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Research Article

Engineering of soil biological quality from nickel mining stockpile using two earth worm ecological groups

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Abstract: Earthworms have the ability in modifying soil biological quality for plant growth. Their ability is mostly depending on its ecological groups. The objectives of the research were to study the influence of two ecological groups of earthworms on soil microbial activity and soil micro-fauna abundance, and to know the potential of soil modified by earthworms as plant growth medium. Eight combination of individual earthworm from epigeic and endogeic groups was applied into pot that was filled by soil from two years of nickel stockpile and each treatment was repeated by five times. The experiment was following complete randomize design procedure. After sixteen days of research, the soil sample from each pot was analyzed for soil FDA activity, number of flagellate and nematodes. Furthermore, one kg of the soil from each pot was taken and every pot was grown by *Paraserianthes falcataria* seedling with the age of five days and continued its growth for two months. The results indicated that the soil FDA activity, number of flagellate and nematodes among treatments were significantly differences. In addition, it indicated the significant differences in dry weight of shoot, root, total plant, and root to shoot ratio of *P. falcataria* seedlings. It concluded that the combination of an individual number of epigeic and endogeic earthworms improved soil biological quality of stock pile, amd most suitable for seedlings growth in nickel mining area.

Keywords: *dry weight, epigeic, endogeic, FDA activity, flagellate, nematode.*

Introduction

The abundance and the activity of soil biota are commonly used as indicators for the soil biological quality which was degraded (Banerjee et al., 2000; Paz-Ferreiro and Fu, 2013). Status of soil biology is related to the capacity of soil ecosystem functioning such as soil decomposition, nutrient recycling and bioturbation (Schloter et al., 2003; Wilkinson, 2008). Soil microbial abundance and activity contribute to the dynamics of soil organic matter, nutrient transformations and soil fertility. Soil fauna activity is stimulated on microbial populations during the process of transformation and release nutrients into the soil solution so that it can be taken up by the roots for the growth of plants (Jouquet et al., 2006; Osler and Sommerkorn, 2007). Soil fauna can act as ecosystem engineering in the relation of soul fauna ability to change the status of soil quality (Jones et al., 1994; Bayon et al., 2011). Earthworms are known as ecosystem engineers in tropical soils (Lavelle, 1996; Kilowasid et al., 2012). Those concepts of earthworms as ecosystem engineers have been applied to the improvement of post-mining land quality (Butt, 2011). Two ecological groups of earthworms, which are epigeic and endogeic often tested for quality improvement and soil fertility in the tropical agro ecosystems (Fragoso and Lavelle, 1992; Griffth et al., 2013). Epigeic earthworms require organic matter in relatively large amount, while endogeic require mineral soil for the survival of life (Lavelle and Martin, 1992).

Epigeic groups get their food by consuming organic material on the soil surface and rarely make a hole into the ground so that little direct impact on the soil structure (Binet and Curmi, 1992; Shipitalo and Bayon, 2004). Unlike the case with the group endogeic organic matter associated with soil particles, releasing cast on the side of the ground and creating a network of holes sub horizontal that can directly influence the structure of the mineral soil (Shipitalo and Bayon, 2004; Zorn et al., 2005). The ability of epigeic to produce cast was more efficient than endogeic (Gajalakhsmi et al., 2001). Cast produced by both groups provide a substrate for the activity of soil microbes to produce biomass as a source of food for micro- and meso fauna soil in the soil food webs (Brown and Doube, 2004; Holtkamp et al., 2011; Kilowasid et al., 2012).

A number of studies showed that endogeic earthworms could be reduced to bulk density, soil organic carbon and nitrogen, and subsequently influence on biomass and activity of microbial (Edwards and Bater, 1992; Pashanasi et al., 1992; Fonte et al., 2010; Ngo et al., 2012). Suthar (2008) showed that microbial activity as measured by substrate-induced respiration rate and dehydrogenase activity in vermicomposting reactor containing two groups of epigeic and anecic was higher than that of epigiec group or anecic group. On the other hand, Palm et al. (2013) found that the abundance of epigeic endogeic increased. Although there is a positive interaction between the abundance of epigeic with endogeic, but a study related to the influence of endogeic and epigeic simultaneously on the quality soil biology in nickel mining area is still neglected.

An understanding of the influence of ecological groups of earthworms on soil quality is very important in the development of post-mining land restoration methods as proposed by Butt (2008) that use earthworms of different ecological groups for restoration of post mining area. Nickel ore is one of the few export commodities mined minerals from Indonesia (Menko Bidang Perekonomian Republik Indonesia, 2011).

Nickel ore extraction activity starts by removing the vegetation cover of the soil surface, peeling away the layers of surface soil (top soil) and pile (stockpiling). The quality of the soil stockpile that characterize nutrient and soil biota populations are low, so it requires soil quality engineering technology that can improve the abundance and activity of soil biota as a key point of functioning soil as a medium growth for post-mining activities (Sheoran et al., 2010).

Objectives of this research were to study the influence of two ecological groups of earthworms on soil microbial activity and soil micro fauna in abundance, and to know the potential of soil modified by them as a medium for plant growth.

Materials and Methods

Study site

The research was held in the nickel mining area operated by PT. Wijaya Inti Nusantara in Torobulu village, District Laeya, South Konawe which is located in the geographical position $04^0$25’51.8” South latitude and $122^0$28’04.5” East longitude and altitude at 29 m above sea level. The field experiment was conducted from March to September 2013 byusing ultramaphic parent material with topography categorized was flat-wavy with 0-15% in slopes. The average rainfall in this area was 1.415mm/year with the air temperature around 29^0C-31^0C and 78%-82% for air moisture.

Collection of earthworms

Endogeic earthworms (*Pontoscollex* sp.) were collected from forest fragment remaining in the area of mining activity, while epigeic earthworms (*Lumbricus* sp.) were collected from area of household organic waste disposal. Soil cores were taken from each spot by using a stainless steel cylinder with 20 cm high and 15 cm in diameter (Kilowasid et al., 2012) and each indivual earthworm was removed from the soil using the hand sorting techniques (Swift and Bignel, 2001). Each ecological group of earthworms was maintained for one month in different plastic container that has been filled with topsoil mixed with cow dung residues produced from biogas installation.

Experiment 1

Experiment 1 was conducted to analyze the effect of earthworm combination ecological groups on soil biological quality indicators. In the study, the average weight of epigeic and endogeic used was 0:57 g per individual. The eight combinations of the abundance epigeic and endogeic were without earthworms; 4 epigeic + 4 endogeic; 0 epigeic + 8 endogeic; 8 epigeic + 0 endogeic; 2 epigeic+ 6 endogeic; 6 epigeic + 2 endogeic; 3 epigeic + 5 endogeic; and 5 epigeic+ 3 endogeic been tested. Each combination was repeated five times, so overall there were 40 pots.

Before the treatment, each earthworm was treaten hungry (emptied their stomach) for 3 hours on the tissue surface then released into each pot (15 cm in diameter and 17 cm in height) which contained 1.5 kg of the stock pile (less than 2 years old) and mixed with 50 g of the cow dung residues, then each pot was watered to saturation and allowed to no water dripping from the pot. Furthermore, each pot was placed randomly following the procedure of completely randomized seeding into the house made of wooden building with a shade of sago leaves. After 16 days of incubation, the soil was removed from each pot and earthworms were separated by hand sorting techniques. A total of 500 g of soil from each pot incorporated and put into a zipper pack and each zipper pack was put in cool box, and then transported to a laboratory for microbial activity analysis, and calculated the number of flagellate and soil nematodes.

Analysis of the total microbial activity used fluorescence in diacetate hydrolysis method from Green et al. (2006). A total 0.50 g of soil suspended into phosphate buffer (pH 7.6) and fluorescence in diacetate acid (FDA) solution. Each soil suspension was incubated for 3 hours and each removed from the incubator space was added 2 ml acetone and divortex for ± 1 min. The suspension was allowed to stand until the soil settle, the supernatant further Erlenmeyer flask was poured into a test tube and centrifuged at 3000 rpm for 5 minute, then the absorbance was measured using a spectrophotometer at a wavelength of 469 nm.

The number of flagellates was estimated by method provided by Adl et al. (2008). A total of 1 g of soil samples were inserted in a 6 cm diameter petri dish, then add 5 ml of water. The suspension was left for 30 seconds and subsequently 15μL suspension was pippeted and transferred in to a haemocytometer chamber placed under a microscope. The flagellates were calculated in the grid size of 0.0025 mm^2.

Nematodes extracted from 100 g of soil by using Baerman funnela modified method (Kleynhans, 1999). After 24 hours incubated, nematodes sieved using a sieve size of 38 μm, nematode in tubes heated in water at a temperature of 70^0C for 2 minutes. The nematodes were re-filtered using 38 μm sieves and preserved with 4 % formalin in vial and stored until the counting was done. Nematodes from every vial poured into a petri dish the next number of individual under a dissecting microscope.

Experiment 2

A total of 1 kg of soil from each treatment was put back into each orignal pot and use to test the effect of modified soil on seedling growth. *P. falcataria* seeds that had been soaked in warm water for 24 hours were planted on media made from rice husk, lime soil and guano mixed. The seedling that was five old days was planted in each pot. Shoot dry weight, root dry weight, and the ratio of root: shoot seedlings were measured at two months after planting. Soil was removed from the roots of each plant using the water flow, then at the root collar of each plant was cut to separate the root from the shoot. Each part of the plant was put into paper bags and then transported to the laboratory. The plant parts were then dried in an oven at a temperature of 40°C, which gradually increased to 50°C during 96 hours. Plant dry weight is expressed as total shoot and root dry weight.

Statistical analysis

Analysis of variance was applied to detect effect of the combination on total microbial activity, number of flagellate and nematode, dry weight of root, shoot, plant, and root: shoot ratio. Homogeneity in variation of each parameter was tested with Levene statistics at the $p > 0.05$ level. Before analysis of variance was applied, the total microbial activity, root dry weight and root: shoot ratio values were transformed with log (x+1), and number of flagellate with ln (x+1). To detect differences among the treatments, DMRT at the $p < 0.05$ level was applied.

Results and Discussion

Soil biological quality

The results of soil FDA activity, number of flagellate and nematodes from stockpiles soil that were treated with a combination of soil epigeic and endogeic abundance was significantly different (Table 1). Significant differences in FDA hydrolytic activity were shown by treatments 2Ep+6EN and 0Ep+0En, while with 3Ep+5En, 3Ep+0En and 0Ep+5En did not show a significant effect. Soil microbial activity in the soil treated with 2Ep+6En tended to be higher than other combinations of the earthworm ecological group.

Data presented in Table 1 show that the number of flagellates in the soil treated with 2Ep+6En, 6Ep+2En and 5Ep+3En were higher than the other treatments. The number of flagellates in 3Ep+ 5En and without epigeic earthworms and endogeic (0Ep+0En) treatment was similar. It also showed a similar effect by 8Ep+0En, 0Ep+8En and 4Ep+4En compared to without earthworms. These results indicated that the interaction of two different ecological groups played an important role on the amount of soil flagellates stockpiling of nickel mining area. The number of nematodes in the soil stockpile treated with various combinations of epigeic earthworm abundance and endogic than without earthworms was similar.

Seedling growth

The plant dry weight (Figure 1A) and shoot dry weight (Figure 1B) on no earthworm's treatment were not significantly different from those of the

4Ep+4En treatment, although the plant dry weight of without earthworms was lower than that of other treatments. The plants dry weights on soil modified with 2Ep+6En, 3Ep+5En and 5Ep+3En were higher than that of the 4Ep+4En treatment. The plants dry weights were not significantly different from those of 0Ep+8En, 8Ep+0En and 6Ep+ 2En treatments (Figure 1A). The shoot dry weight on soil modified by 4Ep+4En was not significantly different from that of other combinations of epigeic and endogeic (Figure 1B).

Table 1. Soil microbial activity, number of flagellate and nematode (mean ± sd, n = 5) in stockpile soil after two weeks treated with two ecological groups of earthworms

Treatments	Soil biological quality parameter		
	Soil microbial activity (mg FDA soil/kg)	Number of flagellate ($x10^9$ ind. soil/100 g)	Number of nematode (ind. soil/100 g)
0Ep+0En (A)	0.538±0.100bc	10.35±1.43c	10.80±1.07b
4Ep+4En (B)	0.826±0.179ab	12.04±1.21c	20.40±2.80a
0Ep+ 8En (C)	0.604±0.073ab	14.35±2.07bc	19.20±2.03a
8Ep+0En (D)	0.769±0.103ab	14.87±1.09bc	20.80±1.46a
2Ep+6En (E)	0.889±0.171a	27.56±2.49a	24.00±1.92a
6Ep+2En (F)	0.727±0.093ab	27.89±2.49a	22.40±2.18a
3Ep+5En (G)	0.871±0.064a	18.34±2.08b	21.20±1.46a
5Ep+3En (H)	0.712±0.066ab	34.10±6.57a	18.00±1.95a

Note: Ep is epigeic; En is endogeic; Number located in front of Ep or En stating individual number of epigeic (Ep) or individual number of endogeic (En). Capital letters located in parentheses specifies the symbol for each combination of individual number for Ep and En. The different letters following the numbers in the same column was shown significantly differences among treatments according DMRT at the $p < 0.05$ level.

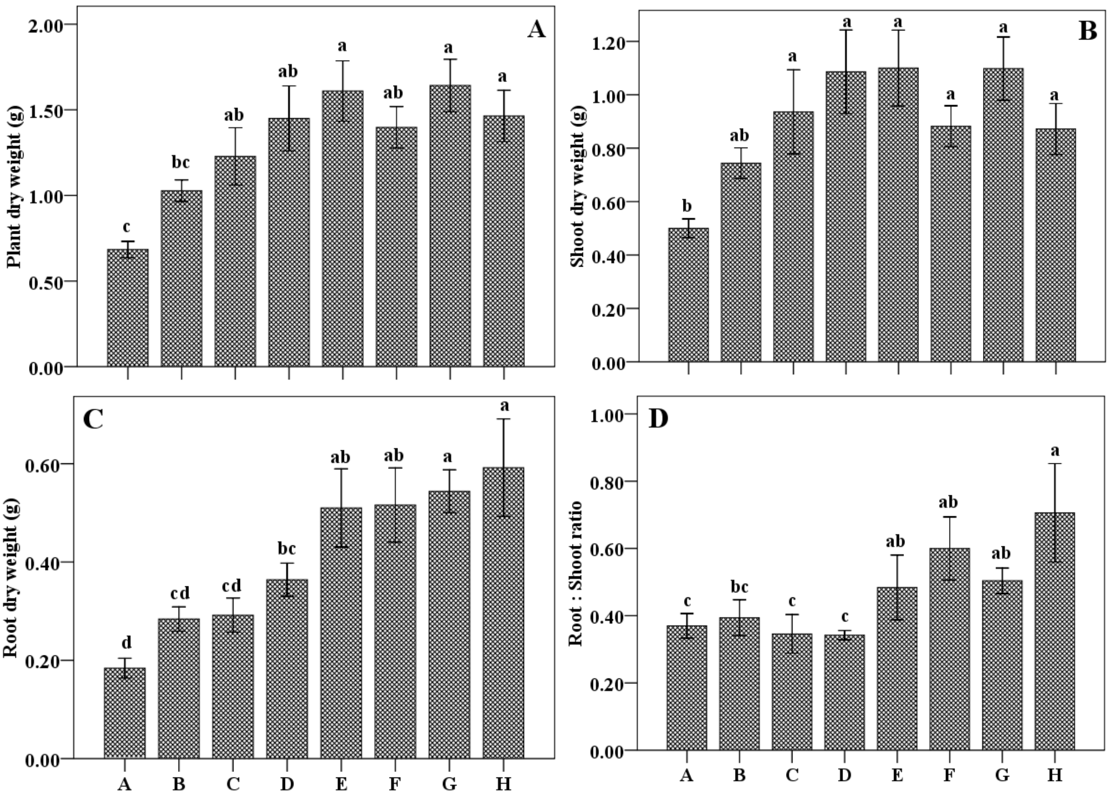

Figure 1. A) Plant dry weight, B) Shoot dry weight, C) Root dry weight, and D) Root: shoot ratio. Note: A is 0Ep+0En; B is 4Ep+4En; C is 0Ep+ 8En; D is 8Ep+0En (D); E is 2Ep+6En (E), F is6Ep+2En; G is 3Ep+5En; and H is 5Ep+3En.Different letters located above each bar shown significantly different according DMRT at the $p > 0.05$ level.

The root dry weight on soil without earthworm treatment was not significantly different from that of 4Ep + 4En and 0Ep+8En treatments. The root dry weight on soil without earthworm treatment was higher than the root dry weight on soil modified by the other treatments of epigeic and endogeic combination (Figure 1C). The root dry weight in the soil modified by 3En+5Ep, 3Ep+5En, 6Ep+2En, and 2Ep+6En treatments were almost similar to other treatments (Figure 1C).

The ratio of root: shoot on the soil without earthworms was similar to that of the soil modified by 4Ep+4En, 0Ep+8En and 8Ep+0En. The ratio of root: shoot on the soil without earthworms, however, was lower than the root: shoot ratio on soil modified by 2Ep+6En, 6Ep+2En, 3Ep+5En and 5Ep+3En (Figure 1D). Biomass allocation to roots (root: shoot ratio) tended to be highest on the soil modified by 5Ep+3En. However, it was not significantly different to the ratio on the soil modified by 2Ep+6En, 6Ep+2En and 3Ep+5En. Biomass allocation to roots that was higher than the allocation to the shoot indicated that growth in the root growing medium modified by 5Ep+3En was better than other treatments. This phenomenon was also shown by the dry weight of roots in the combination 5Ep + 3En which tended to be higher than other treatments.

Discussion

The soil biota plays an important role in the soil function as a medium for plant growth. Functional role of soil biota includes decomposition, nutrient recycling, formation and mineralization of soil organic matter and soil aggregation (Sanchez et al., 2003). In the context of rehabilitation of mined land quality, the abundance and activity of soil microbes and fauna can be used to analyze the success of reclamation practices (Dunger and Voigtländer, 2005; Boyer et al., 2011).

The results of this study showed that the variation of combination epigeic and endogeic earthworms presented differences in FDA activity, flagellate and nematode in stockpile soil from nickel mining area (Table 1). This fact indicated that earthworms play an important role in facilitating access to soil microbial organic matter that has been applied and create environmental conditions that allow growth and soil microbial activity (Scheu, 1990; Ponmani et al., 2014). These results also supported the findings of previous studies that the composition of epigeic and endogeic earthworm greatly affected soil microbial activity and number of flagellate and nematode (Zang and Hendrix, 1995; Li et al., 2002). Epigeic earthworms leave and consume organic matter in the upper soil, being endogeic leave in the soil and consume soil organic matter (Zhang and Hendrix, 1995). Currently, the published articles generally reported the influence of epigeic or endogeic earthworms on the abundance of flagellates, while the studies of the effect of the combination of two ecological groups of earthworm are still rarely reported. For example, Aira et al. (2003) reported that endogeic earthworms (*Allolobophora caliginosa*) reduced the number of flagellate, in contrast, endogeic (*Allolobophora molleri*) increased the number of flagellates in soil.

In other studies, Aira et al. (2008) found that effect of epigeic earthworms (*Eisenia foetida*) on the abundance of flagellates was not significant. Aira et al. (2003) found the number of nematodes in earthworm casts was lower than in the soil around the cast. The number of bacteriovorus nematodes was higher than other nematodes in cast *Allolobophora molleri*. Dominguez et al. (2003) found that the presence of earthworms (*Eisenia andrei*) reduced number of bacteriovorus and fungivorous nematodes during vermicomposting. Inoculation of *Metaphire guillelmi* (enecic) and corn residue application could increase the individual number of nematodes in the soil (Tao et al., 2009). Loranger-Merciris et al. (2012) indicated that *Pontoscollex correthrurus* was not reduced on root parasitic nematodes. According on this study the possibility of combination of epigeic earthworms and endogeic could be used to stimulate and to increase the flagellates and nematodes numbers in the nickel stockpile soil.

Soil biological quality parameters including microbial activity, flagellate and nematodes could be used as an indicator of soil capacity to support plant growth (Riches et al., 2013). Soil microbial activity indicated by FDA activity, abundance of protozoa and nematodes associated with the rate of release of nutrients into the soil solution available is taken up by the roots for the growth of plants (Osler and Sommerkorn, 2007; Holtkamp et al., 2011). These two facts were indicated that a growing medium modified with 5Ep+3En stimulated root and development growth (Zaller, 2007). From all growth parameters tested showed that biological quality of the soil stockpile modified by combined epigeic and endogeic strongly support plant growth. This fact indicated soil produced by engineering activity from a combination of epigeic and endogeic earthworms could be used to increase plant growth (Parfitt et al., 2005; Sheoran et al., 2010).

Conclusion

This study concluded that the composition of epigeic and endogeic earthworms are most important in ecological restoration on biological quality of stockpile soil in ore nickel mining activity area. Stockpile soil was modified by two ecological groups of earthworms (epigeic and endogeic) as a growing medium that is appropriate for the post-ore nickel mining land revegetation programs.

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