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Enhancing fungal diversity in ex-coal mine soils through tillage and organic waste

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Abstract. *Zainudin, Roeswatwati D, Sutanto A, Ikhwan A, Kesumaningwati R. 2025. Enhancing fungal diversity in ex-coal mine soils through tillage and organic waste. Biodiversitas 26: 3516-3527.* This study investigates the effects of tillage depth, urban organic waste application, and incubation period on fungal diversity in soils from former coal mining sites in Samarinda, East Kalimantan, Indonesia. Using a Box-Behnken design within Response Surface Methodology (RSM), three factors, tillage depth (5-15 cm), incubation period (60-120 days), and organic waste application (20-60 tons ha⁻¹) were tested across 17 treatment combinations. Fungal populations were quantified using Potato Dextrose Agar (PDA) through serial dilution and colony counting. Fungal richness increased significantly with incubation time; four species were found at 60 days (*Penicillium* sp., *Aspergillus fumigatus* Fresen., *Corynespora* sp., *Aspergillus niger* Toph.), six at 90 days (*Aspergillus flavus* Link., *A. fumigatus*, *A. niger*, *Gliocladium* sp., *Trichoderma* sp., *Penicillium* sp.), and seven at 120 days (*A. flavus*, *A. fumigatus*, *Rhizoctonia* sp., *A. niger*, *Gliocladium* sp., *Trichoderma* sp., *Colletotrichum* sp.). Diversity indices also improved with time. At 60, 90, and 120 days, Shannon-Wiener index (H') values were 1.369, 1.691, and 1.846 (medium), dominance (D) values were 0.294, 0.197, and 0.174 (low), and Margalef index (Dmg) were 1.365, 1.698 (low), and 2.502 (medium). Evenness values ranged from 0.944 to 0.987, indicating an almost even fungal distribution. Statistical analysis revealed that only the incubation period significantly affected fungal population density ($p < 0.05$), while tillage depth ($p = 0.2654$) and organic waste application ($p = 0.6662$) did not. The resulting regression model was: Total fungal colony = $1024 + 2.12A + 5.63B + 1.6900C$. This study contributes novel insights into the integrated effects of soil management practices on fungal diversity in tropical post-mining soils, an area previously underexplored. The application of RSM allowed optimization of interacting variables. The findings reinforce the ecological role of fungi as bioindicators and agents for soil restoration, emphasizing incubation duration as a key driver in enhancing microbial recovery for sustainable land reclamation.

Keywords: Fungal diversity, incubation period, post-mining soil, soil rehabilitation, urban organic waste

INTRODUCTION

Soil microbial biodiversity, particularly fungi, plays a critical role in sustaining terrestrial ecosystem functions. Fungi contribute significantly to the decomposition of organic matter, nutrient cycling, and the formation of soil structure. Their enzymatic capabilities enable the breakdown of complex plant residues, such as lignin and cellulose, thereby enhancing soil fertility and promoting plant productivity. Furthermore, fungi establish symbiotic associations such as mycorrhizae that facilitate nutrient uptake, particularly phosphorus, thus supporting plant health and ecological resilience (Abadi et al. 2022; Fujasi et al. 2025). In addition to their roles in nutrient dynamics, fungi improve soil aggregation, increase water retention, reduce erosion, and serve as sensitive bioindicators of soil health.

Fungal diversity is especially important in disturbed ecosystems, where soil structure, chemical composition, and organic matter content are often severely degraded. Post-mining landscapes, such as those in Samarinda, East Kalimantan, Indonesia, exemplify this degradation. Open-

pit coal mining, the predominant method in the region, has caused extensive soil disturbance through the removal of topsoil, compaction of subsoil layers, and accumulation of toxic heavy metals (Hartati and Sudarmadji 2022). These changes lead to reduced soil fertility, loss of microbial diversity, and diminished potential for natural ecosystem recovery. Soils in these areas typically exhibit low organic matter content, poor structural integrity, and elevated metal concentrations, all of which inhibit fungal colonization and activity. Nevertheless, certain fungal species display resilience under such adverse conditions, indicating their potential roles as both indicators and agents of soil restoration.

Understanding fungal diversity in post-mining soils is essential for informing effective ecological restoration strategies. Fungi can support soil rehabilitation through their capacities in bioremediation and functional recovery. Several studies have shown that fungal diversity tends to increase with the age of reclamation; however, the dominant fungal groups often remain unchanged despite ecological succession (B et al. 2022). This indicates that while reclamation age positively influences community

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 Ali Ikhwan

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Keywords: Fungal diversity, incubation period, post-mining soil, soil rehabilitation, urban organic waste

INTRODUCTION

Soil microbial biodiversity, particularly fungi, plays a critical role in sustaining terrestrial ecosystem functions. Fungi contribute significantly to the decomposition of organic matter, nutrient cycling, and the formation of soil structure. Their enzymatic capabilities enable the breakdown of complex plant residues, such as lignin and cellulose, thereby enhancing soil fertility and promoting plant productivity. Furthermore, fungi establish symbiotic associations such as mycorrhizae that facilitate nutrient uptake, particularly phosphorus, thus supporting plant health and ecological resilience (Abadi et al. 2022; Pujati et al. 2025). In addition to their roles in nutrient dynamics, fungi improve soil aggregation, increase water retention, reduce erosion, and serve as sensitive bioindicators of soil health.

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pit coal mining, the predominant method in the region, has caused extensive soil disturbance through the removal of topsoil, compaction of subsoil layers, and accumulation of toxic heavy metals (Hartati and Sudarmadji 2022). These changes lead to reduced soil fertility, loss of microbial diversity, and diminished potential for natural ecosystem recovery. Soils in these areas typically exhibit low organic matter content, poor structural integrity, and elevated metal concentrations, all of which inhibit fungal colonization and activity. Nevertheless, certain fungal species display resilience under such adverse conditions, indicating their potential roles as both indicators and agents of soil restoration.

Understanding fungal diversity in post-mining soils is essential for informing effective ecological restoration strategies. Fungi can support soil rehabilitation through their capacities in bioremediation and functional recovery. Several studies have shown that fungal diversity tends to increase with the age of reclamation; however, the dominant fungal groups often remain unchanged despite ecological succession (Ji et al. 2022). This indicates that while reclamation age positively influences community

richness and complexity, certain resilient fungal taxa maintain ecological dominance across stages.

Recent studies on post-coal mining soils have identified Hypocreales (28.57%), Pleosporales (19.18%), Ascomycota (12.61%), Sordariales (12.20%), Agaricales (3.75%), Pezizales (1.74%), Mortierellales (1.54%), Capnodiales (1.51%), Eurotiales (1.35%), and Cantharellales (1.22%) as the predominant fungal groups, many of which include species with endophytic, saprotrophic, or mycorrhizal functions that are ecologically significant in soil recovery processes. These dominant taxa play important roles in nutrient cycling, organic matter decomposition, and plant-microbe symbiosis, making them critical indicators of soil health and restoration progress in degraded landscapes (Xie et al. 2023).

Despite these findings, the mechanisms driving these patterns remain unclear, especially regarding how specific environmental factors such as organic inputs, soil depth, and microbial interactions influence fungal succession in tropical post-coal mining soils. Most existing studies are focused on temperate or semi-arid zones, leaving a significant knowledge gap in the understanding of fungal community dynamics in tropical degraded landscapes. In this regard, Response Surface Methodology (RSM) provides a robust statistical approach for evaluating and optimizing multiple interacting variables simultaneously. RSM has been successfully applied in microbial ecology to assess the influence of parameters such as pH, temperature, and incubation period on microbial community development (Chen et al. 2022), yet its application remains underutilized in post-mining fungal ecology. Addressing this gap is critical for designing effective, site-specific reclamation protocols that enhance microbial diversity and ecosystem resilience.

This study explores the combined effects of soil tillage, urban organic waste application, and incubation period on fungal colony development in post-coal mining soils in Samarinda. By employing RSM, this research aims to identify the optimal conditions for enhancing fungal diversity and to analyze the interactions among these management practices. The findings are expected to inform more effective strategies for improving soil biological quality and fostering ecological recovery in tropical post-mining environments.

Despite the growing recognition of the ecological significance of fungi, research on integrated soil management in tropical post-mining environments remains limited. Most existing studies have focused on single factors or have been conducted in temperate regions. To date, few studies have systematically examined the

synergistic effects of tillage, organic amendments, and incubation period on fungal diversity using multivariate optimization approaches in tropical post-mining contexts.

By addressing this gap, the present study contributes valuable insights into fungal community responses under managed soil restoration strategies. The results will support the development of sustainable reclamation practices that harness microbial biodiversity to promote long-term ecosystem recovery. Ultimately, this research underscores the role of fungi not only as bioindicators of soil health but also as active agents in restoring the functionality, structure, and resilience of degraded tropical soils.

MATERIALS AND METHODS

Site description

This research has been conducted for 5 months, from August to December 2024. The location for sampling is in Samarinda City, East Kalimantan, Indonesia. The geographically ex-coal mining site is located at 00°29'34" South Latitude and 117°10'80" East Latitude. The initial characteristics of post-coal mining soil are presented in Table 1.

Procedures

Urban organic waste was obtained from Samarinda City, East Kalimantan, a location of significant importance. The materials for the analysis of soil fungal populations consisted of PCA Media (Plate Count Agar), soil samples, 70% alcohol, lactophenol, methylene blue, NaCl 0.85%, crystal Violet, iodine, safranin, 96% alcohol, hydrogen peroxide (H₂O₂), an Aquades. The laboratory work was carried out at the Laboratory of Plant Pests and Diseases, Faculty of Agriculture, Universitas Mulawarman, Samarinda, Indonesia. The research involved the following steps: selecting the study location, collecting soil samples, sterilizing equipment and materials, preparing Potato Dextrose Agar (PDA) medium, isolating and identifying fungal species, and purifying cultures.

Soil tillage

The soil tillage treatments were implemented at depths of 5, 10, and 15 cm in alignment with the treatment combinations outlined in Table 3. Tillage activities took place on raised plots with dimensions of 1 m by 2 m, separated by 30 cm to ensure adequate soil aeration and facilitate effective site preparation.

Table 1. Chemical properties of organic waste and post-coal mining soil before application

	Characteristics							
	pH	Org C (%)	N (%)	C/N	P	K	CEC (me 100 ⁻¹ g)	BS (%)
Soil	5.64	2.82	0.18	15.45	0.000114 %	0.044 %	13.64	96.33
Organic waste	9.50	16.41	1.72	9.54	4.13 ppm	4.23 ppm	-	-

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Application of organic waste

The organic waste utilized in this experiment comprised freshly sourced organic fraction of municipal solid waste, including discarded food items, vegetable remains, and fruit scraps. This organic material, originating from diverse sources, represents a heterogeneous mixture without a standardized composition. Variability in its constituent components—such as moisture content, nutrient levels, and decomposition rates—reflects the complexity typical of unprocessed urban organic waste. The waste was incorporated directly into post-mining soils that had been tilled to the predetermined depths. It was evenly blended into the soil at the specified tillage depth to ensure consistent distribution across treatment plots.

Following application, the treated soils were incubated for durations of 60, 90, and 120 days, in accordance with the experimental framework established by the Response Surface Methodology (RSM). This incubation phase enabled meaningful interaction between the organic inputs and the soil matrix, thereby supporting microbial activity, promoting natural decomposition processes, and enhancing nutrient availability through improved biogeochemical cycling.

Sampling method

Soil sampling was conducted randomly on treatment plots (raised beds) according to the treatment combinations outlined in Table 3. Sampling was carried out at the end of each designated incubation period. For example, for the 60-day incubation period, samples were collected from treatment runs 1, 3, 14, and 15. These runs represent specific combinations of tillage depth, incubation period, and organic waste application that were incubated for 60 days. Similar procedures were followed for the 90-day and 120-day incubation periods.

For each treatment run, one composite sample was collected. This composite was derived from five subsamples taken from different points within the same treatment bed to ensure spatial representation. The five subsamples were thoroughly mixed to produce a single homogeneous composite sample per run. This standardized method of composite sampling minimized spatial variability and provided a representative sample for laboratory analysis. This randomized and systematic sampling approach ensured adequate representation of each treatment combination and minimized sampling bias across the experimental design.

This experiment employs three factors: the dose of urban organic waste, tillage depth, and incubation period. The experimental design utilized Box-Behnken using Response Surface Methodology, with the first numerical factor being tillage depth, ranging from a minimum of 5 cm to a maximum of 15 cm. The second factor is the incubation period (in days), with a minimum of 60 days and a maximum of 120 days. The third factor is the dose of urban organic waste, ranging from a minimum of 20 tons ha⁻¹ to a maximum of 60 tons ha⁻¹ (Table 2). The response variable measured is the total fungal colony count. A total of 17 treatment combinations were derived from the formulation process, which will subsequently be optimized using the Response Surface Methodology (RSM) (Table 3).

Table 2. Experimental factors and their levels used in the Box-Behnken design

Factors	Level		
	-1	0	1
Soil tillage (cm)	5	10	15
Organic waste (tons ha ⁻¹)	20	40	60
Incubation period (days)	60	90	120

Note: Each row represents a specific experimental run based on the Box-Behnken design, showing the combination of three factors: Factor 1 - soil tillage depth (cm), Factor 2 - incubation period (days), and Factor 3 - organic waste application rate (ton ha⁻¹)

Table 3. Experimental runs and treatment combinations based on the Box-Behnken design

Run	Soil tillage (cm)	Incubation period (days)	Organic waste (tons ha ⁻¹)
1	10	60	60
2	15	90	20
3	10	120	60
4	10	90	40
5	10	90	40
6	10	90	40
7	5	120	40
8	15	120	40
9	15	60	40
10	10	60	20
11	10	120	20
12	10	90	40
13	10	90	40
14	15	90	60
15	5	90	60
16	5	60	40
17	5	90	20

Fungal isolation and identification

Fungal isolation was conducted using soil samples collected from each experimental plot, resulting in a total of 17 samples. A total of 1 kg of soil was collected from each treatment based on the experimental design using Response Surface Methodology (RSM). Samples were taken from four different points within each bed and subsequently composited into a single 1 kg composite sample. The samples were then packed in plastic bags and labeled according to their respective coordinates. Thereafter, the samples were transported to the laboratory in a cool box to ensure the viability and preservation of the microbial communities present in the soil.

This process was followed by the determination of the total microbial population using standard procedures for microbial analysis. The method employed for quantifying microbial populations was the plate count method, utilizing a colony counter. One gram of soil was diluted with 9 mL of distilled water and subjected to serial dilution up to 10⁻⁶. These dilutions were used to determine the population levels of the respective microbial groups (Rosfiansyah and Sopialena 2018).

For fungal population analysis, dilutions at ratios of 10⁻³ and 10⁻⁴ were used, with Potato Dextrose Agar (PDA) as the growth medium. The samples were incubated for

three days. Fungal isolation and enumeration were conducted in two replicates per sample to ensure the reliability and consistency of the results. Representative fungal colonies were subsequently purified and subjected to identification based on morphological characteristics (e.g., colony texture, color, and spore structure). The CFU per gram of soil was calculated using the following formula:

$$N = \frac{\Sigma C}{[(1 \times n^1) + (0.1 \times n^2) \times d]}$$

Where: N: Number of colonies per mL or g of product, ΣC : Sum of all colonies on all plates counted, n_1 : Number of plates in first dilution counted, n_2 : Number of plates in second dilution counted, d: Dilution from which the first counts were obtained

Purification was carried out on fungal colonies exhibiting distinct morphological characteristics. After inoculation and growth on fresh media, purification was performed by aseptically transferring the clean hyphal tips or uncontaminated parts of the colony using a sterile inoculating needle to new media. This subculturing process was repeated five times to obtain a pure fungal culture that was homogeneous and free from contamination.

Biodiversity index

The biodiversity parameters assessed in this study included several standard ecological indices: the Shannon diversity index, dominance index, Margalef richness index, and the evenness index (Pratiknyo and Setyowati 2020; Mulya et al. 2021; Wardhana et al. 2022; Dewi et al. 2023; Kesumaningwati et al. 2024).

Shannon diversity index

The Shannon diversity index reflects species diversity based on both abundance and evenness, formula:

$$\text{Shannon index} = - \sum_{i=1}^s \left[\frac{n_i}{N} \ln \frac{n_i}{N} \right]$$

Calculated using the formula that considers the proportion of each species ($p_i = n_i/N$, where, n_i is the number of individuals of species i , and N is the total number of individuals in the sample). The interpretation criteria are: Shannon diversity ≤ 1 : low diversity, $1 <$ Shannon diversity < 3 : moderate diversity, Shannon diversity ≥ 3 : high diversity

Dominance index (D)

The dominance index (D) measures the extent to which a community is dominated by one or a few species. It reflects the probability that two individuals randomly selected from a sample belong to the same species, formula:

$$\text{Dominance index (D)} = \sum_{i=1}^s P_i^2$$

Where: S: The total number of species in a sample/community, p_i : Proportion of species i to total species.

With criteria 0-0.30 for low, 0.31-0.60 for medium, and 0.61-1.00 for high diversity

Margalef richness index

This index used to estimate species richness, calculated based on the number of species (S) and the total number of individuals (N) in the community, formula:

$$\text{Margalef richness index (Dmg)} = \frac{s - 1}{\ln(N)}$$

Where: S: The number of species observed, N: The total number of individuals observed. With criteria $Dmg < 2.5$ for low, $2.5 > Dmg > 4$ for medium, and $Dmg > 4$ for high level of richness

Evenness index (E)

Measures how evenly individuals are distributed across different species, calculated using the ratio of the Shannon index to the natural logarithm of species richness ($\ln S$), formula:

$$\text{Evenness index (E)} = \frac{\text{Shannon Diversity}}{\ln S}$$

Interpretation criteria: 0.00-0.30: low evenness, 0.31-0.60: moderate evenness, 0.61-1.00: high evenness. In all indices, S refers to the total number of species observed, and $\sum p_i = 1$ (the sum of all species' relative abundances equals one)

Analysis data

The experimental data were analyzed using Design-Expert 13. Analysis of Variance (ANOVA) was performed to assess the significance of each model term. Model adequacy was evaluated using the coefficient of determination (R^2), adjusted R^2 , predicted R^2 , and lack-of-fit tests.

Response surface and optimization

Response surface plots and contour diagrams were generated to visualize the interaction between the variables and identify the optimal conditions for maximum fungal colony growth. The optimal point predicted by the model was then validated experimentally.

RESULTS AND DISCUSSION

Ecological succession in soil fungal communities

The incubation of soils from former coal mining sites demonstrated a dynamic trajectory of fungal community succession, which offers insight into microbial functional shifts during early-stage ecosystem recovery. At 60 days, early-colonizing taxa such as *Chrysonilia sitophila*, *Rhizoctonia* sp., *Aspergillus fumigatus* Fresen., and *Aspergillus niger* Tiegh. were dominant (Table 4; Figure 1). These fungi are known r-strategists, typically fast-growing, opportunistic saprotrophs that thrive in disturbed environments rich in labile organic matter (Qu et al. 2022). Their presence reflects a microbial response to fresh

organic amendments, exploiting easily degradable substrates with minimal enzymatic investment. Among them, *C. sitophila* has been reported to colonize nutrient-rich substrates rapidly but is often short-lived due to its narrow enzymatic capabilities (Chen et al. 2020). The remarkable environmental adaptability of *Aspergillus* species enables their persistence across a broad range of temperatures, limited water availability, and fluctuations in soil pH and oxygen levels. The interactions between *Aspergillus* species and both biotic and abiotic environmental factors play a critical role in determining the types and functional roles of secondary metabolites synthesized, including aflatoxins, gliotoxin, patulin, cyclopiazonic acid, and ochratoxin (Nji et al. 2023).

Table 4 shows that as incubation progressed to 90 and 120 days, the fungal community structure shifted toward fungal groups with K-strategist taxa, such as *Trichoderma*, *Gliocladium*, and *Penicillium*. These fungi exhibit higher functional diversity, including the ability to degrade lignocellulosic compounds, produce secondary metabolites, and act antagonistically against soil-borne pathogens. This shift reflects a more stable and competitive soil microbial environment. For instance, *Penicillium* has been utilized through inoculation into plants or soil to support ecological restoration in post-mining areas (Zhao et al. 2021b). *Penicillium* and *Trichoderma* fungi are known to activate plant defense responses against pathogens, as well as stimulate plant hormone activity regulated by endogenous mechanisms to promote plant growth. Therefore, the final stage of restoration is characterized by an increased abundance of *Penicillium* and *Trichoderma* species (Hou et al. 2024).

The change in fungal species composition occurring along the succession process leads to a gradual shift in dominant species with the increasing age of coal mine spoil tips. A progressive increase in the relative abundance and density of the genus *Penicillium* Link, 1809 characterizes this (Iliushin et al. 2022a).

Roy et al. (2023) found that even after more than 50 years of recultivation, the diversity of fungal species in post-mining soils had not fully recovered to pre-mining levels. Nevertheless, the functional attributes of the fungal

community showed substantial recovery, closely resembling the original pre-disturbance conditions. Post-mining soils require revegetation to support the recovery of soil fungal communities, as revegetation activities contribute to the improvement of soil properties. Changes in soil nutrient status induced by different revegetation types are the primary factors influencing the diversity and composition of fungal communities (Zhu et al. 2022).

Functional dominance and bioremediation potential of *Aspergillus* spp.

The consistent dominance of *A. fumigatus* and *A. niger* across all incubation periods underscores their ecological plasticity and metabolic efficiency in degraded, nutrient-poor environments. These fungi produce a broad suite of extracellular enzymes capable of degrading cellulose, hemicellulose, and lignin, allowing them to persist even as substrate complexity increases (Ginting et al. 2024). Additionally, their ability to solubilize phosphate and synthesize indole-3-acetic acid (IAA) suggests a dual role in both organic matter decomposition and facilitation of plant-microbe interactions (Shelton et al. 2022). These traits are particularly valuable in post-mining soils, where fertility is often limited and vegetation establishment is a key goal of reclamation.

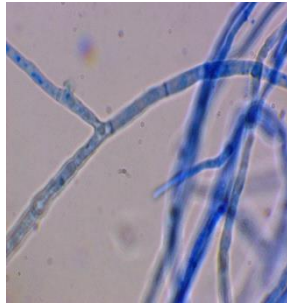
However, the prolonged dominance of *Aspergillus* spp. may indicate that the soil ecosystem remains in an early or arrested successional stage. While these fungi are effective decomposers, their dominance could suppress the establishment of more functionally diverse fungal taxa, resulting in a simplified community structure with limited resilience. This may be exacerbated by environmental filtering caused by uniform amendment types (e.g., high-carbon, low-nitrogen urban waste) or abiotic stressors typical of post-mining soils (e.g., low moisture, high compaction). To promote ecological maturity, reclamation strategies should aim to diversify organic inputs and promote plant-soil feedbacks that enhance microbial heterogeneity and resilience.

Table 4. The abundance (%) and the fungal diversity during the organic waste incubation period in mine soil

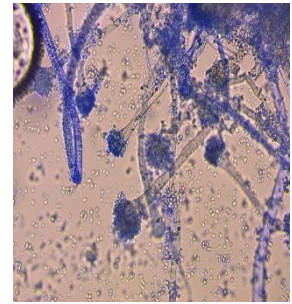
Incubation period (days)	Phylum	Family	Genus	Species	Fungi abundance (%)
60	Basidiomycota	Ceratobasidiaceae	<i>Rhizoctonia</i>	<i>Rhizoctonia</i> sp.	5.0
	Ascomycota	Aspergillaceae	<i>Aspergillus</i>	<i>Aspergillus fumigatus</i> Fresen.	7.5
	Ascomycota	Sordariaceae	<i>Neurospora</i>	<i>Chrysonilia</i> sp.	5.0
90	Ascomycota	Aspergillaceae	<i>Aspergillus</i>	<i>Aspergillus niger</i> Tiegh.	5.0
	Ascomycota	Aspergillaceae	<i>Aspergillus</i>	<i>Aspergillus fumigatus</i>	10.0
	Ascomycota	Aspergillaceae	<i>Aspergillus</i>	<i>Aspergillus niger</i>	12.5
	Ascomycota	Aspergillaceae	<i>Aspergillus</i>	<i>Aspergillus flavus</i> Link	2.5
	Ascomycota	Nectriaceae	<i>Fusarium</i>	<i>Fusarium</i> sp.	7.5
	Ascomycota	Aspergillaceae	<i>Penicillium</i>	<i>Penicillium</i> sp.	2.5
	Ascomycota	Hypocreaceae	<i>Trichoderma</i>	<i>Trichoderma</i> sp.	10.0
120	Ascomycota	Hypocreaceae	<i>Gliocladium</i>	<i>Gliocladium</i> sp.	5.0
	Ascomycota	Aspergillaceae	<i>Aspergillus</i>	<i>Aspergillus flavus</i>	5.0
	Ascomycota	Aspergillaceae	<i>Aspergillus</i>	<i>Aspergillus fumigatus</i>	2.5
	Basidiomycota	Ceratobasidiaceae	<i>Rhizoctonia</i>	<i>Rhizoctonia</i>	5
	Ascomycota	Glomerellaceae	<i>Colletotrichum</i>	<i>Colletotrichum</i> sp.	2.5

Ascomycota	Aspergillaceae	<i>Aspergillus</i>	<i>Aspergillus niger</i>	7.5
Ascomycota	Hypocreaceae	<i>Gliocladium</i>	<i>Gliocladium</i> sp.	2.5
Ascomycota	Hypocreaceae	<i>Trichoderma</i>	<i>Trichoderma</i> sp.	2.5

Days 60 soil incubation duration



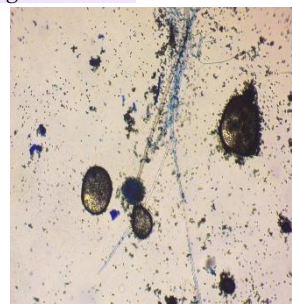
Rhizoctonia sp.



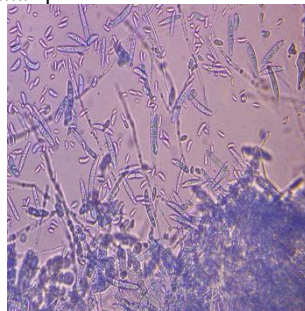
Aspergillus fumigatus Fresen.



Chrysonilia sp.

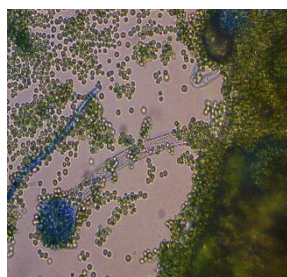


Aspergillus niger Tiegh.

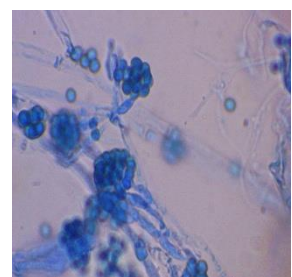


Fusarium sp.

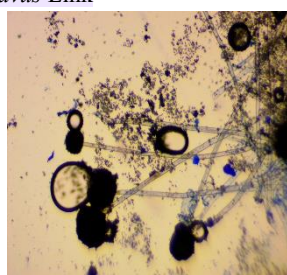
Days 90 soil incubation duration



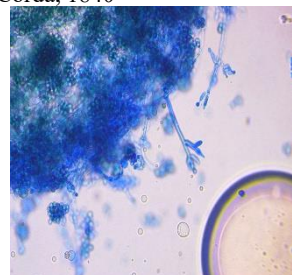
Aspergillus flavus Link



Gliocladium Corda, 1840



Aspergillus niger Tiegh.

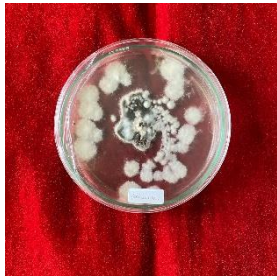


Trichoderma sp.

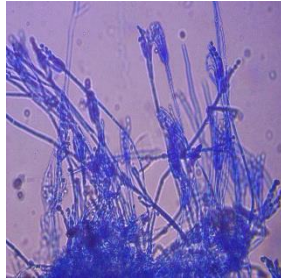
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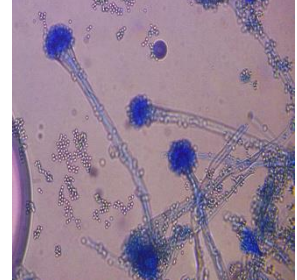
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Penicillium sp.



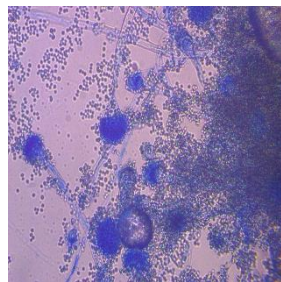
Aspergillus fumigatus Fresen.



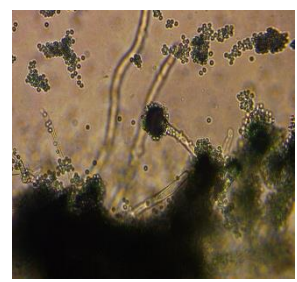
Days 120 soil incubation duration



Aspergillus fumigatus Fresen.



Aspergillus flavus Link



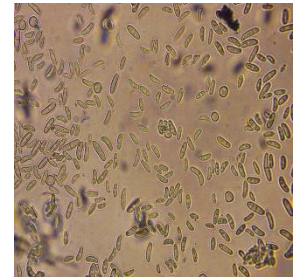
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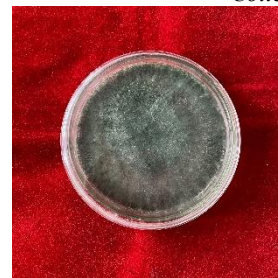
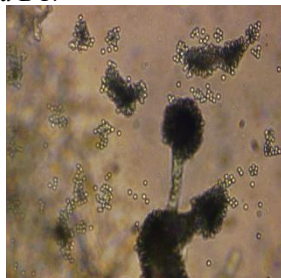
Rhizoctonia DC.



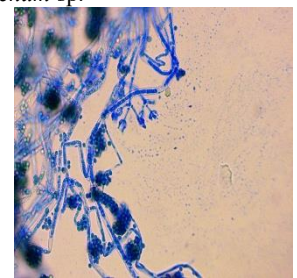
Colletotrichum sp.



Aspergillus niger Tiegh.



Gliocladium sp.



Trichoderma sp.

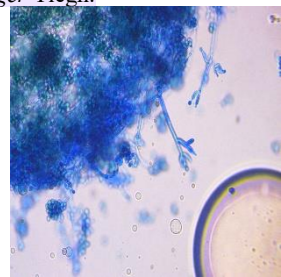


Figure 1. Microscopic and macroscopic visualization of fungal growth from ex-coal mining soil cultured on PDA media

Pathogenic fungi: A constraint on ecological recovery

The sporadic presence of *Rhizoctonia* sp. and *Colletotrichum* sp. during incubation potentially constrains soil health and vegetation recovery. *Rhizoctonia solani* J.G.Kühn, detected at both 60 and 120 days, is a persistent soil-borne pathogen with sclerotia that enable long-term

survival under adverse conditions (Akber and Fang 2024). Its resurgence may result from unsterilized organic waste inputs or legacy inocula in the soil seedbank. Ecologically, its presence can disrupt early root colonization by beneficial fungi and reduce plant establishment rates, thereby delaying ecosystem succession.

Colletotrichum sp., detected only at 120 days, is known to respond strongly to specific microclimatic cues such as high humidity and warm temperatures (Peralta-Ruiz et al. 2023). Its late appearance suggests environmental filtering may be enabling latent pathogen activation as soil conditions improve. If unmanaged, such pathogens may create negative feedback loops by inhibiting vegetation growth, thus reducing litter inputs and slowing microbial diversification. Integrating disease-suppressive composts, beneficial antagonists like *Trichoderma*, or implementing crop rotation with resistant species may be necessary to mitigate these risks in reclamation projects.

Methodological considerations and statistical interpretation

The non-significant effects of tillage and organic waste treatments on fungal diversity warrant further scrutiny. One plausible explanation is the limited range of variation in treatment intensities. For instance, tillage may have been too shallow to alter soil structure meaningfully, or the organic waste applied may have been chemically homogeneous, limiting its influence on microbial niches. Moreover, microbial responses are often non-linear and context-dependent, and the relatively short duration of incubation (120 days) may not have allowed for full microbial adaptation or competitive exclusion to manifest.

Additionally, the spatial heterogeneity of post-mining soils, coupled with relatively low replication, may have contributed to high variability in microbial measurements, reducing statistical power. Future research should adopt factorial designs with expanded treatment gradients, replicate across heterogeneous soil microsites, and incorporate molecular tools such as metagenomics or qPCR to better capture subtle shifts in microbial function and community.

Fungal diversity dynamics during organic waste incubation

The observed Shannon diversity index (H') values 1.369 at 60 days, 1.691 at 90 days, and 1.846 at 120 days (Figure 2) indicate a progressive increase in fungal community diversity throughout organic waste incubation. All values fall within the "medium" diversity category, yet the upward trend suggests an ongoing ecological transition toward a more functionally diverse fungal community.

This pattern aligns with ecological succession theory, where microbial diversity increases over time as labile substrates are depleted and replaced by more recalcitrant compounds, fostering niche differentiation and competitive interactions (Moitinho et al. 2022). The rise in H' suggests that early-stage colonizers were gradually accompanied or replaced by more specialized decomposers, reflecting shifts in both resource availability and environmental conditions such as moisture, pH, and nutrient status (Sui et al. 2022).

The Simpson dominance index (D) has a value range between 0 and 1 (Table 5). Values near zero signify a balanced community with no single species dominating, indicating ecological stability. In contrast, values closer to one suggest dominance by one or a few species, which may

reflect ecosystem imbalance and potential ecological stress (Safaie et al. 2024). In this study, the Simpson dominance index (D) for soil fungal communities under various incubation periods remained low, implying that the community structure was relatively stable and showed no evidence of ecological disturbance.

The Margalef species richness index (D_{mg}) ranged from 1.365 to 2.502, which is generally classified as indicative of low to moderate species richness. This indicates that the soil fungal community exhibited increasing diversity with longer incubation periods. These values may suggest that the ecosystem is beginning to show signs of recovery.

The analysis of the evenness index (E) revealed that the values for soil fungal communities ranged between 0.944 and 0.987. These figures correspond to classifications of near-even to fully even distribution, reflecting a high degree of evenness within the community. This implies that the abundance of individuals among the species was relatively balanced, with no species exerting dominance over the others.

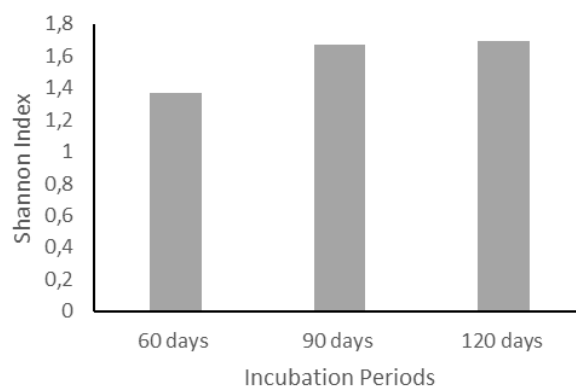


Figure 2. Shannon diversity index of fungal communities in post-coal mining soil across different incubation periods

Table 5. The fungal biodiversity profile during incubation periods

Index biodiversity	Incubation periods (days)		
	60	90	120
Species level			
Shannon diversity	1.369 (medium)	1.691 (medium)	1.846 (medium)
D	0.259 (low)	0.197 (low)	0.174 (low)
D_{mg}	1.365 (low)	1.698 (low)	2.502 (medium)
E	0.987 (evenly distributed)	0.944 (almost evenly distributed)	0.949 (almost evenly distributed)

Note: For D (Simpson dominance index): 0.00-0.30 (low), 0.31-0.60 (medium), 0.61-1.00 (high); For Shannon diversity index: ≤ 1 (low), $1 < \text{Shannon diversity} < 3$ (medium), ≥ 3 (high); For D_{mg} (level of richness): < 2.5 (low), $2.5 > D_{mg} > 4$ (medium), > 4 (high); For E (Evenness index): 0.26-0.50 (less even), 0.51-0.75 (relatively even), 0.76-0.95 (almost evenly distributed), 0.96-1.00 (evenly distributed)

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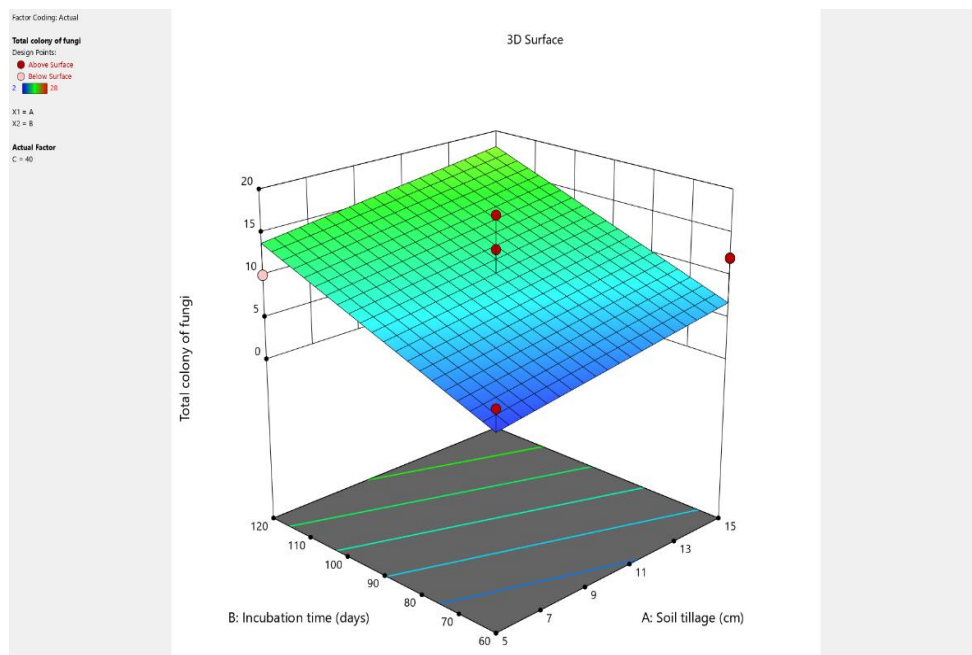


Figure 3. Three-dimensional surface plot of total fungal colony count

Table 6. Effects of soil tillage, incubation period, and urban organic waste on the total colony of fungi

Run	Factors			Response of total fungi colony (cfu g ⁻¹)	
	Soil tillage (cm)	Incubation period (days)	Urban organic waste (tons ha ⁻¹)	Actual	Predicted
1	10	60	60	9.00	5.61
2	15	90	20	6.00	11.36
3	10	120	60	24.00	16.86
4	10	90	40	8.00	10.24
5	10	90	40	2.00	10.24
6	10	90	40	17.00	10.24
7	5	120	40	10.00	13.74
8	15	120	40	12.00	17.99
9	15	60	40	12.00	6.74
10	10	60	20	3.00	3.61
11	10	120	20	28.00	14.86
12	10	90	40	5.00	10.24
13	10	90	40	13.00	10.24
14	15	90	60	11.00	13.36
15	5	90	60	5.00	9.11
16	5	60	40	5.00	2.49
17	5	90	20	4.00	7.11

Effect of soil tillage, urban organic waste, and incubation period

The experimental design and response data are presented in Table 6. The highest total fungal colony count was observed under the soil tillage treatment with a depth of 10 cm, an incubation period of 120 days, and an application of 20 tons ha⁻¹ of urban organic waste. In contrast, the lowest colony count was recorded at the same depth with a 90-day incubation period and a higher application rate of 40 tons ha⁻¹. However, variations in organic waste dosage resulted in differences in total fungal colonies. Table 7 shows the predictability of response variables for linear models, and Table 8 shows that factor C (organic waste) had a p-value of 0.6662 (p > 0.05), indicating that organic waste did not

have a significant effect on the total fungal colony count. This may be due to inappropriate dosage and the short duration of the study. As explained by Gryta et al. (2020), excessive application of organic fertilizers can reduce microbial diversity and activity. Additionally, Xu et al. (2020) reported that the timing of organic waste application is crucial, with long-term organic fertilization having a greater impact on important microorganisms such as Actinobacteria, Proteobacteria, and Ascomycota.

Factor A (soil tillage) also did not have a significant effect on the total fungal colony count (p = 0.3654), which is presumably due to moisture loss resulting from tillage activities that subsequently affect fungal colonies. According to Zhao et al. (2021a), Li et al. (2020a), and Li et al.

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(2020b), soil management through minimum tillage and no-tillage systems is considered the most effective cultivation practice for enhancing microbial populations.

A differing opinion was expressed by Zainudin and Kesumaningwati (2021a,b), who stated that microbial communities decline as a result of soil management practices that lead to the loss of organic matter, as observed in annual crop fields, mixed gardens, and post-coal mining soils. In contrast, factor B (incubation period) showed a significant effect on total fungal colonies, with a p-value of 0.0275 ($p < 0.050$) (Figure 3). This finding is consistent with the study by Dong et al. (2022), which demonstrated that the incubation period has a significant effect on fungal diversity. The total fungal colony count increased with longer incubation periods. The highest number of colonies, totaling 17, was recorded after 90 days of incubation at a tillage depth of 10 cm. The addition of organic waste to soil remains a beneficial fertilization practice, as it helps maintain soil organic carbon, increases phosphorus and potassium levels, and strengthens the microbial community (Mora-Salguero et al. 2025).

The regression equation derived from the analysis is as follows:

$$\text{Total colony of fungi} = + 10.24 + 2.12A + 5.63B + 1.0000C$$

Where: A = Soil tillage, B = Incubation period, C = Urban organic waste

Optimization

The Design-Expert software was employed to optimize the regression equation for predicting the response within the experimental domains (Niyomvong et al. 2022). The optimization parameters for the soil incubation process were established based on the criteria outlined in Table 9. The primary optimization criterion was the total fungal colony count, which serves as an indicator of soil microbial activity. The optimization process yielded 16 potential solutions with a maximum desirability of 52.4%. The optimal conditions for the soil incubation process were determined to be soil tillage volume of 15 mL, an incubation period of 103 days, and an application of urban organic waste at a rate of 36.5 tons ha⁻¹. Under these conditions, the total fungal colony count was predicted to reach 14.712 colonies. (Table 10).

Table 7. Predictability of response variables for models

Source	Degrees of freedom	Sum of squares	Mean square	Report F	Prob. > F
Model	3	297.25	99.08	2.41	0.1135
Error	4	146.00	36.50		
Lack of fit	9	387.81	43.09	1.18	0.4703
Total corrected	16	831.06			
R ²	0.3577	R ² adjusted	0.2094		

Table 8. Type, intercept, and coefficient of regression (equation model) for soil tillage (A), incubation period (B), and urban organic waste (C) for improving the ex-coal mining soil characteristics

Source	Total colony of fungi (Linier)		95% CI low	95% CI high
	Coefficient	p-value		
Intercept	10.24		6.88	13.59
A-Soil tillage (cm)	2.12	0.3654	-2.77	7.02
B-Incubation period (days)	5.63*	0.0275	0.7305	10.52
C-Urban organic waste (tons ha ⁻¹)	1.0000	0.6662	-3.89	5.89
Lack of fit	387.81	0.4703		

Note: ANOVA analyzed the data. Coefficients followed by * indicate significant effects ($p < 0.05$) on the total population of fungi in mining soil

Table 9. Optimization criteria derived from Box-Behnken design using Response Surface Methodology (RSM)

Factors	Goal	Limit	Weights	Importance (1-5)
Soil tillage (cm)	In range	5-15	1-1	3
Incubation period (days)	Maximized	60-120	1-1	3
Urban organic waste (tons ha ⁻¹)	Maximized	20-60	1-1	3
Response				
Total Fungi (CFU/g)	Maximized			3

Table 10. Physicochemical characteristics of compost derived from palm oil waste

Characteristics	Predicted mean	95% PI low	95% PI high
Total population of microorganisms	14.712	-0.534262	29.9586

Table 11. Optimized treatment formula for enhancing total fungal colony count in post-coal mining soil based on Response Surface Methodology (RSM)

Soil tillage (cm)	Incubation period (days)	Urban organic waste (tons ha ⁻¹)	Total colony of fungi (cfu g ⁻¹)	Desirability
15	103.5	36.5	14.7	0.524

Table 11 presents the optimal solution formula obtained from the RSM design, in which soil tillage, incubation period, and organic waste at 15 cm, 103.5 days, and 36.7 tons ha⁻¹, respectively, resulted in a total fungal colony count of 14.7 CFU g⁻¹, with a desirability value of 0.524.

In conclusion, this study underscores the pivotal role of temporal dynamics, in facilitating the recovery of fungal communities in post-coal mining soils. Although the immediate effects of organic waste application and soil tillage on fungal colony abundance were minimal, the pronounced influence of incubation time indicates that fungal recolonization is a gradual and ecologically complex process. These findings highlight the necessity of adopting long-term rehabilitation strategies that account for the temporal nature of microbial community development. From a land rehabilitation standpoint, the results suggest that extending and managing incubation periods as part of reclamation protocols can effectively promote the re-establishment of beneficial soil fungi. This is particularly pertinent in tropical mining regions, where microbial-driven functions such as nutrient cycling, organic matter decomposition, and ecosystem stabilization are essential to sustainable land recovery. Moreover, the combined use of organic amendments (e.g., compost derived from agricultural waste) and native or well-adapted fungal strains holds substantial promise for enhancing bioremediation efficacy, improving soil physical structure, and mitigating toxic residues often associated with mining activities. With respect to future microbial research, these findings call for targeted investigations into fungal taxa with high bioremediation potential. Further studies should examine the synergistic interactions between these fungi, organic inputs, and evolving soil physicochemical properties across temporal scales. In addition, molecular and functional analyses of fungal communities focused on traits linked to ecological resilience and succession can inform the development of specialized fungal inoculants tailored for tropical post-mining landscapes. Such research directions will contribute to more effective, ecologically grounded, and context-specific strategies for the restoration of degraded soils on a global scale.

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