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Research Article

Increasing the abundance of microorganisms in a regosol soil using biopelet fertilizer composed from biochar, chicken manure, and shrimp waste to increase soil fertility

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Abstract

Results of previous research have proven that soil organic matter (humic and biochar compounds) can increase and maintain soil fertility and protect environmental resilience. The purpose of this study was to increase the abundance of microorganisms in young soil (regosol) with biopelet fertilizer composed of a combination of biochar, chicken manure, and shrimp waste to improve soil fertility. The experimental design used was a randomized block design with two factors, namely the composition and dosage of biopelets. The compositions of biopelet were 70% biochar, 15% chicken manure and 15% fish waste (B1); B2: 50% biochar, 25% chicken manure and 25 % fish waste (B2): and 20% biochar, 40% chicken manure and 40% fish waste (B3).. The dosage used was four levels (0, 2.5, 5, and 10 t/ha). The results showed that the application of biopelet fertilizer to the regosol soil reduced soil pH from slightly alkaline to near neutral, and the addition of up to 10 t biopelet / ha increased soil organic C content from 1.17% to 1.72%, as well as increasing the availability of N, P, and K nutrients. Improvement in pH, organic-C, and soil macronutrients was followed by an increase in the abundance of soil microorganisms, especially bacteria.

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Introduction

Soil is a very complex system, and its components interact. Microorganisms in the soil are very abundant and affect many processes in the soil. Microorganisms in the soil are part of the biological characteristics of soil which are very important and easy to change compared to soil chemistry and physics, but there are still not many problems revealed compared to the fields of soil chemistry and physics. Several research results indicate that a decrease in soil fertility has a direct impact on the abundance of soil microorganisms (Nanganoa et al., 2019). Likewise, the results of previous research have proven that soil organic matter is closely related to soil microorganisms and soil fertility and environmental resistance (Novak et al., 2016; Mierzwa Hersztek et al., 2020; Winarso et al., 2020). On the other hand, Indonesia's efforts to maintain food self-sufficiency and make Indonesia a world food barn by 2045 are largely determined by land availability (Rusono et al., 2015; Mulyani and Agus, 2017). One of the expansions of land for agriculture is young or sandy soils. It has been a long time since sandy lands have had quite many constraints for farming but have had to be used or managed in agricultural land extension programs (Whitten, 1987; Schulz et al., 2019). This soil is generally less fertile because the main factors are very low water retention capacity, coarse texture, low organic matter content (Fahmi, 2010; Liniger and Studer, 2019) and very low nutrients content (FAO, 2015).

There have been several research results that can be used to increase soil fertility (regosol) by adding organic matter and especially biochar, such as Mohammadi et al. (2011), Wang et al. (2015), Dietrich et al. (2020), Adekiya et al. (2020), and Winarso et al. (2020). Tomczyk (2020) stated that biochar has a high potential in improving soil fertility. Biochar has the potential to (1) reduce pollution due to its high CEC and specific surface area, (2) improve soil fertility by increasing soil pore volume, and (3) improve carbon sequestration because of its carbon and ash contents. Research results from Holík et al. (2019) indicated that the long-term use of organic and inorganic fertilizers did not significantly affect microbial structure and enzyme activity. The organic C content varies from 1.01 to 1.33% (including low status). Soil microorganisms (particularly those that are beneficial) are rarely evaluated (Miransari, 2016; Wang et al., 2020).

The aim of this research was to increase the abundance of young soil microorganisms (regosol) by a combination of biochar biopelet fertilizers, chicken manure, and shrimp waste to increase soil fertility.

Materials and Methods

This research was conducted in 2019 in the greenhouse of the Soil Science Study Program, the Soil Fertility Laboratory, and the Soil Biology Laboratory Faculty of Agriculture, University of Jember.

The materials and equipment used were regosol soil (planting medium, with the characteristics presented in Table 1), chicken farm waste, fish waste, sugarcane waste, and molasses (biopelet adhesive); while the tools used were agricultural tools, meat grinders and presses, cone chamber or "kon tiki" (Schmidt and Taylor, 2014) burners for making biochar and several laboratory measuring instruments (Spectrophotometer, AAS, and pH-meter).

The soil used for this study was a regosol soil collected from Gumukmas, Jember, East Java, Indonesia with the following characteristics, pH = 7.64, total N = 0.13%, available P = 0,19 ppm, exchangeable K = 1.01 cmol/kg, organic-C = 1.17%, fungi population = 2.348 × 10^4 cfu/g, bacteria population = 3.422 × 10^5 cfu/g, and total microorganism = 7.211 × 10^7 cfu/g.

The experimental design used was a factorial randomized block design consisting of two factors, namely, the composition and dosage of biopelet fertilizers, each of which was repeated 3 times. The first factor was the composition of biopelet fertilizer (mixtures of biochar, chicken manure and fish waste) consisting of three levels, namely: B1: 70% biochar, 15% chicken manure and 15% fish waste; B2: 50% biochar, 25% chicken manure and 25 % B3 fish waste: 20% biochar, 40% chicken manure and 40% fish waste. The second factor was the dosage of biopelet fertilizer consisting of four levels, namely: D0: 0 tha; D1: 2.5 t/ha; D2: 5 t/ha; and D3: 10 t/ha.

Preparation of biochar

Biochar was made by burning sugar factory waste in the form of sugarcane waste. Before burning, the material was dried to accelerate combustion and reduce smoke. Combustion used a pyrolysis furnace in the form of an iron cone and a lid which is often called a "kon-tiki". When the burning process is closed, the furnace is closed and the combustion is stopped when the material has turned into charcoal, not ash. The dried sugarcane waste was chopped and put into the iron cone up to 1/2 part and then wood or litter was added to make the sugar cane waste easy to burn.. The combustion process took 4-5 hours with a high temperature of 500-700°C. The charcoal produced was then cooled down by pouring water on the charcoal.

Preparation of bio-pellet fertilizer

Biopelet fertilizer was made from a mixture of sugarcane blotong biochar or sugar factory waste, chicken manure, and fish waste. The fish waste used was mostly fish bones and heads. Fish waste and chicken manure were sun-dried until the water content was relatively stable. After drying, the wastes were pounded into flour to obtain a relatively homogeneous material for forming pellets. Likewise, the biochar was ground to become flour. Biochar flour, fish waste and chicken manure flour were mixed thoroughly by adding a small amount of molasses as an adhesive. Biopelet fertilizer was made with the ratio of biochar: chicken manure: fish waste of 70:15:15; 50:25:25; and 20:40:40 (% weight). The chemical characteristics of biopelet fertilizers of several combinations are presented in Table 2.

Table 2. Chemical properties of biopelet fertilizers and their combinations.

Fertilizer Variables	**Biochar: chicken manure: fish waste**		
	70:15:15	**50:25:25**	**20:40:40**
pH	7.22	7.41	7.12
Total N (%)	1.65	3.08	3.85
Available P (ppm)	0.5	1.24	1.51
Exchangeable K (%)	0.75	0.9	0.83
CEC cmol/kg	33.2	30.8	19.6
Organic-C (%)	20.8	24.57	29.4

Based on the chemical characteristics (pH, total N, available P, exchangeable K, and organic-C) the three combinations meet the standard requirements for organic fertilizers from the Regulation of the Minister of Agriculture of the Republic of Indonesia Number 70 /PERMENTAN/SR.140/10/2011. Fertilizer material was formed to biopelet using a meat grinder. The diameter of 5 cm pellet is shown in Figure 1, which consisted of 3 combinations of biochar: chicken waste: fish waste, i.e. a) 70:15:15; b) 50:25:25; and c) 20:40:40. The biopelet fertilizers that have been perfectly formed were then air-dried for 3-5 days until they are ready for use.

(a) (b) (c)

Figure 1. Combined biopelet fertilizer of biochar: chicken manure: fish waste a) 70:15:15; b) 50:25:25; and c) 20:40:40.

Results and Discussion

Improved soil chemical characteristics

Soil physical properties such as texture, structure, density, porosity and others were not evaluated in this study because the results of previous research, namely the treatment of biopelet fertilizers that had been given to ultisol soil, were not significantly different. Likewise by several experts, one of them is Novak et al. (2016) that the statement of the results of soil analysis in 5 areas with different management, soil depth 0-10 and 10-20 cm, soil density, macroporosity, microporosity, total porosity, average diameter weighted average, aggregate stability index, and particle size of organic matter; not significantly different between regions. This shows that physical characteristics are relatively not easy to change.

In contrast to the results of research by Nanganoa et al. (2019), which evaluated different and relatively fixed land use systems for the long term in the humid tropics of Cameroon, namely secondary forests, oil palm plantations, banana plantations, sugarcane plantations, and rubber plantations on several physicochemical properties. Soil and abundance of macrofauna indicate that organic matter and numbers of macrofauna are higher in secondary forest compared to other land use types.

Soil chemical and biological properties tend to change faster or be more sensitive (Winarso et al., 2020; Penn and Camberato, 2019) compared to soil physical properties which are relatively fixed, especially in Entisol (Regosol). Therefore, evaluation of the chemical and biological properties of soil by treating Regosol with biopelet adding was carried out specifically to evaluate chemical properties, which often affect other soil properties and plant growth and the abundance of soil microorganisms. The chemical properties are pH, organic-C, CEC, total N, available P, and exchangeable K (available K) as well as the population biology of fungi, bacteria and total microorganisms by the method as shown in Table 1. The variable of chemical properties of this soil is very important, and influencing other soil properties will be discussed in relation to the activity of soil microorganisms or soil biological properties. Soil chemical properties variables are also often used as a basis for assessing soil fertility or soil quality which is also closely related to soil functions and ecosystem services (Bünemanna et al., 2018).

The results showed that the treatment of entisol soil (regosol) with biopelet fertilizers (a combination of biochar, chicken manure, and fish waste) could reduce the soil pH. The original soil pH value was 7.63 or slightly alkaline (Table 1) decreased for all treatments, as shown in Figure 2. Figure 2 (a) shows that there are more and more biopelet fertilizers with the facilities presented in table 2 (chemical properties and combination of biopelet fertilizers) will lower soil pH. Based on the calculation, the average decrease in pH or increase in soil acidity was 0.01 for every addition of 1 t/ha of biopelet fertilizer. The decrease in soil pH of a regosol has reached a neutral pH range or slightly above 7, which is generally relatively suitable for the growth of most plants. The decrease in soil pH by biopelet fertilizer treatment makes the soil better, especially when it is related to the availability of P, which is more available in a pH range of slightly below 7 (Winarso et al., 2011). The decrease in soil pH is due to the release of organic acids resulting from the decomposition of organic matter (biopelet fertilizers), which has a stronger effect than the bases released during the decomposition process (Winarso et al., 2016).

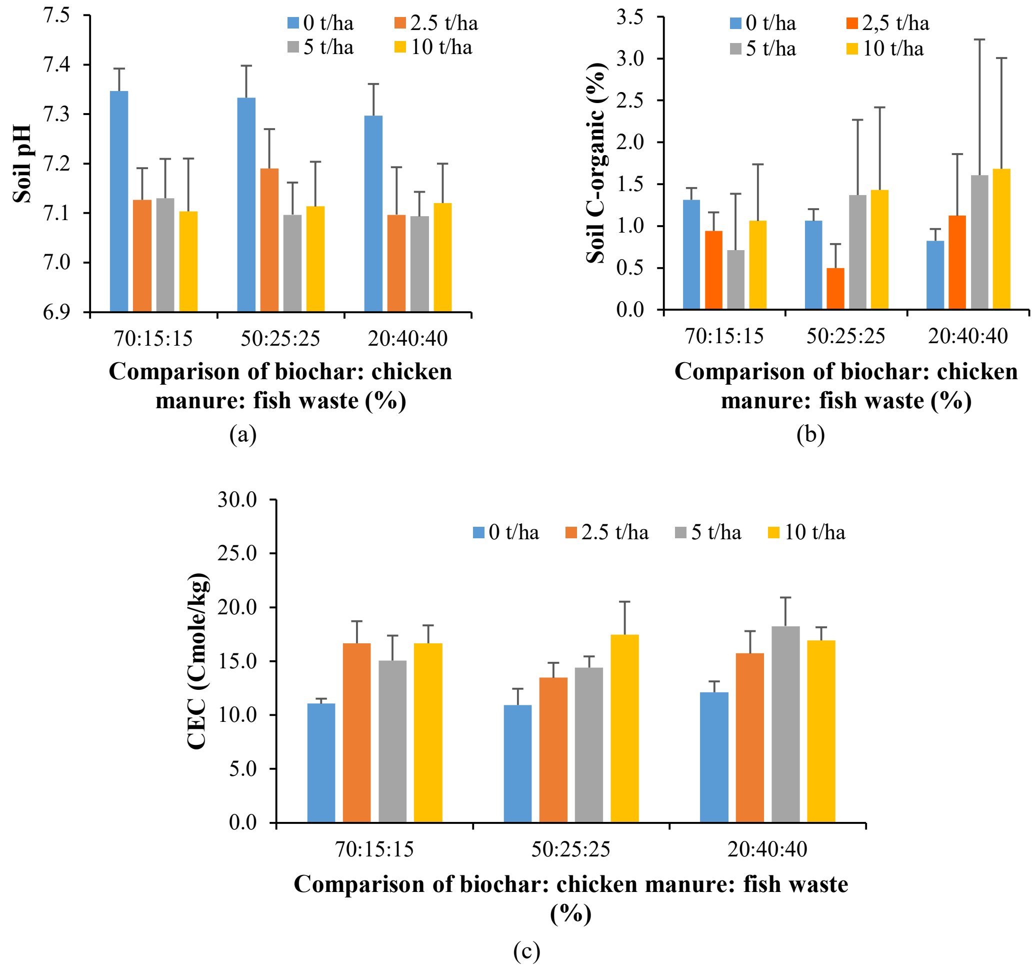


Figure 2. Addition of biopelet (biochar, chicken manure, fish waste) to regosol soil with various compositions for pH, organic-C content, and soil CEC.

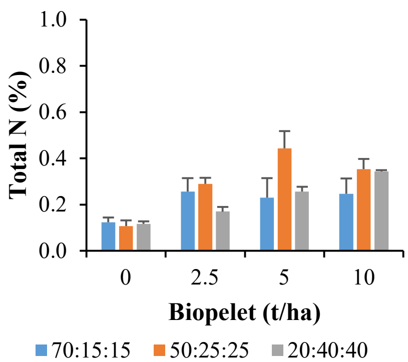
The addition of biopelet fertilizers up to 10 t/ha on regosol soil with low soil organic-C content (1.17%) increased the organic-C content of the soil, although it was still below 2%. The high organic-C status is very important because the organic-C in the soil can influence other soil properties for the better. The physical properties of the structure and the ability of the soil to store water, CEC, C/N, and the ability to support the soil are influenced by soil organic-C content (Latifah et al., 2018; Hailegnaw et al., 2019; Nelson and Su, 2010). Soil organic-C content also affects the activity of soil microorganisms (Wu et al., 2019; Rao et al., 2019) as a source of foodstuffs. Gmach et al. (2020) stated that the part of soil organic matter that is very active so that it affects other soil properties, but this small amount is categorized as generally dissolved organic matter (mostly as low molecular weight substances, such as organic acids, sugars, and amino acids).

The combination of biopelet fertilizers with different proportions of biochar, chicken manure and fish waste appeared to have different effects on soil C content (Figure 2b). The application of biopelet fertilizer with the ratio of 20:40:40 gave a higher increase in soil organic-C compared to the ratio of 70:15:15 and 50:25:25. However, in general, the increase in the addition of biopelet increased the soil organic-C content, especially in the combination ratio of 50:25:25 and 20:40:40 compared to 70:15:15. The difference in the effect of this combination ratio was because each material had a different decomposition speed; organic matter with low C/N decomposes and releases available nutrients faster than organic matter with a high C/N ratio (Sun et al., 2019).

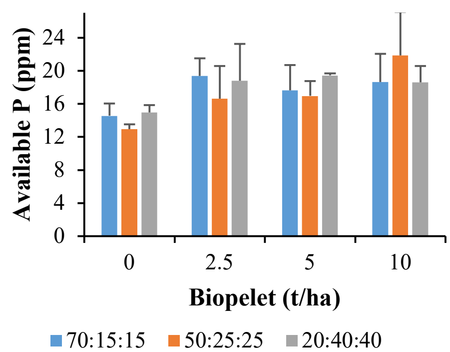
Regosol CEC status was low, namely 9.6 cmol/kg (Table 1), and the treatment of biopelet fertilizers could not increase CEC of the soil (Figure 2d c). This is understandable because regosol has a coarse texture (dominated by sand) and low levels of organic matter. Factors affecting soil CEC are pH, organic matter, and clay/texture (Hailegnaw et al., 2019). The results of this study showed that the CEC value of regosol soil was very significantly related to soil C content with the value of r = 0.688 **, meaning that the higher the organic matter content, the higher the soil CEC. The average increase in the CEC value of the regosol soil was 0.17 cmol/kg for every 1% increase in soil organic-C. Apart from organic-C, CEC was also significantly influenced by the pH value of the soil, namely with the value of r = -0.720 **. t Every decrease of 1 pH unit would increase the soil CEC by 2.08 cmol/kg. The pH value of regosol soil for this study was slightly alkaline or above 7.63 and decreased to a neutral range. Based on many research results (Parfitt et al., 1995; Khorshidi and Lu, 2017), the value of CEC is influenced by soil pH, organic-C content, number and type or type of clump. The very low clay content in this soil did not significantly affect the soil CEC. Regosol soil texture is dominated by sand fraction.

Giving biopelet (a combination of biochar, chicken manure, and fish waste) to regosol soil also increased total N, available P, and exchangeable K of the soil (Figure 3). The increased availability of N, P, and K nutrients was caused by the biopelet materials have high levels of CEC (Table 2). The increase of total N was very significant (63.34%) with the addition of 10 t biopelet fertilizer/ha. This increase especially occurred in the addition of combination biopelet in the ratio of 50:25:20. Based on the soil fertility value, the total N-content of this soil is already in the medium category. The addition can increase to high status if the total N-soil content reaches above 5%. The availability of P and exchangeable K also increased, although the increase was not as large as N. The increase in the availability of CEC elements needed by plants is relatively high, which greatly determines the growth of plants and microorganisms (Mohammadi et al., 2011; Mierzwa Hersztek et al., 2020).

Improvements in the pH, organic-C, and CEC values of regosol soils were also followed by improvements in the availability of NPK macro nutrients. The application of biopelet fertilizers increased the total N-content of the soil, which increased according to the increase in the addition of biopelet fertilizers (Figure 3a). The increase in total N content in the soil tends to lower the soil pH (Qiao et al., 2018) because in the process of change (oxidation) it releases nitrate ions which in the reaction will release H ions (the cause of acidity). The relationship between the decrease in pH of regosol soil by the addition of biopelet fertilizers was very close to the increase in soil total-N (inversely proportional to the relationship), with r = -0.56 **.



(a)



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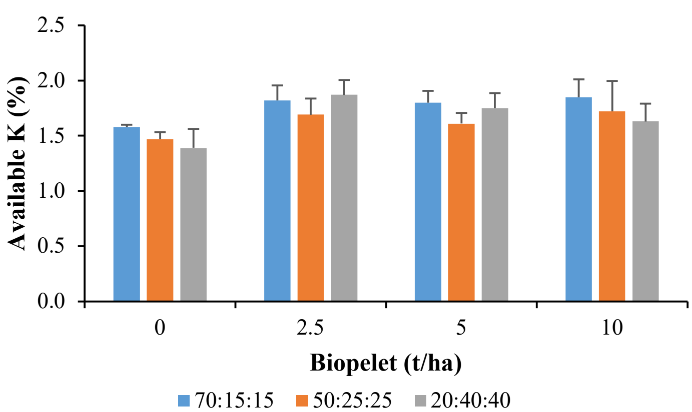


(c)

Figure 3. Effect of addition of biopelet (biochar, chicken manure, fish waste) to regosol with various compositions on total N, available P, and available K of the soil.

The increase in total N was shown by the soil available P and available K, which also increased with the addition of biopelet fertilizers (Figures 3b and 3c). Increasing the availability of P in the soil was closely related to a decrease in soil pH, with r = -0.49 ** (inversely proportional). This is in accordance with the results of research conducted by Penn and Camberato (2019) and Winarso et al. (2011). However, many studies have shown that a decrease in pH will reduce the levels of soil bases, including available K (Hailegnaw et al., 2019; Rosenstock et al., 2019). In this study, the availability of K increased with decreasing soil pH with r = -0.48 ** (inversely proportional). The diversity of increasing soil available K by biopelet treatment (biochar, chicken manure, fish waste) is presented in Figure 3c.

Improvement of soil properties (pH, organic-C, CEC, and available NPK) as described above will increase or open up opportunities for annual planting frequency in the same land and increase crop production. In addition, the results of this study can also be made to modify the fertilizer material according to the conditions at the farming location so that it can be applied to young soils (regosol), which are widely distributed in Indonesia and in the world.

Abundance and activity of soil microorganisms

Results of this study showed that the abundance of microorganisms in the soil increased according to the increase in the dose of biopelet, especially up to an additional 5 t/ha (Figure 4).. Amina et al. (2012) obtained the same results in their research in 3 locations with different management or planting systems. The increased abundance of microorganisms proves that the application of biopelet fertilizers can improve the ecosystem or the living environment of microorganisms and increase the availability of food sources for microorganisms. Furthermore, after evaluating the abundance of bacteria and fungi, the increase in total soil microorganisms was due to the increase in the abundance of bacteria compared to fungi (Figure 4). This is most likely due to the relatively high pH above 7; as stated by Wang et al. (2015), pH is a strong driving factor in influencing bacterial growth. When connected with the chemical properties of the soil that have been described previously, it shows that the application of biochar, chicken manure, and fish waste can improve or increase the chemical properties and availability of regosol soil NPK nutrients and will further increase the abundance of soil microorganisms.

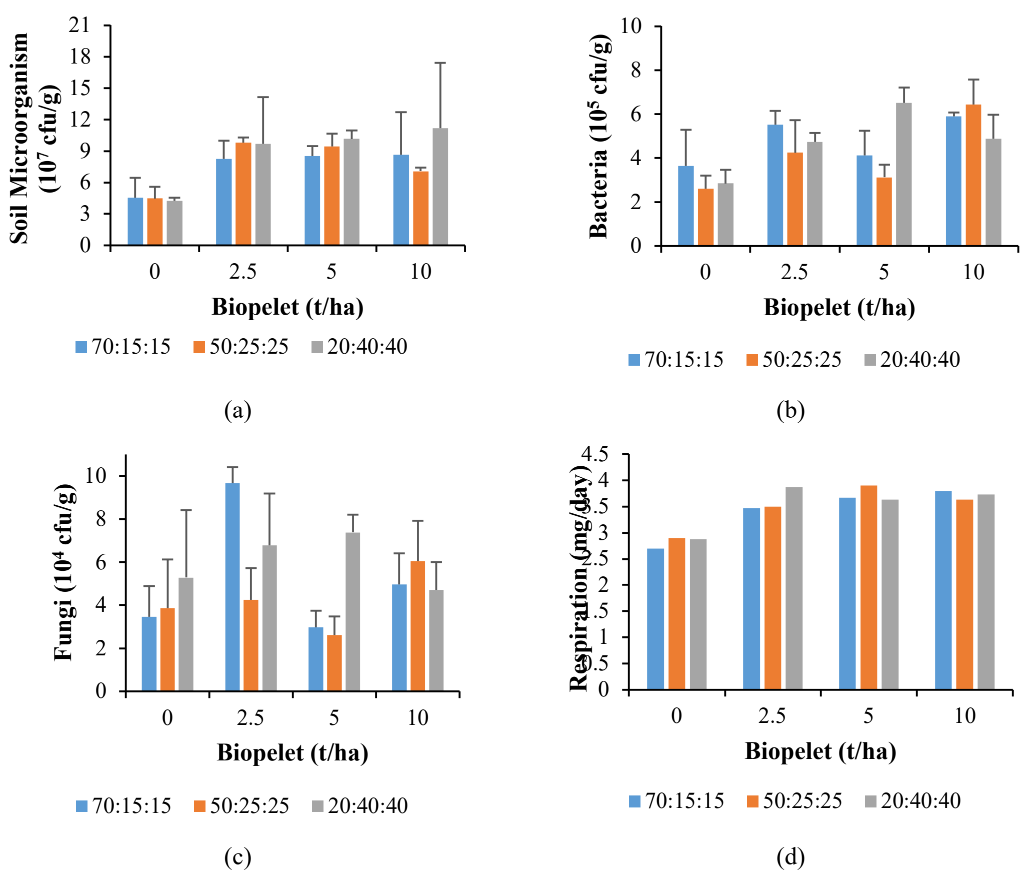
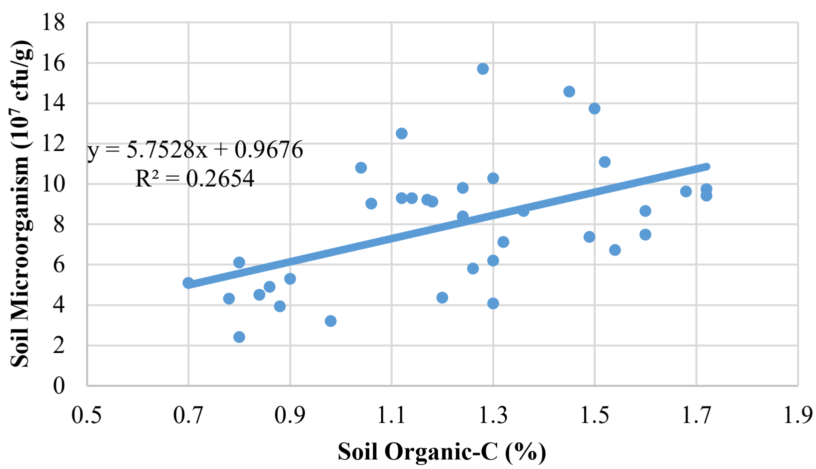


Figure 4. Effect of addition of biopelets (biochar, chicken manure, fish waste) to regosol with various compositions on the abundance of soil microorganisms.

The abundance of soil microorganisms is influenced by the soil organic-C content as a food source (Mohammadi et al., 2011). The availability of nutrients, especially NPK, will also increase growth and development (Phat et al., 2019). The increased abundance of soil microorganisms also increased microbial activity based on soil respiration data (Figure 4). The higher the abundance of soil microorganisms, the higher their activity or respiration (Elbl et al., 2019). Some experts strongly believe that the abundance of microorganisms in the soil is very important in improving soil and ecosystem quality. Wang et al. (2020), who conducted a short-term evaluation of microbial composition and diversity of cover crops, showed that multi-mix better than a single mix. Likewise, Castellano-Hinojosa and Strauss (2020) reviewed that interactions between soil microorganisms, nutrient cycles, and plants will enable the management of a more effective and sustainable annual cropping system. Microorganisms play an important role in the C cycle, N (N fixation, nitrification, and denitrification), and the P cycle (Sahu et al., 2017; Jacoby et al., 2017).

Correlation of soil chemical properties with abundance of soil microorganisms

It has been explained above that the treatment of biopelet fertilizers with a combination of biochar, chicken manure and fish waste can increase levels of organic-C and soil CEC (despite high variations) and, in turn, reduce the pH of the regosol soil, which was slightly alkaline (7.63). The increase in the chemical properties of the soil was followed by an increase in the availability of macro nutrients of NPK, especially the N-total soil, as shown in Figure 5. Likewise, the improvement of the chemical properties of regosol soil also occurred in the biological properties of the soil as indicated by the variable total abundance of microorganisms, bacteria, and fungi.



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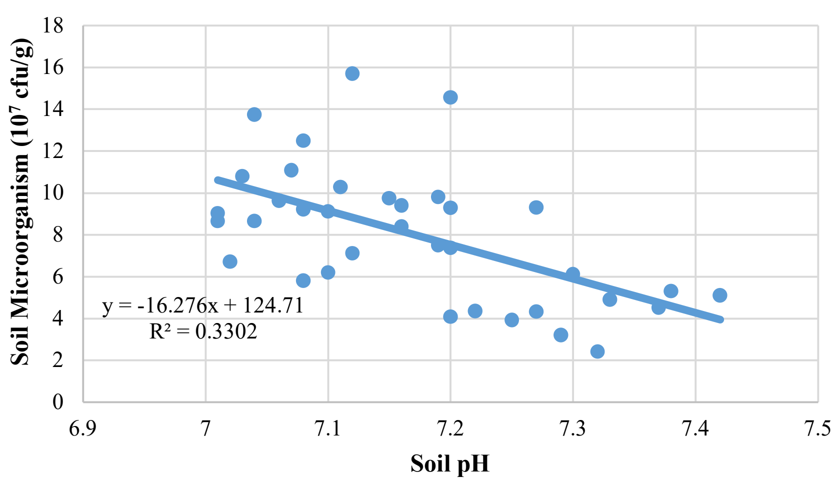


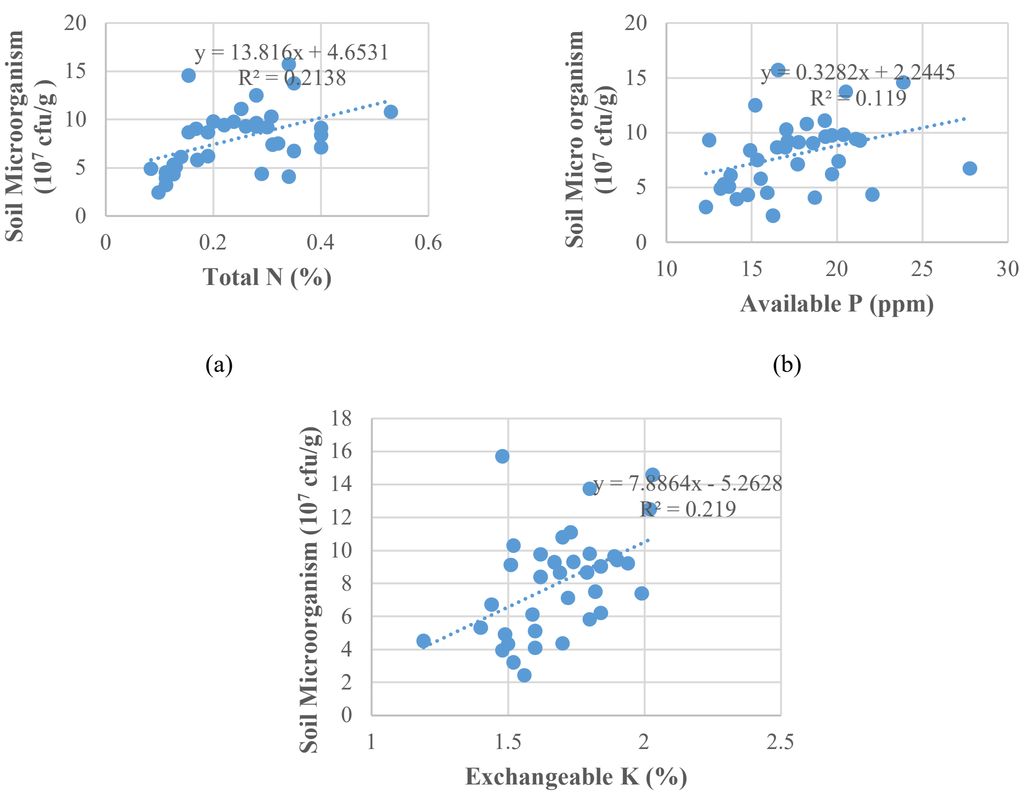
(b)

Figure 5. Relationship between pH (a) and organic-C (b) of regosol fertilized with biopelet fertilizer (biochar, chicken manure, fish waste) with soil microorganisms.

These variables, both chemical and biological, do not stand alone but are interrelated or correlated. The abundance of soil microorganisms is closely related to several soil chemical properties. The application of biopelet fertilizers directly increased soil organic-C with a significant relationship (r = 0.52 **) with the formula of Y = 5.75X + 0.97, where Y is the total soil microorganism (10^7 cfu/g), and X is soil organic-C (%) (Figure 5a). Based on this equation, every 1% increase in soil organic-C will be followed by an increase in soil microorganisms of 6.72 10^7 cfu/g. This indicates that the soil organic-C variable can be used as a dominant indicator for the evaluation of soil productivity or soil fertility. Dissolved organic matters (mostly as low molecular weight substances, such as organic acids, sugars, and amino acids) are sources of C for soil microorganisms (Gmach et al., 2020).

The correlation of soil pH or soil acidity with total soil microorganisms was also high (Figure 5b). The initial pH of the regosol soil was above 7 and decreased after being treated with biopelet fertilizers. The correlation between the two variables indicates that the decrease in soil pH will be followed by the increased abundance of soil microorganisms (r = -0.57**). Based on this data, soil pH can also be used as a dominant indicator for the evaluation of soil productivity or soil fertility in regosol soils. Increasing the availability of macronutrients also has a significant contribution to increasing the total soil microorganisms (Figure 6). The increase in soil total N directly increased the total number of soil microorganisms, especially bacteria. The N element which is taken by these microorganisms comes from N in organic compounds so that if the soil organic matter contains high N, it will directly give N to the microorganism. In addition, some of the N products will be released in the form available to plants (Coyne and Mikkelsen, 2015; Kafle et al., 2019).

This increase also occurred in P and K and had a significant correlation with the total soil microorganisms. When viewed from the slope of the relationship line between total soil microorganisms (illustrating the increase in total soil microorganisms per unit addition of elements) with N, P and K elements, it shows that the highest increase was observed for total N followed by exchangeable K and available P. This indicates that the increase in soil total N will be followed by the largest increase in total soil microorganisms when compared to exchangeable K and available P. The form of the relationship is a linear or straight line, and the values of r (size of the relationship) of N and K are almost similar, followed by P. The increase in soil microorganism in relation to the availability of nutrients is presented in Figure 6.



(a) (b) (c)

Figure 6. Relationship between total-N, available P, and exchangeable K in regosol soil fertilized with biopelet (mixtures of biochar, chicken manure, fish waste) with soil microorganisms.

There is a good correlation between the abundance and activity of soil microorganisms, and the chemical properties of these soils open the opportunity for the use of microorganism abundance and activity variables for rapid evaluation of soil fertility or health. This is possible because the analysis of soil microbiological (biological) variables is relatively shorter in time for the analysis than the chemical or soil physics variable.

Conclusion

The abundance of microorganisms in regosol increased according to the increase in the dose of biopelet (a combination of biochar, chicken manure, and fish waste), especially up to an additional 5 t/ha; the abundance of bacteria was greater than that of fungi. Increasing of the abundance of microorganisms in the soil is closely related to the increase in organic-C levels and the availability of NPK nutrients as well as a decrease in soil pH, which was originally slightly alkaline.

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