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ORIGINAL ARTICLE

Temporal dynamics in diversity and composition of the air mycobiome and dominant allergenic fungi in the subway environment

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Abstract

Analyzing and monitoring air microbiomes in the subway environment has a great impact on public health, especially in urban cities. In this study, temporal distribution profile of air mycobiome at Bangkok's Sukhumvit subway station (MRT-SKV) was explored over 12 samplings during December 2021–November 2022 by the environmental DNA-metabarcoding approach. The top 5 most abundant fungal genera identified were *Yarrowia*, *Cladosporium*, *Hortaea*, *Cutaneotrichosporon*, and *Leptobacillum*. Among the 496 genera found, 24 genera could be considered "core mycobiome" of the MRT-SKV air, as they were consistently found in ≥85% of the samples. Many members of the core mycobiome constituted human commensal genera, but several of them can elicit allergic reactions in humans and thus pose a potential health risk. *Cladosporium* was the most abundant allergenic fungi found in the MRT-SKV, which is in contrast to *Malassezia*, *Ustilago*, or *Aspergillus* being the most abundant allergenic fungi in subway environments in other countries. The fungal compositions in the subway environment differed temporally, and the abundances of allergenic fungi were the highest in samples obtained during the first and seventh samplings (done in December 2021–June 2022). These compositional variations were most likely influenced by air quality variables, including particulate matter (PM), temperature, and relative humidity, which showed seasonal variations. Finally, certain fungal genera were shown to co-occur together in co-occurrence network. Many of the allergenic fungal genera formed networks more prominently in rush hour than in other traffic hours. The co-occurrence network suggested associations among certain fungal genera, such as the association between *Malassezia–Nigrospora*, *Alternaria–Aspergillus*, and *Aspergillus–Cutaneotrichosporon* in the MRT-SKV bioaerosols. The implied associations between these fungi are worth further investigation since they may point toward their impact on human health (as they are opportunistic fungi found in human).

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Our results thus facilitate understanding the ecological and health impacts of fungal components of the subway air environment.

KEYWORDS
allergenic fungi, metabarcoding, metagenomic DNA, mycobiome, subway air environment

1 | INTRODUCTION

It is widely accepted that exposure to bioaerosols can have profound health effects on humans, particularly those with respiratory issues (Fan et al., 2017). The spread of airborne particles sometimes has a significant effect on health, as it can lead to the development of allergies, cancer, and toxic effects (Douwes et al., 2003). These bioaerosols usually contain a diverse assemblage of microorganisms. Studies on air microbiomes in terms of their community composition as well as their impacts on human health have been conducted across environments such as indoor environments (Mbareche et al., 2022), hospitals (Stockwell et al., 2019), and outdoor environments (Yang et al., 2022). With the increasing numbers of people living in urban areas (United Nations, 2018), public transportation networks such as trains, buses, and subways are a regular point of interaction interface for billions of people, allowing exchanging of microorganisms with each other, as well as with transit surfaces and air. Especially, in cities around the world, a high number of daily commuters use the subway as their choice of transportation (Robertson et al., 2013). The subway systems are semi-open indoor environments suitable for the transmission of airborne microbes including fungi, bacteria, and viruses between humans and between humans and environments (Fujiyoshi et al., 2017; Hernández et al., 2020). Therefore, indoor air quality in the subway system has globally drawn much attention due to its public health importance (Matz et al., 2014). Moreover, it has been demonstrated that humans themselves are a significant source of bioaerosol emission in the subway environment through respiratory activity, coughing, skin contact, and skin shedding as daily commuters leave their microbial clouds and interact with other passengers (Fan et al., 2017; Noti et al., 2012). In addition, microorganisms may be transmitted among commuters via fomites (Fan et al., 2017; Qian et al., 2014).

A number of filamentous and yeast fungi have been found in the air and on surfaces of the subway systems in several cities around the world including New York City, St. Petersburg, Tehran, Beijing, Tokyo, Athens, Busan, and Seoul using both culture-dependent technique such as morphological characterization on agar plates (Bogomolova & Kirtsideli, 2009; Cho et al., 2006; Fan et al., 2017; Hoseini et al., 2013; Hwang et al., 2016; Kawasaki et al., 2010; Kim et al., 2011) and culture-independent molecular approaches such as metabarcode and shotgun metagenome sequencing (Grydaki et al., 2021; Kim et al., 2022; Robertson et al., 2013). Fungal species may propagate inside the subway itself (if the conditions are appropriate for fungal growth), or they may be brought into the subway system from outside environments. The fungal kingdom is an intrinsic and essential component of the global environment, with the number of species estimated to be 3.8 million (Hawksworth & Lücking, 2017). Because of the ubiquity and diversity of fungal communities (mycobiomes) in the environment, daily human exposure to fungi is inevitable. Certain fungi reside commensally on various mucosal surfaces of human body, such as the oral cavity, the lungs, the intestines, the vaginal tract, and the urinary tract (Ackerman & Underhill, 2017; Bandara et al., 2019; Hamad et al., 2017; Hamm et al., 2020; Ward et al., 2018). In addition, some fungal species have been found from the human skin, breast, and breastmilk (Heisel et al., 2019; Santus et al., 2021; Zhu et al., 2020). Among more than 100,000 fungal species identified, a few hundred of them act as opportunistic pathogens infecting susceptible hosts with immunocompromised conditions (Simon-Nobbe et al., 2008). Some fungi are capable of producing mycotoxins, mostly nonvolatile, secondary metabolites of low molecular weight such as aflatoxins (produced by *Aspergillus flavus* and *Aspergillus parasiticus*), ergot alkaloids (produced by *Claviceps* spp., *Aspergillus fumigatus*, and *Penicillium chermesinum*), and ochratoxins (produced by *A. ochraceus*, *A. alliaceus*, *A. terreus*, *P. niger*, and *P. viridicatum*) (Simon-Nobbe et al., 2008). Mycotoxins can impair the immune system and have neurotoxic, mutagenic, carcinogenic, and teratogenic effects.

Importantly, a broad panel of allergic reactions can result from the inhalation and ingestion of fungal spores and hyphae or the contact with mycelial cells that result in type I (IgE-mediated), type III (IgG mediated), and type IV (T-cell mediated) hypersensitivity, with the type I hypersensitivity being the most common (Vitte et al., 2022). Type I allergic reactions include asthma, atopic dermatitis, rhinitis, conjunctivitis, and urticaria. Type II hypersensitivity occurs with exposure to the mannan of the cell wall of *Candida* and *Aspergillus*, whereas type III hypersensitivity includes allergic alveolitis and bronchopulmonary aspergillosis (ABPA) (Vitte et al., 2022). Important allergy-causing fungal genera include *Alternaria*, *Aspergillus*, *Candida*, *Cladosporium*, *Curvularia*, *Epicoccum*, *Fusarium*, and *Penicillium* in the phylum *Ascomycota*, *Calvatia*, *Coprinus*, *Ganoderma*, *Malassezia*, *Pleurotus*, *Rhodotorula*, and *Psilocybe* in the phylum *Basidiomycota*, *Mucor*, and *Rhizopus* in the phylum *Mucoromycota*, and *Phytophthora* and *Plasmopara* in the phylum *Oomycota* (Fukutomi & Taniguchi, 2015; Simon-Nobbe et al., 2008).

As fungi are common and abundant microbes suspended in the air with 30–50 Tg of fungal materials are estimated to be emitted into the earth's atmosphere each year (Fröhlich-Nowoisky et al., 2012; Heald & Spracklen, 2009), fungal materials represent one of the main sources of organic aerosol dispersed in the air. Airborne fungal cells and spores have been implicated as the causes of respiratory allergy, and there are evidences indicating the elevated counts of fungal spores in the environment can lead to higher numbers of

asthma cases (Crameri & Blaser, 2002; Elbert et al., 2007; Horner et al., 1995; Lanier et al., 2012). Respiratory allergy to fungi can be found in up to 6% of human population and up to 30% of atopic individuals (Kurup et al., 2000). Large or aggregated fungal conidia and spores are usually deposited in the upper respiratory tract, whereas small spores can reach the lung.

Since fungi can pose health risks to human beings including subway passengers, human exposure to fungi in the subway system should be a significant public concern, especially as many fungi can thrive in a tropical climate with hot and humid conditions in Thailand. Bangkok, the capital of Thailand is designated as one of the megacities by the UN (United Nations, 2019), where approximately 11 million people currently live in the metropolitan area (Bangkok and its vicinities) and the number of population is still increasing (Digital Government Development Agency, 2021). To serve the residents within the Bangkok metropolitan region, the metropolitan rapid transit (MRT) subway system is one of the public transportation systems that conveniently connects to other mass transit systems in Bangkok. There are currently 54 stations along the MRT route (Bangkok Expressway and Metro, 2018), and the MRT Sukhumvit (MRT-SKV) station was the busiest station in Bangkok with an annual ridership of around 35 million (Bangkok Metro Network, 2023) due to it being in the heart of Bangkok's business and shopping district and being an interconnecting point to the sky train system. Even though there is a recent interest in the bacteria communities and their functions in the Bangkok MRT environment (Siriarchawatana et al., 2023), the fungal communities in the Bangkok MRT environment have not been extensively examined and the information on airborne fungi was sorely lacking. Thus, the exploration of fungal compositions and their dynamics over time will immensely aid in monitoring and management of the airborne fungi in the subway station, which potentially impact the health of millions of passengers.

Taking advantage of environmental DNA-metabarcoding technology coupled with next-generation sequencing, in this study, we examine bioaerosols collected in the MRT-SKV station in Bangkok, Thailand over 12 months (December 2021–November 2022). In addition, comparing fungal communities at the MRT-SKV in Bangkok to those at the subway station other highly populated cities can greatly facilitate greater understanding of spatial distribution of fungal communities in the subway environment. To this end, bioaerosol samples collected at the MRT-SKV could be assessed and compared to the samples collected at the same period at Dongzhimen station, one of the busiest subway stations in Beijing, P.R. China. The station is an interchange station for Line 2, Line 13 and the Capital Airport Express of the Beijing Subway and is also the only underground station on Line 13 subway. Furthermore, the fungal compositions identified at the MRT-SKV were also compared to the metagenome data from subway samples in China (Hong Kong), Norway, Sweden, the United Kingdom, and the United States collected by the International Metagenomics and Metadesign of Subways and Urban Biomes (MetaSUB) Consortium. In this study, we aimed to (i) explore the compositions of fungal communities found in the MRT-SKV air environment and the temporal shift of fungal taxa at approximately 30-day sampling interval over 12-month period, as well as comparing the fungal communities at the MRT-SKV to those in the subway stations of other cities, (ii) explore the diverse allergenic fungi in the subway system, (iii) investigate the influence of environmental factors, including various air quality variables, on the composition of the airborne fungal communities, and (iv) explore fungi that co-occur and most likely interact in the subway environment.

2 | MATERIALS AND METHODS

2.1 | Air sampling

Air samples were collected at MRT-SKV station, which is the busiest station of the Bangkok Metro system, and it serves the blue line of the MRT subway system, as well as being a connection point to the BTS sky train system (Bangkok Expressway and Metro, 2023). The station situates at the intersection of the Sukhumvit and Asoke-Montri Road in downtown Bangkok. The MRT-SKV contains three entrance/exits. The samplings took place at the platform level, which is three-story below ground underneath the access level and the concourse level (Figure S1 in Appendix S1). The platform level contains a central platform, with access to both the inbound train (toward Lak Song) and outbound train (toward Tha Phra). During air sample collection, the air sampler was placed near the escalators in the middle of the train platform. The samplings were performed on the last Monday of the month during December 2021 to November 2022 (Table S1 in Appendix S2), so that the interval between sampling days was approximately 30 days. For each sampling, the samples were collected in 1-h intervals continuously during rush hour (RH, 7 a.m. to 9 a.m.), transitional hour(TH, 9 a.m. to 11 a.m.), and non-rush hour (NRH, 11 a.m. to 1 p.m.) to obtain a total of six samples per day. The RH was designated to be 7 a.m. to 9 a.m. according to the time period in the morning when the frequency of trains to serve commuters is approximately twice as high as in other time period (Bangkok Expressway and Metro, 2018). Thus, a total of 72 bioaerosol samples were collected over the sampling period. Furthermore, at the Dongzhimen station in Beijing, P.R. China, bioaerosol samples were also collected during December 2021–November 2022. The samplings were synchronized with the samplings performed at the MRT-SKV to perform on the same day. The bioaerosol collection was conducted in the exhaust air channel of Line 2 of the subway system (Figure S1 in Appendix S1). The exhaust fan aided in greatly increasing the collecting speed of the air sampler (>100 L/min); therefore, the sampling time was reduced to 10 min with three replications.

Air samples were collected on polyethersulfone (PES) (Pall Corporation, USA) filter membranes with 0.45-μm pore size using the developed "AirDNA" sampler consisting of a portable ventilation fan (PVF) and bioparticle-filter mounting plates allowing the use of up to 27 filter membranes simultaneously to capture bioaerosols (Harnpicharnchai et al., 2023). After 1 h of sampling, the filter membranes were collected and stored in ice box until they were returned to the laboratory. The membranes were then stored at −20°C until further processing.

Air quality variables including particulate matters (0.3, 0.5, 1.0, 2.5, 5.0, and 10 μm), ambient temperature (AT), relative humidity (RH), dew point (DP), and wet-bulb temperature (WB) were measured and recorded every 30 min using the RS-9880 Data Logging Air Quality Meter (RS Components, Bangkok, Thailand) during air sampling.

2.2 | Filter processing and genomic DNA extraction

The filter membranes were washed three times by vortexing in phosphate buffer saline (PBS) pH 7.2 with 0.01% Triton X-100 for 30 s prior to water-bath sonication at room temperature for 1 min following the protocol by Luhung et al. (2021). The vortex-sonication process was repeated twice. After washing, the filter solution was concentrated onto 0.2-μm pore size PES filter membrane (Pall Corporation, USA) using vacuum pump. DNA was then extracted following the protocol described by Crump et al. (2003) and our pervious study (Siriarchawatana et al., 2023). Final DNA solution was measured the concentration by Qubit 2.0 (Invitrogen, USA) using the High Sensitivity double stranded DNA (HS dsDNA) kit. In addition, at Dongzhimen station in Beijing, air samples were also collected once a month during December 2021–November 2022. Genomic DNAs were also extracted from air samples obtained at the Dongzhimen Station using the same DNA extraction method detailed above.

2.3 | Amplicon metabarcode sequencing

The fungal ITS2 region was amplified from the extracted metagenomic DNAs with primers IT3F (5′ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCATCGATGAAGAACGCAGC) and ITS4R (5′ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCTCCGCTTATTGATATGC) (White et al., 1990) that included Illumina overhang adapter. Amplification of fungal ITS2 region was performed in a 50 μL PCR reaction mixture containing 5 μL DNA template, and a final concentration of 1 × PCR buffer (containing 1.5 mM $MgCl_2$), 0.5 μM of each primer, 200 μM of each dNTPs, and 0.5 U Phusion® High-Fidelity DNA Polymerase (Thermo Fisher Scientific, Massachusetts, USA)., thermocycling consisted of initial denaturation at 95°C for 3 min, 34 cycles of denaturation at 95°C, 30 s; annealing at 61°C, 30 s and extension at 72°C, 30 s; and a final extension at 72°C for 5 min. The PCR products were confirmed by 1% agarose gel electrophoresis and purified with the GeneJet Gel Extraction Kit (Thermo Fisher Scientific, Massachusetts, USA) and eluted with dH_2O.

2.4 | Sequence processing and fungal ASV taxonomic assignment

The raw sequence reads of ITS2 were processed and assembled using DADA2 pipeline (Callahan et al., 2016). The assembled sequences were classified into amplicon sequence variants (ASVs). The taxonomic assignment of fungal ASV was performed using "classify-hybrid-vsearch-sklearn" function in QIIME2 (Bolyen et al., 2019) with UNITE's fungal ITS sequences (Nilsson et al., 2019).

Fungal genera were assigned as allergenic fungi according to allergen nomenclature approved by the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee (www.allergen.org) and those allergenic fungi that were listed in Knutsen et al. (2012).

The trophic modes and fungal guilds of fungal genera were predicted by mapping against FUNGuild database (Nguyen et al., 2016) and FungalTraits database (Flores-Moreno et al., 2019). Living niches of fungal genera that can be mapped with fungal guilds are shown in Table 1. The fungal genera were broadly categorized into "plant associated," "animal associated," or "animal and plant associated." Genera whose fungal guild were not associated with plant or animal were categorized as "other pathotroph–saprotroph," while genera that were not found in FUNGuild and FungalTraits database were labeled as "unknown."

2.5 | Diversity analysis

Alpha diversity of air samples was assessed using Shannon's diversity index and Chao1 richness estimator. Shannon's index was calculated using "vegan" R library (Oksanen et al., 2022) and Chao1 richness estimator was calculated using "fossil" R library (Vavrek, 2011). Beta-diversity analysis were performed by using Bray–Curtis dissimilarity using "vegan" packages.

The probability densities of core mycobiome of the MRT-SKV which were found in ≥85% of all samples, were analyzed and visualized using "mia" and "miaViz" R library (Ernst et al., 2022). Horizon

TABLE 1 Living niches broadly mapped with fungal guilds.

Living niches	Fungal guilds
Plant associated	Plant Pathogen, Epiphyte, Root Associate Biotroph, Algal Parasite, Bryophyte Parasite, Plant Parasite, Endomycorrhizal, Leaf Saprotroph, Plant Saprotroph, Ericoid Mycorrhizal, Ectomycorrhizal, Wood Saprotroph, Endophyte
Animal associated	Animal Pathogen, Dung Saprotroph, Animal Associated Biotroph, Insect Parasite, Nematophagous, Animal Endosymbiont, Endosymbiont
Other pathotroph–saprotroph	Undefined Saprotroph, Fungal Parasite, Soil Saprotroph, Litter Saprotroph, Lichen Parasite, Lichenized

plot of allergenic fungi were visualized using "biomehorizon" R library (Fink et al., 2022).

In order to compare the differences in airborne fungal communities of subway systems, metabarcoding data from Dongzhimen station (Beijing, China) was obtained by the same methods as the MRT-SKV data. In addition, available metagenomic sequence data of subway samples from five countries including the United Kingdom, Norway, China (Hong Kong), Sweden and the United States were obtained from Bioproject ID# PRJNA561080 (Leung et al., 2021). Linear discriminant analysis Effect Size (LefSe) analyses were performed to distinguish differential fungal genera across different countries using MicrobiomeAnalystR (Dhariwal et al., 2017).

2.6 | Assessing the effect of air variables on airborne fungal communities

Difference of air variables across months and across traffic hours (RH, TH, and NRH) was analyzed using one-way ANOVA and Tukey's post-hoc tests. In order to determine the impact of air variables on fungal diversity, distance decay of dissimilarity of fungal communities and distance of variable were calculated. Rolling correlation was performed to assess the effect between air quality variables and abundance of allergenic fungal genera using "NonParRolCor" R library with window length = 24, which consist of samples obtained from four consecutive samplings.

2.7 | Construction of temporal co-occurrence patterns of fungal communities

All MRT-SKV samples were managed into 27 subsamplings according to rolling window of 4 months. Each subsampling contained eight samples of each traffic hours (RH, TH, and NRH) within a period of 4 consecutive month. Fungal genera which had prevalence lower than 50% were removed for each sampling. The pairwise Spearman's rank correlation of fungal genera abundance for each sampling was calculated using "Hmisc" R library. The significant correlation pairs (p value <0.05) which appear more than four subsampling in the same traffic hour were used to construct the co-occurrence network for the RH, TH, and NRH. The correlation between network density and air quality variables was calculated "rcorr" function in "Hmisc" R library.

3 | RESULTS

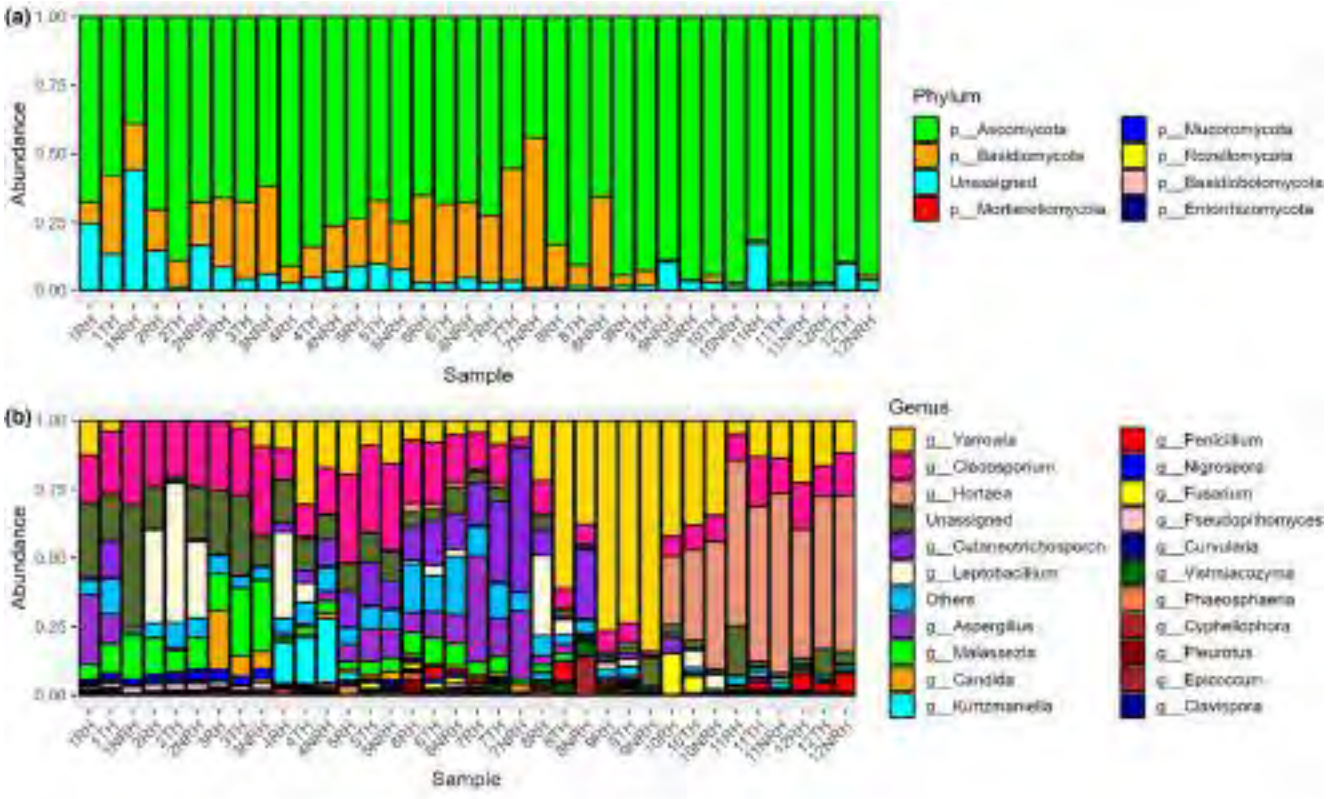
3.1 | Characterization of fungal communities found in the MRT-SKV environment

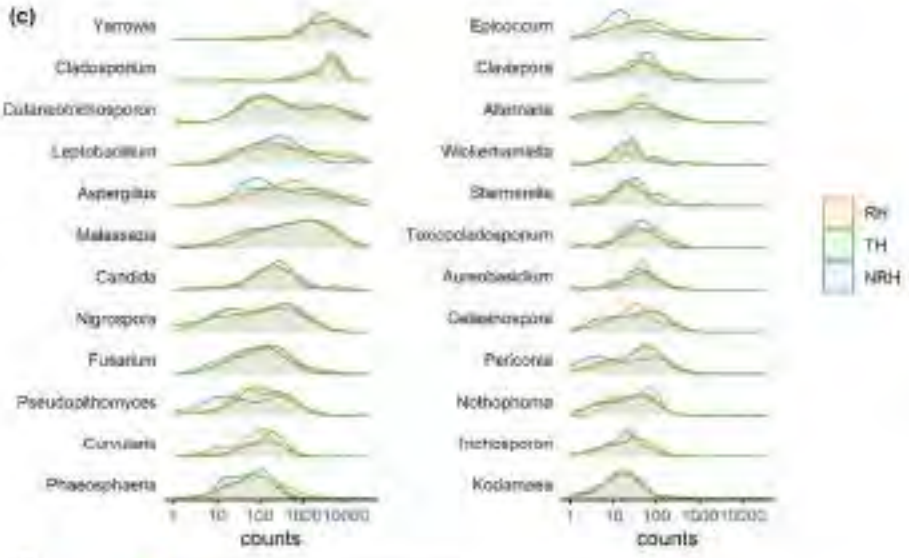
3.1.1 | Fungal composition and diversity in the MRT-SKV bioaerosols

Fungal compositions of the bioaerosol samples collected at the Sukhumvit subway station (designated MRT-SKV) were assessed. Taxonomic analysis using the ITS2 metabarcoding sequences showed that the samples collected at MRT-SKV at approximately 30-day interval during December 2021–November 2022 were mainly represented by seven phyla as shown in Figure 1a. *Ascomycota* (average relative abundance 69.4%) was the most abundant fungal phylum in all samples, followed by *Basidiomycota* (19.8%), *Mortierellomycota* (0.03%), *Basidiobolomycota* (0.006%), *Mucoromycota* (0.0055%), *Rozellomycota* (0.001%), and *Entorrhizomycota* (0.002%). At the genus level, there were 496 genera detected in the MRT-SKV bioaerosols. The abundance levels of these fungal genera were found to differ among samples collected at different sampling time. Among the five most abundant fungi identified, *Yarrowia* was the most abundant fungal genus detected (20.05%), followed by *Cladosporium* (16.68%), *Hortaea* (12.93%), *Cutaneotrichosporon* (6.81%), and *Leptobacillum* (6.06%), (Figure 1b). Besides being highly abundant, *Cladosporium* was also highly prevalent as they were found in all bioaerosol samples obtained from the MRT-SKV, whereas *Yarrowia*, *Hortaea*, *Cutaneotrichosporon*, and *Leptobacillium* were found in 87.5%, 79.2%, 98.6%, and 86.1% of the collected samples, respectively (Table S2 in Appendix S3). These genera, with the exception of *Hortaea*, were members of the 24 highly prevalent genera that could be considered "core air mycobiome" of the MRT-SKV as they were found in ≥85% of all samples across the sampling period (Table S2 in Appendix S3). The prevalence of the members of the core mycobiome across the traffic RH (7–9 a.m.), TH (9–11 a.m.), and NRH (11 a.m.–1 p.m.) was also assessed. Across different traffic hours, most of these core genera exhibited similar abundance levels in RH, TH, and NRH (Figure 1c), with the exception that *Epicoccum* was more abundant in the RH and TH than in the NRH. Furthermore, regarding the persistence of the fungi found in the bioaerosols across the sampling period, the fungal taxa that could be identified with short-term or long-term persistence are shown in Figure 1d. It can be seen that a majority of the detected fungi had short-term persistence and were found in samplings with 30-day interval. However, fungi with long-term persistence throughout all samplings could also be identified. Those identified fungi with long-term persistence were "core mycobiome" of the MRT-SKV mentioned above.

The fungal species diversity and richness at the MRT-SKV were assessed by the Shannon diversity index and Chao1 richness estimator, respectively. The values of the Shannon diversity index were the highest for the sixth and seventh samples obtained in May and June 2022, respectively, and the lowest for the eleventh sample obtained in October 2022 (Figure 2a). Similar to the Shannon diversity index, Chao1 richness estimator index had the highest values for the sixth and seventh samples (obtained in May and June 2022, respectively; Figure 2c). There were no significant differences in Shannon diversity indices and Chao1 richness estimators between samples obtained at different traffic hours (Figure 2b,d).

Beta-diversity analysis was performed using non-metric multidimensional scaling (NMDS) to evaluate the dissimilarities of fungal communities in the bioaerosol samples from different collection days (occurring at approximately 30-day interval) (Figure 3a). The results showed that the samples collected from the MRT-SKV in the same sampling day at different hours appeared to be clustered closely with one another. The pairwise dissimilarity plot and distance-decay


(a)
Abundance
Sample
Phylum
p__Ascomycota
p__Basidiomycota
Unassigned
p__Mortierellomycota
p__Mucoromycota
p__Rozellomycota
p__Basidiobolomycota
p__Entorrhizomycota
(b)
Abundance
Sample
Genus
g__Yarrowia
g__Cladosporium
g__Hortaea
Unassigned
g__Cutaneotrichosporon
g__Leptobacillium
Others
g__Aspergillus
g__Malassezia
g__Candida
g__Kurtzmaniella
g__Penicillium
g__Nigrospora
g__Fusarium
g__Pseudopithomyces
g__Curvularia
g__Vishniacozyma
g__Phaeosphaeria
g__Cyphellophora
g__Pleurotus
g__Epicoccum
g__Clavispora


(c)
Yarrowia
Cladosporium
Cutaneotrichosporon
Leptobacillium
Aspergillus
Malassezia
Candida
Nigrospora
Fusarium
Pseudopithomyces
Curvularia
Phaeosphaeria
Epicoccum
Clavispora
Alternaria
Wickerhamiella
Stemonitis
Toxicocladosporium
Aureobasidium
Cellulosporina
Periconia
Nothophoma
Trichosporon
Kodamaea
counts
counts
RH
TH
NRH

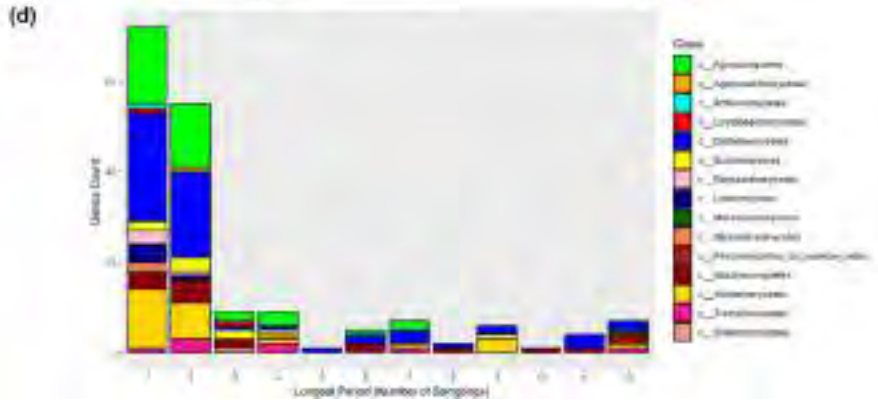

(d)

FIGURE 1 Diversity of fungal compositions detected in air samples of the MRT Sukhumvit (MRT-SKV) station revealed by ITS2 metabarcoding. Relative abundances of fungal compositions are shown at (a) phylum and (b) genus levels. The sample name denoted the sampling ID (the order of sampling performed at approximately 30-day interval) and hours of samplings during December 2021–November 2022 (see Table S1 in Appendix S2). For example, 1RH denotes sample obtained from the first sampling (performed in December 2021) during RH. RH, TH, and NRH denoted samples obtained in rush hour (7–9 a.m.), transitional hour (9–11 a.m.), and non-rush hour (11 a.m.–1 p.m.), respectively. The probability density of the top 24 highest abundant genera in different traffic hours is shown in (c) with orange line (RH), green line (TH), and blue line (NRH). The longest period (according to numbers of samplings) that fungal taxa at class level could be identified from consecutive MRT-SKV air samples are shown in (d).

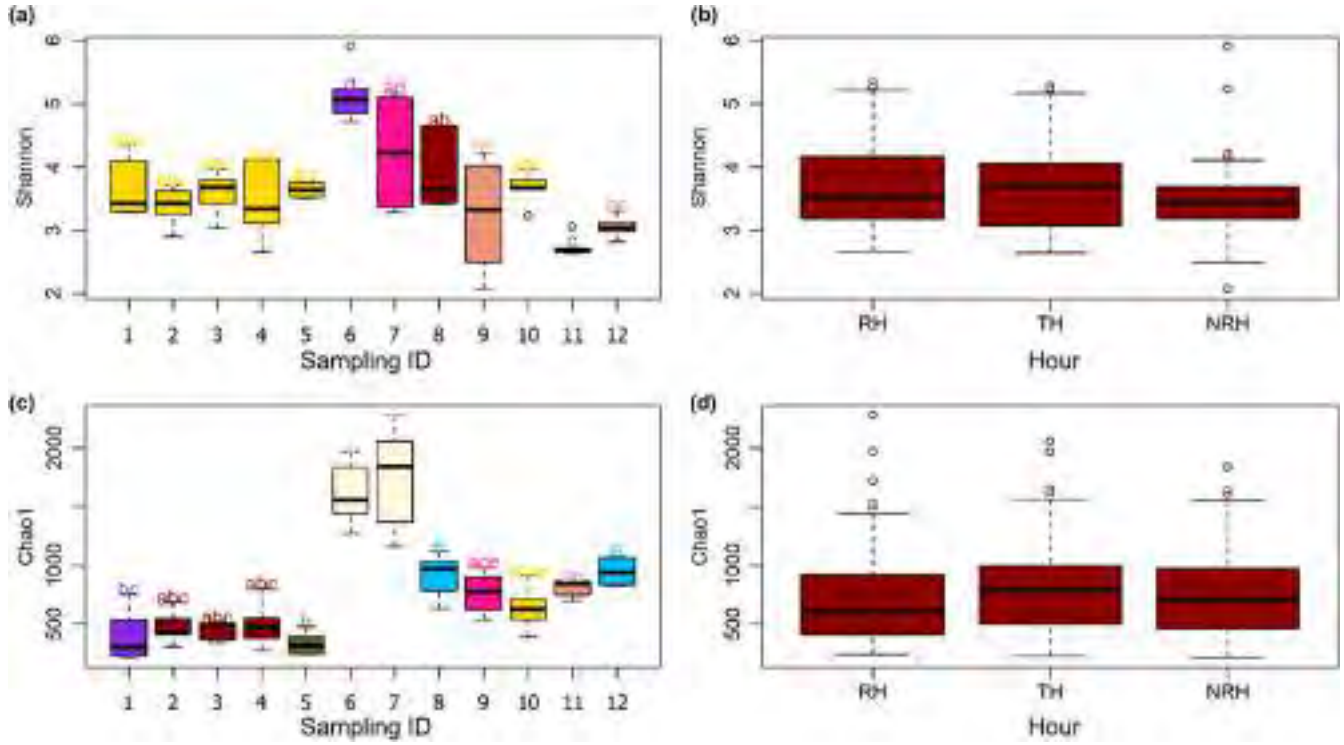


FIGURE 2 Fungal species diversity and richness at the MRT-SKV assessed by (a) Shannon's diversity index of fungal community across different sampling days, (b) Shannon's diversity index of fungal community for rush hour (RH), transitional hour (TH), and non-rush hour (NRH), (c) Chao1 diversity index of fungal community across different sampling days, and (d) Chao1 diversity index of fungal community for RH, TH, and NRH.

analysis based on the Bray–Curtis dissimilarity index revealed that the higher temporal distance appeared to be fundamentally associated with the reduced similarity between fungal communities in the MRT-SKV bioaerosols (Figure 3b,c), as shown by the further apart the sampling days, the higher degree of dissimilarity.

In addition to the fungal communities at the MRT-SKV, fungal communities present in bioaerosol samples from the Dongzhimen station in Beijing's subway system were also analyzed. *Ascomycota* (73.8%) was the dominant phyla found at the Dongzhimen station, followed by *Basidiomycota* (26.1%) and *Mucoromycota* (<0.1%) (Figure S2 in Appendix S3). At the genus level, 202 genera were found across the sampling period at the Dongzhimen station. In contrast to Bangkok MRT-SKV, *Aspergillus* (29.33%) was the most abundant genera detected at the Dongzhimen station, followed by *Cutaneotrichosporon* (17.58%), *Cladosporium* (11.79%), *Penicillium* (10.67%), and *Yarrowia* (10.59%).

LEfSe revealed that the abundance of 15 fungal genera identified from the MRT-SKV was significantly different from those of the Dongzhimen subway (Figure 4). Among these genera, 14 genera were highly enriched in the MRT-SKV (p value <0.05), including *Leptobacillium*, *Malassezia*, *Candida*, *Nigrospora*, and *Pseudopithomyces*. On the other hand, only the genera *Rhinocladiella* was found to be enriched in the Dongzhimen station (p value <0.05) compared to the MRT-SKV.

The metagenome data from subway and urban air samples in China (Hong Kong), Norway, Sweden, the United Kingdom, and the United States collected by the International Metagenomics and Metadesign of Subways and Urban Biomes (MetaSUB) Consortium (NCBI Sequence Read Archive under BioProject ID# PRJNA561080) were also included to compare the fungal composition with those identified from Bangkok MRT-SKV. A total of 63 genera were commonly found in bioaerosols from Thailand and the five countries investigated (Table S3 and Figure S3 in Appendix S3). These genera included *Alternaria*, *Aspergillus*, *Aureobasidium*, *Candida*, *Curvularia*, *Fusarium*, *Malassezia*, *Penicillium*, *Pleurotus*, and *Yarrowia* that were found in high abundance in the MRT-SKV. The most

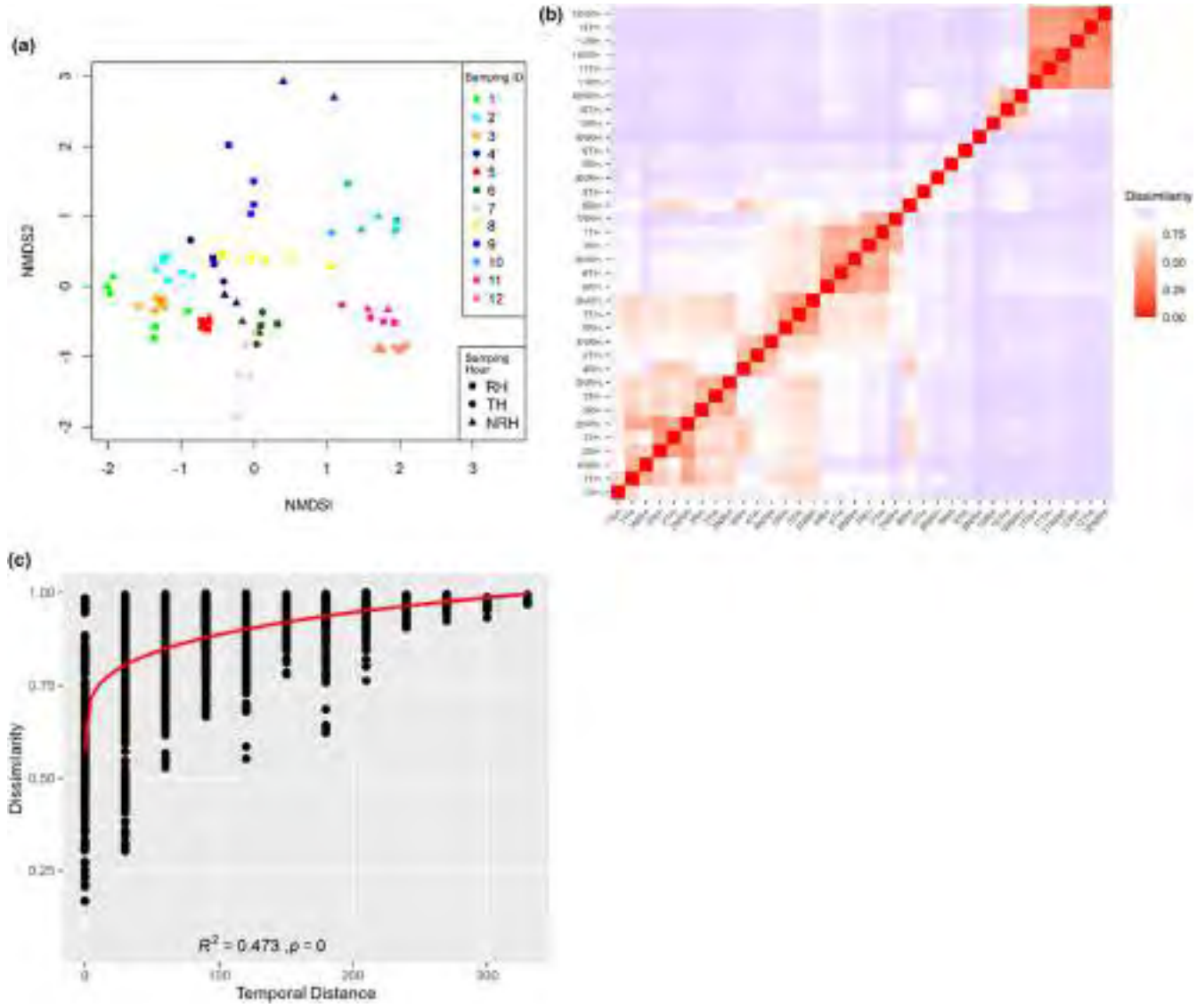


FIGURE 3 Dissimilarity analyses of fungal community in the bioaerosol samples at MRT-SKV station across different collection days. (a) Non-metric multidimensional scaling (NMDS), (b) pairwise dissimilarity, and (c) temporal distance-decay in fungal communities were shown. The sample name denoted the sampling ID (1, 2, 3, ..., 12) and hour of samplings conducted in rush hour (RH), transitional hour (TH), and non-rush hour (NRH) during the sampling period in December 2021–November 2022.

abundant genera in Hong Kong included *Malassezia*, *Schizophylum*, and *Candida*. In Norway, the abundant genera included *Malassezia*, *Botrytis*, and *Aspergillus*, whereas the abundant genera in Sweden included *Malassezia*, *Alternaria*, and *Botrytis*. *Malassezia*, *Ustaligo*, and *Botrytis* constituted major fungal genera in the United Kingdom. In the United States, *Ustaligo*, *Malassezia*, and *Alternaria* were the abundant fungi identified in Denver, while *Malassezia*, *Alternaria*, and *Aspergillus* were the abundant fungi identified in New York City.

3.1.2 | Allergenic fungal genera found in the MRT-SKV air environment

Since exposure to some fungi in the environment can lead to health concerns, especially allergic reactions, the fungal genera capable of inducing allergies were explored (Figure 5). We focused on fungi capable of producing allergens according to systematic allergen nomenclature approved by the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee (www.allergen.org) and allergenic fungi listed in Knutsen et al. (2012). A majority of fungal genera found at the MRT-SKV that can cause allergic reactions were those involved in IgE-mediated allergy, including *Alternaria*, *Aspergillus*, *Candida*, *Cladosporium*, *Curvularia*, *Epicoccum*, *Fusarium*, *Penicillium*, *Pleospora*, *Saccharomyces*, *Stachybotrys*, *Stemphylium*, and *Trichophyton* in the phylum *Ascomycota*, and *Malassezia* and *Rhodotorula* in the phylum *Basidiomycota* (Figure 5a).

Cladosporium, *Malassezia*, and *Aspergillus* were the main allergy-inducing fungi present in the bioaerosol samples collected

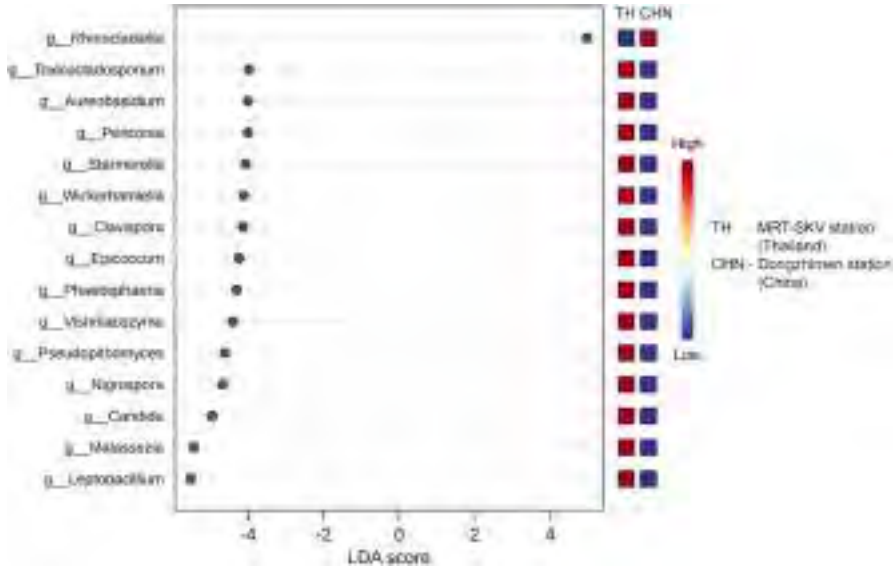


FIGURE 4 Differentially abundant fungal genera between Bangkok MRT-SKV station and Dongzhimen subway station assessed by the Linear discriminant analysis Effect Size (LEfSe). TH and CHN refer to the relative abundance of each differentially abundant fungal genus in the MRT-SKV station (in Thailand) and the Dongzhimen station (in P.R. China), respectively. The relative abundance is shown with heat map colors ranging from low abundance (blue) to high abundance (red). An absolute LDA score greater than 4 and with a p value <0.05 were used to determine significantly different abundance genera between the Bangkok MRT-SKV station and Dongzhimen subway station.

at Sukhumvit MRT station. Overall, the abundance of allergenic fungi was higher in the first seven samples (obtained in months of December 2021–June 2022) than in later samples (Figure 5a,b), although individual genus distributed differently in different samples. For example, while *Cladosporium* and *Malassezia* were more abundant in the first through seventh samples than in other samples, *Aspergillus*, *Alternaria*, *Stachybotrys*, and *Trichophyton* were predominant from the fifth to the eighth samples (obtained in April–July), and *Fusarium* and *Penicillium* were found mostly in the eleventh and twelfth samples (obtained in October and November).

Many of the allergy-causing fungi identified from the MRT-SKV, including *Alternaria*, *Aspergillus*, *Candida*, *Curvularia*, *Fusarium*, *Penicillium*, *Malassezia*, *Rhodotorula*, *Saccharomyces*, *Stemphylium*, and *Trichophyton* were also found in subway stations in China, Norway, Sweden, the United Kingdom, and the United States (Table S3 and Figure S3 in Appendix S3), implying that these may be common allergenic fungi in subway stations in many parts of the world. However, the abundance of these allergenic fungi differed among countries. *Cladosporium* was the most abundant allergenic fungi found in the MRT-SKV in Thailand, whereas *Malassezia* was the most abundant allergenic fungi found in subway stations in China (Hong Kong), Norway, Sweden, the United Kingdom, and the United States (New York City). Furthermore, *Ustilago* and *Aspergillus* were the most abundant allergenic fungi in USA's Denver and China's Beijing subway stations, respectively. *Beauveria* was the only allergy-inducing genus that was found in China, Norway, Sweden, the United Kingdom, and the United States, but not found in Thailand subway in this work.

3.1.3 | Different living niches, trophic modes, and environmental guilds of MRT-SKV air mycobiome

Fungal genera found in the MRT-SKV air samples were characterized according to their living niches, trophic modes, and environmental guilds as shown in Figure 6. For simple classification according to living niche, the fungi identified in the MRT-SKV may be broadly classified as either plant-associated, animal-associated, or other pathotrophs/saprotrophs. The results showed that a majority of fungi found in the MRT-SKV air environment samples were plant associated (Figure 6a), except that the bioaerosols in the seventh samples obtained in June were dominated by animal-associated fungi (especially *Cutaneotrichosporon*) and those in the ninth sample obtained in August were dominated by other pathotrophs/saprotrophs, (especially *Aspergillus*). When the trophic mode of fungal communities in different samples was assessed, fungi that were both pathotrophs and saprotrophs were found with high abundance in the first through seventh samples (obtained in December 2021–June 2022), whereas saprotrophs were found abundantly in the eighth through tenth samples (obtained in July–September), and pathotrophs were found abundantly in the eleventh and twelfth samples (obtained in October–November) (Figure 6b).

The environmental guilds of fungi obtained from the MRT-SKV could be divided into categories such as animal pathogens, plant pathogens, wood saprotrophs, undefined saprotrophs, endophytes, and so on, based on FUNGuild and FungalTraits assignments (Nguyen et al., 2016), and each fungal genera may be associated with a single guild or multiple guilds. It was found that, overall, the MRT-SKV air samples consisted of fungi with

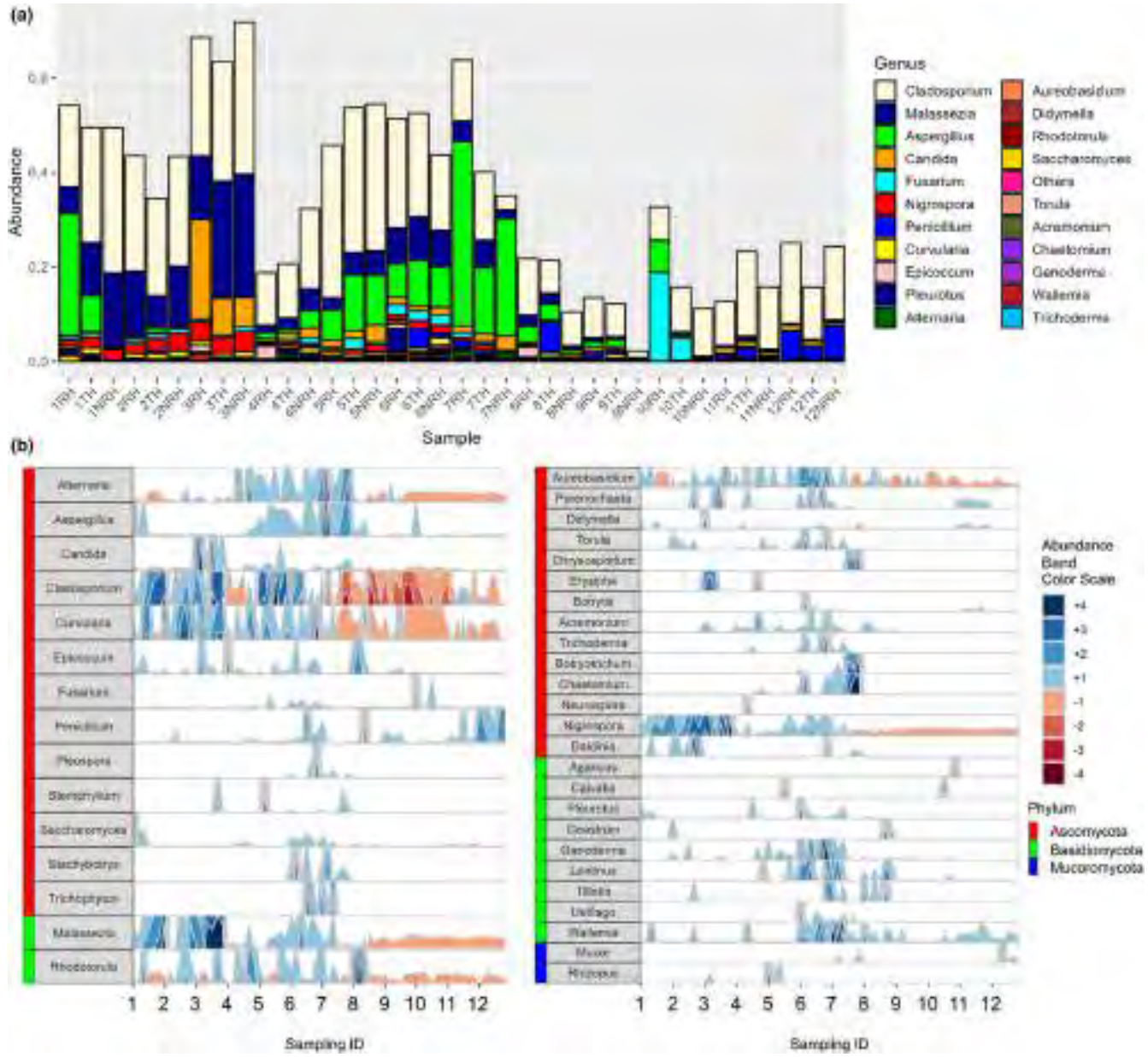


FIGURE 5 Distribution of allergenic fungal genera found in the MRT-SKV in different samples obtained at approximately 30-day interval shown as (a) relative abundance and (b) horizon plot displaying difference of relative abundance value from the median value. For the horizon plot in (b), when the relative abundance was above the median value, it would be shown in blue color. On the other hand, when the relative abundance was below the mean value, it would be shown in red color. The left panel in (b) showed horizon plot for allergenic fungi capable of producing allergens according to systematic allergen nomenclature (www.allergen.org), and the right panel showed horizon plot for additional allergenic fungi that were listed in Knutsen et al. (2012).

diverse functional guilds (Figure 6c). Saprotrophs, especially undefined saprotrophs and plant pathogens, contributed to the largest proportions of identified fungal genera in all MRT-SKV samples, followed by animal pathogens, wood saprotrophs, and endophytes. Meanwhile, the most abundant guild of fungal genera in the air samples of the Dongzhimen station was animal pathogens, followed by undefined saprotrophs, plant pathogens, and endophytes. Examples of the undefined saprotroph fungal genera identified from MRT-SKV were *Yarrowia* and *Nigrospora* belonging to the families *Phanerochaetaceae* and *Dipodascaceae*, respectively, whereas examples of plant pathogens found were *Hortaea* and *Curvularia* in the families *Teratosphaeriaceae* and *Pleosporaceae*, respectively. Examples of animal pathogens found were *Cutaneotrichosporon* and *Clavispora* in the families *Trichosporonaceae* and *Metschnikowiaceae*, respectively. It can also be seen that the fungal compositions in the tenth, eleventh and twelfth samples obtained in September–November 2022 were especially dominated by plant pathogens, whereas those in the seventh sample obtained in June were largely animal pathogens, and those in eight and ninth samples (obtained in July

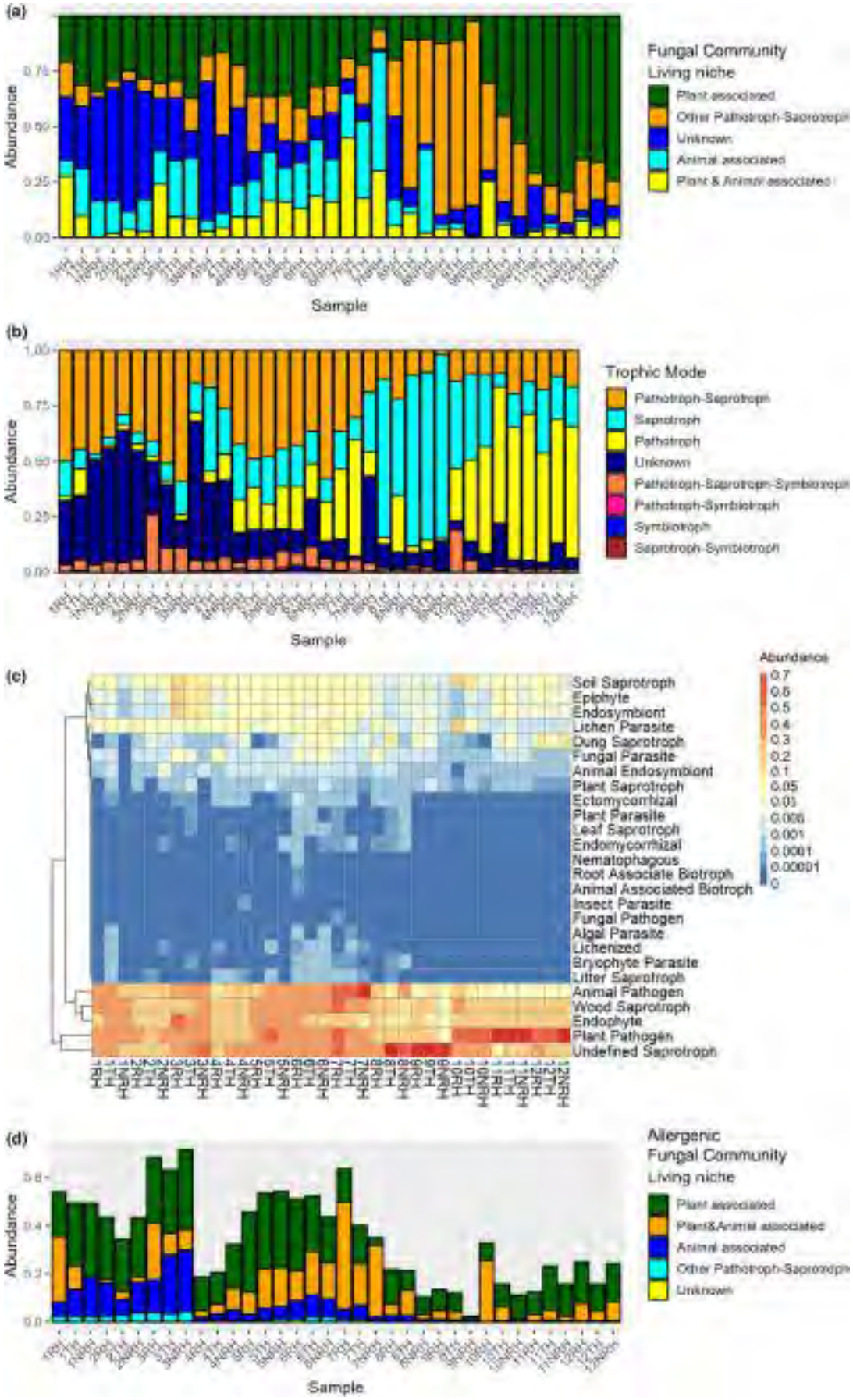


FIGURE 6 Characterization of fungal genera found in the MRT-SKV according to (a) living niche, (b) trophic mode, and (c) environmental guilds; (d) living niche of allergenic fungi found in the MRT-SKV.

and August) were chiefly dominated by undefined saprotrophs (Figure 6c).

With a focus on allergenic fungi, a majority of the identified fungal genera capable of causing allergy could be classified as plant associated (e.g., *Cladosporium*, *Curvularia*, and *Epicoccum*) or both plant and animal associated (e.g., *Alternaria*, *Aspergillus*, *Candida*, *Fusarium*, *Penicillium*, and *Rhodotorula*) that were found predominantly throughout the sampling period (Figure 6d). Some fungal genera associated with animals, especially *Malassezia* and *Cutaneotrichosporon*, were found mainly during the first and seventh samplings performed in December 2021–June 2022.

3.2 | Relationship between air quality variables and fungal communities

Air quality variables, including AT, RH, DP, WB, and particulate matter (PM) with a diameter <10 μm, were measured during samplings at the MRT-SKV. The ANOVA analysis with Tukey's test revealed that the number of particulate matters of all sizes recorded at the MRT-SKV did not show a significant difference across the sampling period investigated (Figure S4 in Appendix S4). In different hours, $PM_{2.5}$ and PM_{10} were significantly higher in RH than in TH and NRH. Interestingly, the amounts of PM of all sizes were lower in the NRH than in other hours. We also found that AT, DP, and WB were significantly higher during the third, tenth, and twelfth samplings (performed in February, September, and November, respectively) than in other samplings, whereas the RH was low during the second and eleventh samplings (performed in January and October, respectively). Across different hours of sampling, AT, RH, DP, and WB were not significantly different in the RH, TH, and NRH.

Distance-decay analysis with the Bray–Curtis dissimilarity revealed air quality variables significantly caused differences in the fungal communities found in the MRT-SKV air environment (Figure 7). The Mantel partial test also showed a significant correlation between the Bray–Curtis dissimilarity and differences in values of air variables (Table S4 in Appendix S4). The Bray–Curtis dissimilarity index was found to correlate with different traffic hours (RH, TH, and NRH) rather than different sampling days.

Remarkably, *Ascomycota* and *Basidiomycota* exhibited different correlations with particulate matters. The correlation analysis revealed that the relative abundance of *Ascomycota* displayed a positive correlation with PM, including $PM_{2.5}$ and PM_{10} (p value <0.05) (Figure 8a,c), whereas the relative abundance of *Basidiomycota* was negatively correlated with PM (Figure 8b,d).

The allergy-inducing fungal genera that displayed a correlation with air quality variables were subsequently assessed. Dynamic (rolling) correlation analysis showed that air quality variables exerted different effects on different allergy-inducing fungal genera over the sampling period (Figure 9). There is a general trend showing the abundance of *Alternaria*, *Aspergillus*, and *Candida* had mostly negative correlations with $PM_{2.5}$ and PM_{10} during the second and eighth samplings (done during January–July 2022), but they displayed positive correlations with $PM_{2.5}$ and PM_{10} during the ninth and eleventh samplings (done during August–October 2022) (Figure 9a,b). *Cladosporium*, *Epicoccum*, *Malassezia*, and *Rhodotoula* had negative correlations with $PM_{2.5}$ and PM_{10} during the second and fifth samplings (done during January–April 2022), but they displayed positive correlations with $PM_{2.5}$ and PM_{10} during the sixth and eleventh samplings (done during May–October 2022). Across the sampling period, *Curvularia* and *Penicillium* showed mostly positive correlations between their relative abundance and $PM_{2.5}$ and PM_{10}, except in the fifth sampling (done in April) when the correlation coefficient had negative values.

Since humidity (RH) and temperature (AT) can play a large role in fungal growth, dynamic correlations between allergy-inducing fungal genera and RH and AT were also accessed (Figure 9c,d). *Candida*, *Curvularia*, *Epicoccum*, and *Malassezia* exhibited a general trend of having mostly positive correlations with RH during the second and sixth samplings (during January–May 2022, which was the dry season in Thailand), and then having negative correlations with RH during the ninth and eleventh samplings (during August–October, which was the wet season in Thailand). Interestingly, *Fusarium* displayed positive correlation with RH during the wet season during August–October 2022 (as well as in March–May during the dry season), in contrast to other fungi that displayed negative correlations with RH during the wet season.

Candida and *Fusarium* had generally positive correlations with AT throughout the sampling periods, with *Candida* exhibiting the highest correlation coefficient during the second and third samplings occurring in January–February (when the temperature in Thailand is usually low), while *Fusarium* exhibited the highest correlation coefficient during the seventh and ninth samplings occurring in June–August. *Alternaria*, *Aspergillus*, *Cladosporium*, and *Malassezia* had negative correlations with AT during the fourth and sixth sampling occurring in March–May (when the temperature in Thailand is usually the highest), and then they displayed positive correlations with AT during the seventh and eleventh sampling occurring in June–October.

3.3 | Co-occurrence network of air mycobiome in the MRT-SKV environment

To explore the airborne fungi that were found together at the same time and may interact with one another in the MRT-SKV environment, the co-occurrence network was constructed to investigate relationships among fungal genera that were found >50% in the RH, TH, and NRH (Figure 10). Interestingly, *Alternaria*, *Aspergillus*, *Aureobasidium*, *Cladosporium*, *Curvularia*, *Epicoccum*, *Fusarium*, *Malassezia*, *Nigrospora*, *Phaeosphaeria*, and *Pseudopithomyces* were members of the core mycobiome of the MRT-SKV bioaerosols that were found in the co-occurrence networks in all hours investigated. *Malassezia* and *Nigrospora* were hubs of the network (having number of degrees greater than the 90th percentile) in every hour, and *Aspergillus* and *Trichosporon* were hubs in both TH and NRH, whereas *Phaeosphaeria* was a hub in the RH and TH.

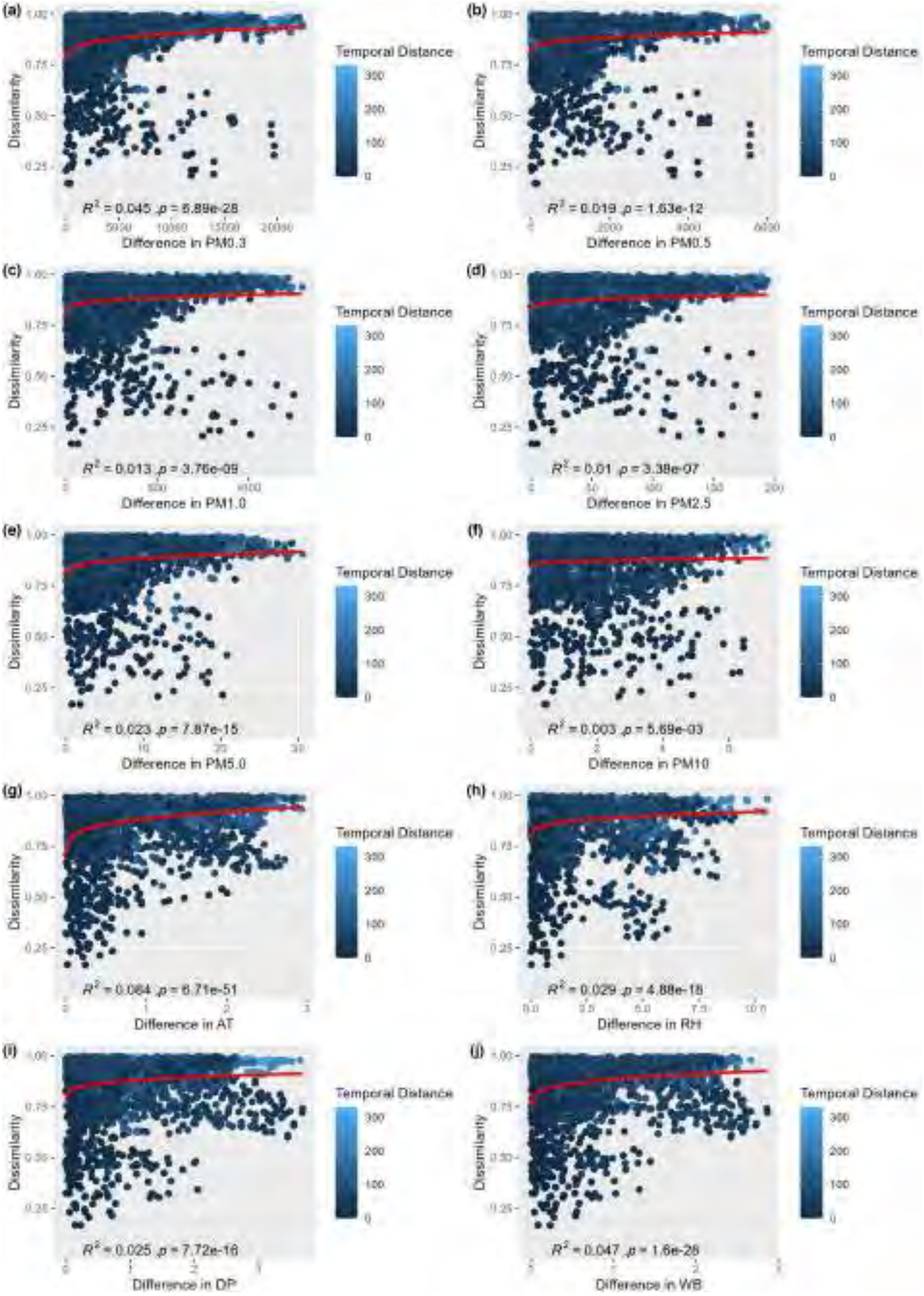


FIGURE 7 The distance-decay analysis showing relationship between the Bray–Curtis dissimilarity and differences in values of air quality variables.

Co-occurrence was seen between fungal genera capable of inducing allergy (shown in bold-type fonts in Figure 10). Especially, co-occurrence between *Malassezia–Nigrospora* pair and co-occurrence between *Alternaria–Aspergillus* pair were identified in all traffic hours. It can also be seen that the number of associations (edges) and density between allergy-inducing fungi was the highest in the RH and lowest in the TH. During the sampling period, co-occurrence between *Malassezia–Nigrospora* was seen most prominently in the RH (>88% prevalent in the RH) than in other hours, whereas co-occurrence between *Alternaria–Aspergillus* was

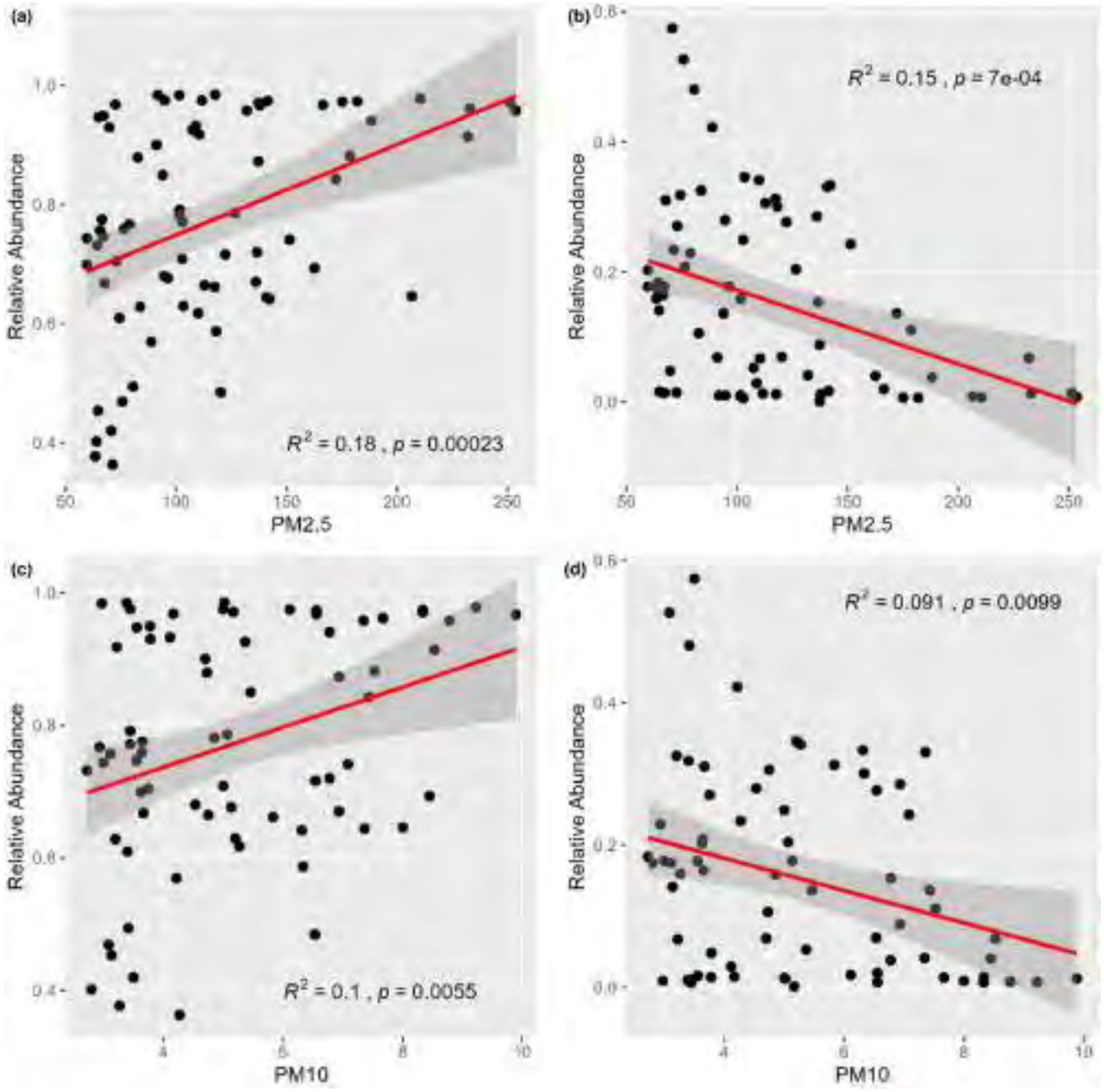
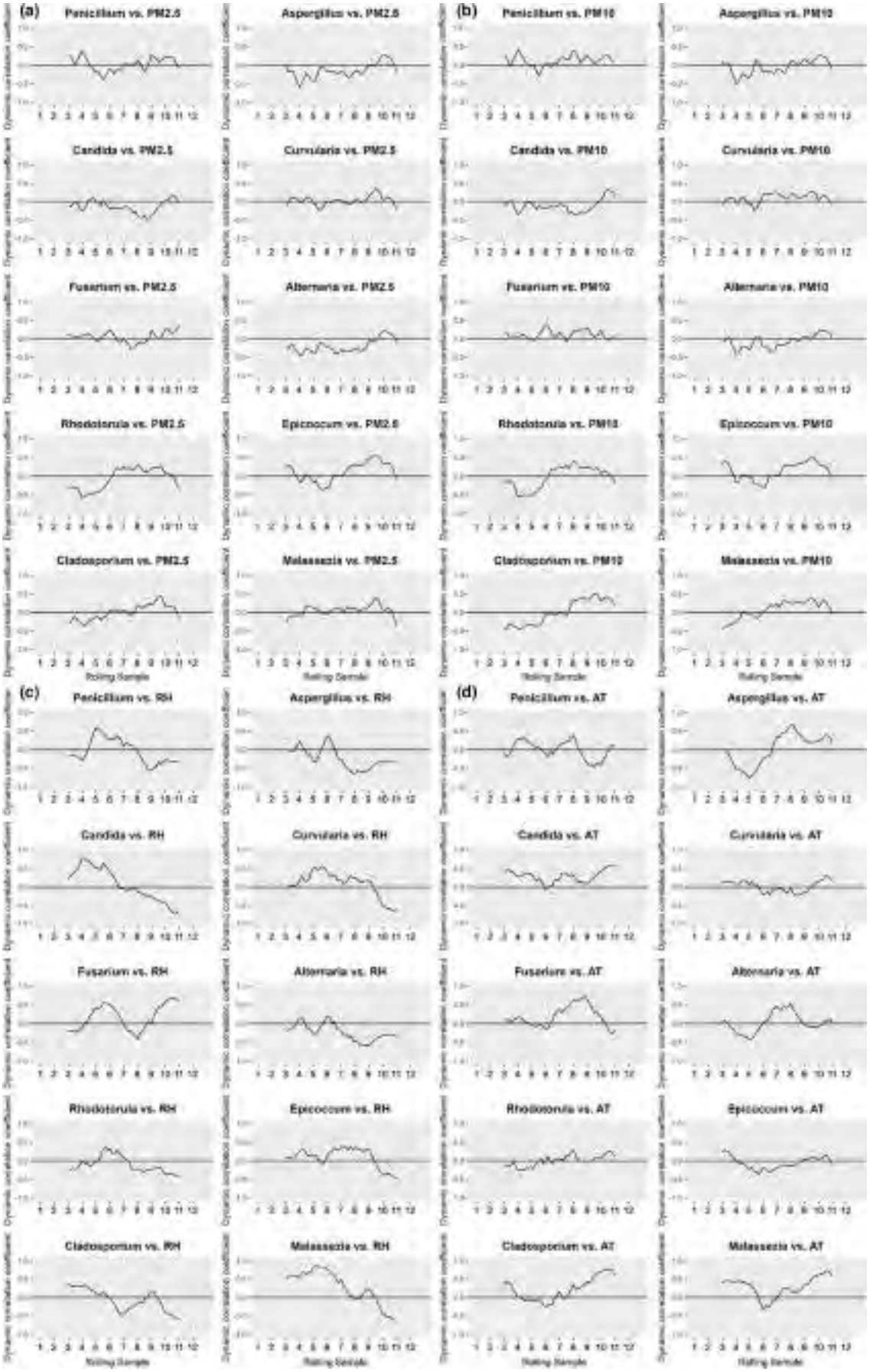


FIGURE 8 Correlations between fungal genera found in bioaerosols obtained at the MRT-SKV and PM. (a) Correlations between *Ascomycota* and $PM_{2.5}$ values, (b) correlations between *Basidiomycota* and $PM_{2.5}$ values, (c) correlations between *Ascomycota* and PM_{10} values, and (d) correlations between *Basidiomycota* and PM_{10} values.

FIGURE 9 Rolling correlations between the diversity of fungal genera capable of inducing allergic reactions and air quality variables in the MRT-SKV. Air quality variables assessed were (a) $PM_{2.5}$, (b) PM_{10}, (c) relative humidity, and (d) ambient temperature. The number on the *X*-axis showed rolling samples with window length = 24 (consisting of samples obtained from four consecutive samplings) and the number on the *Y*-axis showed dynamic correlation coefficients.

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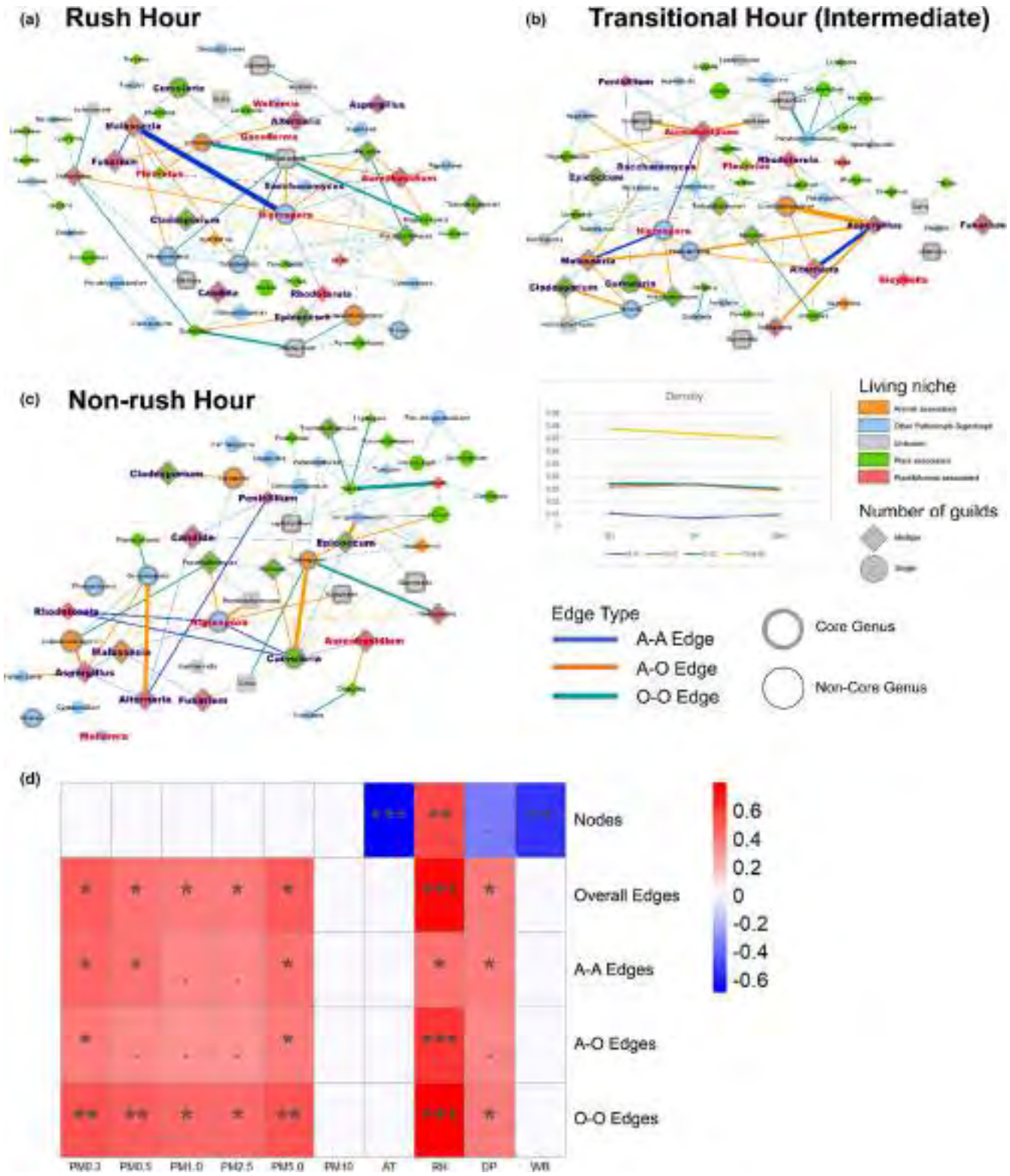


FIGURE 10 Co-occurrence networks of fungal genera in (a) rush hour, (b) transitional hour, and (c) non-rush hour. (d) Correlation of the number of edges and nodes in the co-occurrence network with air quality variables. Members of the core mycobiome in the MRT-SKV were shown with bold borders. Fungal genera that could be classified as plant associated, animal-associated, both plant and animal associated, and other pathogens and saprotrophs were shown in green, orange, red, and blue circles (associated with single guild) or diamonds (associated with multiple guilds), respectively. Allergenic fungal genera were written with bold-type font, with those capable of causing type I allergy shown in blue letters, whereas those causing other types of allergy shown in red letters. A–A edges are edges between allergenic fungi, A–O edges are edges between allergenic fungi and non-allergenic fungi, and O–O edges are edges between non-allergenic fungi. Edges that were highly prevalent (found >77%) are shown with thick lines. Correlations with p value <0.001, <0.01, <0.05, and <0.1 are shown with ***, **, *, and. symbols, respectively.

most prominent in the transitional hour (>77% prevalent in the transitional hour).

The fungal genera capable of inducing allergy were also found to co-occur with non-allergenic genera. During the sampling period, co-occurrence between *Aspergillus* (allergenic fungi)–*Cutaneotrichosporon* (non-allergenic fungi) and co-occurrence between *Curvularia* (allergenic fungi)–*Pseudopithomyces* (non-allergenic fungi) were seen most prominently in the transitional hour (>77% prevalent in the transitional hour) than in other hours. In addition, co-occurrences between *Alternaria* (allergenic fungi)–*Gelasinospora* (non-allergenic fungi), between *Curvularia* (allergenic fungi)–*Trichosporon* (non-allergenic fungi), and between *Epicoccum* (allergenic fungi)–*Sterigmatomyces* (non-allergenic fungi) were most prominent in the NRH (>77% prevalent in the NRH). In addition to the prominent co-occurring fungi mentioned above, the association between *Nigrospora* (allergenic fungi)–*Pseudopithomyces* (non-allergenic fungi) was found with >55% prevalence in every hour. Interestingly, the non-allergenic genera that were shown to correlate with allergy-inducing fungi the most (containing the highest degrees) included *Cystobasidium*, *Phosphoremia*, *Trichosporon*, and *Wickerhamiella*.

Co-occurrences between non-allergenic fungi were also observed. In the rush hour, prominent co-occurrence between *Trichosporon*and *Wickerhamiella* was identified (>77% prevalent in the RH). In addition, co-occurence between *Knufia*–*Phlebia* was observed (>77% prevalent in the NRH).

To further investigate environmental factors that may influence interactions between fungi that co-occur in the same hours, we explored the correlation of the number of edges in the co-occurrence network with air quality variables (Figure 10d). The number of nodes showed positive correlations with RH, but contained negative correlations with AT, DP, and WB. The number of edges (including edges between allergenic fungi, edges between allergenic and non-allergenic fungi, and edges between non-allergenic fungi) were positively correlated with RH, DP, and PM with size <10 μM ($PM_{0.3}$–$PM_{5.0}$). The results indicated that these air quality variables significantly affected the association among fungi that co-occur in the MRT-SKV air environment.

4 | DISCUSSION

4.1 | Temporal shift in the composition of airborne fungi communities in the MRT-SKV

This investigation of the fungal compositions in the MRT-SKV bioaerosols, employing ITS2 culture-independent metabarcoding approach coupled with bioinformatics analysis, is the first characterization of mycobiome characterization in a public subway station in Bangkok. This profiling of fungal diversity in the subway station is important not only because of fungal-mediated health concerns in built environments but also the need to understand the dynamic interaction among fungal species and the relationship between mycobiome and built environment, especially as there is an increasing reliance on public transportation in Bangkok. Furthermore, airborne fungal spores and mycelia can lead to several respiratory diseases such as allergy and asthma. The fungal flora of the MRT-SKV was found to consist of mainly *Ascomycota* and *Basidiomycota* during the investigated period in December 2021–November 2022 (Figure 1), consistent with the predominance of these two phyla being predominant fungi in the biosphere. These phyla were also reported to be dominant phyla found in the subway systems in New York City (Robertson et al., 2013), Athens (Grydaki et al., 2021), Seoul (Hassan et al., 2022), and Busan (Kim et al., 2022). A total of 63 genera, mostly in *Ascomycota* and *Basidiomycota* phyla, were commonly found in bioaerosols from Thailand and from the five other countries investigated (Table S3 and Figure S3 in Appendix S3), suggesting a majority of these genera are commonplace around the world.

Although the major phyla found in the MRT-SKV were relatively consistent across the sampling period spanning 12 months, distinct community patterns at the genus level could be observed in our samples obtained from different samplings. Notably, the fungal species diversity and richness at the MRT-SKV bioaerosols obtained from the sixth and seventh samples collected in May and June were higher than those of other samples. The high abundant of airborne fungal communities in these samples might result from the markedly jump in the numbers of subway passengers (Bangkok Expressway and Metro, 2023), most likely as a consequence of the major ease of COVID-19 travel restrictions starting from May 1, 2022 (Ministry of Foreign Affairs, 2022; TAT Newsroom, 2022). Interestingly, the fungal compositions found in different samplings could be generally grouped into three periods, (a) during the first and seventh samplings (December 2021–June 2022), (b) during the eighth and tenth samplings (July–September 2022), and (c) during the eleventh and twelfth samplings (October–November 2022) (Figures 1b and 3), which the fungal compositions changed from being dominated by *Cladosporium*–*Malassezia*–*Cutaneotrichosporon* genera in the first period to being dominated by *Yarrowia* in the second period, and *Hortaea* in the third period of samplings. This temporal shift in fungal compositions may be, at least in part, due to the seasonal effects as the country is under the influence of the "wet" southwest monsoon from mid-May to September and the "dry and cool" northeast monsoon from mid-October to mid-February (Songnuan et al., 2018; Thai Meteorological Department, 2010). This seasonal effect brought about the change in the climate that most likely affect the fungal composition in the air. For example, a high abundance of *Cladosporium* during the time of relatively low humidity in Bangkok (November–May) seen in this study and in a report by Songnuan et al. (2018) was similar to another report stating that *Cladosporium* tends to thrive in dry weather, especially when it is windy (Hjelmroos, 1993; Kasprzyk et al., 2016). However, the seasonal effect should still be explored in more detail with many factors involved (e.g., wind speed and precipitation) to understand its impact on the mycobiome of the MRT-SKV.

The COVID-19 situation can also have a large impact on the fungal composition and abundance in subway's bioaerosols, especially after

following practice of frequent cleaning and disinfection. Intensive hygiene, as well as increased antibiotic usage and other medication, during the COVID-19 pandemic are thought to negatively affect microbial diversity and accelerate microbiota loss (Finlay et al., 2021). On the other hand, the number of several pathogenic fungi may increase due to the stress on health care facilities causing the strain on the focus and resources needed to prevent and treat certain fungal infection. An example was shown by the report that there was a dramatic rise in the infection cases of *Candida auris*, a normally opportunistic yeast which is difficult to treat, in the United States in 2021 (Lyman et al., 2023). In addition, wearing face mask can influence the distribution of fungal species. A study by Park and colleagues found that when fungal species were isolated from face masks during the COVID-19 pandemic in Japan, a greater number (2.4 times) of fungal colonies were obtained from the outer-side than the face-side of the masks, which is in contrast to a greater number of bacterial colonies being isolated from the face-side of the masks (Park et al., 2022). A longer mask usage also significantly increased the number of fungal colonies but not the number of bacterial colonies. Their study showed that fungal cells such as *Alternaria*, *Aspergillus*, *Cladosporium*, *Malassezia*, and *Mucor* could adhere to the face marks. Thus, these fungal cells can potentially be transferred from the masks to hands and to subway surfaces such as handrails, kiosk buttons, and waiting chairs, especially as *Alternaria*, *Aspergillus*, *Cladosporium*, and *Malassezia* are the core fungal genera found in the MRT-SKV's bioaerosols. It should also be noted that, in this study, the samplings were performed at approximately 30-day interval during the 12-month period. At each sampling day, air samples were collected during the RH, TH, and NRH (for a total of 6 h in the day). Thus, the obtained data allowed both long-term (approximately every 30 days) and short-term (6-h period) time-series analysis of fungal compositions at the subway environment. The long-term analysis allowed investigation of discrete changes in fungal compositions over the 12 samplings, and the short-term analysis allowed investigation of dynamics in fungal compositions across different hours. However, as the presence of fungi in the subway station are thought to be jointly influenced by human sources and environmental sources (Adams et al., 2015), some degree of variation in the fungal compositions is expected to occur from 1 day to another, even within the same month. This day-to-day variation could be mitigated by multiple days of sampling within the month to allow for obtaining samples that well represent the fungal compositions within the month, enabling a fine-resolution picture of the fungal communities in the subway bioaerosols.

Among the fungal genera identified, 24 of these genera were considered "core mycobiome" of the MRT-SKV air environment, as they exhibited high level (≥85%) of prevalence throughout the sampling period. Members of the MRT-SKV core mycobiome that also exhibited high abundance level included *Yarrowia*, *Cladosporium*, *Cutaneotrichosporon*, *Leptobacillium*, *Malassezia*, and *Aspergillus*. The saprophyte *Yarrowia* constituted the most abundant genera found in the MRT-SKV bioaerosols. It comprises mostly oleaginous yeasts that have been studied extensively, especially as source of nutritional and bioactive compounds such as unsaturated fatty acids useful for industrial applications (Jach & Malm, 2022). *Yarrowia* is widespread in nature and can be isolated from a variety of environments such as soil, oil-contaminated soil, and seawater, as well as various food products such as meats, dairy products, and wines. The yeast is also found to reside commensally in the adult respiratory system, cutaneous nodules, and the digestive tract (Irby et al., 2014). *Cladosporium* was another abundant genus found in all air samples collected from the MRT-SKV. Besides being reported as the most abundant fungi in outdoor and indoor air worldwide as it can be found naturally in soil and on plant materials such as fading or dead leaves (Fröhlich-Nowoisky et al., 2012; Li, 2007), *Cladosporium* was also common fungi detected in indoor air in Bangkok (Kallawicha et al., 2019; Songnuan et al., 2018). *Cutaneotrichosporon* species are basidiomycetous yeasts often isolated from human and animal hosts as well as clinical samples, and they may act as opportunistic human pathogens (Aliyu et al., 2020). Many species in this genus are also oleaginous with the capability of lipid biosynthesis. *Leptobacillium* is another prominent fungal genus detected in the MRT-SKV. It belongs to the family *Cordycipitaceae* and has been reported to be entomopathogenic fungi that are parasites to many insects including mites (Casique-Valdés et al., 2022). *Malassezia* is a Basidiomycete and can be classified as animal pathogens or undefined saprotrophs. This genus is commonly known as single-cell yeast found commensally on the skin of humans and other animals and can sometimes act as opportunistic pathogens (White et al., 2014). It tends to colonize the skin of the head–neck–face region. *Aspergillus* constitutes a large group of common saprophytic fungi that can be isolated from soil, air, and plant materials, and it represents a dominant indoor pathogen (Merad et al., 2021).

The diversity of the identified fungi in the MRT-SKV can stem from spatial dependency and source–sink carry-over effect. Since skin-associated fungi (including *Aspergillus*, *Candida*, *Cutaneotrichosporon*, and *Malassezia*) were the abundant airborne fungi identified from the MRT-SKV, this suggested that human is one of the sources of airborne fungi in the subway system (Adams et al., 2015; Hospodsky et al., 2012). Besides the human-associated fungi, the MRT-SKV air environments also contained fungi that could be found in various environmental sources (e.g., compost, soil, outdoor air, and sediments), including *Yarrowia*, *Cladosporium*, *Fusarium*, and *Nigrospora*. Our results implied that the predominant fungi of MRT-SKV were human commensal flora or those present in outdoor environments that human beings carried into the subway system, and the abundance of these fungi depended on the season.

4.2 | Dynamics of allergenic fungi found in different samples of the MRT-SKV bioaerosols

Many fungal species are known to produce many types of allergens and cause allergic reactions in humans, especially type I (IgE), type III (IgG), and type IV (T cells) hypersensitivity, mostly through exposure to fungal spores (Simon-Nobbe et al., 2008). An investigation

of the distribution of allergenic fungi in the subway station is indispensable for effective preventative measures that reduce commuters' exposure to fungal allergens and are beneficial for allergic diagnostic treatment. A majority of fungal genera found at the MRT-SKV that were allergenic were members of *Ascomycota* (*Alternaria*, *Aspergillus*, *Candida*, *Cladosporium*, *Curvularia*, *Epicoccum*, *Fusarium*, *Penicillium*, *Pleospora*, *Saccharomyces*, *Stachybotrys*, *Stemphylium*, and *Trichophyton*) and *Basidiomycota* (*Malassezia* and *Rhodotorula*), and a large fraction of these genera were plant-associated or both plant- and animal-associated fungi (Figure 6). The high abundance of *Cladosporium* found throughout the sampling period in this study suggested that they represented a significant proportion of aerogens in the MRT-SKV air environment and could severely affect asthmatic commuters and patients with respiratory diseases. In addition, the increased level of *Penicillium* in winter period (approximately October–November) and *Aspergillus* in summer period (approximately April–June) was similar to the results found in Spain (de Ana et al., 2006). The relatively higher abundance of *Aspergillus* in summer may be partly because some of the *Aspergillus* species, including *A. fumigatus*, are considered thermotolerant and capable of growth at high temperatures up to 55°C that approach the upper limit for eukaryotes (Bhabhra & Askew, 2005). Many of the allergy-causing fungi (e.g., *Alternaria*, *Aspergillus*, *Candida*, *Curvularia*, *Fusarium*, *Penicillium*, *Rhodotorula*, *Saccharomyces*, *Stemphylium*, and *Trichophyton*) were not only identified from the MRT-SKV, but they were also detected in subway stations in China (Hong Kong), Norway, Sweden, the United Kingdom, and the United States (Table S3 in Appendix S3). This suggested that several allergenic fungi are common in subway stations in many parts of the world. This is unsurprising as these fungi are normally found in both outdoor and indoor environments worldwide.

Among the allergenic fungi found in the MRT-SKV, it has been reported that *Alternaria*, *Aspergillus*, *Cladosporium*, *Epicoccum*, and *Penicillium* have frequently been implicated in causing allergic asthma and allergic rhinitis, whereas *Malassezia* and *Saccharomyces* have been implicated as the cause of atopic dermatitis more than other allergic reactions (Simon-Nobbe et al., 2008). Allergic patients can be polysensitized with hypersensitivity to various fungi with symptoms of allergic rhinitis, asthma, and atopic dermatitis due to exposure to various fungal allergens. Examples of allergens produced by the fungi in the MRT-SKV may include Alt a 1, Cla h 8 (an NADP-dependent mannitol dehydrogenase), Asp f 1 (a ribonuclease), Pen b 13 (an alkaline serine protease), and Mal f 6 (putative cyclophilin) identified in *Alternaria* (e.g., *A. alternata*), *Cladosporium* (e.g., *C. herbarum*), *Aspergillus* (e.g., *A. fumigatus*), *Penicillium* (e.g., *P. citrinum*), and *Malassezia*, (e.g., *M. Furfur*), respectively (Simon-Nobbe et al., 2008). Allergic patients may also have cross reactions to allergens from several fungal species.

In addition to several fungal species being allergenic, many fungi also contain mycotoxins and occasionally infectious components that confer health impact on human beings (Mousavi et al., 2016). Many of the identified fungi were human commensal as they can be found on various mucosal surfaces on/in human bodies. However, they may become opportunistic pathogens that act on immunocompromised hosts. For example, *Cladosporium* can inhabit the oral cavity, lung, vagina, and intestine, while *Malassezia* can be found on the skin, as well as in the oral cavity, urinary tract, and breast milk (Santus et al., 2021).

4.3 | Environmental shaping of airborne fungal communities in the MRT-SKV

Differences in air quality variables can both directly and indirectly impact the concentration, structure, and taxonomy of air microbiome (Andrew et al., 2016). The accumulated data of air quality variables in this study showed the possible effect of PM and other air quality variables on fungal diversity over the sampling period. Our results resulted suggested that differences in values of PM, RH, and AT affected the dissimilarity in compositions of the fungi. Dong and colleagues reported that temperature, RH, and wind speed were key elements influencing airborne fungal diversity (Dong & Yao, 2010), as these factors might influence the fungal spores concentration and fungal growth. Remarkably, our analysis revealed that the abundance of *Ascomycota* found in the MRT-SKV positively correlated with PM including $PM_{2.5}$ and PM_{10}, while *Basidiomycota* negatively correlated with PM (Figure 8). Our results may indicate the association of fungal spores with particulate matters, similar to the report by Sun et al. (2021) showing fungi could attach to particles in the air, and fungal richness and diversity in hazy samples were significantly higher than those in non-hazy samples. As the spore sizes of the *Ascomycota* have been reported to be generally larger than those of the *Basidiomycota* (Elbert et al., 2007; Yamamoto et al., 2012), our findings suggested that the *Ascomycota* spores may attach more efficiently with PM than *Basidiomycota* spores do.

Different fungal genera in the MRT-SKV had different degrees of correlation with different air quality variables. For example, while *Fusarium* exhibited a positive correlation with RH during the ninth and eleventh samplings (performed in August–October, wet season in Thailand), *Candida*, *Curvularia*, *Epicoccum*, and *Malassezia* displayed mostly negative correlations during this period (Figure 9). This implies that *Fusarium* can grow better than *Candida*, *Curvularia*, *Epicoccum*, and *Malassezia* under highly humid conditions in the wet season in Thailand, consistent with findings that *Fusarium* spores are produced and mature when RH was ≥85%, and the spore production and maturation increase with RH (Manstretta & Rossi, 2016). The general trend of many fungi (including *Alternaria*, *Aspergillus*, *Cladosporium*, *Epicoccum*, and *Malassezia*) having negative correlations with AT during March–May (fourth and sixth samplings), which was the hottest period of summer in Thailand, implied that the temperature in the summer was too high to favorably induce growth and spore formation of these fungi, and the temperature in the cooler months in Thailand (especially August–February) would be better suited for the growth of these fungi in Thailand. This is consistent with the finding that the concentrations of fungal spores in Thailand usually peak around August–November (Songnuan et al., 2018).

It is also most likely that multiple air variables simultaneously affect the abundance of airborne fungi. For example, the release of *Cladosporium* spores is dependent on fluctuations in humidity and triggered particularly by a rapid drop in humidity with warm temperatures (e.g., sunny days following previous rainfalls; Knutsen et al., 2012). Sporulation of *Alternaria* is dependent on light rain or heavy dew and the spore counts are higher in warm temperatures. However, its airborne spore levels have been shown to decrease with high precipitation level (Knutsen et al., 2012). The results also showed that, although fungi require moisture for growth, different fungal genera and species may have different optimal range of RH that allows a high rate of growth (Bonner & Fergus, 1960). Other meteorological factors, such as wind speed and level of pollutants in the air, may also affect the abundance of different fungal genera. For example, the presence of hydrocarbon pollutants may favor the growth of certain fungi that can utilize these hydrocarbons for growth.

4.4 | Contrasting co-occurrence patterns among the fungal genera in different traffic hours

Investigation of a fungal co-occurrence network approach revealed the existence of several connected fungal genera in MRT-SKV. Our co-occurrence results demonstrated that the networks were complex and dynamic, indicating that the association between fungi found in the MRT-SKV bioaerosols was not static and involved many interplays among many fungi present in the environment. The co-occurrence among airborne fungi in the Bangkok subway station harbored more positive links than negative links. The positive interaction among these genera indicated they had cooperative interactions that can be mutualism or altruism.

The allergenic fungal genera were also present prominently in the co-occurrence networks. Among different hours, a majority of these genera were found to co-occur in higher abundance in the RH than in other hours. As the RH was associated with the high number of commuters and an increase in the amounts of PM, this suggested that the more allergenic fungi are brought into the subway system, the more interactions they formed with other fungi. Particular close attention should be paid to *Aspergillus*, *Aureobasidium*, *Cladosporium*, *Curvularia*, *Epicoccum*, *Fusarium*, *Malassezia*, and *Nigrospora*, which are core members of the MRT-SKV air environment that co-occurred with other fungal genera in all hours investigated. The association between *Malassezia* and *Nigrospora* was found with high prevalence in all hours, with the association being particularly prominent in RH. The association between them may involve lipid metabolism and utilization, as *Malassezia* species are commensal basidiomycete yeasts generally found on human and animal skin, and they are lipophilic and lipid-dependent yeasts (Triana et al., 2017), whereas several *Nigrospora* species are oleaginous endophytic fungi that can accumulate lipids and could be isolated from various oleaginous plants (Rossi et al., 2011). *Aspergillus* spp. and *Alternaria* spp. were also found to co-occur predominantly in the network. Both of these genera harbor allergy-inducing species that are abundant in both indoor and outdoor environments. They are also fungal taxa found colonizing animal's oral, pulmonary, intestinal, or cutaneous locations. An example of interaction between *Aspergillus* and *Alternaria* was shown when spores of *Alternaria alternata* and *Aspergillus parasiticus* were co-inoculated in sunflower seeds (Dalcero et al., 1989). Even though *A. alternata* did not affect the colonization rate of *A. parasiticus* on the seeds, a remarkable decrease in aflatoxin production by *A. parasiticus* was observed, indicating *A. alternata* could inhibit aflatoxin biosynthesis, degraded the aflatoxins, or competed with *A. parasiticus* for aflatoxin biosynthesis precursors. In the co-occurrence network, an association was also seen prominently between *Aspergillus* (allergenic fungi) and *Cutaneotrichosporon* (non-allergenic fungi), which might partly reflect the commensality of both fungal genera on/in the human body. In addition, as several species of *Cutaneotrichosporon* were considered oleaginous, their production of fatty acids may influence the growth and metabolism of *Aspergillus*. For example, fatty acids were found to take part in the regulation of the development, seed colonization, and mycotoxin production by *Aspergillus* spp. (Calvo et al., 1999). The probable interactions among these fungi suggested by the co-occurrence network can be further studied in more details focusing on their impact on human health, as these fungi can act as opportunistic pathogens, and their impact on biotechnology applications in industry, especially as *Nigrospora* and *Cutaneotrichosporon* are recognized as oleaginous fungi.

The co-occurrence among allergenic fungi in the MRT-SKV environment was also found to be positively affected by RH and PM with sizes <10 μm (Figure 10d). The result suggested that even though humidity may exert little or mostly negative effect on certain fungi (e.g., *Alternaria* and *Aspergillus* in Figure 9d), it exerted a significant effect on the association among fungi in mycobiome communities as a whole. Thus, to gain a more complete understanding of the relation between air variables and fungi, we need to take into account the interactions among all fungi in the mycobiome.

5 | CONCLUSION

In summary, this work revealed temporal changes in the diversity and abundance of fungi in Bangkok's subway station. *Ascomycota* and *Basidiomycota* were found to be dominant fungal phyla similar to those found in many subway stations in other countries. Twenty-four fungal genera were considered "core mycobiome" of the MRT-SKV air environment, as they were identified with high levels of prevalence (≥85%) throughout the sampling period. The core genera included *Yarrowia*, *Cladosporium*, *Cutaneotrichosporon*, *Leptobacillium*, *Malassezia*, and *Aspergillus*, which were also found with high abundance levels. A temporal shift of abundant fungal genera was observed, with *Cladosporium*, *Malassezia*, and *Cutaneotrichosporon* displaying high abundance during samplings done in December 2021–June 2022. Then, *Yarrowia* and *Hortaea* were dominant during samplings done in July–September 2022 and October–November

2022, respectively. This temporal shift in fungal compositions may be, at least in part, due to the seasonal effects. Many fungal genera capable of inducing allergic reactions were identified, including *Alternaria*, *Aspergillus*, *Candida*, *Cladosporium*, *Curvularia*, *Epicoccum*, *Fusarium*, *Malassezia*, *Penicillium*, *Pleospora*, *Rhodotorula*, *Saccharomyces*, *Stachybotrys*, *Stemphylium*, and *Trichophyton*, with *Cladosporium* being the most abundant allergenic fungi found in the MRT-SKV. The fungal compositions in the MRT-SKV bioaerosols were also affected by several air quality variables including PM, temperature, and RH. For example, the abundance of *Ascomycota* was positively correlated with PMs, whereas *Basidiomycota* abundance showed a negative correlation with PMs. Finally, certain fungal genera were shown to co-occur during the sampling hours. Many members of the core mycobiome formed a hub of co-occurrence network in all sampling hours and allergenic fungal genera formed networks more prominently in RH than in other hours. Our results showed that the detection and characterization of fungi in atmospheric aerosol samples of the subway station by DNA analysis facilitate understanding the impact of these fungi on the health of millions of commutes and can further help in elucidating the regional and global spread and diversity of fungi.

AUTHOR CONTRIBUTIONS

SI and LE conceived idea. SI acquired funding and supervised the study. SI, LE, LC, PH, SL, XZ, and JM designed main experiments. PH, PS, PP, SL, SM, XZ, JM, and SI collected samples. PP and PH performed genomic DNA extraction and ITS metabarcode library preparation. PS, SL, and SI carried out bioinformatics work. PH, PS, SL, XZ, JM, YG, LC, and SI analyzed and interpreted the data. PH, PS, and SI provided original draft of the manuscript and all authors contributed to the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data are deposited and are publicly available at the NCBI Sequence Read Archive under Bioproject PRJNA953786.

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