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Diversity of myxomycetes and their occurrence on different substrata of three agroecosystems in Southern Mindanao, Philippines

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Abstract

Monotypic vegetation types, such as agricultural plantations, provide various suitable substrates that are excellent for the isolation of myxomycetes. This study aimed to determine the diversity of myxomycetes across different agroecosystem microhabitats. Specifically, it aimed to identify myxomycetes up to the lowest possible level of classification, determine the diversity of myxomycetes, and determine the relationship between myxomycete occurrence on different substrata in different agroecosystems. The substrates collected from the five different agroecosystems included aerial litter, ground litter, twigs, and bark. The collected samples were incubated in a moist chamber to allow the development of plasmodia and fruiting bodies of the myxomycetes. All grown myxomycetes were described under the stereoscope for identification. A total of 24 myxomycete species were identified from the cacao, Lanzones, rubber, and rambutan agroecosystem sites at the University of Southern Mindanao Agricultural Research and Development Centre (USMARDC). Eleven of these species thrived in the rambutan plantation; however, the abundance of myxomycetes was documented in the durian plantation. Among the substrates used, bark yielded 48 individuals of *Arcyria cinerea*, accounting for most of the counts. Based on the logistic regression, the probability of a species' presence in an agroecosystem is nearly 14 times higher in durian than in rubber and 50% higher in bark than in other substrates. This study concluded that agroecosystems may sustain the establishment of myxomycetes in a variety of habitats and diversity in plantations of high-value crops.

Keywords – Diversity – fruiting bodies – myxomycetes – occurrence – species – spores

Introduction

The plasmodial slime molds (or myxomycetes) are phagotrophic eukaryotic organisms that exhibit characteristics of both protozoa and fungi (Dagamac & Dela Cruz 2015). Their life cycle is divided into two phases: a fungus-like phase that results in fruiting bodies bearing spores, and an animal-like phase that generates plasmodia from the fusion of myxamoeba and swarm cells (Keller & Snell 2002). The current classification for myxomycetes (slime molds).

Eumycetozoa in the supergroup Amoebozoa and Myxogastrids at higher levels (Adl et al. 2019). Myxomycetes are prevalent protist predators that consume bacteria and other

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microorganisms, making a significant contribution to nutrient cycling in terrestrial ecosystems. It is usually found on a variety of dead plant substrates, including bark, twigs, aerial and ground leaf litter, vines, lianas, and even decaying inflorescences or fruit, in both temperate and tropical ecoregions (Dagamac & Dela Cruz 2015). The most important phase in the biotransformation of organic substrates into useful, degraded wastes is the microbiological process of composting. Soil fertility is increased using those microbial compost components, which promote sustainable agriculture.

Over the past few decades, research on Slime molds (myxomycetes) in tropical Southeast Asia has steadily increased. The Philippines garnered 159 species by the following authors research studies: Dagamac & Dela Cruz (2015), Macabago et al. (2017), Bernardo et al. (2018), Buisan et al. (2019), Buisan et al. (2020). Thailand has 145 species compiled by Ko Ko et al. (2010) and Dagamac et al. (2017). In Indonesia, which has 119 species reported by Farr (1990), Andrade et al. (2016), and Rosing et al. (2011), while the Republic of Singapore has 76 investigated by Rosing et al. (2011). In Myanmar, 67 reported by Ko Ko et al. (2013), and Laos a 44 also reported by Ko Ko et al. (2013). Most of myxomycete research studies in Vietnam were conducted in the southern region (Novozhilov et al. 2018), with few studies being conducted in the other areas of the nation.

Recently, attention has shifted toward understanding myxomycetes diversity in agroecosystems and where agricultural substrate creates distinct microhabitats. The study by Redeña-Santos et al. (2017) was the first to document the association of myxomycetes with agricultural plantations, including *Camelia sinensis*, *Dimocarpus longan*, and *Psidium guajava*. In the Philippines, Alfaro et al. (2014) investigated leaf surfaces of agricultural plantations such *as Saccharum officinarum* as a microhabitat for myxomycetes, and Buisan et al. (2020) studied and documented myxomycetes associated with banana plantations and rice litter. These findings show that the agricultural plantation can support diverse assemblages of myxomycetes.

Despite the growing body of literature, the diversity of myxomycetes in agroecosystems of Southern Mindanao remains poorly documented. In particular, no comprehensive research has been conducted within the University of Southern Mindanao Agricultural Research and Development Centre (USMARDC), a site characterised by various plantations of economically important crops. Therefore, this study was carried out to identify myxomycetes species occurring in selected agroecosystems, assess their diversity and abundance in various plantations, and determine the myxomycetes association between various substrates in agricultural plantations. This research contributes to the growing knowledge of myxomycetes (slime moulds) ecology in tropical agricultural environments and to the potential agroecosystem to support microhabitat biodiversity.

Materials & Methods

Study Area

The research was conducted at the University of Southern Mindanao Agricultural Research and Development Center (USMARDC), Kabacan, North Cotabato (N 7°01'60" E 124°52'60"), characterized by tropical climate conditions with temperatures ranging from 22.78°C to 32°C. Sampling was undertaken in five agroecosystem plantations: durian, rambutan, lanzones, cacao, and rubber.

Sampling Procedure

Sampling followed the protocol outlined by Redeña-Santos et al. (2018), with modifications. In each agroecosystem, three 5×5 m subplots were established. Four substrata—bark (BK), twigs (TW), aerial leaf litter (ALL), and ground leaf litter (GLL)—were purposively collected. A total of 60 samples per plantation were collected, comprising five replicates per substrate type per subplot, yielding 300 samples across all sites. Substrates were stored in labeled paper bags and air-dried for two weeks before culturing.

Fig. 1 – Sampling area: USMARDC, USM, Kabacan Cotabato, showing the location of the five agricultural plantations (durian, cacao, lanzones, rubber and rambutan) where samples were collected.

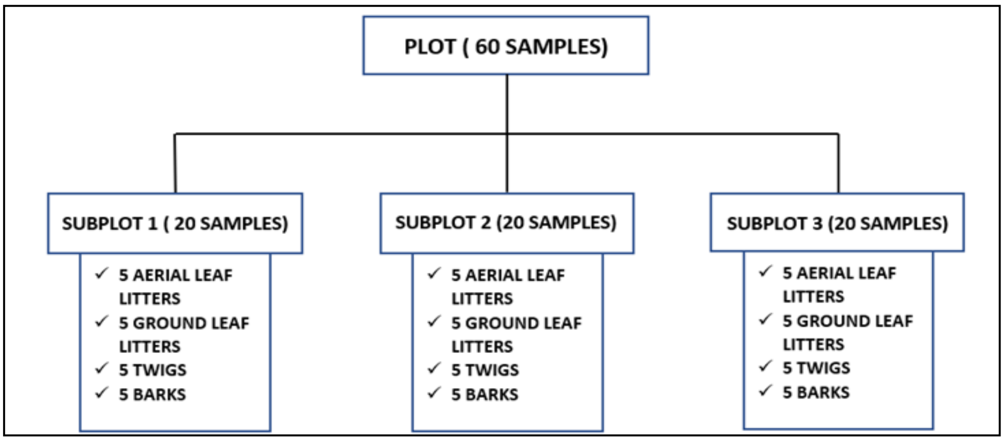

Fig. 2 – Sample collection per plot.

Culturing of myxomycetes using the Moist Chamber Technique

The moist chamber technique, as described by Stephenson & Stempen (1994), was used with slight modifications. Substrate samples were cut into 1×1-inch pieces, placed in Petri dishes lined with two layers of tissue paper, and saturated with distilled water overnight. Excess water was drained, and the petri dishes were incubated under ambient light at 29–30°C for 8–10 weeks. Moist chambers were monitored twice weekly for the development of plasmodia and/or fruiting bodies.

Keeping the culture moist, distilled water was sprayed occasionally. The moist chamber that developed fruiting bodies was initially separated to avoid pest infestation; the fruiting bodies that occurred on substrates were isolated and placed within the improvised box. This improvised box was made of cardboard with 2 x 1.5 inches. This is more convenient for the mass isolation of fruiting bodies.

Characterization and identification of myxomycetes

Fruiting bodies were observed under a stereomicroscope (OMNIBUS SZS1) to describe morphological characteristics, including color, type, shape, and presence of lime. Internal features, such as columella, hypothallus, and attachment to the calyculus, were recorded. Species identification was initially performed using online taxonomic resources and nomenclatural databases and subsequently validated by experts. Voucher specimens were stored in the herbarium of the Department of Biological Sciences, College of Science and Mathematics, University of Southern Mindanao.

Data Gathering

Organic matter content Analysis

Air-dried substrate samples were pulverized and analyzed for organic matter content at the CMU Soil and Plant Analysis Laboratory. Substrate morphology was examined, including leaf texture, bark thickness, and surface roughness.

Characterization of Substrate Morphology/Texture

The collected substrate was observed, and its overall structure was recorded by describing its morphological characteristics such as leaf texture, twigs and barks thickness, and surface texture.

Statistical Analysis

Species diversity indices were calculated based on the number of positive cultures. The Jaccard Similarity Coefficient (JSCI) was used to determine species similarity across substrata and agroecosystems. Logistic regression was performed to analyze the relationship between myxomycete occurrence and substrate/agroecosystem variables.

Results

A total of 300 moist chambers were established from all the samples collected in different sampling areas. Among these, 218 harbor myxomycetes. Fruiting bodies successfully emerged in 132 chambers, while the remaining positive cultures remained in the plasmodial stage throughout the incubation period. Microscopic examination of the fruiting bodies from the different agroecosystems revealed a total of 145 myxomycete individuals representing 24 species, dominated by *Arcyria cinerea* (74) (Fig. 3). Interestingly, this species was recorded from all of the agroecosystem plantations except the rubber plantation. Species richness varied among the five agroecosystems. The rambutan plantation support the highest number of species (11), followed by Durian (9), lanzones (8), Cacao (5), and rubber (4) (Fig. 4).

The Jaccard Similarity Coefficient analysis revealed generally low similarity in species composition among agroecosystems plantation (Table 1). Moderate similarity values were observed between the plantation of cacao-lanzones (0.2), rubber-rambutan (0.3), cacao-rubber (0.4), and durian-lanzones (0.4). While paired plantations such as durian-cacao, durian-rubber, durian-rambutan, cacao-rambutan, lanzones-rubber, and lanzones-rambutan exhibited a strong divergent myxomycete population as indicated by a 0.1 Jaccard Similarity Coefficient value (Table 1).

Table 1 Jaccard Similarity Coefficient value between agro-ecosystems.

Agroecosystems	Jaccard coefficient value
Durian-Cacao	0.1
Durian-Lanzones	0.4
Durian-Rubber	0.1
Durian-Rambutan	0.1
Cacao-Lanzones	0.2
Cacao-Rubber	0.3
Cacao-Rambutan	0.1
Lanzones-Rubber	0.1
Lanzones-Rambutan	0.1
Rubber-Rambutan	0.3

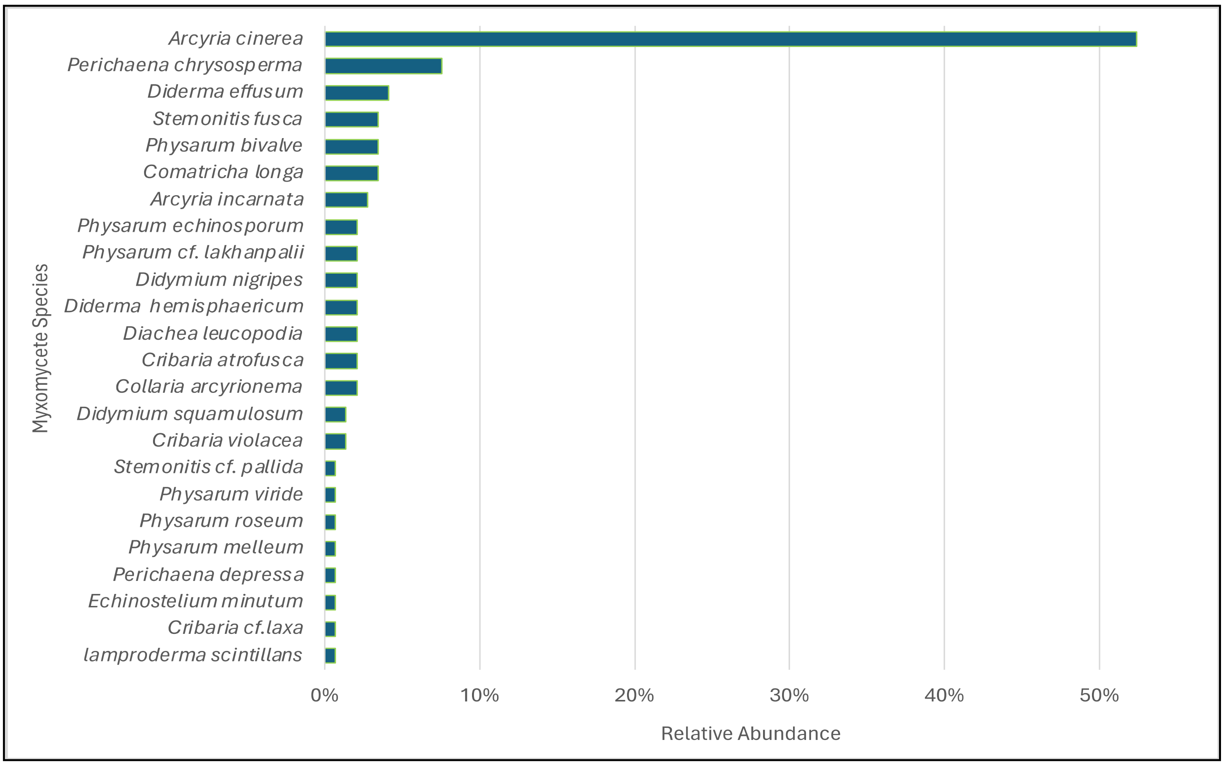

Fig. 3 – Relative abundance of myxomycetes identified from Durian, Rubber, Cacao, Lanzones, Rambutan agroecosystems.

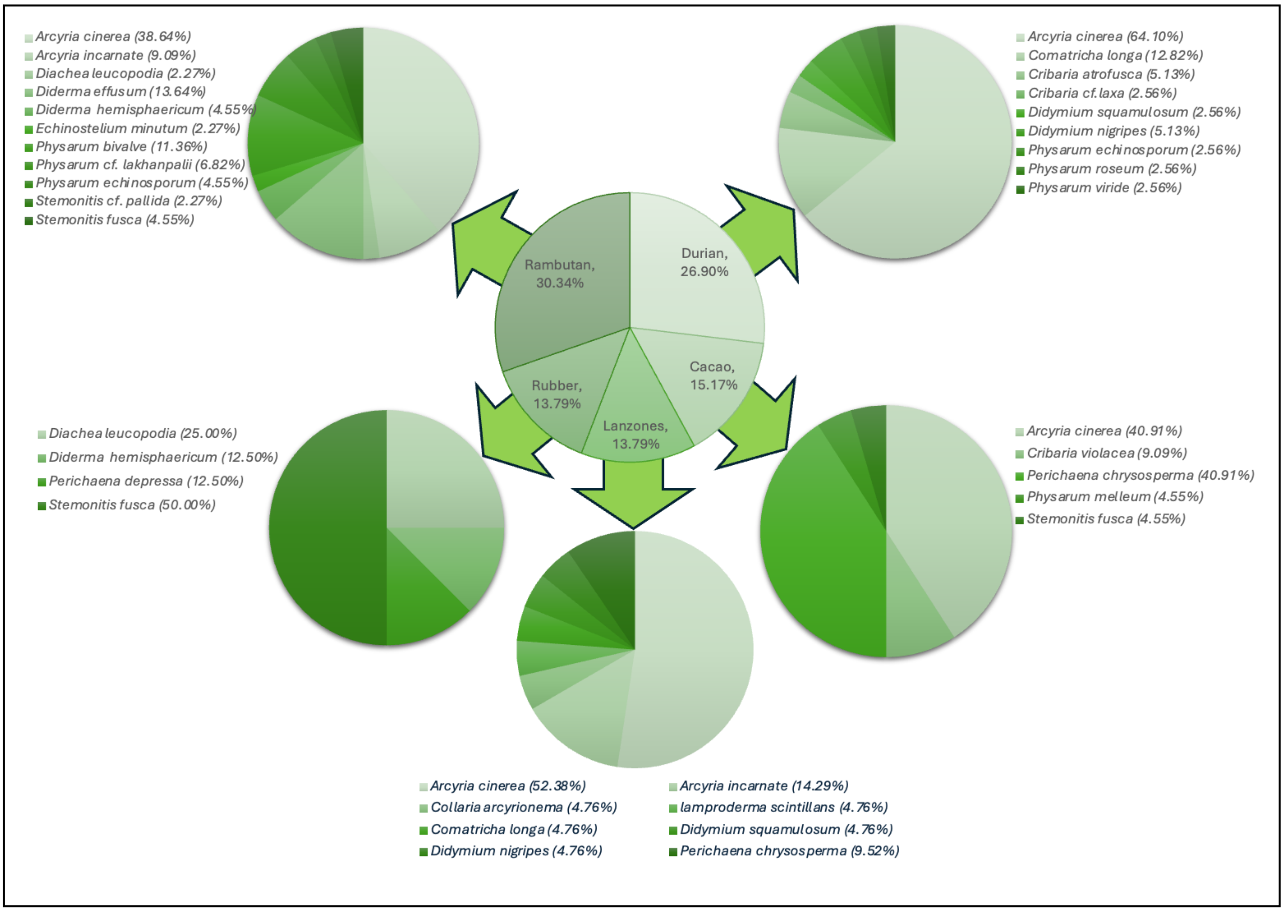

Fig. 4 – Myxomycete species were identified from the different agroecosystems.

Moreover, the Jaccard Similarity Coefficient analysis between substrates revealed that most of pairs showed a similarity coefficient value of 0.2, including aerial leaf litter-ground leaf litter, aerial leaf litter-twigs, aerial leaf litter-bark, ground leaf litter-twigs, and twigs-bark. Ground leaf litter -bark exhibited a strong divergent myxomycete population as indicated by the 0.1 Jaccard Similarity Coefficient value (Table 2).

Table 2 Jaccard Coefficient Values of the different substrates in pairwise comparison.

Substrates/Plots	Jaccard coefficient value
Aerial leaf litter-Ground leaf litter	0.2
Aerial leaf litter-Twigs	0.2
Aerial leaf litter-Bark	0.2
Ground leaf litter-Twigs	0.2
Ground leaf litter-Bark	0.1
Twigs-Bark	0.2

Given a set value of components, the logistic regression (Table 3) shows that the probability of a species occurrence was approximately three times higher in the aerial litter than in twigs. The bark substrate also indicated that the likelihood of species presence is 50% higher in bark than in twigs. At the same time, the species' occurrence is 40% lower on the ground than on twigs. In the agroecosystem, cacao plantations showed eight times higher probability of species occurrence compared with rubber. The likelihood of a species being present in durian is nearly 14 times higher than in rubber. In lanzones, this means that the chance of a species presence in lanzones is two times higher than the probability of a species presence in rubber. In rambutan, the chance of a species presence is seven times higher than the probability of a species presence in rubber.

In terms of organic matter (OM), which has a positive association with myxomycete occurrence, the result revealed that for a unit increase in organic matter, the chance of a species' presence rises by 19%.

Table 3 Logistic regression in four different substrates and five agroecosystems.

Variables in the Equation						
	B	S.E.	Wald	df	Sig.	Exp(B)
Constant	-8.882	4.146	4.589	1	.032	.001
Twig			24.914	3	.000	
Aerial leaf litter	1.205	.537	5.038	1	.025	3.338
Bark	.403	.513	.617	1	.432	1.496
Ground leaf litter	-.908	.477	3.617	1	.057	.403
Rubber			32.274	4	.000	
Cacao	2.095	.545	14.772	1	.000	8.126
Durian	2.617	.570	21.090	1	.000	13.691
Lanzones	.781	.406	3.698	1	.054	2.183
Rambutan	2.007	.501	16.051	1	.000	7.445
OM Analyses	.175	.079	4.884	1	.027	1.192

Discussion

This study provides a new database for myxomycetes species diversity, distribution, and their substrate associations in the various agroecosystems of Southern Mindanao. Twenty-four species were recorded throughout five agricultural plantations indicating that managed agricultural environments may sustain diverse myxomycete communities. These findings suggested the growing evidence that agroecosystems can remain important habitats for myxomycetes, as long as microhabitats and substrates are available. The abundance occurrence of *Arcyria cinerea* identified in this study is supported by other studies that characterize this species as environmentally adaptable and can sporulate across various microhabitats (Pecundo & Dela Cruz 2023).

Its widespread occurrence in different plantations shows its adaptability to diverse substrate types. The absence of *Arcyria cinerea* in the rubber plantation may be explained by the substrate characteristics specific to rubber plantations. These may include the smoother bark surfaces and particular leaf morphology, which may result in the limitations of spore development and distribution of myxomycetes. The variation in species abundance and diversity among different agroecosystems suggested that the different plantation affects myxomycete populations. The rambutan plantation harboured the highest species diversity, whereas the durian plantation included the largest population of myxomycetes individuals. This pattern suggests that the different agricultural plantations show varying of microhabitat diversity, litter accumulation, and moisture retention. Redeña-Santos et al. (2017) and Rojas et al. (2014) in their research study shows that plantations with thicker bark, leathery leaves, and rough surfaces, such as durian and rambutan plantations, are likely to promote the entrapment of spores, which enables the development of plasmodial and myxomycetes occurrences. The type of substrate markedly affected the occurrence of myxomycetes (slime molds). Bark exhibited the highest myxomycetes species, supporting the assumption that the corticolous myxomycetes thrive and reproduce on the bark of Plantations (Novozhilov et al. 2017).

Meanwhile, the Ground leaf litter indicate a lower diversity of myxomycete species. Ground leaf litter substrates often remain its moisture content for extended durations potentially benefiting fungal competitors and hindering spore dispersal. Hence, limiting the growth of myxomycetes (Novozhilov et al. 2006, Dagamac & Dela Cruz 2015). The higher presence of myxomycetes in aerial leaf litter compared to ground leaf litter illustrates the significance of moisture stability for determining species distribution.

The low Jaccard similarity indices reported across agroecosystem plantations and substrate types (microhabitats) indicate the presence of myxomycete species assemblages across different habitats. The low overlap indicates that myxomycete presence is significantly influenced by substrate morphological structure rather than being uniformly distributed across plantations. Comparable patterns of habitat specific assemblages have been documented in tropical forests and agricultural plantations across several locations (Schnittler et al. 2002, Rojas & Stephenson 2012).

The logistic regression analysis results indicate the effect of both substrate and agroecosystem plantation on myxomycete (slime molds). The higher likelihood of species presence in aerial leaf litter compared to twigs may be due to lower moisture levels that promote spore retention and sporulation. Bark substrates showed the highest probability to harbored myxomycetes, which is rational given their structural complexity and ability to serve as a stable microhabitat. The markedly highest percentage of species presence in durian, cacao, and rambutan plantations compared to rubber highlights the influence of plant morphological characteristics on the composition of myxomycete species in agroecosystems.

The occurrence of myxomycetes correlated with increased organic matter, suggesting that surfaces abundant in organic matter may support higher abundances of microbial prey and improve conditions for plasmodial growth. This finding supports the previous research emphasizing the significance of organic resources in supporting myxomycete populations within decomposer ecosystems (Dagamac & Dela Cruz 2015). This study illustrates that agroecosystems in Southern Mindanao can support the richness and diversity of myxomycete species. The type of plantation, substrate, and quantity of organic matter affect species occurrence and distribution.

The results indicate that the ecological significance of agroecosystems as reservoirs of microbial biodiversity promotes further study across wider spatial scales to enhance the research study of agroecosystem plantation contribution to the conservation of myxomycetes richness and diversity.

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