
MACROFUNGAL DIVERSITY AND ABUNDANCE IN THREE DIFFERENT FOREST TYPES IN CLOUDBRIDGE NATURE RESERVE, COSTA RICA

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ABSTRACT

This research focuses on the macrofungal diversity and abundance in three forest types in a cloud forest in Costa Rica. Fungi play a key role in the ecosystem and they are beneficial for man. The purpose of this research is to compare the species diversity and species richness of the different forest types and through this, to get a deeper knowledge of the resilience and biodiversity of the cloud forest. The tree forest types are: planted, young, secondary forest; young, natural regenerated secondary forest, and primary, old growth forest. The forests are located in the Talamanca mountains of Costa Rica, at an altitude ranging from 1500 to 2200 meters. Data is collected in the rainy season of 2022, between the months of May and August. Plots of 10 by 10 meters were used. Within them, the macrofungi species were counted and identified. Along this is recorded on which substrate they are growing, the soil pH level and the canopy coverage. Substrate is looked at to assess on which fungi grow most frequent and if this varies per forest type. For soil pH and canopy coverage is researched whether there is a relation between these factors and fungal diversity and abundance. For the diversity and abundance, three different statistical tests were used; Chi squared, Shannon Wiener index and the Simpson index. Results show a significant correlation between forest type and fungal abundance and diversity. With the young, natural regenerated forest containing the highest biodiversity and the primary, old growth forest having the highest abundance. This bellies the hypothesis "*The macrofungal diversity and abundance is higher in the primary forest than in the secondary forest*". A positive correlation is found between soil pH and macrofungal diversity-and abundance. There was no correlation found between canopy coverage and macrofungal diversity-and abundance. Of all fungi, the majority of 53% is found to only grow on wood. The species found in all forest types are *Mycena aculata*, *Campanophyllum proboscideum*, *Xylaria polymorpha*, *Xylaria hypoxylon* and *Cookeina venezuleae*. The most frequent in all forest types were *Xylaria polymorpha* and *Xylaria hypoxylon*. Common families are; Mycenaceae, Ganodermaceae and Xylariaceae.

1. INTRODUCTION

In this research, a comparison will be made in the macrofungal diversity and abundance in three different types of cloud forest in Cloudbridge nature reserve in Costa Rica.

Cloud forests are mountainous forest systems that only make up 2.5 percent of the world's tropical forests. They form on areas where the canopy intersects with the clouds. (Hence the name.) This intersection makes for a lot of rainfall. They only occur when local climatic conditions, elevation, and distance from the sea are just right. These rare and unique forests are characterized by a green mossy understory and a high quantity and diversity of ferns, lichens and orchids. The uniqueness of the ecosystem makes that there is a high level of endemism.

Fungi are an often overlooked, underestimated and understudied kingdom within forestry and ecology. However, it fulfills one of the most important roles in the ecosystem: decomposition. Together with bacteria and micro-organisms, fungi are responsible for decomposition. Allowing plants to take up nutrients that would otherwise get lost and essentially cleaning up the forest. Another role fungi fulfill in the ecosystem is nutrient fixation. Not all plant species absorb minerals and nutrients easily, fungi help with this through the ability to fix these elements and transfer them through various symbiotic relationships. Fungi also create mycorrhizal networks, which trees use to share nutrients and communicate via their roots. Fungi help soils absorb water better and they are an important food source for insects and other animals. *"Despite the importance of macrofungi in nutrient cycling and succession in forest ecosystems, our current understanding of species diversity, community structure, and dynamics in macrofungi remains limited."* (Ferrer & Gilbert, 2003). Questions remain about environmental effects on macrofungi. Little is known about how and where fungi can be conserved. (Bhagwat & Watkinson, 2005) (Svenning et al., 2018) What the effects are of environmental- and dispersal processes on macrofungal community structures. It is also not understood if community assembly differs between different functional guilds of macrofungi, e.g. soil, wood and leaf litter fungi. By obtaining this knowledge, the place of fungi in the ecosystem can be demystified. (Ferrer & Gilbert, 2003).

The goal of this research is to investigate the fungal diversity and species richness of three different types of cloud forest. And through this, to get a deeper knowledge of the resilience and biodiversity of the cloud forest. Assuming that macrofungal diversity is an indicator and a supporter of biodiversity. (Lodge & Cantrell, 1995). A small difference in diversity and abundance of mushrooms between the three sites can indicate a high resilience. Meaning the forest quickly regained its diversity and species abundance after reforestation. A higher diversity in the old growth forest could indicate that the young forest is still in the process of recovery. A fast recovery would be wishful for the reserve, as they are focused on getting a high nature value in the park. A faster recovery in the planted areas would also indicate fruitfulness of planting.

2. PROBLEM STATEMENT

The field of fungi is very under researched. Many species of fungi are still undiscovered and there is very limited knowledge about less common species of fungi, their distribution and community structures. Because of the mysteries around this topic, not much can be done for their protection. This is becoming problematic, for the research of fungi is of growing importance, as many macro fungi are becoming extinct and facing threat of extinction because of habitat destruction. (Anand & Mathur, 2014)

Aside from this, the research of fungi can benefit humanity. Fungi are proven to help with combatting the effects of climate change, plastic pollution, oil pollution and they can be used for their wide arrange medicinal properties. (Sheldrake, 2021)

As mentioned in the introduction, cloud forests are unique ecosystems which are very rare. Its vulnerability due to its sensitivity to changes in climate and its housing of unique species makes cloud forests of great importance for protection. Obtaining more knowledge about this ecosystem, its dynamics and its resilience, can give more insight on how much pressure (be it from agriculture, tourism or forestry) it can handle. This can lead to a more considered interaction between man and the ecosystem.

2.1 HYPOTHESIS

It is predicted that the macrofungal diversity and abundance higher is in the primary forest than in the secondary forest.

2.2 RESEARCH QUESTIONS

The goal of this research is to investigate the species diversity and species richness of three different types of cloud forest.

The main question this research aims to answer is: "Is there a significant difference in diversity and abundance of macrofungi between the three forest types: primary, old growth forest; secondary planted, young forest and secondary natural regenerated, young forest?"

Sub questions that will help to provide a clear cut answer to the main question are:

- *What external factors contribute to the difference in macrofungal diversity?*
- *Is there a difference in macrofungal diversity (species richness) between the forest types?*
- *Is there a difference in abundance between the forest types?*
- *Is there a relationship between soil pH and macrofungal diversity?*
- *Is there a relationship between canopy coverage macrofungal diversity?*
- *What are the species most often found?*
- *On what substrates are the most species found?*

3. METHODS AND MATERIALS

3.1 AREA DESCRIPTION

Data is collected in Cloudbridge nature reserve, a private reserve with an altitude ranging from 1500 to 2200 meters. It is located in the Talamanca mountains of Costa Rica, in the province of San Jose. The park just recently came in existence. In 2002, 255 hectares of pasture and cropland, along with 28 hectares of primary forest is bought by the reserve. Most of the forest of the area consists of very young, secondary cloud forests. The Cloudbridge organization is dedicated to conserving and restoring the cloud forest. Parts of the forest have been planted to speed up the process of regrowth.

Plots of 10 x 10 meters are set out, in which mushroom species are identified, counted and noted how many times they are seen. There are four plots in every forest type. Each plot is visited twice within a span of three months. This is done to combat the influence the weather can have on the mushroom growth.

THE CLIMATE

Cloudbridge is located in a tropical montane forest characterized by a green, mossy understory and a fog due to its intersection with the clouds (cloud forest). Cloud forests occur between an elevation of 1500 to 3500 meters above sea level.

Annual and seasonal rainfall patterns in cloud forests range from 500 to 6000 mm per year. Cloud forests are found on the point where clouds and mist are frequently in contact with mountain slopes. Due to this, it rains nearly every day of the year. Although there is a distinct rainy season between the months of April and November, in which it starts raining in the early afternoon and usually goes on for the rest of the day. The average monthly temperatures range from 10 to 27 °C, being the highest in the months of May and July but having little variation throughout the year. Fungi generally need a temperature above 15 °C to form sporocarps.

Water is one of the main factors that influence the success in mushroom growth. High soil and air humidity (80-85% RH) stimulates the growth of the sporocarps. (Bellettini et al., 2019) The consistent, humid climate creates a great habitat for many different species of fungi of which fruiting bodies can be seen all throughout the year, although they are much more found in the rainy season.

GEOLOGY

The main soil textures are: Sandy-clay, Silty-clay, Fine silt and Silty. According to available ArcGIS data, the soil is classified as Utiisol. This is an umbrella term for red-clay soil types that are common in the south of the United States, Africa, Asia and South America.

The soil is seen as the ultimate product of continuous weathering of minerals in a humid, temperate or tropical climate. It is known to have a low Ph. According to Bellettini et al. a pH between 4 and 7 is optimal for mycelium growth of oyster mushrooms and for the forming of their basidiocarps (mushrooms), between 3.5 and 5 is optimal.

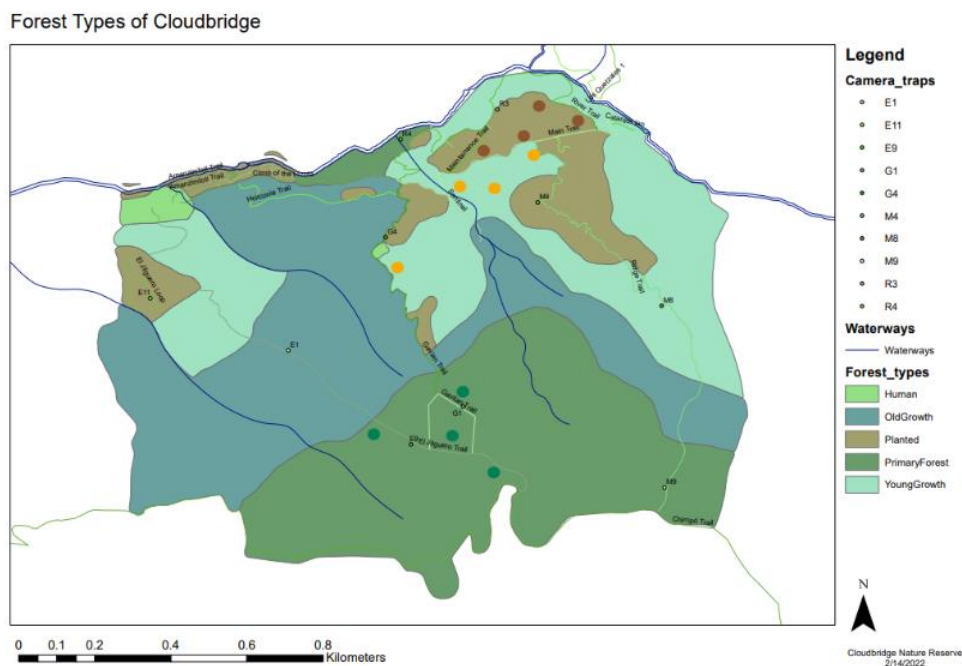
LOCATION

In this figure, a map of the cloudbridge reserve is seen wherein a distribution of the plots is shown. The plots are chosen to be evenly distributed when possible and to be around the same altitude. As can be seen, the plots in the primary forest are relatively far out.

According to maps.me the altitude of plots in the primary forest is around 1900 meter, while the other plots are at 1700 meter altitude. As can be seen in (figure 1) the plots are distributed in the following way.

- Green dots: plots primary forest
- Brown dots: plots planted
- Orange dots: plots natural regenerated

FIGURE 1



3.2 FIELDWORK METHODS

To answer the first research question: "What external factors contribute to the difference in macro-fungal diversity?" A literature study is done. This also helped building up the rest of the research.

Plots are set out in three different forest types within the Cloudbridge reserve: old growth (primary) forest, planted secondary forest and naturally regenerated secondary forest. Each forest type contains four plots, evenly spread where possible. Plots are sized 10 x10 meters, as this was mentioned in previous literature and turned out to give good sample sizes. Within these, all epigeous (above ground) fungi above 1 cm in diameter will be identified and counted and it will be noted how many times they are seen (frequency).

Mushrooms are essentially the 'fruit' of the fungus, they do not always show. When they do, they are triggered by environmental factors such as falling trees, climatic changes or changes in soil temperature or moisture. To minimize the influence of these factors on the data, each plot will be visited two randomized times in between the months of May and August. This data will be put next to each other to also see potential differences or similarities. After all data is collected, it will be added up per forest type. This way, forest sites can be easily compared.

Data is collected in the rainy season, starting in the end of May, it is expected to encounter the most mushrooms from this time on. Mushroom growth is very sensitive to weather so timing the surveys is important. The best time is after a period of rain with some sunny intervals (Wild, 2022). Specimens are collected for identification and are counted. Sources used for identification are google lens, books (Halling et al., 2003) websites, (Macrofungi of Costa Rica, n.d.)

The plots are set out with use of maps.me. on this app, altitudes are easy to read and plot locations can be saved. The plots location of the plots is decided from the map beforehand. Plots are named and numbered to avoid confusion. PH tests of the soil will be taken in each plot, to look for a correlation between this and macrofungal diversity. Soil is collected in tree corners of each plot, then the soils will be dried and the pH will be taken with a pH meter. With the canopy app, a photo is taken directed at the sky, in three corners of each plot. Out of this, a mean will be calculated. This measures the canopy coverage. A relation can be investigated between canopy cover and species richness.

FIELD MATERIALS

Field materials needed are; phone with maps.me, canopy app and a camera, area map, tape measure, machete, marking tape, sharp knife, containers, pen and paper, soil pH tester and identification books.

3.3 STATISTICAL ANALYSIS

For the statistical analysis is looked at frequency, abundance and diversity. Next to this, is assessed whether there is a correlation between pH,- canopy coverage and species richness- and abundance. The following methods are used.

ALPHA, BETA, GAMMA DIVERSITY

This method is commonly used to determine biodiversity in terms of species richness. Alpha diversity refers to the total amount of species per habitat or specific area, in this case, forest type. Gamma diversity is the total diversity of a region, in essence, the total amount of species found. Between sample comparison is called Beta diversity, it can be defined as the ratio between gamma and alpha diversities.

CHI SQUARED, SHANNON WIENER AND SIMPSON

A chi squared test is used to find weather there a significant association between forest type and the number of individuals found per species. Shannon-Wiener- and Simpson index are used to measure species diversity within the sites. For the factors canopy coverage and soil pH is looked whether there is a significant correlation between these factors and species richness and abundancy. This is done by creating graphs and looking at the R^2 value of the correlation.

4. RESULTS AND DISCUSSION

To conclude which factors are of influence for fungal growth, it is assessed what factors other research papers incorporate. In study by Bellettini et al. about external factors that influence the growth of the oyster mushroom. Factors included are: substrate (leaf litter/ wood/ soil), soil type, parent material, altitude, soil pH, soil temperature, soil and air humidity, light intensity, vegetation type/ plant association and forest type. A study by O’Hanlon and Harrington included substrate, forest type, other tree species, parent material and soil pH. “Wet season macrofungi of the Caribbean slope in Monteverde, Costa Rica” by Herbst (2008) gives how the factors included in the research (soil pH , elevation and canopy coverage) correlate to the fungal diversity and abundance.

A total of 90 species of fungi were found, (Gamma diversity). 49 of which were found in the natural regenerated plots, 36 species in the planted plots and 38 species in the old growth forest (Alpha diversity). A significant difference is found in the macrofungal diversity between the different forest types. A calculation of the Beta diversity (see table 1) shows the closest relation in terms of species composition between the planted site and the old-growth forest.

TABLE 1

	Site 1	Site 2	site 3	
Site 1				
site 2	57			
site 3	63	56		

A significant difference in macrofungal diversity is found between the three forest types. The Simpson index (table 2) shows a biodiversity of respectively 0.928 0.868 0.906 in the natural regenerated, the planted- and the old growth forest.

The Shannon-Wiener index in (table 2) shows a biodiversity of 3.228 - 2.749 - 2.733 respectively in the natural regenerated-, the planted- and the old growth forest. Natural regenerated forest being (>3.0 =) biodiverse. Both index’s show the highest macrofungal diversity in the natural regenerated, secondary plots.

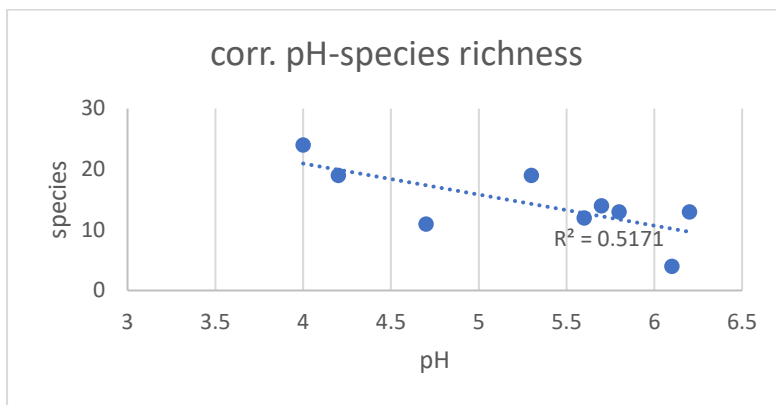
Table 2

	Nat.	plant	ogf
Simpson_1-D	0.928	0.8688	0.9063
Shannon_H	3.228	2.749	2.733

The results of the chi-squared test indicate that there is a significant association between forest type and the number of individuals found per species (abundance). The chi squared test gives ($\chi^2 = 444.61$, $df = 98$, $p < 0.05$). A count of the total amount of specimens per forest site gives of 312 specimens in the natural regenerated forest, 258 in the planted forest and 401 in the old growth forest. The old growth forest does not have a higher macrofungal diversity, however it does have the highest macrofungal abundance. With a difference of 89 with the planted forest, while there is only a difference of 54 between the planted and non-planted secondary forests.

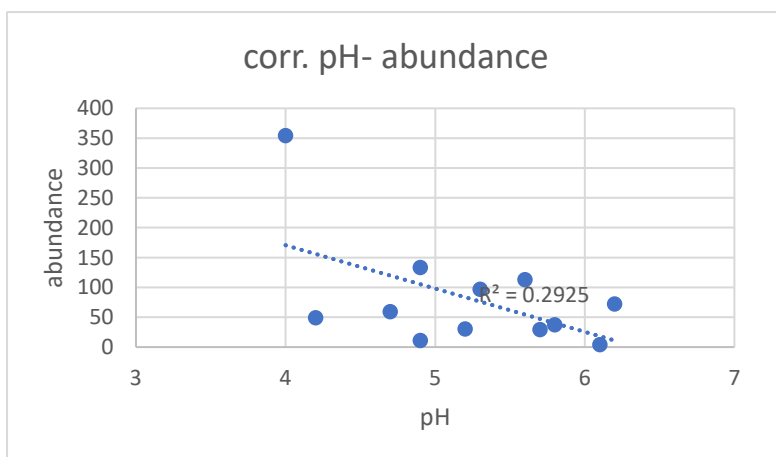
A significant, negative correlation is found between pH and species richness ($R^2 = 0.5171$). The R^2 value of the pH-diversity correlation gives a correlation of 52%. As can be seen in figure 2, the plot with the lowest pH is found to have the highest species richness (24 specimens). The plot with the second highest pH had the lowest species richness with only 4 specimens found in two visits. The average soil pH differed little per forest site. The average soil pH is 5.6 in the natural regenerated forest, in the planted forest this is 5.3 and in the old growth forest the average is 4.8.

FIGURE 2



When looked at the correlation of the macrofungal *abundance* and soil pH, the R^2 value is only 29%. This being a lot lower, it still can be stated that for both diversity and abundance that there is a significant negative correlation with the soil pH. It is thought that one outlier messed with this result. The plot counted 354 specimens while the average without this outlier would be 57. Another interesting result is that the diversity and abundance graphs show similar trendlines and the plots with the highest diversity are also the ones with the highest abundance. (See figure 2 and 3.)

FIGURE 3



Between canopy coverage and species richness, a negative correlation is found ($R^2= 0.154$), although the results show insignificant. The same results show for the correlation between canopy coverage and abundance. A study in park Monte Verde by Herbst (2008) found similar results in correlation for both pH and canopy coverage.

The species that were present in *all* forest types are: *Mycena Aculata*, *Mycena Xylaria polymorpha*, *Xylaria hypoxylon*, *Cookeina venezuleae*, *Campanophyllum proboscideum* and *Mycena californiensis*. To show this in more detail what species were most often found per forest site, a list of common species per forest type can be seen below in table 3. It can be seen that *Xylaria hypoxylon* (antler fungus) among most frequent is in all forest sites and *Xylaria polymorpha* (dead man's fingers) in all but the planted forest site. Most frequent families are; Mycenae, Ganodermataceae, Xylariaceae.

TABLE 3

Natural regenerated

	individuals	frequency
Xylaria polymorpha	48	12
Xylaria hypoxylon	54	4
Gymnopus lodgeae	12	5
Cookeina venezuelae	10	5
Calocera viscosa	26	7
Gymnopus lodgeae	12	5
Crepidotus applanatus	11	3

Planted

	individuals	frequency
Cyatus poeppigi	80	1
Echinoporia aculeifera	37	4
Mycena inclinata	21	8
Mycena sp.	13	12
Lycoperdon pyriforma	10	4
Campanophyllum proboscideum	7	3
Xylaria hypoxylon	6	2

Old Growth

	individuals	frequency
Campanophyllum proboscideum	50	10
Annulohypoxylon multiforme	50+	2
Stereum hirsutum	50+	4
Polyporus versicolor	50+	1
Xylaria hypoxylon	50+	9
Xylaria polymorpha	46	4

Of found species, one species was found to be parasitic on other fungi, 2,3% was found growing only on (leaf)litter, 2,3% was found to grow on leaf litter and wood , 19,3% found to only grow in the soil, 21,5% grow on all three substrates (soil, (leaf)litter and wood) and 53% was found to grow only on wood.

5.

The results of the data-analysis show that there is a significant difference in mushroom diversity and abundance between the tree forest types. The Shannon wiener index shows a high biodiversity in the natural regenerated forest. While the other two forest types, both very close to each other in terms of biodiversity, fall short. The Simpson index concludes a high biodiversity in both the natural regenerated and the old growth forest. From these results, it can be concluded that there is a significant difference in macrofungal diversity between the forest types. With the natural regenerated, young growth forest containing the highest diversity. This bellies the hypothesis: *“The macrofungal diversity and abundance is higher in the primary, old growth forest.”*

The R² value of the pH-diversity correlation gives a correlation of 52%. However, when looked at the correlation of the macrofungal *abundance* and soil pH, the R² value is only 29%. This being a lot lower, it still can be stated that for both diversity and abundance, there is a significant, negative correlation with the soil pH. For canopy coverage and macrofungal diversity-and abundance, no correlation is found. This might be due to fault in the methodology, it is thought that, to get an accurate representation of the canopy coverage, more pictures per plot are needed. Due to lack of time this was not possible.

The old growth forest does not have a higher macrofungal diversity but does have notably higher macrofungal abundance. In all forest types, the majority of macrofungi species grow on wood (53%). This being said, It would be helpful to investigate the role dead wood plays for macrofungal diversity and abundance.

The results indicate that the macrofungal diversity of the natural regenerated, secondary forest higher is than expected. This can indicate that its resilience is high. Meaning that the biodiversity re-established itself within a relative short period of time. For this claim to be made is more research needed. The result can also be because of external factors that are not included in this research, like; soil temperature, soil humidity, dead wood, weather, altitude, or parent material or due to limited sample size.

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APPENDIX

PICTURES UNIDENTIFIED SPECIES







SPECIES LIST

Apioperdon pyriforme
Armillaria mellea
Armillaria ostoya
Ascobolus sp.
Asterophora lycoperdoides
Astraeus hygrometricus
Auricularia auricula-judae
Auricularia auricularia-judae
Biscogniauxia marginata
Bolbitius vitenillus
Calocera cornea
Calocera viscosa
campanophyllum proboscideum
Cantharellus subalbidus
Cavicipitaceae
Cerocorticium confluens
Chondrostereum purpureum
Clavaria zollingeri
Cookeina venezuelae
Cortinarius iodes
Crinipellis scabellus
Cyatus poeppigi
Earliella scabrosa
Echinoporia aculeifera
Entoloma murrayi
entoloma sericeum
entoloma sp.
Exidopsis calcea
Galerina hypnorum
Galneria sp.
Ganoderma lingzhi
Geoglossum glutinosum
Granoderma sp.
Gymnopus dryophilus
Gymnopus lodgeae
Hygrocybe acutoconica
Hygrocybe conica
Hyphoderma occidentale
Hypoderma sp.
Hypoxylon multifforme
Irpex lacteus
Junghuhnia sp.
Lycoperdon pyriforme
Marasmius berteroi
Marasmius perlongispermus
Marasmius siccus
Marasmius sp.
Mucronella calva
Mycena acicula
Mycena californiensis
Mycena Californiensis
Mycena galericulata
Mycena inclinata
mycena leptcephala
Mycena margarita
Mycena sp. (dark grey)
Mycena sp. (light grey)
Mycena sp. (white)
Omphalina pyxidata
Peniophora sp.
Physisporinus vitreus
pleurotus pulmonarius
Podostroma alutaceum
Podostroma cornu-damae
Polyporus versicolor
Prenectria oligospora
Psathyrella gracilis
Pseudohydnum gelatinosum
Ramaria zippelii
Rickenella fibula
Schizophyllum amplum
Sebacina concrescens
Steccherinum ochraceum
Stereum hirsutum
Stereum subtomentosum
Strobilurus conignoides
Strobilurus sp.
Terana caerulea
Trametes versicolor
Tremella mesenterica
Trichoglossum hirsutum
Tyromyces kmetii
unidentified sp
unidentified sp.
Xerula hispida
Xylaria culleniae
Xylaria hypoxylon
Xylaria polymorpha