BIODIVERSITAS
Volume 25, Number 7, July 2024
Pages: 2921-2930

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250713

Assessment of nematode and microbial diversity and screening of entomopathogens in revegetated post-coal-mining land in Kutai Kartanegara District, East Kalimantan, Indonesia

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Manuscript received: 22 December 2023. Revision accepted: 7 July 2024.

Abstract. *Sopialena, Rosfiansyah, Suryadi A. 2024. Assessment of nematode and microbial diversity and screening of entomopathogens in revegetated post-coal-mining land in Kutai Kartanegara District, East Kalimantan, Indonesia. Biodiversitas 25: 2921-2930.* Post-coal-mining management should strive toward site revegetation. The success of such revegetation efforts is largely determined by the soil's fertility status, which encompasses physical, chemical, and biological components. Biological fertility is indicated by the presence and abundance of soil microbes and nematodes. The objective of this study was to identify the diversity of nematodes and soil microbes in ex-coal-mining land revegetated with oil palm in Kutai Kartanegara District (East Kalimantan, Indonesia), to screen for entomopathogenic nematodes and microbes, and to analyze their relationships with the chemical fertility status of the land. As a control, non-mining land was also investigated. The results showed that microbial diversity and density (including fungi and bacteria) in the ex-coal-mining area were significantly lower compared to the control. Additionally, a decrease in microbial diversity and density was found in deeper soil layers of both post-mining and non-mining lands. In contrast, soil nematodes were more abundant in post-mining land, both in terms of diversity and density, and were also more plentiful in the deeper soil layers. Among the nematodes and microbes studied, only *Heterorhabditis* and *Steinernema* (nematodes), *Trichoderma* (fungi), and Bacillaceae (bacteria) were identified as entomopathogens, causing death in *Tenebrio molitor* L. larvae. Overall, soil fertility in ex-coal-mining land was observed to be lower, characterized by decreased soil pH, basic saturation, Soil Organic Carbon (SOC), and nutrient content, which contributed to a reduced microbial community compared to non-mining land.

Keywords: Entomopathogen, Kutai Kartanegara, nematodes, post-coal mining, soil microbes, *Trichoderma*

INTRODUCTION

Coal mining activities in Kutai Kartanegara District, East Kalimantan, Indonesia, have experienced rapid growth in recent years, with both large-scale and small-scale operations, including those with mining authorities. However, this expansion has come at a significant cost to the environment, resulting in widespread damage. This damage manifests in the form of arid and infertile post-mining lands or artificial "craters" with unclear purposes. While some efforts have been made to rehabilitate these ex-coal-mining lands, they remain sporadic and insufficient, allowing environmental degradation to outpace recovery efforts.

Rehabilitating these lands is essential to mitigating the adverse environmental impacts of coal mining. The success of land recovery can be evaluated by assessing the diversity of soil microbes, which serves as an indicator of biological fertility. Soil microbes play a pivotal role in converting potential chemical fertility into actual fertility. Moreover, their activities gradually enhance the physical properties of the soil, further improving physical fertility (Cui et al. 2021). However, the restoration of ex-coal-mining lands faces numerous challenges, including the need to address soil degradation, erosion, and biodiversity loss resulting from mining activities (Ahirwal et al. 2016). To address these challenges, it is crucial to consider the role of soil microbes, including entomopathogenic microbes, which are an integral part of soil ecosystems (Boucias and Pendland 2018).

The roles of nematodes and microbes in ex-mining lands are fundamental to ecological restoration efforts, improving soil health, supporting plant growth, and maintaining ecological balance. These microorganisms can significantly impact the success of revegetation and restoration projects in post-mining landscapes. The presence of nematodes and microbes in the soil benefits plant health during revegetation endeavors (Guo et al. 2021; Li et al. 2022). Furthermore, these microorganisms support plant establishment and growth. Healthy and robust plant growth, in turn, stabilizes the soil, prevents erosion, and enhances biodiversity, all of which are important for the overall success of revegetation projects (Mensah 2015). The presence of microbial diversity in mining-affected areas can be influenced by factors such as soil type, climate, land management practices, as well as the host plants' root exudate composition (Sasse et al. 2017; Santoyo 2022). Consequently, understanding and managing the diversity of these microorganisms are critical for the success of revegetation efforts.

Entomopathogenic microbes, which encompass various taxonomic groups, including bacteria, fungi, nematodes, and viruses, are known to regulate insect populations and play a vital role in ecological processes (Deka et al. 2021). One of the most remarkable contributions of entomopathogenic microbes during revegetation is their role as natural pest controllers (Vega et al. 2009). These microorganisms serve as natural enemies of various insect pests that could harm newly planted vegetation (Shrestha et al. 2020). By infecting and eliminating these pests, entomopathogens help regulate insect populations and protect the restored vegetation (Rozpądek et al. 2019). In regions where entomopathogens thrive, the risk of pest outbreaks is reduced, safeguarding the overall health of the revegetated land (Koskella et al. 2020). Entomopathogenic microbes, such as fungi and bacteria are natural antagonists of insects and play a pivotal role in regulating insect populations in terrestrial ecosystems. While extensively studied for their potential in biological pest control in agriculture, their utilization in ecological restoration, particularly in ex-coal-mining land revegetation, remains a relatively unexplored area, and a promising avenue for research and practical implementation. The diversity and abundance of entomopathogenic microbes are subject to environmental factors (Fatu et al. 2020). Factors such as soil physicochemical properties, which can fluctuate significantly in mining-affected areas, play pivotal roles in shaping microbial community structure (Mudrak et al. 2021).

The objective of this study was to identify the diversity of nematodes and soil microbes on revegetated ex-coal-mining land, to investigate for entomopathogenic nematodes and microbes, and to analyze their relationships with the chemical fertility status of the land.

MATERIALS AND METHODS

Experimental sites

The study was conducted at two locations, in post-coal mining and non-mining areas, in the Pendingin Subdistrict of Sanga-Sanga, in Kutai Kartanegara District, East Kalimantan Province, Indonesia. Oil palm plantations were planted in a post-coal mining area (00°40'29.7"S 117°16'09.8"E), while rubber plantations were cultivated in a non-mining area (00°41'41.3"S 117°15’54.9"E) used as a control.

Sample collection

The sampling method used in this study was the standard method of ISPM (International Standards for Phytosanitary Measure). Soil samples were taken randomly at ten sampling points at each experimental site. The soil samples were taken at four levels of depth (0-30 cm, 30-60 cm, 60-80 cm, and 80-100 cm) from the soil surface. After removing the gravel and plant debris, soil samples were composited for each level of soil depth, and 1 kg of soil was collected for microbial extraction and identification of fungi, bacteria, and nematodes in the laboratory. In addition, physicochemical properties of soil were also analyzed in this study.

Fungal and bacterial isolation and identification

For both fungal and bacterial isolation, 10 g of soil samples were dissolved in a liter of distilled water. Then 1 ml of soil solution was taken and diluted in steps from 10^{-1} to 10^{-3} to determine the CFU value of each sample, by repeating 2 times each dilution from three dilution levels. An inoculating needle was dipped in the soil solution, and then inoculated on Potato Dextrose Agar (PDA) medium to culture fungi, while another inoculating needle was dipped in the same solution and applied to Nutrient Agar (NA) medium to culture bacteria. After one week of culturing for fungi, or two days for bacteria, the microbes were isolated and observed under a microscope to identify the type, color, and shape of individual bacteria and fungi, as well as that of their colonies, following Hucker's procedure with minor modifications (Hucker 1921). The number of microorganisms (fungi and bacteria) present in each test sample as a weighted average of two consecutive dilutions was calculate using the following equation (Matuurin and Peeler 2001):

$$N = \frac{\sum C}{[(1\ x\ n_1) + (0{,}1\ x\ n_2)\ x\ (d)}$$

Where: N: Number of colonies per ml or g of product; ΣC: Sum of all colonies on all plates counted; n1: Number of plates in first dilution counted; n2: Number of plates in second dilution counted; d: Dilution from which the first counts were obtained

Entomopathogenic fungal and bacterial screening

Entomopathogenic fungi and bacteria were selected using mealworm (*Tenebrio molitor* L.) larvae 30-40 days of age (Figure 1). One milliliter of each fungal and bacterial isolate was inoculated to mealworms. Only fungi and bacteria that could cause mortality to mealworms were considered entomopathogens in the present study.

Isolation and identification of nematodes

Nematode isolation was carried out using the Baermann funnel method with minor modifications. This method was considered the most effective in nematode isolation (van den Hoogen et al. 2020).

Figure 1. Mealworm (*Tenebrio molitor* L.) larvae at 30-40 days of age

Briefly, a silicone hose was attached to a funnel, then the end of the hose was tied using a gauze rubber band and placed on top of the funnel. The Baermann funnel apparatus was installed in a horizontal position without buckling the silicon hose. Fifty grams of soil for each sample was placed on the filter paper, and the soil was wrapped to cover its entire surface. The water was added slowly into the funnel to submerge the sample, until the sample was saturated, to create a moist environment that stimulated nematodes to migrate toward the water source. The funnel was incubated for 3×24 hours and the water released from the bottom of the funnel. The water was collected in a film tube/vial. The collected water then was passed through a fine sieve to separate the nematodes from the water and debris. Up to 1 drop of formalin (5%) was added and fixed for 15 minutes.

Entomopathogenic nematode screening

Entomopathogenic nematodes were screened by bait trap using mealworms. The mealworm larvae (3-5 larvae) were placed in a plastic container and mixed with ~150 g of moist soil containing nematodes. The container was then inverted so that the larvae were covered with soil, then placed at room temperature.

After 3-4 days, if larval mortality was observed, the larval cadaver was removed and rinsed with sterilized distilled water. Entomopathogenic nematode trapping was then conducted using the White trap method to release entomopathogenic nematodes from the cadaver's body. Nematodes were observed and counted using a stereo microscope. Identification of soil nematodes and entomopathogenic nematodes to genus level was carried out through morphological observations.

Physicochemical analysis of soil

Samples were analyzed for their chemical and physical properties, including organic C, total N, available P (Bray), available K (Morgan), Ca^{++}, Mg^{++}, K^{+}, Na^{+}, CEC (Cation Exchange Capacity), Base Saturation (BS), Al saturation, pH, and soil texture. The soil physicochemical analysis was conducted based on the general procedure developed in the Soil Laboratory, Faculty of Agriculture, Universitas Mulawarman, Samarinda, Indonesia.

Data analysis

Data collected were analyzed descriptively to uncover the role of soil microbes in supporting the recovery of post-coal-mining land and relationship between the diversity of soil microbes and chemical fertility status of the study site.

RESULTS AND DISCUSSION

Fungal diversity and density

Result showed that higher fungal diversity was observed in the non-mining land compared to the ex-mining land, with five fungal genera found in non-mining, and four genera in ex-mining soil (Figure 2 and Table 1). The fungal diversity in ex-coal mining and non-mining land was different in every soil layer observed in this study (Table 1). *Penicillium* sp., which was found in every soil layer of post coal-mining land, was not present in the non-mining land soil. On the other hand, *Mucor* sp., which was found in the non-mining land soil, was absent in coal-mining land soil. In addition, *Aspergillus* was found abundant in almost all soil layers in both ex-coal-mining and non-mining land. Fungal density in non-mining land soil was also significantly higher (ranging from 2.6-7.2×10^5 cfu.g^{-1}) than in soil of ex-coal-mining land (ranging from 1.4-3.8×10^5 cfu.g^{-1}) at every soil depth layer. The deeper the soil layer, the fewer fungal populations were found. Among all the fungi identified in the ex-mining and non-mining land, only *Trichoderma* was screened as an entomopathogenic fungi. *Trichoderma* was observed in the digestive system of deceased *T. molitor* mealworm larvae.

Table 1. Fungal diversity and density at different soil depth layers, and screening of entomopathogenic fungi in ex-mining and non-mining land in Pendingin, Sanga-Sanga subdistrict, Kutai Kartanegara District, Indonesia

Soil depth layers (cm)	Fungal population density (cfu/g)	Genera/species
Non-mining land		
0-30	7.2×10^5	*Aspergillus, Trichoderma*, Penicillium*
30-60	5.4×10^5	*Aspergillus, Trichoderma*, Penicillium*
60-80	3.2×10^5	*Trichoderma*, Pythium, Penicillium*
80-100	2.6×10^5	*Aspergillus, Penicillium*
Post-coal-mining land		
0-30	3.8×10^5	*Aspergillus, Trichoderma **
30-60	2.2×10^5	*Aspergillus niger, Trichoderma**
60-80	1.8×10^5	*Aspergillus* sp., *Mucor* sp.
80-100	1.4×10^5	*Aspergillus niger, Aspergillus flavus*

Note: *Entomopathogenic fungi

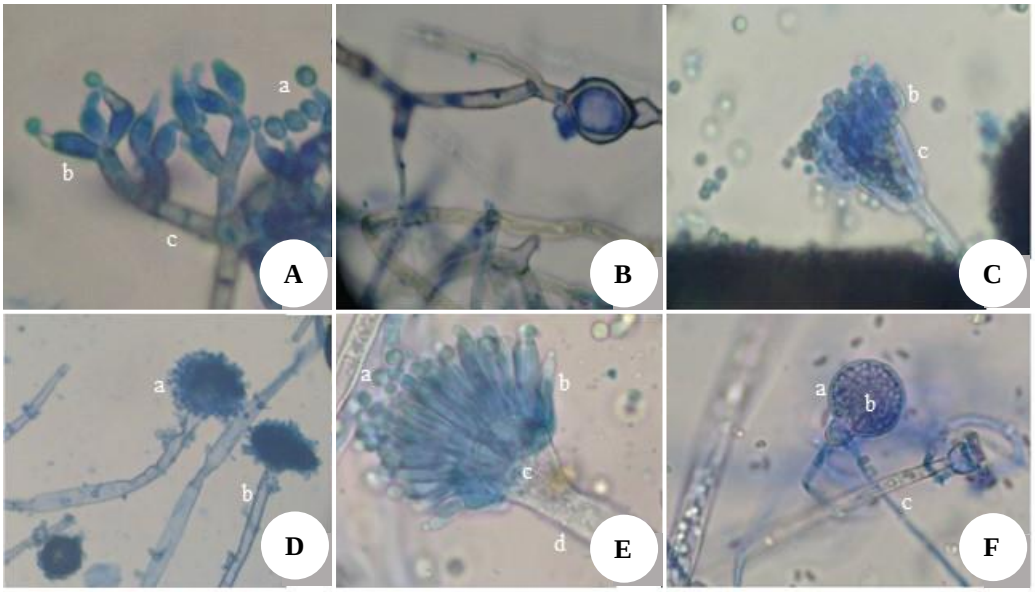

Figure 2. Fungal diversity: A. *Trichoderma*: conidia (a), hyalin (b), conidiophore (c); B. *Pythium*: oogonium (a), hyphae (b); C. *Penicillium*: conidium (a), phialide (b), metula (c), stipe (d); D. *A. niger*: conidia (a), conidiophore (b); E. *A. flavus*: conidia (a), phialide (b), vesicle (c), stipe (d); F. *Mucor* sp.: sporangium (a), sporangiophore (b), hyphae (c)

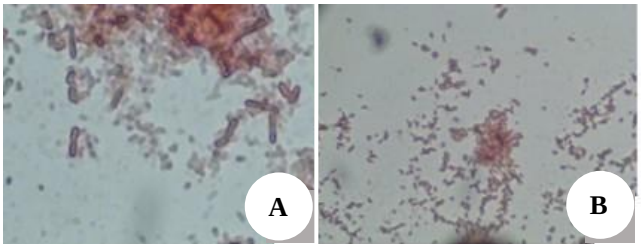

Figure 3. Bacteria (A. Bacillaceae, and B. Azotobacteraceae)

Entomopathogenic bacteria

Two bacterial families, Azotobacteraceae and Bacillaceae, were observed in the soils of both non-mining and ex-coal-mining land (Figure 3). However, only Azotobacteraceae was present in the deeper subsoil layers (60-80 cm or 80-100 cm) of the ex-coal-mining land. Interestingly, all Azotobacteraceae observed in this study were identified as coccus Gram-positive bacteria, except in the ex-coal-mining land, in which coccus Gram-negative Azotobacteraceae were found at the soil depths of 30-60 cm and 60-80 cm. The bacterial population was denser in non-coal-mining ($4.2\text{-}9.6\times10^6$) compared to ex-coal-mining land ($1.4\text{-}5.1\times10^6$). Bacterial population decreased along with soil depth at both experimental sites (Table 2). In this study, only Bacillaceae bacteria, observed in the digestive system of deceased *T. molitor* larvae, were identified as entomopathogenic bacteria in both ex-mining and non-mining lands.

Entomopathogenic nematodes

A high genetic diversity of nematodes was observed in both non-mining and ex-mining land. Among the fifteen genera identified (Figure 4), six—namely *Heterodera*, *Heterorhabditis*, *Hoplolaimus*, *Pratylenchus*, *Radopholus*, and *Rotylenchus*were present in both non-mining and ex-coal-mining lands. Conversely, *Dorylaimus*, *Meloidogyne*, *Rotylenchulus*, and *Tylenchus* were found only in non-mining area, while *Longidorus*, *Rhabditis*, *Steinernema*, and *Xiphinema* were exclusive to ex-mining land. The number of nematode populations was notably higher in ex-coal-mining compared to non-mining land. It was also observed that the deeper the soil layer, the greater the number of isolated nematodes, as found in both non-mining and ex-coal-mining land (Table 3). Despite the high nematode diversity, only *Heterorhabditis* and *Steinernema* were identified as entomopathogenic nematodes.

Physicochemical properties of soil

In general, soil nutrient content was higher in non-mining land compared to post-coal mining land (Table 4). There was also a variation in the nutrient content at different soil depth layers. Most of the nutrients were more abundant in the subsoil compared to the topsoil, as observed in the available K, Ca++. Mg++, K++, and Na+, as well as base saturation in non-mining land. In contrast, the reverse phenomenon was observed in post-coal-mining land.

Aluminum content was found to be higher in post-coal-mining than non-mining land. Moreover, the aluminum content tended to decrease with increasing soil depth on non-coal-mining land, while tending to be higher in the deeper soil layers on post-coal-mining land. The pH status of non-mining and post-coal-mining land was in the range of 4.02-4.9 and 3.19-4.19, respectively, showing higher acidity in post-coal-mining land.

Soil texture was determined by the proportion of mineral components in the soil. Among these mineral particles, silt, rather than clay or sand, was the dominant soil texture in topsoil and subsoil of both non-mining and post-coal-mining land. However, the relative proportions of different mineral particles (sand, silt, and clay) varied in each soil layer, resulting in distinct soil textures.

Table 2. Bacterial diversity and density at different soil depth layers, and screening of entomopathogenic bacteria in ex-mining and non-mining land in Pendingin, Sanga-Sanga subdistrict, Kutai Kartanegara District, Indonesia

Soil depth layers (cm)	Bacterial population density cfu/g	Family
Non-mining land		
0-30	9.6 x 10^6	- Coccus, Gram (+), Azotobacteraceae - Bacilli, Gram (+), Bacillaceae*
30-60	5.5 x 10^6	- Coccus, Gram (+), Azotobacteraceae - Bacilli, Gram (+), Bacillaceae*
60-80	4.5 x10^6	- Coccus, Gram (+) Azotobacteraceae - Bacilli, Gram (+), Bacillaceae*
80-100	4.2 x 10^6	- Coccus, Gram (+): Azotobacteraceae - Bacilli, Gram (+): Bacillaceae
Post-coal-mining land		
0-30	5.1 x 10^6	- Coccus, Gram (+); Azotobacteraceae - Bacilli, Gram (+); Bacillaceae*
30-60	4.9 x 10^6	- Coccus, Gram (+) Azotobacteraceae - Bacilli, Gram (+); Bacillaceae*
60-80	1.6 x 10^6	- Coccus, Gram (+); Azotobacteraceae
80-100	1.4 x 10^6	- Coccus, Gram (+); Azotobacteraceae

Note: *Entomopathogenic bacteria

Table 3. Nematode diversity and density at different soil depth layers, and screening of entomopathogenic nematodes in ex-mining and non-mining land in Pendingin, Sanga-Sanga Subdistrict, Kutai Kartanegara District, Indonesia

Soil depth layers (cm)	Nematodes Population/ kg soil	Genus
Non-mining land		
0-30	1.1 x10^2	*Heterodera, Meloidogyne, Tylenchus, Heterorhabditis**
30-60	1.2 x 10^2	*Heterorhabditis*, Pratlyenchus, Heterodera, Radopholus, Hoplolaimus*
60-80	4 x 10^2	*Pratylenchus, Heterodera*
80-100	2 x 10^2	*Rotylenchus, Rotylenchulus, Dorylaimus*
Post-coal-mining land		
0-30	5.1 x 10^2	*Heterodera, Xiphinema*
30-60	7.0 x 10^2	*Heterorhabditis*, Steinernema, Radopholus, Rhabditis*
60-80	8.0 x 10^2	*Steinernema*, Hoplolaimus, Rotylenchus*
80-100	9.2 x 10^2	*Pratylenchus, Longidorus*

Note: *Entomopathogenic nematodes

Discussion

The greatest concern regarding land degradation impacts of post-mining areas for the agriculture sector is the reduction of biodiversity and soil fertility. Mining activities often lead to the destruction of natural habitats and ecosystems, resulting in the deracination and decline of plant and animal species, and leading to a loss of biodiversity that also negatively affects agricultural productivity. In addition, post-mining land undergoes physical and chemical changes that render it unsuitable for agriculture, such as reduced soil porosity and aeration due to the alteration of soil structure, a lack of organic matter and nutrients due to the topsoil removal, introduced contaminants such as heavy metals and toxic chemicals, and altered soil pH—more acidic or alkaline—which can all be detrimental to plant growth (Prince et al. 2018; Janečková et al. 2023).

Efforts to address the loss of arable land in post-mining areas involve comprehensive reclamation and rehabilitation practices that aim to restore soil fertility, structure, and water-holding capacity, and to repair the quality of the environment and ecosystem, as mentioned in the regulation issued by the Minister of Energy and Mineral Resources Regulation 7/2014 and Regional Regulation East Kalimantan 8/2013 (Yulianingrum 2023). These efforts may include topsoil replacement, revegetation, and ongoing monitoring to ensure the land can support agriculture practices. Furthermore, to achieve normal criteria for fertile soil supporting agricultural activities, maintenance of microbial community structures is very important.

Microbial entomopathogen diversity and density

In this study, it was observed that the nematode and soil microbial diversity in post-coal-mining land clearly differed to that in the non-mining land. Fungal diversity and density in post-coal-mining land soil was considerably lower compared to the non-mining land. This situation was also observed in soil bacteria, which showed low biodiversity in post-coal-mining land even after the completion of the reclamation process. A significant reduction of microbial diversity due to coal mining

practices after land restoration was also observed in a study conducted by de Quadros et al. (2016). In their study, post-coal-mining land had undergone different durations of restoration (3-19 years), which involved replacing the soil layers, applying limestone to raise the pH to 6.0, and revegetating the land with grasses. Even after the completion of the reclamation process after many years, the composition of the microbial diversity and biomass of post-mined sites was significantly lower than that of the control (unaltered sites). Similar results were also found in different studies comparing the microbial diversity and density in reclaimed and revegetated post-coal-mining sites with that in undisturbed sites, both of which reported significant declines in microbial community structure and diversity (Li et al. 2014; Upadhyay et al. 2014).

A reduction of microbial (bacterial and fungal) diversity and density was also observed in the deeper layers of soil in both the post-coal-mining and non-mining-land, in which the microbes decreased as the depth of the soil layer increased. A similar observation was studied by Upadhyay et al. (2014), who reported that rhizospheric soils showed greater diversity in both bacteria and fungi compared to non-rhizospheric soil. This outcome is supported by recent studies concluding that soil microbial diversity decreases along with the soil depth (Guo et al. 2021; Wang et al. 2021).

It is well known that topsoil is the most biologically active and favorable layer for microorganisms. Compared to the subsoil layer, topsoil contains the highest concentration of organic matter, providing food and nutrients to microorganisms (Bahram et al. 2017). In addition, the uppermost layer of soil, also known as A-horizon, is well-aerated and has adequate moisture content and moderate temperatures, providing a suitable environment for a wide range of microorganisms (Liu et al. 2019; Aguado et al. 2023).

Figure 4. Nematodes (left to right: A. *Hoplolaimus*, B. *Pratylenchus*, C. *Tylenchus*, D. *Heterorhabditis*, E. *Steinernema*, F. *Dorylaimus*, G. *Xiphinema*, H. *Heterodera*, I. *Rhabditis*, J. *Radopholus*, K. *Longidorus*, L. *Rotylenchus*, M. *Rotylenchulus*, N. *Helicotylenchus*, O. *Aphelenchus*)

Table 4. Physicochemical properties of soil in non-mining and ex-coal-mining land in Kutai Kartanegara District, East Kalimantan, Indonesia

Soil parameters	Units	Non-coal mining land		Ex-coal mining land					
		R1 0-30	R2 30-60	R3 60-80	R4 80-100	S1 0-30	S2 30-60	S3 60-80	S4 80-100
Carbon organic	%	7.65	4.64	4.14	3.41	3.80	3.74	1.38	0.86
Nitrogen total	%	0.41	0.24	0.51	0.14	0.17	0.15	0.16	0.19
Ratio C/N	-	18.66	19.33	8.12	24.36	22.35	24.93	8.63	4.53
Available P (Bray1)	ppm	19.39	12.60	13.38	20.26	5.11	4.03	0.96	4.12
Available K (Morgan)	ppm	110.45	214.54	170.21	234.33	209.22	81.56	82.09	143.28
Al^{3+} (Morgan)		4.00	2.20	2.10	3.10	2.00	6.80	5.10	8.40
H^{+} (Morgan)		3.46	1.80	1.70	2.66	1.66	6.20	4.46	4.26
Ca^{++}	meq/100 g	0.63	1.67	0.83	1.19	1.95	0.79	0.52	0.61
Mg^{++}	meq/100 g	0.48	1.25	0.68	0.98	1.01	0.48	0.37	0.49
K^{+}	meq/100 g	0.30	0.65	0.48	0.91	0.67	0.20	0.26	0.40
Na^{+}	meq/100 g	0.04	0.38	0.28	0.29	0.34	0.11	0.02	0.12
CEC	meq/100 g	8.91	7.95	6.07	9.13	8.16	8.58	10.73	14.28
BS	%	16.3	49.7	37.4	36.9	48.7	18.4	10.9	11.3
Al Saturation	%	44.89	27.67	34.60	33.95	26.21	46.64	47.53	58.82
pH	–	4.02	4.62	4.54	4.90	3.56	3.35	4.19	3.19
Clay	%	14.77	36.54	20.07	23.21	28.72	16.54	23.94	32.85
Silt	%	34.48	58.18	55.30	35.43	67.66	64.75	33.70	35.54
Sand	%	50.75	5.28	24.63	41.36	3.62	18.71	42.36	31.61
Texture*		SiCL	SiCL	SiL	L	SiCL	SiL	L	CL

Note: *Soil Texture, SiCL: Silty Clay Loam; SiL: Silty Loam; CL: Clay Loam; L: Loam

Furthermore, there is an interaction between microorganisms and plant roots, which are more abundant in the top layer of soil. Plant roots release root exudates and provide energy to many soil microorganisms, thereby contributing to a more active microbial community (Zhalnina et al. 2018; Zhou et al. 2020). The vegetation types, plant species, and climate all influence the soil microbial diversity and community composition (Li et al. 2022; Sopialena and Palupi 2017).

One of the key roles of microorganisms in agriculture is as agents of biological control, a process that harnesses natural enemies to suppress pest populations. The use of biological control agents is considered the most prudent manner of controlling pests as it can reduce our reliance on chemical pesticides, and because it is a long-term, specific pest targeting, environmentally friendly, cost-effective approach that is safe for human health and supports sustainable agriculture (Boro et al. 2022). Therefore, besides observing the genetic diversity and density of the microorganisms in the ex-mining and non-mining land, this study also focused on screening entomopathogenic nematodes and microbes as potential biological control agents for suppressing insect populations. Insects are the largest and most diverse group of organisms included as one of the most common and economically significant types of plant pests (García-Lara 2016), causing significant damage to many crop plant species (Manosathiyadevan 2017).

Among all microbes, only *Trichoderma* (fungi) and Bacillaceae (bacteria) were identified as entomopathogenic microorganisms in the present study, in both ex-mining and non-mining lands. *Trichoderma* can indirectly act as an entomopathogen by producing insecticidal secondary metabolites, antifeedant compounds, and repellent metabolites (Poveda 2021). This is due to *Trichoderma*'s ability to produce metabolites such as citric acid (Vinale et al. 2012), as well as enzymes like urease, cellulase, glucanase, and chitinase (Carlile et al. 2001), which can negatively affect insects and even cause death when they enter the body. On the other hand, bacteria from the Bacillaceae family, particularly *Bacillus thuringiensis*, have received significant attention as entomopathogens. This bacterium is capable of producing proteinaceous protoxin crystals with insecticidal properties. When ingested by insects, these crystals disintegrate in the stomach and are broken down by host proteases to produce an active poison known as endotoxin, which ultimately leads to insect death (Deka et al. 2021).

In contrast to the fungi and bacteria, nematodes were found to be more abundant in the post-mining than in the non-mining land in terms of their diversity and density. Additionally, more nematodes were observed in the deeper soil layers. Most related studies have observed that the vertical distribution of nematode communities decreases from the topsoil layer to the subsoil layer (Cheng et al. 2021). This variation in soil nematode communities may be explained by the distinct characteristics of soils at different layers (Liu et al. 2022). The differences in soil nematode diversity and density at different soil depths observed in this study might be influenced by soil variables. The amount of nematode diversity varied in different experimental sites (Ilieva-Makulec et al. 2015). Results showed that nematode generic richness was higher in the topsoil of one site, while in a different habitat, nematode diversity was significantly lower in the topsoil and highest in the subsoil. Overall, it is likely that nematode abundance and distribution are influenced by many factors, such as soil properties, climate and environmental conditions, land use and management, plant species and root exudates, predation and competition, and succession and ecosystem

development (Ilieva-Makulec et al. 2015; Zhang 2020; Cheng et al. 2021; Li et al. 2022).

Nematodes may be classified into three main groups: saprophages (living on dead organic matter), predators (feeding on small animals, including other nematodes), and parasites (infesting insects, animals, human, fungi and higher plants) (Pant et al. 1998). Eleven of the fifteen nematodes identified in this study, namely *Heterodera*, *Meloidogyne*, *Tylenchus*, *Pratlyenchus*, *Radopholus*, *Hoplolaimus*, *Rotylenchus*, *Rotylenchulus*, *Xiphinema*, and *Longidorus* were plant-parasitic nematodes. *Meloidogyne*, *Radopholus*, and *Rotylenchulus* were the top three genera of plant-parasite nematodes observed in East Kalimantan (Suyadi and Rosfiansyah 2017). *Dorylaimus* and *Rhabditis*, on the other hand, were non-plant-parasitic nematodes. *Dorylaimus* functions as an omnivore in the rhizosphere, and *Rhabditis* as a bacterivore. In this study, only *Heterorhabditis* and *Steinernema* were identified as entomopathogenic nematodes, as also observed in the previous studies (Bhat et al. 2020; Julià et al. 2023).

Relation of soil physicochemical properties and microbial community

The physicochemical properties of post-mining soil were considerably different from those of non-mining soil in this study. The different characteristics of post-mining land arise from the significant alterations in soil properties, structure, and chemical composition due to mining activities. The mining process disrupts the natural soil structure, leading to soil compaction, which reduces pore spaces and affects water infiltration and root penetration (Hermansyah et al. 2021). Additionally, the removal or burying of topsoil (due to improper reclamation processes) results in a significant reduction in the organic content of the soil (especially in the root growth layer), leading to nutrient loss and further diminishing soil fertility. Furthermore, the mining process can expose rocks and minerals that, when weathered, can alter the soil's pH, leading to acid mine drainage and significantly lowering the soil pH (Herman et al. 2024). These conditions make the soil ecosystem in post-mining land unconducive for microbial communities, which contrasts with the conditions in non-mining land.

Soil is a medium for plant growth and also acts as an environment for microbial growth, determining the quality of growing soil microbes and plants. The interactions between soil microbes and plants play a crucial role in soil health and fertility. Fertile soil is typically a source of organic matter for microbial activity. Soil status encompasses a wide range of physical, chemical, and biological properties that influence the habitat and conditions for soil microorganisms. A healthy soil status with sufficient organic matter and nutrient availability ensures that microorganisms have the resources they need to thrive (Kaur et al. 2022). The presence of high organic matter in soil is characterized by several physicochemical parameters, such as pH levels, Cation Exchange Capacity (CEC), Soil Organic Carbon (SOC) content, and nutrient content, especially of nitrogen, phosphorus, and potassium (Dandwate 2020).

It was also observed that soils in both post-coal mining and non-mining land were found to be acidic, with minor variations at every soil depth layer. However, soil pH in the post-mining land was considerably lower, in the range of 3.19-4.19, compared to that in the non-mining land (4.02-4.9). Related to the lower pH in post-mining land was the aluminum content, which was higher than that in the non-mining land as acidic soils enhance aluminum solubility and availability. Furthermore, soil pH is positively correlated with another soil property, Basic Saturation (BS), which was observed to be higher in the non-mining land.

Soil pH strongly influences microbial communities. Acidic or alkaline soils can limit the diversity and activity of microorganisms, impacting nutrient availability and overall soil. It is a critical factor that affects the activity and diversity of soil microorganisms. Different microbial species have specific pH preferences, and soil pH can influence their metabolic activities. Soil pH outside the optimal range for specific microbes can limit their growth and activity (Wang et al. 2019). Therefore, maintaining an appropriate pH level is essential for a diverse and active microbial community.

Soil organic matter was higher in non-mining land in this study, as characterized by the higher Soil Organic Carbon (SOC) and nutrient content than that of post-coal-mining land, as well as by the pH level. Higher organic matter content is associated with high SOC, which, as carbon is a fundamental component of organic materials, mainly occurs via the microbial breakdown, partial decay, and conversion of deceased organic materials (Lefèvre et al. 2017). This activity is co-related with nutrient availability; that is, as organic matter decomposes, it releases nutrients into the soil, contributing to the soil's overall fertility and health (Gerke 2022).

Overall, soil physicochemical properties play a significant role in shaping the composition and activity of the microbial community (Dasgupta and Brahmaprakash 2021). A larger microbial community was observed alongside the higher physicochemical soil properties of the non-mining land, rather than in the post-coal-mining land. Changes in these properties can influence the composition and activity of microbial community, subsequently impacting nutrient cycling, organic matter decomposition, and overall soil fertility (Leiva et al. 2020; Kaur et al. 2022). Fertile soil is characterized by a well-balanced and diverse microbial community that supports plant growth by providing essential nutrients and improving soil structure. The composition and diversity of the soil microbial community are important factors in maintaining soil health that also support plant growth and health (Wang and Li 2023). The increasing microbial population (both in diversity and abundance) causes the dynamics of the soil to improve and become healthier naturally (Bertola et al. 2021). The ability to change soil biological properties in a positive direction can increase the population of microbes, including entomopathogenic microbes, which benefit plant growth and reduce the need for artificial fertilizer and pesticide use.

In conclusion, the results showed that microbial diversity in post-coal-mining land differed from that in

non-mining control. The entomopathogenic fungal and bacterial diversity and density in ex-coal-mining soil was considerably lower compared to that in the non-mining land. The microbial (bacterial and fungal) diversity and density were lower in deeper layers of soil in both post-coal-mining and non-mining land, and tended to decrease soil layer depth increased. In contrast to fungi and bacteria, entomopathogenic nematodes were observed to be more abundant in the post-mining than in non-mining land in terms of their diversity and density. Additionally, nematodes were more abundant in the deeper layers of soil. Soil fertility was found to be lower in ex-coal-mining land, as characterized by several soil physicochemical properties, of which the lower soil pH, basic saturation, Soil Organic Carbon (SOC) and nutrient content detected likely supported a smaller microbial community compared to the non-mining land.

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