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Myxomycete ecology in urban areas: rapid assessment from two cities

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Abstract

Urban ecology has been an understudied topic in myxomycete research. For that reason, the present investigation aimed to generate data related to myxomycetes in urban areas of the Neotropical region. With an approach of two discrete experiments, one centered on ground-based information and one on air-based data, results showed that myxomycetes can be valuable organisms for microbial ecology assessments in urban centers. Data from these experiments showed that, using a simple and classical laboratory-based method of detection, the number of records seemed to be affected by the degree of urbanization, which also had an effect on pH values, but the number of species seemed to be more associated with site-specific characteristics. Airborne propagules of myxomycete dispersion, captured using substrates exposed to outdoor conditions, indicated that air currents may play a role on the distribution of myxomycetes in urban conditions, potentially affecting the process of ecological data generation. The results obtained herein are useful to demonstrate that myxomycetes can be studied in urban centers and that more systematic approaches could generate relevant data in the context of climate change, green cities, and urban biodiversity monitoring.

Key words – airborne – Colombia – Costa Rica – city – ground litter – Neotropics – slime molds

Introduction

Urban ecology is an active topic of research that has been considered in multiple international agendas in recent years (see IPCC 2019). Since living organisms respond to the pressures created by urban environments, ecological patterns associated with them are incredibly useful for monitoring purposes. As such, ecological quantification and integrated analyses have been used to study pollution patterns (Yuan et al. 2020), hydrology (Holder & Gibbes 2017) and socio-historical anthropogenic transformations (Colding & Barthel 2017).

As part of this movement, scientists have coined new terms to refer to species associations with urban centers (see Grant et al. 2011), studied scale-dependent patterns (Egerer et al. 2017) and integrated temporal variables in their analyses (Schröder et al. 2018). It has been said that urban ecology is the single topic that will have the greatest impact on the development of theoretical and applied ecology in the years to come (Barot et al. 2019). Urban ecology has become a true multidisciplinary field and cannot be ignored for reaching the complex goals needed to sustain the modern lifestyle.

Myxomycetes have been recorded in cities before (i.e. Ya-Fen et al. 2005) and recent studies

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have shown that urban centers can be interesting spaces to test ecological hypotheses on these organisms (Hosokawa et al. 2019). Attempts to study the effect of air pollution on myxomycete assemblages (Wrigley de Basanta 2000) and fragmentation-dependent biodiversity (Rojas et al. 2016) have demonstrated that myxomycete responses to urban pressures can be quantified for hypothesis testing. However, these investigations have really been designed for purposes other than urban ecology assessments and have provided data indirectly.

Even though myxomycete information based on the reproductive stage (i.e. sporocarps) provides an incomplete scenario for biodiversity purposes (Schnittler et al. 2017), it does represent direct evidence of myxomycete presence. As such, for ecological studies attempting to address simple methodologies to monitor urban dynamics, myxomycete sporocarps are extremely useful for quantification. Moreover, being these the main structures of the reproductive stage, some of their morphological characters can be used for functional characterization (see Rojas & Valverde 2015), which allows long-term evaluation of the stimulus-response dynamics.

The present study has been designed to address the potential that myxomycete data can have for functional interpretation of microbial dynamics in urban centers. Based on two cities located in the Neotropical region and two main experimental approaches, the data contained herein is relevant to demonstrate that myxomycetes can be integrated in the urban ecology movement. Since these are microscopical organisms, we also attempt to demonstrate that urban microbial ecology has relevance for monitoring purposes in a rapidly changing world.

Materials & Methods

The present study consisted of two experiments conducted between 2017 and 2019. The first experiment was carried out in the Great Metropolitan Area of San José, an urbanized region within a northwest-southeast long tectonic depression surrounded by mountains in Central Costa Rica. This area occupies about 6% of the territory but is inhabited by more than 70% of the people in that country and it has a population density close to 1800 individuals/km^2. For this experiment, the two urban centers of San José (abbreviated as SJO) and Cartago (CTG) were chosen based on their size and geographical location (Fig. 1). These cities are part of an urban continuum, but they are separated by the continental divide at the Ochomogo mountain saddle and represent the Pacific and Caribbean slopes, respectively.

In each city, a central sampling point (defined either as SJO C or CTG C) was selected based on topography and elevation. From this point, the distance to the urban border was calculated in different directions and averaged. Then, other six sampling points were defined in three main directions (abbreviated as 1, 2 and 3) at similar elevations to the central point. These six points were divided in two zones, located at 2.5 times and 5 times (abbreviated as A and B) the respective average distance between the central point and the urban border. As such, each city was represented by seven sampling points where a series of 20 samples of ground litter were collected for a total of 140 samples in each case. In all instances, the material was obtained during the same day, and collected at the edge (between 1-2 m) of public roads. For contextual purposes, the ecological condition of the environment where samples were collected was considered as "heavily disturbed"; however, the Normalized Difference Vegetation Index (NDVI) and the percentage of both green areas (quantification of forest cover) and constructions (quantification of urbanization) within a 1 km radius around each sampling point were calculated from Landsat satellite imagery (USGS).

This experiment was set up to evaluate the effect of two types of urban centers on myxomycetes associated with ground substrates. Beyond potential differences in the presence/absence of species and their relative frequencies, the focus of this experiment was the evaluation of general patterns of data recovery using a standard recording methodology and the spatial design explained earlier. For this, all 20 samples from each sampling point were used to generate the same number of moist chamber cultures using the methodology of Stephenson & Stempen (1994). With this method, the material was placed on petri dishes previously lined with filter paper and water was added. After 24 h, the excess water was poured off the plate, pH values were obtained and myxomycetes were recorded within the first three months, although no other pH values were recorded. All moist chambers were studied simultaneously. Myxomycete fruiting bodies were extracted, identified, and deposited in the Herbarium of the University of Costa Rica (USJ).

For the second experiment, we aimed to evaluate data recovery from myxomycetes in urban air, and the Metropolitan Area of the Aburrá Valley in Colombia was selected. This area is very similar to the studied region in Costa Rica in terms of elevation and climate, but it has a northeast-southwest configuration. The area is also located within a tectonic depression surrounded by mountains and it is home to approximately 4 million people, making its population density close to 3400 individuals/km^2. In this area, a determination of the predominant wind directions was made using data from the Olaya Herrera and José María Córdoba airports and two sampling points were established in residential neighbourhoods in both a windward location (Copacabana, abbreviated as COP) and a leeward position (Envigado, ENV). Two more sampling points were established in Rionegro (RIO), a windward location on a higher elevation plateau, east of the Aburrá Valley, with typically higher wind speed; and in Medellín Sur (MDE), a leeward location in a heavily industrialized section of the metropolitan area, with taller buildings and more traffic than the residential locations (Fig. 1). The estimation of NDVI values as well as percentage of constructions and forested areas within a 1 km radius around each sampling point was also carried out in this case.

In all four sampling points, a series of six sterile mesh bags (HDPE, 0.27 mm pore size) containing autoclaved ground litter were set up on the four fronts of residential buildings aligned with the four different cardinal directions. These 24 bags per sampling point were placed within the first 20 m of vertical distance from the ground and left in the same position for three months. In a similar manner to Stephenson & Rojas (2020), these bags containing dead plant material served as natural traps for airborne spores of myxomycetes (referred to herein as spore traps). All bags were recollected after being exposed to the air and used to make one moist chamber culture per sample. All cultures were maintained at room conditions for four months and checked constantly for myxomycetes. After extracting and identifying the specimens, they were deposited in the Herbarium of the University of Antioquia (HUA).

In the case of both experiments, results were based on the presence data obtained with moist chamber techniques using the morphological species concept. As such, the nomenclatural system of Lado (2005-2020) was used. To analyze the data, in the software PAST, v4.01 (Hammer et al. 2001), an alpha value of 0.05 was used on all occasions for the evaluation of the respective null hypothesis. After the determination of normality, different tests such as ANOVA or t-test were used in the case of continuous variables depending on the number of groups to be evaluated. Parametric correlations were established using Pearson's r value and the respective coefficient of determination. Finally, the calculation of both the Simpson´s and the Shannon's diversity indices was carried out for ecological comparisons.

Results

Both experiments yielded positive results showing the presence of myxomycete activity in the studied urban centers. In the case of the experiment in Costa Rica, a total of 347 records in 32 species were made. From these, 289 records in 29 species were observed in the sampling points associated with San José and 58 records in 12 species were obtained from material associated with Cartago (Table 1). In both urban centers, forest coverage and urbanization showed an opposite pattern (r = -0.89 in average) and the latter demonstrated to be negatively correlated with the number of records (see Fig. 2). Interestingly, urbanization showed a strong positive correlation with the number of species in San José, but a weak correlation in Cartago.

No differences in the diversity indices were observed between the respective urban centers and the two outward zones, except for the calculation of the Shannon Index between SJO C and SJO A (t = -2.4, $d.f$ = 45.4, P = 0.02, see Fig. 3). However, diversity profiles were all different between zones across cities. Species such as *Physarum compressum* and *Diderma hemisphaericum* were observed in all urban zones of both cities, but species such as *Physarum cinereum* and *Perichaena chrysosperma* were only recorded in the outer zones.

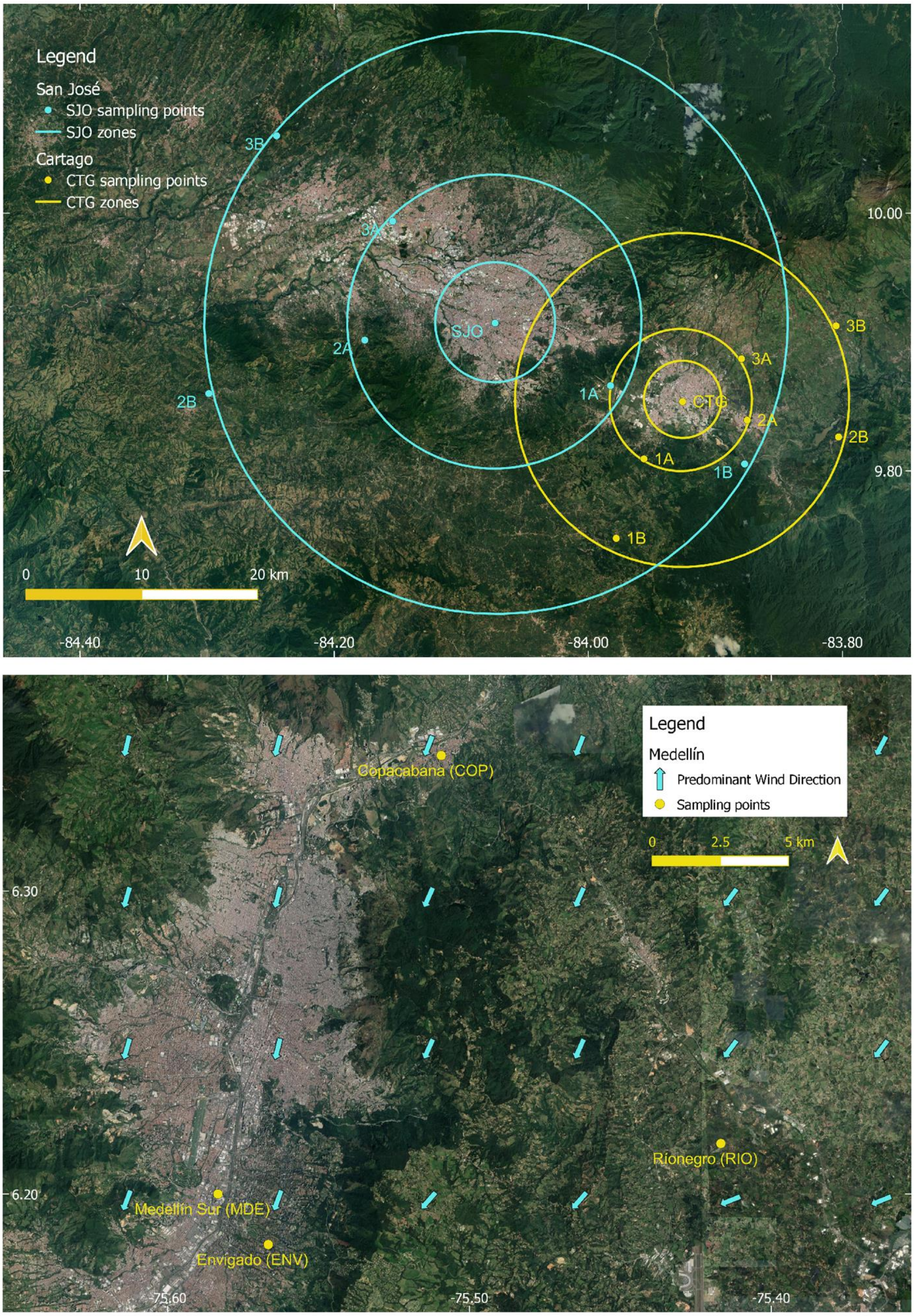

Fig. 1 – Satellite images of the two areas where the experiments were carried out. Top, experiment one in "Central Valley" – Costa Rica; radial arrangement of sampling points in both San José (SJO) and Cartago (CTG). The inner radius has been abbreviated as A, whereas the outer radius as B referring to the relation of the distance between the urban centroids and the urban borders as explained in Material & Methods. Bottom, experiment two in Valle de Aburrá – Colombia; location of sampling points in relation with the configuration of the urban area and indication of the predominant direction of winds in the general area.

Table 1 Number of records per species observed in the three main urban zones associated with San José and Cartago – Costa Rica, along with ecological summary values and quantification of urbanization. For abbreviations see Materials & Methods

Species	**San José (SJO)**			**Cartago (CTG)**		
	SJO C	**SJO A**	**SJO B**	**CTG C**	**CTG A**	**CTG B**
Arcyria afroalpina		3	3			
Arcyria cinerea	5	10	12	1	1	
Arcyria denudata		1				
Comatricha nigra			1			
Comatricha pulchella		2	1			
Comatricha tenerrima						1
Didymium bahiense	3	12	7		1	9
Didymium clavus	1	2	3			
Didymium difforme	5	19	18		1	
Didymium dubium		2				
Didymium iridis	1	3	4			
Didymium minus	2	10	6		1	
Didymium squamulosum		11	25			
Diachea leucopodia	1	2				
Diderma hemisphaericum	2	5	36	2	1	2
Fuligo intermedia					1	
Hemitrichia pardina	1	1	1			
Lamproderma scintillans		3	10			
Perichaena chrysosperma		2	3		1	2
Perichaena depressa		3	1			
Perichaena pedata	1		3			
Perichaena vermicularis		1	1			
Physarum auriscalpium		1				
Physarum bivalve		1				
Physarum cinereum		1	2		2	1
Physarum citrinum	1		1			
Physarum compressum	3	10	11	1	14	14
Physarum didermoides		2				1
Physareum melleum			1			
Physarum pusillum			5			
Physarum spectabile						1
Stemonitis fusca			1			
Number of records (avg/sampling point)	26	107 (35.7)	156 (52)	4	23 (7.7)	31 (10.3)
Number of species (avg/sampling point)	12	23 (7.7)	23 (7.7)	3	9 (3.0)	8 (2.7)
NDVI	0.30	0.40	0.49	0.35	0.38	0.45
Average forest cover (%)	5	18	45	15	26	48
Average urbanization cover (%)	53	24	11	75	25	15
Simpson's Diversity Index	0.89	0.90	0.87	0.63	0.63	0.71
Shannon's Diversity Index	2.44	2.60	2.54	1.21	1.26	1.50

In contrast, no species were recorded in the urban centers only. The pH values recorded in urban centers were the lowest (6.28±0.71) and the increasing progression towards the outermost zones (6.42±0.65 for A, 6.92±0.36 for B) was significant (F (2,207) = 37.6, P<0.0001). The number of records was found to be highly correlated with pH values (r^2 = 0.97). In San José, route 2 (heading southwest) was associated with the highest number of species and records, whereas route 3 (heading northwest) was the poorest and did not show any differences with the urban center. In Cartago, route 2 (heading southeast) showed an increment in the number of records and was associated with the highest number of species.

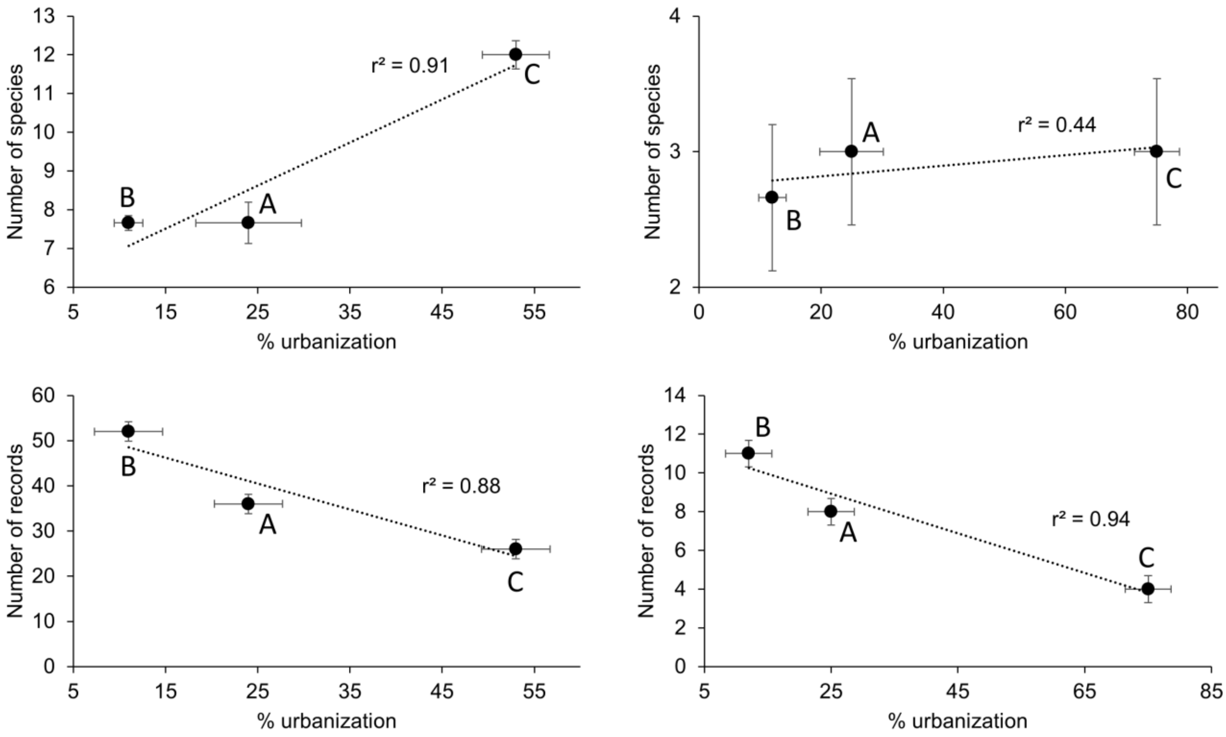

Fig. 2 – Patterns associated with the number of species (top) and records (bottom) in relation with urbanization (by means of spatial quantification of buildings) for San José (left) and Cartago (right). The values of the coefficient of determination and the variability associated with the urban zones are shown.

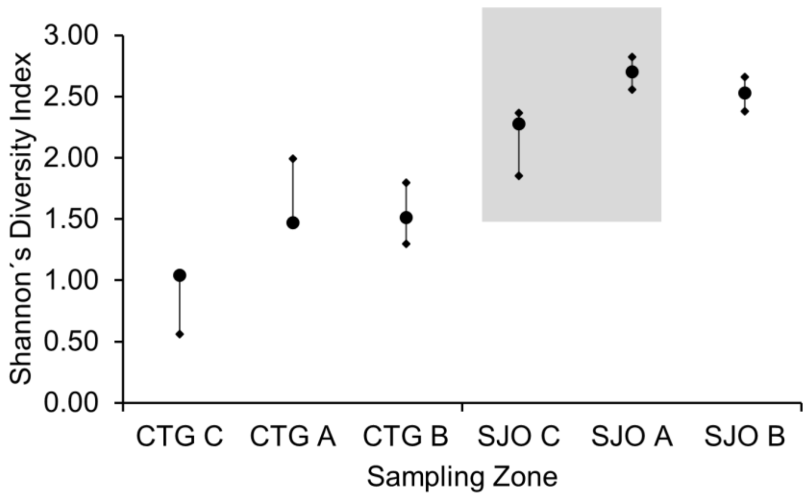

Fig. 3 – Average values of the Shannon's Diversity Index and associated range for the three sampling zones in the two evaluated cities in Costa Rica. The gray square shows the only significantly different combination. For abbreviations and coding see Material & Methods.

In the case of the second experiment, 51 (53.1%) of the total number of substrate samples evaluated in the laboratory showed myxomycete activity, but only 18 of them (18.8%) yielded fruiting bodies. In the latter, a total of seven species were identified in three of the four sampling points (Table 2). The number of non-fruiting plasmodia associated with the moist chambers was high in comparison with the number of records from fruiting bodies. Interestingly, when both values were considered, Rionegro showed the lowest incidence of myxomycete activity, whereas both Envigado and Medellín Sur were associated with the highest values. The percentage of positive moist chambers relative to the total number of samples per location showed the same pattern. Not more than one myxomycete or plasmodium was observed per sample.

When the cardinal directions associated with the spore traps were analyzed, the highest myxomycete activity was observed in the samples exposed to both the east and north directions, with 17 (33% of all positive samples) and 15 (29%) positive ones. Samples exposed to the south and west directions accounted for 21% and 15% of all positive samples, respectively. No difference in the number of positive samples per cardinal direction was observed. However, when the data was analyzed in each sampling location, clearer patterns were observed. In Copacabana, 76% (10 of 13) of the samples exposed to east and north showed activity, a similar value to 64% (11 of 17) and 43% (7 of 16) for Envigado and Medellín Sur, respectively. In Rionegro, four out of the five positive samples were exposed to the north and east, with three of them (60%) belonging to the east facing group (Table 3).

Table 2 Number of records per species observed in the four sampling points in the Valle de Aburrá – Colombia, along with the number of plasmodia and relative positive activity observed in 24 moist chambers. A summary of urbanization characteristics of each place is also provided. For abbreviations see Materials & Methods.

Species	**Sampling point**			
	COP	**ENV**	**MDE**	**RIO**
Arcyria cinerea		4	2	
Comatricha nigra			2	
Comatricha tenerrima			1	
Didymium bahiense			2	
Perichaena depressa			1	
Stemonitis axifera	1			
Stemonitis fusca		2		
Plasmodia	10	10	8	5
Percentage of positive moist chambers (out of 24)	54	71	67	21
Number of species	1	2	5	0
Number of records	1	6	8	0
NDVI	0.02	0.17	-0.02	0.32
Average forest cover (%)	15	30	5	30
Average urbanization cover (%)	75	40	85	10

Table 3 Number of records (including plasmodia) of myxomycetes associated with samples exposed to the four cardinal directions in all sampling points in the Valle de Aburrá – Colombia.

Sampling point	**Cardinal direction of exposure**			
	East	**North**	**South**	**West**
Copacabana (COP)	4	6	0	3
Envigado (ENV)	5	6	4	2
Medellín Sur (MDE)	5	2	6	3
Rionegro (RIO)	3	1	1	0

Discussion

Myxomycetes are clearly present in urban environments. From a distributional point of view, this is not surprising since such observation has been previously made in both plant material and in aerial conditions (see Wrigley de Basanta 2000, Hosokawa et al. 2019, Oh et al. 1998, Surratt & Levetin 2005). However, this is the first focused study on myxomycetes in Latin American cities, and, as such, it is a relevant contribution to the ecology of these microorganisms in the Neotropics, and to the potential of this type of information for environmental monitoring. Previous studies have already demonstrated that myxomycetes are present in a wide range of ecological situations

(Rollins & Stephenson 2011) and that they are resilient to aspects of city environments such as heavy metal pollution (Setälä & Nuorteva 1989); but pattern recognition in urbanized settings is largely absent in most studies on myxomycetes, and by default, their urban ecology is still poorly documented.

In the case of the experiment in Costa Rica, it is interesting to note that the degree of urbanization influenced the number of records but not the number of species. As such, results suggested that the presence/absence of species is site-specific and closely linked to landscape-level aspects. A similar observation had been previously made in the Costa Rican Central Valley (see Rojas et al. 2016), where most differences in the species composition of floricolous myxomycete assemblages had been associated with the structure of the vegetation within a study site, regardless of its location within the urban context. Interestingly, also supported by previous research, the number of records of myxomycetes using the moist chamber technique was heavily influenced by the availability of suitable substrates (see Stephenson et al. 2020), which in the case of the urban environments studied herein, likely increased with vegetation complexity in the outer zones of the studied cities.

It was interesting to note that substrate pH also increased from the urban centers to the outer zones, presumably linked to vehicle density and emissions (see Shi et al. 2017), but the degree of change was small and did not seem enough to affect ecological patterns. Most myxomycete species associated with ground litter in Costa Rica can form fruiting bodies in wider ranges of pH values (see Schnittler & Stephenson 2000, Lado & Rojas 2018), although it is known that bark species are more susceptible to pH changes (Wrigley de Basanta 2004). In this sense, pH could have been a secondary factor accounting for differences in the results observed herein, but the degree of urbanization was much likely the primary driver of differences. This effect could be explained by limited substrate availability and simpler habitat complexity within cities (see a review on the topic from Liu et al. 2016). As such, it would be valuable to assess this hypothesis in a future project focusing on urban settings.

Interestingly, even though the experiment in Costa Rica was carried out using ground substrates, the fact that two southern sampling point routes (SW bound in San José and SE bound in Cartago) showed the highest values of the ecological estimators evaluated, suggested that wind could also play a role in the distribution of potential propagules (i.e. myxomycete spores). In that valley, the wind comes from the northeast and normally has a southwest/south direction. With the data from the second experiment in Colombia, it seemed that such observation was not completely illogical. After all, suspended particles in the Aburrá Valley, spores included presumably, are pushed away from windward (north) to leeward (south) areas (Rave et al. 2008). As observed in the results, the two windward sampling points of Copacabana and Rionegro were the ones with 1) the lowest number of observations, 2) the lowest number of positive moist chamber cultures and 3) the lowest values of recorded species. In contrast, the two leeward sampling points in Envigado and Medellín Sur, showed the highest values for all these parameters. It is clear that wind is not the only factor that can be related with the distribution of data in the present study but playing such an important role in the distribution of organisms, it is likely the primary driver of results.

However, it seemed that the difference in outcomes between Envigado and Medellín Sur could be related with two separate factors that also differ between these two locations. Both industrialization and convective currents have been shown to be higher in the Guayabal area, where the Medellín Sur sampling point was located, than in Envigado (see Rave et al. 2008, García et al. 2008). Industrialization is linked with taller and hotter buildings (i.e. industrial chimneys), higher albedo and warmer surfaces, which create constant convective currents moving the air vertically, which in contrast, are buffered by the higher percentage of forested areas in Envigado. As such, even though higher values of species richness and record abundance could be expected in these two southern locations, site-specific turbulence primarily caused by constant air mixing in Medellín Sur could partially explain the more even distribution of records in all four cardinal directions in this sampling point [presumably in a similar manner to the study of Policina & dela Cruz (2020)]. Data from the two windward sampling points seemed to support such observation, since in both locations those samples exposed to the cardinal direction opposite to the direct influence of the wind, yielded zero myxomycete activity.

Our results are very limited to make strong conclusions, but certainly suggested potential effects of urban landscape settings on myxomycete data. The importance of the present study is to bring back the topics of urban ecology and myxomycetes together in a moment where urban assessments are important for climate change and green city design. It would be valuable to plan future studies with higher density of sampling points and quantification of other parameters such as the temporality and speed of wind direction. However, the experiments presented herein demonstrated that the use of simple techniques such as the spore trap in urban contexts could be valuable for monitoring purposes. Recent studies (i.e. Stephenson & Rojas 2020) have found that mosses could be more suitable than leaves as the primary substrate to capture airborne spores, and this simple modification of the protocol used in the present investigation could generate improved results.

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