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Research

Unveiling The Diversity and Ecological Roles of Macrofungi in Ayer Hitam Forest Reserve, Selangor, Malaysia

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ABSTRACT

The biodiversity of macrofungi in remaining lowland dipterocarp forest in highly urbanization pressures is infrequently studied, even though their significant impact on the ecosystem of forested and non-forested habitats. Therefore, this research endeavours to unravel the diversity of macrofungi from different substrates in the Ayer Hitam Forest Reserve located in Selangor, Malaysia. Through a combination of field surveys, the study seeks to contribute valuable insights into the complex relationships between macrofungi and the surrounding habitats where macrofungal community structure was mainly influenced by substrate richness and microclimates. Sporocarps of macrofungi were collected from selected sites using opportunistic sampling methods. A total of 333 sporocarps were obtained and identified based on morphological and molecular analysis. Basidiomycota prevailed in the Ayer Hitam Forest Reserve where 27 species belonging to 14 families were successfully identified. Saprophytic fungi (22 species, 81.5%) dominated the areas, and a small number of mycorrhizal (1 species, 3.7%) and parasitic (4 species, 14.8%) fungi were found. Based on species diversity, order Polyporales is the highest occurrence species in the sampling areas with a Shannon-Weiner Index value of 2.103 and Simpson Index value of 0.954, Evenness Index value of 0.601, making it the most abundant order containing *Microporus* species, The findings are expected to enhance our knowledge of the biodiversity of macrofungi from different substrates in Ayer Hitam Forest Reserve, which can lead to conservation efforts and promote the ecological significance of macrofungi in tropical forest ecosystems.

Key words: Basidiomycetes, macrofungi, mycorrhizal, parasites, saprophytes, urban

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INTRODUCTION

The overall diversity and health of ecosystems are enhanced by macrofungi, which perform crucial ecological roles in their habitats. Despite their significance in nutrient cycling as an organic decomposer (Kinge *et al*., 2017; Santamaria *et al*., 2023), and symbiotic relationships (Hyde *et al*., 2018), the diversity of macrofungi associated with different substrates in this forest reserve are still unclear. Additionally, certain macrofungi cause decay in healthy plants due to their parasitic behavior (Tapwal, 2013). Unlike mycorrhizal macrofungi, which have symbiotic relationships, parasitic macrofungi obtain nutrients from their host plants, often causing harm or disease in the process. Apart from their previously mentioned functions, certain macrofungi are advantageous due to their nutritional and medicinal qualities, which are why many people eat them and use them as traditional medicines (Samsudin & Abdullah, 2019).

Despite the wide range of ecological functions and advantages that macrofungi offer, this amazing class of organisms is currently under threat from various factors,

including human activity. Rapid development and urbanization are considered the most dangerous human endeavors as they harm macrofungi and their natural habitats. Environments affected by rapid development typically encompass a variety of features such as parks, shopping centers, residential neighborhoods, highways, medical facilities, and many more, which undergo significant changes. Furthermore, urban habitats are typically characterized by severe environmental factors, including reduced organic matter, high temperatures, restricted soil volume, high pH, and heavy metal concentrations (Shuhada *et al*., 2020).

The ongoing disturbance brought on by unending land expansion may eventually result in an ecological imbalance. Consequently, research on habitats that are close to urban areas is becoming increasingly important for protecting biodiversity and, concurrently, for balancing conservation efforts with the inevitable development in these areas.

Rapid development and industrialization activities have led to the conversion of most newly developed urban areas in Malaysia. As a result of the considerable changes that have occurred, these regions now harbor unique ecological niches that are home to new species. Modifications also apply to Puchong, Selangor. In addition to being the only lowland dipterocarp forest in the Klang Valley to remain in perfect condition, Ayer Hitam Forest Reserve is special because it borders the major cities of Shah Alam, Kuala Lumpur, Petaling Jaya, and Cyberjaya. Although urbanization benefits the region economically, it also brings about several unwanted risks. Changes in land use and emissions, such as air, noise, and water, are the main effects of urbanization.

Through meticulous field surveys and ecological studies, this research aims to determine the diversity of macrofungi from various substrates in the Ayer Hitam Forest Reserve located in Selangor, Malaysia. Understanding the complex relationship between macrofungi and the broader ecosystem is essential for effective conservation and management strategies. Moreover, the findings of this research may contribute valuable insights into the border field of tropical forest ecosystems.

MATERIALS AND METHODS

Study site and specimen collection

This study was carried out at Ayer Hitam Forest Reserve, which is located at 3°00'32"N 101°38'38"E in Puchong, Selangor, Malaysia. A sampling series from November 2021 to June 2022 was conducted using opportunistic sampling methods (Dulay *et al*., 2020) to collect and record all macrofungi found at the sampling sites. The fruiting bodies samples were placed in paper bags and were marked with the specimen number, date, and coordinates. Humidity, temperature, light intensity, and pH of the substrate of the samples were recorded Humidity, temperature, light intensity, and pH of the substrate of the samples were recorded using HTC-2 digital temperature humidity meter, infrared thermometer, portable lux meter, and soil pH tester respectively. Based on their hosts or substrates *in situ*, the specimens were categorized as saprophytes, mycorrhiza, or parasites according to Yusran *et al*. (2022).

Morphological characterization

The specimens were photographed, and the morphological characteristics were recorded. The macroscopic features of the sporocarps such as fruiting body forms, cap color, surface, margin, and edge (Parlucha *et al*., 2021) were recorded. The hymenophore type (gilled, pores, or teeth) and attachment to the stipe (stem) were also observed. Other features that were noted included stipe cross-section, annulus (veil), color and shape, surface, attachment position, and type of stipe attachment on the substrate (Leonard & Fechner, 2010). A ruler was used to measure the height and width of each sporocarp specimen. The samples were placed in an ice box to maintain their freshness and subsequently taken to the lab for additional analysis.

Species identification based on Internal Transcribed Spacer (ITS) sequence analysis

The fresh sporocarps were placed in sterile mortar and ground using liquid nitrogen (Möller, *et al*., 1992). The genomic DNA of the pure culture was extracted by using UltraClean Microbial DNA Isolation Kit (MO BIO, Carlshad, CA, USA). Internal transcribed spacer (ITS) region was amplified using universal primers ITS1 (5′- TCCGTAGGTGCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTAT TATTATGC-3″) (White *et al*., 1990). The PCR reaction mixture was conducted in a total volume of 20 µL containing 1 ng DNA, 100 mM of each primordial, 0.1 U/µL Taq DNA polymerase, 2.5 mM $MgCl_2$, and 0.2 mM dNTPs. The amplification reaction occurred with a thermal cycler C1000 Contact (BioRad, USA). White *et al*. (1990) system was used for thermal cycling conditions, with minor modifications. Initial denaturation at 95°C for 30 sec, followed by 35 denaturation cycles at 95°C for 10 sec, annealing at 59°C for 15 sec,

extension for 30 sec at 72°C and final extension for 5 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 later be visualized under a UV trans-illuminator to view the amplicon size. All the PCR products were purified by using the QIAquick Gel Extraction Kit (QIAGEN, USA) by following the instructions of the manufacturers.

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

After species identification based on the ITS gene was completed, the potential ecological roles of the fungal species were determined using information available in the "Checklist of Fungi of Malaysia" (Chua *et al*., 2012).

Species Diversity Index

The diversity of fungi present in the Ayer Hitam Forest Reserve, Shannon-Wiener, Simpson, and Evenness index was calculated using the formula (Ifo *et al*., 2016).

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

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

$$D = \sum n_i(n_i - 1)/N(N - 1)$$

where the Simpson index D, n_i is the number of individuals in species i, and N is the total individuals of all species

$$J = \frac{H'}{H_{max}}$$

where the Pileou Evenness index, value of J ranges from 0 to 1. Higher values indicate higher levels of evenness. At maximum evenness, J = 1.

Significant and correlation analysis

The comparison of microclimates between fungal orders and microclimates between substrates was analyzed based on descriptive statistics and the non-parametric analysis of variance (Kruskal-Wallis ANOVA) using 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

Potential ecological roles of macrofungi in Ayer Hitam Forest Reserve

Studies on macrofungi are scarce where forest reserves are surrounded with urbanized setting ecosystems. Nonetheless, recognizing the value of biodiversity and its preservation, numerous organizations have expressed interest in assessing and preserving biodiversity on campus (Karun *et al*., 2018; Putra *et al*., 2020). Some individuals plant avenue trees or trees with therapeutic and nutritional properties and establish gardens and arboretums to cultivate native, uncommon, and threatened tree species. These types of landscapes produce a lot of plant debris from dead roots, old bark, and woody leaf litter. These all end up serving as the main substrates for the development and maintenance of macrofungi.

A total of 333 sporocarps which include 27 species of macrofungi from 14 families were collected for this study (Table 1). All samples were identified to the species level based on ITS sequence analysis. Every sequence that was acquired was deposited to GenBank (Table 1). Based on the GenBank

database, the ITS sequence exhibited a high percentage of similarity, ranging from 78% to 100%. About 81.5% of the groups were made up of saprophytes, 14.8% were made up of parasites, and the least was mycorrhizae fungi, 3.7% with only 1 species (Table 1).

The results agreed with research conducted in the Philippines' Southern Luzon (Parlucha *et al*., 2021) and Northern Ethiopia (Alem *et al*., 2021), where saprophytes were found to make up the largest percentage of the macrofungi gathered in both investigations. The most active decomposers of litter, saprophytic fungi, greatly contribute to the cycles of carbon and nitrogen as well as other soil nutrients (Chen *et al*., 2018). As a result, saprophytic fungi were common and abundant. Fungi classified as saprophytes are incapable of generating their nourishment. To live, they consume decomposing and dead materials. Saprotrophs by nature frequently grow on a variety of substrates, mainly on wood, soil, and leaf litter (Karun *et al*., 2018). Because of their various ecological roles, mycorrhizae and parasitic fungi are typically found in lower abundance in forests than saprophytic fungi (Tedersoo *et al*. 2010; Lindahl and Tunlid, 2015). Mycorrhizae develop symbiotic associations with plants, assisting in nutrient absorption and promoting plant growth, which is critical for forest ecosystems. Parasitic fungi, on the other hand, may harm plants, which causes a balance in their abundance to prevent undue damage to forest flora (Delavaux *et al*., 2023).

Table 1. The GenBank accession number of deposited fungal isolates based on ITS

No.	Species ID	Orders	Families	ITS Accession No.	Species Similarity (%)	Substrate	Potential Ecological roles*
1	*Climacodon dubitativus*	Polyporales	Phanerochaetaceae	OR178470	99.12	Dead wood	Parasite
2	*Gloeoporus dichrous*	Polyporales	Irpicaceae	OR178471	88.89	Dead wood	Saprophyte
3	*Coriolopsis retropicta*	Polyporales	Polyporaceae	OR178472	99.52	Dead wood	Saprophyte
4	*Trametes strumosa*	Polyporales	Polyporaceae	OR178473	78.99	Dead twigs	Saprophyte
5	*Russula brunneoviolacea*	Russulales	Russulaceae	OR178474	93.95	Unknown tree	Mycorrhizae
6	*Gymnopus androsaceus*	Agaricales	Omphalotaceae	OR178475	81.47	Leaf litter	Saprophyte
7	*Clitopilus sinoapalus*	Agaricales	Entolomataceae	OR178476	97.08	Leaf litter	Saprophyte
8	*Microporus vernicipes*	Polyporales	Polyporaceae	OR178477	95.75	Dead twigs	Saprophyte
9	*Panus velutinus*	Polyporales	Polyporaceae	OR178478	99.35	Soil	Saprophyte
10	*Ganoderma australe*	Polyporales	Polyporaceae	OR178480	82.14	Living tree trunks	Parasite
11	*Marasmius cystidiatus*	Agaricales	Marasmiaceae	OR178481	94.49	Dead wood	Saprophyte
12	*Microporus xanthopus*	Polyporales	Polyporaceae	OR178482, OQ158863	99.04 97.69	Dead twigs	Saprophyte
13	*Microporus affinis*	Polyporales	Polyporaceae	OQ158874	97.72	Dead twigs	Saprophyte
14	*Schizophyllum commune*	Agaricales	Schizophyllaceae	OR178483	99.84	Dead wood	Saprophyte
15	*Ganoderma williamsianum*	Polyporales	Polyporaceae	OR178484	99.68	Dead wood	Parasite
16	*Termitomyces striatus*	Agaricales	Lyophyllaceae	OR178485	98.00	Termite nest	Saprophyte
17	*Lactarius subserifluus*	Russulales	Russulaceae	OQ158864	95.42	Leaf litter	Saprophyte
18	*Marasmius guyanensis*	Agaricales	Marasmiacea	OQ158866	99.06	Dead wood	Saprophyte
19	*Skelotocutis delicata*	Polyporales	Polyporaceae	OQ158867	88.40	Dead wood	Saprophyte
20	*Entoloma flavovelutinum*	Agaricales	Entolomataceae	OQ158870	99.68	Soil	Saprophyte
21	*Trametes sanguinea*	Polyporales	Polyporacea	OQ158872	100	Dead wood	Saprophyte
22	*Favolus acervatus*	Polyporales	Polyporacea	OQ158865	89.38	Dead wood	Saprophyte
23	*Trametes flavida*	Polyporales	Polyporacea	OQ158868	90.35	Dead twigs	Saprophyte
24	*Podosypha gillesii*	Polyporales	Meruliaceae	OQ158871	81.72	Dead wood	Saprophyte
25	*Cortinarius salor*	Agaricales	Cortinariaceae	OQ158873	82.56	Soil	Saprophyte
26	*Ridigoporus microporus*	Polyporales	Meripilaceae	OQ158875	86.97	Dead wood	Parasite
27	*Hymenochaete iodina*	Hymenochaetales	Hymenochaetaceae	OQ158869	94.33	Dead wood	Parasite

* Potential ecological roles were determined based on Chua *et al*. (2012).

Species diversity indexes of macrofungi in Ayer Hitam Forest Reserve

Most of the saprophytes were derived from dead wood and soil. *Panus velutinus* and *Entoloma flavovelutinum* from Polyporaceae and Entolomaceae, respectively are great examples of saprophytic fungal species that live in the soil (Table 2). On the other hand, the fruiting bodies of the Polyporaceae species of *Trametes* and *Microporus* are saprophytic fungal genera that inhabit fallen branches and dead wood. They can endure low-moisture conditions during the dry season and have a stiff, leathery texture (Parlucha *et al*., 2021). According to Lynch and Thorn (2006), saprotrophs can break down a range of resistant organic substrates present in certain land-use systems, including natural and planted trees. The abundance of polyporoid fungi in the area can be attributed to the numerous old branches of AHFR, which offer ideal growth conditions for these fungi. This result is consistent with another finding that found greater diversity in disturbed areas among saprotrophic macrofungi (Ye *et al*., 2019).

Table 2. Diversity index of Basidiomycete fungi according to their order classification

Species diversity	Polyporales	Agaricales	Russulales	Hymenochaetales
Shannon Wiener Index	2.103	0.762	0.544	0.105
Simpson Index	0.954	0.709	0.824	0
Pielou's evenness	0.601	0.347	0.304	0

Four species of parasitic fungi were found in the Ayer Hitam Forest Reserve. Temperature seasonality, host abundance, and make-up of host species assemblages are among the factors that affect the diversity of parasitic fungal species in an area (Zhu *et al*., 2023).

Moreover, parasitic fungi can only flourish on trees when the presence of nutrients. Thus, the diversity of these kinds of fungi has been limited by a variety of hosts (Stallman & Robinson, 2022). *Ganoderma* species typically lead hybrid lives that combine necrotrophic and biotrophic lifestyles. Initially, during the biotrophic phase, the pathogenic species starts to colonize and infect the intact host plant cells. The primary goal of biotrophs is to preserve their host cells so they can absorb food (Bahari *et al*., 2018). Following that, the species enters the necrotrophic phase, in which the plants' cell walls are severely damaged, enabling the fungi to infect and survive saprotrophically (Chong *et al*., 2017). The diseased trees eventually withered away. Worldwide, there are over 80 species in the genus *Ganoderma*. Many of these species are native to tropical climates (He *et al*., 2022). Basal stem rot (BSR), a devastating disease in Southeast Asian (SEA) oil palm plantations, primarily in Malaysia and Indonesia, is primarily caused by *Ganoderma* species (Paterson, 2019; Shokrollahi *et al*., 2021).

Russula brunneoviolacea (31.8%) was the only species of mycorrhizal fungus found in this study, making it the least common species (Figure 1). Mycorrhiza refers to the relationship between specific soil fungi and the fine roots of almost all forest plants. Plants with better access to nutrients provide these fungi with sugars, and occasionally the fungi themselves provide water (Sridhar & Karun, 2019). Furthermore, plants' capacity to absorb potassium, phosphate, and nitrogen is enhanced by the presence of mycorrhizal fungi (Mohammadzadeh & Pirzad, 2021). Typically, mycorrhizal fungi are found in forests. This was because humans supplied all the necessary nutrients for trees in disturbed or ornamental areas, so fungi were not needed to aid them. These results are unique to Malaysia, especially to Selangor, where no reports of the presence of *Russula* species linked to avenue trees have been found. According to earlier reports, fungi belonging to the Russulaceae family are ectomycorrhizal symbionts with higher plants and trees were linked to Orchidaceae plants, such as *Chamaegastrodia inverta* and the canopies of *Areca catechu* (Pecoraro *et al*., 2020; Parlucha *et al*., 2021).

Based on the samples' order classification, the fungal diversity was computed. To compare the occurrence of each class found in Ayer Hitam Forest Reserve, the diversity of all the samples was calculated by using the Shannon-Weiner Index (SWI), Simpson Index (SI), and Evenness Index (EI) (Table 3). With a diversity index of 0.105 for the order Hymenochaetales*, Hymenochaete mirocyla* is the least abundant species, while the order Polyporales has the highest occurrence of species in the region, with the SWI and SI of 2.103 and 0.954, respectively, making it the most abundant order containing *Microporus* species. Meanwhile based on EI, the Polyporales order, has the greatest distribution of *Microporus* species in the region and the least amount of Hymenochaetales species.

Fig. 1. Macrofungi obtained from Ayer Hitam Forest Reserve, Malaysia. A, *Favolus acervatus*; B, *Climacodon dubitativus*; C, *Russula brunneoviolacea*; D, *Ganoderma williamsianum* ; E, *Gloeoporus dichrous*; F, *Termitomyces striatus*; G, *Entoloma flavidum*; H, *Marasmius cystidiatus*; I, *Ganoderma aurales*; J, *Coriolopsis retropicta*; K, *Panus velutinus*; L *Trametes flavida* ; M, *Ridigoporus microporus;* N, *Marasmius guyanensis*; O, *Trametes sanguinea*.; P, *Microporus xanthopus*; Q, *Clitopilus sinoapalus*; R, *Hymenochaete iodina;* S, *Podoscypha gillesii;* T, *Microporus affinis*; U, *Skeletocutis delicata*; V, *Schizophyllum commune*; W, *Gymnopus androsaceus*; X, *Lactarius subserifluus*; Y, *Trametes strumosa* ; Z, *Cortinarius salor*; AA, *Microporus vernicipes*

Table 3. Diversity index of Basidiomycete fungi based on Basidiomycete substrate

Diversity Index	Order	Substrates			
		Wood	Twigs	Leaf	Soil
Shannon-Wiener Index	Polyporales	0.941	1.145	0	0.017
	Agricales	0.491	0	0.168	0.102
	Russulales	0	0	0.526	0.017
	Hymenochaetales	0.105	0	0	0
Simpson Index	Polyporales	0.879	0.929	0	0
	Agaricales	0.525	0	0.692	0.714
	Russulales	0	0	0.816	0
	Hymenochaetales	0	0	0	0
Evenness Index	Polyporales	0.356	0.396	0	0
	Agaricales	0.447	0	0.153	0.093
	Russulales	0	0	0.327	0
	Hymenochaetales	0	0	0	0

The prevalence of Basidiomycetes may be attributed to the availability of trees and leaf litter as substrate as well as the high humidity and moisture that promote the rapid growth of macrofungi (Priyamvada *et al*., 2017, Rudawska *et al*., 2022).

To make a comparison based on the diversity of substrate, which is shown in Table 3, the majority of these macrofungi were dominated by wood and twig rotters which come from Polyporales order, indicating an ecological hazard to dipterocarps and other valuable forest species (Bolhassan *et al*., 2012, Niego *et al*., 2023). The second number of species are found in the order Agaricales, with most of them also from wood rotten and leaf litter decomposer species like *Schizophyllum commune*, *Marasmius guyanensis*. *Cortinarius salor*, *Marasmius cystidiatus*, and *Gymnopus alliifoetidissimus*. Comparatively, according to the Evenness Index, a greater proportion of the species in each area are found in the Agaricales order, with the majority originating from the *Schizophyllum commune*, which grows prolifically on dead rubber trees. Tree-dwelling macrofungi are common due to their ability to survive greater temperatures and the fact that their toughness deters herbivorous animals (Couceiro *et al*., 2022). Additionally, Order Polyporales was observed to be the most common macrofungus in six Aeta tribe settlements in Tarlac, Pampanga, and Zambales (Alem *et al*., 2021). More species of macrofungi were found to grow in the substrate litter, including dead and rotten leaves, tree branches, and rotten wood, compared to those growing in the soil. Local dispersal sources have a significant impact on the colonization patterns of rotten wood fungi, and successful colonization may necessitate large-scale spore deposition (Chen *et al*., 2018).

Comparison between fungi orders, substrates, and microclimate

Comparison of microclimatic variations reveals non-significant differences (Kruskal-Wallis ANOVA, $p>0.05$) among different fungal orders (Table 4). It can be inferred that humidity, temperature, light intensity, and substrate pH do not significantly influence the spatial distribution of fungal orders across the sampled sites. This observation aligns with the notion that any discernible effects in this context may be attributed to random chance rather than systematic influences. However, microclimate effects appeared to be more pronounced across different fungi substrates (Table 4). Among the microclimatic factors, light intensity and pH values were significantly varied ($p<0.05$) among fungi substrates. This highlights the pivotal role of light intensity and pH of substrates in shaping the fungal distribution patterns within the sampled sites. The significance of these variables suggests a non-random association, implying that variations in light intensity and pH contribute meaningfully to the observed differences in the collection of fungi across distinct substrates. This nuanced understanding adds depth to our comprehension of the intricate interplay between microclimatic elements and fungal distribution dynamics in the examined ecological context.

The relationship between fungi community structure, substrates, and microclimates is further visualized in the nMDS plot (Figure 2). The plot shows that Polyporales, Agaricales, Russulales, and Hymenochaetales are separated into different groups. The association between each fungal order with specific substrates is also evident, although their correlation with microclimate was less apparent. Fungi from order Polyporales, which is associated with wood and twig substrate, appeared to be influenced by pH. Therefore, pH value of 6.3, the environment would be considered almost neutral about the pH preferences and tolerances observed in the studies on Polyporales. Different fungi species have specific pH requirements for optimal growth. Some fungi thrive in acidic conditions, while others prefer neutral or alkaline environments, which can influence nutrient availability and enzyme activity in fungal

metabolism (Senanayake *et al.*, 2016; Min *et al.*, 2023). Fungi produce enzymes to break down complex organic materials present in wood. The activity of these enzymes is pH-dependent. The pH level can affect the efficiency of enzyme function, impacting the ability of fungi to digest and utilize wood as a nutrient source (Gallo *et al.*, 2022).

The correlation between fungal orders and microclimate is shown in Table 5. Among the microclimates, humidity, and temperature had a moderate positive correlation (r=0.623, p<0.05). Among the fungal orders, Polyporales appear to be significantly correlated with pH (r=0.307; p<0.05).

Table 4. Comparison of microclimates between different fungal orders and different substrates. * = significant at p<0.05

Microclimates		Fungi order				*p*-value
		Polyporales	Agaricales	Russulales	Hymenochaetales	
	n	33	9	6	1	
Air Humidity (%)	x̄	70.6	70.5	71.3	68	0.592
	±SD	8.4	8.4	8.4	-	
Temperature	x̄	28.4	28.3	28.4	28.4	0.813
	±SD	0.9	0.8	0.8	-	
Light intensity (lux)	x̄	3748.3	3910.8	3979.1	3450	0.258
	±SD	1310.6	1143.7	1062.7	-	
pH of substrates	x̄	6.3	6.3	6.3	6.3	0.304
	±SD	0.2	0.2	0.2	-	
Microclimates		Substrates				*p*-value
	n	Wood	Twigs	Soil	Litter	Tree
Air Humidity (%)	x̄	70.6	70.5	69.5	71.0	79.0
	±SD	8.3	8.6	7.9	8.4	-
Temperature	x̄	28.4	28.4	28.2	28.4	29.8
	±SD	0.9	8.6	0.8	0.8	-
Light intensity (lux)	x̄	3472.2	3933.8	3957.8	4007.4	3020.0
	±SD	1297.5	1124.0	1099.1	1065.3	-
pH of substrates	x̄	6.3	6.3	6.3	6.3	6.4
	±SD	0.2	0.2	0.2	0.2	-

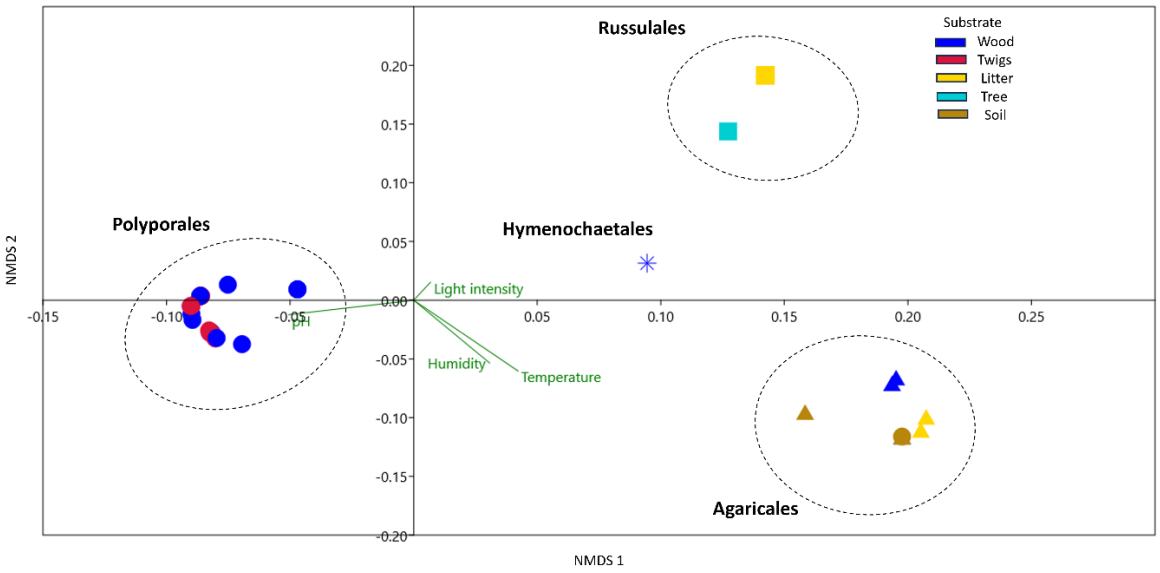

Fig. 2. Non-metric Multidimensional Scaling (NMDS) plot visualizing the relationship among fungi community structure (shapes), substrates (shape colors), and microclimates (green lines). Fungi samples are indicated by different shapes according to their orders: filled circle = Polyporales; filled triangle = Agaricales; filled square = Russulales; asterisk = Hymenochaetales.

Table 5. Pearson correlation between fungal orders and microclimate. Values in bold = significantly correlated with $p = 0.05$

	Humidity	Temperature	Light intensity	pH	Polyporales	Agaricales	Russulales
Humidity							
Temperature	**0.62292**						
Light intensity	-0.10485	0.15803					
pH	-0.13901	-0.067607	0.15332				
Polyporales	-0.041273	-0.0075368	-0.11564	**0.30693**			
Agaricales	0.27134	0.22353	0.03115	-0.020016	**-0.5067**		
Russulales	-0.20486	-0.18936	0.057209	-0.16756	**-0.43368**	-0.14781	
Hymenochaetales	-0.03919	0.0055281	0.0013801	-0.018399	-0.17599	-0.059984	-0.051339

The environment influenced the abundance of macrofungi. Most of the species that have positive effects on macrofungal abundance were observed at high relative humidity. According to Jang and Hur (2014), mushrooms prefer humidity above 82%. However, the abundance of macrofungi and light intensity were negatively correlated. According to Tomao *et al*. (2020), decomposition is less favored in areas with closed canopies, which typically have low intensity due to the dense foliage of trees and shrubs. Decomposition is slowed by high canopy cover as it blocks out light, moisture, temperature, and rain. As species richness increased in mature, closed forests, (Arenas *et al*., 2021) observed a similar pattern, with sporocarp abundance doubling in younger, open stands.

However, saprotrophic fungi have been discovered to benefit more from canopy cover when certain substrates are available (Castaño *et al*., 2018). According to Nakamura *et al*. (2017), the dynamics of forest ecosystems and the creation of habitats are influenced by the tree canopy. In response to light intensity, trees can either stimulate or inhibit the growth and spread of understory species such as macrofungi (Thomaes *et al*., 2013). The effects of soil moisture on macrofungi are detrimental. As Goldmann *et al*. (2015) reported in similar findings, anaerobic soil conditions in waterlogged stands are intolerant of basidiomycetes. While macrofungi have limited fruiting due to low rainfall, certain species can also be inhibited by excessive moisture (Ren *et al*., 2021).

CONCLUSION

A total of 333 basidiocarps were identified in the study, all of which were attributed to the phylum Basidiomycota. These basidiocarps exhibited three potential distinct ecological roles: saprophytic, mycorrhizal, and parasitic. Saprophytic fungi displayed the highest species diversity, totaling 22, while mycorrhizal fungi exhibited the lowest diversity with only one species. Notably, four parasitic fungi were found coexisting symbiotically with various tree species. The decomposition of wood and dead twigs emerged as the most diverse substrates, highlighting the significant contribution of the Polyporales order to species diversity. The Ayer Hitam Forest Reserve was identified as a conducive environment for a wide array of macrofungi, with favourability for saprophytic macrofungi. Although only four species of parasitic fungi were identified, their presence is deemed potentially hazardous to the forest's trees. The influence of microclimates, specifically concerning light intensity and substrate pH, appears to exert significant effects on the distribution of macrofungi. However, the study highlights the considerable significance of pH conditions and evolving climatic conditions as pivotal determinants of prospective biodiversity reduction in macrofungi. Emphasizing the promotion of diverse tree species in forest reserve ecosystems becomes imperative, given that various macrofungi exhibit specific associations with particular tree species. This objective can be realized through judicious urban planning and the implementation of strategic tree-planting initiatives.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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