72°08’15” W. Camera: 24 km.

Grilli, G., Longo, S., Huais, P. Y., Pereyra, M., Verga, E. G., Urcelay, C., & Galetto, L. (2017). Fungal diversity at fragmented landscapes: synthesis and future perspectives. *Current Opinion in Microbiology; Environmental Microbiology * CRISPRcas9, 37*, 161-165. 10.1016/j.mib.2017.09.003

Halme, P., Heilmann-Clausen, J., Rämä, T., Kosonen, T., & Kunttu, P. (2012). Monitoring fungal biodiversity – towards an integrated approach. *Fungal Ecology, 5*(6), 750-758. 10.1016/j.funeco.2012.05.005

Halme, P., & Kotiaho, J. S. (2012). The importance of timing and number of surveys in fungal biodiversity research. *Biodiversity and Conservation, 21*(1), 205-219. 10.1007/s10531-011-0176-z

Harms, H., Schlosser, D., & Wick, L. Y. (2011). Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. *Nature Reviews Microbiology, 9*(3), 177-192. 10.1038/nrmicro2519

Hartmann, M., Niklaus, P. A., Zimmermann, S., Schmutz, S., Kremer, J., Abarenkov, K., Lüscher, P., Widmer, F., & Frey, B. (2014). Resistance and resilience of the forest soil microbiome to logging-associated compaction. The ISME Journal, 8(1), 226-244. 10.1038/ismej.2013.141

Henke, C., Jung, E., & Kothe, E. (2015). Hartig' net formation of Tricholoma vaccinum-spruce ectomycorrhiza in hydroponic cultures. *Environmental Science and Pollution Research, 22*(24), 19394-19399. 10.1007/s11356-015-4354-5

Jin, X., Ruiz Beguerie, J., Sze, D. M., & Chan, G. C. F. (2012). Ganoderma lucidum (Reishi mushroom) for cancer treatment. *The Cochrane Database of Systematic Reviews, (6):CD007731. doi*(6), CD007731. 10.1002/14651858.CD007731.pub2

Jonsson, L., Dahlberg, A., Nilsson, M., Zackrisson, O., & Karen, O. (2003). Ectomycorrhizal fungal communities in late-successional Swedish boreal forests, and their composition following wildfire. *Molecular Ecology, 8*(2), 205-215. 10.1046/j.1365-294x.1999.00553.x

Karwa, A., & Rai, M. K. (2009). Tapping into the edible fungi biodiversity of Central India. *Biodiversitas (Surakarta), 11*(2)10.13057/biodiv/d110209

Karwehl, S., & Stadler, M. (2016). Exploitation of Fungal Biodiversity for Discovery of Novel Antibiotics. In M. Stadler, & P. Dersch (Eds.), *How to Overcome the Antibiotic Crisis: Facts, Challenges, Technologies and Future Perspectives* (pp. 303-338). Springer International Publishing. 10.1007/82_2016_496

Kogel, K., Franken, P., & Hückelhoven, R. (2006). Endophyte or parasite – what decides? *Current Opinion in Plant Biology; Biotic Interactions / Edited by Anne Osbourn and Sheng Yang He, 9*(4), 358-363. 10.1016/j.pbi.2006.05.001

Kuo, M.*MushroomExpert.com.* Retrieved October, 2022, from https://www.mushroomexpert.com/

Lange, L. (2014). The importance of fungi and mycology for addressing major global challenges. *IMA Fungus, 5*(2), 463-471. 10.5598/imafungus.2014.05.02.10

Maqbool, Z., Hussain, S., Imran, M., Mahmood, F., Shahzad, T., Ahmed, Z., Azeem, F., & Muzammil, S. (2016). Perspectives of using fungi as bioresource for bioremediation of pesticides in the environment: a critical review. *Environmental Science and Pollution Research, 23*(17), 16904-16925. 10.1007/s11356-016-7003-8

Martinez-Garcia, L. B., De Deyn, G. B., Pugnaire, F. I., Kothamasi, D., van der Heijden, M G A, Sub Plant-Microbe Interactions, & Plant Microbe Interactions. (2017). Symbiotic soil fungi enhance ecosystem resilience to climate change. *Global Change Biology, 23*(12), 5228-5236. 10.1111/gcb.13785

Molina, R., O'Dell, T., Luoma, D., Amaranthus, M., Castellano, M., & Russel, K. (1993). *Biology, ecology, and social aspects of wild edible mushrooms in the forests of the Pacific Northwest*. U.S. Dept. of Agriculture, Forest Service, Pacific Northwest Research Station.

Monroy, L. A. V., Cauich, J. R. C., Ortega, A. M. M., & Campos, M. R. S. (2021). Chapter 5 - Medicinal plants as potential functional foods or resources for obtaining anticancer activity

metabolites. In M. R. S. Campos, & A. M. M. Ortega (Eds.), *Oncological Functional Nutrition* (pp. 161-194). Academic Press. 10.1016/B978-0-12-819828-5.00005-X

Moore, D., Gange, A. C., Gange, E. G., & Boddy, L. (2008). Chapter 5 Fruit bodies: Their production and development in relation to environment. *British Mycological Society Symposia Series, 28*, 79-103. 10.1016/S0275-0287(08)80007-0

Nakayama, M., Imamura, S., Taniguchi, T., & Tateno, R. (2019). Does conversion from natural forest to plantation affect fungal and bacterial biodiversity, community structure, and co-occurrence networks in the organic horizon and mineral soil? *Forest Ecology and Management, 446*, 238-250. 10.1016/j.foreco.2019.05.042

National Centers for Environmental Information. (2022). NOAA Climate Data Online. National Oceanic and Atmospheric Administration. https://www.ncdc.noaa.gov/cdo-web/

Oksanen J et al (2016) Vegan: community ecology package. R package version 2:4–1 https://CRAN.R-project.org/package=vegan

Pérez-Moreno, J., Guerin-Laguette, A., Rinaldi, A. C., Yu, F., Verbeken, A., Hernández-Santiago, F., & Martínez-Reyes, M. (2021). Edible mycorrhizal fungi of the world: What is their role in forest sustainability, food security, biocultural conservation and climate change? *Plants, People, Planet, 3*(5), 471-490. 10.1002/ppp3.10199

Rombach, M., & Dean, D. L. (2023). Exploring Key Factors Driving Urban Foraging Behavior in Garden and Non-Garden Locations. *Foods, 12*(5)10.3390/foods12051032

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Stephens, R. B., Remick, T. J., Ducey, M. J., & Rowe, R. J. (2017). Drivers of truffle biomass, community composition, and richness among forest types in the northeastern US. Fungal Ecology, 29, 30-41. 10.1016/j.funeco.2017.05.004

Subramanian, C. V. (1995). Mushrooms: Beauty, diversity, relevance. *Current Science, 69*(12), 986-998. http://www.jstor.org/stable/24097287

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N. S., Wijesundera, R., Ruiz, L. V., Vasco-Palacios, A., Thu, P. Q., Suija, A., Smith, M. E., Sharp, C., Saluveer, E., Saitta, A., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Põldmaa, K., . . . Abarenkov, K. (2014). Global diversity and geography of soil fungi. *Science, 346*(6213), 1256688. 10.1126/science.1256688

Tedersoo, L., Bahram, M., & Zobel, M. (2020). How mycorrhizal associations drive plant population and community biology. *Science, 367*(6480), eaba1223. 10.1126/science.aba1223

Tomao, A., Antonio Bonet, J., Castaño, C., & de-Miguel, S. (2020). How does forest management affect fungal diversity and community composition? Current knowledge and future perspectives for the conservation of forest fungi. *Forest Ecology and Management, 457*, 117678. 10.1016/j.foreco.2019.117678

Tucaković, I., Barišić, D., Grahek, Ž, Kasap, A., & Širić, I. (2018). 137Cs in mushrooms from Croatia sampled 15–30 years after Chernobyl. *Journal of Environmental Radioactivity, 181*, 147-151. 10.1016/j.jenvrad.2017.11.004

Usman, M., Ho-Plágaro, T., Frank, H. E. R., Calvo-Polanco, M., Gaillard, I., Garcia, K., & Zimmermann, S. D. (2021). Mycorrhizal Symbiosis for Better Adaptation of Trees to

Abiotic Stress Caused by Climate Change in Temperate and Boreal Forests. Frontiers in Forests and Global Change, 4, 742392. 10.3389/ffgc.2021.742392

van Dijk, H., Awana Onguene, N., & Kuyper, T. W. (2003). Knowledge and Utilization of Edible Mushrooms by Local Populations of the Rain Forest of South Cameroon. AMBIO: A Journal of the Human Environment, 32(1), 19-23. 10.1579/0044-7447-32.1.19

van Der Heijden, Marcel G. A., & Horton, T. R. (2009). Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. *Journal of Ecology, 97*(6), 1139-1150. 10.1111/j.1365-2745.2009.01570.x

van Der Heijden, Marcel G. A., Wiemken, A., & Sanders, I. R. (2003). Different arbuscular mycorrhizal fungi alter coexistence and resource distribution between co-occurring plant. *New Phytologist, 157*(3), 569-578. 10.1046/j.1469-8137.2003.00688.x

Yang, Z. L., Weiß, M., Kottke, I., Oberwinkler, F., Nehls, U., Guttenberger, M., & Hampp, R. (1999). Amanita. In J. W. G. Cairney, & S. M. Chambers (Eds.), Ectomycorrhizal Fungi Key Genera in Profile (pp. 201-230). Springer Berlin Heidelberg. 10.1007/978-3-662-06827-4_8