

Species composition and environmental associations of macrofungi in Sungai Tua Forest, Malaysia

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ABSTRACT

Fungi play an important role in the nutrient cycle and in maintaining ecosystem stability in the forest environment. Environmental conditions and plant communities can significantly influence macrofungal diversity. Therefore, studying fungal communities is essential for developing effective forest management strategies, as fungi are key components of forest resilience and health. The study aimed to determine the diversity and ecological roles of macrofungi in Sungai Tua Forest, Selangor, Malaysia. Macrofungi were collected using an opportunistic sampling method, and species identification was conducted based on morphological and molecular analyses. A total of 454 macrofungal specimens were collected and identified as 27 species belonging to 18 genera, seven orders, and two phyla. Agaricales was the dominant order, followed by Polyporales, while Dacrymycetales, Pezizales, Xylariales, Russulales, and Gomphales were each represented by a single species. Sungai Tua Forest has high diversity values, with a Simpson index of 0.9485, a Shannon-Weiner index of 1.9788, and Pielou's evenness index of 0.5938. Correlation analysis between fungal orders and environmental parameters shows that light intensity, temperature, and humidity were associated with the distribution of fungal groups, indicating that these factors influence macrofungal diversity.

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1. INTRODUCTION

Human activities, including logging, farming, and recreation, greatly impact forest ecosystems, including fungal populations. The diversity and community structure of fungi are essential markers of the resilience and health of forests because they are involved in carbon storage, nitrogen cycle, and plant-soil interactions (Robinson et al., 2020; Tomao et al., 2020; Wong et al., 2021). Despite their ecological importance, fungal biodiversity remains understudied in many tropical regions, particularly within recreational forests in Malaysia. The effects of recreational activities on fungal diversity and community composition are still poorly understood. Compared with other forms of disturbance, recreational forests experience pressures such as soil compaction, litter removal, and pollution, which may later affect fungal communities in distinct ways (Li et al., 2019; Tomao et al., 2020). Addressing this knowledge gap is crucial for developing comprehensive conservation and management strategies that recognize fungal biodiversity as a fundamental component of forest ecosystem health and resilience.

Research on fungal biodiversity in Malaysia's recreational forests is needed to advance disturbance ecology

and tropical forest resilience, thereby improving ecological understanding, informing forest management, and strengthening conservation strategies. Diverse fungal communities, particularly wood-decaying fungi, are vital for nutrient cycling and ecosystem functioning. According to Hattori et al. (2012), Malaysia hosts several rare and potentially threatened fungal species, including *Antrodiella xanthochroa*, *Rigidoporus malayanus*, and *Flabellophora velutinosa*, which are often associated with old-growth forests that are increasingly threatened by habitat loss and logging. Consequently, documenting fungal diversity in disturbed and semi-disturbed forest environments is critical to preventing species loss and ensuring long-term ecosystem sustainability.

Despite this importance, baseline information on the species composition and environmental associations of macrofungi in Malaysia's recreational forests remains limited. Most existing studies have focused on protected or undisturbed forests, while forests subjected to continuous recreational activities have received comparatively little attention. The lack of detailed understanding of how environmental factors and recreational disturbance influence macrofungal communities constrains the ability to assess ecosystem health, identify conservation priorities, and

implement effective forest management strategies.

The implications for forest management are significant. Findings from fungal biodiversity studies can guide infrastructure planning, such as trail and campsite placement, to minimize ecological disturbance and protect areas with high fungal diversity. Human activity in recreational forests can disrupt sensitive fungal habitats, but informed management practices can mitigate these impacts. Furthermore, understanding fungal biodiversity supports sustainable tourism by promoting a balance between recreation and conservation. Reducing anthropogenic disturbances helps maintain fungal diversity and preserves ecosystem integrity (Hattori et al., 2012; Kerfahi et al., 2014).

Regular evaluation of fungal diversity and ecological roles is a key component of effective conservation and sustainable forest management in Malaysia's recreational areas. Continuous research provides essential data on fungal species composition and ecosystem interactions, supporting evidence-based conservation strategies (Bukhori et al., 2020). Long-term monitoring of fungal communities enables forest managers to detect ecological changes, assess ecosystem health, and implement adaptive management strategies to mitigate the impacts of land conversion, deforestation, and recreational pressure (Kerfahi et al., 2014; Masum et al., 2017).

Sungai Tua Forest, a dipterocarp hill forest in Batu Caves, Selangor, Malaysia, is an ideal site for investigating macrofungal diversity in a recreational forest environment, as the area experiences high levels of anthropogenic disturbance, particularly from a resort offering various visitor amenities. Despite its ecological importance, the macrofungal diversity in Sungai Tua Forest has not been comprehensively studied. Therefore, the present study aims to characterize the diversity and ecological roles of macrofungi in this forest.

2. MATERIALS AND METHODS

2.1. Study site and specimen collection

This study was conducted in Sungai Tua Forest, Selangor, located at coordinates N3.7531, E102.6525 (Figure 1). The forest is a dipterocarp hill forest and serves as a recreational area with moderate to high human activity due to a nearby resort. Macrofungal samples were collected using an opportunistic sampling method following the procedure described by Etikan et al. (2016). This method is commonly applied in macrofungal biodiversity studies because fungal fruiting bodies occur sporadically and are strongly influenced by microhabitat conditions and seasonal rainfall, making systematic plot-based sampling less effective for detecting species presence. Sampling was conducted along accessible forest trails to maximize the detection of visible fruiting bodies while minimizing disturbance to the surrounding vegetation. A

total distance of approximately 2.47 km was surveyed across 51 pre-determined sampling sites distributed along these trails. Field surveys were conducted twice between December 2023 and October 2024 to capture temporal variation in macrofungal fruiting associated with seasonal changes. Although opportunistic sampling can effectively document macrofungal diversity across heterogeneous habitats, it may introduce detection bias and fail to adequately capture species with sporadic fruiting or outside the study period.

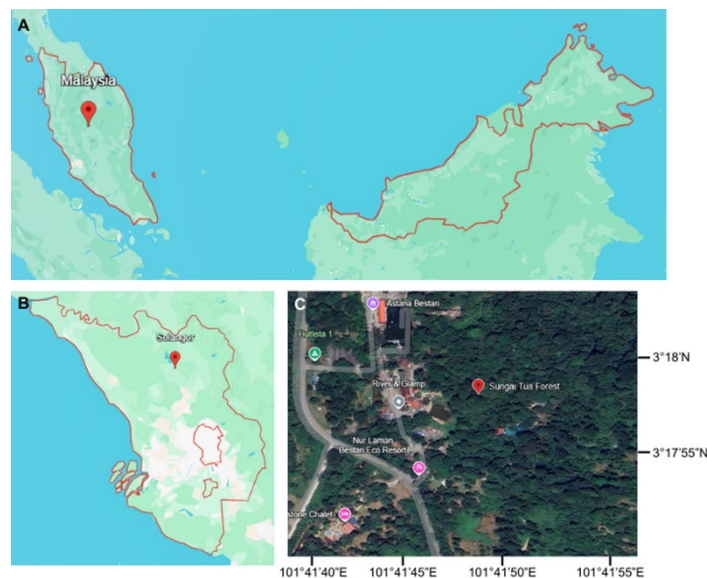


Figure 1: Map of study areas at Sungai Tua Forest, Selangor, Malaysia. Map of Malaysia (A), Selangor state (B), and Sungai Tua Forest (C) with coordinates.

Environmental parameters, including air humidity, soil pH, air temperature, substrate temperature, and light intensity, were recorded at each sampling site. Air humidity and temperature were measured using an HT607 temperature and humidity meter. Soil pH was determined with a Takemura DM-15 soil pH meter, while substrate temperature was measured using an HT650C infrared thermometer. Light intensity was measured with a Milwaukee MW700-WP waterproof LUX meter, and coordinates were obtained from Google Maps. These parameters were considered important factors influencing macrofungal growth and diversity.

2.2. Morphological characterization

The size of each macrofungal sporocarp sample was measured, and its morphological characteristics, including the form, apex, surface, and margin of the pileus and annulus, were documented. Observed features, including lamella attachment and arrangement, stipe attachment to the cap, form, surface, and substrate attachment, were documented following the method of Leonard (2010). Sporocarps were carefully removed from their substrates using a hand shovel. Collected samples were placed in paper bags, stored in a chiller box to preserve freshness, and later transported to the

laboratory for further analysis.

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

For sample preparation, excess dirt and substrate were removed from the specimens using a brush. A fresh fungal specimen was then cut into small pieces and ground in liquid nitrogen using a sterilized mortar and pestle. The resulting fungal powder was immediately transferred into a cryovial using a spatula. The DNA extraction process was performed using the UltraClean® Microbial DNA Isolation kit. The DNA extracts were stored at -20 °C until further analysis.

A 40 µL master mix containing reagent 5x Green GoTaq Buffer, 2 mM dNTPs, 2.5 mM MgCl₂, 10 mM primer ITS1, 10 mM primer ITS4, 0.2 µl GoTaq® Flexi DNA Polymerase (Promega, USA), sterile distilled water, and 20 ng DNA template was mixed before the amplification began. The primers used for this process were ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') as forward primer and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') as reverse primer (White et al., 1990). The cycle begins with an initial denaturation at 95°C for 30 seconds using a Professional Standard Thermocycler. Thereafter, 35 cycles of denaturation at 95°C for 10 seconds, annealing at 59°C for 15 seconds, and extension at 72°C for 30 seconds. The last extension is five minutes of warmth at 72°C.

The PCR products were visualized using gel electrophoresis. The amplified band was viewed under ultraviolet light using Enduro GDS software. The successful amplification of the ITS region between 500 - 700 bp was observed. A 100 bp DNA ladder was used in this process. All PCR products were purified using the QIAquick Gel Extraction Kit (QIAGEN, USA) according to the manufacturer's instructions.

The PCR-purified products were sent to the Next Gene company for sequencing. All sequences were analyzed, completed the Basic Local Alignment Search Tool (BLAST), and deposited in the GenBank database (National Center of Biotechnology Information at <https://www.ncbi.nlm.nih.gov>).

2.4. Species diversity index

Fungal species diversity was determined using the Shannon-Wiener Index (Shannon & Weaver, 1949). The index was used to estimate the species diversity within a community. In addition, the Simpson Index was used to measure species diversity. According to Simpson's (1949) diversity index, the probability that two randomly chosen individuals belong to the same species increases as diversity decreases. When there is no diversity (one species), there is a one percent chance that two randomly chosen individuals will be identical. Pielou's

evenness index (Pielou, 1966) can be used to illustrate how even a community is. The term "species evenness" describes the distribution of species within an ecosystem.

The formula for Shannon-Wiener Index (Shannon & Weaver, 1949) is:

$$H = -\sum_{i=1}^S p_i \times \ln p_i$$

Where:

p_i = proportion of individuals of species i ,
 $\ln$ = natural logarithm, and
 S = summation of species richness.

The formula for the Simpson Index (Simpson, 1949) is:

$$D = \sum_{i=1}^S \frac{1}{n_i(n_i-1)}$$

Where:

n_i is the number of individuals in species i ,
 N = total number of individuals of all species,
 $n_i/N = p_i$ (proportion of individuals of species i), and
 S = summation of species richness.

The formula for Evenness Index (Pielou 1966) is:

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

Where:

H is the value from the Simpson Index, and
 $\ln$ = natural logarithm.

2.5. Significance and correlation analysis

Correlation analysis was used to identify associations between macrofungi diversity and physical parameters, including temperature, substrate pH, light intensity, and humidity. The analysis was determined using the Pearson correlation test (Pearson, 1896). PAST software version 4.03 was used to perform the correlation.

3. RESULT AND DISCUSSION

3.1. Ecological roles of macrofungi in Sungai Tua Forest

A total of 454 samples of sporocarps were collected at Sungai Tua Forest, Selangor, Malaysia, from varieties of substrates, including dead wood, soil, leaf litter, dead twigs, and bamboo. Twelve species were found under the order Agaricales, while 10 species were found under the Polyporales, as shown in Table 1. Only a single species was found belonging to orders Dacrymycetales, Gomphales, Pezizales, Russulales, and Xylariales.

Table 1: The GenBank accession number of deposited fungal isolates based on Internal Transcribed Spacer (ITS) sequence analysis.

No.	Species ID	Orders	Number of collected sporocarps	ITS Accession no.	Similarity (%)	Substrate	Ecological roles
1.	<i>Calocera viscosa</i> (Pers.) Fr.	Dacrymycetales	18	NA	NA	Dead wood	Saprophyte
2.	<i>Clitopilus sinoapalus</i> S.P.Jian & Zhu L.Yang	Agaricales	18	OR178476	100	Dead wood	Saprophyte
3.	<i>Cookeina tricholoma</i> (Mont.) Kuntze	Pezizales	1	NA	NA	Dead twigs	Saprophyte
4.	<i>Coprinellus disseminatus</i> (Pers.) J.E.Lange	Agaricales	28	PQ787862	100	Wood	Saprophyte
5.	<i>Coprinopsis nivea</i> (Pers.) Redhead, Vilgalys & Moncalvo	Agaricales	17	NA	NA	Soil	Saprophyte
6.	<i>Coprinopsis lagopus</i> (Fr.) Redhead, Vilgalys & Moncalvo	Agaricales	4	NA	NA	Soil	Saprophyte
7.	<i>Cyathus crassimurus</i> H.J. Brodie.	Agaricales	12	PV052456	100	Dead twigs	Saprophyte
8.	<i>Daldinia eschscholtzii</i> (Ehrenb.) Rehm	Xylariales	18	PV052458	100	Dead wood	Saprophyte
9.	<i>Earliella scabrosa</i> (Pers.) Gilb. & Ryvarden	Polyporales	25	PQ350351	100	Dead wood	Saprophyte
10.	<i>Gymnopilus lepidotus</i> Hesler	Agaricales	4	PQ350350	100	Soil	Saprophyte
11.	<i>Lactarius subserifluus</i> Longyear	Russulales	21	OQ158864	100	Wood	Mycorrhizae
12.	<i>Lentaria surculus</i> (Berk.) Corner	Gomphales	3	PP375112	100	Bamboo	Saprophyte
13.	<i>Marasmiellus candidus</i> (Fr.) Singer	Agaricales	2	NA	NA	Dead twigs	Saprophyte
14.	<i>Marasmius cystidiatus</i> S.A. Sharafudheen & Manim	Agaricales	4	OR178481	100	Wood	Saprophyte
15.	<i>Marasmius guyanensis</i> Mont.	Agaricales	23	OQ158866	100	Leaf litter	Saprophyte
16.	<i>Microporus xanthopus</i> (Fr.) Kuntze	Polyporales	23	PQ185527 PQ803334	100	Wood	Saprophyte
17.	<i>Mycena adscendens</i> Maas Geest.	Agaricales	5	NA	NA	Dead twigs	Saprophyte
18.	<i>Mycena polyadelpha</i> (Lasch.) Kühner	Agaricales	15	NA	NA	Leaf litter	Saprophyte
19.	<i>Rigidoporus microporus</i> (Sw.) Overeem	Polyporales	23	PQ864759 OQ158875	100	Wood	Parasite
20.	<i>Schizophyllum commune</i> Fr.	Agaricales	42	OR178483	99.84	Wood	Saprophyte
21.	<i>Trametes polyzona</i> (Pers.) Corner	Polyporales	20	PQ787864	100	Wood	Saprophyte
22.	<i>Trametes pubescens</i> (Schumach.) Pilát	Polyporales	33	NA	NA	Wood	Saprophyte
23.	<i>Trametes strumosa</i> (Fr.) Zmitr., Wasser & Ezhov	Polyporales	9	PV052457 OR178473	100	Wood	Saprophyte
24.	<i>Trametes sanguinea</i> (Klotzsch) Pat.	Polyporales	3	PQ787863	100	Wood	Saprophyte
25.	<i>Trametes cubensis</i> (Mont.) Sacc.	Polyporales	20	PV052459	100	Wood	Saprophyte
26.	<i>Trametes</i> sp. 1	Polyporales	22	NA	NA	Wood	Saprophyte
27.	<i>Trametes</i> sp. 2	Polyporales	41	NA	NA	Wood	Saprophyte

NA = Not available; identification was completed based on morphological characterization.

The dominance and widespread distribution of wood-decaying fungi, such as *Trametes* species, suggest that decomposition processes and nutrient cycling are actively maintained within the ecosystem. These findings highlight the importance of ongoing fungal biodiversity assessments in tropical forests, particularly in underexplored areas such as Sungai Tua Forest. In addition to their role in decomposition, macrofungi form essential symbiotic relationships with plants, most prominently through mycorrhizal associations. In these relationships, fungi colonize plant roots, enhance minerals, and water uptake, and increase host tolerance to heavy metals and other environmental stresses (Hu et al., 2022). By strengthening plant–soil interactions and supporting beneficial

soil microorganisms, mycorrhizal fungi significantly contribute to plant growth, survival, and diversity, thereby improving overall forest ecosystem stability. Furthermore, they enhance plant resistance to root pathogens, promoting the health of the plant community (Hu et al., 2022). Similar patterns have been observed in other Malaysian macrofungal studies. For instance, a macrofungal survey conducted at the UiTM Pahang campus revealed unexpectedly high diversity in an urban environment, with 62 species representing two phyla and 23 families, dominated by saprophytic Basidiomycota. The predominance of saprophytic and mycorrhizal fungi, together with the very low occurrence of parasitic species, suggests that the campus provides a favorable and

ecologically balanced habitat, underscoring the need for continuous monitoring to support urban fungal conservation (Mat Shah Fee et al., 2023).

The dominance of saprophytic fungi within the Sungai Tua Forest is consistent with findings from other humid tropical forests in Southeast Asia, such as the Lentang Forest Reserve and the Ayer Hitam Forest Reserve (Noordin et al., 2024a,b), where wood-decaying Basidiomycota are predominant. Similar observations have been made in selectively logged forests in Borneo (Kerfahi et al., 2014), indicating that moderate disturbances may promote opportunistic saprotrophs that can swiftly colonize dead organic matter. Nevertheless, when contrasted with undisturbed dipterocarp forests, the comparatively low occurrence of ectomycorrhizal taxa might suggest changes in forest structure or a decrease in the availability of mature host trees.

Recreational pressure, including soil compaction, litter removal, and canopy changes, may indirectly affect fungal community composition by reducing the abundance of sensitive mycorrhizal taxa while promoting the growth of resilient saprophytic species. Thus, the dominance of Polyporales may indicate a forest system with active turnover of woody debris and moderate anthropogenic disturbance.

3.2. Species diversity indexes of macrofungi in Sungai Tua Forest

Analysis at the taxonomic level indicates that the order Polyporales is the most diverse and abundant fungal group in the study area. This order recorded the highest order-level Shannon-Wiener Index ($H = 1.409$; Table 2), indicating high species richness with a relatively even distribution among species. The high Simpson Index (0.881) further confirms the dominance of Polyporales within the fungal community, while the Evenness Index suggests that no single species within this order is overwhelmingly dominant.

These findings are consistent with those of Noordin et al. (2024a,b), who reported Polyporales as one of the most diverse orders in the Ayer Hitam Forest Reserve and the Lentang Forest Reserve in Malaysia. Notably, this order includes multiple species of the genus *Trametes*, which are well-known wood-decaying fungi that play a critical role in lignin degradation and nutrient recycling in forest ecosystems. Through their contribution to nutrient cycling, macrofungi support plant growth and enhance overall forest productivity (Hu et al., 2022). The high frequency and wide distribution of Polyporales underscores their ecological importance in the study area.

In contrast, the order Pezizales exhibited the lowest diversity, with a Shannon-Wiener Index of 0.013. This suggests extremely limited representation, with *Cookeia*

tricholoma being the least abundant and possibly the only species recorded from this order in the present study. This may be attributed to ecological factors such as habitat specificity, seasonality, or limited sampling coverage, which might have affected the detectability of Pezizales.

With a diversity index of 0.013 for the order Pezizales, *Cookeia tricholoma* is the least abundant species. In contrast, the order Polyporales has the highest species richness in the region (Figure 2), with SWI and SI of 1.499 and 0.881, respectively, making it the most abundant order containing *Trametes* species (Table 3). Meanwhile, based on EI, the Polyporales order has the greatest distribution of *Trametes* species in the region. Values of zero SI and EI recorded for certain orders indicate either the presence of only a single species or the absence of recorded species on specific substrates, rather than reflecting a true lack of diversity.

3.3. Comparison between fungi orders, substrates, and microclimate

The correlation analysis between ecological parameters and fungal orders provides insight into the environmental drivers shaping fungal community composition in Sungai Tua Forest. Although Polyporales showed a positive correlation with both light intensity and temperature, the strength of these relationships was relatively low (Table 4). However, across microclimates (Table 5), a moderate, statistically significant positive correlation was found between Polyporales and light intensity ($r = 0.479$, $p < 0.05$). Temperature also showed a moderate correlation ($r = 0.337$, $p < 0.05$), suggesting that microclimatic factors influence the occurrence and activity of certain fungal orders, particularly those involved in wood decomposition. In addition, the Kruskal-Wallis test was used to compare differences in order and substrates across microclimatic conditions. This approach allows for the statistical validation of the observed differences, moving beyond mere reliance on descriptive statistics.

The relationship between Polyporales and both light intensity and temperature indicates that these fungi flourish in conditions that are most conducive to their growth and enzymatic functions. Specifically, elevated light intensity may promote photosynthetic activity in plant species linked to them, increasing organic matter availability, which in turn aids the proliferation of ligninolytic fungi. Similarly, temperature affects metabolic rates and enzyme activity, which, in turn, affects the efficiency of lignin and substrate degradation. This suggests that changes in environmental conditions could significantly impact the ecological roles of Polyporales in forest ecosystems.

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

Species diversity	Polyporales	Agaricales	Gomphales	Russulales	Dacrymycetales	Xylariales	Pezizales
Shannon-Wiener Index	1.409	1.201	0.033	0.142	0.128	0.128	0.013
Simpson Index	0.883	0.868	0	0	0	0	0
Pielou's evenness	0.588	0.483	0	0	0	0	0



Figure 2: Some of the macrofungi obtained from Sungai Tua Forest, Selangor, Malaysia. A, *Schizophyllum commune*; B, *Microporus xanthopus*; C, *Cyathus crassimurus*; D, *Marasmius cystidius*; E, *Lactarius subserifluus*; F, *Rigidoporus microporus*; G, *Cookenia tricholoma*; H, *Calocera viscosa*; I, *Marasmius guyanensis*; J, *Lentaria surculus*; K, *Coprionopsis lagopus*; L, *Coprionellus disseminatus*; M, *Earliella scabrosa*; N, *Daldinia eschscholtzii*; O, *Trametes sanguinea*; P, *Trametes strumosa*; Q, *Trametes polyzona*; R, *Clitopilus sinoapalus*; S, *Marasmiellus candidus*.

Table 3: Diversity index of Ascomycete and Basidiomycete fungi orders according to substrates.

Diversity Index	Order	Substrates				
		Wood	Twigs	Leaf	Soil	Bamboo
Shannon-Wiener Index	Polyporales	1.499	0	0	0	0
	Agaricales	0.520	0.170	0.306	0.206	0
	Gomphales	0	0	0	0	0.033
	Russulales	0.142	0	0	0	0
	Dacrymycetales	0.128	0	0	0	0
	Xylariales	0.128	0	0	0	0
	Pezizales	0	0.013	0	0	0
Simpson Index	Polyporales	0.881	0	0	0	0
	Agaricales	0.636	0.550	0.577	0.507	0
	Gomphales	0	0	0	0	0
	Russulales	0	0	0	0	0
	Dacrymycetales	0	0	0	0	0
	Xylariales	0	0	0	0	0
	Pezizales	0	0	0	0	0
Evenness Index	Polyporales	0.625	0	0	0	0
	Agaricales	0.473	0.154	0.278	0.188	0
	Gomphales	0	0	0	0	0
	Russulales	0	0	0	0	0
	Dacrymycetales	0	0	0	0	0
	Xylariales	0	0	0	0	0
	Pezizales	0	0	0	0	0

Table 4: Comparison of microclimates between different fungal orders and various substrates. * = significant at p<005.

Microclimates		Fungi order							p-value
		Polyporales	Agaricales	Russulales	Xylariales	Gomphales	Pezizales	Dacrymycetales	
	N	219	174	21	18	3	1	18	
Air Humidity (%)	$\bar{x}$	72.786	74.842	78.050	69.050	73.900	76.500	66.800	0.576
	$\pm$ SD	5.852	6.104	12.657	3.182	0	0	0	
Temperature (°C)	$\bar{x}$	32.518	29.837	29.650	34.300	29.100	31.600	28.900	0.460
	$\pm$ SD	5.662	4.444	2.616	3.253	0	0	0	
Light intensity (lux)	$\bar{x}$	41966.23	1332.947	351.000	10768.50	8967.000	352.00	19633.00	0.237
	$\pm$ SD	79928.39	1809.054	162.635	13367.85	0	0	0	
pH of substrates	$\bar{x}$	6.123	5.942	6.350	6.450	6.000	6.800	6.000	0.358
	$\pm$ SD	0.525	0.548	0.778	0.212	0	0	0	

Microclimates		Substrates					p-value
		Wood	Twigs	Leaf	Soil	Bamboo	
	n	364	20	42	25	3	
Air Humidity (%)	$\bar{x}$	72.72	77.44	74.38	73.53	73.90	0.614
	$\pm$ SD	6.19	5.78	5.01	6.93	0	
Temperature (°C)	$\bar{x}$	31.91	30.36	29.50	29.800	29.10	0.617
	$\pm$ SD	5.03	3.68	2.86	8.521	0	
Light intensity (lux)	$\bar{x}$	30376.91	514.86	2436.25	1349.25	8967.0	0.459
	$\pm$ SD	68200.25	240.90	3585.53	1258.32	0	
pH of substrates	$\bar{x}$	6.16	6.04	6.30	5.35	6.0	0.115
	$\pm$ SD	0.48	0.42	0.12	0.92	0	

Table 5: Pearson Correlation analysis between fungal orders and ecological parameters in Sungai Tua Forest, Selangor. Values in bold = significantly correlated with p alpha = 0.05.

	Agaricales	Dacrymycetales	Gomphales	Pezizales	Polyporales	Russulales	Xylariales	Light intensity	Temperature	pH of substrate
Agaricales										
Dacrymycetales	-0.10242									
Gomphales	-0.10242	-0.021277								
Pezizales	-0.10242	-0.021277	-0.021277							
Polyporales	-0.5322	-0.11056	-0.11056	-0.1105						
Russulales	-0.12317	-0.025587	-0.025587	-0.0255	-0.13295					
Xylariales	-0.1386	-0.028792	-0.028792	-0.0287	-0.14961	-0.034625				
Light intensity	-0.22525	0.17644	0.1224	-0.1006	0.47966	-0.14367	0.15655			
Temperature	-0.29533	-0.066027	-0.059241	0.02197	0.33759	-0.097848	0.10644	0.34816		
pH of substrate	-0.08057	-0.016285	-0.016285	0.18301	-0.13016	-0.0222	0.14946	-0.3204	0.012422	
Humidity	0.05375	-0.16894	0.012202	0.07426	-0.18325	0.27498	-0.12316	-0.4421	-0.32745	0.17291

While the statistical analysis demonstrated significant correlations between Polyporales and environmental factors, the ecological implications are profound. The interaction between light, temperature, and fungal activity emphasizes the critical role of these variables in shaping community dynamics and nutrient cycling in ecosystems.

These results are further supported by non-metric multidimensional scaling (nMDS), which clearly segregates fungal communities by taxonomic order, substrate type, and associated microclimatic conditions. The clustering pattern reveals three distinct groupings, with Polyporales occupying the right side of the plot, where they are primarily associated with wood substrates and positively influenced by light intensity and pH. This aligns with the ecological role of many Polyporales species, such as *Trametes* species, which are efficient lignin-degraders that thrive in illuminated, moderately warm environments.

The relationship between fungal community structure, substrates, and microclimates is further visualized in the nMDS plot (Figure 3). This plot illustrates three distinct separations of fungal communities by taxonomic order, substrate, and environmental factors. The Polyporales are clustered on the right, predominantly inhabiting wood, while the Agaricales are located on the lower left, where they are more frequently found in wood and soil. Other fungal orders, including Xylariales, Russulales, Gomphales, and Pezizales, are found in the central region, reflecting a broader diversity of substrate utilization. This clustering pattern suggests that different fungal orders have specific substrate preferences. Analysis of microclimates shows a positive relationship between pH and light intensity in Polyporales, as indicated by the arrows pointing towards this group. In contrast, humidity is more closely related to Agaricales. A moderate influence of temperature was observed along the nMDS1 axis. The distribution and clustering of data points suggest that both substrate characteristics and environmental factors play significant roles in determining fungal community composition.

Research has shown that favorable light conditions can significantly increase the yields of several macrofungi by affecting their metabolic activities and reproductive cycles (Hu et al., 2022). Additionally, the metabolic rates of macrofungi are significantly influenced by temperature, which is a crucial environmental factor. Most fungi exhibit optimal growth and reproduction within specific temperature ranges, typically between 20°C and 30°C. However, exposure to extreme temperatures can suppress fungal activity and impair spore germination. Furthermore, high humidity is vital for fungal development, as it reduces water evaporation and enhances spore germination. Studies indicate a significant relationship between humidity and the prevalence of fungi, with higher

relative humidity often associated with greater richness and diversity of macrofungal species (Hu et al., 2022; Joshi et al., 2022; Noordin et al., 2024a).

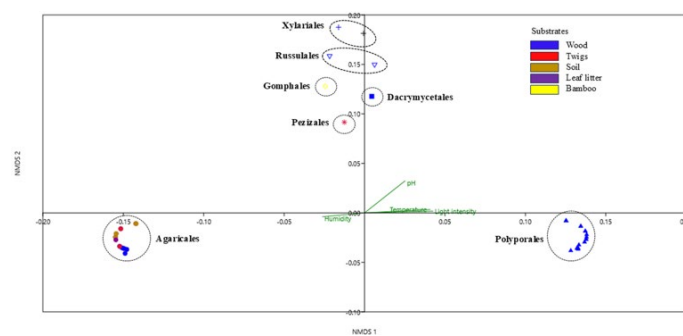


Figure 3: Non-metric Multidimensional Scaling (nMDS) plot visualizing the relationship among fungal community structure (shapes), substrates (shape colors), and ecological parameters (green lines) in Sungai Tua Forest, Selangor. Different shapes indicate fungal samples according to their orders: filled circle = Agaricales; filled triangle = Polyporales; filled square = Dacrymycetales; diamond = Gomphales; inverted triangle = Russulales; asterisk = Pezizales; plus = Xylariales.

4. CONCLUSION

A total of 454 macrofungal sporocarps belonging to the phyla Basidiomycota and Ascomycota were documented in this study. The dominance of saprophytic fungi, particularly from the orders Polyporales and Agaricales, highlights their key role in decomposition and nutrient cycling within forest ecosystems. Correlations between macrofungal orders and environmental parameters suggest that variations in environmental conditions and human activities may potentially influence fungal diversity. These findings highlight the importance of incorporating macrofungal diversity into forest conservation and management frameworks, particularly in areas subject to tourism and development. Long-term monitoring is recommended to capture temporal changes better and inform adaptive management strategies.

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