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Species diversity and ecological roles of macrofungi in Lentang Forest Reserve, Pahang, Malaysia

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Abstract. *Md Noordin NA, Zainudin NAIM, Khairuddin NH, Yusof MT. 2024. Species diversity and ecological roles of macrofungi in Lentang Forest Reserve, Pahang, Malaysia. Biodiversitas 25: 2615-2622.* Macrofungi are high-value forest components with functionally significant roles in the forest ecosystem, however, fungi and their functions remain among the least studied. Therefore, this study aims to unravel the current diversity of macrofungi in Lentang Forest Reserve, located in Pahang, Malaysia to provide data for comparison with other ecosystems. Macrofungal sporocarps were collected from selected sites using convenience sampling. A total of 332 sporocarps were identified based on morphological and Internal Transcribed Spacer (ITS) sequence analysis. Ascomycota and Basidiomycota predominated in the Lentang Forest Reserve, with 28 genera representing 8 families were successfully identified. A Shannon diversity index of 0.454 indicated high fungal diversity in the sampling location. Microclimatic changes do not significantly affect fungal orders and substrates across the studied sites, suggesting random chance rather than systematic influences. The samples of Polyporales had the highest amounts, which showed that microclimate factors affected the make-up of the fungal fruiting community. In harsh microclimates, tough-fleshed fruit bodies were more common. The Agaricomycetes class exhibited the highest species presence, accounting for 89.09% of the identified species. The findings of this study can serve as a valuable checklist for future research on fungal distribution in tropical regions and will significantly contribute to biodiversity conservation efforts.

Keywords: Basidiomycota, forest reserved, fungal biodiversity, fruiting body, Internal Transcribed Spacer (ITS), macrofungi

INTRODUCTION

Fungal diversity is one of the most important indicators of forest health and biodiversity (Rakić et al. 2023). Fungi are fundamental elements participating in nutrient cycling, symbiotic relationships, and decomposition processes of healthy and balanced ecosystems. Ecologically, fungi can be divided into saprophyte, parasite, and symbiotic (mycorrhizal) species groups (Nithiyaa et al. 2012; Nacua et al. 2018; Parlucha et al. 2021). Most terrestrial fungi are saprophytes or symbionts with other organisms, but some of them are plant and animal pathogens (Sun et al. 2019; Nazri et al. 2020).

Understanding the diversity of fungi helps to assess ecosystem health and provides insights into interactions of fungi such as mycorrhizal associations or pathogenic relationships of crops. Many fungi produce bioactive compounds with pharmaceutical properties that have been used in traditional medicine and hold promise for future therapeutic developments (Gafforov et al. 2023). By exploring the diverse fungal species present in the forest, researchers can uncover novel compounds with antimicrobial, anticancer, or immunomodulatory properties that may lead to the development of new drugs or biotechnological products.

Scientists and conservationists have emphasized the importance of recognizing fungi as key components of ecosystems and have called for targeted management actions to safeguard fungal species. These efforts include preserving old-growth forests, maintaining habitats to meet the specific requirements of threatened fungal species, and leaving deadwood and unfertilized grasslands to increase diversity (Da Silva et al. 2019). One positive impact of these conservation efforts is the increased awareness and understanding of the vital roles that fungi play in supporting the environment and biodiversity. By engaging citizen science, raising public awareness, and involving more mycologists in conservation initiatives, there has been progress in assessing and prioritizing global fungal diversity for conservation action (Purahong et al. 2022). Furthermore, the expansion of scientific approaches, such as DNA metabarcoding, has enhanced the monitoring of fungal populations and trends, aiding in the identification and preservation of threatened species (Erinjery et al. 2018; Tedersoo et al. 2022).

Tropical areas are well-known for their high diversity of flora and fauna. However, fungal diversity in tropical region has not been fully discovered and documented even in Malaysia's protected forests. Over the past five years, conservation efforts to protect fungal diversity in protected forests in Malaysia have focused on integrating fungal conservation with broader biodiversity conservation strategies. Overall, recent conservation efforts in Malaysia have highlighted the need to protect fungal diversity alongside

plant and animal, emphasizing the interconnectedness of these kingdoms in maintaining healthy ecosystems. By aligning conservation goals with collective efforts, increasing research initiatives, and engaging a broader community in fungal conservation, there has been a positive shift towards safeguarding fungal diversity in protected forests, thereby benefiting biodiversity and enhancing ecosystem resilience.

Lentang Forest Reserve, located in the state of Pahang, Malaysia is a recreational forest reserve with a river running across the entire rainforest area. This forest reserve has a high diversity of fungi which not only reflects the health of the forest but also evidence of the complex web of life that thrives within its boundaries. The presence of a wide variety of macrofungal species indicates a rich and complex ecosystem where each organism plays a unique role in sustaining the overall balance and functioning of the forest. Moreover, fungi in the Lentang Forest Reserve include saprophytes, with some species forming mycorrhizal symbiotic relationships with plants. This intricate underground network of fungal hyphae connects individual plants, creating a community-wide communication system that enhances plant health and the resilience of the forest ecosystem.

This study aims to discover the diversity of macrofungi and understand their role in tropical rainforests in Lentang Forest Reserve. By exploring the species diversity of macrofungi in the reserve, this research not only contributes to scientific knowledge but also provides valuable insights for conservation and sustainable management strategies. It underscores the importance of recognizing fungi as integral components of ecosystems and emphasizes the need for their inclusion in biodiversity assessments and conservation initiatives. On top of that, documenting fungal diversity is essential for conservation efforts, ensuring the preservation of endangered species and their habitats.

MATERIALS AND METHODS

Study period and area

Sampling of macrofungi sporocarps (fruiting body) was conducted in January 2020 in the Lentang Forest Reserve, Bentong, Pahang located at the geographical coordinates of 03°39' N and 101°89' E. The Lentang Forest Reserve is a large natural preservation area located near the Karak Highway and the southwestern outskirts of Bentong City in Pahang, Malaysia (Figure 1). This reserve represents hill dipterocarp forest with elevation of 300-500 meter above sea level (m asl) with average annual rainfall of 2500-3000 mm. Annual temperature variation is around 27-32°C with 60-80% range of humidity.

Data collection procedure

The sampling method employed was the convenience sampling method (O'Dell et al. 2004). Convenience sampling is a non-probability sampling technique where samples are collected because of their easy accessibility and proximity to the researcher. Macro-morphological characteristics and habitat information including humidity, temperature, and substrate type, which comprise wood, twigs, and soil of the mushrooms, were observed in situ and recorded. The humidity and temperature of the samples were recorded using the HTC-2 digital temperature humidity meter. Each sample was placed in a paper bag along with field notes detailing the collection date, environmental conditions, characteristics, location, and number of specimens. All macrofungi samples were brought to the Mycology Laboratory in the Department of Biology, Faculty of Science at Universiti Putra Malaysia for fungal isolation and identification.

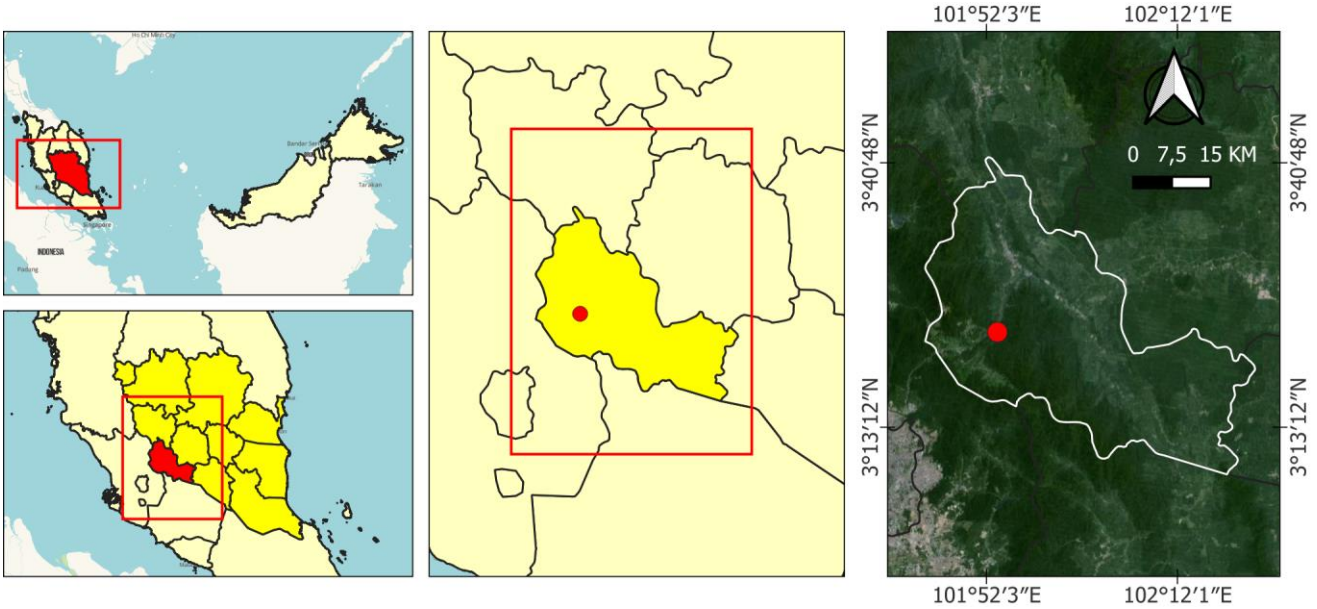

Figure 1. Map of study area in Lentang Forest Reserve, Bentong, Pahang, Malaysia

Morphological characteristics

Measurements of various parts of the mushrooms were recorded, and their morphological features were observed. The following parameters were used for the identification of mushroom specimens: pileus shape, pileus apex, pileus surface, pileus margin, lamellae attachment, lamellae arrangement, stipe attachment to the cap, stipe shape, stipe surface, stipe attachment to substrate, and annulus (Queensland Mycological Society 2011).

Internal Transcribed Spacer (ITS) sequence analysis

For species identification, the Internal Transcribed Spacer (ITS) region was used, utilizing primers ITS1 (5′-TCCGTAGGTGCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTAT TATTATGC-3′′) (White et al. 1990). The fresh sporocarp cutting samples were placed in a sterile mortar and ground using liquid nitrogen (Möller et al. 1992). The genomic DNA from the powdered sporocarp was extracted using UltraClean Microbial DNA Isolation Kit (MO BIO, Carlshad, CA, USA).

Polymerase Chain Reaction (PCR) amplification was conducted using a thermal cycler C1000 Contact (BioRad, USA). The PCR mixtures in a total volume of 50 µL contained 30 ng DNA, 100 mM of each primordial, 0.05 U/µL Taq DNA polymerase, 4 mM $MgCl_2$, and 0.4 mM of each dNTPs. The PCR procedure followed the thermal cycling conditions outlined by Adeniyi et al. (2018), with minor modifications. The protocol began with an initial denaturation at 95°C for 5 min, followed by 30 denaturation cycles at 95°C for 1 min, annealing at 59°C for 1 min, extension for 2 min at 72°C and final extension for 7 min at 72°C.

The successful amplification of the ITS region between 500-700 bp was observed. A 100 bp DNA ladder was used in this process. The gel was later visualized under a UV transilluminator to observe the amplicon size. All the PCR products were purified using QIAquick Gel Extraction Kit (QIAGEN, USA), following the manufacturer's instructions.

The PCR-purified products were sequenced using the ABI3730XL sequencer from MyTACG Bioscience Company. All sequences were analyzed, using the Basic Local Alignment Search Tool (BLAST) in the GenBank database, and subsequently deposited into the GenBank database (National Center of Biotechnology Information at https://www.ncbi.nlm.nih.gov).

Fungal diversity using Shannon-Weiner Index

The diversity of fungi present in the Lentang Forest Reserve was assessed using the Shannon-Weiner index, calculated using the below formula (Ifo et al. 2016):

$$H' = -\sum_{i=1}^{R} ln\,(pi)$$

Where: *H'*= Value of Shannon-Weiner Index, *pi* = Proportion of species, *R* = Number of species in community

Pielou's evenness *J'* (Pielou 1975) was calculated to measure species evenness for each community, expressed by the Shannon information scaled by the maximum information as follow:

$$J' = \frac{H'}{\ln(S)'}$$

Where: *H*' represents the observed value of Shannon index, and S is the total number of species observed. The value of *J'* ranges from 0 to 1, with larger values representing more even distributions in abundance among species.

Statistical analysis

The comparison of microclimates between fungal orders and substrates was analyzed using descriptive statistics and non-parametric analysis of variance (Kruskal-Wallis ANOVA) with the Statistical Package for Social Science (SPSS). The relationship between fungal orders and microclimates was analyzed using Pearson's correlation analysis. The relationship between fungal orders, substrates, and microclimates was further determined using pairwise Bray-Curtis dissimilarities and visualized in the non-metric multidimensional scaling (NMDS). All correlation analyses were performed using PAST software version 4.03.

RESULTS AND DISCUSSION

Macrofungi species in Lentang Forest Reserve, Bentong, Pahang

A total of 332 samples of macrofungi sporocarps (fruiting bodies) were collected from Lentang Forest Reserve, Bentong, Pahang. All the fruiting bodies samples were successfully identified as belonging to the phyla Ascomycota and Basidiomycota. In total, 28 genera of macrofungi were identified, including *Agaricus*, *Amouroderma*, *Auricularia*, *Annulohypoxylon*, *Cantharellus*, *Cookeina*, *Cyathus*, *Entoloma*, *Favolus*, *Ganoderma*, *Geastrum*, *Hexagonia*, *Hygrophorus*, *Hymenopellis*, *Lactarius*, *Laetiporus*, *Lepiota*, *Marasmiellus*, *Microporus*, *Mycena*, *Oligoporus*, *Phellinus*, *Polysporus*, *Russula*, *Trichaptum*, *Trametes*, and *Xylaria* species (Figure 2). Ten species were successfully identified through morphological and ITS sequence analysis. These species included *Cyathus striatus*, *Cookeina tricholoma*, *Favolus acervatus*, *Ganoderma williamsianum*, *Hexagonia tenuis*, *Hygrophorus agathosmus*, *Marasmiellus candidus*, *Microporus xanthopus*, *Russula leucocarpa*, *Trichaptum durum*, and *Trichaptum fuscoviolaceum*. Based on the closest matches, the ITS sequence showed a high percentage of similarities between 97 to 100% with the GenBank database. Approximately 89.3% of the groups consisted of saprophytic fungi, while mycorrhizal fungi made up 7.1%. Parasitic fungi constituted the smallest proportion, accounting for 3.6% with only one species (Table 1).

Figure 2. Fruiting body of macrofungi obtained from Lentang Forest Reserve, Malaysia: A. *M. candidus*; B. *C. tricholoma*; C. *M. xanthopus*; D. *G. williamsianum*; E. *Nigrohirschioporus durus*; F. *Trichaptum fuscoviolaceum*; G. *Hygrophorus* sp.; H. *C. striatus*; I. *R. leucocarpa*; J. *F. acervatus*; K. *Polyporus* sp.; L. *H. tenuis*; M. *Agaricus* sp.; N. *Entoloma* sp.; O. *Lepiota* sp.; P. *Phellinus* sp.; Q. *Xylaria* sp.; R. *Trametes* sp.; S. *Lactarius* sp.; T. *Geastrum* sp.; U. *Cantharellus* sp.; V. *Auricularia* sp.; W. *Annulohypoxylon* sp.; X. *Mycena* sp.; Y. *Hymenopellis* sp.; Z. *Laetiporus* sp.; AA. *Oligoporus* sp.; AB. *Amauroderma* sp. Scale bar: 1 cm

Table 1. Ecological roles of macrofungi recorded in Lentang Forest Reserve, Bentong, Pahang, Malaysia

Species	Substrate	Ecological roles	ITS accession no
Laetiporus sp.	Wood	Saprophyte	-
Agaricus sp.	Wood	Saprophyte	-
Marasmiellus candidus	Wood	Saprophyte	MW661077
Cantharellus sp.	Root	Saprophyte	-
Phellinus sp.	Wood	Saprophyte	-
Lactarius sp.	Wood	Mycorrhizae	-
Oligosporus sp.	Wood	Saprophyte	-
Russula leucocarpa	Soil	Mycorrhizae	MW661076
Microporus xanthopus	Wood	Saprophyte	MW661081
Hygrophorus sp.	Soil	Saprophyte	-
Cookeina tricholoma	Wood	Saprophyte	MW661086
Hymenopellis sp.	Leave	Saprophyte	-
Mycena sp.	Wood	Saprophyte	-
Xylaria sp.	Wood	Saprophyte	-
Geastrum sp.	Wood	Saprophyte	-
Auricularia sp.	Wood	Saprophyte	-
Annulohypoxylon sp.	Wood	Saprophyte	-
Favolus acervatus	Wood	Saprophyte	MW661078
Polyporus sp.	Wood	Saprophyte	-
Cyathus striatus	Wood	Saprophyte	MW661079
Hexagonia tenuis	Wood	Saprophyte	MW661080
Ganoderma williamsianum	Wood	Saprophyte	MW661083
Trametes sp.	Wood	Saprophyte	-
Lepiota sp.	Soil	Saprophyte	-
Amouroderma sp.	Soil	Saprophyte	-
Entoloma sp.	Soil	Saprophyte	-
Nigrohirschioporus durus	Wood	Saprophyte	MW661084
Trichaptum fuscoviolaceum	Wood	Saprophyte	MW661085

Table 2. Diversity of macrofungi based on classes found in Lentang Reserve Forest using Shannon-Weiner diversity index (H') and Pielou species diversity index (J')

Class	Number of species	Percentage (%)	H'	J'
Agaricomycetes	49	89.09	0.102	0.114
Pezizomycetes	2	3.64	0.120	
Sordariomycetes	3	7.27	0.190	
Total	55	100	0.866	

The findings align with previous studies conducted in Gunung Korbu, Perak, Malaysia (Bakray et al. 2020), Philippines' Southern Luzon (Parlucha et al. 2021), and Northern Ethiopia (Alem et al. 2021), where saprophytes fungi predominated among the collected macrofungi in both studies. Saprophytic fungi, as the primary decomposers of litter, play a crucial role in the cycling of carbon, nitrogen, and other essential soil nutrients (Chen et al. 2018). As a result, saprophytic fungi have become widespread and prolific. Unlike other organisms, saprophytic fungi cannot produce their own food. Instead, they rely on consuming decaying organic matter for survival. These saprotrophs naturally thrive on a variety of substrates, including wood, soil, and leaf litter (Karun et al. 2018).

Species diversity of macrofungi in Lentang Forest Reserve, Bentong, Pahang

Fungal diversity was assessed based on the class category of the samples. The class with the highest species presence was Agaricomycetes, comprising 89.09% of the total diversity, followed by Sordariomycetes at 5.45%, Pezizomycetes at 3.64%, and Ascomycetes at 1.82%. To compare the occurrence of each class found in Lentang Forest Reserve, the diversity of all the samples was calculated using Shannon-Weiner Index (Table 2). The Sordariomycetes class exhibited the highest occurrence of species, with a diversity index of 0.160. This indicates that Sordariomycetes is the most abundant class in the area, primarily represented by species from the *Xylaria* and *Annulohypoxylon* genera. However, despite having the highest number of samples, the Agaricomycetes class has a lower diversity index compared to Sordariomycetes and Ascomycetes. The most frequently encountered species within Agaricomycetes are from the *Ganoderma* and *Microporus* genera.

A comparison of microclimatic changes revealed no significant differences among different fungal orders (Kruskal-Wallis ANOVA, $p>0.05$) (Table 3). Therefore, it can be concluded that factors such as humidity, temperature, light intensity, and substrate pH do not significantly influence the spatial distribution of fungal orders and substrates across the studied sites. This discovery supports the idea that any detectable impacts in this setting can be attributed to random chance rather than systematic influences. This sophisticated insight deepens our understanding of the complex relationship between microclimatic factors and fungal dispersion patterns in the studied ecological setting. Additionally, the Polyporales order exhibited the highest abundance among the collected samples. This finding aligns with Krah et al. (2022), who observed that microclimatic variables influence the composition of fungal fruiting communities, with tough-fleshed fruit bodies being more prevalent in severe microclimates.

The correlation between fungal orders and microclimate is shown in Table 4. Among the fungal orders, only Pezizales showed a strong positive and significant correlation with humidity ($R=0.75105$; $p>0.05$). The relationship between fungal community structure, substrates, and microclimate is further illustrated in the NMDS diagram (Figure 3). The illustration shows that Polyporales, Agaricales, Russulales, and Pezizales are grouped distinctly. The association between each order of fungi and specific substrates shows minimal correlation.

Polyporales, primarily found on wood and twig substrates, seem to be the next closest order to potentially being influenced by temperature and humidity. These results contradict the Pearson correlation results, which indicate that only Pezizales are significantly affected by humidity. This discrepancy is likely caused by the scope of the study which may not be fine-grained enough to capture microclimatic variations, as fungal communities can exhibit local variations that may not be apparent on larger scales. According to Pouska et al. (2016), stem thickness influences fungal species composition by affecting temperature and humidity conditions.

Table 3. Comparison of microclimates between different fungi orders and various substrates

Microclimates	n	Fungi order					Substrates			
		Polyporales	Agaricales	Russulales	Pezizales	p-value (Kruskal-Wallis)	Wood	Twigs	Soil	p-value (Kruskal-Wallis)
		53	17	3	8		18	18	4	
Humidity (%)	x̄	79.3	78.7	81.0	93	0.44	79.9	80.1	81.0	0.659
	±SD	4.0	3.6	-	-		0.8	8.6	-	
	Min	75.0	75.0	-	-		57.0	57.0	-	
	Max	86.0	86.0	-	-		86.0	86.0	-	
Temperature (°C)	x̄	29.1	29.2	29.2	26.3	0.197	29.1	29.1	26.4	0.217
	±SD	0.9	0.7	-	-		0.9	8.6	-	
	Min	27.8	27.8	-	-		26.9	26.9	-	
	Max	30.1	30.1	-	-		30.1	30.1	-	

Table 4. Pearson correlation between fungi orders and microclimate

	Humidity	Temperature	Polyporales	Agaricales	Russulales	Pezizales
Humidity		-0.28349	-0.10013	-0.4918	-0.00301	**0.75101**
Temperature	-0.28349		0.49999	0.052709	-0.57088	-0.59843
Polyporales	-0.10013	0.49999		-0.48813	-0.33899	-0.33899
Agaricales	-0.4918	0.052709	-0.48813		-0.14399	-0.14399
Russulales	-0.0030057	-0.57088	-0.33899	-0.14399		-0.1
Pezizales	0.75101	-0.59843	-0.33899	-0.14399	-0.1	

Note: Values in bold indicate a significant correlation with p alpha = 0.05

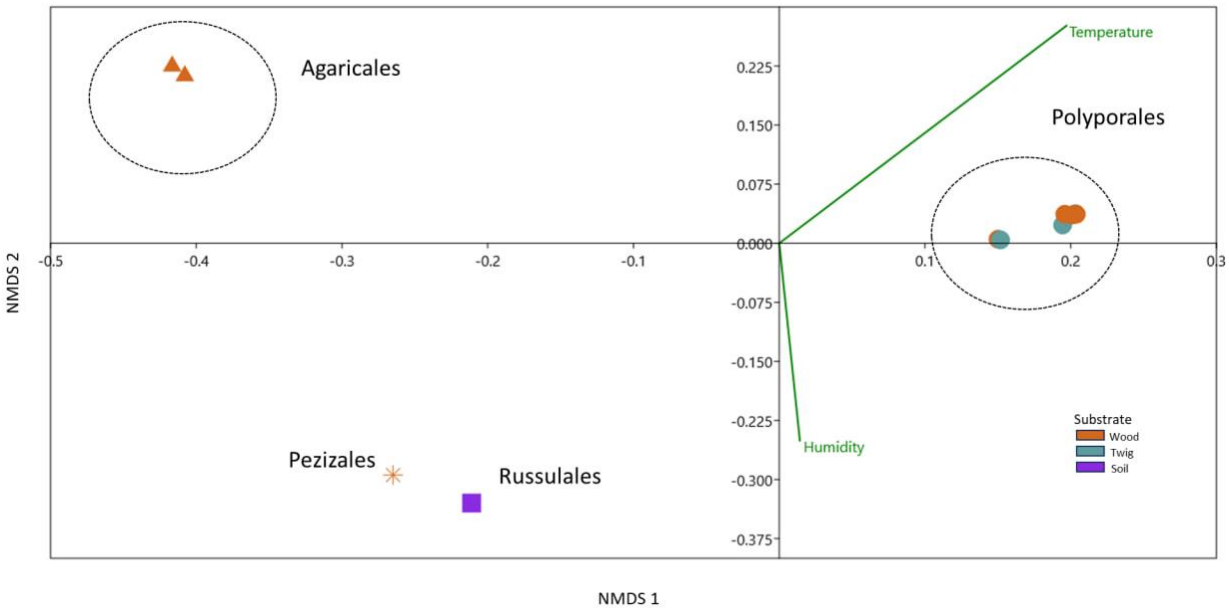

Figure 3. Non-metric multidimensional scaling plot visualizing the relationship among fungi community structure (shapes), substrates (shape colors), and microclimates (green lines). Fungi samples are indicated by different shapes according to their orders: filled circle = Polyporales; filled triangle = Agaricales; filled square = Russulales; asterisk = Pezizales

The kingdom Fungi plays a crucial role in maintaining ecosystem balance and significantly influences human activities and well-being. As mentioned by Mandal et al. (2023) and El-Ramady et al. (2022), wild macrofungi constitute a diverse range of natural resources. They are renowned for their medicinal, nutritional, and economic value, contributing to food security for some local communities. Similarly, microfungi play a crucial role in the microbial decomposition of plant litter in ecosystems. Therefore, both morphological and molecular identification techniques are essential for accurately identifying species. This comprehensive characterization is important not only for confirming known species but also for discovering new ones.

The prevalence of Basidiomycetes may be attributed to the abundance of trees and leaf litter serving as substrate, along with the high humidity and moisture levels that promote the rapid growth of macrofungi (Priyamvada et al. 2017; Rudawska et al. 2022). The majority of these macrofungi were leaf-litter decomposers and wood rotters, indicating an ecological hazard to dipterocarps and other valuable forest species (Hattori et al. 2012; Dulay et al. 2020; Niego et al. 2023). High diversity of fungi might expect in some forest types (e.g. lowland rainforest) compared to other forest types (e.g. spruce forest). The largest number of species are found in the orders Agaricales and Polyporales, with most being soil-dwelling species like *Russula* sp., *Amauroderma* sp., *Entoloma* sp., *Lepiota* sp., and *Hygrophorus* sp. Due to their ability to survive in higher temperatures and the fact that their toughness deters herbivorous animals, tree-dwelling macrofungi are common (Halbwachs et al. 2017). In six Aeta tribe settlements in Tarlac, Pampanga, and Zambales, Philippines the order Polyporales was identified as the predominant macrofungus (Alem et al. 2021). Similarly, in Malaysia, *Polyporus* sp., *Microsporus* sp., and *Favolus* sp. were found at Gunung Korbu, Perak, and accounted for the greatest number of specimens collected (Bakray et al. 2020).

In comparison to the growth of macrofungi in soil, researchers unearthed a richer diversity of macrofungi species thriving amidst the substrate litter, a habitat comprising decayed leaves, tree branches, and decomposing wood. The colonization patterns of rotten-wood fungi are profoundly shaped by local dispersal sources, with ample spore deposition often serving as a prerequisite for their successful establishment (Chen et al. 2018). This illustrates the uniform distribution of various species samples across a specific area. During the rainy and early dry seasons, macrofungi diversity peaks in high-altitude regions. The heightened diversity at higher altitudes can be attributed to lesser human interference compared to the extensive forest degradation observed in low-altitude areas, largely due to the establishment of oil palm and rubber plantations, cocoa farms, and food crop fields (Sharma et al. 2023). The majority of succulent and elaborately adorned large fungi are documented during the rainy season, which offers optimal conditions for their proliferation. This season is conducive for production since there is ample moisture, temperature, optimum relative humidity, and adequate light intensity to facilitate the fungi to decompose dead organic matter (Kinge et al. 2017; Wang et al. 2022).

In conclusion, 332 macrofungi sporocarps were discovered in this study, all of which belonged to the phylum Basidiomycota and Ascomycota. These sporocarps manifest three distinct ecological roles: saprophytic, mycorrhizal, and parasitic. Among them, saprophytic fungi exhibited the highest species diversity, comprising 25 distinct species, while parasitic fungi demonstrated the lowest diversity, represented by a solitary species. Notably, two mycorrhizae fungi were discovered living symbiotically with a variety of tree species. The most diverse substrates were found to include wood disintegration and dead twigs, highlighting the significant contribution of the Polyporales order to species diversity. The Lentang Forest Reserve emerges as a conducive habitat for a diverse array of macrofungi, particularly favoring saprophytic species. Furthermore, our observations suggest that microclimatic factors, notably humidity and temperature, wield considerable influence over macrofungi distribution. The study emphasizes the importance of promoting diverse tree species within urban forest ecosystems as a means to counteract the decline in macrofungal biodiversity, a trend heavily influenced by temperature and climatic variations.

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