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Quercus humboldtii as a study case in the tropical Andes**

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Tesis, presentada como requisito para obtener el título de:
(Biólogo)

Directora
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How ectomycorrhizal communities vary from natural to urban ecosystems: *Quercus humboldtii* as a study case in the tropical Andes

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Abstract

Urban landscapes are becoming more important due to the accelerated population growth and increasing urbanization. Urban ecosystems serve as home to diverse plant and fungal communities. However, studies focusing on the diversity and structure of biological communities are uncommon in this habitat. In Colombia, *Quercus humboldtii* Bonpl. is a conspicuous ectomycorrhizal species present in tropical montane forests that hosts a high diversity of ectomycorrhizal fungi in its roots. *Quercus humboldtii* is commonly used as an urban tree in Bogotá, but the ectomycorrhizal communities of this species have not yet been studied in urban ecosystems. We studied how the ectomycorrhizal fungal communities associated with this tree change between natural and urban ecosystems. Roots of 24 trees were sampled in two sites, Chicaque Natural Reserve (natural) and three neighborhoods of Bogotá city (urban). Using Illumina sequencing, the ITS1 region of all root associated fungi was amplified and analyzed using both OTUs and ASVs bioinformatics pipelines. We found 949 OTUs in Bogotá and 514 OTUs in Chicaque. We didn't find significant differences in the species richness between Bogotá and Chicaque sites based on Fisher's alpha or species-accumulation curves. In Chicaque communities, the most abundant genera were *Russula* and *Lactarius*, while *Scleroderma*, *Hydnangium*, and *Trechispora* were highly abundant in Bogotá. An NMDS analysis showed that samples from the natural site had a significantly different community composition compared with urban trees. Our results highlight the importance of *Quercus* trees as reservoirs of ectomycorrhizal fungal diversity in Bogotá.

Keywords Ectomycorrhizal fungi · *Quercus humboldtii* Bonpl. · Community structure · Rural vs Urban communities

Introduction

Mycorrhizas are associations between specialized soil fungi and the roots of plants (Brundrett 2002) where the host plant supplies the mycorrhizal fungi with carbohydrates and receives nutrients in return (Taiz et al. 2015). These associations also help protect plants against pathogens and biotic and abiotic stress conditions (Halling 2001). The two main types of mycorrhizal associations are arbuscular mycorrhizas (AM) and ectomycorrhizas (ECM). Arbuscular mycorrhizal associations are widespread worldwide and are the most ancient type of mycorrhiza (Brundrett 2002). Ectomycorrhizas are characterized by an external mycelial layer called the mantle (that covers the surface of root tips) and by the Hartig net (that are hyphae that grow between the root cortical cells) (Halling 2001). In contrast with AM, evolution of ECM is more recent and this association is predominant in boreal, temperate and some tropical forest ecosystems (Steidinger et al. 2019).

The richness and abundance of ECM fungi in tropical forests usually have its peak in montane forests. These forests are at medium elevations and have high levels of precipitation, intermediate temperatures, and high density of ECM host trees (Corrales et al. 2018). Due to their enzymatic capabilities and high growth rates, ECM fungi can control some ecosystem processes like soil carbon storage and soil nutrient availability (Corrales et al. 2018; Vasco-Palacios et al. 2019). However, the ecology and ecosystem function of tropical ECM fungi are not fully understood.

Urban landscapes are becoming more important as a habitat for wild species due to the accelerated population growth and increasing urbanization. An important part of these urban landscapes are urban ecosystems, understood as those areas of vegetation, such as parks, gardens, and road separators found in cities (Stevenson et al. 2020). These ecosystems serve as home to plant and fungal communities, among other groups, however, it is a hostile environment due to factors such as soil compaction, air pollution and limitation of root development (Olchowik et al. 2020). Additionally, other human interventions can alter fungal community composition and structure directly, influencing plant communities or soil via fertilization or watering (Baruch et al. 2020).

Few studies had focused on the structure of fungal communities in urban ecosystems; and even for ECM fungal communities there is little available information considering that ECM plants are common in urban areas (Stevenson et al. 2020).

Soil variables, such as levels of phosphorus (P), nitrogen (N), and N:P ratio, are factors that can affect soil fungal communities. A study by Baruch et al. (2020), about the composition and structure of soil fungal communities within different urban ecosystems (sports fields, community gardens, parklands, and young/old revegetation) in Australia, found that soil fungal diversity and community structure differed among urban ecosystems due to differences in soils, vegetation, and management practices. In addition, a study focusing on ECM communities associated with *Quercus spp.* in rural and urban sites in Manhattan (Kansas, USA), found that edaphic variables like P, levels of heavy metals, and organic matter were the most influential factors in the composition of the ECM communities (Jumpponen et al. 2010). Also Olchowik et al. (2020) in a study in Warsaw (Poland) found that ECM richness didn't differ between healthy trees growing in parks or streets. However, they found that healthy trees growing in streets and parks have a significantly higher ECM richness and colonization than unhealthy trees growing in the same streets. They also found that concentrations of Na, Cl, and Pb in the soil have a negative effect on the ECM colonization (Olchowik et al. 2020).

Quercus is a genus with approximately 500 species of trees and shrubs with a global distribution, mainly in the temperate northern hemisphere. being a conspicuous member of the forests of North America, Europe, and Asia (Manos et al. 1999). This genus has great ecological and economic importance because it is a source of food and habitat for wildlife, and can have socio-economic benefits such as the provision of wood and food for human communities (Aldrich & Cavender-Bares 2011; Pagano & Lugo 2019). *Quercus* forms associations with ECM fungi that contribute to the plant's nutrition and intervene in soil biogeochemical processes.

There are few studies focusing on *Quercus* ECM fungal communities in the tropics (García-Guzmán et al. 2017; Morris et al. 2008; Waring et al. 2016). In dry tropical forests dominated by *Quercus spp.* in Costa Rica, Desai et al. (2016) found a high diversity of ECM fungi, dominated by Russulaceae and Thelephoraceae and with a low prevalence of Ascomycota. These results are

similar to those obtained by Morris et al. (2008) who found that the ECM fungal communities in a tropical forest of Mexico were dominated by the families Russulaceae, Cortinariaceae, Inocybaceae, and Thelephoraceae. In Colombia, *Quercus humboldtii* Bonpl. is a conspicuous species present in montane forests and is the most abundant ECM host tree in the country (Rendón 2020). Several studies based on fruiting body collection had shown that *Q. humboldtii* hosts a high diversity of ECM fungi. From the 172 species of ECM fungi reported for Colombia, 116 are associated with *Quercus* monodominant forest (Pagano & Lugo 2019; Peña-Venegas & Vasco-Palacios 2019). The most species-rich lineages of ECM fungi reported for *Q. humboldtii* forests are: Boletaceae, Amanitaceae, and Russulaceae (Pagano & Lugo 2019; Peña-Venegas & Vasco-Palacios 2019; Vargas and Restrepo 2019).

Due to its beauty and ecological importance, *Q. humboldtii* is commonly used as an urban tree in Colombia. However, the ECM communities associated with this species have not yet been studied in urban ecosystems. This study explores the community composition of root-associated fungi of *Quercus humboldtii* (Fagaceae) using high-throughput amplicon sequencing of the fungal ITS1 region in urban and natural ecosystems. We aim to determine how the composition of the ECM fungal communities associated with *Q. humboldtii* changes with biotic and abiotic variables. Due to gradients of air pollutants, changes on soil variables due to application of fertilizers and presence of pets, and other differences in cultivation techniques of urban trees, we expect to find differences in the composition of ECM fungal species associated with *Q. humboldtii* between natural and urban ecosystem and also among urban sites. We also expect that communities from urban ecosystems will have a greater abundance of fungal taxa adapted to high levels of N pollution.

Methods

Study area

We selected two study sites located in the andean mountain range of Colombia in the Cundinamarca province. The first site was the Chicaque Natural Reserve (4°36'22" N, 74°18'17" W) located in the eastern andean cordillera. The reserve has an extension of 308,88 ha approx. and it is part of the La Playa watershed, located between the municipalities of Soacha and San Antonio (Colparques 2017; Bernal & Guevara 2019). The reserve presents an altitudinal gradient from 2000

to 2720 m.a.s.l., with an average temperature of 14.5 °C, and a bimodal rainfall regime with the highest rainfall between March-May and October-November (Colparques 2017; Rivera and Córdoba 1998). The reserve has a natural *Q. humboldtii* monodominant forest which is characterized by soils with a deep organic horizon (Colparques 2017).

The second study site was the city of Bogotá, specifically sampling was performed in the districts of Puente Aranda (4 ° 36'45 " N, 74 ° 06'24 " W; 4 ° 36'09 " N, 74 ° 06'55 " W) and Teusaquillo (4 ° 40'14 " N, 74 ° 5'35 " W) (Supplementary Material, Fig. S1). Bogotá has 7'181,469 inhabitants and is located at an average altitude of 2640 m.a.s.l (DANE 2019). The average temperature is 14 °C and the annual rainfall distribution has a bimodal regime with an average annual rainfall of 840 mm (IDIGER 2021). The district of Puente Aranda has a higher incidence of air pollutants with a monthly average of 20.24 for NO₂, 17.35 for PM_{2.5}, and of 40.08 for PM₁₀ while in Teusaquillo the monthly averages are slightly lower with 17.93 for NO₂, 15.16 for PM_{2.5}, and 25.9 for PM₁₀ (SISAIRE, <http://sisaire.ideam.gov.co/ideam-sisaire-web/consultas.xhtml?est=8216¶m=NO2>)

In Teusaquillo, the sampled parks are open to the public and the entry of pets is allowed. Street divider samples taken in this locality were collected next to the highway Avenida 50 (AV), with high vehicular traffic that produces high levels of atmospheric pollutants. In the district of Puente Aranda, three of the urban parks sampled were parks where animals are allowed to enter (CSM, PCR, PLC); and from Ciudad Montes Park (PM), a park that has restricted hours for visitors, surveillance, tree maintenance, and pets are not allowed.

Root and soil collection

A total of 24 oak trees were sampled, twenty individuals of *Q. humboldtii* were sampled in Bogotá (urban area) and four were sampled in Chicaque (rural area). Specifically for Bogotá, eight trees were located in urban parks in Puente Aranda, seven trees were located in urban parks of Teusaquillo, and five trees were located in a street divider in Teusaquillo. Ectomycorrhizal roots of the selected oaks were excavated up to 1m from the trunk to the fine roots, making sure that they belonged to the target tree. Fine roots were stored in plastic bags and refrigerated after collection. Each sample was carefully cleaned with distilled water under stereoscope and cut into 2cm long

pieces, 10 pieces (20cm) of root samples from each tree were stored in 2X CTAB buffer in -20°C until DNA extraction. In addition, soil samples were collected under each sampled tree (except for samples collected in Chicaque) and analyzed for texture (% of sand, silt and clay), pH, total nitrogen (%N), available phosphorus (P, mg/Kg), C:N ratio, base saturation percentage (SB), Mg (cmol(+)/Kg), Na (cmol(+)/Kg), K (cmol(+)/Kg), and Ca (cmol(+)/Kg), in the National Laboratory of Soils of the Instituto Geográfico Agustín Codazzi (Bogotá, Colombia).

DNA extraction and amplification

The DNA extraction of the samples was done using the cetyl-trimethylammonium bromide (CTAB) 2× protocol following (Gardes & Bruns 1993). The ITS1 region was PCR-amplified and sequenced on Illumina HiSeq2500 PE250 (Novogene Bioinformatics Technology Co. Ltd., Beijing, China), using the fungal specific primers ITS5-1737F (GGAAGTAAAAGTCGTAACAAGG) and ITS2-2043R (GCTGCGTTCTTCATCGATGC).

Bioinformatics

Sequences were processed with two different pipelines using OTUs (Operational Taxonomic Units) and ASVs (Amplicon Sequence Variants) to compare how different bioinformatics methods can influence community diversity metrics. For the OTUs bioinformatics, the Illumina Mi-Seq data was analyzed using AMPtk v1.3.0 (Palmer et al., 2018) following the previously published protocol Corrales et al. (2020). In brief, the MiSeq reads were demultiplexed following the documentation established by AMPtk 1.3.0. (<https://amptk.readthedocs.io/en/latest/pre-processing.html>) and sequences with a length less than 150 bp were discarded. Sequence reads were clustered at 97% similarity to generate operational taxonomic units (OTUs). OTUs were assigned to taxonomic classifications using the taxonomy algorithm in AMPtk (Palmer et al. 2018) against the UNITE database Version 02.04.2020. (Abarenkov et al. 2020). All non-fungal OTUs and OTUs with fewer than 10 sequences were excluded from the dataset. Furthermore, the ecological guild of each OTU per site was determined using the FungalTraits database (Pöhlme et al. 2020) based on their genus classification.

For the ASVs bioinformatics, the Illumina Mi-Seq data was processed using DADA2 version 1.16.0 package (Callahan et al. 2016) in R version 4.0.4 (R Core Team 2020) following the DADA2

ITS Pipeline Workflow (https://benjjneb.github.io/dada2/ITS_workflow.html). The primers were identified and subsequently removed using *cutadapt* v3.2 (Martin 2011). Subsequently, the *filterAndTrim* function of DADA2 was used with the following parameters (maxN = 0, maxEE = 2, truncQ = 2, minLen = 50). Sequence error and dereplication rates were performed using the *learnErrors* and *derepFastq* functions respectively using the default parameters. Chimeras were removed using the *removeBimeraDenovo* function, applying the consensus method. Taxonomy was assigned against the UNITE database Version 02.04.2020. (Abarenkov et al. 2020) with the dynamic grouping thresholds, using the Naïve Bayesian Classifier (Wang et al. 2007) implemented in the *assignTaxonomy* DADA2 function. In this step, the taxonomy levels from phylum to genus were assigned. All taxa other than fungi and with less than 10 sequences were excluded from the data set. Finally, the ecological guild of each ASV per site was determined using the FungalTraits database (Pöhlme et al. 2020) based on their genus classification.

Statistical analyses

Statistical tests were carried out in R v4.0.4 (R Core Team 2020). Alpha diversity was calculated by site using Shannon and Fisher indices using the *phyloseq* R package v1.30 (McMurdie et al. 2013). The alpha diversity was compared among sites using ANOVA and checked for normality using the Shapiro-Wilk test. Species accumulation curves were performed for OTUs and ASVs using the *Vegan* v2.5-7 (Oksanen et al. 2017) in R. We also constructed abundance bar plots and heatmaps using genus and family level relative abundance per site using the *phyloseq* package in R.

To compare fungal community species composition among sites, a Nonmetric Multidimensional Scaling (NMDS) was performed for the following groups: 1) Chicaque, 2) Avenida 50, 3) Puente Aranda parks, and 4) Teusaquillo parks. In addition, we run an ADONIS analysis to test statistical differences in community compositions among sites using Euclidean distance and 1000 permutations (Anderson et al. 2011; Oksanen et al. 2008). After the ADONIS analysis, the soil variables that had a statistically significant effect on the structure of the communities were added to the NMDS. The results were considered significant at $p < 0.05$. The urban soil variables were compared among sites using ANOVA.

Results

A total of 4'778,648 valid reads were obtained with an average of 230,583 reads per sample. A total of 735,516 reads were recovered for Chicaque and 4'043,132 reads from Bogotá. Total number of OTUs was 1,154 and 4'687,262 reads were assigned to OTUs (98%). We found 949 OTUs in Bogotá and 514 OTUs in Chicaque. On Avenida 50, there were 129,093 Ascomycota and 668,750 Basidiomycota OTUs. In Chicaque there were 147,370 Ascomycota and 306,213 Basidiomycota OTUs. In Teusaquillo parks, there were 115,205 Ascomycota and 1'169,188 Basidiomycota OTUs. In Puente Aranda parks, there were 198,301 Ascomycota and 1'314,139 Basidiomycota OTUs.

ANOVAs of the soil variables for the three urban sites show that only soil variables that showed significant differences among sites were Ca/K, (Ca+Mg)/K, Ca/Mg, and Mg (Table 1). Boxplots for specific differences among sampling sites are in Supplementary Material (Fig. S2).

Table 1. The average and standard deviation (SD) of the analyzed soil variables. The variables that showed significant differences in the ANOVA are represented by a * ($p < 0.05$).

Variables	Site						
	Avenida 50		Puente Aranda parks		Teusaquillo parks		
	mean	SD	mean	SD	mean	SD	
DBH	42.70	10.91	51.25	42.37	48.64	25.12	
Sand %	63.62	7.36	59.13	7.83	65.10	8.41	
Clay %	9.78	2.19	11.33	3.26	8.86	2.92	
Silt %	26.60	6.73	29.55	9.74	26.04	7.28	
pH	5.06	0.47	5.01	0.41	4.52	0.59	
Organic C	6.70	1.52	6.11	1.85	5.75	1.26	
P	83.49	57.30	234.76	342.98	134.96	90.54	
CIC	31.30	10.26	39.73	12.98	35.78	6.94	
Mg	0.65	0.17	1.50	0.77	0.69	0.35	*

Na	0.27	0.30	0.38	0.54	0.54	0.65	
N	0.79	0.18	0.72	0.22	0.67	0.15	
Mg/K	1.18	0.67	1.25	0.47	0.70	0.45	
C total	9.11	2.07	8.31	2.52	7.82	1.71	
C:N	0.15	0.13	11.59	0.07	11.60	0.04	
CICE	8.53	2.66	11.09	5.69	6.01	2.45	
Ca	6.02	2.64	6.63	3.94	2.72	2.37	
K	0.67	0.29	1.20	0.41	1.08	0.31	
B.T.	7.61	2.98	9.70	5.33	5.03	2.69	
SB	28.66	18.62	27.13	14.78	14.86	8.86	
Ca/Mg	9.81	5.17	4.73	2.78	3.61	1.65	*
Ca/K	11.07	8.56	5.65	2.70	2.61	1.98	*
(Ca+Mg)/K	12.25	9.11	6.90	2.98	3.31	2.33	*

Species accumulation curves revealed that the sampling effort was sufficient to reach a plateau when using OTUs (Fig. 1A). However, in the case of the ASVs sampling effort wasn't enough to have a complete representation of species richness and reach a plateau (Fig. 1B). The Alpha Fisher diversity index didn't show significant differences among the four sampling areas (Fig. 2A). However, the Shannon index showed significant differences between the urban communities of Puente Aranda and Teusaquillo parks and the rural site of Chicaque (Fig. 2B).

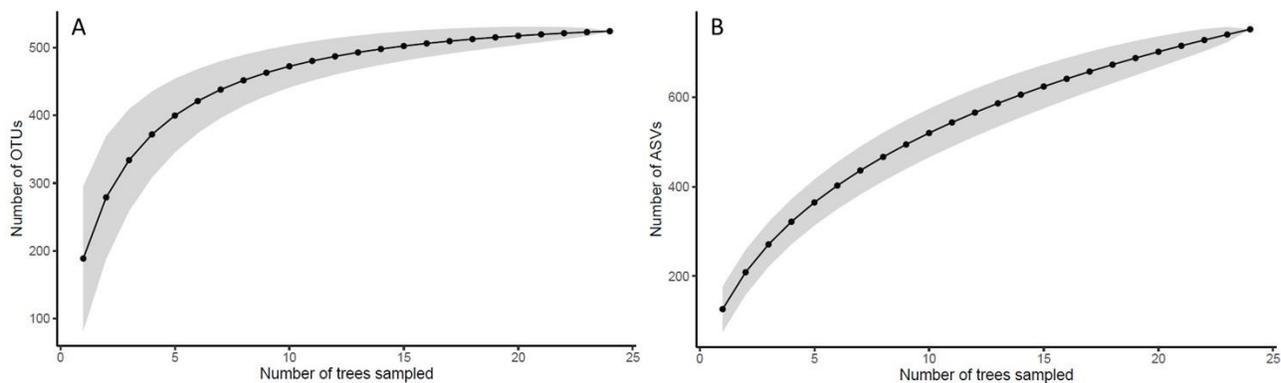


Fig. 1. Species accumulation curves for A) OTUs and B) ASVs.

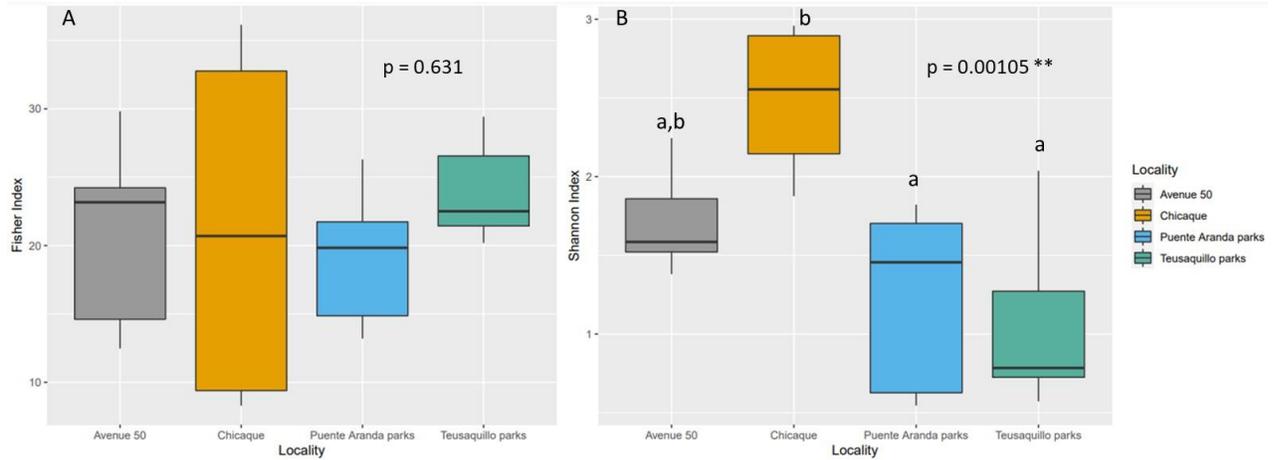


Fig. 2. Alpha diversity indices boxplots for OTUs for the four sampled localities. A) The Alpha Fisher diversity index. B) Shannon index.

The 15 most abundant genera based on the OTUs classification are found in Fig. 3A. *Scleroderma* was the most abundant genus in Puente Aranda and Teusaquillo parks, while in Chicaque the most abundant genera were *Russula* and *Lactarius* and in Avenida 50 the most abundant genera were *Hydnangium* and *Trechispora*. Within the 15 most abundant families (Fig. 3B), Sclerodermataceae had a high abundance in the samples from Puente Aranda and Teusaquillo parks while Russulaceae was the most abundant family in Chicaque and Hydnangiaceae and Hydnodontaceae in Avenida 50. The most abundant genera and families classified by ASVs are found in Supplementary material (Fig. S6). The heatmap of genera classified by OTUs shows very similar results as the ones in the abundance bars (Fig. 4).

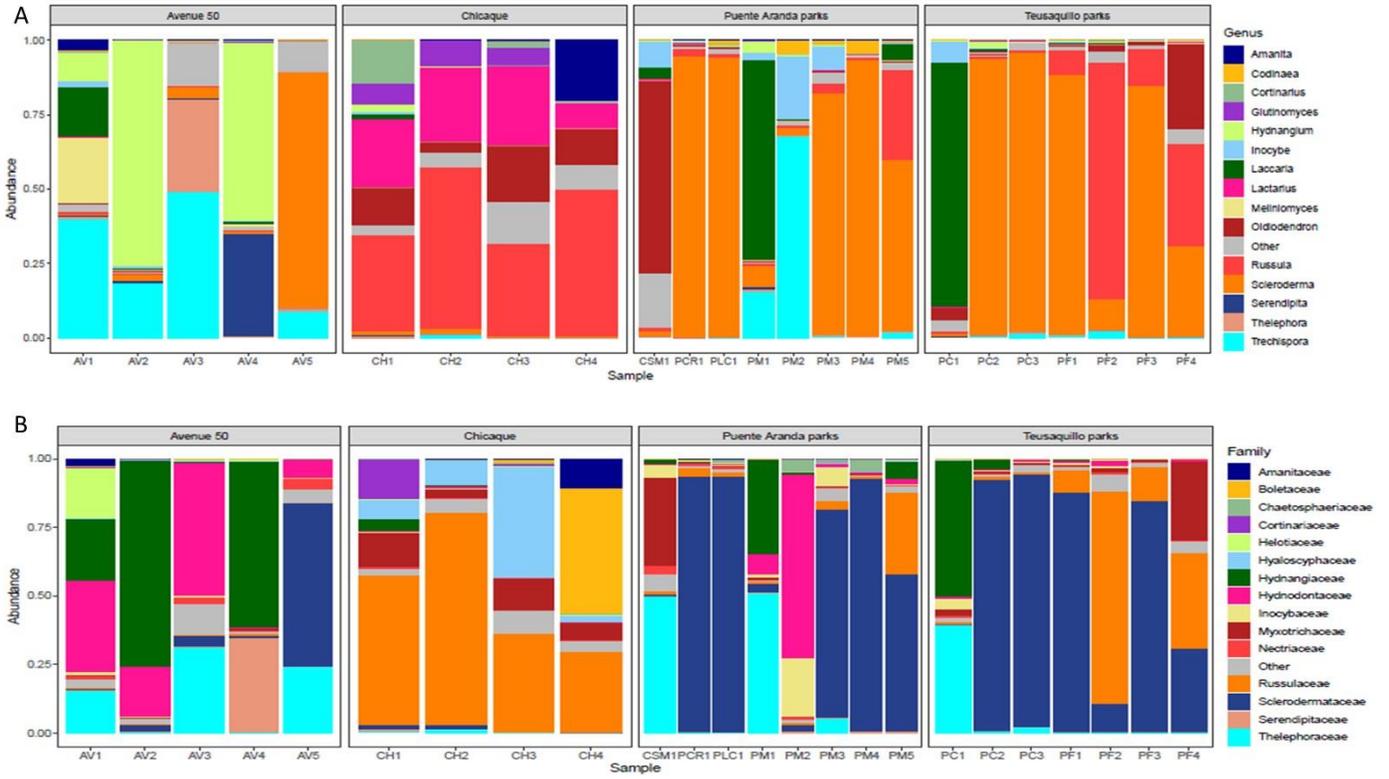


Fig. 3. Genus and family abundance bars classified by OTUs. A) Top 15 most abundant genera. B) Top 15 most abundant families.

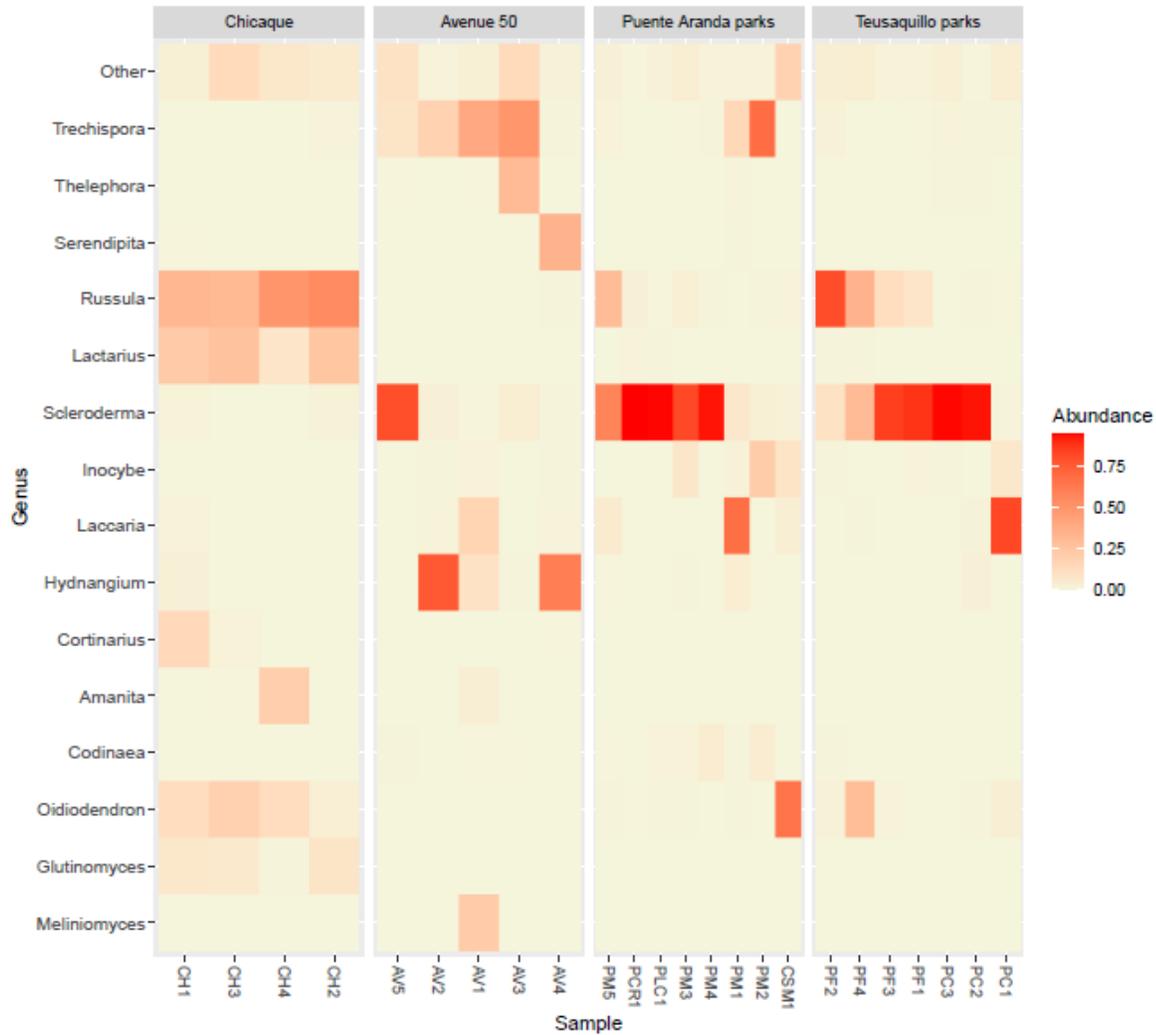


Fig. 4. Heatmap based on genera abundance classified by OTUs.

The NMDS analysis showed a clear separation of the samples from Chicaque in one cluster but also shows that the urban samples separate in two clusters. In these two urban clusters, the samples do not clearly cluster by sampling locations (Fig. 5A). Analysis of the soil variables shows that DBH, sand %, silt %, N, Na, and Mg/K were statistically significant correlated with the structure of the communities based on OTUs. However, none of these variables seems to clearly explain the separation between the two urban clusters (Fig. 5B). The same pattern for separation of samples was found when using ASVs data, however the significant environmental variables were DBH, clay %, and Mg (Fig. S10).

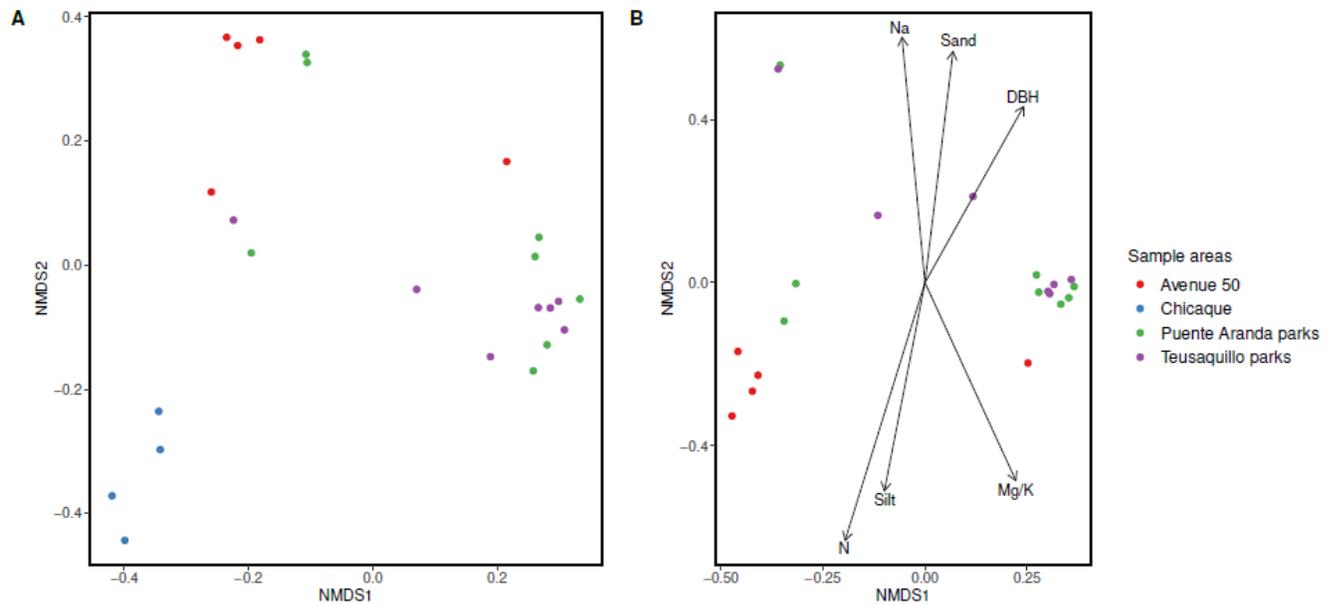


Fig. 5. Nonmetric Multidimensional Scaling (NMDS) of fungal communities classified by OTUs. A) Ordination between all rural and urban sample sites, and B) Ordination of only urban samples with soil variables that showed a statistically significant correlation based on ADONIS analysis.

A total of 437 OTUs from urban samples were analyzed by FungalTraits to determine their primary lifestyle. The primary lifestyle with the most reads was ECM (66%) followed by wood saprotroph (8%), soil saprotroph (4%), root endophyte (2%), and plant-pathogen (1%) (Fig. 6A). 19% of the sequences were not classified within any functional group and were represented as "Unknown" (Fig. 6A). Similarly, 219 OTUs from rural samples were analyzed and, also, ECM was the primary lifestyle with the higher percentage (54%) followed by soil saprotroph (10%), root endophyte (5%), and 29% of "Unknown" (Fig. 6B).

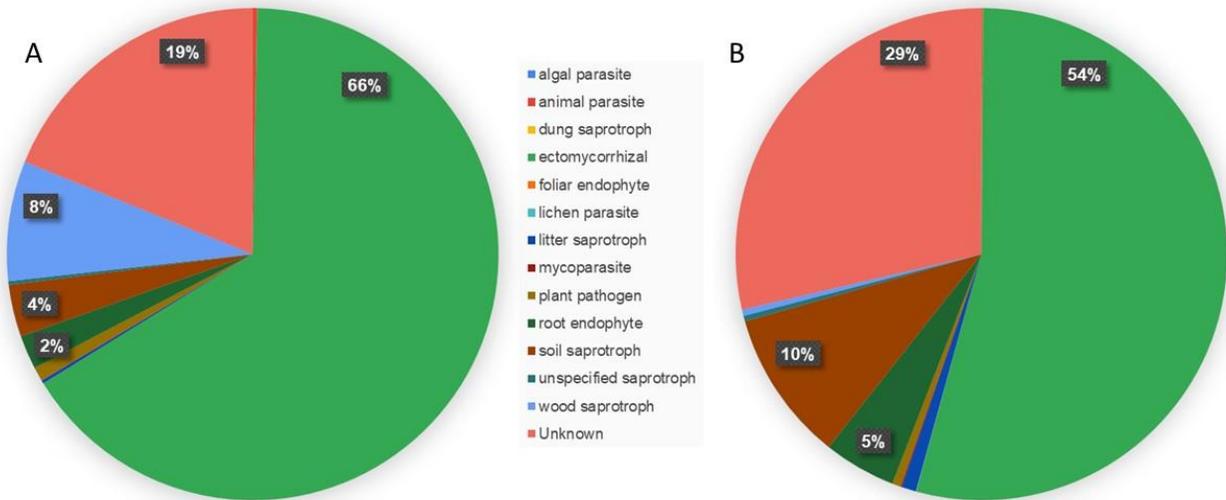


Fig. 6. Classification of OTUs primary lifestyle classification based on the FungalTraits database for A) urban samples, and B) rural samples.

Discussion

The present study explores the structure and species composition of the ECM fungal communities associated with *Q. humboldtii* in urban ecosystems of Bogotá and how these communities vary when compared with those of a nearby rural ecosystem. This comparison allows the identification of biotic and abiotic variables that may be important in structuring ECM fungal communities in tropical urban systems.

Even though we didn't find a significant difference in the species richness between rural and urban samples we observed a trend to a higher species richness in the Chicaque communities compared to Bogotá. This was reflected by the Shannon index but not by the Alpha Fisher index that showed a high degree of variability (Fig. 2). The species accumulation curves per site (Supplementary Material, Fig. S4) did not show a clear separation between the sampled sites probably due to low replication. Previous studies from temperate ecosystems had found significant differences in species richness between rural and urban communities. For example, Baxter et al. (1999) studied forest stands dominated by *Quercus rubra* and *Q. velutina* Lam. in New York State, USA. They found a higher average richness of ECM fungi in rural samples compared to urban samples. Also, Ochimaru & Fukoda (2007) found that the diversity of ECM fungi in forests dominated by *Q.*

myrsinaefolia, was significantly lower in urban and suburban samples than in rural samples of the Kanto District (Japan).

Our NMDS analysis showed three clusters of samples, one of which only consisted of samples from Chicaque and the other two clusters were composed of urban samples. However, the soil variables that had a statistically significant effect on the structure of the communities (N, sand %, DBH, Na, silt %, and Mg/K) didn't seem to clearly explain the separation between the two clusters of urban samples. Similar studies have found that the composition of the ECM fungal communities is different between urban and natural sites with pH, P, and ammonia being the soil variables most correlated with the ordination (Martinová et al. 2016). However, our study didn't include soil samples from our rural site that could have helped explain the differences among clusters.

The composition of ECM fungal communities has been shown to be affected by multiple environmental factors such as water availability, temperature, soil nutrients, or anthropogenic factors like deposition of N or heavy metals, urbanization, heat islands, and edge effects (Baruch et al. 2020; Jumpponen et al. 2010; Martinová et al. 2016). In addition, the use history of the sites can result in different fungal communities (Karpati et al. 2011). According to the silvicultural management guide for Bogotá, urban trees are fertilized with high concentrations of P and K in their first year of transplantation and later with fertilizers rich in N to favor the development of roots and foliage (Mahecha et al. 2010). These high nutrient loads could explain the strong changes in species composition between urban and rural sites. In addition to fertilization, disturbances such as cultivation, gardening and watering that are often applied to urban trees in Bogotá, can disrupt mycelial networks and species interactions. Also, the introduction of exotic plant species like *Pinus* spp., *Eucalyptus* spp., and *Acacia* spp. and their associated fungi can also disrupt fungal communities of native species.

The fragmentation of the landscape and the sealing of soil caused by urbanization reduces the survival of fungal communities because it limits the dispersal of spores and hinders the growth of fruiting bodies (Martinová et al. 2016). *Quercus humboldtii* is a tropical monodominant species that usually grows in high densities hosting high density populations of its associated ECM fungi. However, in urban sites the interactions between soil and vegetation can break or change the

interactions of fungi with other biotic and abiotic factors (Baruch et al. 2020). For this reason, it is important to continue to investigate the biotic or abiotic factors structuring communities of ECM fungi in urban ecosystems.

We also found that tree DAP was significantly correlated with the ECM community composition suggesting that tree age could significantly alter the symbionts species present in *Q. humboldtii* roots. Previous studies have found that fungal communities vary with time of tree establishment. For example, Baruch et al. (2020) compared different types of urban ecosystems (sports fields, gardens, parklands, young revegetation sites, and old revegetation sites) and found that those sites that have a longer time of vegetation establishment (> 10 years) have higher ECM fungi richness, while soil traits alone did not influence fungal communities. Bearing this in mind, the differences found in the soil features among our urban sites should be complemented with studies on time of establishment and specific management practices that each tree has received (such as inoculums used) to explain the differences between the two urban clusters.

The high abundance of *Scleroderma* found in urban sites is consistent with the results of other studies such as Karpati et al. (2011) carried out in a metropolitan area of New Jersey comparing undisturbed forests and disturbed urban sites. In this study, the authors found the presence of *Scleroderma* in all urban sites, suggesting that this genus is typically associated with disturbed ecosystems. Also, consistent with our results, *Russula* and *Lactarius* have been found to be good indicators of natural ecosystems due to their high abundance in undisturbed sites (Karpati et al. 2011; Martinová et al. 2016). A high number of ECM species of the Russulaceae family have been found in several rural sampling sites in Colombia based on fruiting body surveys, including the department of Cundinamarca (to which the city of Bogotá belongs) (Vargas and Restrepo 2019).

Two fruiting bodies were found in the sampled urban sites corresponding to a specimen of the genus *Laccaria* and *Scleroderma*, the were latter identified based on morphology and the ITS region as *Scleroderma bovista* (Corrales unpublished data). Many species of the genus *Scleroderma* are used as inoculant for commercial plantations of various tree species due to their ability to form ectomycorrhizae and to protect their host plant against pathogens (Pinzón Osorio & Pinzón Osorio 2018). *Scleroderma bovista* has a wide distribution, with several reports in Europe

and the Americas. This species has been reported in urban and rural areas of Colombia as ectomycorrhizal (Pinzón Osorio & Pinzón Osorio 2018). Therefore, the high abundance of *Scleroderma* in urban trees could also be caused by inoculation with an exotic species. This hypothesis will need to be tested in future studies.

The genus *Laccaria* is a typical ECM taxa reported in plant communities on all continents (except Antarctica, Kropp & Mueller 1999). Some taxa of the genus *Laccaria* have been reported as pioneer species, frequently found in disturbed sites (Kropp & Mueller 1999) and has also been reported as a nitrophilic taxa (associated to high nitrogen conditions) in temperate and tropical forests (Corrales et al. 2020; Lilleskov et al. 2011). Given these previously reported functional characteristics it is not surprising to find it at high abundances in our urban sampling sites (Fig. 4)

In general, the results between OTUs and ASVs were very similar and no substantial differences were seen between the community patterns of root-associated fungi obtained with the bioinformatics pipelines. The only difference found was that when using OTUs the sampling effort was sufficient to reach a plateau in the species accumulation curves while using the ASVs, the sampling effort was not enough to reach a plateau. The results of the diversity indices, NMDS, and classification of functional groups, did not differ between OTUs and ASVs. Depending on the method used, some differences were found in the top 15 most abundant families and genera. For example, *Tomentella* was excluded from the top 15 when using OTUs, but was included when using ASVs. However, both methods showed the same community patterns and coincided in most of the abundant families and genera for all sampling sites.

In Colombia, the species composition and richness patterns of ECM fungi are still understudied and so far, very little research has focused on the role of tropical ECM fungi species in nutrient cycling and forest structure in natural and urban sites. Therefore, we must continue studying urban mycorrhizal communities to promote the inclusion of this component when implementing environmental management plans and ecological restoration programs. The high number of disturbances in urban ecosystems has a large effect on soil characteristics, which can affect soil fungal communities. It is necessary to take into account other abiotic factors such as the specific management practices of vegetation and the use of ECM fungal species as inoculants to explain

the changes in composition of ECM fungi communities within urban ecosystems. Additionally, the use of OTUs or ASVs does not have substantial differences when studying communities of root-associated ECM fungi.

Acknowledgements. Special thanks to the Universidad del Rosario for its financial and academic support during the research through the “Fondo para la Financiación de Trabajos de Grado - Facultad de Ciencias Naturales, Universidad del Rosario”. We thank David Escobar and Nelly Maldonado from the Chicaque Natural Park for their support during fieldwork. Collections were made under the ANLA License Resolution 530 of May 27, 2014.

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