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Substrate Preference Determines Macrofungal Biogeography in the Greater Mekong Sub-Region

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Abstract: The availability and the quality of substrates are important drivers of macrofungal biogeography, and thus macrofungal species occurrence is potentially dependent on the availability of different substrates. However, few studies have explored the properties of macrofungal substrates and assessed the relationship between macrofungal diversity and substrate diversity at a landscape level. To address this issue, we conducted a landscape-scale survey of basidiocarp substrates in the Greater Mekong Subregion (GMS). A total of 957 macrofungal species distributed across 73 families and 189 genera were collected. Substrates of these macrofungi were categorized into four main groups (namely, litter, soil, root, and rare substrates) and referenced into 14 sub-substrate types (such as branches, leaves, and fruit). The results revealed that 50% of the observed macrofungal species were symbiotrophs living in ectomycorrhizal association with plant hosts, 30% were saprotrophs decomposing plant litter, 15% lived in soil organic matter, and 5% lived in rare substrates. The most abundant root symbiotic fungi were members of *Russula*, whereas most litter saprotrophic fungi belonged to *Marasmius*. Macrofungi commonly favored a single substrate. This specificity was not affected by changes in vegetation or climate. Less than 1% of macrofungi (e.g., *Marasmius* aff. *maximus*) could live on multiple substrates. Most of these unusual macrofungi were characterized as highly mobile and were generally found in successional areas. In secondary forests, our survey indicated that significant correlations exist between substrate preference and taxonomic diversity, reflected as higher substrate diversity generally accompanied by higher macrofungal diversity. In conclusion, substrate preference is an important factor driving macrofungal composition and distribution in the GMS. Macrofungi that thrive on multiple substrates constitute pioneer groups that have an important role in establishing macrofungal communities in new habitats. These observations have furthered our understanding of how substrate preferences could explain macrofungal biogeography.

Keywords: saprotrophic fungi; wood fungi; mycorrhizal fungi; Greater Mekong Subregion

1. Introduction

Macrofungi constitute an important part of terrestrial ecosystems. They account for a high proportion of species diversity and are key players in ecosystem processes [1–6]. They live on diverse substrates, e.g., litter and soil, where they attach, grow, and extract nutrients [7,8]. Macrofungi tend to

specialize in one of a range of substrates depending on their life history, colonization, and decomposition abilities. Hereafter, this specialization is referred to as a preference. Substrate decomposition is an important process in which macrofungi acquire energy and nutrients. Therefore, substrate availability could be a critical factor in explaining the influence of vegetation on macrofungal diversity, abundance, and distribution [6,7,9].

Macrofungi can be grouped into three major ecological types, which are demarcated by substrate preference. Saprotrophic fungi (SAC) are the major macrofungal decomposers, which have varying degrees of specificity to substrates such as humus, fallen leaves, fruits or catkins, standing or fallen wood, bark of standing trees, or animal excrement [10,11]. In contrast, symbiotic fungi (biological symbiotic macrofungi (BYC), ectomycorrhizal macrofungi (ECM)) mainly utilize the carbon resources of their host plant and thus are associated with the roots of symbiotic hosts [12–14]. Another major group—parasitic or pathogenic fungi (PAC)—are known to colonize living plants or insects. These fungi produce enzymes that can puncture living plants or animals, which sometimes kills them, to extract nutrients and energy from these hosts [15,16]. Major macrofungal groups have substrate preferences due to their ecological niches. However, some macrofungal species have been found to undergo changes in ecological type. For example, some SAC have been found to act as PAC in highly disturbed areas [17]. Additionally, some SAC may have the potential to transform into BYC, which suggests that some fungal species could live both symbiotic and saprotrophic lifestyles [18–22]. Changes in substrate utilization are still hypotheses for these fungal species because the possibility has not yet been explored in the field. Therefore, researchers should explore whether or not extant fungal species can change from SAC to BYC (or the other way around) based on their substrate utilization in different areas.

Macrofungal taxonomic diversity varies across landscapes and can be affected by vegetation and climatic factors [2–4,6,22]. The environmental factors that drive these patterns could include dispersal-limiting factors for wind or animal-dispersed fungi, host species specificity for mycorrhizal fungi, or litter quality and quantity for saprotrophic fungi [23]. Substrate diversity could directly mediate the impacts of vegetation and climate on fungal taxonomic diversity. For example, forests produce more woody substrates, while grasslands produce more leafy material. Tropical forests produce a greater diversity of wood, leaves, and insect residues than temperate forests [24–26]. Additionally, in some disturbed areas, human activities could produce additional substrates, such as stem litter and damaged plant materials, which have the potential to cause changes in macrofungal composition [27,28]. However, there have been few studies that have explored the link between substrate diversity and macrofungal genetic diversity.

The Greater Mekong Subregion (GMS) represents a landscape across China, Laos, and Thailand that harbors significant diversity in climate, vegetation type, and habitat and supports thousands of recorded mushroom genera and species. The goals of our study were to compare the diversity and the composition of fungal communities across the varied vegetation types of the GMS and to test for correlations with substrate preference. We aimed to (1) survey macrofungal substrate preferences; (2) explore whether macrofungi can use multiple substrates; and (3) test the correlations between substrate diversity and fungal taxonomic diversity.

2. Methods and Materials

2.1. Sampling Area and Study Plots

The sampling locations are shown in Figure 1, with site information displayed in Table 1. These five sites were divided into three climate zones: temperate, subtropical, and tropical. Zhongdian and Baoshan have temperate climates and are located in northwest Yunnan Province. Mengsong has a subtropical and tropical climate and is in south Yunnan Province. Chiang Rai and Oudomxay have tropical climates and are located in north Thailand and Laos, respectively. In this study, 66,400 m^2 (20 m × 20 m) plots were established: (1) 9 plots in Chiang Rai, Thailand (1-TI); (2) 12 plots in Oudomxay, Laos (2-LS); (3) 15 plots in Mengsong, China (3-MS); (4) 15 plots in Baoshan, China (4-BS); and (5) 15 plots in

Zhongdian, China (5-ZD). Four vegetation types were included in this study: primary forest, secondary forest, plantation forest, and grassland. Detailed site information regarding location description, vegetation composition, and climate characteristics is provided in Table 1.

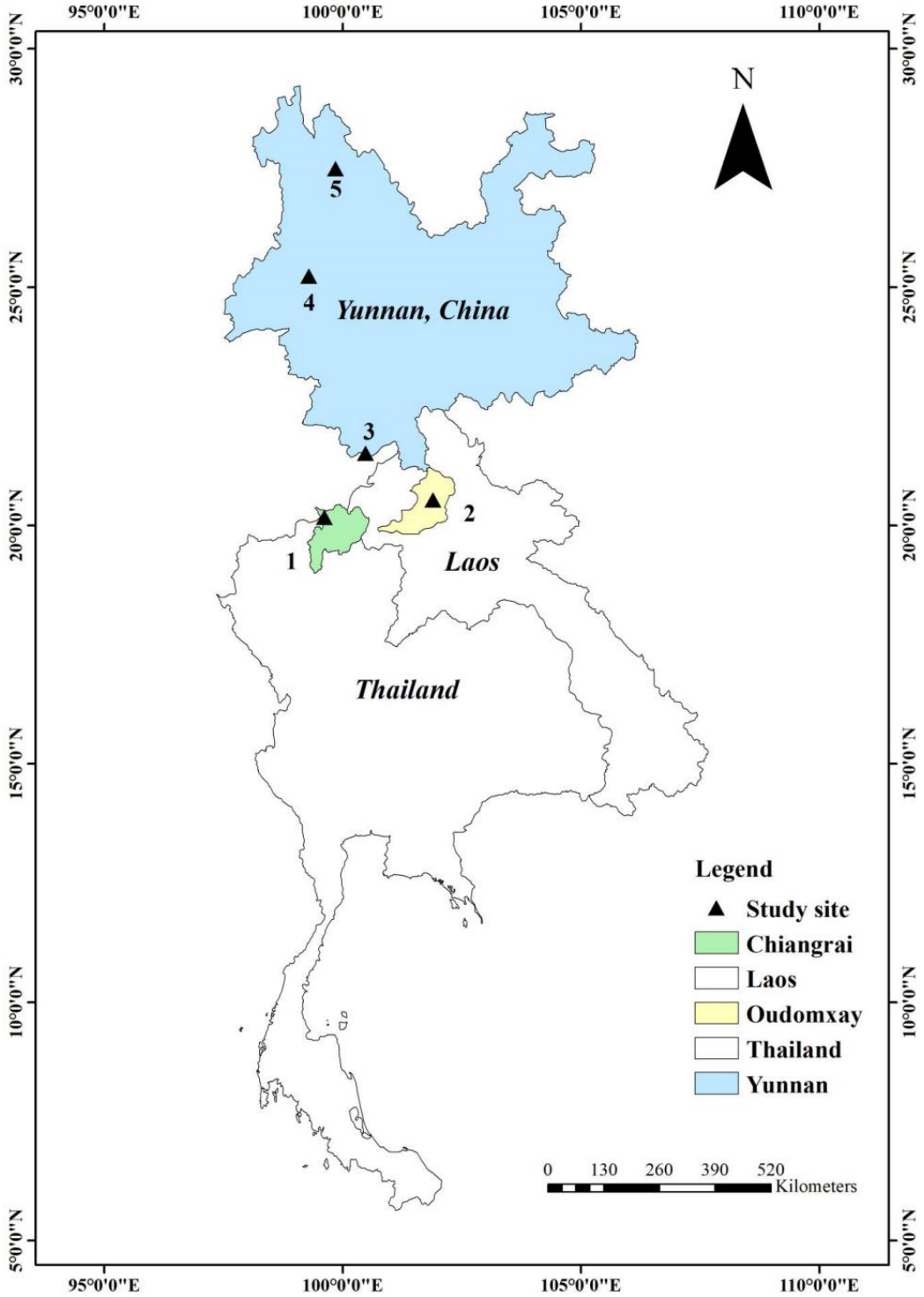

Figure 1. The study sites in the Greater Mekong Subregion (GMS) region. 1: Chiang Rai; 2: Oudomxay; 3: Mengsong; 4: Baoshan; 5: Zhongdian.

Table 1. Background information for each study site: Global Positioning System (GPS) coordinates, elevation (m), climate type, mean monthly temperature (MT, June–October 2014), mean monthly rainfall (MR), and dominant tree species.

Study Site	Location	GPS Coordinates (Long., Lat.)	Elevation (m)	MT/MR (°C/mm)	Climate	Dominant Tree Species
A-ZD (15 Plots)	Zhongdian, China	99.8633 °E, 27.4733 °N	3100–3300	12.3/82.4	Temperate	*Pinus densata*, *Picea likiangensis*, *Rhododendron rubiginosum*
B-BS (15 Plots)	Baoshan, China	99.2882 °E, 25.2566 °N	2400–2600	21/127.2	Temperate	*Pinus armandii*, *Pinus yunnanensis*, *Castanopsis orthacantha*, *Quercus rehderiana*
C-MS (15 Plots)	Mengsong, China	100.4898 °E, 21.4946 °N	1500–1700	25.7/164.4	Subtropical	*Syzygium brachythyrsum*, *Xanthophyllum flavescens*, *Macaranga henryi*, *Cryptocarya hainanensis*, *Myrsine seguinii*, *Anneslea fragrans*
D-LS (12 Plots)	Oudomxay, Laos	101.8967 °E, 20.5335 °N	700–900	25.4/232.7	Tropical	*Castanopsis* spp., *Lithocarpus* spp., *Cephalostachyum virgatum*, *Dendrocalamus strictus*, *Oxytenanthera paviflora*, *Coffea arabica*
E-TL (9 plots)	Chiang Rai, Thailand	99.62305 °E, 20.16833 °N	980–1300	27/211.3	Tropical	*Lithocarpus elegans*, *Castanopsis tribuloides*, *Castanopsis diversifolia*, *Castanopsis calathiformis*, *Hevea brasiliensis*, *Coffea arabica*, *Pinus kesiya*

2.2. Macrofungal Species Sampling and Recording of Substrate Observations

Macrofungal surveys in Mengsong, Chiang Rai and Oudomxay were carried out once a week during the rainy season from May to September of 2014. The sites at Zhongdian and Baoshan were sampled once a week from July to September of 2014 [29]. The sampling times were adjusted according to the timing of the local rainy season. All macrofungal basidiocarps (2 cm high) were collected. Fresh specimens were photographed, and observations of the substrates and habitats on which they were found were recorded in the field. Once the mushrooms were collected, samples were wrapped in aluminum foil or kept in a box separate from rare specimens to avoid mixing and damaging the samples. The collected samples were dried in an electric drier at 40 °C until dry and then stored in a sealed plastic bag [7,30]. The dry specimens were deposited in the Herbarium of the Kunming Institute of Botany (HKAS), Chinese Academy of Sciences, China.

2.3. Macrofungal Identification and Classification of Substrates

Macrofungi were identified as morphospecies with the aid of monographs and guides books [7,9,10,16,24,26,29] according to the specimens' macro- and micro-morphological characteristics. Macrofungal nomenclature followed that of the Index Fungorum. In this study, we identified 88% of macrofungal specimens to species level and the remaining 12% to genus level. Here, the substrates were identified as the matter to which the macrofungi were attached. Based on our observations of substrates in the field and on previous research [7], we identified 14 different substrate types and grouped them into four types: litter, soil, root, and rare. "Litter" included woody litter (log wood, branch wood, and living tree wood) and leaf litter (dead leaves, rotten leaves, fallen fruits, and twigs). "Soil" included mineral soil and organic soil. "Root" referred to living tree roots that could form a symbiotic association with the macrofungi. "Rare" included dung, macrofungal fruiting bodies, insects, lichen, and termite nests. Less than 5% of the samples were classified this way. Definitions of substrate type and macrofungal ecological type are shown in Table S1.

2.4. Substrate Utilization Analysis

Substrate utilization was analyzed based on the relative abundance of macrofungi growing on a given substrate (RAMs) [31], which was calculated using the following formula:

$$\text{RAMs}(\%) = \frac{\text{Msp}}{\text{Mtot}} \times 100$$

where RAMs represents the relative abundance of a macrofungal species on a specific substrate, Msp is the count for a given macrofungal species found on the defined substrate, and Mtot is the total number of macrofungi, which may belong to several different species found on the defined substrate. The total number of genera and species was calculated for each unique substrate.

2.5. Correlation Between Macrofungal Taxonomic Diversity and Substrate Utilization

We identified 14 different substrate types, and we calculated the macrofungi-substrate utilization diversity using data from individuals in each substrate in order to evaluate the differences between functional diversity and taxonomic diversity. To avoid any significant confounding effects from vegetation type when testing the correlation, we only considered our data from secondary forests, which was the dominant vegetation type across the GMS. The Fisher's alpha diversity and the species richness index were calculated for the macrofungi and the substrate in each study plot [32–34]. The proportion of each substrate relative to all other substrates in the study site was calculated for each study site. The Kruskal–Wallis rank sum test was used to test for differences in macrofungal diversity, substrate diversity, root (%), litter (%), and soil (%) between different study sites. The relationship between soil saprophytic fungi and ECM fungi was analyzed using Spearman's correlation test [35], and the linear relationship was plotted. RStudio-0.99.902 was used for the statistical analysis and to create the figures (https://www.rstudio.com/) [36].

3. Results

3.1. Substrate-Specific Composition of Macrofungi

A total of 975 species of macrofungi in 180 genera and 75 families were recorded in the GMS. Root macrofungi were the predominant substrate-specific group (around 50% of the observed samples), followed by macrofungi living on litter (35%), soil (17%), and rare substrates (3%) (Figure 2). The group of macrofungi (ectomycorrhizal fungi) that colonized roots was composed of 443 species belonging to 46 genera and 26 families (Table 2). The species were generally found in temperate areas and formed ectomycorrhizal relationships with the roots of pine or fir trees. *Russula* was the most abundant macrofungal group observed among the root macrofungi (Figure 2), and *Laccaria laccata* represented the most abundant species in this group (Table 2).

The macrofungi found in litter were predominantly saprotrophic fungi and made up the highest proportion of the community composition in tropical and subtropical sites in Mengsong and Thailand. There were 342 macrofungal species living on litter that belonged to 91 genera and 45 families (Figure 2). *Marasmius* was the most abundant macrofungal genus found among litter decomposers. Log wood macrofungi (species on dead wood on ground) was the biggest sub-group (220 species), followed by dead leaf macrofungi (86 species) (Figure 2). *Amauroderma rugosum* was the most commonly observed wood (including log wood and tree wood) macrofungus, while *Marasmius* aff. *siccus* was the dominant macrofungus living in dead leaves (Table 2).

Soil and rare-substrate macrofungi had a narrower distribution area and weaker seasonality than root or litter macrofungi. These macrofungi mainly appeared in subtropical areas with highly disturbed vegetation, such as the secondary and plantation forest in Mengsong. A total of 171 soil macrofungal species were recorded in this category, representing 52 genera and 25 families, all of which were saprotrophic decomposers that had colonized organic layers (Figure 2 and Table 2). Members of the genus *Entoloma* predominantly lived in the soil, with *Clitopilus apalus* being the

most frequently recorded species. An additional 30 macrofungal species occurred on rare substrates, including coprophilous (dung fungi), entomogenous (insect fungi), and parasitic fungi (fruiting-body fungi). *Stropharia semiglobata* was the most abundant species living on dung (Table 2).

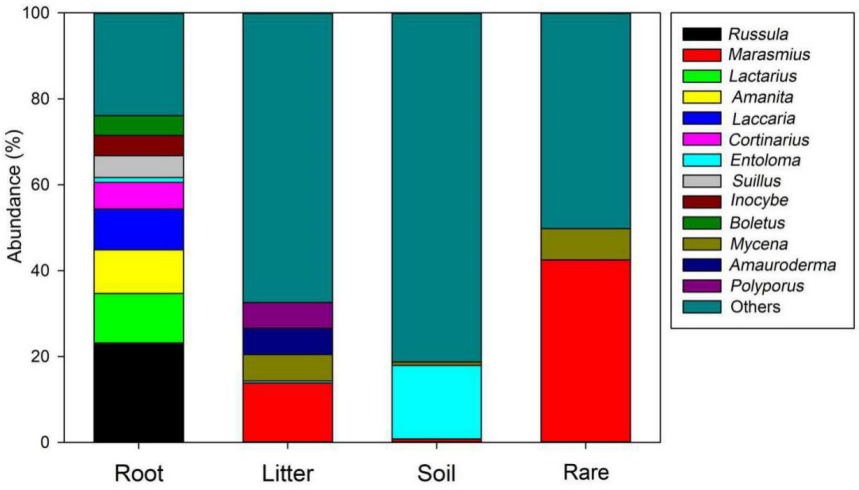

Figure 2. Relative abundances of dominant macrofungal genera collected in the GMS (Zhongdian, Baoshan, Mengsong, Thailand, and Laos) for each substrate category (root, litter, soil, and rare).

Table 2. The classification of macrofungal species growing on each substrate collected in the GMS. There were 15 substrates recorded. Dominant fungal species are those that had the highest individual abundance in each substrate. Fungal ecology types (ECT) of recorded macrofungal species had four sub-types: saprophytic macrofungi (SAC) that decompose substrate to obtain nutrients; parasitic macrofungi (PAC) that obtain nutrients directly from the host; biological symbiotic macrofungi (BYC) that obtain nutrients from a mutually beneficial symbiotic system; ectomycorrhizal macrofungi (ECM) that obtain nutrients from tree roots. Each substrate and sub-group are defined with identification methods and substrate characteristics in Table S1.

Major Substrate Category	Substrate Subgroup	Family	Genus	Species	Dominant Fungal Species	ECT
Root	Root (R)	54	97	443	*Laccaria laccata*	ECM
Litter	Total Litter	45	91	342		
	Log wood (LW)	45	78	220	*Amauroderma rugosum*	SAC
	Dead leaf (DL)	24	36	86	*Marasmius* aff. *siccus*	SAC
	Rotten leaf (RL)	10	15	27	*Collybia* aff. *dryophila*	SAC
	Branch wood (BW)	2	2	2	*Mycena lactea*	SAC
	Fruit (FT)	2	2	2	*Auriscalpium vulgare*	SAC
	Tree wood (TW)	4	4	4	*Hypholoma fasciculare*	SAC
	Twig (TG)	11	15	35	*Marasmius chordalis*	SAC
Soil	Total Soil	25	42	172		
	O-Soil (OS)	22	39	167	*Clitopilus apalus*	SAC
	M-Soil (MS)	3	3	5	*Leucocoprinus fragilissimus*	SAC
Rare	Total Rare	10	15	30		
	Dung (DG)	5	9	9	*Stropharia semiglobata*	SAC
	Fungal fruiting body (FB)	2	2	2	*Hypomyces* sp.	PAC
	Insect (IT)	4	6	8	*Ophiocordyceps nutans*	PAC
	Lichen (LN)	2	2	2	*Lichenomphalia* sp.1	BYC
	Termite-nest (TN)	2	2	9	*Termitomyces bulborhizus*	BYC

3.2. Macrofungal Species Occupying Multiple Substrates

At the genus level, most macrofungi occupied only one substrate. However, 5% of the macrofungi sampled in our study occupied two or more types of substrate. Of all observed macrofungi, 26 macrofungal genera had two substrates. Of these genera, 17 lived in both the soil and on

the litter. *Phaeocollybia* was the only genus found in both the roots and the soil. Seven macrofungal genera, including *Entoloma* and *Ramaria*, were found on three different substrates. Besides being commonly found in litter and soil, these macrofungi could occasionally be found in roots or rare substrates, such as dung or insects (Figure 3A and Table 3A). Litter was the most diverse substrate category, including seven different substrates types ranging from leaves to dead wood. Moreover, 20% of litter macrofungi could live on the high lignin-containing woody substrates, dead leaf (DL) and log wood (LW). We also collected *Psathyrella*, *Marasmiellus*, *Mycena*, *Marasmius* and *Coprinus* from rotten leaf (RL) and twig (TG) (Figure 3B and Table 3B). Overall, macrofungi able to live on more than three substrates (multiple-substrates macrofungi) accounted for a lower percentage of fungal species (10%) than did two-substrate fungi (20%) or single-substrate macrofungi (70%). These results indicate that substrate-specificity is the norm in these macrofungi. Even for a typical multiple-substrates macrofungi such as *Mycena*, individuals are most commonly found in DL (90% of observed individuals), whereas only 10% were found in LW, RL, or TG.

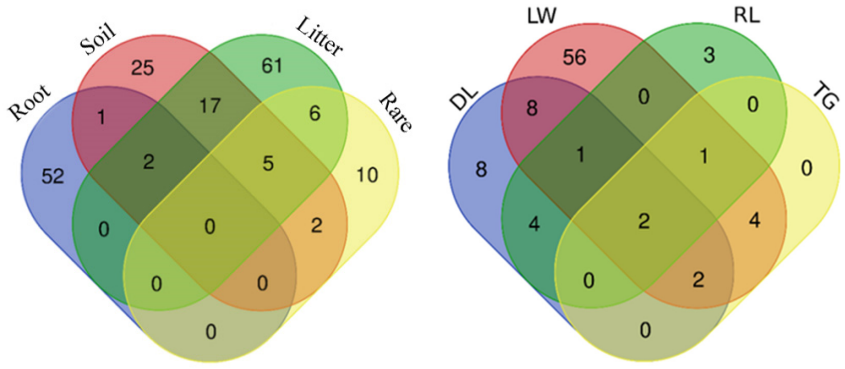

Figure 3. Venn analysis of substrate utilization of each macrofungal genera collected in the GMS by (**A**) substrate category and (**B**) substrate subgroup in the category of litter. Each substrate is represented by a single color with the number of genera present in each, while the number in overlapping colors represents the count of macrofungi present in the multiple substrates whose colors overlap there. For help with substrate subgroup identification, see Table S1.

Table 3. Substrate category (**A**) and substrate subgroup in litter category (**B**). Amount of genera recorded in (**A**) and (**B**), and dominant genera in (**A**) and (**B**).

A: Category	Genus	Dominant Genus	B: Substrate Subgroup in Litter Category	Genus	Dominant Genus
Litter Root Soil	2	*Entoloma*	DL LW RL TG	2	*Mycena*
Litter Soil Rare	5	*Marasmius*	DL LW RL	1	*Psathyrella*
Root Soil	1	*Phaeocollybia*	DL LW TG	2	*Marasmiellus*
Litter Soil	17	*Clitocybe*	LW RL TG	1	*Coprinus*
Litter Rare	6	*Favolaschia*	DL LW	8	*Hohenbuehelia*
Soil Rare	2	*Conocybe*	DL RL	4	*Clitocybe*
Root	52	*Heimioporus*	LW TG	4	*Cyathus*
Litter	61	*Echinoporia*	DL	8	*Aphelaria*
Soil	25	*Megacollybia*	LW	56	*Antrodiella*
Rare	10	*Multiclavula*	RL	3	*Lycoperdon*

Note: Substrate subgroup in Litter: Dead leaf = DL, Log wood = LW, Rotten leaf = RL, Twig = TG.

3.3. Correlation between Macrofungal Taxonomic Diversity and Diversity of Its Substrates

In secondary forests, macrofungal taxonomic diversity formed a bell-shaped distribution pattern from temperate to tropical climate zones, peaking in Mengsong and Oudoxmxay (Figure 4A,B). Alpha diversity was highest in Mengsong ($p < 0.05$), while species richness was highest in Laos ($p < 0.05$) (Figure 4A,B). Fungal species richness was slightly lower in Mengsong but still 50% higher than in other sites. Due to the high heterogeneity, the increase of species richness in Mengsong was not statistically significant. A similar bell-shaped distribution pattern was also found for substrate diversity, with a

peak in Mengsong. However, there were differences between taxonomic and substrate diversity in the tropical areas (Thailand and Laos), which had higher taxonomic and lower substrate diversity than the other sites (Figure 4C).

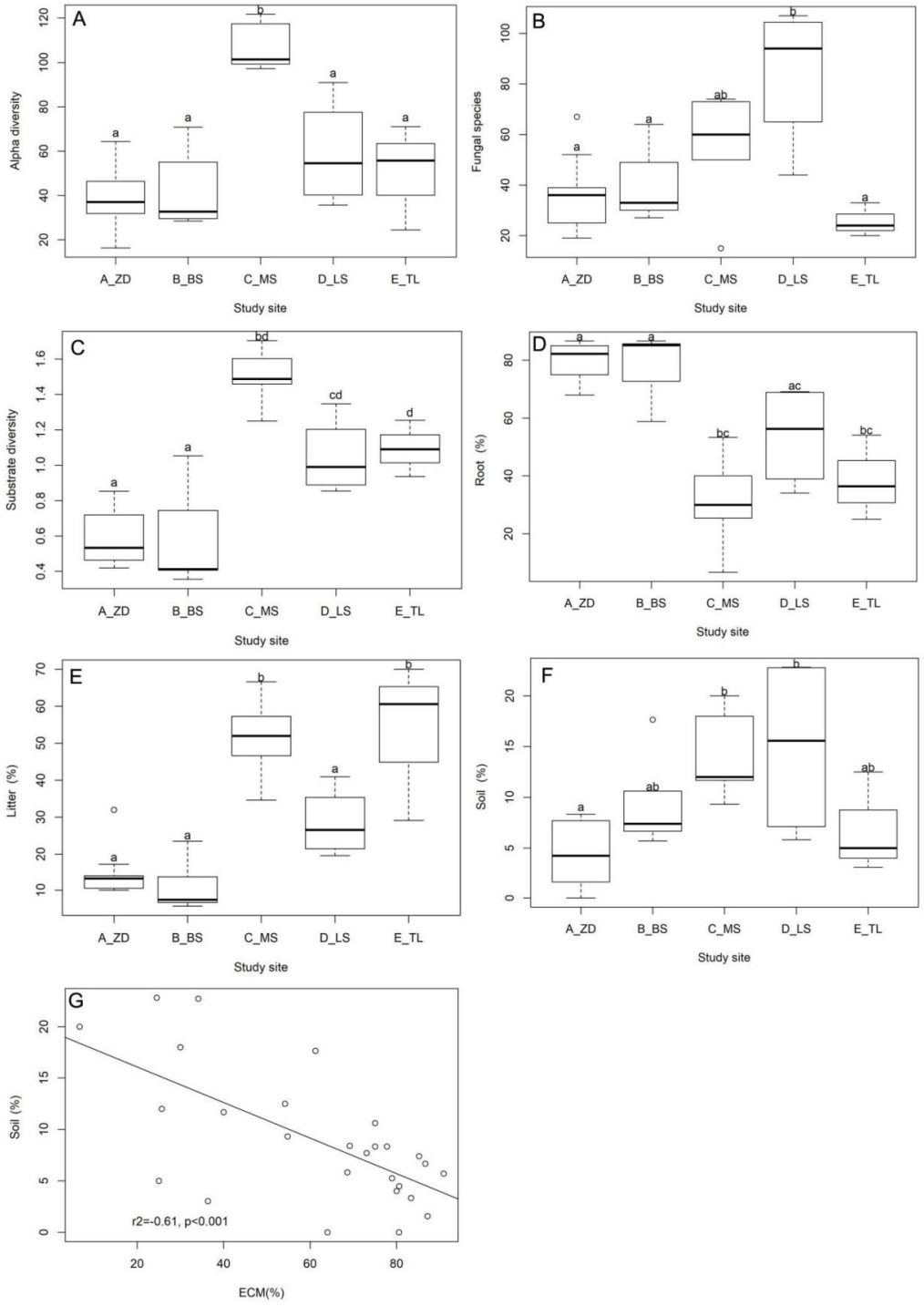

Figure 4. Macrofungal taxonomic diversity (**A**), species richness (**B**), and substrate utilization diversity (**C**) for macrofungal species collected in the GMS. For each site, we calculated the taxonomic diversity based on morphological characteristics and calculated the substrate diversity based on the macrofungi count in each of the 15 substrates (Table S1). Relative abundance of macrofungal species in each substrate category across all sampling sites (**D**–**F**). The correlation between soil macrofungi and ectomycorrhizal fungi was also calculated (**G**). ZD: Zhongdian, BS: Baoshan, MS: Mengsong, LS: Laos, TL: Thailand. The lower-case letters indicate statistical significance.

Root fungi were more abundant in temperate areas, whereas litter fungi were more abundant in subtropical and tropical areas (Figure 4D,E). Macrofungi living on soil were less abundant than either litter or root fungi (117 species vs. 800 species) (Table 2). The minimum abundance of soil fungi was highest in the subtropical forests of Mengsong, and thus the soil fungi achieved lower counts in strictly temperate and tropical areas (Figure 4F). Changes in macrofungal abundance in the soil followed the same pattern as general macrofungal diversity changes across the sites. Additionally, a significant negative correlation was observed between the abundance of soil fungi and ectomycorrhizal fungi ($r^2 = 0.61$, $p < 0.001$) (Figure 4G).

4. Discussion

4.1. Substrate Specificity Drives the Biogeography of Macrofungi

The majority of macrofungal species in this study lived on a single type of substrate (substrate-specific) and were limited in their distribution by substrate availability. Root macrofungi, most of which are ectomycorrhizal fungi, live on plant roots and are dependent on plant photosynthesis for carbon acquisition [37]. *Laccaria laccata* and *Russula albida*, the predominant root fungi in the GMS, were always associated with tree species belonging to Pinaceae, Fagaceae, and Betulaceae and had similar biogeographic distribution patterns to these host species. Due to their requirements for carbon and nutrients, substrate specificity for saprotrophic fungi is related to variations in the chemical properties of their substrates, such as lignin and cellulose ratio, pH, and metal content [38]. Saprotrophic macrofungi differ in their decomposition ability depending on their enzyme composition because each enzyme enables the digestion of a specific compound in the substrate. Previous studies on typical substrate-specific saprotrophic macrofungi (namely, white and yellow soft root fungi) have attributed their substrate specificity to their variation in enzyme activity. White root fungi release laccase and peroxidase to break down lignin, whereas yellow root fungi mainly release cellulose to decompose substrates with a low lignin to cellulose ratio [21,39–42]. Across the study landscape, we did find that some saprotrophic fungi are able to switch to another substrate type with similar C/N or lignin/cellulose ratios (e.g., wood vs. branch) but not to one with a very different composition (e.g., wood vs. leaves). Some parasitic macrofungi and symbiotic macrofungi were also present on rare substrates (insects and termite nests) when these host insects were available [26,43,44]. To our knowledge, our study is the first investigation into macrofungal substrate specificity on a landscape level. Previous studies have shown how vegetation, climate, and soil properties influence macrofungal biogeographic distributions, but they did not consider the effect of substrates [23]. We propose that these previously-studied factors also indirectly determine macrofungal distribution at a landscape scale by affecting substrate composition and availability.

4.2. Multiple-Substrates Macrofungi form Pioneer Fungal Communities

Contrary to our expectations, only a few macrofungi (seven genera) were observed to occupy more than three substrates, and we did not observe any macrofungi that occupied both root and litter substrates. Recently, it was proposed that ectomycorrhizal fungi may be able to shift life strategies and adopt a saprotrophic role because the expressions of laccase and peroxidase genes have been observed in some mycorrhizal fungi. However, there has been no direct evidence of such changes, and thus this theory awaits confirmation in the field [45,46]. In our study, fungi inhabiting multiple substrates were either saprotrophic macrofungi or parasitic macrofungi and only occupied substrates with similar qualities, such as both wood and branches. Multiple-substrates macrofungi were primarily found in just two substrates but were sometimes found on a third substrate. For example, fungi from *Marasmius* and *Psilocybe* genera harbored species that could be found in soil, litter, and rare substrates. Their ability to colonize a broader set of substrates could be due to their diverse enzyme activity and high carbon and nutrient efficiency [47–49].

We mainly found multiple-substrates macrofungi in highly disturbed areas, such as secondary forests. Substrate quality and quantity frequently differ in such disturbed areas. One limitation of macrofungi is that they cannot actively seek their substrate in the way that animals forage actively. Therefore, the substrate resources that are available to macrofungi may be random and finite. Macrofungi that can use different substrates generally have another competitive advantage, too. These fungi also tend to produce wind-dispersed spores that spread over large areas and may be deposited in open-canopy forest areas. This method of using natural forces results in a random distribution, and helps the macrofungi obtain novel substrate resources. Light availability can also encourage some saprotrophic macrofungi to adopt a parasitic macrofungi lifestyle in order to colonize the pioneer plants that also thrive in forest gaps [17]. Therefore, we propose the potential for both a shift in substrate selection and in between life strategies (saprophyte vs. pathogen) due to altered substrate availability in disturbed areas.

4.3. Substrate Diversity is Correlated with Fungal Taxonomic Diversity

Within secondary forests, which formed the major vegetation type of our study of the Greater Mekong Subregion, macrofungal diversity did not follow the same distribution patterns as plant diversity (Figure 5). Macrofungal diversity was generally higher in subtropical forests and lowest in temperate and tropical forests, which was similar to the distribution of substrate diversity. This result suggests that substrate diversity may be an important factor in determining macrofungal diversity [8,50]. Root fungi and litter fungi showed contrasting distribution patterns as the dominant fungal groups in temperate and tropical forests, respectively (Figure 5). Temperate forests had a higher abundance and diversity of ectomycorrhizal tree species compared to tropical and subtropical forests [29,51,52]. Thus, it is expected that they would support a higher presence of root-associated fungal species [53].

Conversely, the relatively low abundance of ectomycorrhizal tree species in tropical and subtropical areas should limit the number of root-associated fungal species within these regions. However, litter composition was more diverse and abundant in tropical areas, whereas aboveground biodiversity of macrofungi was higher in temperate areas [23]. This high substrate availability in tropical and subtropical areas is probably the result of a favorable climate, high vegetation diversity, and an ideal soil environment. The high temperature and precipitation levels provide a suitable environment for fungal growth and enzyme activities, thus supporting a larger fungal community. This further enables a greater diversity of substrates to be utilized [54–56].

Interestingly, we found that macrofungal substrate diversity was highest in subtropical areas. These subtropical areas served as buffer zones between areas of root or litter macrofungi domination and harbored larger amounts of soil and rare macrofungal species than temperate and tropical areas (Figure 5). Similar patterns of distribution have been shown in past studies using molecular analyses [29,57,58]. The higher substrate diversity in subtropical areas can be explained by the interactions between vegetation and climate factors. In subtropical areas, litter decomposition is slower than in tropical forests, which results in a thick organic layer in these forests that is suitable for macrofungal growth [59–61]. Also, human activities frequently cause forest disturbance in the subtropical GMS. In a previous study conducted in the Mengsong area, which contains several secondary forests, forest disturbance was reported to have a significant effect on litter production and decomposition [62]. We also found that saprotrophic and parasitic macrofungi were more diverse in disturbed areas, such as secondary and plantation forests [17]. Plant diversity was not correlated with substrate diversity, yet there was a similar trend between substrate diversity and fungal diversity, which might account for the lack of relationship between the biogeography of plant and macrofungal species.

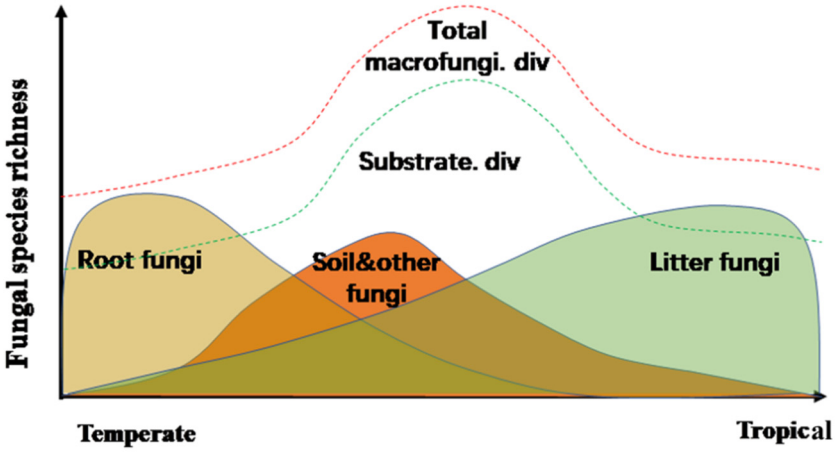

Figure 5. Conceptual diagram of the distribution patterns of substrate-specific fungi in secondary forests across latitudinal gradients. We compared substrate diversity with total fungal taxonomic diversity. The x-axis represents climate zones ranging from the temperate to the tropical zone. The y-axis represents macrofungal species richness. Our survey indicated that macrofungi that colonized the soil, the litter, and the root showed geologically-separated distribution patterns. Litter fungi dominated in the tropics, whereas root fungi dominated in temperate zones. Macrofungi living on soil and rare substrates were mainly found in subtropical areas near the tropics, where there was also higher macrofungal taxonomic diversity (total macrofungi. div) and substrate utilization diversity (substrate. div).

5. Conclusions

Based on our investigation of macrofungal substrate utilization in the GMS, we found that macrofungi are most commonly associated with a single substrate. This substrate specificity appears to be a direct driver of macrofungal biogeography. A few macrofungi were present on multiple substrates. These have the potential to act as pioneer groups in disturbed habitats. Within secondary forests, we found similar rates of change along latitudinal gradients in both taxonomic and substrate diversity. Soil macrofungal diversity could potentially be used as an indicator of total fungal taxonomic diversity. Overall, research into substrate preferences is a promising new direction for the study of the natural distribution of macrofungi and their response to environmental disturbances.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/10/10/824/s1, Table S1: Substrate types used in this study and how these were distinguished.

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