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The myxomycetes of Costa Rica's Metropolitan Area: Association with air quality and chemical attributes of tree bark

Los mixomicetes del Área Metropolitana de Costa Rica: asociación con la calidad del aire y los atributos químicos de la corteza de los árboles

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RESUMEN

Antecedentes: Los mixomicetes son amebas que se dispersan por esporas y pueden ayudar a responder preguntas ecológicas en microorganismos. Aunque se han estudiado en bosques de Costa Rica, su presencia en zonas urbanas ha sido poco investigada.

Objetivo: Documentar mixomicetes en la mayor zona urbana del país en función de la calidad del aire y algunos elementos químicos en corteza de árboles.

Métodos: Durante dos años se muestrearon 12 sitios urbanos con dos protocolos de aislamiento. Se recolectaron datos de calidad del aire y composición química de corteza. Se evaluaron correlaciones entre variables y los indicadores de respuesta registrados, incluyendo el pH.

Resultados y conclusiones: Se identificaron 32 morfoespecies, con mayor biodiversidad en el sur del área urbana. Se hallaron correlaciones moderadas con niveles de cobre, zinc y variables de calidad del aire. Las regresiones múltiples mostraron alta correlación con el Índice de Diversidad de Shannon. Se confirmó un núcleo urbano de especies y se propuso que su presencia en sistemas urbanos puede deberse a controles ecológicos tanto primarios como secundarios.

Palabras clave: ecología comunitaria, distribución, modelaje, mohos deslizantes acelulares, protistas urbanos

ABSTRACT

Background: Myxomycetes are spore-dispersed amoebae that can help answer ecological questions about microorganisms. Although they have been studied in Costa Rican forests, their presence in urban areas has been poorly investigated.

Objective: To document myxomycetes in the country's largest urban area in terms of air quality and selected chemical elements in tree bark.

Methods: Twelve urban sites were sampled over two years using two isolation protocols. Data on air quality and bark chemical composition were collected. Correlations between variables and recorded response indicators, including pH, were evaluated.

Results and conclusions: Thirty-two morphospecies were identified with greater biodiversity in the southern part of the urban area. Moderate correlations were found with copper and zinc levels and air quality variables. Multiple regressions showed a high correlation with the Shannon Diversity Index. An urban core of species was confirmed, and it was proposed that their presence in urban systems may be due to both primary and secondary ecological controls.

Keywords: community ecology, distribution, modelling, urban protists, slime molds

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INTRODUCTION

The myxomycetes, or myxogastrids, are a group of amoeboid organisms present in all ecosystems across the planet (Stephenson *et al.* 2008). These life forms are unique among protists because their reproductive structures are visible to the naked eye. Any other group known to have macroscopic structures on earth (i.e., metazoa, embryophyta, fungi) developed such capacity through multicellularity, but myxomycetes did so by developing complex intracellular controls (Schnittler *et al.* 2022). These organisms disperse by forming spores within reproductive structures that resemble microscopic fungi and are able to colonize distant areas across the planet (Tesmer and Schnittler 2007). As such, endemicity in myxomycetes is very rare (Dagamac *et al.* 2017), and ecological requirements of the different species are responsible for shaping the visible composition of myxomycete assemblages (Schnittler *et al.* 2022) worldwide.

The ecological documentation of urban myxomycete assemblages has been poorly performed and only a handful of studies are available in mainstream scientific literature (Wrigley de Basanta 2004, Hosokaga *et al.* 2019). This phenomenon is quite likely the result of most myxomycete researchers focusing on either taxonomic or pure biodiversity matters. Despite that, there have been some efforts in the last decade intended to document urban patterns of occurrence and associated ecological pressures in the Neotropics (Rincon *et al.* 2021, Rojas *et al.* 2022). As a product, we have seen that both the degree of urbanization and presence of conurbations in cities promote higher biodiversity values, and that the direction of winds can be related with the species distribution. However, at the moment, no data related to any potential connection between either airborne or surface-deposited urban pollution and myxomycetes are available for any location.

Myxomycete data, particularly information on the reproductive stage, can be very useful to evaluate the effects of climatic (Schnittler *et al.* 2015) or anthropogenic (Bernardo *et al.* 2018) phenomena on ecological strategies of survival in microorganisms. Due to a robust basic documentation of myxomycetes in Costa Rican forests (Rojas *et al.* 2018), this country represents a good starting point for considerations oriented towards anthropogenic systems. In urban settings, this information can also be used to generate distribution models that along with environmental data, could provide meaningful insights into microbial dynamics in urban environments. The present study represents the first step towards such modelling, using systematically collected figures and a standard isolation technique. As such, our objective was to document the presence of myxomycetes in Costa Rica's largest urban area in the context of air quality data and chemical values associated with the substrates upon which these organisms occur.

MATERIALS AND METHODS

This study was carried out in the Great Metropolitan Area of Costa Rica (shortened herein as Metro Area), where both the capital and most populated cities in that country are located. Within such a region, a series of 12 locations, geographically spaced to encompass the inner core of the urbanized area, were selected and studied during the period between 2021 and 2023. Starting in the northeastern most section and following a zig zag pattern to the south and west (Figure 1), these locations corresponded to the jurisdictions of Moravia (abbreviated herein as MO), San Pedro de Montes de Oca (SP), Desamparados (DE), San Sebastián (SS), Barrio México (BM), Santo Domingo de Heredia (SD), Heredia (HE), Lagunilla de Heredia (LA), Escazú (ES), Santa Ana (SA), Belén (BE) and Río Segundo de Alajuela (AL).

In each of these locations, during 2021 and 2022, a series of six bark samples from randomly selected trees were collected to evaluate myxomycete occurrence. Of those, three samples corresponded to the outer bark, also known as cork, and three to the inner bark, or phelloderm. All samples were collected using a pocketknife and placed in individually labeled paper bags that were immediately taken to the laboratory for processing. The pocketknife was cleaned with 96% alcohol between sites. During 2023, in the same locations, a series of spore traps were placed at heights between 2-3 m from the ground and left outdoors for three months before they were taken back to the laboratory. These traps were made in the manner described by Rincón-Marín *et al.* (2021) with a 0.27 mm pore size HDPE plastic mesh and autoclaved leaves and were designed to capture and support the colonization of airborne propagules of myxomycetes.

In all cases, sporocarps of myxomycetes were isolated from plant material using the moist chamber culture technique described by Wrigley de Basanta and Estrada-Torres (2022), which included the determination of the pH values associated with each sample. For this process, all 288 samples of bark, from two consecutive years, and 120 leaf samples from spore traps were studied in a laboratory with controlled temperature and air humidity (22-23 °C, 45-60 %) at the Instituto de Investigaciones en Ingeniería (Universidad de Costa Rica) in San Pedro de Montes de Oca. All moist chamber cultures were investigated for a period of three months and myxomycete occurrence was defined either by the presence of plasmodia or sporocarps. In the latter case, the reproductive structures were extracted and glued onto pasteboard boxes that were dried out in the same laboratory conditions. Upon this process, all specimens were identified and deposited in the Luis Fournier Origgi Herbarium (USJ) of the Universidad de Costa Rica. All the scientific names conform to the nomenclature of the Nomen Eumycetozoa (Lado 2022-2025).

Within the period of study, air quality data were also collected on a monthly basis for one year, in each of the studied locations using an EG B078ZS8RVL Portable Air Monitor. All assessments were conducted in the morning following a consistent site order. During this process the concentration of the particulate matter commonly known as PM1.0, PM2.5, and PM10 along with the Air Quality Index (AQI) were obtained. Also, the concentrations of formaldehyde (HCHO) were measured. The AQI is a widely used indicator of air pollution developed by the EPA in the United States in the 1970s (Horn and Dasgupta 2024). High values of all these metrics are linked with poor air quality and several health issues. At the same time, in 2022 pooled samples (i.e., from different places and heights but all mixed up in one larger sample) of bark were obtained from the same trees where the cork and phelloderm samples were collected. These secondary samples were air dried under laboratory conditions and then grounded up for elemental analysis (N, P, Ca, Mg, K, S, C, Fe, Cu, Zn, Mn, B, Cd, Pb) at the Centro de Investigaciones en Agronomía of the Universidad de Costa Rica. Nitrogen and carbon were measured by dry combustion using a C/N autoanalyzer, whereas P, Ca, Mg, K, S, Fe, Cu, Zn, Mn and B were analyzed by inductively coupled plasma (ICP) atomic emission spectrometry. Cadmium and lead were analyzed by ICP mass spectrometry. In urban conditions, tree bark is a good depository of chemical pollution. The complete dataset of average element concentrations in bark and air quality parameters along with all myxomycete records compiled herein was published in The Knowledge Network for Biocomplexity (Rojas and Solano Arguedas 2024) and it is freely available for research.

For data analysis, all 288 samples from the bark assessments were combined into one dataset because the decision to do the yearly surveys simply corresponded to a strategy intended to increase the representativeness of the data. The 120 samples of the spore trap survey were considered a separate dataset since such recording strategy was intended to provide data related to airborne propagules. In both cases, the results of the field surveys were used to calculate, for each sampling location: 1) the percentage of samples resulting in positive records of myxomycetes, by considering all plasmodia and sporocarp occurrences together; 2) the number of species, 3) the number of records, and 4) the Shannon's diversity index. These four estimates were considered the response variables for analysis.

An initial correlation approach was used to evaluate individual relationships between either air quality or bark chemistry values and response variables. This approach was carried out using the Spearman's Rho correlation (ρ) estimator only for the subdataset of bark samples. A similar approach was taken with the litter/spore trap subdataset, but in this case, only air quality values were used. In both cases, only moderate to high correlations with estimators greater than 0.40 (or -0.40 in the case of negative associations) were considered. Following this step, for the bark subdataset, a series of combinations of environmental variables was used to construct multiple regressions using the most significant response variables from the previous step. This approach was taken to observe the effect of adding multiple variables on the correlation value and, more importantly, on the coefficient of determination associated with the relationship. In this case, the Pearson's correlation and determination coefficients were used. Multicollinearity was accounted for, and only non collinear variables were used for multiple correlations.

After this, the data from the bark sampling was di-

vided into the two bark depth categories: cork and phelloderm. This step was considered herein to evaluate, first, which myxomycetes were present in the inner bark layer; and second, to analyze the relationship between environmental variables and the same response metrics but recalculated for the correct data partition. Similar to a previous step, in this case, a combination of moderately correlated variables was used to construct multiple regression models in order to evaluate the combined effect.

Finally, several datasets from previous studies carried out in different parts of the Neotropics were used to calculate both the average pH of the cultures and the number of species per sample. This approach was carried out to evaluate the relevance of pH values to explain species diversity in myxomycetes, a highly used argument in myxomycete ecology for which limited testing has been conducted using Neotropical data. For this section, both the coefficient of correlation and the coefficient of determination were calculated to analyze the observed relationships. The pH values of the two subdatasets analyzed herein were compared with three similar datasets obtained outside Costa Rica's Metro Area in Hojancha, Turrialba and Bagaces. A Kruskal-Wallis test was used to compare all datasets, and a posthoc Dunn test was used to identify the group with different values. In these cases, an alpha value of 0.05 was used to test the null hypothesis of no differences in the pH values.

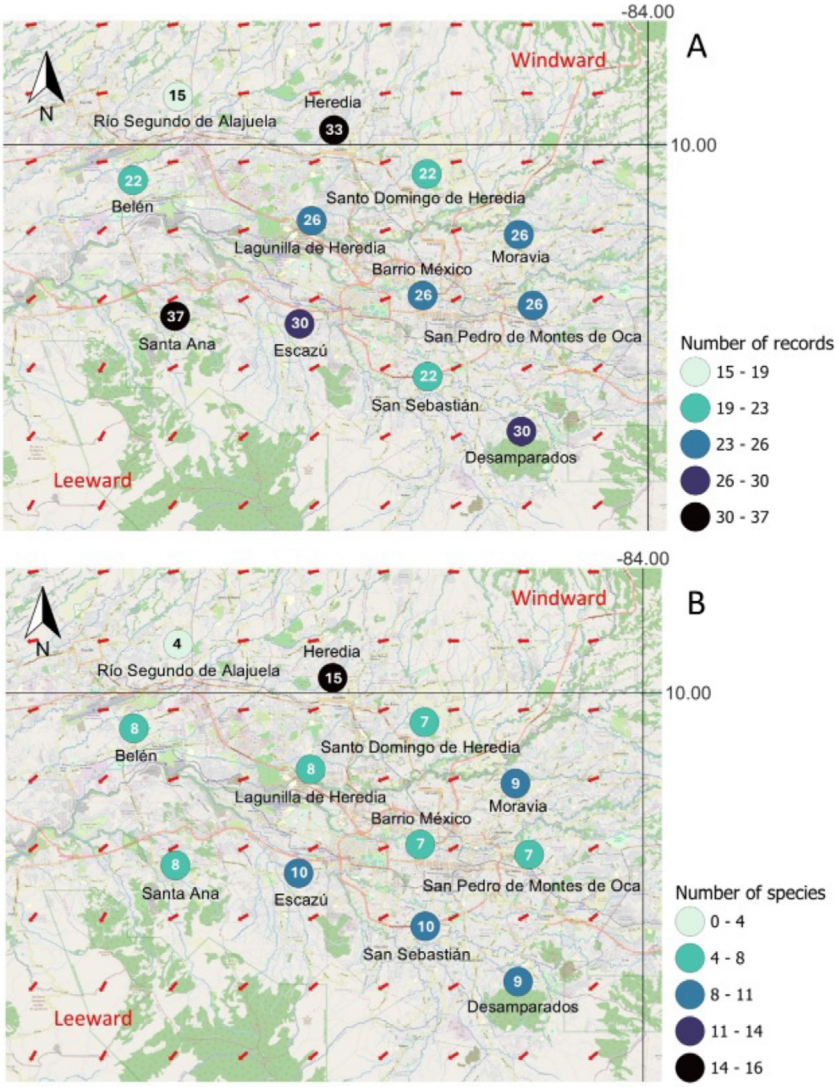

Figure 1. Map of the number of records (A) and number of species (B) of myxomycetes in each sampled location within Costa Rica's Metro Area showing the main direction of the prevailing winds and both windward and leeward areas.

RESULTS

A total of 32 morphospecies of myxomycetes from 136 identified records were obtained (Table 1). Of the total, 22 morphospecies were observed only on bark samples, five on litter material and the other five were recorded on both. The highest number of unique species, six of them, was recorded in SA, followed by MO and LA with four species each. The average percentage of positive moist chamber cultures for the spore trap assessment was 60 %, with a range oscillating between 50 % for AL, BE and SD and 80 % for SS and LA. Similarly, the average percentage of positive moist chamber cultures observed in the bark was 56 %, with a range between 29 % for AL and 87 % for HE (Table 2).

Overall, and as a general trend, the highest numbers of records and species were observed in HE, SS, DE, ES and SA. Contrastingly, the locations with the lowest values were SD and AL. The Shannon's Diversity Index followed a similar pattern. A geographical overview of the number of species and number of records observed herein showed that, in general, the southern locations in the Metro Area were more productive (Figure 1), which in terms of wind direction coincided with the leeward section of the so called "central valley".

Of all the individual correlations observed on the bark subdataset, the number of species and the Shannon's Diversity Index were the two response variables that showed both the highest number of associations and the highest value for the Rho coefficient. The number of species was moderately and positively correlated with copper (ρ=0.43) and negatively correlated with PM1.0 (ρ=-0.60), PM2.5 (ρ=-0.61), PM10 (ρ=-0.58), HCHO (ρ=-0.45) and AQI (ρ=-0.60). Copper was the only variable correlated with the number of records (ρ=0.53). The Shannon's Diversity Index was positively correlated with both copper (ρ=0.41) and zinc (ρ=0.51) and negatively correlated with PM1.0 (ρ=-0.53), PM2.5 (ρ=-0.58), PM10 (ρ=-0.61), HCHO (ρ=-0.56) and AQI (ρ=-0.57). For the litter/spore trap subdataset, the same index was moderately correlated with PM1.0 (ρ=0.44).

The strongest multiple regression was found for the copper+PM2.5 model explaining bark species richness, with a value of r=0.91 (adjusted r^2=0.81). The model of copper+PM10 explaining Shannon's Diversity Index also resulted in a moderately high correlation (r=0.83, adjusted r^2=0.62). Finally, the model of zinc+PM10 explaining Shannon's Diversity Index showed a correlation value of r=0.73 (adjusted r^2=0.43).

Seventeen species of myxomycetes were recorded in the inner layer of bark, or phelloderm (Table 1), from which *Clastoderma debaryanum*, *Diderma hemisphaericum*, *Lamproderma scintillans*, and *Physarum crateriforme* were recorded only in such bark type. When the two bark layers were separated into different datasets, the external layer or cork, showed moderate correlations between both the number of records or the number of species and copper (ρ=0.56 and ρ=0.47, respectively). In the internal layer of bark, or phelloderm, moderate correlations were found between the number of records and nitrogen (ρ=0.52), as well as between the number of species and both nitrogen and copper (ρ=0.71 and ρ=0.48, respectively). The most significant multiple correlation was found for the model of nitrogen and copper explaining the number of species in the phelloderm (r=0.62, adjusted r^2=0.38).

The pH evaluation using other Neotropical datasets resulted in a pattern of increasing the number of species per sample as the values of pH increased as well (Figure 2). In this case, both the coefficients of correlation and determination demonstrated a good relationship between variables (r=0.75, r^2=0.56). When we compared the pH values of the two subdatasets analyzed herein with three datasets from outside Costa Rica's Metro Area, significant differences were observed (H=546.1, P<0.0001), with the highest dissimilarities obtained for comparisons with the Metro Area subdatasets (P<0.0001 in all cases). The average pH values for the two urban datasets were 5.68±0.25 and 6.11±0.47 for litter and bark, respectively. The average values for the non-urban datasets were 7.36±0.57, 6.81±0.56 and 6.75±0.34 for Turrialba, Bagaces and Hojancha, respectively.

Table 1. Records of myxomycetes in the Costa Rican Metro Area arranged by species and location. Scientific names with an asterisk represent species found in phelloderm

Species	MO	SP	DE	SD	BM	SS	HE	LA	ES	AL	BE	SA
Arcyria afroalpina	1											
Arcyria cinerea *	1	2	4	3			2	1	2	2	2	
Arcyria insignis				1							1	
Clastoderma debaryanum *	2											
Claustria didermoides												*1*
Comatricha nigra		1							1		1	
Comatricha tenerrima									1			
Cribraria violacea *					2							*1*
Didymium bahiense *			1			1	1					
Didymium difforme *	1		2				4	3				
Didymium clavus								2				*2*
Didymium minus			1		2	3	1		1		2	
Didymium squamulosum												*1*
Diderma hemisphaericum *				1								
Echinostelium minutum *		1		1				3				
Hemitrichia minor												*1*
Lamproderma scintillans *			1					1				
Licea biforis *					1	5						
Licea pedicellata *	9		2		2	2	3	1	3			*1*
Macbrideola scintillans												*1*
Ophiotheca chrysosperma *	1				2	2	5	1		1		*2*
Ophiotheca pedata *								1		1		*1*
Perichaena corticalis *							1				2	
Perichaena depressa						1						
Physarum album	1											
Physarum cinereum							1					
Physarum compressum *			1									
Physarum crateriforme *											2	*4*
Physarum javanicum					1							
Physarum pusillum								2				
Stemonitis fusca *				1					1			
Stemonitopsis hyperopta *		1										*1*
Unidentified plasmodia	7	11	9	11	10	10	15	11	11	5	11	*6*
Perichaena depressa						1						
Physarum album	1											
Physarum cinereum							1					
Physarum compressum *			1									
Physarum crateriforme *											2	*4*
Physarum javanicum					1							
Physarum pusillum								2				
Stemonitis fusca *				1					1			
Stemonitopsis hyperopta *		1										*1*
Unidentified plasmodia	7	11	9	11	10	10	15	11	11	5	11	*6*

Table 2. Values of all four response variables arranged by location within Costa Rican Metropolitan Area

Species	MO	SP	DE	SD	BM	SS	HE	LA	ES	AL	BE	SA
Number of species on litter	3	1	2	1	2	3	2	5	2	3	2	*3*
Number of records on litter	7	6	5	5	6	8	6	11	5	5	5	*7*
Shannon's Diversity Index on litter	0.9	0.0	0.6	0.0	0.7	1.1	0.5	1.6	0.6	1.3	0.8	*1.1*
Positive cultures using litter (%)	70	60	50	50	60	80	60	80	50	50	50	*60*
Number of species on bark	7	7	8	6	7	6	9	7	8	4	6	*10*
Number of records on bark	17	13	17	13	13	19	28	15	18	7	16	*15*
Shannon's Diversity Index on bark	1.7	2.0	2.1	1.7	2.1	1.8	2.0	1.9	1.9	1.5	1.6	*2.5*
Positive cultures on bark (%)	71	46	58	54	42	63	88	54	63	29	58	*50*

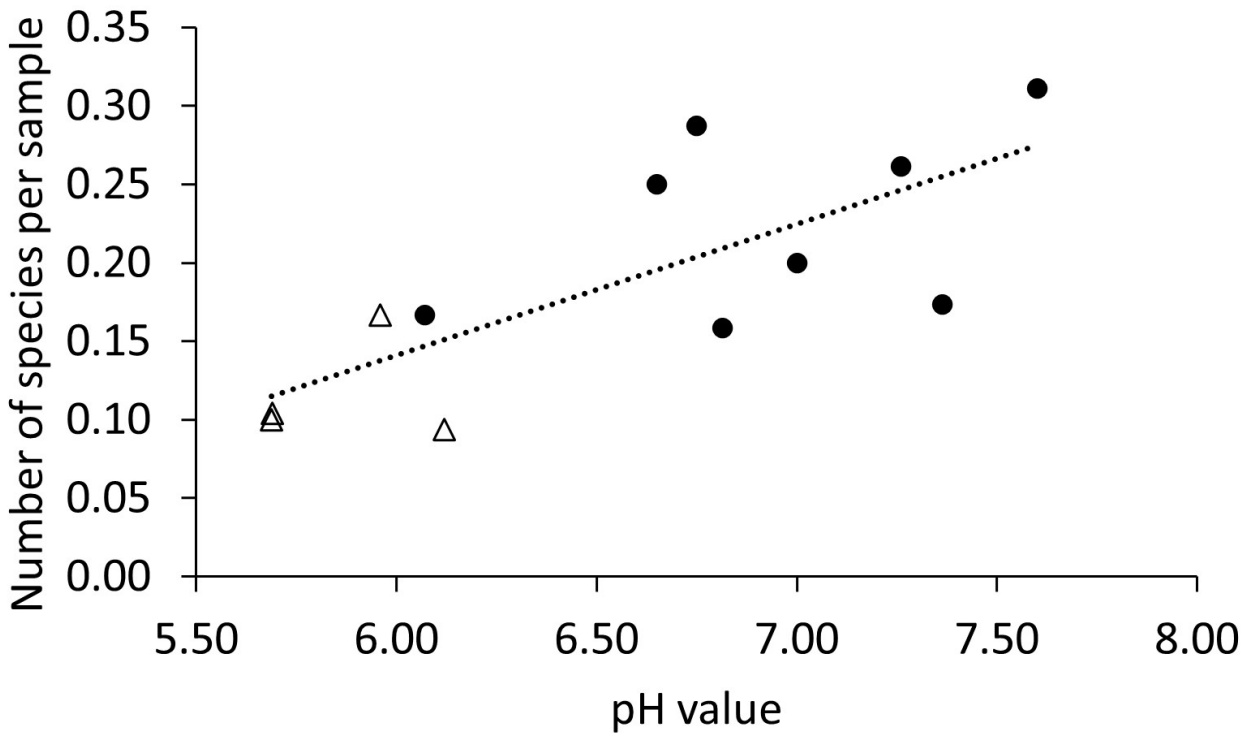

Figure 2. Relationship between substrate pH and the number of species of myxomycetes per sample for 12 Neotropical datasets, including the subdatasets considered in the present study (filled circles) and two other urban datasets (unfilled triangles).

DISCUSSION

Myxomycetes are ecologically versatile organisms capable of thriving across a wide range of habitats, including urban ecosystems. The presence of plant biomass seems to be the key element associated with their occurrence (Stephenson *et al.* 2008) and urban systems, despite many differences with undisturbed settings, still contain plenty of such niches for organisms like myxomycetes to colonize. The effect of urban conditions on the quality of microhabitats, though, seems to affect the structure of myxomycete assemblages (Stephenson *et al.* 2020) and the results of the present study point in that direction as well.

There is one previous report of myxomycetes in Costa Rica's Metro Area (Rojas *et al.* 2022), but that study was intended to compare assemblages between the geographical center of the urban extent and several other locations representing the outer limits of the general urban landscape. In that investigation, a total of 12 species were identified for a location in downtown San José, from which, ten species were also found in the present investigation. Technically, the evaluation of Rojas *et al.* (2022) and the present study were expected to have more or less dissimilar assemblages because they focused on different microhabitats (Novozhilov *et al.* 2022), but they revealed a core of species, including *Arcyria cinerea*, *Physarum compressum*, and most species within the genus *Didymium*, that were consistently found in both studies. This result indicates compositional stability within the myxomycete assemblage of the urban landscape.

A relevant aspect of the present investigation is that it expanded the number of known myxomycetes in the studied urban extent to 32 species, increasing the previous value by 167 %. As a comparison, both the studies of Hosokawa *et al.* (2019) in Sidney, Australia as well as Stephenson and Novozhilov (2021) in the Metro Area of Northwest Arkansas yielded 26 species, showing that the tropical assemblage observed herein was slightly more complex. This result, however, can be related to sampling intensity as well. In this sense, even though the reported increase is a clear byproduct of previous under sampling in urban situations within Costa Rica, it showcases the importance of studying urban systems from an ecological perspective. Currently, it is quite relevant to determine stochastic and deterministic processes affecting life in cities (Ossola *et al.* 2021) and within that framework, urban life should be studied to determine baseline information for future biodiversity monitoring and sustainable urban planning. The presence of myxomycetes in urban systems, for instance, has been associated with the degree of forest cover (Rincon-Marín *et al.* 2021), highlighting the relevance of green areas within the urban extent.

As observed herein, response variables like the number of species, the number of records and the Shannon Diversity Index were generally high on the southern side of the Metro Area, which coincides with previous observations made by Rojas *et al.* (2022). In that study, the locations outside of the urban extent in the direction of Santa Ana, Mora and Puriscal (in a southwestern direction from downtown San José) showed a high number of records and the highest number of species in comparison to the eastern and northwestern directions. As such, there seems to be agreement that the area close to Santa Ana, also sampled herein, is a good location for myxomycetes to occur. Among other factors, this observation could be related to the direction of the wind (Santa Ana is in the leeward zone) and to the legal protection of the nearby Cerros de Escazú, the conservation buffer that has controlled the urban expansion in that part of the city (Rodríguez Soto *et al.* 2022). For future studies, however, it would be interesting to maintain a fixed directionality of the spore traps to control such a factor.

The high results observed in HE and the low results obtained for AL and SD are also quite interesting. According to Morera-Beita *et al.* (2018), Heredia (HE) has seen a decrease in the fragmentation of natural areas with a consequent increase in biological connectivity since 2000, Santo Domingo de Heredia (SD) has experienced the opposite trend, and the area of Río Segundo de Alajuela (AL) is just recovering from very high fragmentation. If there is a link between this aspect and the presence of myxomycetes, which was also supported by the Santa Ana data, the likely mechanism would be associated with the contention of habitat loss. As such, the presence of green areas in urban systems would be associated with higher levels of substrate availability, perhaps the most relevant factor explaining the absence of myxomycetes (Rojas and Doss 2014). This idea is also backed up by data from the desertic city of Chiclayo, in Perú, where the

increase in available microhabitats observed with the greening of the urbanized area (in the form of public parks or small forested zones) was linked to higher myxomycete occurrence values compared to native desertic systems where fewer substrates were present (Rojas *et al.* 2022).

In this way, a secondary level of ecological control associated with the quality of the microhabitats, would be responsible for compositional aspects. In this direction, the potential link between copper levels and myxomycetes is enigmatic since no connection has been previously observed other than the capacity of one species, *Lycogala epidendrum*, to bioaccumulate this metal (Kryvomaz 2015). This element seems not to affect the presence of myxomycetes, but it could be interesting to focus future research on other ecological effects (e.g., related to reproductive strategies). A previous study of myxomycete biodiversity on leaves from different trees in the central region of the United States revealed that the lowest number of species and number of records were associated with the leaves that contained the most copper (Goad and Stephenson 2013). However, the results presented in the present investigation showed a positive correlation between biodiversity metrics and copper levels on bark. In a long-term study of myxomycete biodiversity in Turrialba, Costa Rica, the highest metrics were also observed in areas with the highest copper annual fluxes (Rojas and Rojas, in press).

At the moment, the myxomycete-zinc relationship could be more informative since more information is available. Stephenson *et al.* (2020), for instance, had already observed high levels of myxomycete occurrence on bark substrates with high levels of zinc, and some species are known to contain up to 250 times the concentration of this metal than substrates in the surroundings (Zhulidov *et al.* 2002). Zinc seems to be associated with the formation of compounds used in the pigmentation of sporocarps (Aldrich 1981), particularly the yellow tones, and thus, the tolerance of myxomycetes to high levels of this metal is suggested to be an adaptation of the group. The concentration of zinc in plants varies depending on ecological and local traits, but most values observed in the present study (16-101 mg/kg) are comparable to the concentration ranges observed in either food plants (1-73 mg/kg) or grasses (6-80 mg/kg) worldwide (Kabata-Pendias 2010).

According to Odukoya *et al.* (2000), zinc levels measured on bark are very good indicators of urban air pollution since this element is an airborne pollutant, and plants can even accumulate Zn at concentrations of 0.1 % (Kabata-Pendias 2010). Interestingly, in the present study, the two highest Zn concentrations were recorded in BM (101 mg/kg), a site located in the central part of the capital city and SS (86 mg/kg), a site located next to a main highway. However, observations from soils contaminated with Zn indicate that, when copper is also present, both metals may inhibit each other in terms of plant absorption (Kabata-Pendias 2010). This observation might indicate a potential direction of further research given the fact that both metals could be related to myxomycetes in urban environments, as seen herein. Despite that, the high diversity values observed in the present study in areas with high levels of zinc (ρ=0.51), understood as high pollution areas, demonstrate that myxomycetes can survive in chemically rough urban conditions. In this sense, one interesting observation of the present study is that *Physarum javanicum* was only recorded in BM, the location with the highest Zn values, although the reasons for this observation are unclear at the moment.

Such chemical tolerance to metals, which have been shown to differ among few species (Kryvomaz *et al.* 2016), cannot be solely responsible for compositional patterns. For the latter, the potential added effect of dispersal, indirectly suggested by the multiple regressions, could be revealed as well. Particulate matter is known to change as a result of different wind patterns (Kelly *et al.* 2012), and lower values of suspended particles are expected in areas with more intense sustained winds. In these areas, the air also carries myxomycete spores that get deposited in plant material, and this constant additive "seeding" effect increases the probability of recording myxomycetes, as shown by experimental data from both temperate and tropical locations (Rojas *et al.* 2020). In this manner, the spore trap data obtained herein might suggest the idea of an airborne strategy of dispersal, that within the urban extent would simply show the level of mobility associated with the different species. Based on the analysis from Tesmer and Schnittler (2007), the capacity for propagules to move back and forth in an area like the one studied herein is perfectly plausible, and interestingly, in the present study, the species with

more spread records either had warted or spinulose spores, a condition that increases their aerodynamic properties.

The presence of myxomycetes in the phelloderm is interesting but not surprising, given the well-known association of species with bark (Mitchell 1977) and could point in the direction of endophytism (Wrigley de Basanta and Lado 2025). The connection observed with nitrogen, however, could demonstrate high potential for future studies. In a recent study of the myxomycete diet within a successional system, Fukasawa *et al*. (2018) observed that myxomycetes tended to be more likely present on woody substrates where fungal colonizers were also present, and that such association could be determined by the increased levels of nitrogen on the substrate due to the presence of fungal hyphae. Since fungal predation by myxomycetes seems to be very common and has been known for almost a century by now (Gilbert 1928), this was the proposed mechanism of action in that study. Based on the results from the present investigation and using the same idea, myxomycetes could easily move into the inner layers of bark looking for hyphae to feed upon, which would then show the indirect association with nitrogen. The direct effect of nitrogen is not supported by experimental data, which has instead demonstrated no link between this element and the occurrence of myxomycetes (Walker *et al*. 2019). However, herein, the highest nitrogen concentrations were found in DE (2.45 %) and LA (2.37 %), where phelloderm-isolated myxomycete species accounted for 86 % (6 of 7) and 77 % (7 of 9) of total identified species in each location respectively. As such, these results emphasize the complex associations between the occurrence of myxomycetes and local microenvironmental traits, possibly involving other unidentified factors as well.

Finally, the observed relationship between myxomycete occurrence and pH levels is very well supported by previous studies (Novozhilov *et al*. 2022). In one of the investigations on the subject, Härkönen and Vänksä (2004) documented an increase in the number of myxomycetes associated with bark and moist chamber productivity in an urban restored system in which average pH values increased from 4.2 to 5.2 between 1974 and 2002. That study showed, however, that pH values on bark in an urban system can be very acidic and that more neutral values in non-urban conditions could promote the production of sporocarps of myxomycetes. Wrigley de Basanta (2004) experimentally manipulated the pH values of moist chamber cultures and came out to the same conclusion. The pH value differences observed in the present investigation indicate that the acidification of substrates is also present in the studied urban area, but pH dissimilarities between urban and non-urban conditions are less pronounced than those from larger cities (Cha and Lee 1991). In an unpublished dataset from the urban area of the Aburrá Valley in Colombia, the same pattern of an increased myxomycetes occurrence with increasing substrate pH was observed.

In synthesis, the study of myxomycetes in urban systems can reveal interesting information about the effects of anthropogenic ecological forces on life forms. The present study has shown that even though these organisms are present in Costa Rica's Metro Area, the distribution of species could be related to both microhabitat availability and niche quality. The presence of green areas or the level of fragmentation due to urbanization as well as the direction of the prevailing winds and the levels of chemical deposition on plant substrates are a few aspects that can be extracted from the analysis carried out in the present study. However, further experimental approaches are necessary to understand the complete dynamics that control the distribution of these organisms within urban environments. For instance, research on the link between myxomycetes and both Cu and Zn would be interesting to investigate for the elucidation of whether those elements are life cycle requirements or if the observed link is simply indicative of chemical tolerance. This and other questions remain wide open at this point. Despite the latter, a relevant aspect of the present investigation is the generation of scientific data, now available for a group of organisms and a subject about which not much has been comparatively investigated in Central America and the Neotropics.

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