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CIIDIR OAXACA

VARIATION IN DIVERSITY AND COMPOSITION OF MACROFUNGAL SPECIES ALONG AN ENVIRONMENTAL GRADIENT IN TEMPERATE AFFINITY FORESTS OF OAXACA, MEXICO.

Variación en la diversidad y composición de especies macrofúngicas a través de un gradiente ambiental en bosques de afinidad templada de Oaxaca, México

MAESTRÍA EN CIENCIAS EN CONSERVACIÓN Y APROVECHAMIENTO DE RECURSOS NATURALES

PRESENTA

Brenda Noemi Pérez Rosas

DIRECTOR DE TESIS:

Dr. Marko Aurelio Gómez Hernández

Santa Cruz Xoxocotlán, Oaxaca.

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INSTITUTO POLITÉCNICO NACIONAL
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ACTA DE REGISTRO DE TEMA DE TESIS
Y DESIGNACIÓN DE DIRECTOR DE TESIS

Ciudad de México, 13 de diciembre del 2020

El Colegio de Profesores de Posgrado de CIIDIR Unidad Oaxaca en su Sesión
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ordinaria No. 10 celebrada el día 9 del mes noviembre de 2020, conoció la solicitud presentada por el (la) alumno (a):

Apellido Paterno:	Pérez	Apellido Materno:	Rosas	Nombre (s):	Brenda Noemí

Número de registro: A 1 9 0 0 0 7

del Programa Académico de Posgrado: Maestría en Ciencias en Conservación y Aprovechamiento de Recursos Naturales

Referente al registro de su tema de tesis; acordando lo siguiente:

1.- Se designa al aspirante el tema de tesis titulado:

"Variación en la diversidad y composición de especies macrofúngicas a través de un gradiente ambiental en bosques de afinidad templada de Oaxaca, México"

Objetivo general del trabajo de tesis:

Determinar la posible variación en diversidad y composición de especies de macrohongos en relación a variables microclimáticas y ambientales

2.- Se designa como Directores de Tesis a los profesores:

Director: Dr. Marko Aurelio Gómez Hernández 2° Director:

No aplica: X

3.- El Trabajo de investigación base para el desarrollo de la tesis será elaborado por el alumno en:

CIIDIR Unidad Oaxaca

que cuenta con los recursos e infraestructura necesarios.

4.- El interesado deberá asistir a los seminarios desarrollados en el área de adscripción del trabajo desde la fecha en que se suscribe la presente, hasta la aprobación de la versión completa de la tesis por parte de la Comisión Revisora correspondiente.

Dr. Marko Aurelio Gómez Hernández
Director(a) de Tesis

2° Director de Tesis (en su caso)

Pérez Rosas Brenda Noemí
Aspirante

Dr. Salvador Isidro Belmonte Jiménez
Presidente del Colegio

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En la Ciudad de Santa Cruz Xoxocotlán, Oax., siendo las 12:00 horas del día 04 del mes de febrero del 2021 se reunieron los miembros de la Comisión Revisora de la Tesis, designada por el Colegio de por el Colegio del Centro de Investigación Interdisciplinario para el Desarrollo Integral Regional, unidad Oaxaca (CIIDIR UNIDAD OAXACA) para examinar la tesis titulada:

"Variación en la diversidad y composición de especies macrofúngicas a través de un gradiente ambiental en bosques de afinidad templada de Oaxaca, México"

de la alumna:

Apellido Paterno:	Pérez	Apellido Materno:	Rosas	Nombre (s):	Brenda Noemí

Número de registro: A 1 9 0 0 0 7

Aspirante del Programa Académico de Posgrado: **Maestría en Ciencias en Conservación y Aprovechamiento de Recursos Naturales**

Una vez que se realizó un análisis de similitud de texto, utilizando el software antiplagio, se encontró que el trabajo de tesis tiene 7 % de similitud. **Se adjunta reporte de software utilizado**.

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El trabajo aporta información novedosa sobre el conocimiento de la diversidad de macrohongos y su asociación con variables ambientales.

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CARTA CESIÓN DE DERECHOS

En la Ciudad de ____Oaxaca_____ el día __05__ del mes de ____febrero_____ el año _2021___, la que suscribe __Brenda Noemi Pérez Rosas_____ alumna del Programa de Maestría en Ciencias en Conservación y Aprovechamiento de Recursos Naturales con número de registro _A190007__, adscrita al Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Oaxaca, manifiesta que es autora intelectual del presente trabajo de Tesis bajo la dirección del _Dr. Marko Aurelio Gómez Hernández_ y cede los derechos del trabajo titulado: **"Variación en la diversidad y composición de especies macrofúngicas a través de un gradiente ambiental en bosques de afinidad templada de Oaxaca, México"** al Instituto Politécnico Nacional para su difusión, con fines académicos y de investigación.

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CONTENT

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Variation in diversity and composition of macrofungal species along an environmental gradient in temperate affinity forests of Oaxaca, Mexico.

Abstract

Diversity into macrofungal communities is tightly related to environmental gradients and changes in microclimatic conditions, being humidity and temperature the most studied variables. However, there is a lack of ecological studies assessing patterns of macromycete diversity along environmental gradients. In this study, alpha and beta diversity were evaluated in macrofungal communities and it was determined if environmental factors were related to the variation of diversity along the study area. The study was carried out in Ayoquezco de Aldama, Oaxaca, four sites were selected in oak-pine, pine-oak and pine forests, and ten permanent 10 x 10 m plots were stablished in each site. Macromycetes were recollected from june to november 2019, recording abundance and species richness. Alpha diversity was measured with the number of species and the True Diversity index, whereas beta diversity was measured using the Chao-Jaccard index.

Air and soil temperature and humidity, canopy openness, aspect, slope and vegetation variables were measured. 617 organisms were recollected, grouped into 187 species, 60 genera, 42 families, 22 ascomycete species and 165 basidiomycetes. The most abundant species were *Russula emetica*, *Craterellus lutescens*, *Dacrymyces* aff. *chrysospermus* and *Gymnopus* aff. *androsaceus*. Oak vegetation showed the highest macromycete diversity, and similitude of macromycete species, followed by sites with pine and pine-oak vegetation. Macromycete richness was mainly related to aspect, slope, and canopy openness.

Results in this study show how the composition of macrofungi species varies as a function of environmental changes; evidencing how important is to understand these relations in the face of environmental problems that could cause the loss of species.

Resumen

En México, Oaxaca es reconocido por su alta biodiversidad. La posición al sureste de las zonas tropicales en el continente americano, lo ha provisionado de una gran variedad de climas, tipos de vegetación y especies de hongos. Aun así, la información acerca de los hongos es escasa, así como estudios de diversidad macrofúngica (Ver: Ramírez, 2017 y Avedaño, 2019). El objetivo de este estudio fue evaluar la diversidad alfa y beta de especies macrofúngicas en bosques de afinidad templada y determinar cómo las variables microclimáticas/ambientales se relacionan con los patrones observados a lo largo del gradiente ambiental. El estudio tuvo lugar en Ayoquezco de Aldama, Oaxaca, se seleccionaron cuatro sitios de estudio en bosques de pino-encino, encino-pino y pino, y establecieron parcelas permanentes de 10X10m en cada sitio. Los macrohongos se recolectaron de Junio a Noviembre de 2019, registrando abundancia y riqueza de especies. La diversidad alfa se midió con el número de especies y el índice de Diversidad Verdadera, mientras que la diversidad beta se calculó con el índice de similitud Chao-Jaccard. Se midieron valores de variables de temperatura de aire y suelo, humedad, abertura del dosel, orientación, pendiente, y variables de vegetación. Se registraron 617 individuos, agrupados en 187 especies, 60 géneros, 42 familias, 22 especies de Ascomicetos y 165 Basidiomicetos. Las especies más
abundantes fueron *Russula emética*, *Craterellus lutescens*, *Dacrymices* aff. *chrysospermus* y *Gymnopus* aff. *androsaceus*. La vegetación de encinos mostró los valores más altos de diversidad de macromicetos y mayor similitud en composición de especies, seguidos por los sitios con vegetación pino y pino-encino. La riqueza de macrohongos se relacionó principalmente con la pendiente, orientación de las parcelas y con la abertura del dosel.

2. Introduction

It has been estimated ca. 5.1 million of fungal species worldwide (Blackwell, 2011), and about 53,000 to 111,000 are estimated to be macrofungal species (characterized by the production of fruit bodies visible to the naked eye) (aMueller et al. 2007). In Mexico, estimations suggest more than 250,000 species of fungi (Guzmán, 1998), and approximately 9,000 to 11,000 of macrofungi (Aguirre-Acosta et al., 2014). Within most terrestrial ecosystems, macrofungi play a main role owning to their interaction with other organisms and abiotic environments of relevance in natural processes (Dighton, 2006) like carbon cycles (Harley, 1971). Also, macromycetes relates with other groups (mainly plant species) as pathogens, where they are detrimental to plant growth and fitness (Termorshuizen, 2014); mutualists, like mycorrhizae helping plants to obtain nutrients and providing defense; and endophytes, improving the absorbance of soil nutrients by plants (Rodríguez et al., 2009).

There is an lack of species inventories and studies on the relationship between the diversity and distribution of macrofungi and environmental factors (Braga-Neto et al,2008, Brown et al, 2006), so the current understanding of fungal species diversity, community structure, and dynamics remains limited (Ferrer and Gilbert, 2003).

Studies suggest that the structure of macrofungal communities along environmental gradients is tightly related to the range sizes of plant distribution (bMueller et al., 2007), changes in vegetation structure, climatic and microclimatic conditions (Gómez-Hernández and Williams-Linera, 2011) as precipitation (Salerni et al., 2002) humidity, temperature (Durall et al., 2006), but this relationships has been scarcely studied (Zhang et al. 2010).

Due to the importance of macromycetes, understanding the factors related to fungal diversity and distribution could be helpful in ascertaining which areas are supporting high diversity or a unique group of fungal species (Gomez-Hernandez et al, 2012), and evaluate the effectiveness of macrofungal species in ecosystem functioning both in natural or urbanized environments (Packham et al., 2002). To assess

biodiversity, alpha (punctual diversity in a site or a particular community) and beta diversity (the turnover of species composition among sites or communities) (Whittaker, 1972) have been used, together these metrics measure the overall heterogeneity of communities in an area (Wilson and Shmida, 1984), and can be compared in spatial and temporal scales (Veech and Crist, 2010).

In Mexico, Oaxaca is recognized for its broad biodiversity. The southern position of tropical zones in the American continent have provided them a variety of climates, vegetation types, and fungal species. Nevertheless, information about fungi is scarce in this region, as well as diversity studies of macromycetes (see Ramirez, 2017 and Avedaño, 2019). The objective of this study was to evaluate the alpha diversity and turnover of macrofungal species in temperate affinity forests and determine how microclimatic variables relate to the observed patterns along the environmental gradient.

3. Methods

3.1 Study area

This study was carried out in Ayoquezco de Aldama, located at 16°41N and 96°47W, in the politic district of Zimatlan de Alvarez, Oaxaca de Juarez, Mexico.

Ayoquezo is located approximately at 1598 masl, seasonally defined with a dry season from March to April and a rainy one from May to October. Its climate goes from warm to temperate with short annual thermal oscillation. Mean annual temperature of 18.9 °C and precipitation of 1409 mm.

The flora in Ayoquezco is represented mainly by oak-pine forests, coniferous forests, and the presence of tree species like the “cuatle”(*Eysenhardtia polystachya*), ”cuachepil” (*Senna septemtrionali*), “tepehuaje” (*Lysiloma acapulcensis*), “cazahuate” (*Ipomea murucoides*), “enebro” (*Juniperus communis*), “sabino” (*Taxodium huegelii*) and “cedro”(*Cedrela odorata*).

3.2 Study sites

Four sites were selected: one site corresponding to oak forests, two for pine-oak forest, and one site in pine forest. In each site there were established 10 permanent plots of 10X10 m, with a separation of about 10 m between plots and 30 m from the forest edge to avoid “edge effect”.

3.3 Macromycete sampling

From june to november 2019, the macromycete samplings were conducted by visiting once a month every study site. It was recorded as one individual organism the sporomes of the same species inside a diameter of 50 cm, and the sporomes growing on the same log or branch. The collected samples were described when fresh by their macro and morphological characteristics, and the identification was based on macro and micro morphological characteristics, searching to reach the higher taxonomic level as possible following keys and reference guides (Guzmán,1977, Largent, 1986, Læssøe, T., and Petersen, J. H. 2019). Species that could not be identified were referred as a morphospecies.

3.4 Microclimatic and environmental variables

Once at each plot, data of altitude, slope and aspect was recorded. Every sampling date in each plot, the air temperature and air humidity were measured using a digital thermohydrometer, the soil temperature was recorded using a soil thermometer. To get soil humidity values, a sample of 200g was taken to calculate the humidity percentage from the difference between fresh and dry weight.

Vegetation structure was characterized at the end of the macromycete sampling period to lessen the disturbance in each plot. It was measured diameter, tree height, and number of woody plants > 10 cm of diameter at breast height (DBH). The variables calculated for vegetation structure were density of trees, average height of trees, and basal area.

3.5 Diversity and species turnover

Alpha diversity of macromycetes in each site was measured with the True Diversity index of first order (qD), (Jost, 2006) and the species richness (number of species). The true diversity index was calculated in R version 3.2.3 (R Core, Team, 2017).

Beta diversity between sites was measured as the turnover of species composition using the Chao-Jaccard non-parametric similitude index, where values close to 1 indicate a higher similitude in species composition and values close to 0 indicate lower similitude. (Chao et al., 2005). The index was calculated using EstimateS (Colwell, 2013).

A cluster analysis based on presence-absent data was carried out into R (R Core, Team, 2017) aiming to localize the similarities in species composition among sites

3.6 Data analyses

Spearman correlation coefficient was performed to identify the microclimatic/environmental variables related to the variation in macromycete diversity. A regression tree was used to determine how these variables affect the variation of diversity among sites.

The relationship between the geographic distance and changes in species composition was calculated using linear regression analyses. A Non-metric

multidimensional scaling (NMDS) was calculated to visualize the distance between each study site according to species composition. The distribution of species in relation to the explanatory variables in each community was determined with a Canonical Correspondence Analysis (CCA).

All the data analyses were carried out in R version 3.2.3 (R Core Team, 2017).

4. Results

A total of 617 fungal individuals were collected, grouped into 187 species, 60 genus, 42 families, which corresponded to 22 ascomycetes and 165 basidiomycetes. The highest number of species was recorded in Site 3, followed by Site 1, Site 2 and Site 4 (Figure 1).

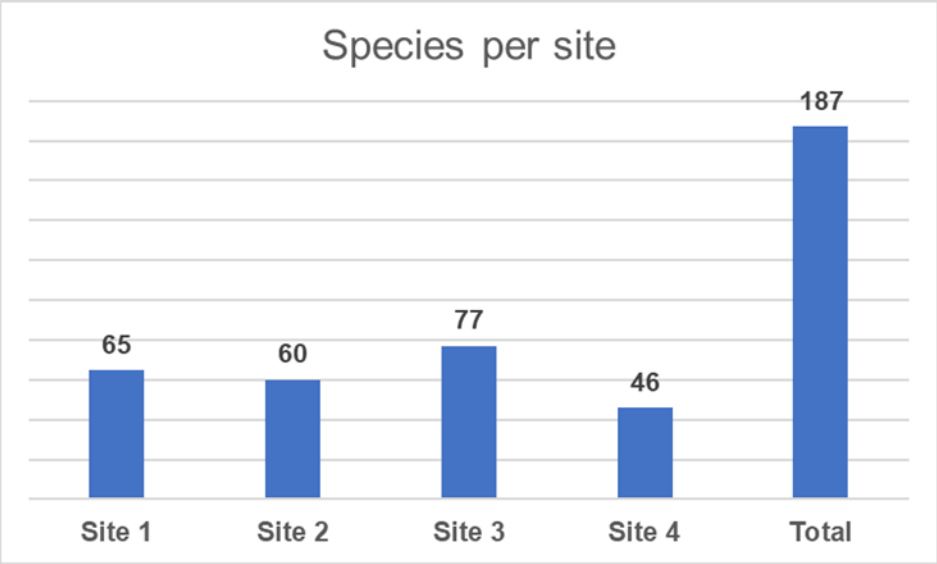

Figure 1 Number of species/morphotypes per study site

The most abundant species were *Russula emetica* (33), *Craterellus lutescens* (27), *Dacrymyces* aff. *chrysospermus* (27), *Gymnopus* aff. *androsaceus* (25), *Mycena* sp.1 (23) and *Gymnopus* sp2. (21) (Figure 2).

The true diversity index (Table 1) showed that Site 1 had the highest value of diversity, followed by Site 3, Site 2 and Site 4.

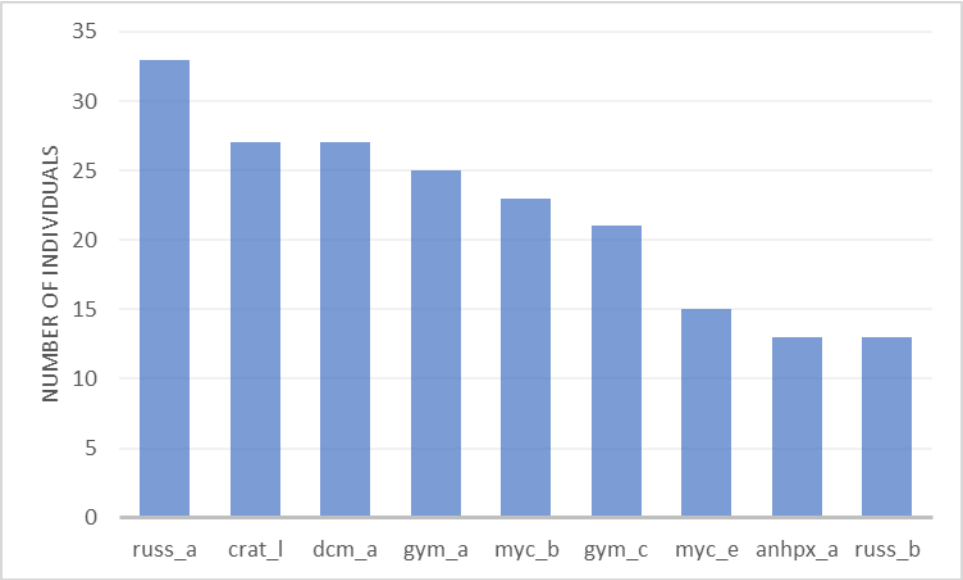

Figure 2 The most abundant species: *Russula emetica* (russ_a), *Craterellus lutescens* (crat_l), *Dacrymyces* aff. *chrysospermus* (dcm_a), *Gymnopus androsaceus* (gym_a), *Mycena* sp. (myc_b), *Gymnopus* sp. (gym_c), *Mycena epipterygia* (myc_e), *Annulohypoxylon thouarsianum* (anhpx_a) and *Russula brunneoviolacea* (russ_b)

Table 1 True diversity of order one (q=1) in each study site.

True diversity	Site 1	Site 2	Site 3	Site 4
q=1	49.83028	36.56908	40.85818	31.17056

Spearman correlations coefficients were statistically significant only for aspect and canopy openness, and the correlation was negative for both variables (Table 3).

Table 2. Spearman correlations coefficients (*p*) between species richness of macromycetes and the explanatory variables. Only significative values are shown.

Variable	*p*	*p*-value
Aspect	-0.4307	0.0055
Canopy openness	-0.432175	0.0053

The Chao-Jaccard abundance-based similarity index Indicated that the species similarity was higher between site 1 and 2, and site 1 and 3 were the less similar (Table 3).

The cluster dendrogram supports the results of Chao-Jaccard similarity index by showing the site 1 and 2 as the sites with the greatest similarity in species composition, followed by site 4, and finally site 3 as the least similar (figure 3).

Table 3 Chao Jaccard abundance-based similarity index.

Pair of sites		**Chao-Jaccard abundance-based similarity**
1	**2**	0.36
1	**3**	0.089
1	**4**	0.136
2	**3**	0.218
2	**4**	0.166
3	**4**	0.260

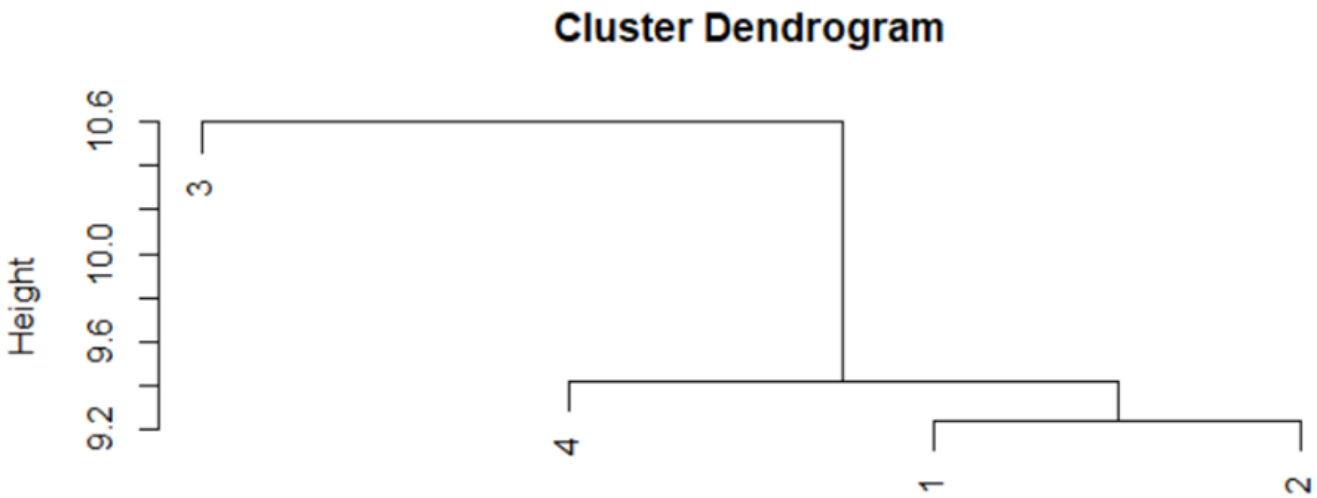

Figure 3 Cluster dendrogram. Presence-absence data for each site.

NDMS showed that site 1 and 2 are closer with regard to the species composition while Site 2 and 4 are the farthest.

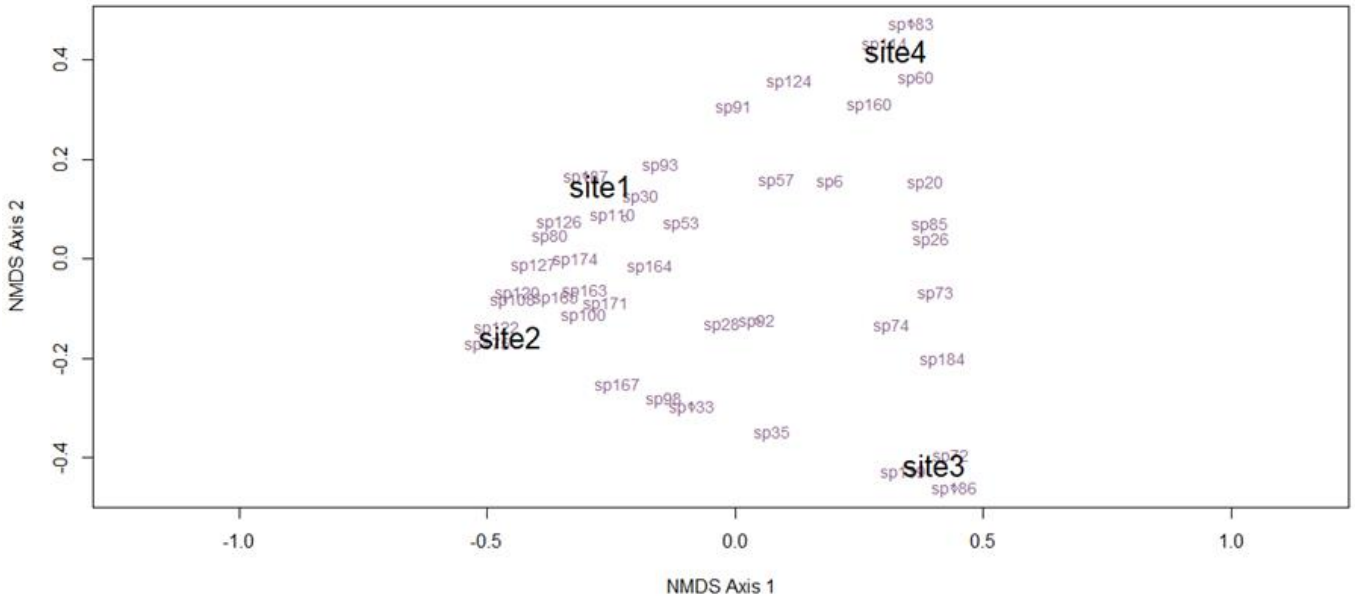

Figure 4 . Non-metric Multidimensional Scaling analysis for macrofungal species complementarity between the four study sites.

Regression tree analysis (residual mean deviance = 40.05) indicated that from a total of 40 plots, species in 42.5% of the plots were influenced by air humidity (AirH) when it was <48.85%, of these samples, 52.99% were influenced by canonpy openness (CanopyOP) <27.56, the other 47.05% were influenced when values were >27.56. These represented the terminal node on the left side of the tree.

When AirH was >48.85%, it influenced the species in 57.5% of the total plots (23 plots). Of this total, values of air temperature >20.983, only were affecting the 26.08% while the 73.91% of the 23 plots were affected by air temperature (AirT) when it had values <20.9833. Soil humidity (SoilH) was affecting the 52.94% and 47.05% of the previous total, when the values were <35.41, and >35.41, respectively.

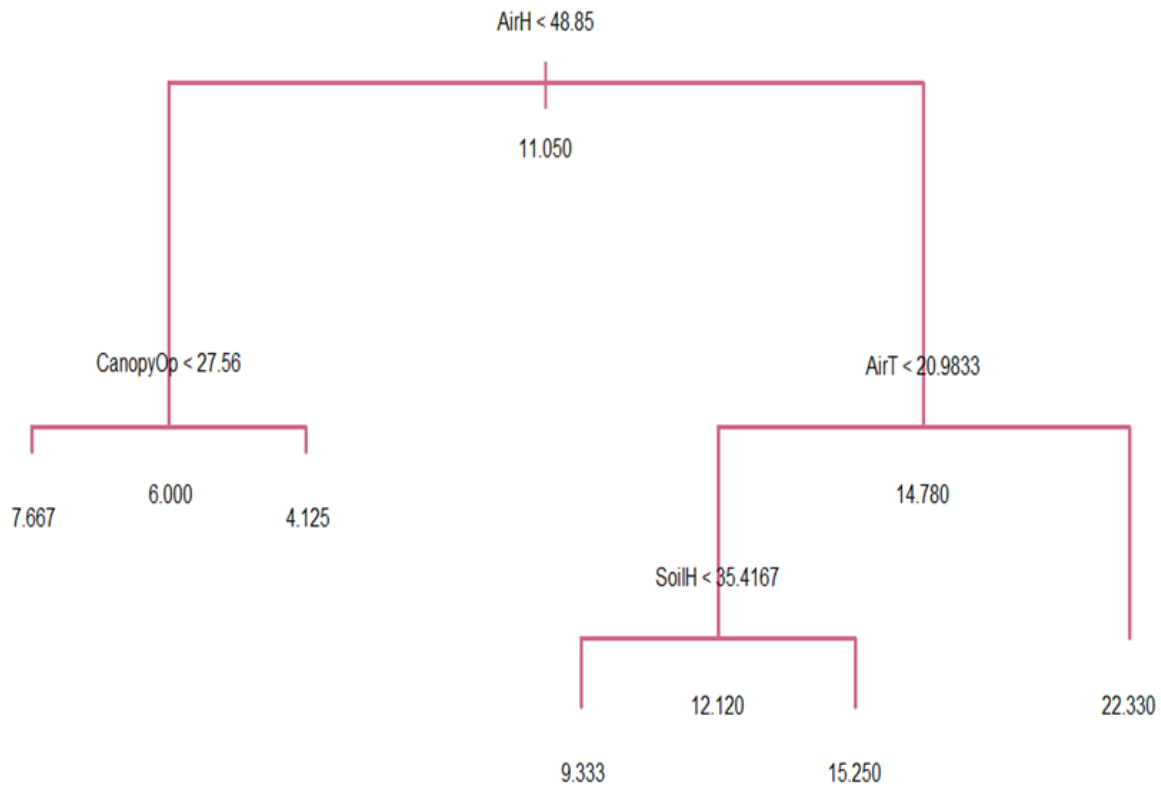

Figure 5. Regression tree. Each partition shows the explanatory variable and threshold at which the partition was made, and the average value of variable effect is indicated at the tips and nodes. Variables are air humidity (AirH), canopy openness (CanopyOp), air temperature (AirT), soil humidity (SoilH).

The CCA of microclimatic and environmental variables was carried out for the 187 macromycete species. The model showed that site 1 and 2 are separated from sites 3 and 4 by the Axis 1 (eigenvalue= 0.7151) and site 3 is separated site 4 by Axis 2 (eigenvalue=6393). Axis 1 (14.85%) and Axis 2(12.83%) represented a cumulative proportion of 27%.

Explanatory variables: AirH (relative air humidity), BA (basal area), MaxH(maximum height), MeanH (average height), SoilH (soil humidity), Slope, CanopyOP (canopy openness), Aspect, SoilT (soil temperature), AirT (air temperature) are displayed as vectors.

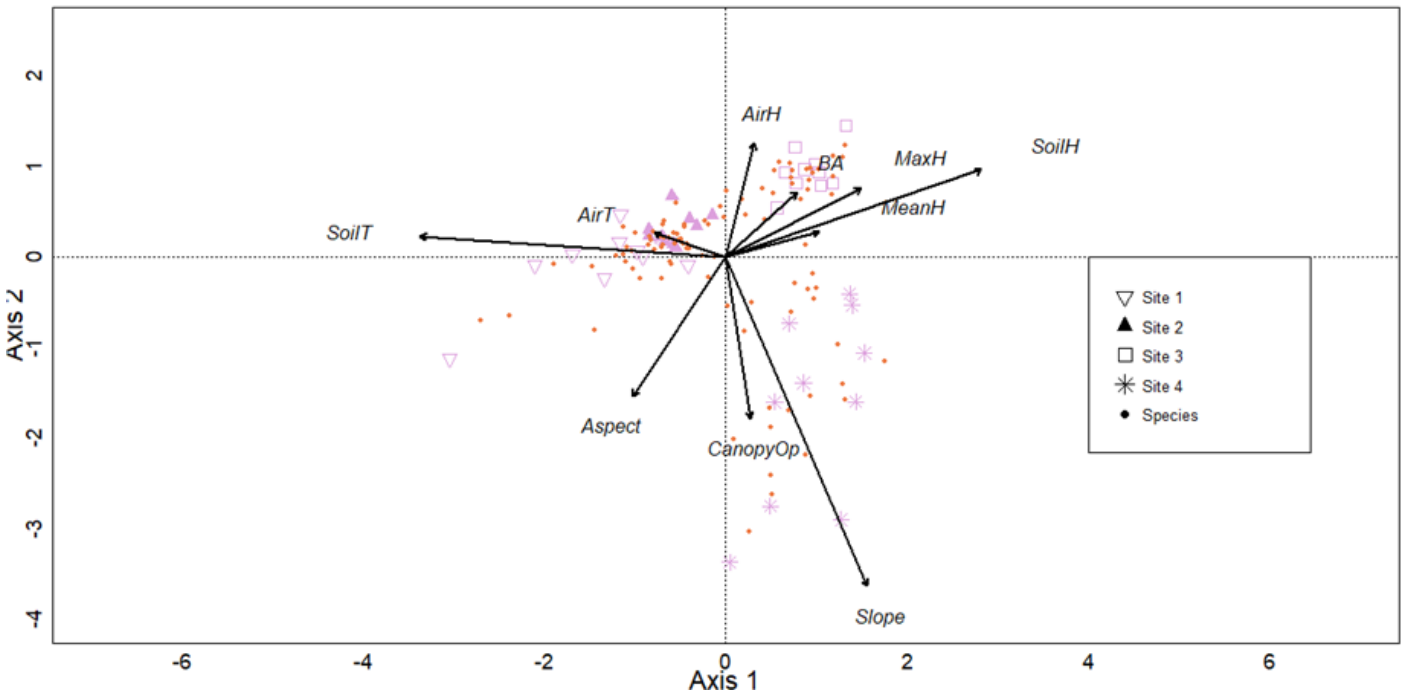

Figure 6. CCA for all recorded macromycetes in study sites Explanatory variables: AirH (relative air humidity), BA (basal area), MaxH(maximum height), MeanH (average height), SoilH (soil humidity), Slope, CanopyOP (canopy openness), Aspect, SoilT (soil temperature), AirT (air temperature)

5. Discussion

In temperate affinity forests, Ectomycorrhizal (EMC) are form by symbiosis with the majority of trees (Goldmann et al, 2015) and most ectomycorrhizal fungi belong to the Basidiomycota class (Clasen et al, 2018), agreeing to our results that shows the basidiomycetes as the most abundant group compared to the ascomycetes. *Russula emetica*, *Craterellus lutescens*, and *Gymnopus* sp. were the most abundant species in our study, agreeing with other studies reporting them among the most abundant ectomychorrizal species found in pine, and pine-oak vegetation. (Rodríguez-Alcantar et al, 2019, Garibay-Orijel et al., 2009)

It has been observed that macromycetes species richness and diversity are mainly related to environmental and microclimatic variables, as well as vegetation structure and woody plant composition along environmental gradients that acts as traits filters (Caiafa et al., 2017; Crowther et al., 2014). Our results from regression tree show that macrofungal species respond to changes in soil moisture. Water content into

soil should not be excessive; it has been reported that excessive moisture can inhibit the macrofungi fructification (O'Dell et al, 1996; Lodge et al., 2004). The air humidity has been reported with a positive effect on fructification, mostly when it is higher than 82% (Shuhada et al.2020), but our results showed macrofungi to be favored with relative air humidity >49%. The air temperature is also related to macrofungi species. It has been reported that species decrease when temperature is above 27°C while the occurrence of most fungi, is found at mean temperature air values (Ghate and Sridhar, 2016, Jang and Hur, 2014)

Canopy openness showed a relevant role on macrofungal diversity due to the positive effect of shading by the canopy surrounding trees into macromycete fructification (Kubartová et al, 2012). Less canopy openness can result in more litter on forest floor, thus providing additional habitats and resources for fungal assemblages (Gabel and Gabel, 2007), meanwhile an open canopy can increase soil exposure to sunlight, increase the soil temperature and decrease soil moisture (Ford et al., 2018) which is not favorable for macrofungal species production.

Aspect was found to be positive and significantly related with macrofungal diversity. It has been observed that factors linked to the topography in a landscape (e. g. soil surface unevenness, slope, aspect) can influence the drainage and evaporation rate of water, wind exposure of the terrain affecting fruit body production and species richness (Gomez-Hernández et al. 2012, Rubino and McCarthy 2003, Nantel and Neumann, 1992).

Chao-Jaccard similarity index, NDMS, and the cluster showed high species turnover between studied sites and the highest species similarity among oak forests. Although geographic distance has been suggested to be related with species turnover between macromycete assemblages, our analysis showed that there is not statistical correlation between species turnover and distance, so the difference could be given by environmental factors (Brown et al., 2006). This environmental and microclimatic values along the gradient drove macrofungal species distribution. Each site of study shows heterogeneous characteristics, in the ordination analysis (CCA) soil temperature, slope, canopy openness, and tree maximum height were the main

factors driving macromycete distribution along the study area. Macromycete distribution is limited by high soil temperatures, primarily for fungi forming mycorrhizae (Parke et al., 1983, Cabtree, 2010). Also, vegetation structure and tree species composition in mature forest are more productive in macrofungi fruti bodies that the young ones (Abrego and Salcedo 2013, Pavithra et al., 2016).

Since the species composition of macrofungi is determined by multiple factors, a sampling year can be considered an underestimation of true diversity and abundance (Ford, 2018). However, this study contributes to macrofungi ecological information associated with temperate forests in Oaxaca. Results can be used to find out the ecosystem's quality and determine the changes in the ecological dynamics (Rojas et al., 2017). Also, the information obtained could be useful in conservation decisions and management recommendations (Dejene et al., 2017) by identifying sites with the richest species, or sites where the distribution of macromycetes is limited (Gabel, 2007). It is recommended improving this kind of ecological studies by increasing sampling efforts and sampling dates, also it is suggested to study patterns of taxonomic, functional, and phylogenetic diversity, as well as carrying out genetic analyses along spatial and temporal scales to explain multiple ecological phenomena (Naeem et al., 2012)

The climate change problematic into temperate forests can be translated in an accelerated decline of macrofungal communities (Jang and Hur, 2014), affecting ecosystem functioning.

6. Conclusions

This study has attempted to state how vulnerable the macromycete communities are to environmental changes, and the importance of conserving forests to avoid disturbing the functioning of ecosystems. The results clearly showed that macromycete species diversity and composition can conspicuity change even in short distances due to the variation of local environmental factors as a function of the large array of habitats and resources provided by the woody plants. It should be of interest in future studies to assess how this variation affects the different functional groups along an environmental gradient. Also, including variables related to soil

characteristics could be useful to better understand the observed patters of diversity and distribution.

Increasing the number of samplings, and number of sites could be a way to improve and strengthen ecological studies on macrofungal communities, also complement this kind of studies with other tools as molecular analysis. Improving and increasing the studies could be useful to take conservation and management decisions of macromycetes species, facing to many environmental problems that could cause the decline of species and diversity.

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