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The Abundance of Soil Microorganisms after Planting Rice with Different Fertilization Systems

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Abstract
Soil microorganisms play a major role in soil properties both physical, chemical and biological. The level of intensive land management with high input in conventional farming systems, especially paddy fields using chemical fertilizers, superior seeds and chemical pesticides can significantly affect the population and composition of soil microorganisms. This study aims to analyze the abundance of microorganisms using different fertilization techniques, by using residues of rice straw and inorganic fertilizers. Both techniques are thought to affect the abundance of soil microorganisms. The research was carried out in Salasaee Village, Soil Biotechnology Laboratory and Soil Chemistry Laboratory of Hasanuddin University. The research method was carried out through a collection of soil samples, identification of morphology and Characteris of Physology and Biochemistry, data analysis was carried out by exploratory descriptive. Based on the results of highest abundance of microorganisms found in third location (LK3) with the organic fertilization system which is 742 x 10^{-6} Cfu / gram, and the lowest abundance is found in first location which is 178 x 10^{6} while in second location with semiorganic system is 381 x 10^{-6} Cfu / gram. Microorganisms abundance of organic fertilizing systems addresses higher yields of inorganic and semiorganic fertilization systems. These different fertilization techniques affect the abundance of soil microorganisms can be seen from the diversity of microorganisms in research location where the organic fertilizing system can maintain the abundance of microorganisms compared to inorganic fertilization systems.
Keywords: Abundance, Mocroorganisms, Fertilization Systems

1. Introduction

The use of land resources still plays an important role in determining the increase in sustainable agricultural productivity (Abedon, 2011). Agricultural productivity with management of incentives such as paddy fields both irrigation and raindrops is constantly threatened in recent years by soil degradation which results in declining soil fertility in terms of its physical, chemical and biological properties. The inappropriate management and land management is the biggest contributor to damage to resources (Turbé et al., 2010). One indicator that can be used as a parameter of soil or land fertility is to see the abundance of soil microorganisms (Damayanti, 2011). Soil microorganisms play a major role in soil properties both physical, chemical and biological. Biologically microoganism is able to provide supporting environmental conditions for the life of other organisms, physically microorganisms play a role in the process of destruction of organic matter (decomposition), chemically soil microorganisms play a role in the availability of nutrients in soil needed by plants. (Widyati, 2013). The level of intensive land management with input is high in conventional farming systems, especially rice fields where it use of synthetic chemical fertilizers, using superior seeds and pesticides can significantly affect the population and composition of soil microorganisms. Therefore, proper and sustainable management of land management is needed to maintain the ecological balance of microorganisms in order to conserve ecosystems that will be able to increase the biodeversity (population) of soil microorganisms. One way that can be done to maintain or increase the population of soil microorganisms is biological management of soil by improving the physical environment of soil microorganisms such as by returning crop residues to production land, where crops can be returned directly to land or processed into compost. (Dos Santos Souza et al., 2018). Utilization of harvest residues such as rice straw either directly or processed into compost, where in the presence of organic material from the harvest this condition will allow the presence of soil microorganisms to be abundant because of the supply of organic material. In compost straw contains live microorganisms which when applied to surface of plant and soil, will inhabit the rizofer or inside plant and encourage growth and increase the supply of essential nutrients from plants. Microorganisms consist of groups of soil fertilizing microbes that function to increase fertilization efficiency, decomposing organic compounds that can supply the nutrients needed by plants (Viability et al., 2013). One indication of the abundance of microorganisms that live in the soil is content of organic matter. Organic matter is an energy source for soil flora and fauna both macro and micro. The addition of organic matter in the soil will increase activity and microbiological population, especially those related to decomposition and mineralization of organic matter. Some microorganisms that play an important role in decomposition of organic matter are fungi, bacteria and actinomycetes (Tilman, 2001).

2. Methodology

2.1 *Research Sites*

This research was carried out in two districts, Salasae Village, Bulukumba, South Sulawesi, which will be implemented in 2018. Research in the laboratory will be conducted at Soil Biotechnology Laboratory and Soil Chemistry laboratory, Department of Soil Science, Faculty of Agriculture, Hasanuddin University.

2.2. *Sampling*

The Soil samples taken from the rhizosphere of rice plants. It's around the rhizosphere which taken as much as 100 gram at a depth of 10 cm to 15 cm using a soil drill. Each soil sample is taken from 3 points for each location. Samples from each point were then put in sterile plastic and composted, then under a laboratory for soil physical and chemical analysis in Soil Science Laboratory.

2.3. *Microorganism Abundance Analysis*

A total of 10 gram of each soil sample was taken to analyze the diversity and abundance of microorganisms through dilution and deterrence methods. Each 10 gram of the sample is dissolved with sterile water so that a soil suspension of 100 ml is obtained. The suspension was rocked using an orbital shaker for 20 minutes at a speed of 150 rpm. The suspension is then diluted immediately in series by ml of soil suspension with 9 ml of sterile water in a test tube so that a dilution of 10-1 is obtained. The 10^{-1} dilution suspension is diluted by mixing 1 ml of a 10^{-1} solution with 9 ml of sterile water in a test tube so that a dilution of 10^{-2} is obtained. Dilution continues until the dilution level is 10^{-2}. For a 10^{-3} dilution to 10^{-5} take 1 ml and then cultured in AKD media. The culture results are observed three days after detention (HSP) assuming all microbes have grown. Colonies that grew in the range of 25-250 colonies were measured with TPC values (total plate count) and obtained using the formula:

$$N = \frac{\sum C}{[(1XN1)+(0,1\ X\ n2)]x\ (d)} \quad \ldots\ldots (1)$$

Description :

N = The Number of Product Colonies, Expressed in Colonies per ml or per gram Colony
∑C = The Number of Colonies in All Counted Cups
n1 = The Number of Dishes at The First Dilution Calculated
n2 = The Number of Dishes at The Second Dilution Calculated
d = The First Calculated Dilution

2.4. *Morphological Identification of Microorganisms*

Morphological identification is carried out both macroscopically and microscopically. The determination of morphological characteristics was based on shape of colony; colony color, colony edge, colony elevation and colony color on culture of Nutrient Agar (NA) and Observations in Microscopes. Characterology of Physology and Biochemistry by way of gram reaction, catalasee test and gram staining.

2.5. *Data Analysis*

Data analysis used explorative descriptive analysis. Descriptive data analysis, aims to provide descriptive data about research subjects based on data from variables obtained from a group of subjects studied and not intended to test hypotheses.

3. Results and Discussion

3.1. Enumeration and Isolation of Soil Microorganisms

The total value of plant count (TPC) abundance of microorganisms in locations with organic fertilization techniques addressed the abundance of soil microorganisms higher than the locations with inorganic and semiorganic fertilization techniques, isolates found more in locations with organic fertilization techniques compared to inorganic and semiorganic fertilization techniques (Table 1).

Table 1. The Enumeration Results of Soil Microorganisms at Sampling Locations

Sites	*TPC (CFU/g)*	*Number of Isolates*	*pH*
LK1	178 x 106	15	6.06
LK2	381 x 106	27	7.04
LK3	742 x 106	34	6,86

Based on the TPC calculation, the highest abundance of microorganisms is found at the third location (LK3) with the organic fertilization system which is 742 x 10^{-6} Cfu / gram, and the lowest abundance is at first location, 178 x 10^{6} while the second is with the semiorganic system 381 x 10^{-6} Cfu / gram. The abundance of microorganisms from organic fertilizing system addressed higher yields of inorganic and semiorganic fertilization systems both in terms of number of TPC calculations (cfu/g) and number of isolates produced. Giving organic matter to soil can stimulate the activity of soil enzymes and microbes, total enzyme activity of

soil depends on extracellular enzymes and number of enzymes in dead and living microbial cells. With the addition of organic matter to the soil, not only millions of microorganisms are added to soil, but microorganisms are also encouraged to multiply (Nurbaity, Herdiyantoro, & Mulyani, 2009). Judging from pH value, in these two locations addressing different pH values, locations with organic fertilization techniques tend to be better with pH values close to neutral compared to inorganic and semiorganic fertilization systems, these indications are in line with lower inorganic TPC and more pH values. The pH range for growth of each group of microorganisms varies greatly. Some microbes can grow in a wide pH range. In general, the optimum growth of microbes occurs at pH 7 and can grow well in the range of pH 5-8 (Ciccyliona & Nawfa, 2012)

3.2. Soil Analysis

The diversity and abundance of soil microorganisms is strongly influenced by the components and physical and chemical conditions of soil. Results Analysis of soil physical and chemical properties is presented in Table 2. Important chemical parameters on the soil can also explain the high and low TPC values at the location of the observation location. The C-organic value of LK1 location which is higher than the two other locations. Table 2 shows that organic matter is actually available in sufficient quantities. However, the rate of decomposition of organic matter by soil microorganisms is classified as low which is characterized by a high value of C / N ratio. A high C / N ratio is an indication that the rate of decomposition of organic matter is low so that the C / N ratio is often used as a fertility parameter. The low rate of decomposition of organic material is also due to low abundance and diversity of soil microorganisms (Fallis, 2013).

Table 2. The Analysis of Several Important Chemical Parameters in Sample Soil

Sites	*C-ORG* (%)	*N-Total* (%)	*Rasio* C/N (%)	*P* (mg/100)	*K* (ppm)
LK1	1.87	0,17	11	49,54	0,61
LK2	1,96	0,14	14	40,07	0,53
LK3	2,18	0,22	9	24,26	0,25

Total P and K for LK1 and LK2 are also much higher than LK3. This shows that fertilization of LK1 and LK2 is in excess levels. Total P levels of soil above 40 ppm are included in high levels and above 100 ppm are in the category of excess P. With the results, the land on LK1 and LK2 actually does not need to be given P fertilizer application because the soil already contains high P. Likewise the total% N value (Table 2) addressing a high enough value compared to LK3, chemical fertilization system with high input can result in decreased land productivity both physical and chemical (Hanafiah, 2007). The elemental value of nutrient in LK3 is in sufficient condition, because it's caused by organic farming system in form of straw compost which is able to provide nutrients in a balanced manner in the soil. Straw compost contains live microorganisms which when applied to surface of the plant and soil, will inhabit rizofer or inside of plant and promote growth and increase the supply of main nutrients from plant. Microorganisms consist of groups of soil fertilizing microbes that function to improve fertilization efficiency, decomposing organic compounds that can supply nutrients needed by plants (Pangaribuan & Pujisiswanto, 2008).

3.3. Abundance of Soil Microorganisms

Isolation of soil microorganisms was taken from the location in Sallasae Village in three locations with different fertilization systems. The isolation results obtained 76 isolates with a percentage of 34% of fungi and 66% of bacteria. Furthermore, fungal and bacterial isolates were grouped based on macroscopic morphological characters on PDA media for fungal isolates and NA media for bacterial isolates

Table 3. The Abundance of Microorganisms

Sites	*The Abundance of Microorganisms*	
	Bacteria	*Fungi*
Lk 1	9	6
LK 2	18	9
LK3	23	11

Based on Table 3 it can be seen that total bacteria on soil with an organic fertilization system (LK3) obtained 23 bacterial isolates and 11 fungi isolates, whereas in an inorganic fertilization system (LK3) total bacterial isolates obtained were 9 bacterial isolates and 6 fungi isolates and and in semiorganic fertilization system (LK2) bacterial isolates were obtained as many as 18 bacterial isolates and 9 fungi isolates. In general, the diversity of soil microorganisms in these three locations is dominated by bacteria. Abundance is dominated by bacteria this is in accordance with opinion (Saraswati & Sumarno, 2008) which states that bacteria are the largest number of organisms in soil and can cover biomass.

3.3.1. Fungi

Identification of fungi is based on morphological characteristics. Characterization includes color and texture of colonies, radial lines and concentric circles on surface of colonies, microscopic karmic characteristics including hyphae, spores, coniodophores, and drospores. Observation of morphological characteristics is done to compare observations directly with reference morphological characters that are based on key to determination (Kohlmeyer & Volkmann-Kohlmeyer, 1991), (Watanabe, 2002) and (Kaewchai, Soytong, & Hyde, 2009) in determining the genus of fungal isolates. Fungus diversity in first location (LK1), second location (LK2) and third location (LK3), addressing different levels of diversity, macrosscopically grouped based on pigmentation variation, colony shape, radial lines and concentric circles on PDA media. The results of identification of 26 fungi isolates were obtained by 5 fungi genera. The five genera have similarities with *Tricoderma. sp., Aspergilus sp., Aspergilus niger sp., Penicilium sp.,* and G*liogdalium sp.*

Aspergilus sp. is the most widely found gift in this study as a total is 11 isolates consisting of first location (LK1) consisting of 4 isolates, the second location (LK2) consists of 4 and the third location of LK3 consists of 3 isolates. *Tricoderma sp.* was the second most fungus found in 7 isolates, where in the first location (LK1) and third location (LK3) there were 2 fungi isolates, while in the second location (LK2) three isolates were found. *Penicilium sp.* and *Gliogdalium sp.* were only found in the third location (LK3), each fungus obtained from three fungi. So the highest abundance of fungi is obtained in third location with an organic fertilization system. The addition of organic matter in soil will increase the activity and population of microorganisms in the soil, especially those related to decomposition and mineralization of organic matter. Some microorganisms that play a role in the decomposition of organic matter are fungi, bacteria and actinomycetes (Roidah, 2013).

3.3.2. Bacteria

Initial characterization of bacteria based on macroscopic and microscopic morphology. Macroscopic characters include based on pigmentation variation, colony shape, margin and colony elevation. Microscopic observations in the form of cells are observed with gram staining. The results of identification of 50 bacterial isolates. All isolates from organic fertilizing systems generally varied from the shape of colonies, the shape and color of colonies by spherical colonies except isolates (LK3.1, LK3.4, LK.3.5, LK3.8, LK3.13, LK3.14 and LK3.15) are rod-shaped, and with varying colors from yellowish, cloudy white, and white, while results of gram test show there are more than gram-positive except for isolates (LK3.1 , LK3.2, LK3.4, LK3.10, LK3.11, LK3.14. LK3.14, LK3.15 and LK3.16) Based on observations characterization of bacteria diversity observed is quite varied. Fertilization systems organically address that this system is able to maintain the diversity and abundance of soil microbes.

In a location with a semiorganic fertilization system, 18 isolates were obtained, based on characterization carried out at second location, the results were generally obtained in form of spherical bacteria except isolates (LK2.1, LK2.2 and LK2.3) which were rod-shaped. Whereas from colony form obtained generally flat form bacteria except at (LK2.3, LK2.6 and LK2.11) which have jagged colonies. While the color is dominated by white bacteria except for isolates (LK2.2, LK2.11 and LK2.14) and yellowish color is obtained in isolates (LK2.11, LK2.16, LK2.17 and LK2.18).

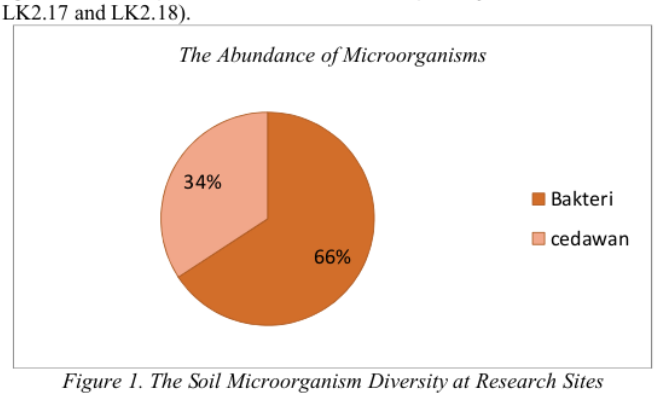

Figure 1. The Soil Microorganism Diversity at Research Sites

Observation results of microbial morphology characterization in locations with fertilization systems inorganic (LK1) of a total of 9 isolates obtained all forms of colonies found were round, with the shape of flat colony edges, except for isolates (LK1.8) whose edges were serrated, and colony color is mostly white except isolate (LK1.3 and LK1.4) and gram test results show gram-positive values in allisolates. The gram staining is used to determine the morphology of bacterial cells and to distinguish gram-positive and gram-negative bacteria. (Ilham, Darmayasa, Nurjaya, & Kawuri, 2014) stated that gram-positive bacteria in gram-colored purple stains are caused by a complex of violet-iodine crystalline dyes which are retained even though given an acetone

alcohol bleaching solution, whereas gram-negative bacteria are red because they are soluble in when administering acetone alcohol blanching solution, it takes safranin red.

Color differences in gram-positive and gram-negative bacteria indicate that there are differences in cell wall structure between the two types of bacteria. The gram-positive bacteria have cell wall structure with thick peptidoglycan content while gram-negative bacteria have high lipid content in cell wall structure (Rahma, 2015).

4. Conclusion

The highest abundance of microorganisms is found in the third location (LK3) with the organic fertilization system which is 742 x 10^{-6} Cfu / gram, and the lowest abundance is in the first location, namely 178 x 10^{6} while in the second one is the semiorganic system which is 381 x 10^{-6} Cfu / gram. Microorgamisem abundance of organic fertilizing systems addresses higher yields of inorganic and semiorganic fertilization systems. These different fertilization techniques affect the abundance of soil microorganisms can be seen from the diversity of microorganisms in the research location where the organic fertilizing system can maintain the abundance of microorganisms compared to inorganic fertilization systems.

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