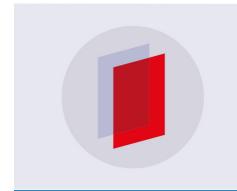
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Soil fauna population during the maize (*Zea maysl.*) growth with the addition of organonitrophos, inorganic fertilizer and biochar

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Abstract. Organonitrophos (OP) is an organic fertilizer which is enriched by nitrogen-fixing and phosphorus solubilizer microbes. This study aimed to determine the effect of the combination dose of OP and inorganic fertilizers as well as the addition of biochar and its interaction on soil fauna (earthworms and mesofauna) in Ultisol soil during the maize growth. This research was conducted at the Integrated Field Laboratory, University of Lampung. The treatment used was a 6×2 factorial with three replications in a randomized block design (RBD). The first factor was the combination dose of OP and inorganic fertilizers consisting of six levels, and the second factor was biochar from rice husk consisting of two levels. Earthworm was examined using "hand sorting" method and soil mesofauna using dry extract "Barlesse-Tulgreen" method. The results showed that during the maize growth, the highest population of earthworms and soil mesofauna as well as the highest biomass of earthworm were found in P5 treatment (100% OP). Application of 100% biochar (B1) decreased the population and biomass of earthworms, on the contrary it increased the population of soil mesofauna. Moreover, Shannon-Weaver Diversity Index of soil mesofauna is not affected by the combined fertilizers and biochar.

1. Introduction

Soil fauna is a fauna that lives on the ground and in the soil. According to the body size, soil fauna can be classified into three groups, namely microfauna (<0.1 mm), mesofauna (0.1–10 mm) and macrofauna (> 10 mm) [1]. The diversity of decomposer type of macrofauna and mesofauna of soil is suspected to be influenced by organic plant material content, decomposer organism and environmental factor. Different chemical composition of the organic matter of the plant makes its decomposition rate also different. Products from the decomposition are nutrients and other mineral elements [2].

In soil systems, the interaction of soil biota seems to be difficult to avoid because soil biota are involved in a lot of food webs in the soil [3]. Soil fauna plays a role in the nutrient cycle and food webs within an ecosystem. Furthermore, soil microbes can serve as sources and sinks of nutrients and their activity and turnover resulting from the decomposition of organic materials are considered to be primary controlling factors in nutrient cycling and availability [4, 5].

Soil in the tropics are generally dominated by infertile soils that have undergone further leaching, so it has a low in organic matter, high acidity and low in nutrients content [6]. Improving soil nutrients

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can be done through the addition of fertilizers either in the form of organic or inorganic as well as the addition of soil amendment such as biochar. Biochar is biological charcoal from incomplete combustion residue and still contains nutrients especially large amount of carbon and decompose in a long time so it can keep moisture and aeration of soil as well as a home ("niche") for soil microorganisms. Application of fertilizer and soil amendment to improve soil productivity and crop production indirectly will also affect the populations of soil fauna. The dynamics or changes of the soil fauna population, especially earthworms and soil mesofauna are important to be studied in relation to the availability of nutrients during plant growth.

At the beginning, organonitrophos (OP) fertilizer is an organic fertilizer which is developed from the decomposition of fresh cow manure and rock phosphate which are available in the Lampung province and it is enriched with nitrogen-fixing and phosphorus solubilizer microbes [7]. However, due to low P content of rock phosphate which was used preliminary, organonitrophos formula continues to be developed to improve its nutrient content by using fresh cow manure, chicken manure, and many agroindustrial wastes eg. solid waste from MSG (monosodium glutamate) industry, coconut husk etc. in order to fulfill Indonesian National Standard for organic fertilizer (SNI) [8]. In order to study the effectiveness of OP as organic fertilizer, it is necessary to do many studies in relation to its effect on soil physical, chemical and biological properties as well as on the growth and crop production. To minimize the use of inorganic fertilizer, combined OP and inorganic fertilizers are also important to be studied in order to get the opportunity for OP as a substitute for inorganic fertilizer.

The beneficial effects of combined organic and inorganic nutrients on soil fertility have been widely studied [9, 10]. Numerous field trials indicate both added benefits and disadvantages of combining nutrient sources. Increased nutrient recovery and residual effects are associated with combined nutrient additions compared with inorganic fertilizers applied alone [11].

Earthworm populations are affected greatly by many of the main agricultural practices; in particular, cultivations, fertilizers, pesticides and crop rotation exert major effects on earthworm activities and communities. Fertilizers either organic or inorganic can affect earthworm populations [12]. Many inorganic fertilizers also contribute indirectly to the buildup of earthworm populations because of increased crop yields and hence increased amounts of crop residues added to the soil.

Soil mesofauna is highly dependent on the availability of energy and food sources for life, such as organic matter and living biomass which are all related to the flow of carbon cycles in the soil. With the availability of energy and nutrients for those soil mesofauna, the development and activity of the soil mesofauna will have good effect and will have a positive impact on soil fertility [3].

This study aimed to determine the effect of the combination dose of organonitrofos and inorganic fertilizers with the addition of biochar on the activity of soil fauna (earthworms and mesofauna) in ultisol soil during the maize growth.

2. Materials and Methods

2.1. Organonitrophos fertilizer, biochar and soil used in the experiment

Organonitrophos (OP) fertilizer has been classified as organic fertilizer based on SNI (Indonesian National Standard) because it has high of total of N, P and K (table 1), but it has low organic-C. Therefore, biochar was used to improve the content of organic-C of OP.Biochar from rice husk was used in the experiment. Biochar was made by using pyrolisator.Biochar has very high of organic-C, Total N, available-P and Exchangeable-K (table 1).

The main soil type of the experimental area is Ultisol [13]. However, the soil has been covered by *Imperata cylindrica* for long time and it was new opened land. Therefore, its fertility quite good in which it has a slightly acid pH, medium content of organic-C, total-N and available-P. Although it still have low in CEC and very low of Exchangeable-K (table 1).

Table 1. Initial chemical analysis of soil, organonitrophos fertilizer and biochar used in the experiment.

Properties	Method	Soil	Organonitrophos	Biochar
pH (H ₂ O)	Electrometry	6.47 (SA)*	5.69 (N)	7.9 (SAA)
Organic-C (%)	Walkey and Black	1.76 (M)	9.52 (L)	14.65 (VH)
Total-N (%)	Kjeldhal	0.28 (M)	1.13 (VH)	0.76 (VH)
Total-P (%)	Olsen	-	5.58 (VH)	=
Total-K (Cmol _{c+} ·kg ⁻¹)	Digestion with HF and	-	0.68 (H)	-
	HClO ₄			
Available-P (mg·kg ⁻¹)	Bray I	6.9 (M)	-	26.83 (VH)
Exch-K (Cmol _{c+} ·kg ⁻¹)	HCl 25%	0.45(VL)	-	1588 (VH)
CEC (me 100·g ⁻¹)	NH ₄ OAc pH7	6.4 (L)	-	-

^{*}Criteria based on [14]: M: moderate, VH: very high, L: low, VL: very low, H:high, SA: slightly acid, SAA: slightly alkalist, N: neutral

2.2. Experimental designs

The field experiment was established in Integrated Field Laboratory of University of Lampung, Lampung Province in Sumatera, Indonesia (5°22'10" S and 105°14'38" E with 146 m above sea level).

Initially land was cleared from the shrubs and weeds. Then, land was tilled by using hoe to mix upper and lower soil layer up to 15–20 cm in order to burying the weeds in the soils. Following by second tillage in which the soil was chopped to have better soil structure. After that, experimental plots were created by 2 m \times 3m as many as 36 plots. Spacing between plots were 0.5 m and between block was 1 m.

A factorial treatment with the applied combination of organonitrophos and inorganic fertilizers (P) as the first factor and the application of biochar (B) as a second factor in a randomized block design was applied (table 2). The treatments were replicated three times.

Table 2.Treatment applied in the experiment as combined fertilizers and biochar.

	Fertilizer combination		Fertilizer dosage				
Treatments	Organic	Inorganic	OP*	Urea	SP-36	KCl	Biochar
	(OP)* (%)	(%)	(kg ha ⁻¹)	(kg·ha ⁻¹)	(kg·ha ⁻¹)	(kg·ha-1)	(kg·ha ⁻¹)
P_0B_0	0	0	0	0	0	0	0
P_1B_0	0	100	0	600	250	200	0
P_2B_0	25	75	1250	450	187.5	150	0
P_3B_0	50	50	2500	300	125	100	0
P_4B_0	75	25	3750	150	62.5	50	0
P_5B_0	100	0	5000	0	0	0	0
P_0B_1	0	0	0	0	0	0	5000
P_1B_1	0	100	0	600	250	200	5000
P_2B_1	25	75	1250	450	187.5	150	5000
P_3B_1	50	50	2500	300	125	100	5000
P_4B_1	75	25	3750	150	62.5	50	5000
P_5B_1	100	0	5000	0	0	0	5000

^{*}OP: Organonitrophos

Either organonitrophos (OP) fertilizer or biochar, according to its treatment, was dispersed into the plots and stirred evenly with the soil. After incubated for one week, maize seeds were planted with plant spacing of 25 cm × 75 cm. Each hole was filled by two seeds and it was only one plant left per hole at 14 days after planting (DAP). The application full dose of inorganic fertilizers (SP-36 and KCl) were carried out at the 14 DAP, while urea was applied twice in which half dose was applied at 14 DAP and the other half dose was applied 60 DAP (the time of entering the final vegetative mass of

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maize). The maize plants were growth properly by watering and maintaining from pests and diseases if necessary.

2.3. Samplings of soil, earthworm and mesofauna

For soil chemical analysis, field soil was collected from each plot before planting (after soil tillage). At each plot, soil samples from three spots were taken by using a soil drill to a depth of 20 cm. Soils were mixed thoroughly, dried and analyzed some of its chemical properties (table 1).

For soil fauna measurement, soil samples were collected at 30, 60, and 104 DAP. Earthworm samples were taken in each plot using wooden box of 50 cm × 50 cm. Then, soil inside the wooden box was dug as deep as 30 cm, placed in the plastic container and the number of earthworm was counted using "hand sorting" method, then its biomass was weighed.

At the same time of earthworm sampling, soil mesofauna samples were also taken from each plot by using ring sample of 5 cm height and diameter of 5.8 cm. Ring samples were buried with the depth of 0–5 cm from the soil surface, then outer space of the ring samples was digged to remove the ring safely. Mesofauna observation was done by soil extraction "Barlese-Tulgreen" method. The identification and quantification of soil mesofauna were carried out by using stereo microscope.

Diversity index of soil mesofauna was calculated by using Shannon-Weaver formula[15], as follow:

$$H' = -\sum pi \ln pi$$

Note: H':Diversity Indeks of Shannon-Weaver

Pi: The number of individual species or groups of fauna in a community per total number of species

2.4. Statistical analysis

All statistical analysis were performed using Minitab Statistical Software version 17 [16]. A factorial analysis of variance (ANOVA) was used to examine the effect of fertilizer, biochar, and their possible interaction on soil fauna. When a significant effect was observed in the ANOVA, Least Significance Difference (LSD) test was performed.

3. Results and Discussion

3.1. The abundance and biomass of earthworm during maize growth

Application of biochar suppressed the population of earthworm during the maize growth for all combined fertilizers (figure 1). Moreover, applying OP with or without inorganic fertilizers (P2, P3, P4 and P5) increased the earthworm population compared to control and 100% inorganic fertilizers (P1). It means that OP has an effect on the earthworm population.

Based on LSD test at 5% level (data not shown), with or without biochar application, the combination of OP and inorganic fertilizers (P_2 , P_3 , P_4 , P_5) have higher earthworm population than 100% inorganic fertilizer without OP (P_1) and control (P_0) at 104 DAP. Application of 100 % OP without inorganic fertilizer (P_5) has the highest earthworm populations compared to others (P_0 , P_1 , P_2 , P_3 , dan P_4). It shows that application of OP alone or its combination with inorganic fertilizers increase the earthworm population.

Figure 2 shows that during the maize growth application of biochar decreased the population and biomass of earthworm, while it increased the population of soil mesofauna. Biochar is more effectively to retain nutrients for its availability for crops than other organic materials such as leaf litter, compost or manure[17]. Based on initial soil analysis (table 2), the organic-C content of biochar is more higher than OP fertilizer, so it can be assumed that the negative interaction between biochar and OP fertilizer is occurred resulting biochar binds organic-C in OP fertilizer so thereby impacting increase in the soil mesofauna population.

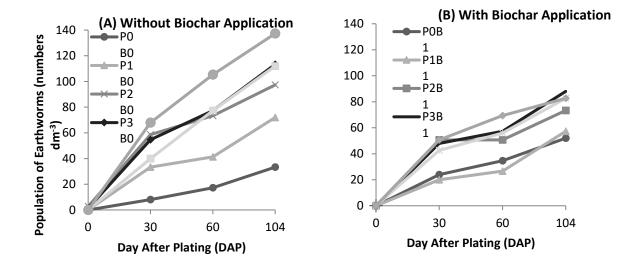


Figure 1. The abundance of earthworm without (left) and with (right) biochar application during the maize growth.

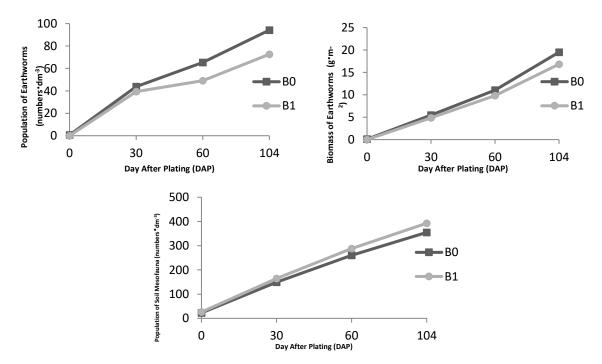
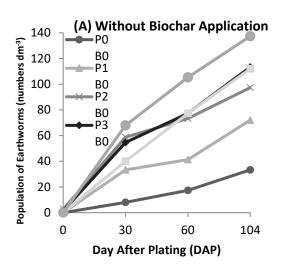


Figure 2. Effect biochar application on the abundance and biomass of earthworm and soil fauna during the maize growth (B0: without biochar, B1: 100% biochar).

According to LSD test at 5 % level (data not shown), during the maize growth, the biomass of earthworm at combination dose of 100% OPand 0% inorganic fertilizer (P5) was higher than others combination dose. While there was no significant difference between biomass of earthworm at control (P0) and others combination of OP and inorganic fertilizers (P1, P2, P3 and P4). The intensive use of chemical fertilizers and not offset by the addition of organic fertilizers can reduce soil properties, especially the biological and chemical properties of the soil [18].

3.2. The abundance and diversity index of soil masofauna during maize growth



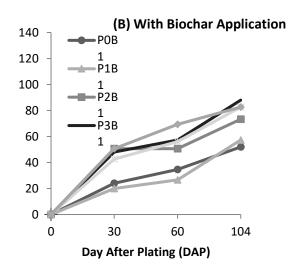


Figure 3. The abundance of earthworm without (left) and with (right) biochar application during the maize growth.

The population of soil mesofauna in all fertilizer combinations always increased with or without biochar application (figure 3). Without the addition of biochar, the highest soil mesofauna population was in the P4 fertilizer combination (75% OP and 25% inorganic fertilizer). On the other hand, with the addition of biochar, the highest soil mesofauna population was in the P2 fertilizer combination (25% OP and 75% inorganic fertilizer). Applying organic fertilizer will add an energy source for the living of soil fauna so that the population of soil mesofauna will also increase. Applying biochar into the soil provides a good habitat for soil microbes because it can increase the availability of phosphorus, total nitrogen and soil cation exchange capacity so that the activity of microorganisms in the soil increases [19].

Shannon Diversity index (H') of soil mesofauna with the combined OP and inorganic fertilizer as well as biochar are presented in table 3 and it belongs to low category [14]. A low diversity index meaning that the dominant soil mesofauna is of a particular species only. It is likely due to the observed soil mesofauna was only grouped into three types which are Collembola, Acarina, and Others (data not shown). Mostly soils in Indonesia are dominated by Collembola and Acarina [20]. There is no difference in the types or the average number of soil mesofauna. Based on some abiotic factors in the study site (data not shown) such as temperature (average of 25.5 °C), pH (average 6.7, neutral category), and organic-C (average 1.5%, low category), so that abiotic factors that are limiting factors in nature are able to provide space and the same source of food, therefore it prevent the occurrence of dominance. A community will have a high species diversity if the community is composed of many kinds with the same or almost the same abundance [21].

Table 3.Shannon-Weaver Diversity Index (H') of soil mesofauna during the maize growth with the application combination of OP and inorganic fertilizers and biochar.

Treatments	Shannon-Weaver Diversity Index (H')			
	30 DAP	60 DAP	104 DAP	
P_0B_0	1.02 a	1.01 a	1.01 a	
P_1B_0	1.01 a	0.98 a	0.94 a	

IOP Conf. Series: Earth and Environmental Science 215 (2018) 012003

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P_2B_0	1.02 a	0.98 a	0.93 a
P_3B_0	1.05 a	1.03 a	1.03 a
P_4B_0	1.00 a	0.96 a	0.97 a
P_5B_0	1.00 a	0.96 a	0.99 a
P_0B_1	0.93 a	0.99 a	0.96 a
P_1B_1	0.84 a	0.86 a	0.89 a
P_2B_1	0.99 a	0.91 a	0.95 a
P_3B_1	1.02 a	0.96 a	1.02 a
P_4B_1	0.89 a	0.92 a	0.97 a
P_5B_1	0.95 a	0.88 a	1.01 a

Note: Numbers followed by small letter in the same column do not significantly different based on Anova test at 5% level.

4. Conclusion

It can be concluded that during the maize growth: (1) The highest population of earthworms and soil mesofauna and the highest biomass of earthworm were found in P5 treatment which was the combination of the highest dose of organonitrophos fertilizer (100%; 5000 kg·ha⁻¹) and the lowest dose of inorganic fertilizer (0%; 0 kg·ha⁻¹); (2) Application of biochar (100%; 5000 kg·ha⁻¹) decreased the population and biomass of earthworms, on the contrary it increased the population of soil mesofauna; (3) application combination of organonitrophos and inorganic fertilizers with biochar did not affect Shannon-Weaver Diversity Index of soil mesofauna.

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