Model Selection for the Key Drivers of Fungal Abundance in a Virginia Mixed-Hardwoods Forest

Phillip LaRue

Introduction

Although lovingly nicknamed “the Forgotten Kingdom " due to their frequency of being overlooked in favor of flora and fauna, fungi play many important roles in the environment they inhabit. This kingdom consists of any species that feeds on organic matter and produces spores and includes mushrooms, yeasts, and molds. Mushrooms generally exist as a network of mycelium that grow beneath the ground and store nutrients, however, fruiting bodies, or mushrooms, are created for the production of spores for reproduction (Branda et al. 2001). While the most obvious role they play is that of decomposers and recyclers of organic matter and nutrients within an ecosystem (Boddy and Watkinson 2011), mycelial networks are highly important for their ability to form symbiotic relationships with plant roots.

Mycelial bodies are the main life stage for most fungi and are responsible for decomposing and storing nutrients from organic matter in the soil. This process makes nutrients such as carbon, nitrogen, and phosphorus readily available for use by primary producers (Boddy and Watkinson 2011). These mycelial networks are quite expansive and can either bore into or grow around the outside of the roots of local plants (Simard and Durall 2004). Once interconnected, symbiotic relationships, or mycorrhizae, can form, which are integral to the survival and development of many trees. In exchange for food in the form of sugars and other organic substances, the mycorrhizae greatly improve the uptake capabilities of plant roots for moisture and stored nutrients by having access to a larger volume of soil (Simard and Durall 2004). Host trees also benefit from an increased stress tolerance for biotic and abiotic factors (Deepika and Kothamasi 2015).

While there are many environmental factors present in forested ecosystems that directly affect the growth of mushrooms and their mycelial networks, the most important factor appears to be soil moisture content. Not only is it a key driver of relative mycorrhizal abundance (Staddon et al. 2003), but the production of fruiting bodies increases linearly with increasing moisture content as well (Flegg 1974). Although the natural extent and optimal content range are mostly unknown (Bridge and Newsham 2009), lab induced conditions elicit opposing optimal moisture ranges at 15-20% (Deepika and Kothamasi 2015) and 20-30% (Iotti et al. 2018). Decomposition rates, and thus the rates of nutrient storage in mycelial networks, also increase linearly with soil moisture content, indicating fungal growth may as well (Pietikäinen et al. 2005).

Increasing levels of light reaching a forest's understory, which are directly related to leaf area index (LAI) and canopy coverage, promote greater mycorrhizal relationships, total root colonization, and mycelial length (Gehring 2002). Soil pH has the reverse effect, however. Fungal growth increases substantially with decreasing pH from a range of 8.5-4.0 (Rousk et al. 2010). These effects appear to depress with pH range, as mycelial growth remains relatively constant across a slightly acidic soil gradient (Richards 1961), while they appear to increase with highly alkaline conditions, which show a negative influence on the nutrient uptake capabilities of mycorrhizae (Richards 1961). Soil moisture content has been shown to increase soil pH, however, this change only occurs at "higher levels" of moisture content, which seems to be a somewhat abstract definition (Zhang and Wienhold 2002). Literature indicates that trees with lower DBH (diameter at breast height) rely more heavily on mycorrhizal interactions for growth and resistance to ecological change (DeForest and Snell 2020), which could imply that fungal presence may increase (at least in proximity to trees) with decreasing DBH. Finally, slope should also influence fungal abundance. North-facing slopes exhibit increased soil moisture, decreased light intensity, and slightly decreased soil pH as compared to south-facing slopes (Chai et al. 2018). Although these trends would suggest that a northern-facing slope would promote greater mycorrhizal growth, the opposite effects were found, as southern-facing slopes produced a higher percentage of root length colonized by arbuscular fungi and hyphae (subunit of mycelium), as well as greater hyphal length density, however, this may have been the result of native plant species composition (Chai et al. 2018).

The primary aim of this study is to determine the most important environmental drivers for the development of mycorrhizal relationships, and while there has been ample research on the various ecological components that affect the production of mycelium and their fruiting bodies, these effects are usually studied in the lab setting and are almost always studied alone without the direct impacts of other factors measured in the same study. Understanding these collective impacts in a natural environment can help narrow this knowledge gap, and increase our understanding of fungal growth conditions, which is important for cultivators and hunters alike. I hypothesize that fungal abundance in a Virginia mixed hardwood forest is driven by various abiotic factors, due to their effects on fungal growth. These effects were measured using a multi-model inference approach to examine the relative importance of these abiotic factors (soil moisture, light intensity, slope aspect, soil pH, tree DBH) as drivers of fungal abundance. In such, I suspect soil moisture will be identified as the most influential factor, since it has well researched impacts on mycorrhizal abundance, decomposition rates, and mushroom growth (Flegg 1974, Staddon et al. 2003, Pietikäinen et al. 2005).

Methods

Study Site:

Pandapas Pond was determined as an ideal study site for this experiment because it is primarily composed of mixed hardwood forests, which exhibit many instances of mycorrhizal relationships, due to their high levels of tree biodiversity (Bender 2018). The general soil found around Pandapas Pond, at least in the first 33 inches of depth, is channery silt loam (Soil survey staff 2021). Within the Pandapas Pond region of Jefferson National Forest, five plots identified as hills with opposing slopes were chosen as the study sites for this experiment. To best minimize variation in the dataset, the time of day and weather conditions were held constant across all data collection from 11:00 AM until 1:00 PM with mostly sunny conditions. Site A data collection began on October 15th (37°16’56”N, 80°28’19”W), site B was on October 22nd (37°17’00”N, 80°28’16”W), site C on October 29th (37°16’53”N, 80°28’10”W), site D on November 5th (37°16’79”N, 80°28’20”W), and site E on November 12th (37°16’57”N, 80°28’02”W). Each site was divided into north and south slopes, which were determined by use of a handheld compass and location via Google Maps.

Procedure:

Data collection was performed on ten deciduous hardwood trees per site, with five being observed on the north slope and five being observed on the opposing south slope, resulting in a total of 50 individuals. Sites were identified by finding a hill with opposing slopes that ran in the same direction as the north-south line of a compass. Data was first collected on the north slope and then proceeded to the south slope once finished. Individual trees were chosen while sweeping from left to right across a slope, and were selected to show variance in fungal abundance. For instance, when trying to determine the trees to observe, some emphasis would be placed on individuals with seemingly greater levels of visible fungal presence if there were already multiple trials with low measured fungal abundance. Fungal abundance was measured for each individual by counting the number of mushrooms growing within four feet of the tree base. Four potential predictors were also measured at each identified individual consisting of: light intensity, soil moisture content, soil pH, and tree diameter at breast height. Light intensity, soil moisture, and pH were measured by placing a Vakdon Upgraded 5-in-1 Soil Tester in the soil as close to the base of the tree as possible, while bypassing the tree's roots. The meter was always placed on the side of the tree facing the bottom of its slope and was submerged until the head of the meter was touching the ground. Each reading was allowed to adjust for approximately five minutes or until the reading went static. Leaf litter and other organic matter present on the top of forest soil was moved aside prior to placing the multimeter in the ground, as this adds moisture to the reading not present in the soil itself. The tree DBH was measured by wrapping diameter tape (D-tape) around the bole of the tree at a height of 4.5 feet. Finally, the species of each observed individual was also noted for future discussion.

Analysis:

Data analysis for this study was performed using R version 4.1.1 (R Core Programming Team 2021). First, a generalized linear mixed model was run using the lme4 package (Bates et al. 2015) with a poisson distribution to test the correlation between our non-normal predictors and fungal abundance. Fungal abundance was the response variable, soil moisture, soil pH, light intensity, slope aspect, and tree DBH were the fixed effects, and site was a random effect. A multi-model inference approach was used to identify the key drivers of fungal abundance using the MuMIN package (Bartoń 2020). Using the "dredge function," model selection was performed across all possible model subsets and ranked using Akaike's Information Criterion (AICc) based on maximum likelihood. Only the null hypothesis and models with ΔAICc < 10 were retained for the purposes of this study. Model averaging was performed based on Akaike weights for all retained subsets to obtain parameter estimates and statistical support. The significance of individual predictor variables was determined using p-values. Additional analyses were performed, one of which was a linear regression using the lme4 package (Bates et al. 2015) with a poisson distribution to test the effects of soil moisture individually on fungal abundance since it was believed to be the most key driver. Finally, a generalized linear hypothesis test was performed using the “Tukey method” under the multcomp package (Hothorn et al. 2021) to test the pairwise differences among light intensities.

Results

Throughout data collection, a total of 252 mushrooms were found. Site A showed the highest counts of fungal abundance, with a maximum of 19 and a mean of 7, while sites D and E showed the lowest counts of fungal abundances with means of about 3 indicating that the random effects of the site would need to be controlled for in additional models (Figure 1).

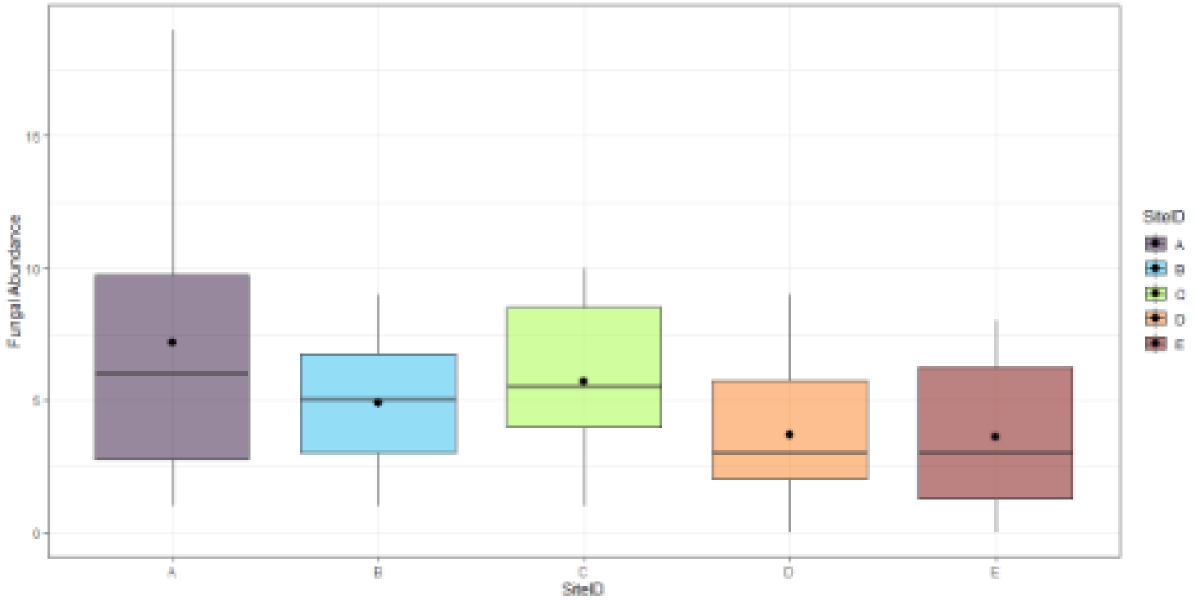

Figure 1. Fungal Abundance across five forest sites of Pandapas Pond in Virginia, where N = 252. Figure 1 shows the differences in fungal abundance between all sites, where the vertical lines represent total range and the boxes represent interquartile range. Mean and median are also displayed by the dots and horizontal lines, respectively.

The model that ranked best from the dredge analysis included soil moisture and light intensity as the most influential predictors of fungal abundance. The model ranking second, which is nearly as well supported as the first (dAIC = 0.07) includes soil moisture, light intensity, and land aspect as the most influential predictors of fungal abundance (Table 2). The six models with dAIC values between 2 and 10 contained various combinations of tree DBH, land aspect, light intensity, soil pH, and soil moisture. The null hypothesis ranked 26th out of 32 models and was much less supported than the various combinations of predictors in higher ranking models (dAIC = 44.01 for comparison between null model and top model) (Table 2).

Table 1. Model selection table including candidate set of top models with dAICc < 10 and null hypothesis.

Model Ranking	Tree DBH	Land Aspect	Light Intensity	Soil pH	Soil Moisture	ΔAICc	Weight
1			positive		0.07718	0.00	0.305
2		positive	positive		0.09519	0.07	0.296
3	-0.00707		positive		0.07607	2.46	0.089
4			positive	0.10040	0.07809	2.49	0.088
5	-0.01060	positive	positive		0.09429	2.50	0.087
6		positive	positive	0.16302	0.09711	2.55	0.085
7	-0.00674		positive	0.09366	0.07694	5.10	0.024
8	-0.01022	positive	positive	0.15587	0.09612	5.14	0.023
26						44.01	8.47E-11

Low light intensity and soil moisture content are the only two predictors determined to be statistically significant (p = 0.000234 and p = 0.000627, respectively). Even though land aspect participated in the second best model (virtually identical to the first) for predicting fungal abundance, neither north-facing slopes or south-facing slopes were significant predictors on their own (p = 0.118261 and p = 0.463530). Tree DBH (p = 0.671917) and soil pH (p = 0.708245) were not significant predictors of fungal abundance.

Table 2. Estimated effects of predictors (light intensity, soil moisture, slope aspect, tree DBH, and soil pH) on fungal abundance based on multimodal averaging across a candidate data set consisting of top model (dAICc < 10). Table contains parameter estimates, standard error (SE), z-values, and p-values for each predictor.

Factor	Estimate	Std. Error	z value	Pr(>|z|)
Low Light	0.734036	0.199524	3.679	0.000234*
Norm Light	0.292726	0.209283	1.399	0.161901

Soil Moisture	0.086137	0.025190	3.420	0.000627*
N Aspect	-0.240572	0.154004	1.562	0.118261
S Aspect	-0.118547	0.161719	0.733	0.463530
DBH	-0.008742	0.020642	0.424	0.671917
pH	0.129805	0.346872	0.374	0.708245

* Significant ($P \leq 0.05$)

Linear regression analysis shows that soil moisture content positively affects fungal abundance (R-squared = 0.495 and p = 2.54E-4)(Figure 2). This effect is relevant to a soil moisture range between 30 and 70 percent. One outlier in the data (abundance = 19) was left and may have contributed to the extremely low p-value obtained from this relationship.

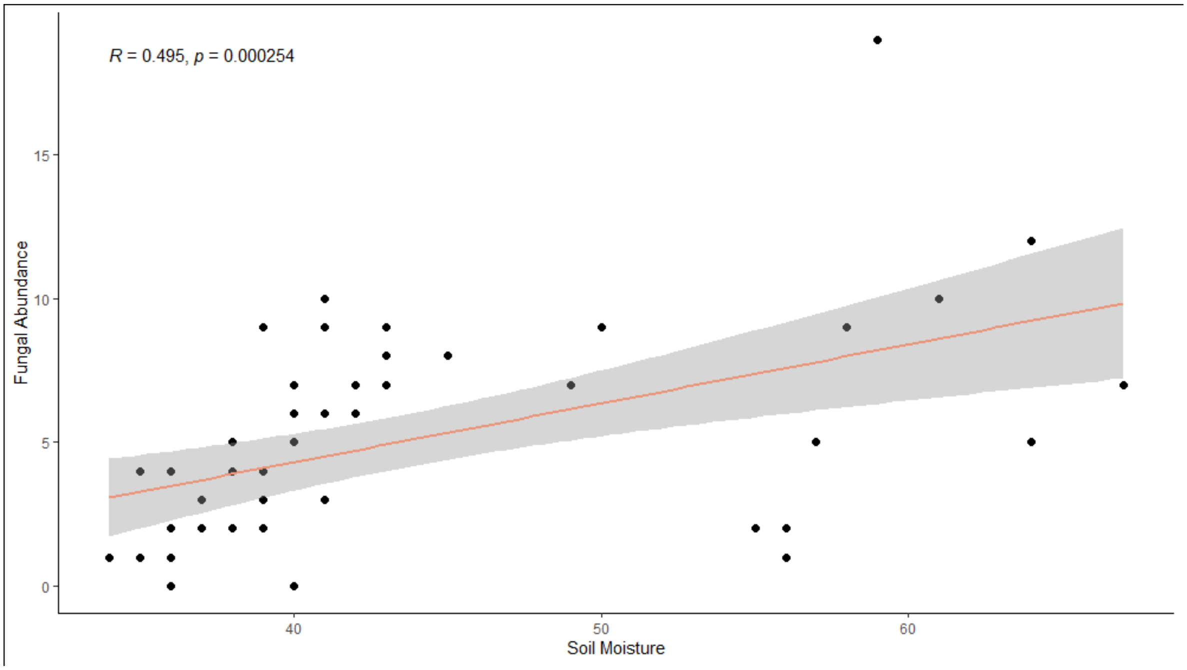

Figure 2. Soil moisture content (percent) effects on fungal abundance (number of fruiting bodies per tree). The trendline shows the results from a linear regression analysis, where the shaded area represents a 95% confidence interval.

The differences between normal light intensity and high light intensity weren't statistically different (p = 0.41669), while differences between low light intensity and high light intensity ($p < 0.001$) were very highly significant and differences between low light intensity and normal light intensity ($p = 0.00141$) were highly significant.

Table 3. Pairwise differences between the categorical measurements of light intensity using the Tukey method. Table contains parameter estimates, standard error (SE), z-values, and p-values for each pair.

Linear Hypotheses	Estimate	Std. Error	z value	Pr(>|z|)
low-high	0.8174	0.1901	4.299	< 0.0001 ***
norm-high	0.3004	0.2040	1.473	0.185859
norm-low	-0.5170	0.1447	-3.574	0.000844 ***

Significant Codes: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Discussion

This study was conducted with the goal of identifying key environmental drivers for the production and congregation of fungi, with the hopes of providing understanding of fungal growth conditions in a natural environment. When accounting for site-to-site variation, two models were determined as statistically significant which consisted of low light intensity, soil moisture, and land aspect. Upon model averaging, however, only soil moisture and low light intensity returned were determined to be significant predictors of fungal abundance. Therefore, low light intensity and soil moisture were the key determinants of mushroom abundance (in that order). Because these environmental drivers were ranked much higher than the null hypothesis and were determined to be significant, my hypothesis was supported that abiotic factors (light intensity and soil moisture) were key driving forces of fungal abundance. My prediction was incorrect, however, as I believed that soil moisture would be the most significant factor, when in fact it was low light intensity.

These results for soil moisture were in line with many previous studies, which suggested that the production of fruiting bodies (Flegg 1974) and decomposition rates increase with soil moisture (Pietikäinen et al. 2005). This would explain why fungal abundance tended to increase with increasing moisture content. Other studies which examined the ideal moisture content ranges (Deepika and Kothamasi 2015, Iotti et al. 2018) were contradicted by the results of this study, as fungal abundance was found to continue to increase between moisture contents of 30-70% (Figure 2). One possible explanation for these differences was that results from the above studies were found under laboratory settings, where moisture content was directly modified through experimentation, whereas our study was observational in nature and performed in natural conditions. Also, these results were found when measuring fungal growth and not fungal abundance. The findings of this study regarding light intensity's effect on the production of mushrooms were very much akin to other studies which measured the effects of light intensity on mycelial growth and total root colonization, both of which increase with decreasing light levels (Gehring 2002). This could provide some evidence that the abundance of fruiting bodies does have some correlation to the abundance of mycorrhizae, although more research would need to be done to affirm this connection. While land aspect was not statistically significant on its own, I believe that its inclusion into the second best model, which was hardly different from the best model (delta = 0.07, Table 2), had to do with the effects that land aspect has on soil moisture and light intensity. Northern facing slopes tend to retain more moisture, while southern facing slopes tend to be xeric and receive much more sunlight (Måren et al. 2015). Thus, land aspect is only recorded as an influential factor during model selection because of the differences in the two primary factors associated with fungal growth.

The site-to-site variation included in data analysis of this study more than likely resulted from temporal differences that occurred from site A to site E. The increasing accumulation of leaf litter as a result of late fall made this method of measuring mushroom abundance much more difficult. Future studies should either choose a more scientific approach to measuring mycorrhizal abundance (i.e. specifically measure fungal root colonization since the end goal is the measure of mycorrhizal relationships), or choose a more prominent time of year for data collection. Although these changes may have had an impact on data collection towards the later trials, accounting for these differences yielded the best model in the model selection table, which holds low light intensity and soil moisture content as the primary environmental factors driving the growth of fruiting bodies.

These results provide supporting evidence for other studies on the environmental factors driving fungal growth and abundance while also introducing a novel aspect of measuring many of these factors at once in a natural environment. A good model for the key drivers of fungal abundance has been created and also adds to the knowledge base as to which environmental influences are more important for the aggregation of fruiting bodies. This information can be employed by those searching for mushrooms or those trying to identify ideal locations for future studies involving mushrooms.

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