

**Research Article**

**Quality of soil from the nickel mining area of Southeast Sulawesi, Indonesia, engineered using earthworms (*Pheretima* sp.)**

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**Abstract**

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Earthworms (*Pheretima* sp.) could survive under abiotic stress soil conditions. Furthermore, their activities as ecosystem engineers allow for the creation of soil biostructures with new characteristics. Therefore, this study aimed to investigate the effect of the abundance of *Pheretima* sp. on the aggregate size, physicochemistry, and biology of the topsoil from the nickel mining area of Southeast Sulawesi, Indonesia. It was carried out by first grouping their abundance into zero, two, four, six, and eight individuals per pot and then carrying out tests. The *Pheretima* sp. were then released onto the surface of the topsoil and mixed with biochar that was saturated with tap water in the pot overnight. The results showed that the abundance of the species had a significant effect on the size class distribution, and aggregate stability of the soil. Furthermore, the size of the soil aggregates formed was dominated by the size class 2.83 - 4.75 mm under both dry and wet conditions. Under dry conditions, three size classes were found, while under wet conditions, there were five size classes. The results also showed that the highest and lowest stability indexes occurred with zero and eight *Pheretima* sp., respectively. Furthermore, the abundance had a significant effect on pH, organic C, total N, CEC, and total nematodes. However, it had no significant effect on the total P, C/N ratio, total AMF spores, and flagellate. The highest soil pH occurred with zero *Pheretima* sp., while with six and two members of the species, the total nematode was at its highest and lowest populations, respectively. Therefore, it could be concluded that the species was able to create novel conditions in the topsoils at the nickel mining area that were suitable for various soil biota.

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**Introduction**

Southeast Sulawesi Province is known as a nickel ore mining area in Indonesia. Generally, rocks at this location are classified into ultramafic formations (Jahidin et al., 2020), and the soil types developed

from these formations are characterized by the following. They include, low in organic matter, available P, cation exchange capacity, exchangeable Ca, exchangeable K, hydraulic conductivity, soil biological activity, and fertility, however, constantly high plasticity (Safiuddin et al., 2011; Tufaila et al.,

2011; Hatfield and Walthall, 2015; Alam et al., 2020). Nickel ore mining activities through a series of soil extraction and removal activities have an impact on landscape changes, loss of natural vegetation, and a deeper decline in soil quality and fertility (Vithanage et al., 2019; Haigh et al., 2020). Therefore, restoration of the soil quality and fertility could be carried out through the rearrangement of soil particles (micro aggregates) into more suitable aggregates (Liu et al., 2019). In the aggregate formation process hierarchically, free primary particles and aggregates measuring  $<20\ \mu\text{m}$  form micro aggregates measuring  $20\text{-}250\ \mu\text{m}$ , while micro aggregates form macro aggregates with sizes of  $> 250\ \mu\text{m}$ . The aggregation process is mediated by soil organic matter and hyphae, which function as binding agents for the soil particles (Six et al., 2004; Tang et al., 2011). The pore space formed between the aggregates could provide microhabitat for soil microflora and fauna that regulate the important process for the soil ecosystem (Jiang et al., 2018). Soil fauna as an ecosystem engineer has the ability to modify the structure and environmental conditions of the soil, and simultaneously modulate the population and activity of soil microbes (Hedde, 2005; Kilowasid et al., 2015a). *Pheretima* sp. could modify the structure of the soil due to their activity of making channels therein and consuming a mixture of organic matter and soil particles, which ultimately leads to the creation of a new soil biostructure (Paz-Ferreiro et al., 2014; Bedano et al., 2019). With regards to size, the soil biostructure formed could be classified into classes 50 - 250, 250 - 500, 500 - 2000, and  $> 2000\ \mu\text{m}$  (Hong et al., 2011). Until now, there are various taxa of the species that are often used in engineering soil structures, restoring soil fertility, and modulating populations of bacteria, arbuscular mycorrhizal fungi, protozoa, and nematodes in soil (Duarte et al., 2014; Martinkosky et al., 2017).

Earthworms that originated from the genus *Pheretima* are distributed in Southeast Asia, including Indonesia (Darmawan et al., 2012). Their activity could stimulate the population growth of arbuscular mycorrhizal fungi and increase the activity of free-living bacteria nitrogenase in the rhizosphere (Zarea et al., 2009). Furthermore, *Pheretima* sp. could survive in soil contaminated with agricultural chemicals (Goto and Sudo, 2018) and Pb/Zn mine tailings (Cheng and Wong, 2008). Previously, Kilowasid et al. (2020) reported the species could be mixed with soil and compost from "komba-komba" (*Chromolaena odorata* L.) pruning engineered to be about 49.24% cast, which contains various morphological types of bacterial. Although *Pheretima* sp. has the opportunity to be used in soil quality and fertility restoration, up till now, studies related to its impact on the modification of the novel soil character from the nickel mining area are still neglected. Therefore the objective of the study is to investigate the aggregate, chemistry, and biology

character of the soil from the nickel mining area engineered using *Pheretima* sp.

## Materials and Methods

This study was conducted in the greenhouse of the Agriculture Faculty, University of Halu Oleo, which is located in the geographical position of  $122^{\circ}31'32.9''$  east longitude (EL),  $04^{\circ}00'33.8''$  south latitude (SL), and 54 m above sea level. Five abundance levels of *Pheretima* sp. were tested, namely without *Pheretima* sp. (labeled C0), two individuals per pot (labeled C1), four individuals per pot (labeled C2), six individuals per pot (labeled C3), and eight individuals per pot (labeled C4). Furthermore, each of the tests was repeated four times in a randomized block design.

Topsoil to a depth of 10 cm from the surface was obtained from the cogongrass vegetation in the nickel ore mining area of PT. WijayaInti Nusantara in Wonua Kongga Village, Laeya District, Konawe Selatan Regency, which is located at  $122^{\circ}27'35.2''$  EL,  $04^{\circ}25'12.4''$  SL, and 54 m above sea level. The soil was then sieved with a sieve measuring 0.5 mm per hole. Furthermore, a total of 3 kg of soil that passed the sieve was put into a plastic pot, of height 15 cm, diameter of 24 cm on the top part, and 18 cm at the below part. The pot also had six small holes below its surface that were covered with plastic gauze and each had a size of  $< 0.2\ \text{mm}$ .

*Pheretima* sp. were collected from the banks of the Konaweheha River which are located at  $122^{\circ}15'43.6''$  EL,  $04^{\circ}00'21.5''$  SL, and at an altitude of 105 m above sea level in Sabulakoa Village, Sabulakoa District, Konawe Selatan Regency. The soil surface covered by the cast was excavated using a hoe, and the worms were separated from the soil using the hand sorting technique. Adult earthworms were reared in containers made of styrofoam and filled with their original soil. They were then transported to the laboratory and maintained till use. During maintenance, feed made from a mixture of compost and manure was given.

The stems and dry twigs of the sengon (*Albizia* sp.) tree were burned in a simple retort made of drums with an incomplete combustion system until biochar was formed. Furthermore, the chilled biochar was extracted, mashed, and filtered using a filter measuring 0.5 mm per hole. About 5% (w/w) of the sieved biochar was mixed with topsoil until a homogeneous mixture was formed in each pot. Then the mixture was saturated using tap water, and the water was allowed to drip from the pot overnight. *Pheretima* sp. which has a clitellum, similar in length and its stomach contents emptied on the surface of the damp filter paper, was released into each pot that had been prepared. The number released was according to the treatment design as mentioned above. After all parts of the *Pheretima* sp. body entered into the mixture of moist topsoil and biochar, the surface for each pot was covered by gauze

with a size of <math><0.2\text{ mm}</math> per hole. The pots were then placed in a greenhouse following a randomized block design procedure. Furthermore, the moisture of the topsoil-biochar mix was maintained by spraying tap water every week. The mixture was then removed from the pot after six weeks of engineered with *Pheretima* sp. (Figure 1). The *Pheretima* sp. removed from the topsoil-biochar mix, and the topsoil-biochar samples were taken for analysis of their physicochemical and biological characteristics which include, the distribution of aggregate size, aggregate stability index, pH, organic C, total  $\text{P}_2\text{O}_5$ , total N, C/N

ratio, and cation exchange capacity (CEC). The distribution of aggregate size and aggregate stability of the soil was determined using a dual wet and dry sieving method (Kemper and Rosenau, 1986; Chisci et al., 1989). Additionally, the soil pH was determined using a pH meter, organic C, using the colorimetric method, total nitrogen using Kjeldahl, total  $\text{P}_2\text{O}_5$  using 25% HCl extract, and cation exchange capacity using the titrimetric method (Motsara and Roy, 2008). The topsoil biological characters determined include total nematodes, arbuscular mycorrhizal fungi (AMF) spore, and flagellates.



Figure 1. Topsoil-biochar mix conditions after engineered by *Pheretima* sp. for six weeks.

Total free-living nematodes in the soil were extracted following a modified Baermen Funnel technique (Kilowasid et al., 2014). Moreover, a total of 250 g of fresh soil samples from each pot were extracted following the Baermen funnel technique for 48 hours. Free swimming nematodes in the water were then filtered using a filter measuring  $38\ \mu\text{m}$  per hole. The nematodes that were stuck on the surface of the filter, were dislodged using water and poured into the Erlenmeyer. Then, the water was heated for 5 minutes until a temperature of  $70^\circ\text{C}$  was ascertained. The dead nematodes, together with the water were then poured into the Erlenmeyer. Furthermore, 5 ml of 70% alcohol and 4 ml of triethanolamine formaldehyde solution were added to the nematode-water mixture and let stand for 1 hour. The nematodes were then poured into a petri dish and counted under a dissecting microscope Bosco using 45 magnifications. AMF spores were extracted using wet sieving and decanting methods followed by differential water/sucrose centrifugation (Snoeck et al., 2010). A total of 50 g of fresh soil samples from each pot were suspended in 1000 ml of water and left to stand for a few seconds. The suspension was then poured into a 70 mesh and 400 multilevel mesh filter, stored in a test tube and added with a sugar solution of concentrations of 20% and 60%. Subsequently, the mixture was stirred and then left to stand. The suspension in the test tube was centrifuged at 2000 rpm for 3 minutes. Therefore, the water separated from the soil solids and was poured over a  $38\ \mu\text{m}$  filter per hole. The water then flowed until the concentration of the sugar solution disappeared. The retained spores were drained using

water into a petri dish, and were counted under a dissecting microscope. Flagellates were then estimated in the heamocytometer chamber and the final abundance was expressed by the number of cells in the initial dilution (Adl et al., 2008).

#### Statistical analysis

Analysis of the effect of treatment on the parameters of the physical, chemical, and biological character of the soil was carried out using analysis of variance. The significant effect of the treatments was then determined using Fisher's exact test at the  $p < 0.05$  level. Whenever there was any significant effect from the abundance of *Pheretima* sp, the Least Difference Test (LSD) was applied at the  $p < 0.05$  level to compare the means of each parameter among the treatments.

## Results

### Aggregate size class distribution

It was discovered that the aggregate size was distributed into classes 2.00 - 2.83, 2.83 - 4.75, and 4.75 - 8.00 mm through dry sieving. Meanwhile, through wet sieving, it was found to be distributed into size classes 0.50 - 1.00, 1.00 - 2.00, 2.00 - 2.83, 2.83 - 4.75, and 4.75 - 8.00 mm (Table 1). Table 1 shows that the significant effect (ANOVA at  $p < 0.05$ ) of the application of *Pheretima* sp on the aggregate distribution through the dry sieving method occurred in size classes 2.83 - 4.75, and 4.75 - 8.00 mm, while there was no significant effect for classes 2.00 - 2.83 mm (ANOVA at the level of  $p > 0.05$ ). Furthermore, the soil aggregates obtained from dry sieving were

dominated by size classes 2.83 - 4.75, 4.75 - 8.00, and 1.00 - 2.00 mm, respectively. The highest percentage of soil aggregates in size class 2.83 - 4.75 mm occurred on the soil treated with C0, and this was significantly different from the percentage of aggregates formed in treatment C4 (LSD test at  $p < 0.05$ ). Meanwhile, the difference with others was not significant (LSD test level of  $p > 0.05$ ). In contrast, the percentage of aggregates formed in the size class 4.75 - 8.00 mm in treatment C4 was the highest and was significantly different from C0 (LSD test at transform  $p < 0.05$ ). Whereas, compared to others, it is not significant (LSD test at transform  $p > 0.05$ ). The difference in the aggregates formed at C2 compared to C0 was significant (LSD test at  $p < 0.05$ ), while for the others,

it was not significant (LSD test at  $p > 0.05$ ). Also, in the size class 2.00 - 2.83 mm, the percentage of the aggregate formed at C1 was the highest, while at C0 it was the lowest. The difference in the percentage at C1 compared to C0 was significant (LSD test at  $p < 0.05$ ), whereas compared to other treatments, it was insignificant (LSD test at  $p > 0.05$ ). However, the difference in the percentage of the aggregates between C4 and C0 treatments was not significant (LSD test at  $p > 0.05$ ). For the size class 2.83 - 4.75 mm, the percentage at C3 treatment was the highest; however, it was not significantly different (LSD test at level  $p > 0.05$ ) compared to C1, C2, and C4, except for C0. Moreover, significant differences were also shown between C1, C2, or C4 versus C0 (LSD test at  $p < 0.05$ ).

Table 1. Distribution of soil aggregates size after application of *Pheretima* sp. on topsoil from nickel ore mining.

Treatments*	Class Size Distribution of Soil Aggregate (%)				
	0.50 – 1.00 mm	1.00 – 2.00 mm	2.00 – 2.83 mm	2.83 – 4.75 mm	4.75 – 8.00 mm
Dry Sieving					
C0	-	-	4.85 a	78.73 a	16.41 b
C1	-	-	6.98 a	71.55 ab	21.47 ab
C2	-	-	6.41 a	70.80 ab	22.79 ab
C3	-	-	6.22 a	69.55 ab	24.22 ab
C4	-	-	5.28 a	65.18 b	29.54 a
Wet Sieving					
C0	9.45 a	6.70 b	15.92 b	67.87 a	0.05 a
C1	10.28 a	9.19 ab	19.61 a	60.78 b	0.13 a
C2	8.38 a	11.45 a	19.44 a	60.60 b	0.13 a
C3	9.71 a	10.03 ab	18.65 a	60.91 b	0.70 a
C4	8.68 a	10.70 ab	18.32 ab	60.70 b	1.59 a

Note: Numbers followed by different letters in the same column show significant differences according to the LSD test at the  $p < 0.05$  level. \*Treatments: C0 = without *Pheretima* sp., C1 = two individuals per pot, C2 = four individuals per pot, C3 = six individuals per pot, C4 = eight individuals per pot.

Based on the results (Table 1) regarding the use of *Pheretima* sp in nickel mining soil media, it was found that the distribution value of soil aggregate through dry sieving and wet sieving was dominated by the particle sizes of 2.83 - 4.75 mm. The distribution value from the dry sieving for particle sizes 2.83 mm - 4.75 mm and 4.75 - 8.00 mm were higher than those from the wet sieving.

#### Stability index of soil aggregate

Figure 2 shows that the application of earthworms had a significant effect on the stability of the soil aggregate from the stockpile in the nickel ore mining activity area (ANOVA at  $p < 0.05$ ). The highest stability index occurred in treatment C0, while the lowest occurred at C4. Furthermore, the difference between the indexes of those treatments was significant at  $p > 0.05$ . However, compared to C1, C2, and C3 it was not significant at  $p > 0.05$ .

#### Soil chemical character

The abundance of *Pheretima* sp. showed a significant effect (at the  $p < 0.05$  level) on pH, organic C, total N,

and CEC in the soil. However, for the total P and C/N ratio, there was not significant at the  $p > 0.05$  level effect. In Table 2, it could be seen that the soil pH of the C0 treatment was the highest; however, for the C3 treatment, it was the lowest. The difference in soil pH between both treatments was significant at  $p < 0.05$ . Significant differences were also shown between treatments C1, C2, or C4 versus C3 (LSD test at  $p < 0.05$ ). Meanwhile, for treatment C0 compared to C1, C2, and C4, there was no significant difference (LSD test at level  $p > 0.05$ ).

#### Soil biological character

The abundance of *Pheretima* sp. showed a significant effect on the total nematodes (at the level of  $p < 0.05$ ), while the total spores of arbuscular mycorrhizal fungi and flagellates were not significant (at the level of  $p < 0.05$ ). The highest abundance of total nematodes occurred in treatment C3, while the lowest occurred in C1 (Table 3). Table 3 shows that the difference in nematode abundance between C3 and C1 was significant at  $p < 0.05$ ; however, compared to C0, C2, and C4 it was not significant at  $p > 0.05$ .

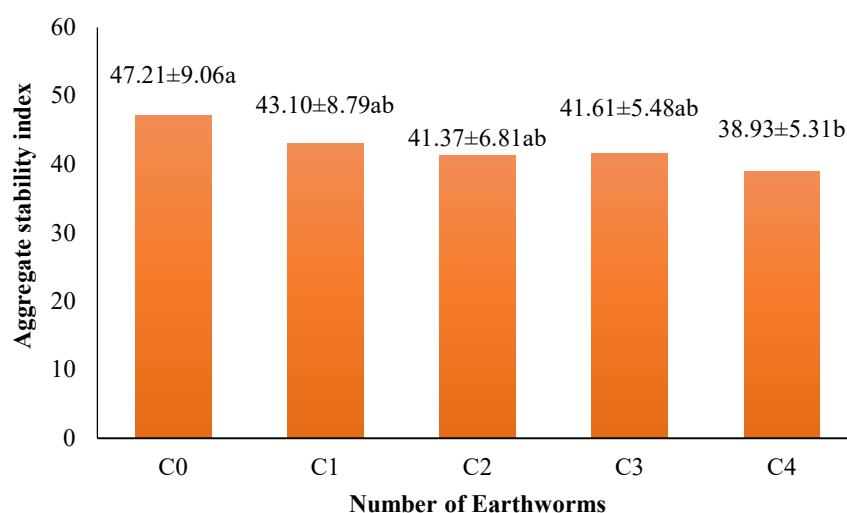


Figure 2. Value of the aggregate stability index after the application of *Pheretima* sp. on the topsoil from the nickel mining area. Note: The number followed by different letters above the bar shows significant differences according to LSD test at the  $p < 0.05$  level. C0 = without *Pheretima* sp., C1 = two individuals per pot, C2 = four individuals per pot, C3 = six individuals per pot, C4 = eight individuals per pot.

Table 2. Soil chemical characteristics of the nickel ore mining area after application of *Pheretima* sp.

Treatments*	pH	Organic C (%)	Total N (%)	P <sub>2</sub> O <sub>5</sub> mg/100g	C/N	CEC (cmol(+)/kg)
C0	7.15 a	0.60 b	0.37 b	12.54 a	1.68 a	1.26 b
C1	7.03 a	0.86 a	0.54 ab	12.88 a	2.17 a	2.02 ab
C2	7.04 a	0.71 ab	0.65 a	9.55 a	1.12 a	3.64 a
C3	6.63 b	0.57 b	0.43 ab	12.96 a	1.52 a	3.13 ab
C4	7.05 a	0.61 b	0.47 ab	13.39 a	1.37 a	3.11 ab

Note: Numbers followed by different letters in the same column indicate a significant difference according to LSD test at the level of  $p < 0.05$ . \*Treatments: C0 = without *Pheretima* sp., C1 = two individuals per pot, C2 = four individuals per pot, C3 = six individuals per pot, C4 = eight individuals per pot.

Table 3. Total spores of arbuscular mycorrhizal fungi, nematodes, and flagellates in the soil from the nickel ore mining area after *Pheretima* sp. application.

Treatments*	AMF Spore (Spore/50 g Soil)	Total of Soil Nematode (Individual/250 g Soil)	Flagellate (x10 <sup>8</sup> Individual/100 g Soil)
C0	60.19 a	3.75 ab	0.78 a
C1	50.44 a	2.81 b	0.53 a
C2	57.37 a	3.25 ab	0.46 a
C3	53.31 a	4.19 a	0.59 a
C4	47.44 a	3.44 ab	0.72 a

Note: Numbers followed by different letters in the same column indicate a significant difference according to LSD test at the level of  $p < 0.05$ . \*Treatments: C0 = without *Pheretima* sp., C1 = two individuals per pot, C2 = four individuals per pot, C3 = six individuals per pot, C4 = eight individuals per pot.

## Discussion

Soil structure is a key indicator of the condition of sustainable soil quality management (Johannes et al., 2019). This is because soil structure has various roles in controlling the function of soil ecosystems, such as regulating water retention and infiltration, organic matter and nutrient dynamics, root penetration, and

soil susceptibility to erosion (Rabot et al., 2018). The structure is formed hierarchically from clay particles (2-20 $\mu$ m) which are cemented by mucus released from the roots and microorganisms to form microaggregates (20-250 $\mu$ m). Moreover, microaggregates undergo coalescence into aggregates (> 250 $\mu$ m) during wetting and drying cycles (Dexter, 1988). The unification process goes through a series of microaggregate

rearrangement, flocculation, and cementation events with particles mediated by organic carbon, biota, ionic bridging, clay, and carbonate (Bronick and Lal, 2005).

The biological process of earthworm activity of mixing clay particles with organic matter produces a soil biostructure (vermicast) which is composed of soil aggregates of different size classes and levels of water stability (Dexter, 1988; Piron et al., 2012; Zanella et al., 2018; Lucas et al., 2019). In this study, five aggregate size classes were analyzed. Under dry conditions, the soil aggregates formed were distributed into sizes 2.00 - 2.83, 2.83 - 4.75, and 4.75 - 8.00 mm. For all treatments, the successive descending order of mass contribution was size class 2.83-4.75 mm ranging from 65-78.33%, classes 4.75 - 8.00 mm ranging from 16-29.54%, and classes 2.00 - 283 mm, ranging from 4.85-6.98% under dry conditions. Furthermore, under these conditions, the contribution of the aggregate mass of class 2.83-4.75 mm that occurred in the soil without earthworm application was the highest (78.73%). However, it decreased significantly with the increasing abundance of *Pheretima* sp up to 65.18%. On the other hand, the mass contribution of the 4.75 - 8.00 mm class experienced a significant increase with the increasing abundance of *Pheretima* sp, while the difference in the mass contribution that occurred on other aggregate sizes was not significant (Table 1). These results explain that naturally, the topsoil aggregate in the nickel ore mining area is dominated by an aggregate size of 2.83-4.75 mm. The significant decrease in the mass contribution of the aggregate size in this class which was accompanied by an increase in the contribution in the class 4.75 - 8.00 mm with the addition of the abundance of *Pheretima* sp. indicates they actively modified the soil aggregation through ingestion and egestion from the soil (Hallam and Hodson, 2020). The increase in the contribution of macroaggregates in line with the increase in the abundance of *Pheretima* sp was probably related to the increase in the number of soil particles consumed by the worms. Soil macroaggregates formed due to the activity of the species had a high content of particulate organic matter between the microaggregates forming the macroaggregate of sizes 4.75 - 8.00 mm. On the other hand, the decrease was related to the dominance of dissolved organic matter between the microaggregates and soil particles forming the aggregate of sizes 2.83 - 4.75 mm (Frazao et al., 2018; Bucka et al., 2019).

The drying-wetting cycle was always followed by the pooling and separation of the soil aggregate into various size classes (Kholodov, 2013). A wetting method is an approach used in detecting the resistance capacity of a macroaggregate structure to water strength (Kholodov et al., 2015). Under the wet conditions, the aggregate was formed and distributed into five aggregate size classes (Figure 2). Furthermore, a significant decrease in mass contribution occurred in the size classes of 2.83 - 4.75 mm and 4.75 - 8.00 mm. The aggregate formed under

the wet soil condition was distributed to sizes 0.50 - 1.00 and 1.00 - 2.00 mm. Additionally, the aggregate mass contribution of 1.00-2.00 mm, 2.00-2.983 mm, and 2.83-4.75 mm increased significantly in line with the abundance of *Pheretima* sp., while the other two size classes were not significant. The increasing abundance of *Pheretima* sp. caused a reduction in the aggregate stability index (Table 2).

Soil aggregate stability indicates the soil's ability to withstand wind erosion, rain blows, water digestibility, and tillage loads. Therefore, soil with a higher aggregate stability value would have a lower level of susceptibility to erosion (Kemper and Rosenau, 1986). The high aggregate stability index in the soil without the application of *Pheretima* sp. indicated that the soil aggregate of the nickel mining area was formed by the binding of multivalent cations ( $\text{Ca}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{Mn}^{2+}$ , and  $\text{Mn}^{3+}$ ) with organic matter (Jakšik et al., 2015; Hu et al., 2018). This fact indicated that *Pheretima* sp. has the ability to disintegrate soil aggregates that are formed naturally by chemical agents. Furthermore, it could bind topsoil particles in the nickel mining area to become aggregates that are more easily dispersed by water and penetrated by plant roots. Fresh aggregate (cast) formed by earthworm activity has lower stability than non-cast aggregate (Mer et al., 2021).

The facts mentioned above show the activity of *Pheretima* sp. being able to modify the topsoil structure of the mining area. The modification of these structures through channels is formed by the movement of earthworms accompanied by the formation of various classes of aggregates that are unstable (Barré et al., 2009; Zhu et al., 2020). The physico-chemical changes associated with the formation of these new structures could include soil pH, C, N, P, C/N, K, and CEC, and these depend on species, soil type, and land use (Sankar and Patnaik, 2018; Bottinelli et al., 2020; Tamartash and Ehsani, 2021). Nadalia and Pulunggona (2020) reported that the soil pH without topsoil at a depth of 0-20 cm is in the range of 5.61 - 6.69 in the mining area of Soroako, South Sulawesi. In this study, all treatments had alkaline soil pH (Table 2). The possibility of this alkalinity is related to the addition of biochar in all treatments. The negative charge surface area of the biochar binding  $\text{H}^+$  increases; therefore, the pH in the soil solution also increases (Li et al., 2013). The results of this study showed that soil pH with the addition of earthworms was lower than without earthworms. This phenomenon is probably related to the *Pheretima* sp's activity in increasing the decomposition of organic matter, based on which soil organic matter increases. This allows the surface area of the biochar to be covered, and the amount of negative surface charge of biochar which is able to bind  $\text{H}^+$  in soil solution to decrease in number; therefore, the soil pH drops (Hailegnaw et al., 2019). The difference in soil pH between *Pheretima* sp abundances was insignificant. These results are in accordance with previous studies

which showed that adding this worm to serpentinite soil with biochar application did not significantly change soil pH (Pingree et al., 2017). This study showed that the CEC of soil without *Pheretima* sp was lower than with the addition of the worm. This indicates that CEC is mutually independent of soil pH (Ross et al., 1991). The increase in soil CEC is related to the content of soil organic matter stored between microaggregates in macroaggregates formed by the worms (García-Montero et al., 2013).

The study by Sheehy et al. (2019) showed that the earthworm *Lumbricusterrestris* increased the potential for agricultural land to store C in macroaggregate-occluded microaggregates. The high organic C content in the soil that is disturbed by *Pheretima* sp's activity is thought to be related to the organic carbon content of microaggregate blankets formed through the activity of the worms in macroaggregates (biogenic), which could reach two times compared to macroaggregates without the worm's activity (Bottinelli et al., 2020). In this study, it appears that their presence with low abundance increases soil organic carbon content compared to their absence. This addition of organic carbon could come from the excretion of the worm's, organic compounds due to the decomposition of organic matter and soil microbial biomass (Sruthi and Ramasamy, 2018; Medina-Sauza et al., 2019). The rate of decomposition of the mineral soil organic matter could be triggered through the presence of the worms (Guo et al., 2019). Through this decomposition, soil organic carbon is emitted in the form of CO<sub>2</sub>, due to the stock of soil organic matter that would continue to be eroded when not accompanied by external input (Middleton, 2020), such as from plant roots and other organic matter. Dechaine et al. (2005) showed that there was a positive correlation between *Pheretima* sp. abundance and the rate of decomposition of organic matter (plant litter). The results show that the soil organic carbon content decreases with the increasing abundance of the species. (Table 2). This indicates an increase in their abundance, which could accelerate the rate of soil organic carbon loss through the decomposition process.

The process of decomposition of soil organic matter is always accompanied by processes of ammonification, nitrification, and denitrification which contribute to nitrogen dynamics in the soil (Araujo et al., 2004). The results show the total nitrogen in the soil supplemented by *Pheretima* sp. was higher than without earthworms, where the amount of total nitrogen decreased with the increasing abundance of the species. This fact agrees with the findings of Ozawa et al. (2005) which showed that the addition of the worms increased total nitrogen and available nitrogen (NH<sub>4</sub>-N and NO<sub>3</sub>-N) in the soil. This addition of total nitrogen may be derived from excreta (mucus and urine) during the worm's activity (Salmon, 2001; Bityutskii et al., 2007) and nitrogen-fixing aerobic microbes associated with those worms (Ozawa

et al., 2005). Although *Pheretima* sp. contribute to total-nitrogen and available nitrogen in the soil, their presence accelerates the rate of nitrogen loss through leaching and denitrification (Parkin and Berry, 1994). Both of these mechanisms contribute significantly to soil total-nitrogen (Domínguez et al., 2004; Braga et al., 2016). Decomposition of soil organic matter and nitrogen mineralization further affect the C/N ratio of mineral soils (Waqar et al., 2019). With time, the C/N ratio of mineral soils with the presence of *Pheretima* sp to those without the presence could be relatively the same and even lower (Fahey et al., 2013). The results also show that the soil C/N ratio for all treatments is similar, and there is even a lower trend with the abundance of the species.

Total phosphorus (TP) in soil, including organic and inorganic P species, was used as a baseline index of soil physicochemical conditions (Zheng et al., 2019). TP, which could be extracted using acid, was used to explain the potency of P to be converted into available phosphate form (Fisher et al., 1998). The TP content in soil depends on the primary P richness of the parent soil, and the impact of modifying factors in the soil (Lemanowicz, 2018), such as the addition of biochar and earthworm activity. The results also showed that the TP content in the soil for all treatments was similar. This fact reaffirms that the availability of P is a suitable indicator of the success of soil quality engineering in the nickel mining area using *Pheretima* sp. as an engineering agent (Wan and Wong, 2004; Bünemann et al., 2018).

Cast aggregates and channels (soil biostructure) created by the worm activity could become suitable microhabitats for other soil biotas (Hirmas and Cooper, 2016). This is because the population of protist, nematodes, bacteria and arbuscular mycorrhizal fungi in this microhabitat is high (Winding et al., 1997; Zarea et al., 2009; Zaller et al., 2013). Variations in microbial, flagellate and nematode populations in the soil are influenced by the abundance and type of *Pheretima* (Ferlian et al., 2019; Demetrio et al., 2019). Kilowasid et al. (2015b) found that the composition of epigeic and endogeic types of this worm's species affected microbial activity, and the population of flagellates and nematodes in the soil from stockpiles in nickel mining areas. Current results, despite the abundance of the worms did not have a significant effect; however, there was a tendency of increasing abundance to reduce the total arbuscular mycorrhizal spores and nematodes in topsoil from the vegetation in the mining area that was applied with biochar. A significant effect appears on the total nematode, which indicates that an increase in the population of the worms could trigger the growth of the total population of the nematode. From these results, further studies are needed regarding the abundance of feeding groups from soil nematodes that have been affected by the activity of *Pheretima* sp. Investigating the abundance of these feeding groups is very helpful in understanding the dynamics of the

abundance of AMF spores and flagellates in the topsoil mixture of nickel and biochar cultivation areas whose character is engineered using the earthworms.

## Conclusion

The abundance of *Pheretima* sp. could change the aggregate size distribution and stability, including the chemical and biological characteristics of the topsoil of the nickel mining area mixed with biochar. The size of the soil aggregate formed was distributed into three size classes under dry conditions. Meanwhile, under wet conditions, it was divided into five size classes. The size class 2.83 - 4.75 mm under these two conditions was the most dominant. Under wet conditions, the aggregate proportions for size classes 2.83 - 4.75 and 4.75 - 8.00 mm decreased, while the smaller size classes, 0.50 - 1.00, 1.00 - 2.00, and 2.00 - 2.83 mm, increased. Additionally, soil aggregate stability decreased with the increasing number of *Pheretima* sp. The soil chemical conditions created after being engineered by the species could form a habitat that is suitable for various soil biota.

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