Annals of Agri-Bio Research **30** (2): 74–84, 2025

Impact Score: **0.4**
(Scopus)

Molecular Evaluation of Soil Fungi in Sport Field and Old Revegetation Sites in Urban Green Spaces, Surabaya, Indonesia

Intan Ayu Pratiwi *, Devida Thalia Salsabella, Annisa Nur Fatimah, Anisa Selvia Widyar Iswara, Mochammad Aqilah Herdiansyah, Sucipto Hariyanto, Bambang Irawan, Thin Soedarti and Muhammad Hilman Fuadil Amin

Department of Biology, Faculty of Science and Technology, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Surabaya 60115, Indonesia
*(e-mail: intan.ayu.pratiwi@fst.unair.ac.id; Mobile: +62-85732501861)

(Received: 27 May 2025; Accepted: 27 August 2025)

ABSTRACT

Fungi are one of the most common microorganisms dominating soil communities. Currently, soil ecosystem has changed due to anthropogenic activities. One of the efforts to stabilize the ecosystem is urban green spaces that covered by vegetation. There are still few studies that discuss the comparison of the presence of fungi in Surabaya Urban Green Space. This study aimed to quantify fungal colony abundance, characterize species composition, and assess the associations between soil physicochemical properties, soil texture, and vegetation with fungal occurrence in both study sites. This research was conducted to reveal differences in the presence of fungi in sport field and old revegetation site. Soil samples were collected and isolated fungi were identified using macroscopic and microscopic characteristics. Dominant filamentous fungi were subjected to further analysis through molecular identification using ITS5 and ITS4 markers. Our results analysis showed that soil sample from sports field yielded 2.61 ± 0.83 log CFU/g, whereas old revegetation site yielded 0.96 ± 0 log CFU/g. Differences in soil physical, chemical structures, and vegetation, influenced the presence of filamentous fungi at both sites. The common fungal genera identified in sport field and old revegetation area were: *Aspergillus, Penicillium, Acremonium, Trichoderma*. *Culvularia* and *Cunninghamela* were found in sport field and *Paelomyces* and *Chrysosporium* isolated from old revegetation area. The dominant species in sports field were *Aspergillus niger* and *Penicillium citrinum*, whereas the dominant species in old revegetation area were *Aspergillus parasiticus, Penicillium shearii*, and *Penicillium simplicissimum*.

Key words: fungi, molecular evaluation, surabaya, urban green space

INTRODUCTION

Microorganisms form most of the biodiversity on earth and can be found anywhere, including soil which is an important ecosystem in the terrestrial biome (Tedersoo et al., 2021). Fungi are one of the microorganisms that dominate many soil communities, playing key roles in various ecological services, such as nitrogen cycling, nutrient balance, and also organic substance decomposition (Orgiazzi et al., 2012; Waring et al., 2013; Pollierer et al., 2015; Ao et al., 2022). Beside of its roles as the ecological services, some study also revealed that several fungi can produced a secondary metabolites compound as their excretion and can be used as medicinal things (Herdiansyah et al., 2024; Zainul et al., 2024). The difference of substrates causes different types of fungi, as well as the difference of environmental conditions such as climate change, soil moisture, temperature, pH of soil, and sunlight intensity (Casu et al., 2024).

The presence of fungi in soil influenced by various factors. Vegetation can influence the presence of fungi in the soil through leaf litter and root exudates which can provide carbon substrates (Santonja et al., 2017). Besides the type of vegetation can influence soil conditions, thereby affecting the type of soil fungi (Canini et al., 2019; Zeng et al., 2020; Han et al., 2021). The distribution of fungi is influenced by different habitat condition, natural habitats showed the most varied genus. Mangrove habitats have the most varied genus diversity compared to land that has changed function. Apart from that, soil chemical factors such as pH, temperature, and humidity can also influence the presence of fungi regarding their adaptability (Fierer et al., 2017; Chen et al., 2020; Buyer et al., 2011; Han et al., 2021). As well as soil physical factors that can influence the ability of fungal mycelium to spread in the soil (Pratiwi and Irawan 2018; Zuev et al., 2019; Xia et al., 2020).

Nowadays, various land ecosystems have experienced urbanization to make human life easier. Some urban spaces are maintained green in the midst of the urbanization process creating Urban Green Space. Urban Green Space can be defined as land in cities that is partially or completely covered by vegetation, such as grass, trees, and other (De Haas et al., 2021).

Several studies have reported the presence of fungal communities in one type of green urban space such as sports ground (Kutovaya et al., 2014), urban forests (Ao et al., 2022), or parks (Ramirez et al., 2014; Zhang et al., 2021). There are still few studies that discuss the comparison of the presence of fungi in more than one type of Urban Green Space. This research was conducted to reveal differences in the presence of fungi in two types of urban spaces, sport field and old revegetation site. Sport field is area with grass monocultures of cultivated lawns and old revegetation site is area have above 10 years of native plant species revegetation (Baruch et al., 2020). The urban space studied is at the Faculty of Science and Technology, Airlangga University, Indonesia, an urbanization area with several lands that have green space. Fungal identification was carried out using two methods, morphological and molecular identification using ITS sequencing for fungal colonies that were found lots in each location. This study aimed to quantify fungal colony abundance, characterize species composition, and assess the associations between soil physicochemical properties, soil texture, and vegetation with fungal occurrence in both study sites. Molecular profiling was conducted to determine fungal density, compare community composition, perform sequencing, and identify species at the molecular level. The findings are expected to provide valuable information on the spatial distribution of soil fungi in Surabaya and to support the advancement of molecular microbiology research on soil microorganisms.

MATERIAL AND METHODS

Soil Collection and Preparation

Soil samples were collected from Faculty of Science and Technology, Airlangga University, Surabaya, Indonesia. The urban green space types were sport field (grass monoculture vegetation) and old revegetation sites (>10 years native plants species revegetation). Samples were taken using purposive sampling by taking 3 plots with 3 repetitions along an imaginary diagonal line on each area. Soil was sampled 500 g each plot from 6 points within 25 cm × 25 cm quadrat, collected and homogenised in a sterilised container. 25 g subsamples were used for fungi analysis until DNA extraction while 250 g was for soil analysis (Wagner et al., 2015).

Soil Sample Analysis

The soil substrate composition test is needed to determine the soil type, as it directly influences soil porosity, which in turn impacts the spread of filamentous fungi hyphae. The soil substrate composition test uses mesh or sieve numbers 5, 10, 40, 60, and 200. Soil designated for the substrate composition test is first placed in a basin and thoroughly saturated with water. The soil deposited on the filter paper is then subjected to oven-drying at 70 °C for a duration of 5–7 days to eliminate moisture content. Subsequent to drying, the weight of the filter paper containing the soil is measured. The weight of soil passing through each mesh size is determined by subtracting the post-oven weight from the initial weight of the filter paper (Husain et al., 2024).

Isolation and Identification

Isolation of fungi from soil samples was conducted using an indirect isolation technique involving sample dilution. A total of 25 g of soil was mixed with 225 mL of physiological saline solution to achieve a 10^{-1} dilution in culture bottles, which were then shaken for 8 h. Subsequently, the samples were allowed to settle. Further dilution to 10^{-2} was achieved by transferring 1 mL of the 10^{-1} dilution into a test tube containing 9 mL of physiological saline solution. This process was repeated until a 10-7 dilution was obtained. To identify morphology of fungi, all isolated fungi were incubated for 7 days at room temperature (23–25 °C) onto Potato Dextrose Agar (PDA). The identification involved the macroscopic character and microscopic characters (Ferdinandez et al. 2021, Sciortino 2017). This character identification needed to get microecological fungal diversity (Tedersoo et al., 2021).

DNA Extraction

The molecular identification of the dominants fungal species was performed. Fungal DNA was obtained from mycelium production. Pure fungal isolates were cultured on potato dextrose agar (PDA) medium slanted in test tubes for 5–7 days at room temperature. Approximately 40 mg of mycelium formed was transferred to Eppendorf tubes containing 300 µL of EDTA. The mycelium was then lysed either by sonication or by grinding. DNA isolation and extraction were conducted using the Promega Kit. The lysed mycelium was centrifuged at 13,000 rpm for 3 min. After centrifugation, the supernatant was discarded, and the pellet was resuspended in 600 µL of Nuclei Lysis Solution and vortexed for 1–3 s. The mixture was then incubated for 15 min at 65 °C. Following

incubation, 3 µL of RNAase was added to the lysate and homogenized by gently inverting the Eppendorf tube 2–5 times. The mixture was incubated for 15 min at 37 °C. After incubation, the sample was left at room temperature for 5 min before the next step. Subsequently, 200 µL of protein precipitation solution was added and vortexed at the highest speed for 20 s. The mixture was centrifuged for 5 min at 13,000 rpm. Following centrifugation, the supernatant containing DNA was transferred to a microtube containing 600 µL of isopropanol at room temperature. The solution was gently inverted until a white thread-like DNA precipitate formed. The solution was then centrifuged at 13,000 rpm for 5 min. The supernatant was discarded, and the DNA was washed with 500 µL of 70% ethanol at room temperature. The DNA was then air-dried and rehydrated with 100 µL of DNA rehydration solution and incubated at 65 °C for 1 h. The isolated DNA was stored in a freezer at −20 °C.

The purity of the isolated DNA was checked using a spectrophotometer. DNA samples and TE buffer or distilled water as a blank solution were loaded into individual wells of a µDropTM Plate and inserted into the Thermo Scientific Multitasker Go device. Absorbance values were read at λ = 260 nm and λ = 280 nm. The ideal DNA purity range is approximately 1.8–2.0, as DNA absorbs light at λ = 260 nm, while proteins and other contaminants absorb light at λ = 280 nm (Wagner et al., 2015).

PCR Amplification

The obtained DNA was amplified using PROMEGA. The gene utilized as a marker for fungi was ITS (Internal Transcribed Spacer). The primers employed for PCR were ITS5 and ITS4. PCR was conducted using a reaction mixture comprising Promega PCR mix cocktail (12.5 µL), ddH2O (7.5 µL), Primer forward (ITS5) (1.25 µL), Primer reverse (ITS4) (1.25 µL), and template DNA (2.5 µL), resulting in a total PCR reaction volume of 25 µL. The DNA amplification steps were as follows: initial denaturation at 94 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 45 s, annealing at 48.7 °C for 45 s, and extension at 72 °C for 30 s, with a final extension step at 72 °C for 10 min.

The amplified products were examined using gel electrophoresis. A volume of 3 µL of 100 bp DNA marker was taken and mixed with 1 µL of loading dye. This mixture was then resuspended and loaded into wells of a 1% agarose gel. Subsequently, 3 µL of the PCR product was loaded into wells containing the 1% agarose gel. Electrophoresis was conducted for 30 min at 50 volts. Following electrophoresis, the gel was stained with Diamond Dye solution for 15 min. Diamond Dye is a fluorescent stain capable of binding to single-stranded DNA, double-stranded DNA, and RNA, thereby facilitating the staining and visualization of nucleic acids within the gel. The stained gel was observed under UV transillu-mination, revealing orange-colored bands.

Sequencing

The successfully amplified PCR products were sent to Korea for Sanger sequencing. The sequencing process is essential for determining the nucleotide base sequence of a PCR-generated DNA molecule. The sequencing results from Singapore, consisting of two nucleotide sequences obtained from PCR with forward and reverse primers, were aligned using BioEdit software. Alignment produced a single sequence of DNA nucleotides, which was then compared with the Basic Local Alignment Search Tool (BLAST) in GenBank to verify the type of fungi. ITS (Internal Transcribed Spacer) sequence divergence values <3% to 5% were used to indicate consistency within the Kingdom Fungi. Acceptable results are those showing a query coverage of 80% with sequence identity of >97%.

Data Analysis

The analysis included enumeration of filamentous fungal colonies, morphological characterization of specimens at both macroscopic and microscopic levels, supplemented by molecular identification based on sequencing data of the most abundant species found in both sites. The initial data included the calculation of the average number of filamentous fungi colonies at each dilution level. Additionally, the total filamentous fungi colony count was determined using the Total Plate Count (TPC) method. The formula for TPC referred to Arantika et al. (2019), which is as follows:

$$\text{CFU/ g} = \frac{\text{Average fungal colony}}{\text{Inoculum volume} \times \text{Dilution level}}$$

Statistical analyses were performed using a t-test (GraphPad Prism 8.0.1) with 95% confidence level to evaluate differences in temperature, pH, and fungal abundance between sport field and old revegetation site. Subsequently, morphological identification was conducted at both macroscopic and

microscopic levels. Molecular identification was only performed dominant isolates with the highest colony counts. Molecular identification began with DNA extraction using the PROMEGA Kit to separate DNA from other substances such as proteins, lipids, and carbohydrates. The purity of the isolated DNA was assessed through DNA purification testing using a spectrophotometer at wavelengths of 260 nm and 280 nm. Acceptable DNA purity ranges from 1.8 to 2. Following this, PCR was conducted to amplify the desired DNA region using the ITS 5F/4R primer pair. The PCR results were visualized through electrophoresis, which separates DNA molecules using a porous gel medium under an electric field. PCR results meeting the criteria were sent to Singapore for sequencing to determine the composition and nucleotide sequence of the extracted DNA. The sequencing results were aligned using BioEdit to obtain the actual DNA nucleotide sequence. Analysis of the alignment results was conducted online using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi accessed on 26 July 2022).

RESULTS AND DISCUSSION

Soil Characteristics

Table 1 shows the physical and chemical properties of the soil in each sampling location. The average temperature in the sports field site is significantly higher than in the old revegetation site (p = 0.0194) because the sports field site is not covered by shade and is exposed to direct sunlight, whereas the old revegetation site is shaded by vegetation shade. Filamentous fungi can grow at temperatures ranging from 1 °C to 35 °C, with an optimum temperature of 25 °C to 30 °C (Andreas et al., 2023; Amorim et al., 2020). The pH in sports field and old revegetation site is not significantly different (p = 0.8017). However, in plot 1, the pH in the sports field is highly acidic, with a value of 3.8. This is because plot 1 is located where water drains, resulting in the accumulation of organic acids and causing the soil pH to become acidic. According to Ma et al. (2022), temperature and pH in nature are not correlated with the diversity of filamentous fungi. The humidity in all plots indicates 100%, which can support the survival of filamentous fungi.

Table 1. Soil physical chemistry at sport field and old revegetation.

Site	Plot	Parameter					
		Temperature (°C)	pH	Moisture (%)	Light intensity	Humidity (%)	Microhabitat
Sport Field	1	30 °C	3.8	100%	2307	51%	Covered in shade, grassy
	2	36 °C	5.6	100%	13,400	56%	Not covered by shade, grassy
	3	32 °C	5.7	100%	3098	53%	Covered in shade, grassy
Old revegetation	1	26 °C	5.0	100%	1194	60.67%	Covered in shade
	2	26 °C	4.8	100%	4271	61.33%	Covered in shade
	3	26 °C	4.8	100%	65,000	60.44%	Not covered by shade

Soil structure is influenced by the soil fraction which can be measured using a mesh. Soil structure affects fungi. The filamentous growth form of fungi is a highly efficient space-filling structure, well adapted for life in spatially heterogeneous environments such as soil, but soil pores ultimately govern how fungal mycelia grow and function within the soil (Ritz and Young, 2004). In turn, fungi can influence soil structure by forming aggregates in the soil. Table 2 shows that the sport field overall has a more compact soil structure than the old revegetation.

Table 2. Soil fraction at sport field and old revegetation sites according to United States Department of Agriculture (USDA).

No. mesh	Percentage (%)		Fraction
	Sport field	Old revegetation	
5	1	4.32	Gravel
10	2	1.82	Very coarse sand
40	3	3.87	Medium sand
60	4	2.66	Fine sand
200	22	20.12	Very fine sand
>200	69	67.21	Silt and clay

Fungal Density

The calculation of the average filamentous fungi colony followed the Angka Kapang Khamir (AKK) which was 10–150 CFU/dilution. Table 3 showed that the Colony Forming Units per gram in samples from the sports field are significantly higher than those in the old revegetation soil. This indicates that the density of molds in the sports field is greater than the density of molds in the old revegetation area. A total of 40.4×10^6 CFU/g of molds were found in the sports field, consisting of 48 isolates, whereas in the old revegetation area, the total colony count was 9 CFU/g, comprising 23 isolates.

Table 3. Fungal density from two sample sites.

Site	Total colony SPC (CFU/g)	T-Value	DF	*p*-Value	Result
Sport field	40.4 × 10	0.04	19	0.970	*p*-value > 0.05 (Accepting H0)
Old revegetation	9				

Further analysis using a *t*-test on the number of fungi in Table 4 also showed that the *p*-value was >0.05. This means that H0 is accepted and the number of fungi in the sports field is higher than in the old revegetation area. These statistical data support the total number of colonies in Table 3, with CFU/g values for fungi in the sports field area higher than those in the old revegetation area.

Fungal Comparison

In the sports field site, 6 genera and 21 species were found, while in the old revegetation area, 6 genera and 20 species were identified. This data indicates that the number of fungal species found in the sports field is greater than the number of fungal species found in the old revegetation area (Table 4)

Table 4. Fungal comparison between sport field and old revegetation using morphology identification.

Fungal genera	Sport field	Old revegetation
Ascomycota	18	19
Aspergillus	10	7
Penicillium	3	5
Paecilomyces	-	2
Acremonium	1	1
Trichoderma	1	3
Culvularia	3	-
Chrysosprorium	-	1
Microsporidia	1	-
Cunninghamela	1	-
Unidentified	2	1

The data shows that four genera were found in both areas. These four genera are: *Aspergillus, Penicillium, Trichoderma, and Acremonium* (Figure 1).

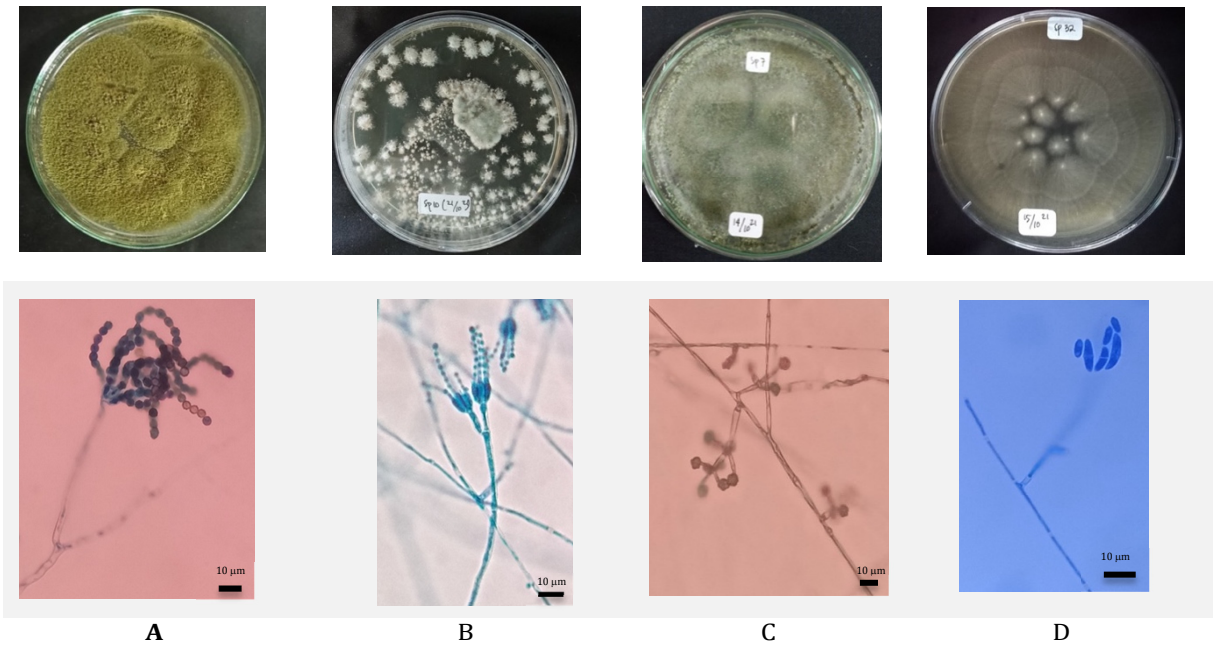

Fig. 1. Macroscopic and microscopic morphology of the genus found in both areas. (**A** = *Aspergillus*; **B** = *Penicillium*; **C** = *Trichoderma*; **D** = *Acremonium*).

Molecular Identification

The results of PCR amplification of the ITS gene for 5 dominant species isolates confirmed by electrophoresis, are shown in Figure 2. All isolate bands appeared at approximately 600bp.

Table 5 shows the results of sequencing to identify the similarity of ITS gene for 5 dominant isolates in 2 sites using BLAST. Isolate code SF-1 had a 98.65% with *Aspergillus niger*, SF-4 had a 98.89% identity with *Penicillium citrinum*, isolate OV-4 had a 98.68% identity with *Aspergillus parasiticus*, isolate OV-6 had a 98,28% identity with *Penicillium shearii*, isolate OV-8 had 100% identity with *Penicillium simplicissimum*.

Fungal communities in the two urban green spaces differed markedly in the abundance and composition. The fungal communities in the sports field support a greater density than in the old revegetation site. This disparity appears closely linked to its vegetation cover. The layer of soil covered by grass makes it easy for soil fungi to decompose the litter, thus creating an adequate supply of nutrients for soil fungi to grow. In contrast, the old revegetation site limuted litter accumulation due to routine leaf removal, reducing organic input to the soil and thereby constraining fungal growth (Ao et al., 2022).

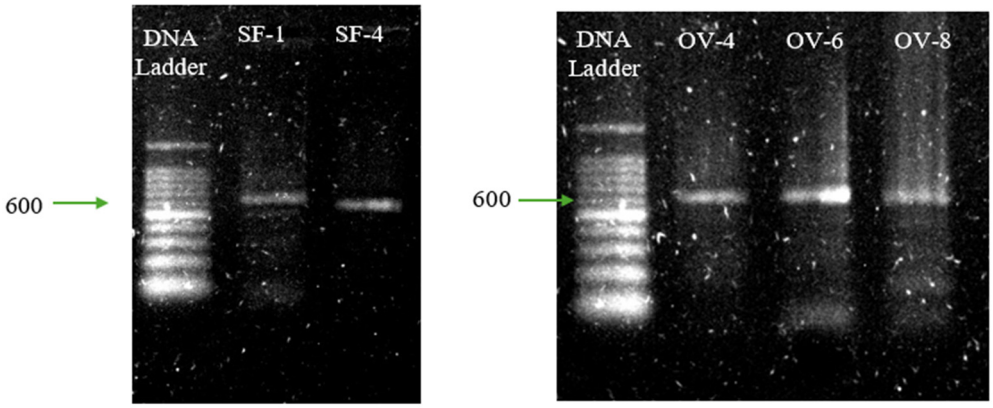

Fig. 2. Conformation of the ITS gene in dominant fungi isolates using electrophoresis methods. (SF-1 = isolate 1 of sport field; SF-4 = isolate 4 of sport field; OV-4 = isolate 4 of old revegetation; OV-6 = isolate 6 of old revegetation; OV-8 = isolate 8 of old revegetation).

Table 5. Similarity of dominant species based on sequencing of the ITS gene using the Basic Local Alignment Search Tools (BLAST) program.

Isolate code	Species	Accession no.	E value	%ID	Query cover (%)
SF-1	*Aspergillus niger* isolate okomu21	MW548418.1	5e-27	98.65%	100%
SF-4	*Penicillium citrinum* isolate UFMGCB9771	KU727718.1	6e-28	98.89%	94%
OV-4	*Aspergillus parasiticus* strain Asp49	MZ158624.1	4e-28	98.68%	100%
OV-6	*Penicillium shearii* CBS290.48	NR_111495.1	1e-22	98.28%	100%
OV-8	*Penicillium simplicissimum* CBS372.48	NR_138290.1	2e-27	100%	100%

Soil texture further shaped fungal distributuion. Soil moisture in both places shows a value of 100%. The sport field's higher proportion of silt and clay enhances water and nutrient retention, creating a more stable microhabitat for the fungal growth. In comparison, the gravel-rich substrate in the old revegetation site results in larger pores, faster drainage, and lower resource availability conditions that can suppress fungal activity and competitive ability (Quigley et al., 2018; Xia et al., 2020).

The soil pH in both places is in an acidic soil position. However, the soil in Old Revegetation shows that it is more acidic than the soil in Sport Fields even though the soil in subplot 1 Sport Field is lower than the soil in Old Revegetation. Fungi are able to grow in soil with a pH of 3–8 but grow optimally at a pH of 5–6. A higher soil pH makes it more likely for fungi to survive and reproduce (Liu et al., 2018; Li et al., 2024). Some genera or species of fungi can have a preference for living at a certain pH. Like *Penicillium*, most of them choose to live in more acidic soil (Yadav et al., 2018). This may be the reason why colonies of the genus *Penicillium* are more commonly found on the Old Revegetation land than on the Sport Field.

Other environmental factors such as humidity can affect the life of filamentous fungi related to the germination of filamentous fungi spores. Filamentous fungi can grow well in air humidity of 40–97%. Another research also reveals that filamentous fungi such as *Aspergillus, Penicillium*, and *Trichoderma* are generally found in environments with humidity conditions of 60–80% (Rodrigues et al., 2020; Pandey et al., 2022; Dove et al., 2023). The optimum soil temperature for fungi that is usually reported is 25–30 °C. However, other research has revealed that above 30 °C, like soil at 35 °C and 45 °C, fungi can still survive and reproduce powerful biocatalyst (Li et al., 2024; Xue et al., 2024).

Despite of these environment contrast, both sites were dominated by the members of the Ascomycota phylum, particulary *Aspergillus* and *Penicillium*. Ascomycota is a phylum of fungi that prevails in soils worldwide (Egidi et al., 2019). Fungi belonging to this particular phylum are present in diverse habitats, such as the rhizosphere and plant structures, where they play significant roles in carbon cycling and the breakdown of organic materials. Additionally, these fungal species are acknowledged for their capacity to enhance plant growth (Ozimek and Hanaka, 2020). *Aspergillus* is the most commonly encountered genus of the phylum Ascomycota at both sites. *Aspergillus* serves as a saprophytic filamentous fungi, contributing to the degradation of organic matter in soil ecosystems (Pfliegler et al., 2020). They play a significant role in the nutrient flow of the ecosystem by aiding in the processing of organic residues and converting them into forms usable by other organisms. *Aspergillus* can secrete toxins known as mycotoxins such as aflatoxin, gliotoxin, ochratoxin, and patulin, making *Aspergillus* potentially pathogenic to plants as the secreted toxins can infect plants. While microorganisms in soil have the capability to undergo microbial biotransformation, detoxification, or degradation of mycotoxins. A diverse range of microorganisms possesses the ability to modify mycotoxins through biotransformation. The study by Raja et al. (2017) also revealed that *Aspergillus* is the dominant filamentous fungi found in campus areas.

The second most dominant genus of filamentous fungi is *Penicillium*. *Penicillium*, a fungus found abundantly across a wide array of habitats globally, exhibits a pervasive presence from terrestrial environments like soil and vegetation to aquatic realms such as air and water. Its versatile distribution underscores its substantial ecological reach and impact (Srinivasan et al., 2020). Similar to *Aspergillus*, *Penicillium* functions as a saprobe in soil by

decomposing organic matter. Research by Chavan and Suresh (2022) shows Penicillium dominates in crop soil. On the old revegetation land, there is vegetation of several trees such as *Ficus Benjamina, Albizia saman, Mimusops elengi, Callistemon citrinus, Ficus elastica, Syzigium cumini. Penicillium* contributes significantly to agricultural soil by its capacity to enhance phosphorus availability, a crucial nutrient for plant development. This is achieved through its production of organic acids such as oxalic and citric acid. These acids serve to acidify select regions of the rhizosphere or function as chelators for cationic partners of phosphate ions, thereby augmenting phosphorus accessibility for plants (Sánchez-Esteva et al., 2016). These fungi also have the potential to enhance crop growth and confer resistance to diseases and adverse environmental conditions. Furthermore, *Penicillium* species possess the capability to break down a variety of xenobiotic substances, including pesticides, phenol, pyrene, and crude oil, which can have advantageous implications for environmental and agricultural studies (Landa Faz, 2021).

Overall, vegetation structure, soil texture, and pH emerged as the primary environmental drives influencing fungal density and diversity in these urban green spaces. These findings underscore the ecological importance of habitat specific factors in shaping soil fungal communities and provide a baseline for further biodiversity monitoring using high-resolution, such as metabarcoding.

CONCLUSIONS

This research observed differences in the number of fungal colonies in two urban green spaces. Fungal colonies in the sports field contained 10.42 × 10 CFU/g, whereas in the Old Revegetation only 9 CFU/g were found. Four genera of fungi were found that were the same between the two places, they are *Aspergillus, Penicillium, Trichoderma*, and *Acremonium. Culvularia* and *Cunninghamela* were only found in Sport Fields, while *Paecilomyces* and *Chrysosporium* were only found in Old Revegetation. Differences in the number of colonies and genera found can be influenced by environmental factors in the two different places such as vegetation, soil texture, and soil pH. Sports fields with vegetated soil floors and a crumblier soil texture are more likely to support fungal growth. For the next research, it's needed to explore another places in Surabaya and also it's needed to do an metabarcoding of the organism founded in this study. Therefore, the biodiversity information of the soil fungi in Surabaya will be richer and informative.

This study found differences in fungal abundance and community composition between two urban green spaces in Surabaya. The sports field had 40.4 × 10 CFU/g colony density compared to the old revegetation site with 9 CFU/g. Four genera found in both sampling site: Aspergillus, Penicillium, Trichoderma, and Acremonium. Certain genera were unique to each site, suggesting that environmental factors influence fungal diversity and density. Future research should include sampling from more green spaces in Surabaya and use metabarcoding methods to identify a wider range of fungal taxa. This approach would improve the city's soil fungal biodiversity database.

Authors Contributions

All authors have contributed equally to the work. All authors reviewed and edited the manuscript. All authors read and approved of the final manuscript.

Funding

This research received no specific grant from a governmental, private, or non-profit organization.

Data Availability

The dataset used for analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgments

We thank Bambang Irawan, for the ITS sequence data and Universitas Airlangga for support us writing this paper.

Competing Interest

All author declared that there is no conflict of interest in this paper.

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