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Documenting the first records of myxomycetes on rice litter of Cotabato, Southern Mindanao, Philippines

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Abstract

Although studies of myxomycetes in plantations have started to appear in the last few years, some agroecosystems with homogenous vegetation remain unexplored. This holds true for rice agroecosystems. A study comparing the occurrence of myxomycetes in organic and conventional rice fields in the province of Cotabato was carried out in rice farms in the municipalities of Kabacan and Midsayap. Ground and aerial litters were randomly collected from rice fields to set up moist chamber cultures. Three cosmopolitan species of myxomycetes are reported in this study, namely *Arcyria cinerea, Diderma hemisphaericum,* and *Perichaena depressa.* Moist chambers set up with rice litter substrates from organic fields showed significantly higher percentage yield than moist chambers with the substrates from conventional rice farms. This study is the first to explore the distribution of myxomycetes in rice agroecosystems of the Philippines and to compare different farming practices.

Introduction

Myxomycetes are known to thrive in ecosystems with a high rate of organic decomposition, playing a quintessential role in nutrient cycling (Rayner & Boddy 1988). This role has been pointed out as the possible reason to why the majority of previous reports on foliicolous (leaf inhabiting) myxomycetes diversity is concentrated and conducted on vegetation with high plant heterogeneity (Redeña-Santos et al. 2017). This remains true with studies in the Philippines showing relatively high myxomycetes diversity in terrestrial ecosystems with heterogenous vegetation (e.g., forest floor litter) (Dagamac et al. 2012; Dagamac et al. 2015; Macabago et al. 2016; Pecundo et al. 2017) in comparison to litter from those with homogenous vegetation (Carascal et al. 2017; Macabago et al. 2017). Thus, specific plant microhabitat associations of myxomycetes are believed to be comparably limited and less understood (Buisan et al. 2019). However, in all of these vegetation studies, one may always expect the presence of cosmopolitan myxomycetes species that thrive on fast decaying organic plant material (Rea-Maminta, et al. 2015; Schnittler et al 2017).

Nonetheless, in recent years, myxomycetes studies in agroecosystems in Southeast Asia gained significant attention (Tran et al. 2008; Alfaro et al. 2015; Redeña-Santos et al. 2017; Buisan et al. 2019). Myxomycetes occurring in agricultural plantations in the Philippines were first reported by Alfaro et al. (2015) in sugarcane plantations, followed by an investigation in banana plantations by Buisan et al. (2019). However, no studies on myxomycetes association with rice agroecosystems have ever been conducted, although the Philippines is one of the top rice producers in the world. The closest investigation ever done was with another grass species with little to no economic importance, *Imperata* sp. (Carascal et al. 2017). This proves that despite the increasing attention to studies of monotypic vegetation, many possible microhabitats – especially agroecosystems – are still left untouched.

Although the number of myxomycetes studies in the Philippines has been increasing during the last decade (Dagamac & dela Cruz 2019), unfortunately, studies in the Southern islands of the archipelago, particularly in Mindanao, are still relatively scarce (Almadrones-Reyes and Dagamac 2018). Moreover, no records of myxomycetes have ever been accounted for rice agroecosystems. The present study was conducted to expand current knowledge on myxomycetes in large agricultural plantations in the country. Additionally, this study aimed to document for the first time the possible impact of different farming practices on the occurrence of myxomycetes.

Materials and Methods

Collection and Preparation of Substrates

Aerial litter (AL) and ground litter (GL) substrate samples were collected from two different municipalities practicing organic and conventional rice farming, respectively. These substrates were composed of a mix of rice leaves and straws. The

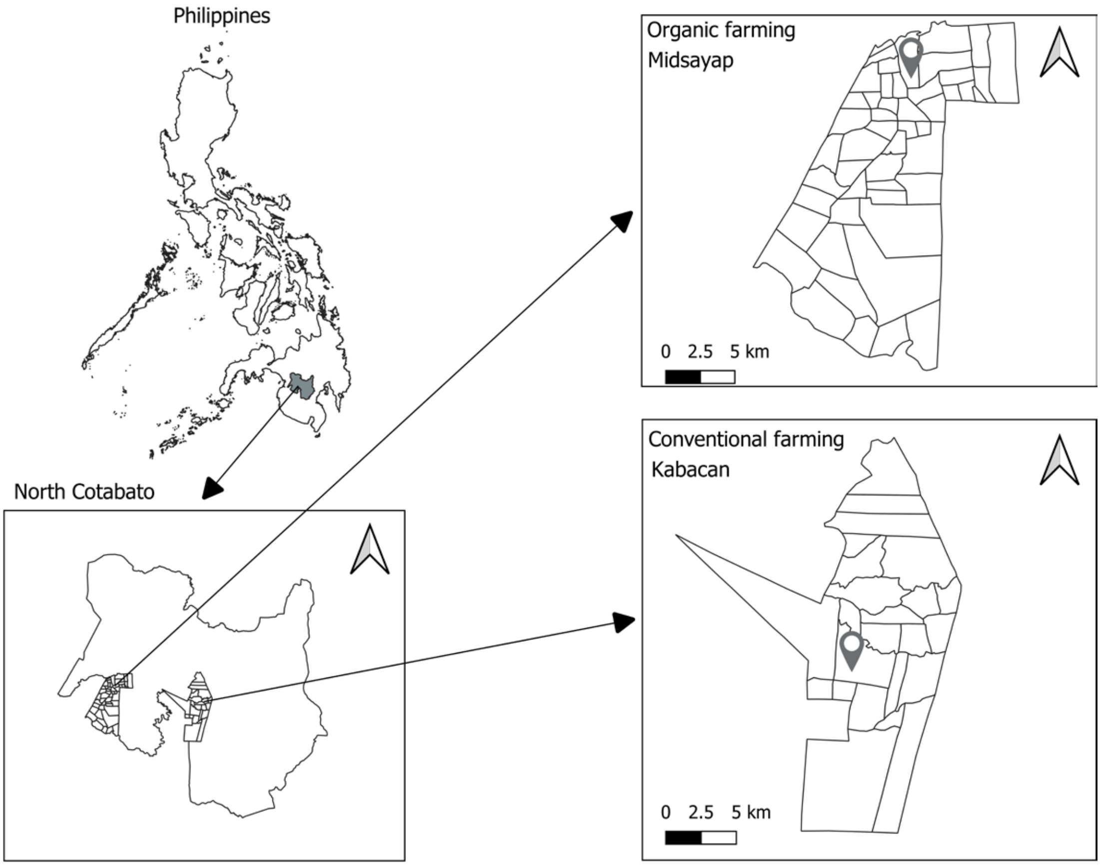

Fig. 1. Study sites: Cotabato Province, Southern Mindanao showing the sampling location of the two rice farms practicing organic and conventional farming.

organic rice farm was sampled in the municipality of Midsayap (7°12’39.1” N, 124°32’45.7” E), while the farm with conventional farming practices was sampled in the municipality of Kabacan (7°07’19” N, 124°49’13”E). Both municipalities are part of the province of Cotabato, situated in Southern Mindanao, Philippines (**Fig. 1**). The seasons are not very pronounced there, with relatively dry periods lasting from November to April, and wet during the rest of the year. The areas experience high annual rainfall which ranges from 1,871 mm/year to 2,876 mm/year, with annual mean temperature ranging from 31.0°C to 33.0°C (Department of Agriculture – Regional Field Office XII 2019).

Prior to collecting the samples, farm owners/personnel were interviewed regarding their farming practices. The farms differ in seedling nursery system, seedling age for transplant, pest and diseases management, common encountered pests, and fertilizer application (**Table 1**). The sampled organic farm practices no fertilizer application and utilizes a practice of water management in dealing with weeds, pests, and diseases. On the contrary, the sampled conventional rice farm applies urea in the fields after transplanting and in regular times as a maintenance until harvest. Unlike the sampled organic farm, the conventional rice farm uses pesticides, herbicides, and fungicides as an intervention to control pests and diseases in the fields.

Three rice fields per farming practice were randomly selected in this study. The sampled fields belonged to the same farm and were approximately 200 m from each other. Samples were collected 4 to 5 weeks after the fields were harvested to ensure the availability of organic decaying litter. A 10 x 10 m plot was established per rice field. Ten samples of AL and GL were randomly collected from each plot making a total of 30 AL and 30 GL per farming practice. All samples were placed in paper bags. The collected substrates were further air-dried for 12 to 14 days prior to establishing moist chamber cultures.

Preparation of Moist Chambers and Identification of Myxomycetes

A total of 120 moist chamber (MC) cultures were prepared following the detailed protocol of Stephenson & Stempen (1994). Each chamber was lined with tissue paper, then a substrate was placed and soaked with distilled water overnight. After 24 hours, the pH of each substrate was measured 3 times with a pH meter (Sartorius, Germany), then MCs were maintained under ambient light conditions at room temperature (25 – 27 °C) for up to 10 – 12 weeks. The MCs were regularly checked every week for the presence of plasmodia and/or fruiting bodies. To keep the MCs moist, distilled water was added at certain occasions. When myxomycete fruiting bodies appeared, they were placed inside herbarium matchboxes and kept frozen overnight to prevent insect infestation. The fruiting body characteristics (type, shape, presence of lime, height, and color) were then described using a stereo microscope (Ceti, Belgium) and were used for initial determination. Microscopic characterization of the fruiting bodies was conducted by placing a single fruiting body in a slide with a drop 10% w/v KOH solution and investigating after 24 hours using light microscope (Olympus, Japan). The specimens were determined using an online database (http://slimemold.uark.edu/) and were verified by the last author. All specimen vouchers were then preserved in the personal collection of the first author.

Calculation of Productivity and Statistical Analysis

Moist chamber productivity was used to evaluate the ability of each microhabitat (aerial and ground rice litter) to support myxomycete growth (Carascal et al. 2017). It was calculated as a percent yield according to Dagamac et al. (2012). A moist chamber that exhibited plasmodia and/or fruiting bodies was considered as a positive record, and was noted

Table 1. Farming practices of the rice farms based on the responses of the farm personnel/manager.

Farming Practice	Organic Rice Farms	Conventional Rice Farms
Seedling Nursery System	Use of seed beds	Wet-bed method
Seedling Age for Transplant	25 days	14–16 days
Source of Water	Irrigation	Irrigation
Pest and Diseases Management	Use of water management practices; Non-use of chemical-based herbicides, pesticides, and fungicides for 19 years	Use of chemical-based herbicides, pesticides, and fungicides
Common Encountered Pests	No common pests	Snails and weeds
Application of Fertilizer	No application	Yes; Urea-based fertilizers

as one positive culture. The number of positive cultures was determined and then divided by the total number of moist chambers prepared. A two-sample Kolmogorov-Smirnov (KS) Test was conducted to compare the proportions of positive cultures from the two municipalities that used different rice farming practices. This was calculated using PAST version 4.01 statistical software (Hammer et al. 2001).

Results

Out of the 120 moist chambers prepared, 38% were positive for myxomycetes (fruiting bodies and/or plasmodia). The percentage of moist chamber cultures with fructifications (20%) was similar to the percentage of cultures with plasmodia only (18%). Moist chamber cultures of agricultural litters obtained from organic rice fields yielded the higher number of positive moist chambers (32) compared to the number of positive moist chamber cultures from conventional rice fields (14) (Fig. 2). The comparison of the proportions of MCs positive for myxomycetes between the two farming systems revealed a significant difference (D = 0.5625; p = 0.0022). In total, organic farms produced 22 / 10 and conventional farms 10 / 4 positive moist chamber cultures of aerial litter / ground litter.

Identification of the collected myxomycetes revealed only three species, all of them cosmopolitan: *Arcyria cinerea* (Bull.) Pers., *Diderma hemisphaericum* (Bull.) Hornem., and *Perichaena depressa* Libert. These species are reported for the first time in rice litter substrates and are presented with some remarks. Mean pH ± SD, min-max values measured on every positive MC are also presented (**Table 2**).

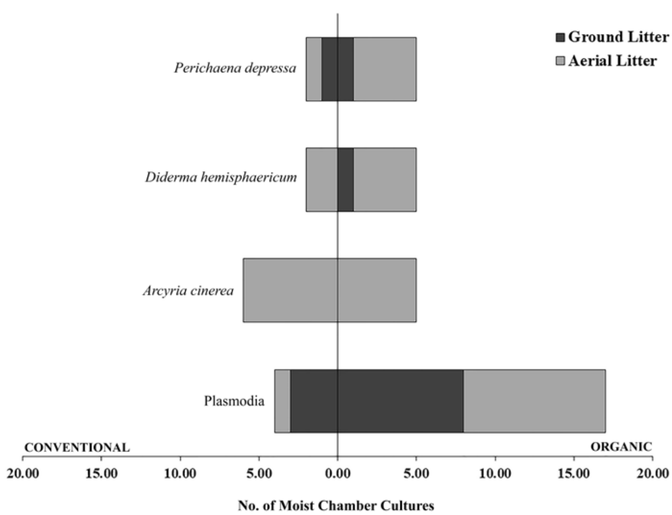

Fig. 2. Bar graph showing the proportion of moist chamber cultures that yielded the three cosmopolitan myxomycete species and plasmodia. The cultures were set up with two different types of rice litter from two farms that utilize different farming practices.

Table 2. The table shows the cosmopolitan species and their mean pH ± SD, min – max values as measured on positive moist chambers.

Species	pH ± SD, min – max
A. cinerea	6.26 ± 0.49, 5.52 – 6.81
D. hemisphaericum	6.58 ± 0.67, 5.48 – 7.66
P. depressa	6.54 ± 0.40, 5.92 – 7.16

Arcyria cinerea (Bull.) Pers.

Sporocarps were clustered in small groups, erect, stalked, cylindrical in shape, and all were pale grey in color. Stalks were concolorous with the sporotheca. Found only in aerial litter samples in both organic and conventional rice farms, a total of 11 moist chamber cultures were positive for *A. cinerea*. Our results show that the fields of both farming practices harbor roughly equal records of *A. cinerea.*

Diderma hemisphaericum (Bull.) Hornem.

Sporocarps were stalked with discoid hypothallus. Sporothecae were also discoid and white in color due to the presence of lime. Seven moist chambers were positive for *D. hemisphaericum*, found both on organic rice fields (5) and conventional rice fields (2). The specimens were found mostly on aerial litter samples.

Perichaena depressa Libert.

Sporocarps were crowded, gregarious and polygonal, with no apprarent hypothallus. Sporangia were markedly depressed with lids intact, and margins exhibiting circumscissile dehiscence. A total of 7 moist chamber cultures were positive for *P. depressa.* This species was found mostly in moist chambers with aerial litter from organic fields.

Discussion

Most of the myxomycetes surveys in the Philippines use litter from forest floors that is produced by heterogenous vegetation. This limits the possibility to reveal foliicolous myxomycetes that could be preferentially associated to specific plant substrates as their main microhabitats. Buisan et al. (2019) pointed out three reasons for this limitation: (1) the impracticality of identifying the leaf litter that is most of the time heavily degraded making it impossible to identify which plant it came from, (2) ground leaf litter is usually composed of a mixture of remnants of different plant species making it difficult to segregate when randomly collected in the field, and (3) most of the scientists conducting rapid assessments of myxobiota have a limited botanical knowledge. Despite these reasons, there is an increasing interest in studying myxomycetes associated with monotypic vegetation. To the best of our knowledge, this paper is the first to report myxomycetes in rice agroecosystems and to compare their occurrences with regards to farming practices.

This study showed a moderately low percentage (38%) of moist chamber cultures positive for myxomycetes either as plasmodia (18%) or fruiting bodies (20%), with only three species revealed. This result is similar to the results of the investigation done for *Musa* sp. litter in Southern Mindanao (Buisan et al. 2019), which gave 31.66% moist chamber productivity – mostly plasmodia – and 5 cosmopolitan species. Furthermore, in comparison to the other study in the Philippines conducted for agricultural sugarcane litter (Alfaro et al. 2015), our percentage yield is comparatively higher (38% vs. 18%). However, our findings contradict the observations made in a similar study of agricultural litter conducted in Thai Nguyen, Northern Vietnam (Redeña-Santos et al. 2017). Their results showed a MC productivity of 90% and a higher myxomycetes occurrence of 165 records for the agricultural plantations of guava, green tea, and longan. The low turn out of positive moist chambers could probably be attributed to the smooth surface of the rice leaves. This is supported by Alfaro et al. (2015) who reported that smooth leaf morphology is not an effective spore trap for myxomycetes, in contrast to leaf litter with thick leathery leaves and hair-like structures studied by Redeña-Santos et al. (2017) in Vietnam. Still, it is interesting to note that rice varies in phenotype depending on the variety (Hu et al. 2013), some exhibit a glabrous or pubescent leaf morphology. This may warrant future investigations comparing myxomycetes associated with leaf litter of rice varieties with different leaf morphology. Because of the low exhaustiveness of the survey, which may have led to the insufficiency to represent a conclusive significance of the differences in myxomycete distribution between aerial and ground litter, the data should be treated with caution. This, however, calls for a more thorough examination, prompting further investigations.

Our results also oppose the findings of Carascal et al. (2017), which evaluated the distribution of myxomycetes in other grass species found in Philippine grasslands. They reported higher productivity despite the homogeneity of the vegetation. However, it should be noted that Carascal et al. (2017) studied the grass species of little to no agricultural importance and the plant community with minimal anthropogenic intervention. Various anthropogenic interventions have been shown previously to reduce myxomycete diversity and abundance (Tran et al. 2008). In turn, rice fields are subject to numerous human interventions (e.g. application of fertilizers and herbicides, and removal of plant materials) that aim to boost crop production, which could affect spore dispersal and viability, especially in conventional rice farms.

The results of this study also show the significant difference in proportions of moist chamber cultures positive for myxomycetes between the two farming practices. It has been previously pointed out that conventional and organic farming systems play a major influence on the composition of microbial communities (Lupatini et al. 2017). Lupatini et al. (2017) also suggested that organically managed systems increase taxonomic and phylogenetic richness, diversity, and heterogeneity of soil microbiota when compared with conventional farming systems, and that composition of microbial communities was altered by soil health treatments. Increased protozoan densities as a result of organic amendments have also been reported before (Treonis et al. 2010). However, sampled organic rice fields in this study do not practice the addition of organic fertilizers and other amendments, hence, the low percentage of positive cultures. Application of organic fetilizers has the potential to increase bacterivorous and other phagotrophic protists (Xiong et al. 2017). Although at this point it would be difficult to conclude if farming practices directly affect the propensity of fructification of other species of myxomycetes, applying for example barcoding approach for species identification of plasmodia (Shchepin et al. 2017) that appear in moist chambers or the more sophisticated eDNA analysis method (Shchepin et al. 2019) for litter and soil samples would help disentangle these speculations.

The occurrence of the myxomycetes reported in this study confirms the broad range of distribution of these species in Southeast Asia (Tran et al. 2008). These records of cosmopolitan species are widely distributed in the Philippines (Reynolds 1981) and other parts of Southeast Asia (Reynolds & Alexopolous 1971; Ko Ko et al. 2010; Ko Ko et al. 2012; Ko Ko et al. 2013). *A. cinerea* can sporulate in a variety of environments and agricultural substrates, such as corn (Tran et al. 2008), banana (Tran et al. 2008; Buisan et al. 2019), sugarcane (Alfaro et al. 2015), and guava (Redeña-Santos et al. 2017). Similarly, *P. depressa* is common in the tropics (Keller & Eliasson 1992) and occurs quite abundantly in different agricultural plantations, especially green tea plantations (Redeña-Santos et al. 2017). To date, there are no records of *P. depressa* in other agricultural litters in the Philippines. *D. hemisphaericum*, on the other hand, was noted to be one of the most common myxomycetes species that has appeared in systematic surveys in the Philippines (Almadrones-Reyes et al. 2019). The species occurs to be quite abundant in mango (Tran et al. 2008), as well as other agricultural plantations, such as green tea, longan, and guava (Redeña-Santos et al. 2017). The occurrence of these cosmopolitan species supports previous suggestions (Buisan et al. 2019) that comprehensive diversity assessments of monotypic vegetation, particularly in agricultural rich areas, are feasible.

The present understanding of the distribution of myxomycetes in the Philippines, especially in Mindanao and in agroecosystems, is far from being complete (Alfaro et al. 2015; Dagamac & dela Cruz 2015, 2019). Many agricultural factors are still in need of further investigations (e.g. climactic variations, seasonal occurrence, use of fertilizers and soil amendments). Economic importance of myxomycetes may also warrant future investigations regarding slime molds and agroecosystems. One interesting idea is the role of myxomycetes as "plant pathogens" as previously reported (Cresenzi et al. 2015; Agra et al. 2018). However, damage to plants was either mechanical, as the result of extensive plasmodial growth due to foraging that eventually smothered the plants, or coincidental (Lee et al. 2008), rather than caused by a true parasitism (Tu et al. 2016). In spite of a few isolated reports, due to no global evaluations have ever been made on the possible economic impact of myxomycetes, the idea of their role as pathogens in agricultural plantations seems highly improbable.

Our results agree with the general pattern that, in spite of the homogeneity of the vegetation like in agricultural plantations, organic decaying leaf litter substrates can harbour at least cosmopolitan myxomycetes species. Considering the results of our study, it seems that agricultural leaf litters can indeed serve as potential microhabitats for myxomycetes. Hence, it would be important to expand myxomycetes studies in different agroecosystems for the Philippines.

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