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An Ecological Analysis of the Elevational Gradient Effect on Mushroom Community Diversity near Andasibe, Madagascar

Zoe Garver
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AN ECOLOGICAL ANALYSIS OF THE ELEVATIONAL GRADIENT EFFECT ON MUSHROOM COMMUNITY DIVERSITY NEAR ANDASIBE, MADAGASCAR

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SIT Madagascar: Biodiversity and Natural Resource Management
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Misaotra betsaka, merci, and thank you!

Abstract

The effect of elevational gradients on biodiversity has been widely studied in the field of ecology (Rahbek, 1995). The evidence supports a trend for highest biodiversity at mid latitudes and decreasing biodiversity as elevation increases (Rahbek, 1997; Grytnes, 2003; Hariharan and Buckley, 2022). This effect has primarily been explained by variation of temperatures and resource availability at different elevations. However, the effect that elevation plays on fungal communities is relatively underrepresented in the literature (Dahlberg, 2001). This study analyzes changes in mushroom diversity across an elevational gradient in Mitsinjo Reserve and Analamazaotra National Park found in Madagascar. Using the Braun-Blanquet method (1965), a total of six plots were selected along a ridgeline present in both parks. Data collection pertained to mushroom and tree communities. Metrics used for data analysis included mushroom species richness, diversity (Shannon Diversity Index (SDI)), evenness, and total tree basal area (BA) at each elevation. Regression models to test for significance were created for all metrics. An additional model was created between total BA and SDI values. Significant results were found for SDI values with an R^2 value of 0.68 and a *p*-value of 0.04. These results are evidence that elevation can be used as an indicator for SDI of mushroom communities. With this knowledge, we can develop more holistic and efficient conservation models for at-risk ecosystems.

Keywords: elevational gradient, mushroom communities, biodiversity, conservation, tree communities

Résumé

Les effets de la pente d’élévation sûr le biodiversité sont assez étudiés dans les domaines d’écologies (Rahbek, 1995). Les résultats des recherches nous montrent souvent que la biodiversité est plus diverse à des élévations moyennes et elle diminue quand ils se montent (Rahbek, 1997; Grytnes, 2003; Hariharan and Buckley, 2022). Ce phénomène est souvent expliqué par la variation des températures et des ressources aux élévations différentes. Par contre, l’effet de la pente d’élévation sûr les communautés des champignons est manqué dans les recherches. Cette étude analyse des changements de la biodiversité des champignons au lieu de la pente d’élévation a le Parc de Mitsinjo et a le Parc National d'Analamazaotra en Madagascar. Au total, il y avait six plateaux de la méthode Braun-Blanquet (1965) qui étaient divisés par les deux parcs. La collection des données est prise en relation des communautés des champignons et des arbres. Les indices pour l’analyse de chaque placeaux ont inclus la richesse, la biodiversité (Shannon Diversity Index (SDI)), l’uniformité des communautés des champignons et la surface terrière totale des arbres.. Les modèles de régressions étaient calculés pour chaque indice. Les résultats significatifs sont trouvées au lieu des SDI avec un résultat de R^2 de 0.68 et un résultat de p de 0.04. Ces résultats indiquent que la pente d’élévation peut être un indicateur pour le SDI des communautés des champignons. Avec ces résultats, nous pourrions développer des modèles de conservation plus efficaces pour le futur.

Les mots clés: la pente d’élévation, les communautés des champignons, la biodiversité, la conservation, les communautés des arbres

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1.0 Introduction

1.1 Elevational Gradient

For many years, the effect elevational gradients have on species communities has been widely studied in the field of ecology (Rahbek, 1995). Species biodiversity is a common variable that is often analyzed to determine how it responds to elevational gradients (Nijs & Roy, 2000). The literature suggests that highest levels of biodiversity for species communities can be found at midlatitudes with a general decrease in diversity as elevations increase (McCain and Grytnes, 2010). Observations of this trend have been attributed to the varying temperatures and resource availability at these elevations. At midlatitudes where temperatures are ideal and resources are abundant, a wider range of ecological niches can be filled, thus increasing biodiversity (Sundqvist *et al*., 2013). This general "humped shaped" species diversity relationship with elevation has been supported by research including Rahbek's 1997 analysis of neotropical birds. Additionally, the trend of decreased biodiversity with increased elevation has been supported by many research projects. For example, a study done in 2003 found that vascular plant species diversity decreased as elevation increased (Grytnes, 2003). This relationship can be explained by the increased species competition that occurs at higher elevations as resources become limited. In addition to flora and faunal communities, elevation has been shown to have an effect on microbe communities. A study by Hariharan and Buckley (2022) concluded that stark differences in microbe soil presence at different points along a steep mountain were due to elevational gradient effects. This large base of research has been used to expand our knowledge and understanding about the effect elevation has on ecosystems. However, despite all this research, there still exists a gap in the literature pertaining to the effect on mycological communities.

Among the limited mycological research associated with ecology, intriguing evidence has been found. A study analyzing the general effects of environmental factors on macrofungal communities found that direct habitat requirements (species present, soil, etc.) are stronger predictors for mushroom community health compared to surrounding habitat fragmentation (Brown *et al.*, 2006). This study made it evident that environmental factors such as elevation could play a big role in macrofungal community makeup. In relation to this question, a study conducted by Siles and Marges in 2016 found a positive correlation between elevation and macrofungal community diversity (Siles & Margesin, 2016). This positive correlation was attributed to the higher concentrations of mineral nutrients found in the soil as elevations increased. These results are intriguing because they do not support the midlatitude and high elevation effects on species diversity outlined in the previous paragraph. Further research in this field is essential to expand mycological knowledge and understanding of environmental factors such as elevation on species communities. This study aims to address this issue by analyzing how elevational gradients affect mushroom community diversity.

1.2 Mushroom Life Strategies

Mycology is the study of fungi and has been significantly underrepresented in research pertaining to ecology (Dahlberg, 2001). The most commonly researched topics regarding mycology concern their many health benefits (Kalač, 2013). Despite their underrepresentation in research, fungi play major roles in our society. Their large presence in many food economies has made them economic advantages for communities around the world (Mapook *et al*. 2022). While it's clear that fungi play an important role in our social lives, the attention to their ecological role is limited.

Similar to all organisms, mushroom life strategies have largely been shaped by their environmental conditions. Fungi species are diverse both in their form and ecological roles (Dias and de Brito, 2017). The three main divisions of fungi include saprotrophic fungi, ectomycorrhizal fungi, and endoparasitic fungi.

Saprotrophic Fungi

Saprotrophs are organisms that receive their nutrients from dead organic matter and are commonly found on dead wood and fallen leaves (Pirot, 2006). This fungi plays a vital role in decomposition and nutrient cycling in the environment. They form complex networks above ground which enable them to control available nutrient levels for other organisms in their environment (Boddy, 1993). Without them, ecosystems would not be as efficient in nutrient cycling.

Ectomycorrhizal Fungi

Ectomycorrhizal fungi receive their nutrients through symbiotic relationships that they form with surrounding vegetation (Pirot, 2006). These relationships are often mutually beneficial because the plant receives nutrients from the fungi and the fungi receives glucose from the plant (Harley, 1971). They are more difficult to see compared to saprotrophic fungi because they carry out the majority of their life cycle underground (Pirot, 2006). This fungi is very beneficial to the health of an ecosystem because of the essential mutualistic relationships it can form with other organisms (Pickles & Simard, 2017).

Endoparasitic Fungi

Endoparasitic fungi receive their nutrients from other living organisms by forming parasitic relationships through their spores (Liu *et al.*, 2009). This sounds problematic for the living organism, but endoparasitic fungi play an important role in natural selection by attacking already compromised organisms (sick, old, etc.). They are not as common to see compared to saprotrophs and mycelium (Pirot 2006).

1.3 Ecological Importance

As outlined above, fungi play many important roles in the environment primarily by acting as nature's decomposers (Dias and de Brito, 2017). They are essential to the health and resiliency of ecosystems. Studies have found that the symbiotic relationship ectomycorrhizal fungi form with forest communities and the presence of specific species have been found to be important indicators for promoting forest health (Harley, 1971). This is achieved through mycelium networks which attach themselves to tree roots underground. The mycelium converts soil nutrients to readily available forms for root uptake, while the roots provide the mycelium with necessary glucose levels for survival. Without this relationship, forest communities are not as resilient (Pickles & Simard, 2017). Due to the limited research in mycology, there are still many different beneficial relationships such as this one that remain unknown to science. Conducting controlled studies will allow us to increase our knowledge of what fungal networks are present in ecosystems and how we can use them to develop effective conservation models.

The incorporation of fungi into habitat restoration and conservation projects have become more common over the past few years (Heilmann−Clausen *et al.*, 2015). This is primarily because of the valuable ecosystem services fungi provide and the increased implementation of holistic approaches to conservation biology. Mycological studies have shown that higher species diversity correlates with increased efficiency in ecosystem functionality (Abdel-Azeem, 2010). Increasing our understanding about how mushroom species react to environmental factors will allow us to incorporate fungi into efficient conservation models.

1.4 Mushroom Research in Madagascar

Madagascar is one of the most distinct biodiversity hotspots in the world and yet there is very little research that has been done in the field of mycology (Ganzhorn *et al.*, 2001). Studies that have been conducted in the country are limited to the extent that many fungi species have yet to be discovered. The existing research primarily covers inventory of the species diversity in the forests surrounding the village of Andasibe which is located in the eastern part of Madagascar (Dumetz 1999; Buyck 2008). A study done by Rivas-Ferreiro *et al.* in 2023 conducted an ecological survey of sporocarps and ectomycorrhizal roots to find that 60% of all fungi and 81% of the ectomycorrhizal samples were endemic to Madagascar. From these studies, it's clear that there is still a lot to be learned about mycological communities in Madagascar. This study aims to expand our knowledge by analyzing how environmental factors influence mushroom species diversity.

1.5 Research Objectives

This research project aims to analyze how environmental factors impact macrofungal community diversity in the surrounding area of Andasibe, Madagascar. Current research has shown that elevations at midlatitudes support higher biodiversity while higher elevations do not. However, the limited research that exists pertaining to fungi counters this claim and has shown that fungal species diversity is positively correlated with elevation. Given the current research, mushroom life strategies, and the complex relationships they form with tree communities, this study aims to provide evidence that will increase our understanding of how macrofungal communities behave in the environment. To test this aim calculations of species richness, diversity, and evenness will be done for six different elevations. Additionally, this study will analyze the relationship between mushroom species diversity and tree density at different elevations to observe how forest communities are related to mushroom communities. The primary research objective of this study is to analyze the differences in mushroom communities across an elevational gradient in the secondary forest near Andasibe, Madagascar to improve knowledge for conservation models and increase our understanding of mycological communities.

2.0 Methodology

2.1 Study Site

The study site for this research project can be found near Andasibe, Madagascar in the Analamazaotra National Park and Mitsinjo Park Reserve. These organizations are located in the central eastern part of Madagascar at a relative elevation of around 950 meters (m) in a tropical rainforest climate (Association Mitsinjo, 2024; Andasibe, Toamasina, Madagascar Climate, 2024). The landscape is composed of a secondary, evergreen forest which contains many endemic flora and fauna species (Wild Madagascar). Mitsinjo Reserve encompasses 1,172 hectares (ha) of protected forest and Analamazaotra Park encompasses 874 ha (Association Mitsinjo, 2024; Protected Areas, 2024). Tourism is popular in these parks among international and local visitors because of its biodiversity which includes twelve lemur species and numerous species of amphibians, reptiles, and birds.

Figure 1. Locality of Andasibe in the central eastern part of Madagascar

Field work was conducted in this area during the month of April (April 9th to April 21st) which is near the end of Madagascar's wet season making it one of the wettest months of the year. Looking at historical data for Andasibe's April climate, the area typically receives an average precipitation of 225.22 mm of rain and has average temperature highs of 28.86°C with lows of 25.48°C (Andasibe, Toamasina, Madagascar Climate, 2024). This is an ideal time of year to conduct mycological research because of its high levels of rain. Field data was collected in nine days over the course of thirteen days (four days off due to scheduling conflicts). Rainfall and daily temperatures for these days can be found in the results section.

2.2 Study Plots

The study sites in Mitsinjo Reserve and Analamazaotra National Park were selected for a number of different reasons. First, there is very limited existing literature on mycological research in Madagascar. However, the studies of these projects have been carried out in the surrounding areas of Mitsinjo Reserve. By choosing the same location, current and future researchers will be able to cross reference data with hopes of expanding our understanding and knowledge of mycological communities in Madagascar. Second, this location was selected due to the presence of a guide, Christin Nasoavina, who is one of the few guides in Madagascar who specializes in local mushroom identification. Working with him was essential to increase confidence in species identification throughout research. Finally, the third main reason for why this location was picked is due to the ideal elevational conditions present in the area. The elevational gradient of the forest ranges from 900-1,050m and contains numerous ridge lines. Study plots were selected in both parks because the elevational lows and highs of an ideal ridgeline were separated by the parks. Otherwise, all plots would've been selected in one park to control for differences in environmental management factors. Given existing research, resources, and environmental conditions, these two parks presented themselves as the most ideal locations to carry out a research project analyzing differences in mycological communities across an elevational gradient.

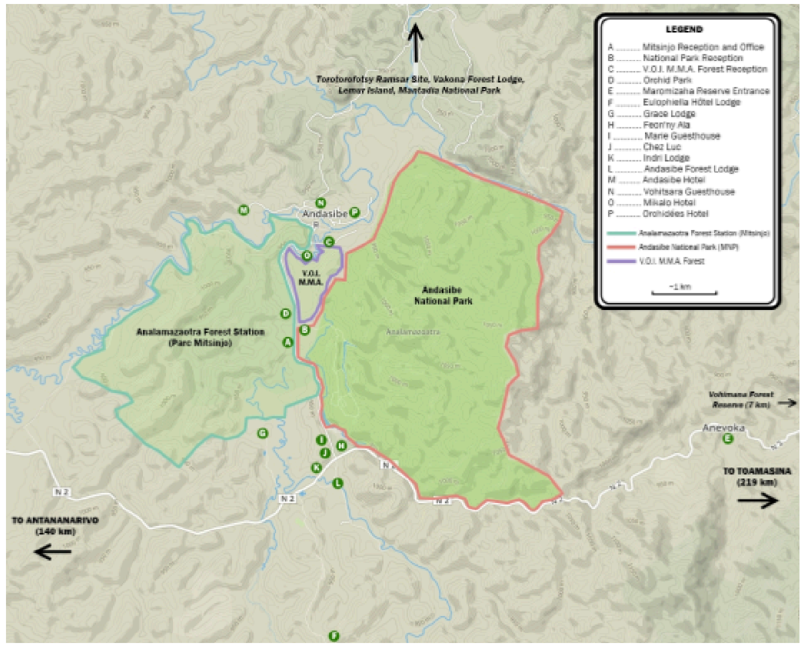

Figure 2. Park divisions in Andasibe. Mitsinjo Reserve is outlined in blue on the left and Analamazoatra is outlined in red on the right. A small, locally run park is outlined in purple.

Within the study area, six 50x20m plots were selected based on their elevational positioning. Prior to going into the field, general locations were selected along the ridgeline that was divided by park boundaries from ~930m to ~1030m in elevation. The specific plot locations were decided upon in the field using a handheld Garmin ETREX 32x GPS and reading its elevational outputs. The six plots were recorded at elevations of 937.5m, 940.5m, 973.0m, 993.0m, 995.5m, and 1002.5m. These values were calculated by taking the average of the two GPS points recorded at each plot (north and south ends). This method was used to account for variation in elevation within plots. For example, at the lowest plot, the GPS points read 937m and 938m which gives an average of 937.5m. The coordinates and elevations of all points were recorded using the GPS in the field and then verified using Google Earth after field work (Google Earth, 2024). There were two plots located in Mitsinjo Reserve (two lowest) and four plots in Analamazaotra National Park.

Given that the variable being analyzed in this study was elevation, it was important that all other variables pertaining to the plots were held relatively constant. All plots were east facing, oriented from south to north, and had inclines ranging from 0°-15°. Comparison between all six plots was possible because forest environments were homogenous. After the general area for a plot was decided upon, a 50x20m plot was set up.

The formation of each plot followed the Braun-Blanquet method (Braun-Blanquet, 1965). This method was used to record an accurate representation of the mushroom and forest communities at each elevation mentioned in the previous paragraph. To create one plot, first, a 50m rope was set up from south to north with GPS coordinates being taken at 0m and 50m. Next, a 20m rope was set up perpendicular to the 50m rope at 10 meter increments. The 20m rope extended 10m to both sides of the 50m rope. The ropes created rough boundaries for the entire plot and rough boundaries for the ten, 10x10m placettes formed within the larger plot. See Figure 3 for visualization. It's important to note that ropes were not set up along the 50m outside boundaries to prevent excessive trampling of biodiversity in the area. After the ropes were set up, data was collected for tree and mushroom communities. Data was recorded by placette, working from placette 1 to placette 10.

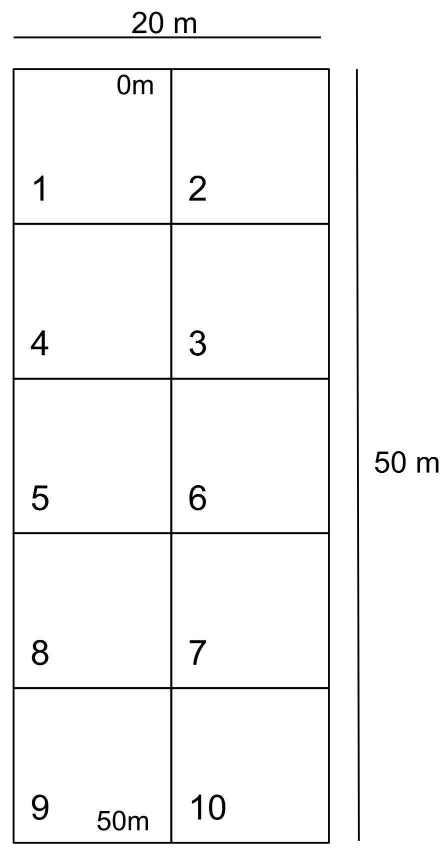

Figure 3. Visualization of Braun-Blanquet Plot. Coordinates were taken at 0m and 50m.

2.3 Data Collection

Within each plot, data collection pertaining to tree composition included diameter at breast height (DBH) and estimated height of trees greater than 10cm in diameter. After all large trees had been recorded, data pertaining to the mushroom community was collected which included each sample's growth matter, description, and species identification to the genus level (when possible). A minimum of 20 minutes was spent per 10x10m placette to look for all visible mushroom samples. This minimum was selected to allow enough time to diligently walk each placette to look for mushrooms. There was no maximum limit because time spent identifying any given individual varied in each placette. Unknown and unidentifiable species were taken note of with a picture and general description. Post-field work identification was done when possible using the internet, or marked as NI (not identifiable) when identification was not possible. Species that weren't identifiable to genus level were recorded to the highest taxonomic level and marked as NA for all other unknown levels.

Mushroom Identification

In mycological research, mushroom identification is achieved through a combination of DNA sequencing, spore printing, and analysis of visual characteristics (Rivas-Ferreiro *et al.*, 2023). Given the limited resources available in this study, mushroom identification was achieved only by taking physical characteristics into account. In addition to certainty in Nasoavina's mushroom identification, many identification keys were used during field work. These included *Champignons comestibles des forêts denses d'Afrique centrale: Taxonomie et identification* by Hugues Eyi Ndong, Jérôme Degreef, and André De Kesel, and *Olatra: Champignons d'Andasibe (Madagascar)* by Paul Pirot. In conjunction with consultation of these books, analysis of the following physical characteristics were used to achieve species identification of each sample.

The following descriptions of mushroom characteristics were derived from *General Aspects of Mushroom Fungi* (Denchev *et al.*, 2013). These characteristics were intensively studied for each sample during data collection.

Size, Color, and Texture

Fungi come in many different sizes, colors, and textures which are helpful in species identification. Colors can range from dark brown to bright yellow. Sizes can range from microscopic to as big as a hand (in Madagascar). Common textures include shiny, matte, dry, wet, sticky, scaly, velvety, powdered, veined, wrinkled, rugged, and uneven. Taking all three of these variables into consideration helps with field species identification.

Shape: Pileus and stipe

The pileus is more commonly known as the cap and the stipe is more commonly known as the foot or stem. These structures are visible above the substrate layer and make up what's often called the "fruiting body" of fungi. There are many different shapes that they can form and are useful for species identification. The different shapes include pileus and stipe, bracket or fan, lobed, trumpetlike, pear, star, phallic, cup or disk, club, and coral shape. Within these different shapes, the pileus and stripe are connected in different orientations. Different attachment orientations include central, eccentric, lateral, or stripless (pileus emerges directly from substrate). Pileus structure can take on many different forms including convex, hemispherical, spherical, ovoid, conical, cylindric, flat, depressed, funnel shaped, campanulate, umbonate, umbilicate, and uplifted.

Hymenophore

The hymenophore is the structure of a mushroom that contains its spores. The most common structures that make up the hymenophore are gills, tubes, spines, or ridges. The attachment of gills can be a good indicator of species classification. Common differences in gill attachments include free (do not connect to stripe), not free (connect to stripe), and decurrent (runs down top of stripe). Analyzing the hymenophore is often an essential determinant of species identification.

Ring and Volva

Additional structures that are useful for infield identification include the ring and/or volva. The ring is a remnant of the partial veil which is a layer of tissue and can be found around the stripe. The volva is a layer of tissue that encloses the mushroom and is often found at the base of the stripe.

2.4 Data Analysis

The three sections of analysis included climate data, mushroom community data, and forest community data. Raw data was noted with pen and paper in the field. After field work was completed, raw data was entered into Microsoft Excel and then transferred over to RStudio to carry out data analysis. Climate data organized daily temperatures and rainfall of Andasibe. For mushroom community analysis, calculations were performed to obtain species richness, Shannon Diversity Index (SDI) (index chosen to measure species diversity), and species evenness at each elevation. When species richness was calculated, NA and NI values were left out to control for potential unknown data. To test for significant differences between elevations, a linear regression model was run on all data metrics. For tree community analysis, total basal area (BA) was calculated for each plot. An additional regression model was run to observe the relationship between SDI and total BA for each elevation. Finally, plots were generated to visualize all data.

3.0 Results

3.1 Climate Data

Temperature and precipitation in Andasibe varied during the field collection dates from April 9th to April 21st. Figure 4 displays the daily high, low, and average temperatures for each day. Table 1 describes precipitation observations for each day.

Table 1. Daily Precipitation Data showing daily precipitation observations

Date	Precipitation
9-Apr	No rain; last rain 4 days before
10-Apr	No rain; sunny
11-Apr	No rain; partly cloudy
12-Apr	No rain; sunny
13-Apr	Rain night before; partly cloudy
14-Apr	No rain; partly cloudy
15-Apr	Rain
16-Apr	Rain
17-Apr	Rain night before; partly cloudy
18-Apr	No rain; partly cloudy
19-Apr	No rain; sunny
20-Apr	No rain; sunny
21-Apr	No rain; sunny

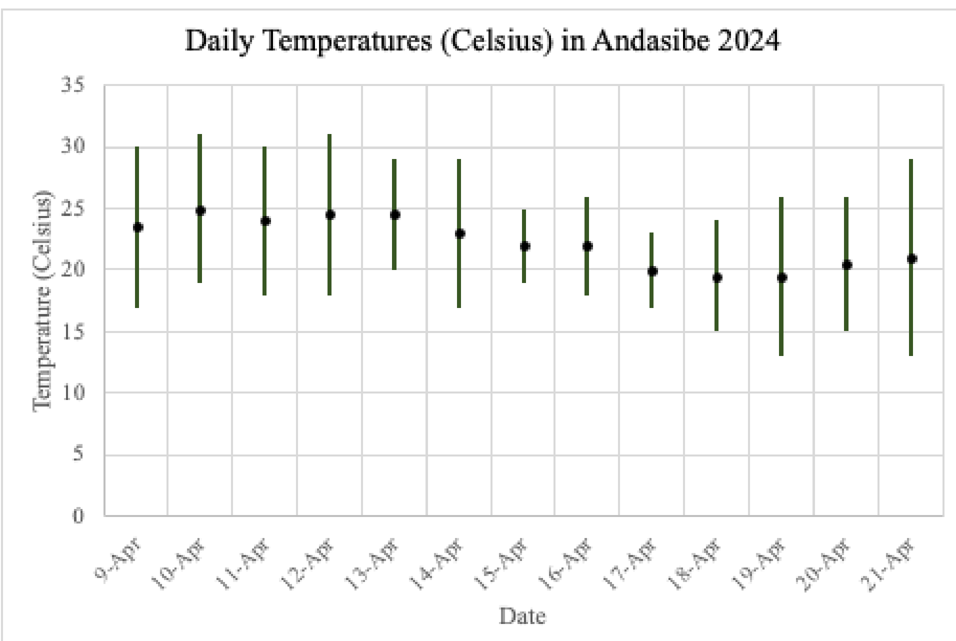

Figure 4. Daily Temperature Data showing daily highs, lows, and averages in degrees Celsius. Data collection occurred on April 9th-13th, 16th-18th, and 21st.

3.2 Mushroom Communities

There were a total number of 1,612 samples recorded across all six plots. Of these 1,613 samples, there were 2 phylum, 5 classes, 12 orders, 31 families, and 40 genus that were observed. Samples were not identified to the species level due to high levels of uncertainty and limited time. The variables that were calculated included species richness, diversity, and evenness of mushroom communities at each elevation. Note that throughout the analysis section, terms that include "species" are referring to genus level classification. For the collected tree data, total basal area (BA) was calculated at each elevation. Regression models were run for all metrics to test for significance. An additional regression line was created between SDI and total BA to test for the relationship between mushroom and tree communities. All calculations were carried out in RStudio.

3.2.1 Species Richness

Species richness measures the number of species present in a given location (Peet, 1974). It's a good metric to use in ecological research to gain an initial sense for what the species makeup looks like for an area. In this study, species richness of mushrooms was calculated for each plot to observe changes across elevation. Values for species richness were obtained by counting the number of genus present in each plot (see Table 2). Within the data set, there were some samples that could not be identified (NI) or could not be identified down to the genus level (NA). These data entries were not included in the species richness calculation because they couldn't be confidently categorized into an existing genus or trusted to count as an entirely separate genus. Highest species richness was found in plots at elevations 937.5m and 993 m with a total of 23 genus observations. The plot at 995.5m elevation had the lowest species richness of 18 genus observations. Figure 5 shows the species richness values plotted against elevation. While there are differences in the species richness values, there were no significant correlations obtained from the regression model.

Table 2. Species Richness Values at each elevation

Species Richness Values	
Elevation (m)	**Species Richness**
937.5	23
940.5	19
973.0	21
993.0	23
995.5	18
1002.5	20

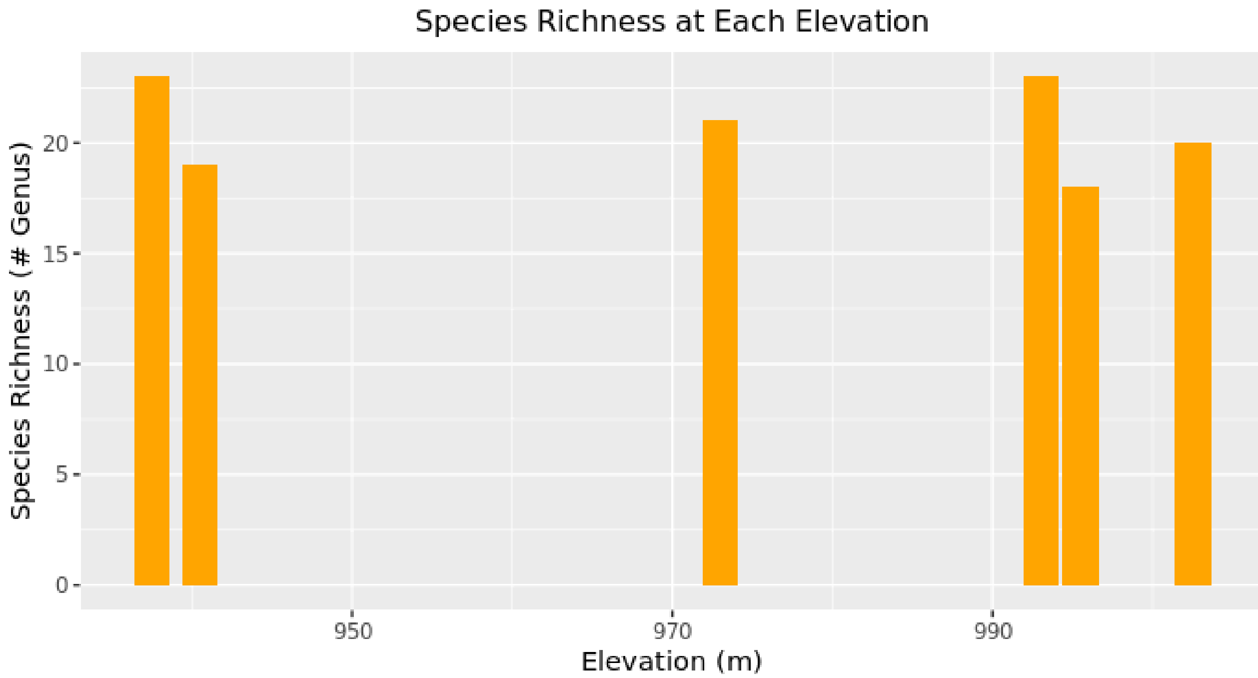

Figure 5. Species Richness at Each Elevation (m). Variation in the data exists, but no significant trends were found.

3.2.2 Species Diversity (Shannon Diversity Index (H))

To determine species diversity in this study, SDI was used. This index is commonly used in ecological studies to determine species diversity because it assumes samples have been taken at random from a large population (Shannon, 1948). The metric uses species richness and relative abundance to calculate a diversity index. The equation is:

$$H = -\sum_{i=1}^{S} p_i ln(p_i)$$

$H = Shannon\ Index$

$S = Total\ Number\ of\ Species$

$p_i = relative\ abundance\ of\ the\ i^{th}\ species$

A small SDI value indicates low species diversity. Similarly, a large SDI value indicates greater species diversity. Shannon indices calculated from the mushroom data set varied from the lowest value of 1.490 at elevation 1002.5m to the highest value of 1.990 at elevation 940.5m All SDI values are shown in Table 3. The SDI values were plotted against elevation as shown in Figure 6. There is a general trend of decreasing Shannon indices as elevation increases. To test the significance of this inverse relationship, a regression model was created between the two variables (Figure 7). The test found an R^2 value of 0.6809 with a significant p-value of 0.0432.

Table 3. Shannon Index Values at each elevation

Shannon Index Values	
Elevation (m)	**Shannon Index**
937.5	1.930
940.5	1.990
973.0	1.591
993.0	1.589
995.5	1.791
1002.5	1.490

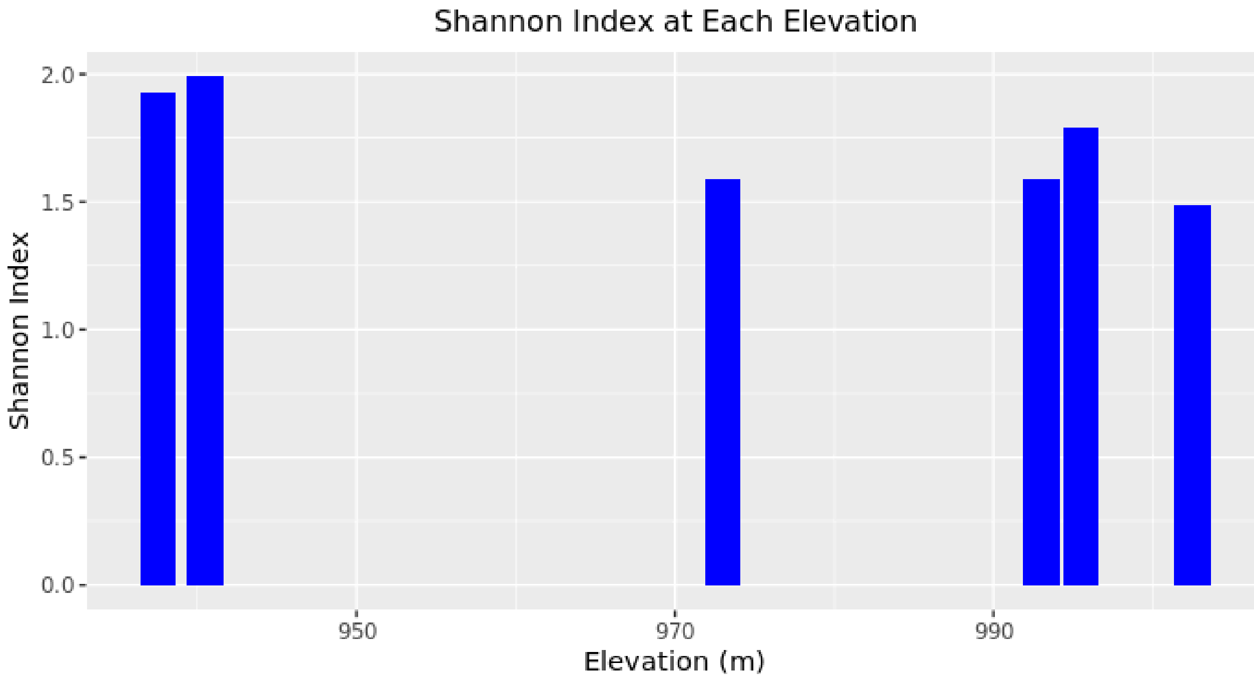

Figure 6. Shannon Index at Each Elevation (m). This plot shows a relative inverse relationship between elevation and SDI. A regression model is shown in Figure 6.

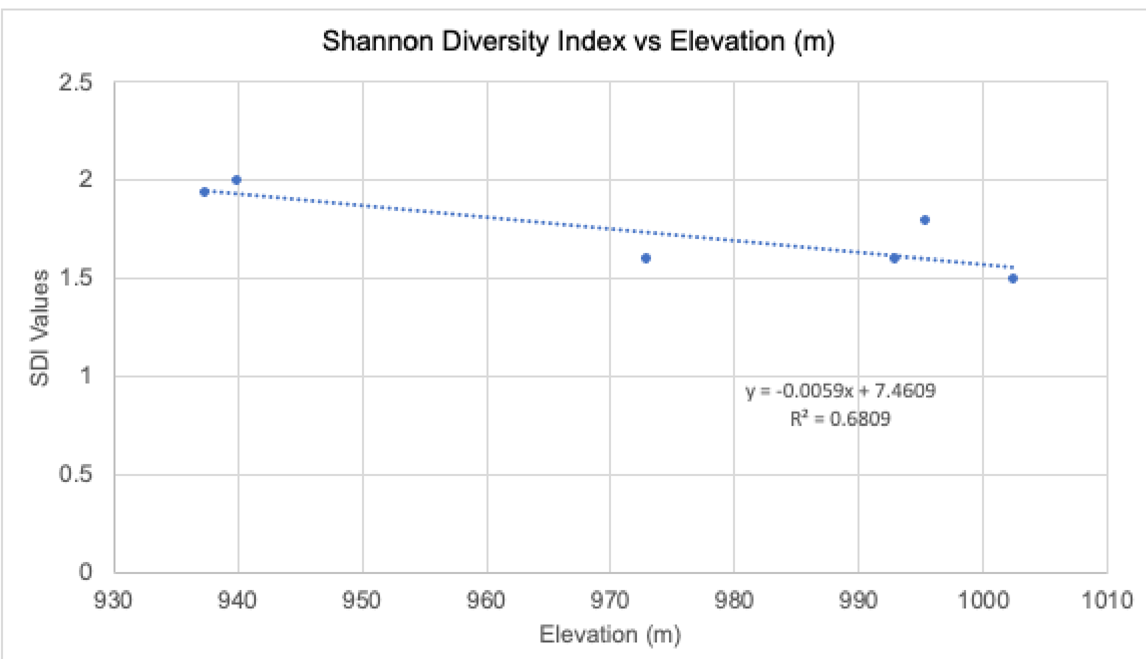

Figure 7. Shannon Index vs Elevation (m) Regression. The correlation obtained an R^2 value of 0.6809 with a significant *p*-value of 0.0432. These results show that elevation is a significant indicator of SDI.

3.2.3 Species Evenness (E)

Species evenness is a measure of how evenly proportioned species are in a given location (Wilsey & Potvin, 2000). This metric is commonly used in ecological research to reveal if there are any dominant or rare species in a community (Moore & Brodie, 2023). The equation for calculating species evenness is:

$$E = Shannon\ Index / Hmax$$

$$Hmax = ln(species\ richness)$$

Species evenness values can vary from 0 to 1. An evenness value of 1 indicates maximum evenness within a community. On the other hand, an evenness value that approaches 0 indicates that there is low evenness and most likely has dominant and/or rare species present within the community. The species evenness values calculated from the data set ranged from the lowest value of 0.497 at 1002.5m to the largest value of 0.676 at 940.5m. These values were plotted against elevation (Figure 8). There is a relative trend in the data that as elevation increases, species evenness decreases. A regression model was created between these two variables and found no significant values.

Table 4. Species Evenness Values at each elevation

Species Evenness Values	
Elevation (m)	**Evenness**
937.5	0.615
940.5	0.676
973.0	0.523
993.0	0.507
995.5	0.620
1002.5	0.497

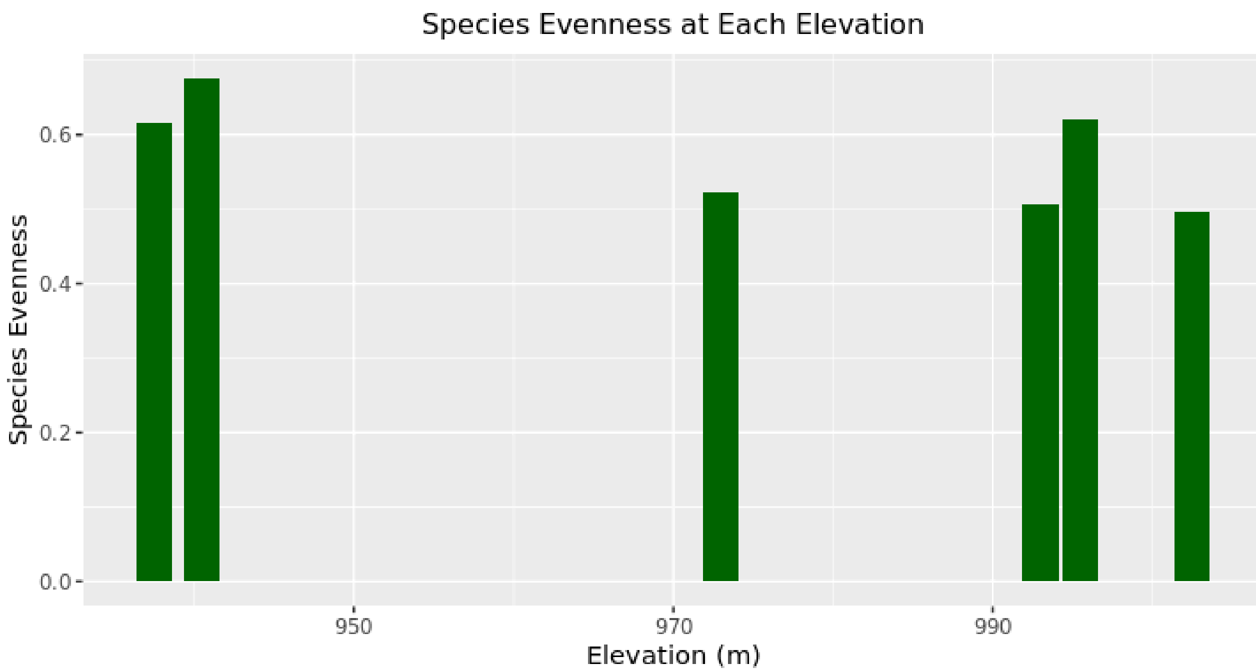

Figure 8. Species Evenness at Each Elevation (m). There was some variation in the data and a small inverse relationship exists (insignificant).

3.3 Forest Communities

3.3.1 Total Basal Area (BA)

Basal area is a metric used primarily in forest ecological studies to calculate how much area a tree takes up in any given location (Contreras 2011). It's a good indicator of how dense a forest community is or how abundant tree species are in an area. The equation for this constant is:

$$BA = pi((DBH/2)^2)$$

$pi = 3.142$

$DBH = diameter\ at\ breast\ height\ (cm)$

BA of all trees in each plot was summed to find the total basal area at each elevation. To convert this result to meters squared, the result was divided by 10,000. Total BA values ranged from 2.101 m^2 at 937.5 m to 4.169 m^2 at 993.0 m elevation. Figure 9 shows total BA values plotted against elevation. There is a slight trend of increasing total BA as elevation increases but no significant trends were found.

Table 5. Total Basal Area Values at each elevation

Total Basal Area Values	
Elevation (m)	**Total BA (m2)**
937.5	2.101
940.5	2.678
973.0	2.835
993.[illegible]	4.169
995.5	2.529
1002.5	3.816

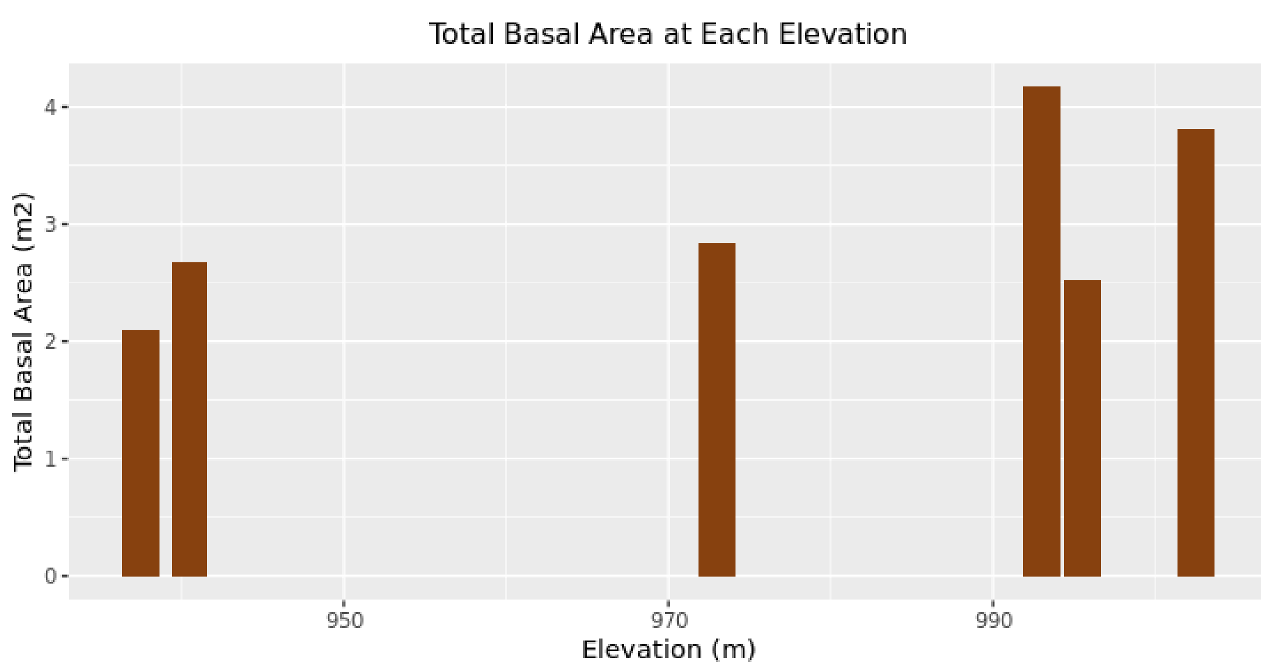

Figure 9. Total Basal Area at Each Elevation (m). There was some variation in the data and a small direct relationship exists (insignificant).

3.4 Mushroom vs Forest Communities

To analyze the relationship between mushroom communities and forest density, a scatter plot and regression analysis was created between SDI and total BA at each elevation. Figure 10 shows this correlation. A general trend can be seen that as total BA increases, SDI decreases. The regression equation supports this trend with a slope of -0.1974*x*. The regression analysis obtained a R^2 value of 0.5971 with an insignificant *p*-value of 0.0716 (Figure 10).

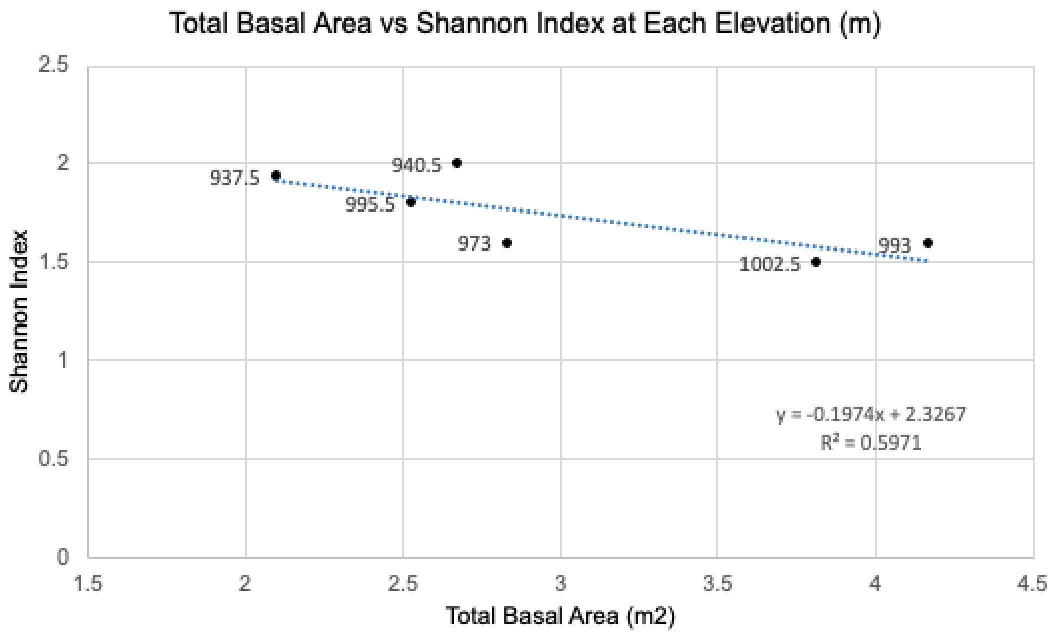

Figure 10. Total Basal Area vs Shannon Index Regression. The correlation obtained an R^2 value of 0.5971 with an insignificant *p*-value of 0.0716. These results show that total BA is not a good indicator of SDI.

4.0 Discussion

4.1 Results Interpretation

This study collected data from mushroom communities and tree communities in plots at different elevations for ecological analysis. Metrics that were calculated included mushroom species richness, SDI, species evenness, and total BA (for trees). Of these four metrics, SDI was the only one that resulted in significant results. The SDI values ranged from 1.490 at elevation 1002.5m to 1.990 at elevation 940.5m with a small inverse relationship. This means that as elevation increased, mushroom diversity decreased. A linear regression model found this relationship to be significant with a R^2 value of 0.68 and a *p*-value of 0.04, indicating that elevational gradient is an environmental indicator of SDI for mushroom communities. Another somewhat notable result of this study is the inverse relationship observed between total BA and SDI at each elevation. The correlation resulted in a R^2 value of 0.597 which indicates that the relationship is not very strong. The significant results obtained from this study pertaining to SDI are evidence that elevational gradients have an effect on mushroom community diversity.

Although the other results were not significant, it's important to note the small trends that were observed for the other metrics. Species richness analysis found a range of species richness from observations ranging from 18-23 genus, but there were no obvious trends. Species evenness values ranged from 0.497 to 0.676. The two lower elevations had relatively higher species evenness values compared to the higher elevation. However, the significance test did not find any significant results, but there was a slight inverse relationship. The total BA of plots ranged from 2.101 m^2 to 4.169 m^2 with variation across elevations. There was no significant trend found in this data set. Despite the fact that there were no significant results in the data, a replication of the experiment with adjustments following the recommendations outlined in the next section may produce more meaningful results. On the other hand, it's possible that elevation doesn't play a significant role in species richness, evenness, or total BA for trees. Nevertheless, all of these ecological metrics increased our understanding of mushroom communities present in the Mitsinjo Reserve and Analamazaotra National Park area.

4.2 Limitations and Recommendations

Despite the significant results obtained, it’s important to take note of the limitations of this study. There are numerous limitations associated with the study site and data collection method outlined below.

Study Site

The limitations associated with the study site include considerations dealing with elevational analysis, time of year, and plot locations. The aim of the experiment was to make confident conclusions based on the effect elevation has on species communities of mushrooms. To do this, the original plan was to divide the ridgeline into three, 50m segments from 900m-950m, 950m-1000m, and 1000m-1050m. Following this division, data would’ve been collected from two plots within each segment. This would’ve increased sample size for comparison between elevations. However, after elevations were checked with Google Earth post field work, it was revealed that they were not consistent with what the GPS had read in the field. Analysis was adjusted to simply compare community diversity across all six plots (as seen in the results section). This comparison was still possible because the Braun-Blanquet method (1965) for data collection ensures that an accurate representation of the forest is taken at any given 50x20m plot. Although the results of this experiment produced significant results by comparing 6 different elevations, it’s recommended that in the future, segments be created and/or more data is collected to increase sample sizes for data analysis.

Additionally, there are limitations associated with the study site as a whole (Mitsinjo Park and Analamazaotra National Park). This is because of the small elevational gradient present in the area that only ranges from roughly 900m to 1050m. Previous research analyzing elevational effects have worked in study sites ranging in elevational gradients from 800m to 1000m (Brown *et al.*, 2006). Although we obtained results that were significant, given that the elevational gradient was so small, we can not be confident that the cause is due to elevation. Other factors might include forest management differences between parks, plot proximity to water sources (rivers/marshes/ponds etc.), and/or forest fragmentation between plots. These factors will be discussed further in the preceding paragraphs. The limited elevation range was known at the beginning of the study, but the site was picked based on the existing mycological research that has been done in the area. This reason was strong enough to keep the study site as is because one of the main goals of this study was to build upon existing mycological research in Madagascar. For the future, it's recommended that a site with a greater elevational gradient be picked to allow confident claims to be made that elevation had a significant effect on results.

Another limitation with the study site is the fragmentation created between the parks both in the literal and management sense. Mitsinjo Reserve is located on the west side of a small road (~10m wide) that transects the forest. Analamazaotra National Park is located on the east side of the same road, directly across from Mitsinjo (see Figure 2 in Methodology). This fragmentation may affect differences in species communities by disrupting ecosystem interaction, thus affecting the significant results that were obtained about species diversity (Fahrig, 2003). Additionally, it should not be ignored that these sites are a part of two different organizations. This most likely has an effect on species diversity because of differences in disruption from tourists and management practices (Vodouhê *et al*., 2010). For example, Mitsinjo Reserve actively conducts research across the park to improve ecosystem health and restore the forest (Association Mitsinjo, 2024). This is done through healthy and responsible reforestation practices which includes the maintenance of a tree nursery. Despite this limitation, both of these parks were selected because they contained the necessary elevational highs and lows for the study to be conducted. Future studies should select a site located within (or entirely out of) one park to control for fragmentation variables.

Finally, a very important limitation about this study is the fact that the forest is a secondary forest restored from an old lumber yard (Association Mitsinjo, 2024). This raises concerns when analyzing species abundance, richness and diversity because of differences in species communities. There is a possible presence and prevalence of invasive tree species that were introduced during the restoration process. Today, eucalyptus and pine are two dominant non-native tree species that can be found in the forests. This ultimately affects mushroom species makeup because of mutualistic relationships formed between specific mushroom and tree species. It's possible that some mushroom species are so successful in the area because of the presence of trees such as eucalyptus and pine. This limitation is important to consider because these invasive tree species were present around the study plots. For example, at elevation 940.5m, there were numerous large pines nearby. As a result, there was a high abundance of *Marasmius* and *Coltricia* genus species in the plot. This likely skewed the species diversity calculated during data analysis. Additionally, the presence of these species likely outcompeted native mushroom species that would have otherwise been present (Charles and Dukes, 2007). For these reasons, we cannot trust the significant results of this study with full confidence. It may be interesting for future research to investigate differences in native and nonnative mushroom species and/or analyze how invasive tree species affect mushroom communities.

Data Collection

There are numerous limitations associated with data collection including the time of year data collection was carried out, using the Braun-Blanquet method (1965), the method used for locating mushroom samples, and flaws in mushroom identification.

The timing of data collection for the project is another limitation associated with data collection for this study. As mentioned in the introduction, April is an ideal time of year because there are relatively high precipitation levels. However, there are times of the year that would be more ideal for mycological research. It's recommended that similar studies be conducted during the months of January, February, and/or March because they are the months with the highest precipitation levels in the Andasibe area (Andasibe, Toamasina, Madagascar Climate, 2024). Given that mushrooms tend to sprout sporocarps with high levels of moisture, this would increase the number of visible mushrooms in the forest and increase the sample size of data. Future studies could analyze species communities in the area over the course of the year because some species may only be present at certain times of the year. Additionally, as mentioned in methodology, data collection occurred in 9 days over the course of 13 days. In future experiments, it's recommended that data be collected without any days off in between to control for temperature and precipitation variations. Despite this limitation, the significant results found during the month of April are beneficial to expanding mycological knowledge in the Andasibe area.

One of the primary limitations associated with data collection pertains to the Braun-Blanquet approach (1965). This method has been widely used to obtain an accurate representation of forest structure. While this was the primary reason for choosing this method, it's not necessarily the most ideal for collecting an accurate representation of mushroom communities in any given area. Despite this limitation, a comparison between mushroom and tree forest composition was obtained.

Furthermore, the use of the method "walking each 10x10m placette" to look for visible mushrooms (outlined in methodology) was not ideal for a comprehensive collection of mushroom species. This method is prone to error including neglect of mushrooms that are too small to see, are hidden by leaves or branches, and/or camouflaged by the surrounding vegetation. For these reasons, there were most likely species samples that were not recorded during data collection which ultimately affected final results. For example, large mushrooms, such as *Russula,* may have been recorded more accurately to their true species count compared to small mushrooms such as *Hymenoscyphus*. To improve data collection, it's recommended that a different approach be used to locate mushroom samples.

Finally, the third limitation of data collection has to do with mushroom identification. Measures were taken to maximize confidence in species identification. These included working with a local mushroom specialist (Nasoavina), using keys, and cross checking species with other sources after field work had been completed. These methods were used due to limited time and resources. However, these methods are prone to subjectivity. Incorrect identification would greatly alter the data collected and analyzed in this study. To increase confidence in the future, it's suggested that samples be collected for DNA analysis. This would dramatically increase confidence in species identification and the conclusions made during the study.

Clearly, there are many limitations associated with the study site. These include study site limitations associated with an unideal elevational range and the plots selected along that range, fragmentation issues between parks, and the persistence of invasive species. Limitations associated with data collection include variables associated with the time of year when collection was carried out, using the Braun-Blanquet method (1965), mushroom location and identification. Future recommendations should be followed to increase confidence in results.

4.3 Moving Forward

This study found statistically significant results that indicate that elevational gradient has an effect on mushroom species diversity. Results found that as elevation increases, SDI decreases. This is interesting because this trend does not support Siles's and Margesin's (2016) conclusions that mushroom diversity increased with elevation. However, the data from this study supports the evidence that elevational increases cause a decrease in biodiversity (Rahbek, 1995). The knowledge gained from this study helps us better understand how fungi react to environmental factors. Increasing our understanding of fungi is essential in planning holistic conservation methods to improve ecosystem health for the future.

The high number of limitations present in this research cannot be ignored. If anything, these limitations show that mycological research is difficult and proper techniques are still being developed for this field of study. Efficient and reliable methods will not be achieved without continued trial and error in research. Increasing knowledge and understanding of the roles and relationships fungi have in the environment will allow us to better implement fungi into restoration projects. The results of this study showed that elevation has an effect on mushroom communities. On a bigger scale, it revealed that mycological research is needed to better help us understand how to improve ecosystem health around the world.

5.0 Conclusion

There is a large base of evidence that elevation can be used as an indicator of species biodiversity (Rahbek, 1995). The common results show that highest biodiversity is present at mid latitudes and generally decreases as elevation increases. However, there is very little research pertaining to fungal communities (Dahlberg, 2001). One study found results that counter the common trends. They found that mushroom diversity increased with elevation as a result of increased nutrients in the soil (Siles & Margesin, 2016). To investigate this further and to expand on mycological research in Madagascar, this study compared mushroom communities across an elevational gradient. Metrics were calculated pertaining to mushroom and tree communities in Mitsinjo Reserve and Anlalmazoatra Parks. Significant regression results were only found between Shannon Diversity Index and elevation. It revealed a statistically significant inverse relationship between the two variables with an R^2 value of 0.68. These results are significant especially because they counter the finding of Siles and Margesin (2016). Important knowledge has been gained from this study by increasing mycological understanding and the need for improvements in mycological methods. Although significant results were found, there are many limitations associated with the study. However, knowledge can be gained from this as mycological research continues to develop. The need for improved methods is essential for the advancement of fungal research and conservation as a whole.

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