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Rapid diversity assessment of litter myxomycete assemblages in the upland and coastal terrains of San Fernando City, La Union, Philippines

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Abstract. *Balaoro-Banzuela RC, Ocenar-Bautista CE, Buebos-Esteve DE, Claudio-Paragas CY, Limbo-Dizon JE, Dagamac NHA. 2023. Rapid diversity assessment of litter myxomycete assemblages in the upland and coastal terrains of San Fernando City, La Union, Philippines. Biodiversitas 24: 2877-2886.* Over the past few years, increasing studies have been on diversity assessments of myxomycetes in the Philippines. Despite these, their utility as bioindicators to evaluate approaches for sustainable ecosystem management remains largely unexplored. Following the ridge-to-reef (R2R) approach, a comparative diversity assessment was conducted between the upland and coastal terrains of San Fernando City, La Union, Philippines. Aerial and ground litter were haphazardly collected and cultivated at ambient conditions using the moist chamber (MC) technique. MCs were highly productive, exhaustively yielding a total of 27 distinct species belonging to 10 different genera. Regarding abundance, 15 species were rare, and four were occasional, common, and abundant. Both species richness and occurrence of rare taxa were higher in the upland terrain than in the coastal terrain. However, several α-diversity indices hinted at no significant difference in species diversity between terrains and substrates. At the community level, clustering analyses and other β-diversity indices revealed that myxomycete assemblages were more similar in terrain than the substrate. This study implicates using myxomycetes as proxy biological models to reflect the ecological influences of plant heterogeneity and human-mediated disturbance. This allows its integration into local policymaking with adherence to R2R management.

Keywords: Biodiversity, intermediate disturbance, microbial ecology, protist, slime molds

INTRODUCTION

Among the eumycetozoans, studies of myxomycetes have been rapidly increasing during the last decade relative to its other taxonomic groups, such as the dictyostelids and the protostelids. Studies of myxomycetes in the Philippines vary from anecdotal species annotations to classical synecology (Dagamac and dela Cruz 2019; Macabago and Stephenson 2021). These studies have covered different vegetations types and many forest islands in the country, ranging from Luzon (Bicol (Dagamac et al. 2017; Macabago et al. 2020), Mindoro (Macabago et al. 2012; Dagamac et al. 2015a), Palawan (Pecundo et al. 2017; Macabago and Stephenson 2021), Pampanga (Dagamac et al. 2014), Pangasinan (Kuhn et al. 2013), Quezon Province (Viray et al. 2014), Zambales (dela Cruz et al. 2010)), the Visayas (Bohol (Macabago et al. 2017), Negros (Alfaro et al. 2014)), and Mindanao (Maguindanao (Buisan et al. 2019), Cotabato (Buisan et al. 2020)). These studies consistently show the following points. Firstly, forest litter harbors a significant number of new records for slime molds in the country (Dagamac et al. 2015b). In comparison to many other substrates, such as barks (Dagamac et al. 2010), grasses (Carascal et al. 2017), and agricultural litters (Buisan et al. 2019), forest litter that is found both on the forest ground and those that are merely hanging still on the aerial parts of tree trunks or forest canopies harbor common myxomycete species that are morphologically clear-cut. Secondly, the heterogeneity of plant communities seems to have a more relevant influence than anthropogenic disturbances (Rea-Maminta et al. 2015), directionality (Policina and dela Cruz 2020), spatial proximity (Bernardo et al. 2018), or natural calamities (Cabutaje et al. 2021). Lastly, fewer accounts for myxomycete diversity have been reported on uncharted ecosystems in the country, i.e., mangal, estuarine, or caves (Limbo-Dizon et al. 2022). That means many ecosystem types that can be described as unique and might harbor undiscovered myxomycetes species can still be tapped to be investigated in the Philippines.

Diversity assessments for myxomycetes have been used to suggest implications that would best understand ecological phenomena that fill the knowledge gap of myxomycetes in the Philippines in terms of biogeographical distribution (Dagamac et al. 2017) and ecological niche requirements (Almadrones-Reyes and

Dagamac 2018). These assessments are used mostly in creating tangible documentation of slime molds for baseline information on the richness of microbial flora in many terrestrial ecosystems in the country (see studies of Pecundo et al. 2021; Eloreta et al. 2020; Dagamac et al. 2014). Despite this, using this microorganism as a candidate species to address possible effective management strategies implemented locally has not been comprehensively explored. This is because most research tackling microbial communities focuses on a single species or at a single habitat and not on a level of diversity and distribution of microbial assemblages at a landscape level. At the latter level, microorganisms' diversity can become proxies to effectively utilize policies to conserve and restore vulnerable ecosystems. It is important to take note that for microorganisms, such as myxomycetes, to be included in an expanded theoretical conjecture of food web at a landscape scale as they conform to a structural and biotic landscape and follow a continuous landscape model that accounts for the presence of multiple substrate or host across a gradient of changing environmental factors (Amend et al. 2022).

Numerous areas in the country have been surveyed regarding myxomycete diversity for the past few years. Nevertheless, the attempt to use myxomycete profiles as bioindicators to assess specifically the approaches in sustainable management using the ridge to reef concept (R2R) is still unexplored. R2R is an approach that takes into account all the environments starting from the upland (ridge) and all the way down to the coastal (reef). Hence for the present study, having litter substrates collected at the upland and coastal terrains in the province of San Fernando City, La Union, utilizes myxomycetes not only for diversity assessments between two terrains but also to implicate slime molds as a prospective bioindicator on the whole ecosystem management strategy in the province, especially now that rapid urbanization and habitat degradation are imminent threats for a possible decline in biodiversity of this green sustainable municipality.

MATERIALS AND METHODS

Study site

San Fernando in La Union province (16.6159° N, 120.3210° E) is the chosen site for isolating myxomycetes for its specialized topography in coastal and upland environments (Figure 1). San Fernando City, La Union, is located in the Ilocos region, about 270 km north of Manila. Being part of a tropical country, San Fernando has an annual temperature that ranges from 24°C to 32°C and has a wet season starting from June to November and a dry season from December until May due to having well-distinct coastal and upland sites which support many microfauna and microflora species.

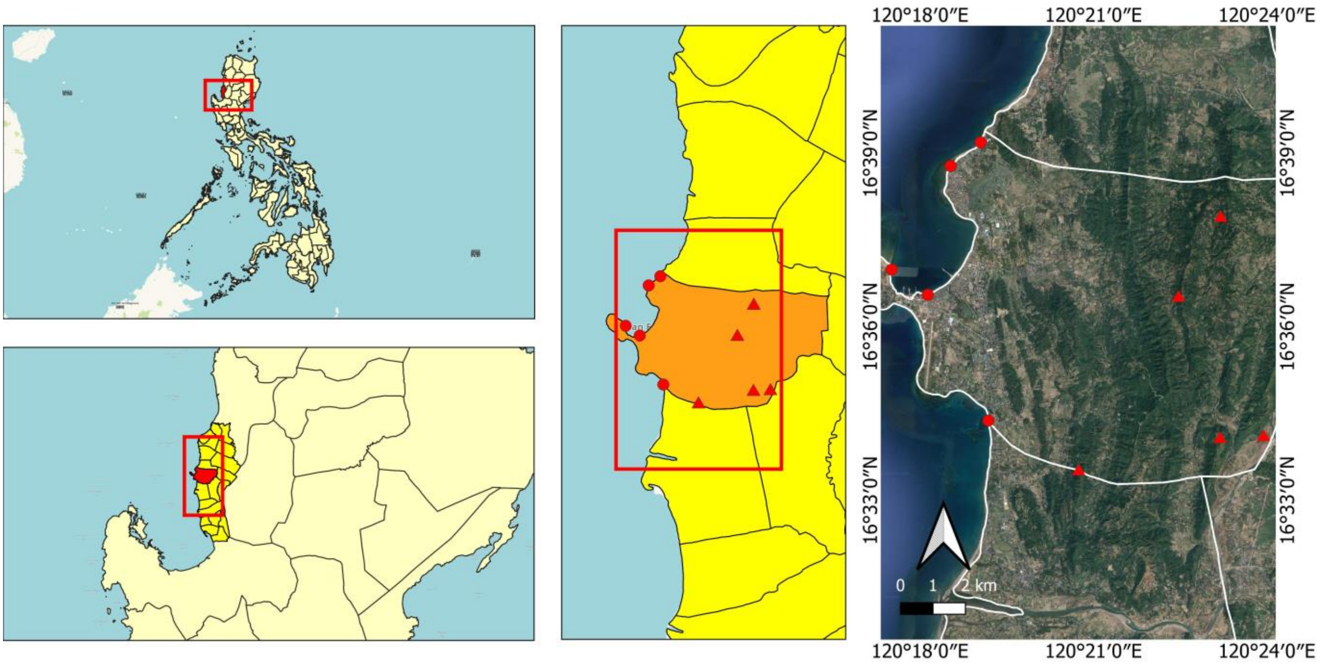

Figure 1. Location of the study area within the Philippines (inset) and geographic map of La Union showing the coastal (circle) and upland (triangle) collection sites

Litter sampling and cultivation procedures

The collection for each terrain was done by having five collecting points. These sample points were accessible and were permitted by the City Environmental and Natural Resource Office (CENRO-San Fernando). Therefore, to avoid experimental bias, sampling sites were randomly selected. A 10m x 10m plot was randomly established to collect three aerial litter and three ground litter for each collection point. This makes 15 aerial litter and 15 ground litter for each terrestrial terrain (upland and coastal) and a total of 30 aerial litter and 30 ground litter for the whole research study. Furthermore, following the set-up of the study by Nguyen et al. (2019), no woody substrates were collected due to the scarcity of this type of substrate in the coastal terrains, hence making only leaf litter samples the priority of collection for this study. All litter samples were air-dried before being kept in paper bags until further use in the laboratory.

The 30 aerial and 30 ground litter samples collected from upland and coastal terrains were cut into smaller strips to fit on a moist chamber set-up described by the protocol of Stephenson and Stempen (1994). Each sample corresponds to a single moist chamber culture for this research study. Moist chambers after being wet for 24 hours, pH measurements were then accounted for, and the 12 weeks observation for plasmodial or fructification commenced. Every once in a while, the moist chambers were sprayed with water to maintain the moist condition until the last week of observation. Moist chambers were maintained under ambient light conditions in the laboratory with an average room temperature of 22-25°C.

Identification and characterization of myxomycetes

All fruiting bodies developed in the moist chambers were removed, allowed to air-dry gradually, and placed in small herbarium boxes with pasteboard trays. The gross morphological features were determined under a dissecting microscope (Olympus, USA) at various magnifications. The diagnostic microscopic features were observed by preparing slides with material mounted in 95% ethanol, following the procedures described by Stephenson and Stempen (1994). Slides were then observed under a brightfield compound microscope (Scanner Objective to OIO, Olympus CX 31). All morphological data were used to identify the myxomycetes to species in most instances and to the genus level when this was impossible. Identifications were made with the use of published literature (Poulain et al. 2011), and currently accepted names were checked and are based upon the online nomenclatural database (Lado 2023) available for eumycetozoans (http://nomen.eumycetozoa.com).

Data analysis

Percent yield was initially calculated to provide an overview of the possible distribution of myxomycetes between the terrains. Any moist chamber culture (MC) that showed some evidence (either plasmodia and/or fruiting bodies) of myxomycetes was considered positive (and thus was recorded as one positive culture). Percent yield was then calculated as the number of moist chamber cultures positive for myxomycetes divided by the total number of moist chamber cultures prepared (Dagamac et al. 2012). To estimate the extent to which the recent rapid survey was exhaustive in terms of species that were recorded in the terrains of the study area, a species accumulation curve for myxomycetes only was constructed based on the records obtained from the collection in the moist chambers, according to the rarefaction formula using the default settings of the program EstimateS (version 9.1; 200 randomizations; http://purl.oclc.org/estimates). The species composition of the collection sites was initially determined simply by recording the different species collected from each terrain to account for their alpha diversity. The occurrence of various myxomycete species in each moist chamber was then calculated. For the calculation, remember that the moist chamber that displayed a fruiting body of a particular species was considered a single positive collection (a taxonomic unit proxy). The recurrent presence of a specific species of myxomycetes in a positive moist chamber was termed its occurrence. A collection was now regarded as an individual unit. The value obtained by dividing the total number of collections for each specific species of myxomycetes by the total number of myxomycetes collected was defined as the relative abundance for each species (Dagamac et al. 2015b). The relative abundance of each species of myxomycetes was interpreted as the abundance index. The computed relative abundance for each species was then translated to an abundance index that was described by Stephenson et al. (1993), namely, rare for species <0.5% of the total number of collections, occasional for species > 0.5% but < 1.5% of the total number of collections, common for species >1.5% but < 3% of the total number of collections, and abundant for species > 3% of the total number of collections. All abundance data for fruit bodies obtained from collecting localities were compared using the Kruskal Wallis test employed in Paleontological Statistics (PAST) software. Abundance-based datasets between terrains and litter sampling types were analyzed in R Studio. The diversity between the terrain (upland versus coastal) and litter (aerial versus ground) was compared using the classical richness indices of Fisher's alpha, the Shannon index (considers richness and evenness), and the first Hill's diversity index (Hill 1973). These indices were tested for significance by a Wilcoxon test. In addition, the most probable abundance distribution model was determined from rank-abundance plots (Whittaker 1965) testing five models following Wilson (1991): Null (fits the broken stick model), Preemption (fits the geometric series or Motomura model), log-Normal, Zipf and Mandelbrot. Both diversity indices and distribution models were calculated in the 'vegan' package of R, using the functions *renyi* and *radfit*, respectively.

The PAST software implemented cluster analysis based on Bray Curtis and Euclidean similarities to infer corresponding beta diversity. Moreover, Venn diagrams show species distribution between terrains and litter types for community analysis. Furthermore, the Coefficient of Community (CC) and Percentage of Similarity indices adapted from Stephenson et al. (1993) were used. The

Coefficient of Community considers the presence or absence of a species, with index values ranging from 0 (the absence of species in either community) to 1 (all species are present in the compared communities).

RESULTS AND DISCUSSION

This study recorded 85% (51 out of 60) positive plates for the occurrence of myxomycetes subsequently to their cultivation in moist chambers. From these positive plates, 14% and 86% yielded plasmodia only and successful fructification, respectively. Comparing the yield of positive moist chambers between the two different terrains, the coastal yielded 45%, whereas the upland yielded 55%. On the other hand, in comparison between the two substrates, the aerial litters exhibited a higher positive yield when compared to the ground litters at 43% and 57%, respectively. A species accumulation curve that compared terrains (Figure 2.A) and litter types (Figure 2.B) shows that there are more species in the upland than in the coastal and that there is a higher number of species in the litter gathered on the ground than in the aerial litter.

The collected samples, as a whole, consists of 27 determined species belonging to 10 different genera (Table 1). The computed relative abundance exhibited four abundant species (*D. hemisphaericum, A. cinerea, D. leucopodia,* & *G. vermicularis*), four common (*D. squamulosum, D. nigripes, P. cinereum,* & *P. M compressum*), four occasional (*D. effusum, P. depressa, E. minutum,* & *P. corticalis*), and 15 rare (*A. afroalpina, A. cinerea var. digitata, A. globosa, A. pomiformis, C. tenerrima, D. iridis, O. pedata, P. echinospora, P. album, P. bivalve, P. echinosporium, P. globuliferum, P. lakhanpalii, P. leucophaeum,* & *P. notabile*) (Figure 3).

The rank-abundance plot (Figure 4) that utilizes different abundance distribution models exhibited similar results for the upland and coastal terrains and aerial and ground litter substrates. Therefore, based on the datasets used for the two different terrains and two litter substrates, the preemption model was the most suitable among the five models used in this plot.

In comparing the different estimators for species diversity (Figure 5), the boxplot exhibits no significant difference in the upland terrain compared to the coastal ecosystem. This can be seen with the corresponding diversity indices showing overlapping plots in the upland than in the coastal (Figure 5.A). Similar results can also be observed in the litter substrate, where a similar trend can be observed (Figure 5.B).

Table 1. Recorded myxomycete occurrences concerning terrains and substrates

Species	Terrain		Substrates	
	Upland	Coastal	Aerial litter	Ground litter
Arcyria afroalpina Rammeloo	1	-	1	-
Arcyria cinerea (Bull.) Pers.	6	8	8	6
Arcyria cinerea var. *digitata* (Schwein) G. Lister	-	1	-	1
Arcyria globosa Schwein.	1	-	-	1
Arcyria pomiformis (Leers) Rostaf.	1	-	1	-
Comatricha tenerrima (M.A. Curtis) G. Lister	1	-	1	-
Diachea leucopodia (Bull.) Rostaf.	3	9	7	5
Diderma effusum (Schwein.) Morgan	1	3	3	1
Diderma hemisphaericum (Bull.) Hornem.	10	8	12	6
Didymium iridis (Ditmar) Fr.	1	-	1	-
Didymium nigripes (Link) Fr.	5	1	5	1
Didymium squamulosum (Alb. & Schwein.) Fr. & Palmquist	8	1	7	2
Echinostelium minutum de Bary	-	2	0	2
Gulielmina vermicularis (Schwein.) Garcia Cunch., J.C. Zamora & Lado	5	7	9	3
Ophiotheca pedata (Lister & G. Lister) Garcia Cunch., J.C. Zamora & Lado	1	-	1	-
Perichaena corticalis (Batsch) Rostaf.	-	2	1	1
Perichaena depressa Lib.	-	4	3	1
Physarina echinospora K.S. Thind & Manocha	1	-	-	1
Physarum album (Bull.) Chevall.	1	-	1	-
Physarum bivalve Pers.	-	1	-	1
Physarum cinereum (Batsch.) Pers.	-	6	4	2
Physarum compressum Alb. & Schwein.	3	2	4	1
Physarum echinosporum Lister	-	1	-	1
Physarum globuliferum (Bull.) Pers.	1	-	1	-
Physarum lakhanpalii Nann. Bremel & Y. Yamam.	-	1	-	1
Physarum leucophaeum Fr. & Palmquist	1	-	1	-
Physarum notabile T. Macbr.	1	-	1	-
Plasmodium white	5	6	4	7
Plasmodium yellow	2	3	1	4

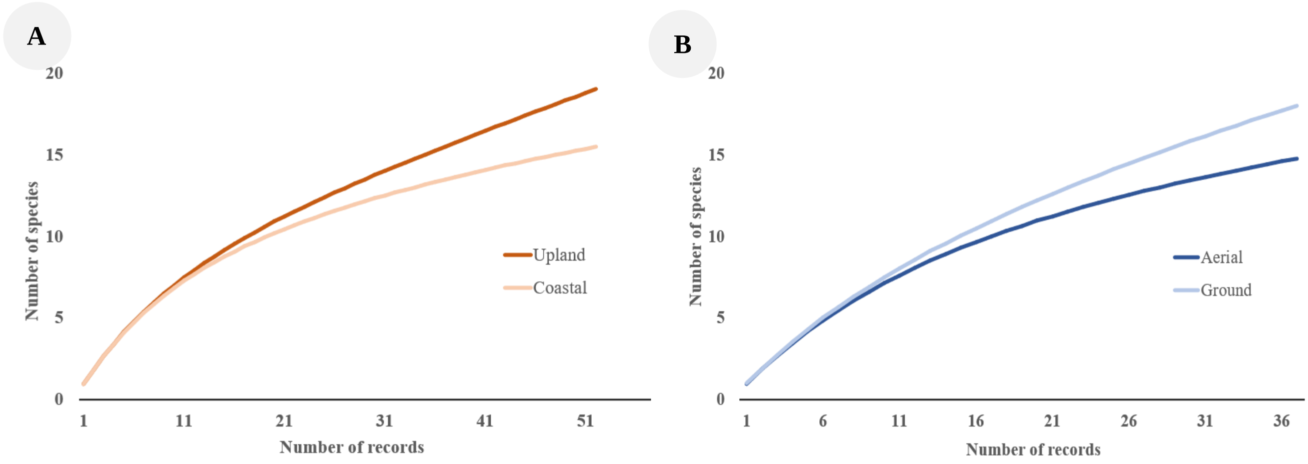

Figure 2. Species accumulation curve of myxomycetes on: A. Two types of terrains (coastal and upland); and B. Litter substrates (aerial and ground)

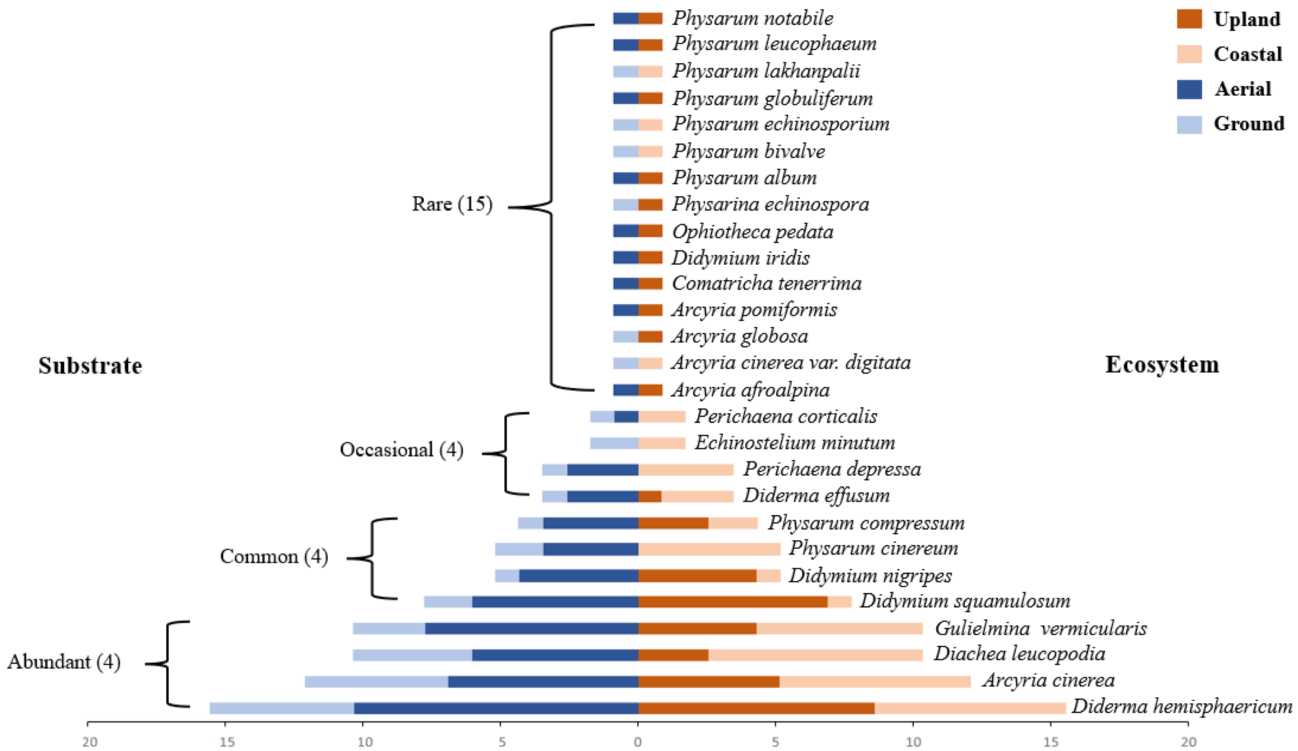

Figure 3. Calculated species abundance between the two ecosystems and two substrate type

In comparing the ecological distances using clustering analysis, the Euclidean (Figure 6.A) and Bray-Curtis (Figure 6.B) dissimilarity indices showed the same assemblages where groupings of myxomycetes were more similar in terms of the terrain rather than of the litter substrate.

A Venn diagram showing two beta diversity and species distribution was made to describe further the species distribution concerning the terrain and litter substrate (Figure 7). The Venn diagram comparing the two terrains shows 11 species exclusive to the upland and seven species belonging only to the coastal. However, regarding similarity, this only showed nine species evident in both terrains. This resulted in a low coefficient of community and percentage similarity values having 0.50 and 0.47, respectively. On the other hand, in terms of litter substrate, this exhibited the same number of species limited for each substrate, having eight species for aerial and eight for ground. With this, 11 shared species were recorded between the two substrates, exhibiting a CC value of 0.58 and a PS value of 0.56.

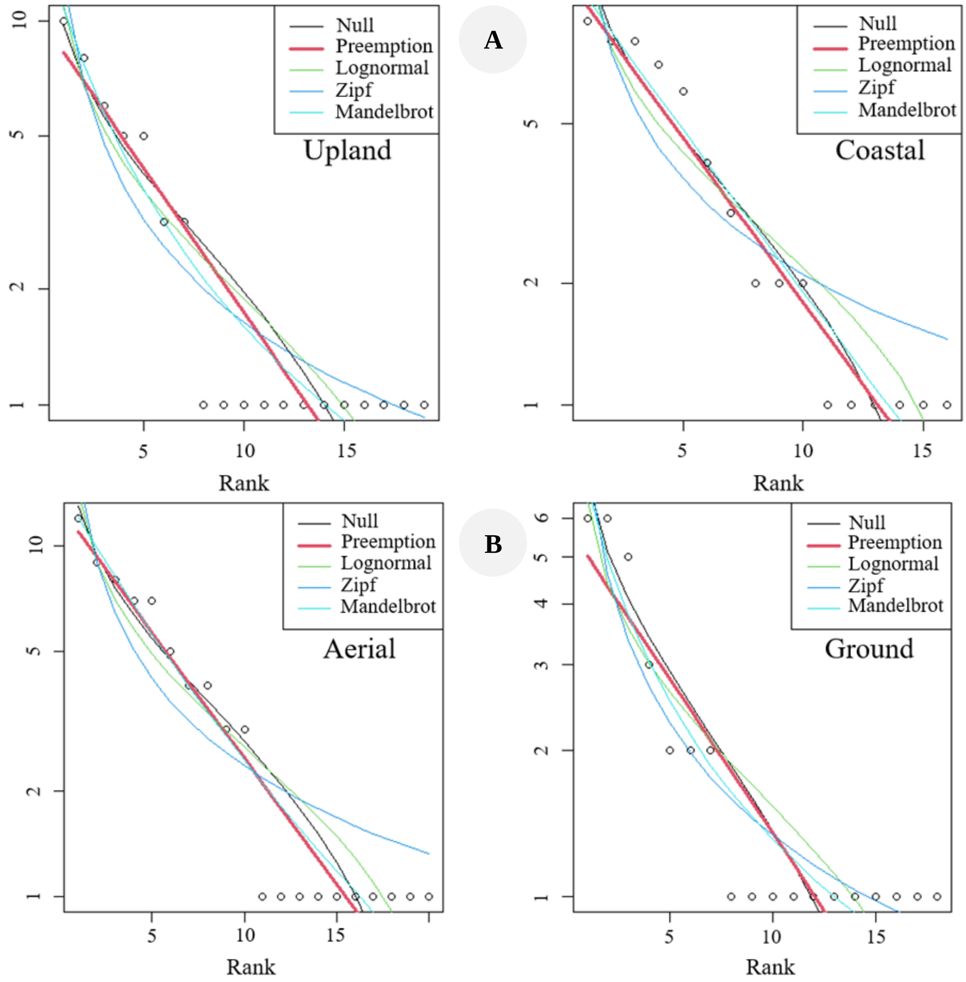

Figure 4. Rank-abundance plot for the abundance of species based on: A. Differing terrains (upland versus coastal); and B. Substrate (Aerial and Ground) fitted with five species distribution models. A thick line highlights the model showing the best fit

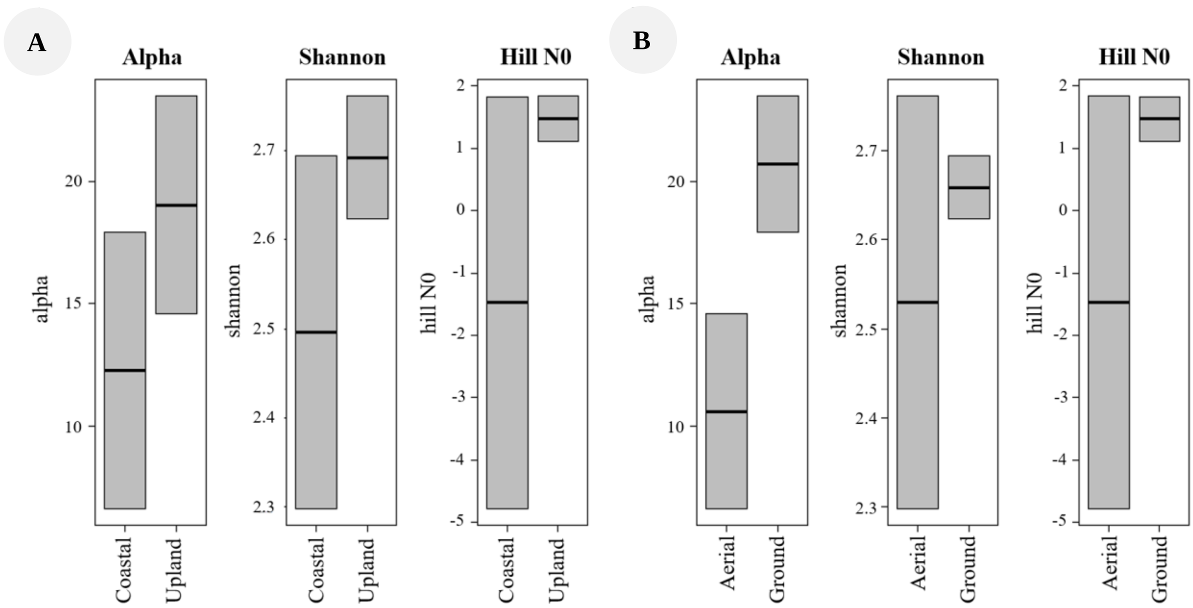

Figure 5. Box plot illustrating three diversity indices (Alpha diversity, Shannon diversity, and N0) based on: A. Terrain (coastal and upland) and B. Litter substrate (aerial versus ground)

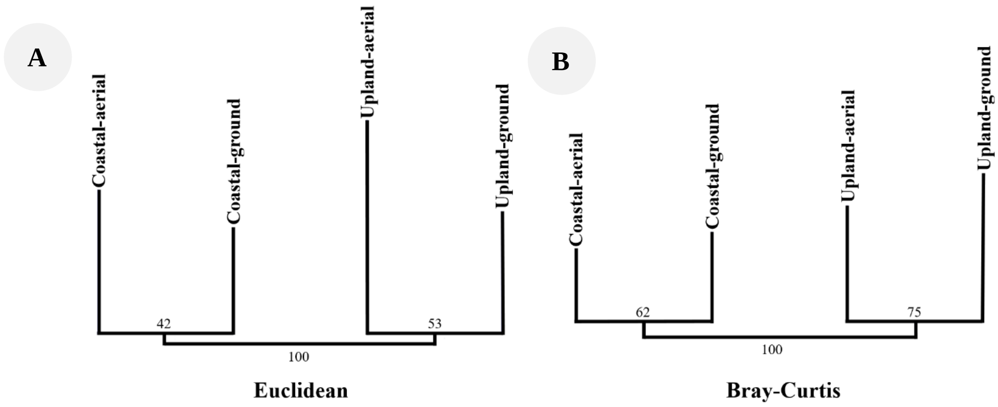

Figure 6. Community clustering analysis of myxomycetes using the bootstrap method on PAST based on: A. Euclidean; and B. Bray-Curtis dissimilarity

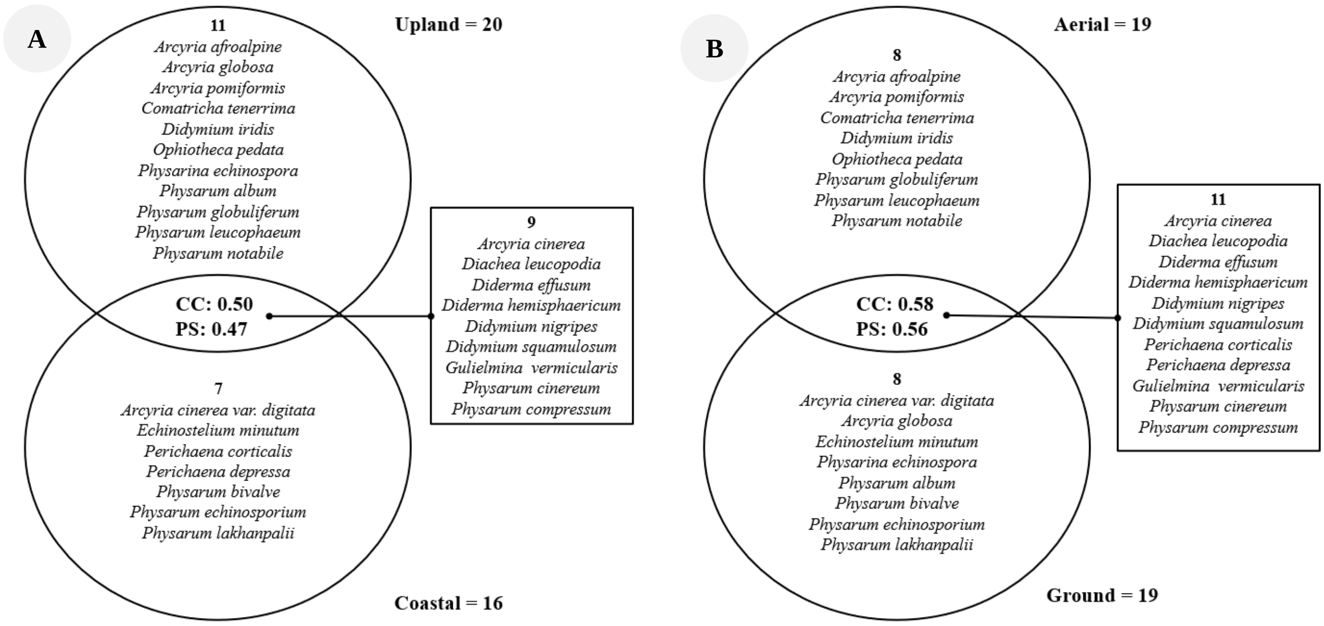

Figure 7. Venn diagram showing the two beta diversity (CC = Coefficient of community and PS = Percentage similarity) and distribution of myxomycetes between: A. Two terrains; and B. Two substrates

Discussion

This rapid synecological study using litter myxomycetes assemblages as a measure to compare the upland and coastal terrains in San Fernando City, La Union, is the first of its kind study conducted in the municipality. Besides understanding myxomycete ecology in the tropics, the presented result herein may serve as a proxy for understanding ridge-to-reef management, an ongoing initiative of local stakeholders promoting environmental sustainability.

The diversity of myxomycetes between the two types of litter (aerial vs. ground) may vary with many factors that can greatly impact its physiological processes for such litter-dwelling microbial flora to thrive. For myxomycetes, however, since these slime molds usually prey on bacteria-where it is evident on litter substrates-the diversity that considers the number of species and evenness of distribution in the locale favors no specific litter substrate. This can be attributed to leaf litter's capacity in any terrain to serve as a suitable microhabitat for myxomycetes. Despite the higher number of possible species (Figure 2), the alpha diversity measures in Figure 5 shows no significant difference between the two litter types. However, despite having no clear significance in diversity, the species composition between the two litter types clearly varies. Species that grow on aerial litter may be due to the ability of the substrates to dry out faster after rain and having better spore dispersal opportunities that cause them to diversify more (Dagamac et al. 2015). On the other hand, species that grow on ground litter may result from the high microbial communities that may potentially reside in the soil and the high amount of dead organic matter (Nguyen et al. 2019). This supposition is exhibited in our result in Figure 7, where eight species belonging to 5 genera were observed in the aerial litter. In comparison, eight species from 4 genera were recorded in the ground litter. Nevertheless, the amount of shared myxomycete species (11 species, Figure 7) between the two litter types is indicative that regardless of whether it is aerial or ground leaf litter-both can provide a suitable environment for many cosmopolitan myxomycetes reported already in the tropics.

At a landscape level, diversity between the upland and coastal terrain differs, too. However, when it comes to ubiquitously found organisms, diversity in the upland terrain is greater than that of the coastal. That is because having a complex yet unique environment and geology, where it is rarely disturbed by factors that cause detrimental effects, houses various species that persisted through the test of time (Gradstein et al. 2008). This is in contrast to the coastal terrain that is frequently disturbed by many factors (DiBattista et al. 2020). This paradigm goes the same way with myxomycetes, where the diversity of these slime molds is also found greater in the upland terrain. Wherein as exhibited in Figure 2, species richness is higher on the uplands than on the coastal terrains. Similar results can also be observed in the study of Macabago et al. (2016), where montane forests yielded higher myxomycetes diversity than coastal forests. Perhaps, the reason for this diversity is much more than that of the unique geomorphological structure of the upland terrain in San Fernando City. This can be attributed to the homogeneity of the biological communities—specifically, the difference in composition of plant communities found in the upland, which are usually made of perennial shrubs and timber trees.

The decrease in diversity from the upland down the coastal terrain is projected with the ridge-to-reef model. For this study, myxomycete assemblages were accounted only at the tail-end of the ridge-to-reef environments, excluding the other ecosystems in-between in the municipality of San Fernando. The result showed the difference in myxomycetes in composition, richness, and evenness. The finding that establishes the upland terrain as being more species-rich than the coastal terrain is consistent with a study by Macabago et al. (2016) and Cabutaje et al. (2021) that reports a higher diversity in sites farther from the seacoast than the ones that are in proximity. Therefore, having said that the outcomes of the present study may be attributed to the fact that the plant communities in the upland hills of La Union are comparatively more heterogeneous than in coastal sites where anthropological disruptions are constantly disrupting vegetation due to tourist congestion, which in turn results in biodiversity loss in plant communities as per status quo. Moreover, Sperandii et al. (2018) provided key findings that designate abiotic, human-mediated disturbances, and landscape factors (in that specified order) as key drivers of plant diversity in coastal dune ecosystems, thus further speculating a higher plant diversity established in upland terrain, which is only intermediately disturbed. The case may be implicit that the heterogeneity of the plant compositions and the abundance of available microhabitats in upland areas of La Union may have contributed to a richer composition of myxomycetes, as established in this study. This supposition is supported by a previous report by Schnittler and Mitchell (2000) which links the actual distribution of myxomycetes to the availability of suitable microhabitats that are apt for their establishment, growth, and development. Ultimately, the pattern of ecology in the ridge-to-rift environments speculatively conforms with the intermediate disturbance hypothesis (Connell 1978), where the highest species diversity, specifically species richness, is established in intermediately disturbed areas where it then follows a descending fashion towards the tail-ends of the disturbance model. Furthermore, myxomycetes diversity in other areas in the Philippines, such as Puerto Galera (Dagamac et al. 2015a) and Bicol (Dagamac et al. 2017), appear to be contiguous with the intermediate disturbance hypothesis (Connell 1978), which presumes the influence of anthropogenic or natural disturbance to myxomycetes assemblages in the localities mentioned above. However, this aspect in the tropics is still not clearly understood, and only a more robust study design is recommended to ascertain if myxomycetes follow such intermediate disturbance hypothesis fully.

With the trends in habitat bioindicators currently being associated with microbial assemblages due to the ease and conventionality in their isolation and cultivation contrary to macroorganisms, slime molds have been a consistent model for many rapid ecological studies focusing on ecosystem management (Novozhilov et al. 2022). Ecological relationships of these slime molds have diverted researchers' attention, specifically studying myxomycetes' ability to be microbial bioindicators towards environmental factors. Therefore, as stimulants of organic matter and effectors of bacterial communities, distinct assemblages of myxomycetes can potentially be associated with physicochemical soil attributes, flora, microbial abundance, and diversity (Fiore-Donno et al. 2016). Indicator alternatives depend on the inherent hypothesis of system dynamics of the study, and so macroenvironmental indicators are also possible subjects for ridge-to-rift research. However, microbial bioindicators are preferred as per the reason for their more convenient techniques for isolation and cultivation compared to macro indicators, which consist of more extensive and time-consuming procedures.

Evidently, with the relatively high yield (85%) of positive plates despite the limited number of litter collected, it can already be denoted that a rapid diversity assessment for myxomycetes utilizing the moist chamber technique is effective. Moreover, their vast numbers in coastal and upland environments, distinction in their interspecies morphology, as well as their role as natural nutrient recyclers, and maintaining balance in their respective ecosystems, myxomycetes are a mainstay when it comes to assessing the quality of their habitat as well as examining the effectiveness of implemented conservation (Rojas and Stephenson 2013). However, it is noteworthy that, albeit not directly affected by these measures, the diversity of myxomycetes assemblages can appraise the intensity of the disturbances experienced by their habitat (Macabago et al. 2017). This is relevant to the site of choice because poorly regulated anthropological activities mainly caused by tourism are very much apparent in La Union. In addition, findings recovered from this study presume that myxomycetes can also be an effective rapid bioindicator to reflect plant heterogeneity and human-mediated disturbances, which allows its integration in reinstating measures with adherence to ridge-to-reef management.

In conclusion comprehensive synecological profiling of myxomycetes assemblages in upland and coastal terrains of San Fernando City, La Union, Philippines, was conducted for this research study. The study has provided insights regarding the use of these profiles as a bioindicator in the ridge-to-reef management of the municipality, as differences in terms of composition, richness, and evenness were shown for both terrains, with the upland terrain as being more species-rich than the coastal terrain in terms of myxomycetes. That being the case, the utilization of these microorganisms demonstrates a promising approach that can facilitate future management decisions and ensure that the area of interest is managed sustainably and effectively.

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