Insights into the profile of soil fungal diversity in Thailand

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Abstract
Soil fungi are diverse and abundant organisms. However, our knowledge of the global diversity and distribution of soil fungi is limited. This study uses the Global Soil Mycobiome Consortium (GSMc) dataset to explore the assortment of soil fungi in Thailand. The study aimed to evaluate the diversity and richness of soil fungi in Thailand, identify the dominant fungal taxa and functional groups present in Thai soils, and examine the impact of land use and fire on the diversity of soil fungi in Thailand. We identified 18,209 OTUs, of which Ascomycota and Basidiomycota were the most abundant in all land conditions. We also found that ectomycorrhizal and soil saprotrophs were primary lifestyles, accounting for around 20%. Our results provide novel insights into the diversity and distribution of soil fungi in Thailand and contribute to understanding soil fungal ecology in tropical regions. The paper also invites collaboration to explore this ecosystem further.

Keywords – Ascomycota, high-throughput sequencing – operational taxonomic unit – soilborne fungi

Introduction
Soil fungi are diverse and abundant organisms that play vital roles in ecosystem functioning and services (Frąc et al. 2018). Fungi are involved in organic matter decomposition, nutrient cycling, carbon sequestration, plant growth promotion, and plant disease suppression (Frąc et al. 2018, Prestt & Roberts 2023). However, our knowledge of the global diversity and distribution of soil fungi is limited, especially in tropical regions where soil fungal diversity is expected to be high (Tedersoo et al. 2014, 2022).

Traditionally, the study of soil fungi has been based on culture-dependent methods (Yasanthika et al. 2022), which are laborious and often underestimate the possible diversity and abundance of soil fungi. With the development of sequencing technologies, high-throughput sequencing (HTS) of DNA markers, such as the internal transcribed spacer (ITS) region and the 18S rRNA gene, has revolutionized the field of soil mycology by allowing the detection and identification of thousands of fungal taxa from soil samples (Tedersoo et al. 2021). HTS can also overcome the challenges of low DNA yield and quality from soil samples by using sensitive and specific PCR primers and amplification protocols (Saeidi et al. 2018). HTS has enabled large-scale

One of the most comprehensive datasets of soil fungal diversity is the Global Soil Mycobiome consortium (GSMc) dataset, which comprises 722,682 fungal operational taxonomic units (OTUs) derived from PacBio sequencing of full-length ITS and 18S-V9 regions from 3,200 plots in 108 countries on all continents (Tedersoo et al. 2021). The GSMc dataset covers various environmental gradients and biomes, providing taxonomic and functional information for each fungal OTU. It is publicly available (https://doi.org/10.15156/BIO/2263453) and can be used to address multiple research questions on soil fungal diversity, biogeography and macroecology. Nevertheless, it is not well-explored at specific geographical locations.

In this study, we used the GSMc dataset to explore the diversity of soil fungi from Thailand, a tropical country with high plant diversity and complex environmental gradients. We compared the soil fungal communities between tropical broadleaf forests, tropical coniferous forests, cropland, village, and fire occurrence. Our main objectives were to: (i) assess the diversity and richness of soil fungi in Thailand, (ii) identify the dominant fungal taxa and functional groups in Thai soils, and (iii) investigate how land use and fire affect soil fungal diversity. It contributes to and gives insights into understanding soil fungal ecology in tropical regions. This paper also invites all interested in soil fungal diversity to collaborate to explore this ecosystem.

**Materials and methods**

We used the GSMc dataset available at the PlutoF data repository (https://doi.org/10.15156/BIO/2263453). The data (OUT table, taxonomic mapping, metadata, and lifestyle) from 34 soil samples collected from the Northern (Mae Hong Son, Chiang Mai, Chiang Rai, and Nan) and Southern (Prachuap Khiri Khan, Surat Thani, Nakhon Si Thammarat, and Krabi) provinces covering different geographic areas was extracted using an in-house R script. Statistical analyses and visualizations of the fungal communities were performed by the MicrobiomeAnalyst web-based tool (Dhariwal et al. 2017) using the default configurations. We analyzed the fungal diversity composition, and the taxonomic results were visualized as a Krona chart using KronaTools 2.8 (Ondov et al. 2011). We also calculated the diversity indices; the alpha diversity (the Chao1 richness) statistic was analyzed and visualized for the fungal communities across all samples. Beta diversity was estimated by computation of the pairwise Bray-Curtis index dissimilarity matrix at the phylum to family level. Dendrograms of fungal community relationships were constructed to estimate beta diversity at the above-mentioned taxonomic ranks. Permutation analysis of variance (PERMANOVA) was performed to compare the centroids and dispersion of the groups. The corresponding R-squared and p-values were calculated to indicate the proportion of variation explained by the model and the significance of the model fit, respectively.

**Results**

**Soil fungal diversity community composition by OTU clustering**

The Thai dataset comprised 18,209 OTUs analyzed for their taxonomic diversity composition. Of the total OTUs, 6,605 (54.4%) were singletons, 2,421 (54.4%) appeared twice, 1,401 three times (45.6%), 936 four times (45.6%), 670 five times (45.6%). Only 6,176 (45.6%) had frequency ranging from 6-10, 3,557 from 11-100, 663 from 101-1000, and only 17 sequences were represented by more than 1,000 sequences, reaching the maximum of 2,095.

Among the phyla, Ascomycota was the most abundant with 9,529 OTUs, followed by Basidiomycota (4,204 OUTs), Fungi Subkingdom incertae sedis (1,074 OUTs), Glomeromycota (896 OTUs), Chytridiomycota (684 OTUs), and Rozellomycota (674 OTUs), Mucoromycota (370 OTUs), Mortierellomycota (309 OTUs), and Zoopagomycota (256 OUTs). Other phyla abundance was represented by less than 100 OTUs: Kickxellomycota (78 OTUs), Monoblepharomycota (40 OTUs), Blastocladiomycota (39 OTUs), GS01 (16 OTUs), Apheleidiomycota (13 OTUs), Basidiobolomycota (9 OTUs), Olpidiomyctca and Entorrhizomycota (5 OTUs),
Calcarisporiellomycota (4 OTUs), Entomophthoromycota (2 OTUs), and Neocallimastigomycota (1 OUT). The total abundance, and that from Ascomycota and Basidiomycota, are represented in Fig. 1, and an interactive figure view can be downloaded from https://rb.gy/i2fir.

At the class level, Sordariomycetes represented 33% of the Ascomycetes and 17% of the total OTUs, followed by Eurotiomycetes (20% and d10%, respectively) and Dothideomycetes (19% and 10%, respectively). In Sordariomycetes, Hypocreales was the most represented order, with Nectariaceae and Hypocreaceae as the dominant families. In Basidiomycetes, Agaricomycetes represented 82% of the OTUs and 19% of the total, with Agaricales representing 33% of Basidiomycetes and 8% of the total OTUs, followed by Thelephorales and Russulales representing 16% and 11% of Basidiomycetes. Other high groups with a high occurrence included Fungi subkingdom incertae sedis, with 6% of the total OTUs, followed by Glomeromycota (5%), Chytridiomycota and Rozellomycota (4%), and Mucoromycota and Mortierellomycota (2%). Less than 2% of OTUs represented other phyla.

Regarding functional groups, the primary lifestyle taxon-rich groups were ectomycorrhizal fungi, soil saprotrophs, wood saprotrophs and litter saprotrophs, unspecified saprotrophs, and plant pathogens. These groups were represented by 1,861 (10.22%), 1,621 (8.90%), 1,130 (6.21%), 1,127 (6.19%), 1,100 (6.04%) and 1,070 (5.88%), respectively. Of the total (18,209 OTUs), 7,181 OTUs (39.44%) were not assigned to any functional guild. We also evaluated fungal secondary lifestyle,
which showed litter saprotroph, foliar endophytes, and root-associated being the most represented guilds, with 1,029 (5.65%), 855 (4.70%), and 819 (4.50%), respectively. Of the total, 13,736, 75.44% were not assigned to a secondary lifestyle (Fig. 2).

![Fig. 2 – Representation of the primary (A) and secondary (B) fungal lifestyles.](https://rb.gy/i2fir)

Further, we evaluated the fungal diversity in function of the land uses and occurrence of recent fires (1-5 years) and no fire (> 5 years). Tropical broadleaf forests accounted for most of the OTUs (12,039 = 66.12%), followed by croplands (4,984 = 27.37%), tropical coniferous forests (3,710 = 20.37%), and villages (824 = 4.52%) (Fig. 3). In all the environments, Ascomycota and Basidiomycota were dominant, with the first representing 52%, 70%, 52%, and 68% of the respective total, while Basidiomycota represented 23%, 19%, 28%, and 15%, correspondingly. At a lower level, Sordariomycetes, Dothideomycetes, and Eurotiomycetes were the dominant classes, with the orders Hypocreales and Eurotiales, representing 18% and 10% of the Ascomycota OTUs in the croplands (Fig. 3). The interactive figure view can be downloaded from [https://rb.gy/i2fir](https://rb.gy/i2fir). Regarding the occurrence of fires, most of the OTUs (14,617 = 80.26) were from non-burned areas, against 4,854 OTUs (26.66%) from burned areas (Fig. 3). As a pattern, Ascomycota represented the majority (52% of the OTUs) and Basidiomycota the following (23% of the OTUs) in the non-burned areas, while in the burned areas, the phyla occurrence was 51% and 27% of the OTUs,
respectively. A pattern similar to the different land uses was observed for the fire occurrence areas. The relative abundance per sample of each taxonomic rank is summarized in Supplementary Figs 1–4 for better visualization.

Fig. 3 – Taxonomic profile of the soil fungi on different land uses. Tropical broadleaf forest (A), tropical coniferous forest (B), cropland (C), village (D), burned (E), and non-burned components in the samples based on a Krona chart. The interactive figure view can be downloaded from https://rb.gy/i2fjr.
**Diversity analysis**

In the analysis of the beta diversity indices, the variation in species composition between different ecosystems or samples for different land use types and the soil fungal communities differed significantly at the order and family levels (Fig. 4). At the order level (PERMANOV; $R^2 = 0.22$; p-value = 0.001), the variations are explained by the axis $x=17.7$ and $y=14.6\%$, respectively. For the family (PERMANOVA; $R^2 = 0.20$; p-value = 0.001), the interpretations justified by the axis $x=17.7$ and $y=12.7\%$, correspondingly. As shown in Fig. 4c, d, cropland, tropical broadleaf forest, tropical coniferous forest, and village all exhibited unique clusters of data points along the first two principal coordinates of our multivariate analysis, indicating distinct patterns of fungal community composition among these different types of land use. These results suggest land use can significantly impact order, family fungal community composition, and beta diversity at lower taxonomic levels.

![Beta diversity of the fungal assemblages associated with the different land use types.](image)

**Fig. 4** – Beta diversity of the fungal assemblages associated with the different land use types. The beta diversity among the different land use types was estimated by a PCoA analysis based on the Bray–Curtis index, with the following parameters: taxonomic level: Phylum, class, order, and family; statistical method: PERMANOVA, experimental factor: biome. The variation in the beta diversity is shown at the phylum (a), class (b), order (c), and family (d) levels. Dots in different colours represent different samples from their respective environments.

The analysis of alpha diversity indices, used to estimate the richness of fungal species, showed variation across different land use types at all taxonomic levels (from phylum to family). However, the Chao1 index, a measure of alpha diversity, was not significantly higher ($p > 0.001$: 0.17774, 0.20671, 0.59909, and 0.50597, respectively). Nevertheless, the tropical broadleaf forest had the highest median Chao1 index value, followed by the tropical coniferous forest, cropland,
and village. This suggests the tropical broadleaf forest has the highest estimated fungal species richness, while the village has the lowest (Fig. 5).

**Fig. 5** – Fig. 1 General pattern of fungal alpha diversity in Thai soils. The alpha diversity among the different land uses was estimated based on the Chao1 index, with the following parameters: taxonomic level: Phylum, class, order, and family; statistical method: T-test/ANOVA, experimental factor: biome. The variation in the alpha diversity is shown at the phylum (a), class (b), order (c), and family (d) levels. As the alpha diversity between land use types was not significantly different (P > 0.05), boxplots lack a letter representing the significance.

**Discussion**

In this study, we investigated the patterns of soil fungal diversity and composition along different land use types and fire occurrences in Thailand. We found that these variables significantly influenced soil fungal diversity and assemblages. We identified 18,209 OTUs, with a significant proportion being singletons or appearing only a few times. Regarding the distribution of OTUs among different fungal phyla, **Ascomycota** was found to be the most abundant, with 9,529 OTUs, followed by **Basidiomycota**, with 4,204 OTUs (Fig. 1). Other phyla, such as Fungi Subkingdom incertae sedis, **Glomeromycota**, **Chytridiomycota**, and **Rozellomycota**, were also represented by a significant number of OTUs. However, some phyla had less than 100 OTUs, indicating lower abundance or diversity within these groups. The pattern of **Ascomycota** and **Basidiomycota** as dominant groups has been observed from the global GSMc dataset (Tedesco et al. 2021) but with different proportions. While Tedersoo et al. (2021) reported 45.7% of **Ascomycota** and 28.3% of **Basidiomycota** worldwide, in Thai soils we observed 49% of **Ascomycota** against 40% of **Basidiomycota** (Fig. 1). Although using a subset of the GSMc dataset, this may be due to fluctuations due to various factors such as changes in climate, soil conditions, and other environmental factors (Talley et al. 2002), endemicity (certain species can be found only in specific regions) (Tedesco et al. 2022), or adaptability to host plants, different soil conditions, and even survive in extreme environments (Rúa et al. 2016, Bazzicalupo 2022).

The high proportion of singletons and low-frequency OTUs suggests that the samples studied may contain a high level of rare or previously uncharacterized fungal taxa; or may include many artefacts (Brown et al. 2015). The presence of a range of other fungal phyla, including Fungi Subkingdom incertae sedis, **Glomeromycota**, **Chytridiomycota**, and **Rozellomycota**, suggests that the studied ecosystems may support a diverse range of fungal taxa. The lower abundance of some fungal phyla, such as **Kickxellomycota**, **Monoblepharomycota**, and **Blastocladiomycota**, indicates that these groups have less diversity or are less adapted to the studied ecosystems. In this regard,
Kickxellomycota is a phylum that has been found in low abundance and diversity (Hurdeal et al. 2021). The same is valid for Monoblepharomycota, another phylum often found in low abundance in various ecosystems (Souza et al. 2022, Wijayawardene et al. 2018). This can be due to the lack of comprehensive taxonomic and evolutionary studies on this group (Wijayawardene et al. 2018). Nevertheless, rare microbial taxa can significantly influence multiple ecosystems; thus, preserving the diversity of these rare organisms can be crucial to the sustainable endowment of ecosystem functioning (Chen et al. 2020, Li et al. 2020). Thus, further research could explore the ecological roles and significance of these rare taxa, the ecological functions and interactions of these phyla within the studied ecosystem, and the ecological roles and interactions within the ecosystem.

Our results showed that soil fungal diversity and richness depend on land use, consistent with previous studies in other regions (Aranguren et al. 2023, Yang et al. 2017, Su et al. 2022). This pattern could be explained by the changes in temperature, moisture, organic matter and nutrient availability, increasing soil acidity, and harsh environmental conditions (Newsham et al. 2017, Liu et al. 2018, Lou et al. 2023, Zeng et al. 2019, Chen et al. 2022, Grishkan et al. 2023). These factors could limit the growth and survival of soil fungi and reduce their niche differentiation and resource partitioning. However, we also found that some fungal taxa, such as Basidiomycota and Ascomycota, were dominant in all the environments (Fig. 3A-D and Supplementary Figs 1-4), suggesting that taxa of these megadiverse groups have adapted to a huge variety of environments and are thus dominant in most ecosystems worldwide. This is more evident in forest ecosystems, where environmental conditions are favourable for fungal diversity and a high number of OTUs was found. Yet, variations in plant composition have also been found to result in different compositions of fungal communities (Wu et al. 2013, Tedersoo et al. 2013, Yang et al. 2022), age (Wu et al. 2013), and biogeographical distributions (Wang et al. 2023, Tedersoo et al. 2022).

Furthermore, our study demonstrates that soil fungal diversity and composition were affected by anthropogenic activities. We found that soil fungal diversity and richness were higher in tropical forests compared with croplands and villages (Fig. 3). This is in agreement with the findings of Tan et al. (2019), who suggest that the urban-to-rural gradient affects fungal composition but not fungal diversity. Soil fungal diversity was similar in planted, naturally regenerated and native forests. Lutter et al. (2023) also reported that soil fungal composition differs from pristine forests and agricultural fields and is more similar in planted, naturally regenerated, and native forests. This could be related to the changes in host diversity, soil temperature, moisture, pH, organic matter, and nutrient content in the different sceneries (Liu et al. 2020). These factors could limit the growth and survival of soil fungi and reduce their niche differentiation and resource partitioning. It could also influence the metabolic activity, growth rate, sporulation and dispersal of soil fungi and alter their competitive interactions and functional roles.

We also found that some fungal taxa, such as Ascomycota and Basidiomycota, showed different habitat preferences, which could reflect their different life strategies, ecological functions and responses to environmental fluctuation. For example, Ascomycota were more diverse and abundant in tropical broadleaf forests, tropical coniferous forests, and croplands, where they can decompose plant litter and organic matter efficiently, thus releasing nutrients for plant growth (Ma et al. 2013, Zeng et al. 2020). Basidiomycota was found to be more diverse and abundant in villages and tropical coniferous forests, suggesting that they adapt to hosts and changes in plant community composition.

Concerning the impact of fire on fungal diversity, the results show that most OTUs were found in non-burned areas, with a smaller proportion found in burned areas (Fig. 3E-F and Supplementary Figs 1–4). These results were also observed in other studies that showed fire affects soil microbiome diversity and functions (Day et al. 2019, Hopkins et al. 2020, Nelson et al. 2022). Alternatively, Vázquez-Veloso et al. (2022) found that burning did not affect fungal richness and diversity, with the composition influenced by the factors of the sites than the occurrence of fires. Our results also showed that Ascomycota and Basidiomycota communities were the least affected, which could be related to the resistance to adverse conditions such as heat, the ability to use nutrients efficiently by breaking down complex compounds, and the ability to develop symbiotic
association with host species. These characteristics could influence the metabolic activity, growth rate, sporulation and dispersal of soil fungi and alter their competitive interactions and functional roles.

A notable observation is the prevalence of ectomycorrhizal and soil saprotroph as primary lifestyles, with 10.22% and 8.90% of the total, respectively. This suggests that these two lifestyles play a significant role in the ecology of soil fungi. On the other hand, a high percentage of OTUs were unassigned to a primary or secondary lifestyle (39.44% and 75.44% of the total, respectively). This indicates that many fungi have not yet been fully characterized in their lifestyles or that their secondary is poorly understood.

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Supplementary Materials

Supplementary Fig. 1 – Taxonomic composition and relative abundance of soil fungi at the phylum level.

Supplementary Fig. 2 – Taxonomic composition and relative abundance of soil fungi at the class level.
Supplementary Fig. 3 – Taxonomic composition and relative abundance of soil fungi at the order level.

Supplementary Fig. 4 – Taxonomic composition and relative abundance of soil fungi at the family level.