

Nonsymbiotic Nitrogen Fixing Microbes and Urease Enzyme Activity in Peatlands Covered by Different Plant Species

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ABSTRACT

Peat soil is a soil that is rich in organic matter, hence contains many essential nutrients, such as nitrogen, to support plant growth. During the vegetative period, plants need a lot of nitrogen as a source of nutrients for plant growth. Other than soil organic matter, nitrogen can also be obtained from N_2 gas in the atmosphere by the Nitrogen Fixing Bacteria. This study aimed to determine the number of non-symbiotic nitrogen-fixing microbial populations on peatlands and their relationship with the urease enzyme activity of peat soil cultivated to sengon (*Albizia chinensis*), petai (*Parkia speciosa*), pineapple and orange. Soil samples were taken from rhizoplane of sengon, petai, pine apple, and orange in Landasan Ulin, South Kalimantan. Six soil samples were taken from each plant in wet season and dry season of 2021 and 2022. The soil samples were transported to the laboratory and used for non-symbiotic nitrogen-fixing microbial and urease enzyme determination. The data obtained were then analyzed to follow the Nested Randomized Complete Design (CRD). The results showed that the number of nitrogen-fixing microbes during dry season was more than that of wet season. The highest number of nitrogen-fixing microbes during dry season was observed in orange rhizoplane, while the highest nitrogen-fixing microbes during wet season was observed in pine apple rhizoplane. The number of nitrogen-fixing microbes had a positive correlation with urease enzyme activity.

Keywords: N_2 Fixer, Peat Soil, Rhizoplane, Urease Enzyme

INTRODUCTION

South Kalimantan's peatlands have the smallest area compared to other Kalimantan provinces. Peatlands are mostly found in areas around Tapin and Hulu Sungai Selatan districts. Peatlands in Kalimantan generally occupy basins and peat swamp plains behind the Barito River.

Peatlands are decreasing over time, one of the causes is land reclamation activities for various purposes including agriculture. In South Kalimantan, according to the Peat Hydrology Area Map (PHM) of the Peat Restoration Agency (PRA), the area of peatland exceeds 103,000 hectares.

The utilization of peatlands in agriculture for cultivation has several physical, chemical and biological constraints and the chemical characteristics of peat soils in Indonesia are quite diverse. The main chemical characteristics of peat soils in Indonesia include their very acidic nature with a pH range of 3-

5, very low exchangeable bases, and very low microelements (Cu, Zn, Mo) which are bound strongly by organic matter and therefore unavailable to plants (Agus and Subiksa, 2008).

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Nitrogen element is a macro nutrient that is needed for plants, Nitrogen element needs for plants can be met by applying N fertilizers such as urea. Before it can be absorbed by plants, urea is first converted into Ammonium through a biochemical reaction catalyzed by the enzyme urease. This enzyme can hydrolyze urea into ammonium (NH_4^+) which then by the process of nitrification will be converted into nitrate (NO_3^-) (Coyne, 1999).

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The soil nutrient cycle involves biological, physical and chemical reactions that are assisted in this process by microorganisms, plant roots and animals in the soil. These biochemical reactions are facilitated by enzymes. Enzymes in soil are mainly sourced. The activity of enzymes in soil can be affected by several factors, such as the type of plant, industrial or plant pollutants, and pesticides such as fertilizers. Fertilizers can be organic fertilizers such as manure or artificial fertilizers (Pankhurst et al., 2002).

The phenomenon of binding nitrogen from the atmosphere is called *diazotrophy* or biological nitrogen fixation, so microorganisms that are able to fix nitrogen are called diazotrophs or nitrogen fixers. Microorganisms whose main function is to supply nitrogen by fixing atmospheric nitrogen can be divided into two groups, namely: (1) free microorganisms, which are not specifically associated with certain plants and (2) microorganisms that are symbiotic with plants. Thus, it is known that there are two biological systems or mechanisms of nitrogen fixation from the atmosphere, namely non-symbiotic nitrogen fixation and symbiotic nitrogen fixation (Hanafiah 2013).

MATERIALS AND METHODS

This research is an experimental study that tests the effect of treatments on observed parameters using a two-factor nested randomised complete block (RAL) environmental design. A survey of peatlands was conducted during the dry season to locate areas covered by *sengon* (*Albizia chinensis*), *petai* (*Parkia speciosa*), orange (*Citrus nobilis*) and pineapple (*Ananas comosus*). In each field, a spot with a peat depth of more than 50 cm was identified. In each field, 4 soil samples were taken at the root zone of each plant, resulting in 16 experimental units (aerobic conditions). Sampling was repeated at the same point during the rainy season (anaerobic conditions).

Soil sampling, soil samples were taken from each *sengon*, *petai*, orange and pineapple plant and placed in a separate plastic sample and labelled. After all soil samples have been taken, they were placed in a cooling box. Performed 2 times, aerobic and anaerobic, and then tested in the laboratory.

Soil samples were then transported to the laboratory for analysis. Non-symbiotic N-fixing

microbial populations were counted by the cup count method using nitrogen-free mineral (NFM) solid media. Soil parameters affecting N-fixing microbial activity were also analyzed, namely C-microbial, urease enzyme activity, ammonium and nitrate. Soil C-microbial was determined by the fumigation incubation method (Jenkinson and Powlson, 1976). Soil ammonium nitrogen (N-NH_4^+) was analyzed by the colometric method (Kempres and Zweers). Soil nitrate (N-NO_3^-) was analyzed by the method of Yang, et al. (1998). Urease activity was determined by the method of Kandeler (1996).

The data obtained in the observations were analysed using Barlett's homogeneity of variance test; if the data were homogeneous, it was continued with the analysis of variance (ANOVA Nested) to determine the effect of treatment using the Anova Excel V-5 program (Blog://mmahbub.wordpress.com/). However, if the data are not homogeneous, data transformations are performed until the data are homogeneous. If the analysis of variance has a real effect, continue the analysis with another test of the mean using Duncan's Multiple Range Test (DMRT) at the 5% level of error. And carry out correlation and regression tests to determine the relationship between the non-symbiotic nitrogen-fixing bacterial population and urease enzyme activity.

RESULTS AND DISCUSSION

Results

Non-symbiotic nitrogen-fixing microbes

The results of the analysis of variance showed that nonsymbiotic nitrogen-fixing microbes in aerobic and anaerobic conditions showed a very significant effect on the total microbial population as indicated by Duncan's Multiple Range Test (DMRT). Nonsymbiotic nitrogen-fixing microbes during aerobic and anaerobic conditions can be seen in Figure 2.

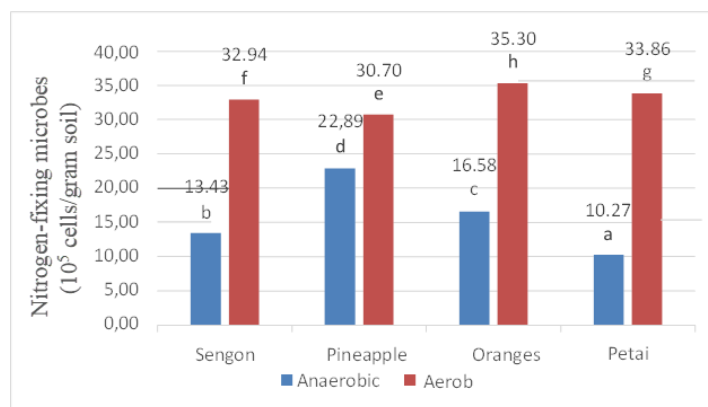


Figure 2. Effect of nitrogen-fixing microbes under aerobic and anaerobic conditions.

The highest total nitrogen-fixing microbes were found in aerobic citrus plants with a value of 35.30 x 10⁵ cells/gram soil and the lowest in anaerobic petai plants with a value of 10.27 x 10⁵ cells/gram soil.

Urease Enzyme

The results of the analysis of variance showed that urease enzyme during aerobic and anaerobic conditions had significantly different effects on the value of urease enzyme (Appendix 2). Duncan's multiple range test (DMRT) is presented in Appendix 2. Urease enzyme during aerobic and anaerobic conditions is shown in Figure 3.

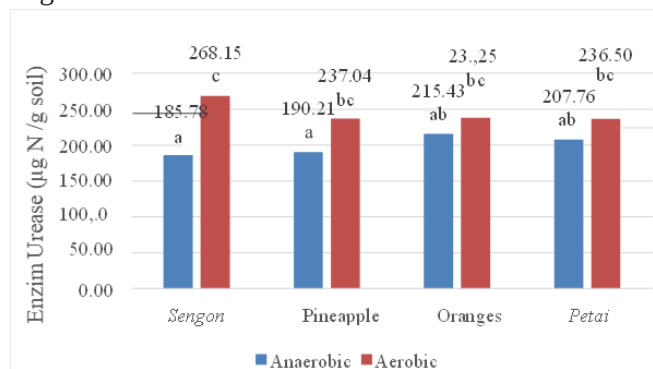


Figure 3. Effect of urease enzyme under aerobic and anaerobic conditions.

The highest urease enzyme was found in aerobic Sengon plants with 268.15 µg N/g soil and the lowest in anaerobic Sengon plants with 185.78 µg N/g soil.

Ammonium

The results of the analysis of variance showed that ammonium in aerobic and anaerobic conditions had a very significant effect on the amount of ammonium (Appendix 3). The difference test of the means using Duncan's Multiple Range Test (DMRT) is presented in Appendix 2. Ammonium under aerobic and anaerobic conditions is shown in Figure 4.

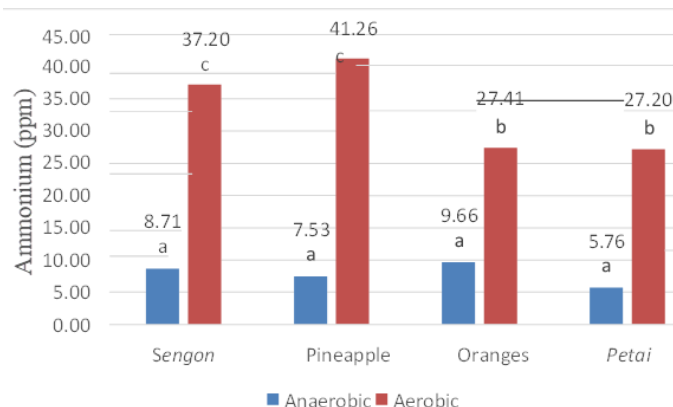


Figure 4. Effect of ammonium under aerobic and anaerobic conditions.

The highest level of ammonium was found in the aerobic pineapple plants with 41.26 ppm and the lowest level in the anaerobic petai plants with 5.76 ppm.

Nitrate

The results of the analysis of variance showed that nitrate during aerobic and anaerobic conditions had a very significant effect on the amount of nitrate (Appendix 4). Duncan's Multiple Range Test (DMRT) is presented in Appendix 4. Nitrate during aerobic and anaerobic conditions is shown in Figure 5.

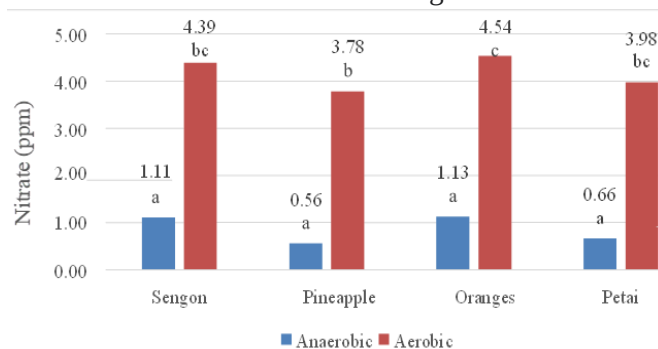


Figure 5. Effect of nitrate under aerobic and anaerobic conditions.

The highest nitrate was found in aerobic citrus plants with 4.54 ppm and the lowest in anaerobic pineapple plants with 0.56 ppm.

Microbial C-Content (C-mic)

The results of the analysis of variance showed that C-mic during aerobic and anaerobic conditions had a very significant effect on the number of C-mic. C-mic during aerobic and anaerobic conditions can be seen in Figure 6.

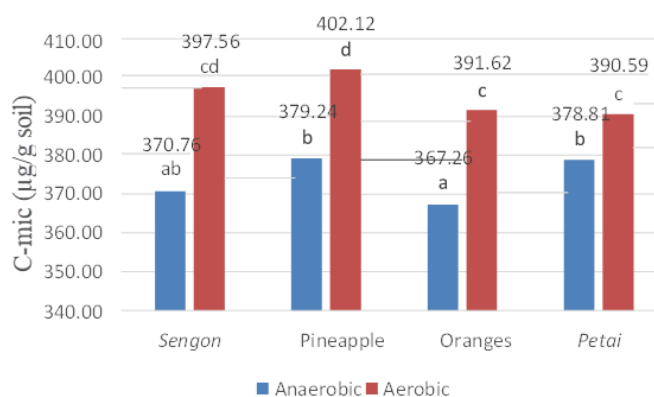


Figure 6. Effect of Cmik under aerobic and anaerobic conditions.

The highest C-mic was found in aerobic pineapple plants with 402.12 µg/g soil and the lowest in anaerobic citrus plants with 367.26 µg/g soil.

Relationship between non-symbiotic nitrogen-fixing microbial population and urease enzyme activity

The correlation and regression analyses were performed on the non-symbiotic nitrogen-fixing microbes and urease enzyme activity data to see the relationship between nitrogen-fixing microbes and urease enzyme under aerobic and anaerobic conditions. The results of the correlation and regression tests between non-symbiotic nitrogen-fixing microbes and urease enzyme activity are shown in Figure 7.

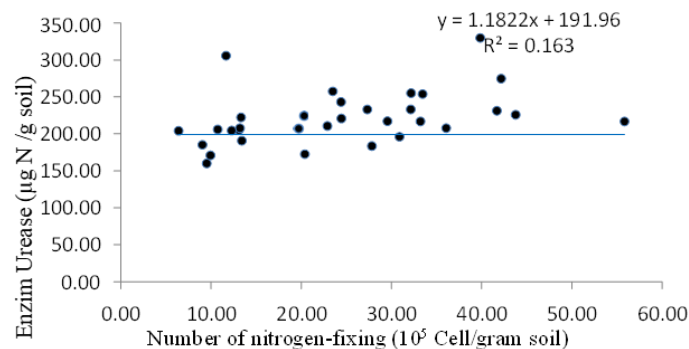


Figure 7. Correlation and regression results between nitrogen-fixing microbes and urease enzyme activity.

The results of statistical analysis of correlation and regression tests showed that total nitrogen-fixing microbes were significantly correlated with urease activity $R^2 = 0.163$. The linear model of the regression relationship (Figure 7) is as follows:
 Urease enzyme activity = $1.1822 \times \text{Population of non-symbiotic nitrogen-fixing bacteria} + 191.96$

DISCUSSION

Non-symbiotic nitrogen-fixing microbial population, ammonium, nitrate, urease enzyme activity and C-microorganisms under aerobic and anaerobic conditions

Nitrogen-fixing bacteria are one of the factors that can increase the rate of nitrogen use in the soil by fixing nitrogen from the air. The results showed that the results of the mean value test showed that the highest population of nitrogen fixing microbes was in aerobic Sengon plants with a value of 35.30×10^5 cells/gram of soil and the lowest in anaerobic Petai plants with a value of 10.27×10^5 cells/gram of soil, low non-symbiotic nitrogen fixing microbes are thought to be due to several factors such as weather, climate, organic matter.

Nitrogen is needed by plants for growth, but there is little in the soil. Nitrogen is taken up by plants as N-NH_4^+ and N-NO_3^- . The average nitrogen content in plant tissue is 2-4% dry weight. In the soil, nitrogen levels vary greatly depending on the management and use of the soil (Rosmarkam and Yuwono, 2002). In addition, nitrogen (N) in the soil is very abundant in the atmosphere in the form of gas (Yuwono, 2008). The

element N in the atmosphere is the dominant element because it constitutes 80% of the existing gas, but this gas form cannot be directly utilised by plants. Its utilisation can only be done with the help of microbial binders (fixation) that convert N_2 into ammonium (NH_4^+) available for plants (Hanafiah, 2013). The results of the mean test showed that the highest amount of ammonium was found in aerobic pineapple plants at 41.26 ppm and the lowest in anaerobic petai plants at 5.76 ppm. The highest amount of nitrate was found in aerobic citrus plants at 4.54 ppm and the lowest in anaerobic pineapple plants at 0.56 ppm.

Soil microbial biomass (C-mic) is a microorganism that can be used as an indicator of soil fertility. According to Wibowo (2013), organic matter plays an important role in soil microbial carbon biomass (C-mic). The greater the presence of organic matter, the higher the microbial activity, so that the soil microbial carbon biomass (C-mic) also increases. The results showed that the mean test showed that the highest C-mic was found in aerobic pineapple plants with 402.12 $\mu g/g$ soil and the lowest in anaerobic citrus plants with 367.26 $\mu g/g$ soil.

The result of urease activity is closely related to the urea content of the soil. The urease enzyme reflects the potential of the soil to convert urea to ammonium (NH_4^+). Urea is not only derived from artificial fertilisers but can also be derived from the secretions of soil fauna (Coyne, 1999). A large number of bacteria, fungi and actinomycetes in the soil are capable of producing this enzyme. The availability of fresh organic matter such as crop residues usually makes urease abundant (Havlin, et al., 2005). The results of the study showed that the highest urease enzyme was found in aerobic sengon plants with 268.15 μg N/g soil and the lowest in anaerobic sengon plants with 185.78 μg N/g soil. The high urease activity is caused by factors such as the high availability of organic matter; the availability of soil organic matter is known to be a source of precursors for enzyme synthesis (Martinez et al., 2007).

Relationship between non-symbiotic nitrogen-fixing microbial population and urease enzyme activity

The model of the relationship between the population of non-symbiotic nitrogen-fixing bacteria

and urease enzyme activity shows a positive correlation, where the more the population of non-symbiotic nitrogen-fixing bacteria increases, the more the urease enzyme activity increases.

According to Tisdale et al. (1985), urease activity increases as the microbial population increases. This enzyme activity is highest in the rhizosphere zone where microbial activity and population is high and is accumulated from plant roots. Based on the research of Tabatabai and Klose (1999) and Siallagan (2004), Cmic content has a positive influence on urease activity because the enzyme component itself is mainly produced by microbes. Soil microbial biomass is the living portion of soil organic matter outside of plant roots and soil fauna, which is larger than the largest amoeba with a volume of approximately 5,103 m^3 (Jenkinson and Ladd, 1981 in Djajakirana, 2003). It is also the main source of enzymes and the best index of microbial activity as a reflection of soil fertility. Soil enrichment with different energy sources will affect the mechanism of production and activity of catabolic enzymes (Gianfreda and Bollag, 1996).

CONCLUSIONS AND SUGGESTIONS

Conclusions

1. The population of nitrogen-fixing microbes in aerobic and anaerobic soil showed significant results where the largest population was in aerobic conditions compared to anaerobic conditions.
2. The population of non-symbiotic nitrogen fixing microbes is directly proportional to the activity of urease enzyme, where the more nitrogen fixing microbes, the more urease enzyme.
3. The high value of nitrogen fixing microbes is due to the high organic matter in the soil.

Recommendation

Further research is needed on the effect of herbicide application at different dose rates to determine the effect specifically on the chemical and biological properties of the soil.

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