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Laura Yesenia Pérez Olivera

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**Survey of macrofungi species richness pattern along an elevational gradient in the Monteverde Cloud Forest**

Laura Yesenia Pérez Olivera

Department of Plant Microbial Biology

University of California, Berkeley

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**ABSTRACT**

Given that there is an extensive diversity in organisms and landscape in Costa Rica, this study focuses on the species richness of macrofungi along an elevational gradient in Monteverde, Costa Rica. I assessed a 200m elevational gradient from 1550m to 1750m of the trails of the Estación Biológica. Using a GPS and altimeter, I recorded the location and elevation of each macrofungi found along the trails. The data gathered from these walks was then used to create a map with ArcMap10.2 that visualizes the species richness along 8 altitudinal bands of 25m of the total elevational gradient. I found a total of 154 organisms and 91 different morphospecies and identified 21 genera. Results indicate that species richness has an overall decrease as elevation increases. However, because there was an increase in the 1675-1700m and 1700-1725m altitudinal bands, this is not indicative of a monotonic pattern. This biogeographical study may therefore only include one piece of the overall trend found on elevational gradient in the Monteverde cloud forest and a pattern may be better assessed along a wider elevational gradient.

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**Riqueza de macrohongos en un gradiente altitudinal en el bosque nuboso de Monteverde****RESUMEN**

Teniendo en cuenta que existe una amplia diversidad de organismos y paisaje en Costa Rica, este estudio se centra en la riqueza de especies de hongos macroscópicos a lo largo de un gradiente altitudinal en Monteverde, Costa Rica. Evalué un gradiente de elevación cada 200 metros desde los 1550m hasta los 1750m en los senderos de la Estación Biológica Monteverde. Usando un GPS y altímetro, registré la ubicación y elevación de cada macrohongo encontrado. Utilicé los datos obtenidos para crear un mapa con ArcMap 10.2 que visualiza la riqueza de especies a lo largo de 8 bandas de 25m de altitud del gradiente total. Encontré un total de 154 organismos y 91 morfo especies diferentes de 21 géneros identificados. Los resultados indican que la riqueza de especies tiene una disminución general a medida que la elevación aumenta. Sin embargo, se ve aumento en las bandas altitudinales de 1675-1700m y 1700-1725m, mis resultados no indican un patrón monotónico. Este estudio biogeográfico puede por tanto incluir sólo una parte de la tendencia general que se encuentra en el gradiente de elevación en el bosque nuboso de Monteverde y el patrón puede ser mejor evaluado a lo largo de un gradiente altitudinal más amplio.

The tropics are defined by abundant climatic, ecological, and geographical diversity. Hence, the tropical rainforests of the world are home to two-thirds of the Earth's floral and fauna biodiversity (Tsui et al., 1998). Within this biodiversity is the kingdom fungi of which 2,000 species are known in Costa Rica, but an estimated 40,000 to 70,000 is believed to exist in this 51.1 billion m<sup>2</sup> country (Mata, 1999). Within the extensive fungi kingdom are macrofungi, which can be seen by the naked eye and are in either the Ascomycota or Basidiomycota phyla. They can be differentiated by their reproductive morphology and microscopic reproductive structures. The typical mushroom that is imagined when we come across the word fungi belongs to Basidiomycota. For fungi to thrive, they need moist environments and plenty of dead matter that they can decompose, as they are key to important ecological processes.

According to the Holdridge life zone the Monteverde Cloud Forest is considered lower montane wet forest. It is physically immersed in a cloud, hence the presence of high relative humidity (RH), reduced sunshine as elevation increases, and increased presence of epiphytes (Foster, 2000). The wetness of the cloud forest is reflected in soil characteristics as they are frequently wet and increase in dampness with increasing elevation. While also being montane, the soils of the cloud forest are often characterized by "low nitrogen values and low decomposition rates" (Foster, 2000). Wet soils and plenty of dead matter substrates, such as tree leaves, branches, and logs, are characteristics of a cloud forest that make it a desirable environment for fungi to grow. Because Monteverde is defined by its cloud forest and high elevations, this study will focus on the species richness of macrofungi along an elevational gradient.

Many biogeographical studies have emphasized a "unimodal" pattern of species richness along an elevational gradient (Cavarzere and Silveira, 2012); this is known as the Mid-domain Effect (MDE). In 2006 there was a study that focused on the species richness of ferns along an elevation gradient in Costa Rica with a discussion on MDE as the elevational pattern of fern species. Watkins' (2006) study on fern richness exhibited the MDE pattern on an elevation gradient of 30 m above sea level (a.s.l.) to 2960 m a.s.l. and peaked at 1000 m a.s.l. MDE defies the traditional elevational gradient pattern which is described as a "monotonic decrease in richness with increasing elevation" and instead describes that species richness distribution along an elevation gradient has a "'humped' distribution with species richness highest near the middle of the gradient, much like the one seen at the 1000 m a.s.l. in Watkins' study (Colwell and Lee, 2000; Watkins et al., 2006)."

Given that the Monteverde cloud forest is defined by its moist environment at high elevations and epiphytic environment, I explored the questions: How does species richness vary across the elevational gradient of the Estación Biológica trails of Monteverde? And does elevation influence a variation of substrate that the macrofungi are found on? I predicted that species richness will display a MDE pattern on the elevation gradient, and that substrate will vary as elevation increases.

## MATERIALS AND METHODS

### *Site and Elevations*

This project focused on a transect-based methodology of assessing fungi biodiversity at a 200m elevation gradient (Schmit, 2005). The elevational study sites assessed are the trails (Sendero Principal, S. Divisido, S. Mirador, S. Cariblancos, S. Jiguero) at the Estación Biológica in Monteverde. The 200m elevational gradient was divided into four 50m altitudinal bands, starting from a 1550m elevation. All trails were visited twice during the data collection time, from May 12, 2016 to May 21, 2016.

### *Data Collection & Analysis*

During my walk on these trails, I used a GPS to record the location and elevation where I observed macrofungi. In case I lost satellite connection, I carried an altimeter for a more accurate elevation measurement. The points documented in the GPS include the macrofungi name (if identified), substrate, and other comments if necessary. All fungi were photographed using a Canon EOS DSLR camera, and some fungi were collected for further assessment of characteristics such as spore prints. The spore prints made during this study were used for identification purposes. They were made by placing the cap, gills, or spores of the mature fruiting body on a piece of paper covered by a glass container or cup to prevent air currents from dispersing the spores. The spores from the spore print could be used for further assessment of fungi by observing under a microscope if necessary.

An excel sheet with GPS number, elevation, substrate, genus and species, longitude and latitude was made for data organization. The points recorded in the GPS were used to create a map using ArcGIS 10.2. This was done by first converting the GPX files of the recorded points to shapefiles that can be used as a layer in ArcGIS. The excel sheet mentioned included a row labeled FID so that the excel sheet (in .csv format) could be joined with the attribute table of the GPS points. Layers of topography, buildings, watersheds, vegetation, hillshade, and streets of Monteverde were obtained from Randy Chinchilla of the Monteverde Institute. I made a simple map by color-coding the points found on the 8 25m elevational bands with a green, yellow, and red gradual color scheme the hillshade, vegetation, watersheds, and building layers.

## RESULTS

A total of 154 macrofungi were documented along the 200m-elevation gradient. There were a total of 81 morphospecies of macrofungi recorded and 21 genera identified. Within this data, a total of 38 recorded macrofungi belonged to the Polyporaceae family, most of which were saprobes, mainly on dead branches and trees. Table 1 shows the observed macrofungi per 25m-altitudinal band. Elevations from 1550m to 1600m, and 1676 to 1700m observed the highest recorded macrofungi.

**Table 1:** 25m-elevational bands and total macrofungi observed at each range

Elevation (m)	Macrofungi recorded
1550- 1575	28
1576-1600	28
1601-1625	14
1626-1650	11
1651-1675	15
1676-1700	27
1701-1725	19
1726-1750	12

There wasn't a fungi species that was found in all 8 25m-altitudinal bands, however, *Mycena speirea* shown in Photo 1 had the highest number of occurrences, 10 times. It was observed along six of the eight-altitudinal belts. It was not observed within the 1650-1675m and 1700-1725 altitudinal belt. The second most common macrofungi observed was *Mariasmus siccus*, which was observed 7 times, and at six 25m-altitudinal belts. The polypore *Trametes versicolor* was observed six times, along 4 altitudinal belts. And there were several species that were only observed once. One particular macrofungi common to Costa Rica, *Lactarius indigo*, was observed twice along the S. Principal, once at the lowest elevation 1550m and the other at the highest elevation 1750m.

**Foto 1.** *Mycena speirea*



**Foto 2.** *Mycena speirea*



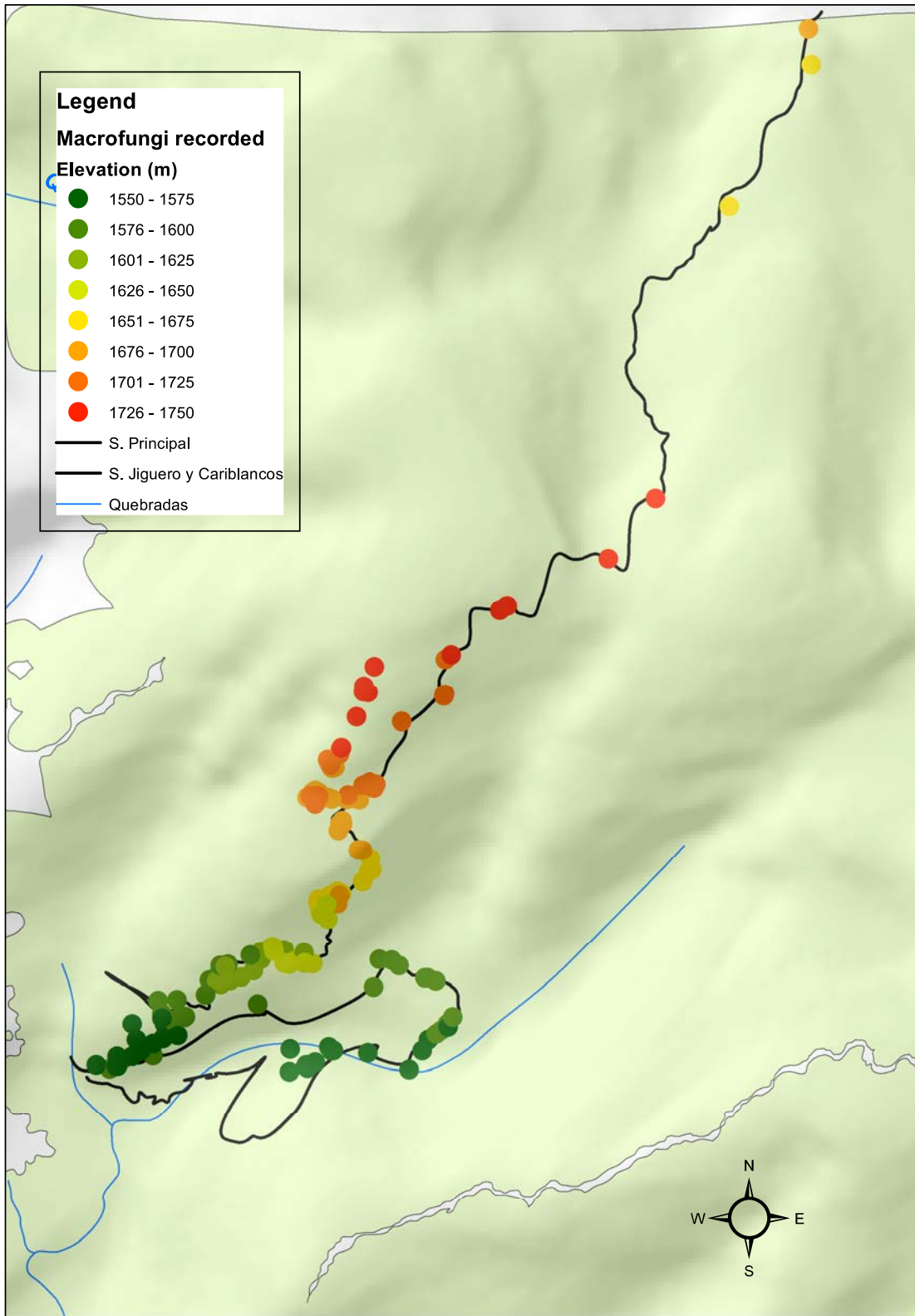
**Foto 3.** *Mariasmus siccus*,



**Foto 4.** *Trametes versicolor*



**Foto 5.** *Lactarius indigo*

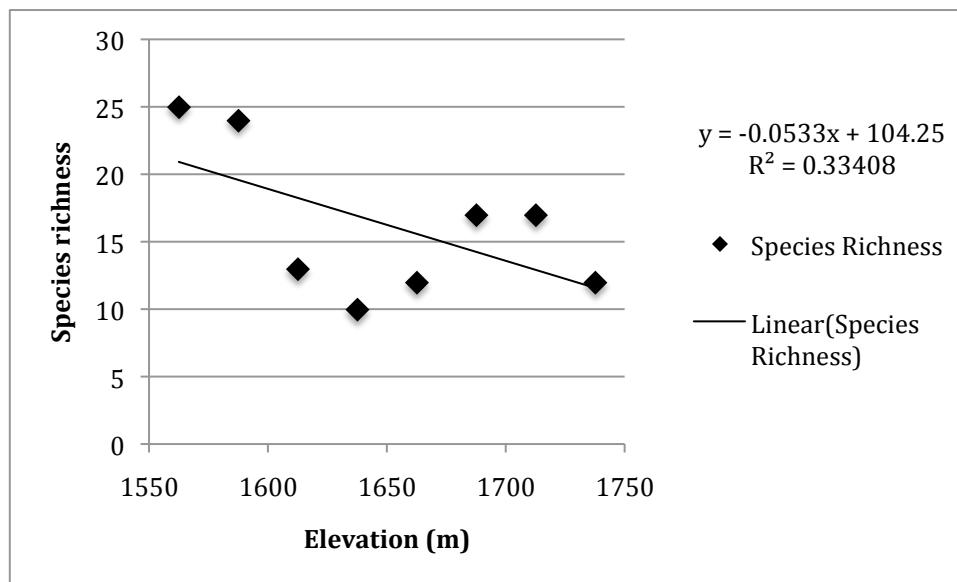


**Figure 1: Map of macrofungi recorded per 25m elevational bands along a 1550 to 1750m elevational gradient of the Monteverde Cloud Forest**

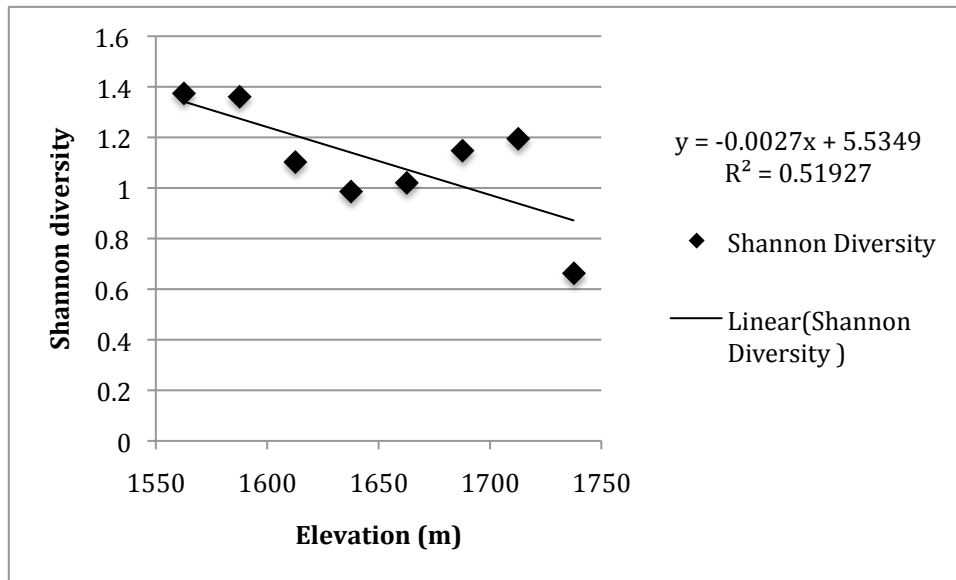


Sendero Principal is the main trail on this map and it ends at the TV towers. Sendero Jiguero and Sendero Cariblanco are next to the Sendero Principal, and parts of the trails are found on elevations lower than 1550m, hence the exclusion of some points that were documented at lower levels.

There was an overall difference in species richness and evenness for each of 25m bands ( $X^2 = 19.714$ ,  $df=7$ ,  $p\text{-value} = 0.006$ ). The Shannon diversity and species evenness (figure 3) further validates this by displaying varying degrees of evenness in the bands with lowest and highest number of species. Species richness varied along the 25m elevational bands with a negative trend as elevation increases. Figure 2 shows this trend. Additionally, Figure 3 displays a negative trend of the Shannon Diversity Index as elevation increases.

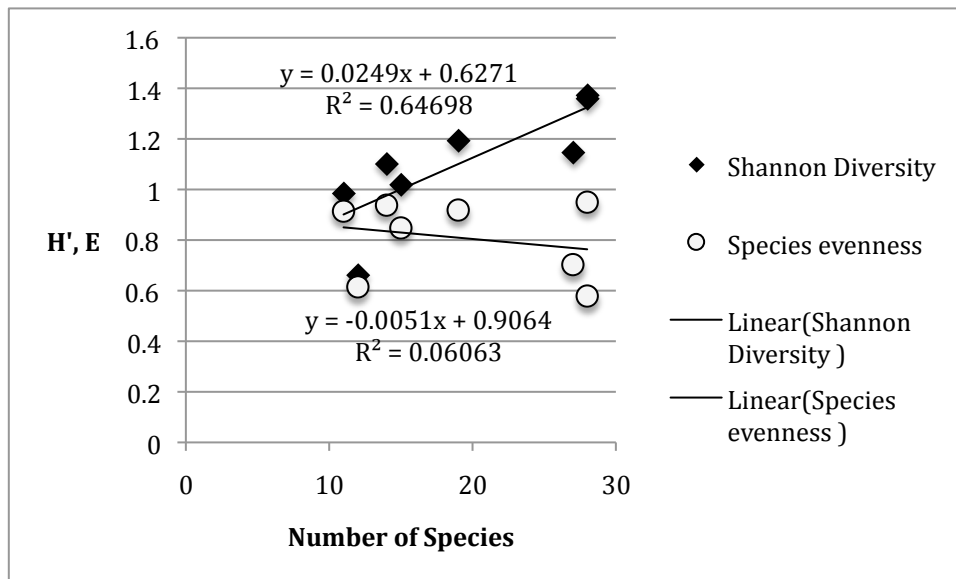


**Figure 2: Species richness per 25m altitudinal band** The graph of macrofungi morphospecies richness found along 25m altitudinal bands. The graph shows a negative trend with decreasing species richness as elevation increases.



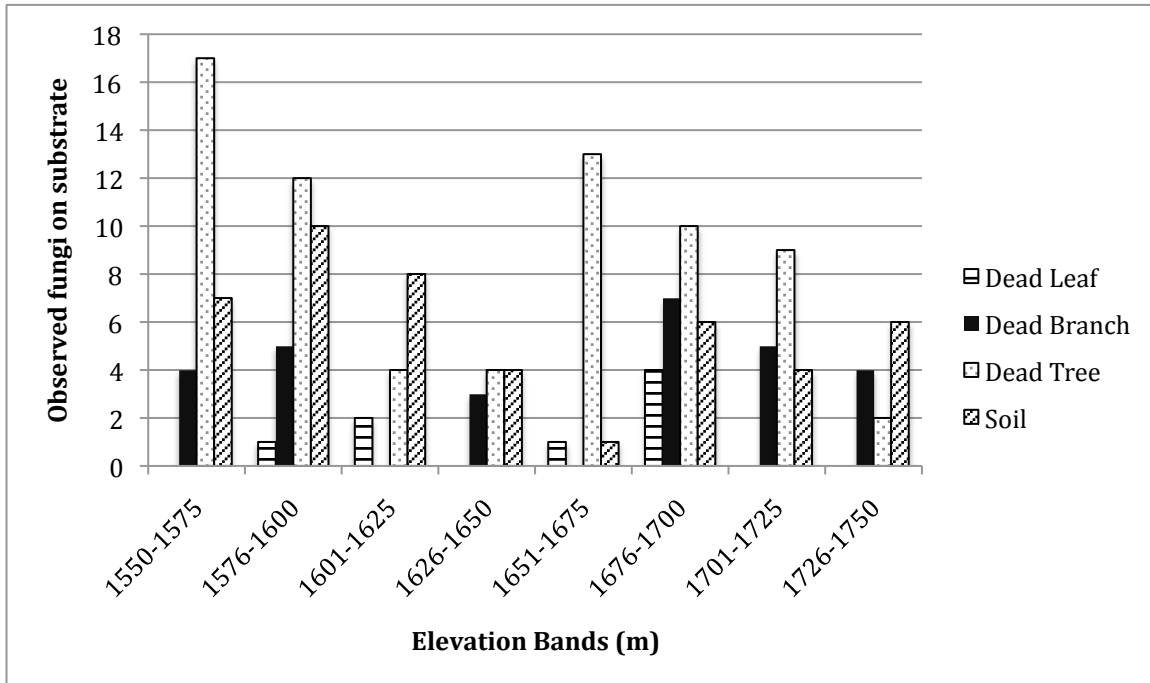
**Figure 3: Shannon diversity index per 25m altitudinal band** The shannon diversity index of the eight 50m altitudinal bands shows a negative trend with elevation. As elevation increases the Shannon Diversity Index decreases.

There is a known trend between the increasing number of species found in an ecosystem and an increase in the Shannon diversity index. The following graph includes species evenness of the 8 altitudinal bands represented by the number of species found at each band.



**Figure 4: Shannon diversity & evenness** This graph shows that diversity increases with increasing number of species, however the species evenness, demonstrates that elevations with the most and least number of species do not display an equal species diversity.

Lastly, figure 4 displays the substrates where macrofungi were found. Dead wood debris dictates substrates found in all but the highest elevational band (1725 to 1750m).



**Figure 5: Substrate presence per 25m elevational band** This graph demonstrates the substrates that macrofungi were found on per 25m elevational band. The lowest elevation (1550 to 1750m) had more macrofungi found on dead wood debris than the highest elevation (1725-1750m), where there were more fungi found on soil vs. woody debris.

## DISCUSSION

This study shows that there is a difference in species richness across the 200m elevational gradient, however it does not plainly display a monotonic decrease in species richness or a MDE pattern. Even though we do not see a MDE pattern, the graph does show that the pattern present is not monotonic across the 25m altitudinal bands because we see an increase of both the richness and diversity index from 1675m to 1700m. The 200m elevational gradient might not be a comprehensive enough gradient to visualize MDE, and it could therefore be better visualized by including an assessment of elevations lower than 1550m. This pattern could have been influenced by the in-between of dry and rainy season during the data collection.

In comparison to Elizabeth Norton's 2013 study of fungi diversity at a 1550m to 1750m elevation of Monteverde, the species richness pattern is completely opposite from Figure 3. Norton concluded that there was an increase of species richness with increasing elevation. Given that Norton's study was conducted during the dry season, and my study was conducted during the beginning of the rainy season, a transitional time for the cloud forest, this could have been a factor for our opposing results. The beginning of the rainy season could have given increased moisture availability at the lower elevations, however, moisture levels are still expected to be higher at increased elevations and hence a better connection to high number of species recorded at lower elevations could be due to the fact that the majority of the fungi were found on dead woody substrates (figure 5). Twenty-one of the twenty-eight macrofungi found at the lowest 25m elevational band

were found on dead wood debris. Castillo et al. (1994) supported that wood decomposer fungi may be adapted to low moisture or high temperatures by studying the growth of *Pycnoporus sanguineus* and *Schizophyllum commune*. In comparison to the highest elevational band (1725 to 1750m) there was a greater number of macrofungi found on soil vs. woody substrates (Figure 5), which can be assumed that is it because of the higher levels of moisture found at higher elevations of the cloud forest.

This study is not conclusive of the species richness of macrofungi found in a 200m elevational gradient, however, it can be a piece to the pattern of an elevational gradient that may be better studied in a broader elevational gradient. Watkins' et al. (2006) study included a 2,930 m elevational gradient, which is significantly wider than the 200m elevational gradient observed at the Estación Biológica. Nonetheless, these results are relevant spatially and temporally to the current event of El Niño that is influencing global temperature increase, longer dry days, hence a longer dry season, and an impact on precipitation levels of the cloud forest. Biogeographical studies on species richness and diversity along an elevational gradient during El Niño events could help us better understand the shifts that there may be in species richness and distribution.

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