The dynamics of functional microbes population in two depths under raru (Cotylelobium spp) stand

D Elfiati*, A Susilowati, C Modes
Faculty of Forestry, Universitas Sumatera Utara, Jl. Tridharma Ujung No.1 Kampus
USU Medan 20155 North Sumatra

*Corresponding author: denielfiati@yahoo.com

Abstract. Raru (Cotylelobium spp) is one of the endemic plants in North Sumatra from the Dipterocarpaceae family. Plant growth is strongly influenced by the condition of the soil environment. Soil provides nutrients to increase plant growth. Soil also a habitat for various microbes that affect plant growth. This study aimed to calculate functional microbial populations under raru stands such as arbuscular mycorrhizal fungi, phosphate solubilizing microbes, and cellulose decomposer microbes. The measurement of the population use plate count methods. Soil samples were taken in composite with 0-5 cm and 5-20 cm in the raru pulut, raru dahanon and raru songal rhizosphere area in Bona Lumban Village, Tukka District, Central Tapanuli Regency North Sumatra. The result showed that a functional microbial population was calculated higher at a depth of 0-5 cm compared to a depth of 5-20 cm. The microbial population of phosphate solubilizing microbes ranged from 10.12x 10^3 to 92.44x10^3 CFU mL^-1 with the largest population found in the raru songal rhizosphere. Cellulose decomposer microbial populations ranged from 6.21x10^3 to 21.55x10^3 CFU mL^-1 with the highest population found in the raru pulut rhizosphere. The highest arbuscular mycorrhizal fungi density in raru dahanon rhizosphere.

1. Introduction
Soil is a complex system with various components that have various functions, mainly due to the activity of soil organisms. Soil microflora plays an important role in soil conditions and plant growth. Bacteria and fungi improve plant performance by increasing mineral solubility in the soil, producing growth hormone and suppressing pathogens [1,2]. Fungi and bacteria are decomposers in the soil, play a role in nutrient mineralization so available and can be taken by plants [2]. The presence of microbes also plays a role in the formation of soil structures with the production of hyphae and polysaccharides that bind soil particles to form stable aggregates. This condition will reduce erosion, increase infiltration and maintain soil aeration [1,3].

Functional microbes are microbes that have certain functions in the soil such as cellulose decomposers and phosphate supply. Cellulose decomposer microbes are microbes that play a role in the decomposition of organic matter, especially those containing cellulose. Microbes involved include genera Pseudomonas, Bacillus, Aspergillus, Penicillium, Mucor, Rhizopus and Trichoderma [2,4,5]. Microbial phosphate supply is microbes that help in providing phosphate for plant growth. Phosphate microbial providers include phosphate solubilizing microbes and arbuscular mycorrhizal fungi. Phosphate is not available in the soil because it is bound by aluminum and iron to acid soils, and its bound by calcium to alkaline soils. The microbes involved are from the genera Pseudomonas, Bacillus, Enterobacter, Penicillium, Fusarium and Aspergillus [6,7]. Arbuscular mycorrhizal fungi are
a symbiosis between fungi and plant roots. Arbuscular mycorrhizal fungi are responsible for removing nutrients, especially phosphorus and increasing water absorption. This association was very important in infertile soils with low phosphorus content [8,9,10].

Raru (Cotylelobium spp) one of the endemic plants in North Sumatra. The types found in North Sumatra are Cotylelobium melanoxylon and C.lancelatum. Generally, the research carried out on Cotylelobium is related to its vegetation, so it is necessary to explore the existence of microbes as supporting plant growth, especially in the rhizosphere region. Rhizosphere is a dynamic area around the roots which is affected by root exudates and the taking of nutrients and water. Microbial populations in the rhizosphere area are higher than those without plants [11].

The study aimed to calculate the total population of soil microbes and functional microbial populations.

2. Material and Methods

2.1. Study site and sampling procedure
This study was conducted at Bona Lumban Village, Tukka District, Central Tapanuli Regency, North Sumatra. This study was conducted from March to July 2018. Soil samples were taken systematically at several points in the depths of 0-5 cm and 5-20 cm around the roots of the raru pulut tree, raru songal (Cotylelobium lanceolatum) and raru dahanon (Cotylelobium melanoxylon). Soil samples are then composted. The samples were transferred to the laboratory and placed in a chilled room at 4-16°C before being analyzed.

2.2. Soil analysis
Soil was analyzed for its chemical properties including soil pH by glass electrode method, organic C with Walkley and Black method, P available with Bray 2 method, total N by Kjehldal method and cation exchange capacity by washing method with ammonium acetate. Analysis was carried out at the Medan North Sumatra Palm Oil Research Center.

2.3. Calculation of microbial population
The measurement microbial population was conducted by viable plate counting method for counting colony form bacteria and fungi propagules. 10 g of fresh soil was put into Erlenmeyer containing 90 mL of physiological solution (8.5 g NaCl in 1 L distilled water) and shaken for 30 min. Soil suspension was diluted ten folds. For cellulose decomposer microbes and phosphate solubilizing microbes, one mL of the 10⁻³, 10⁻⁴, and 10⁻⁵ diluted solution was added with ten mL of nutrient agar at 50°C, then incubated for three days. After incubation, the number of microbes was counted use the Quebec colony counter [12]. The number of microbes was calculated using equation 1.

$$\Sigma \text{colony mL}^{-1} = \Sigma \text{colony} \times \text{dilution factor}$$ (1)

2.4. Calculation of arbuscular mycorrhizal fungi spore density
The spores of arbuscular mycorrhizal fungi were extracted using a filter pour technique and continued with centrifugation techniques [13]. Spore density is calculated per 50 g of soil samples.

2.5. Data analysis
The data of population were analyzed using ANOVA with factorial complete random design. If F count is greater than F table, then the test continued by using DMRT at 5% significant level.

3. Result

3.1. Soil analysis results
The results of soil analysis showed that the soil in the raru tree rhizosphere had very acidic to acidic soil pH, moderate to very high organic C content, very low to low P available, low to moderate total N content and cation exchange capacity including low criteria (Table 1). Nutrient content at a depth of 0-
5 cm tends to be higher than a depth of 5-20 cm, although some are still at the same criteria. In general, soil pH will affect the availability of nutrients and the presence of microbes in the soil. Nutrients are generally available at pH 6-7, while the presence of microbes depends on the type. In acid soils, fungi are more dominant, and at alkaline pH, bacteria are more dominant. The availability of nutrients also affects the presence of microbes in the soil, because soil microbes need nutrients for their survival [14]. Microbes in the soil are mostly heterotrophic, so they need organic compounds as energy and carbon sources. Based on the results of the nutrient content analysis, it can be stated that the soil in the raru tree rhizosphere has low of fertility.

| Raru types | Depth (cm) | pH  | C organic (%) | Total N (%) | P available (ppm) | CEC (me/100g) |
|------------|------------|-----|---------------|-------------|------------------|-------------|
| Raru Pulut | 0-5        | 4.6 | 4.96          | 0.25        | 3.08             | 11.40       |
|            | 5-20       | 5.2 | 3.41          | 0.18        | 0.55             | 8.95        |
| Raru       | 0-5        | 4.5 | 5.64          | 0.27        | 8.88             | 12.42       |
| dahanan    | 5-20       | 4.4 | 3.80          | 0.20        | 4.45             | 10.01       |
| Raru Sognal| 0-5        | 4.7 | 3.03          | 0.19        | 1.23             | 9.53        |
|            | 5-20       | 4.4 | 2.36          | 0.15        | 7.29             | 8.26        |

3.2. Microbial Population

3.2.1. *Arbuscular mycorrhizal fungi spore density*. The density of arbuscular mycorrhizal spores fungi on soil under raru stands with a depth of 0-5 cm were 50 spores, 83 spores and 107 spores per 50 g of soil in raru pulut stands, raru sognal and raru dahanan. At a depth of 5-20 cm, only one spore per 50 g of soil was found under sognal stands (Table 2). The occurrence of differences in spore density caused by the ability of plants to associate with arbuscular mycorrhizal fungi and the influence of environmental conditions. Indirectly can also be caused by differences in root exudates produced by each plant [18].

The existence of arbuscular mycorrhizal fungi provides benefits to plants with the ability to provide nutrients, especially phosphorus to plants and the ability to uptake water in the depth of the soil. The presence of hyphae causes plants to reach deeper layers so that plants infected with arbuscular mycorrhizal fungi can live in environmental stress conditions. The arbuscular is a finely branched hypha that transfers nutrients from the soil to the root system. Arbuscular mycorrhizal fungi have the ability to symbiosis with most land plant families [10].

3.2.2. *Phosphate solubilizing microbial population*. The total population of phosphate solubilizing microbes on soil under raru stands at 0-5 cm depth were 41.8x10^3 Colony Forming Unit (CFU) mL^-1, 25.76x10^3 CFU mL^-1 and 92.44x10^3 CFU mL^-1, while at depth 5-20 cm respectively 10.12x10^3 CFU mL^-1, 11.25x10^3 CFU mL^-1 and 15.07x10^3 CFU mL^-1 is found in raru pulut, raru dahanan, and raru sognal. The highest population was found on raru sognal rhizosphere (Table 3). The population at a depth of 0-5 cm is higher than the population at a depth of 5-20 cm. This is related to the presence of organic material as an energy source and a carbon source for phosphate solubilizing microbes. Phosphate solubilizing microbes in the soil are mostly heterotrophic, so they require organic material as a carbon and energy source. Phosphate solubilizing bacterial population is higher than phosphate solubilizing fungi population, because generally in the soil the bacterial population was higher than the fungi population. Phosphate solubilizing microbes play a role in the supply of phosphorus nutrients which are often bound to soil components so that they cannot be taken by plants. Phosphate solubilizing microbes increase the availability of phosphorus nutrients by producing organic acids. These organic acids then form a chelate with aluminum, iron, and calcium which bind phosphorus. The microbial population of phosphate solubilizing in the soil ranged from 10^4 to 10^6 CFU g^-1 soil, so population of phosphate solubilizing microbes on the soil under the raru stand was relatively low. The
amount and type of organic acids produced by phosphate solubilizing affect the effectiveness of each microbe [15, 16,17].

**Table 2. Density of arbuscular mycorrhizal fungi per 50 g of soil in the rhizosphere raru tree**

| Raru types   | Depth (cm) | Spore density |
|--------------|------------|---------------|
| Raru pulut   | 0-5        | 50            |
|              | 5-20       | 0             |
| Raru dahanon | 0-5        | 107           |
|              | 5-20       | 0             |
| Raru songal  | 0-5        | 83            |
|              | 5-20       | 1             |

**Table 3. Population of microbial phosphate solubilizing under raru stands (CFU mL⁻¹)**

| Raru types | Depth (cm) | Phosphate solubilizing bacteria (x10³) | Phosphate solubilizing fungi (x10³) | Phosphate solubilizing microbes (x10³) |
|------------|------------|----------------------------------------|-------------------------------------|---------------------------------------|
| Raru pulut | 0-5        | 32.12                                  | 9.68                                | 41.80                                 |
|            | 5-20       | 9.32                                   | 0.80                                | 10.12                                 |
| Raru dahanon| 0-5        | 21.49                                  | 4.27                                | 25.76                                 |
|             | 5-20       | 10.25                                  | 1.00                                | 11.25                                 |
| Raru songal| 0-5        | 90.27                                  | 2.17                                | 92.44                                 |
|             | 5-20       | 15.07                                  | 0.00                                | 15.07                                 |

3.2.3. **Cellulose microbial population.** The total population of cellulose decomposer microbes at 0-5 cm depth were 21.55x10⁹ CFU mL⁻¹, 19.13x10⁹ CFU mL⁻¹, and 13.65x10⁹ CFU mL⁻¹, whereas at a depth of 5-20 cm the total population cellulose decomposer microbes were 11.35x10⁹ CFU mL⁻¹, 6.21 CFU mL⁻¹ and 9.03x10⁹ CFU mL⁻¹ under the raru pulut, raru dahanon and raru songal stand respectively. The highest population was found in raru pulut rhizosphere (Table 4). Cellulose decomposer microbial population was higher in 0-5 cm depth than 5-20 cm depth. Cellulose decomposer microbes are microbes that can hydrolyze cellulose through their cellulase enzyme activity. The more microbial populations in the soil, the faster the decomposition process will occur. The decomposition process will produce nutrients that can be taken by plants, where the process is strongly influenced by the presence of microbes and environmental conditions [19].

**Table 4. Population of cellulose decomposer microbes under raru stands (CFU mL⁻¹)**

| Raru types | Depth (cm) | Cellulose decomposer bacteria (x10³) | Cellulose decomposer fungi (x10³) | Cellulose decomposer microbes (x10³) |
|------------|------------|--------------------------------------|----------------------------------|-------------------------------------|
| Raru pulut | 0-5        | 12.44                                | 9.11                             | 21.55                               |
|            | 5-20       | 2.68                                 | 8.67                             | 11.35                               |
| Raru dahanon| 0-5        | 12.20                                | 6.93                             | 19.13                               |
|             | 5-20       | 3.20                                 | 3.01                             | 6.21                                |
| Raru songal| 0-5        | 7.38                                 | 6.27                             | 13.65                               |
|             | 5-20       | 3.20                                 | 5.83                             | 9.03                                |

4. **Discussion**

The existence of microbes are very important for soil fertility, productivity, and sustainability of ecosystems. Microbial diversity directly affects crop productivity by affecting plant growth and nutrient and water uptake. Microbial diversity is one indicator of soil health [1,9,14].
Statistically, the type of raru, soil depth and interaction between the two no significant effect on the microbial population of phosphate solubilizing and cellulose decomposer microbes, while the spores of arbuscular mycorrhizal fungi gave a significant effect (Table 5).

**Table 5. Statistical recapitulation of test results**

| Source of diversity | Microbial population of phosphate solubilizing | Cellulose decomposer microbial population | Density of Spores of arbuscular mycorrhizal fungi |
|---------------------|-----------------------------------------------|------------------------------------------|-----------------------------------------------|
| Raru types          | ns                                            | ns                                       | *                                             |
| Depth               | ns                                            | ns                                       | *                                             |
| Interaction         | ns                                            | ns                                       | *                                             |

ns = not significant, *significant at 5% level

Although statistically, the effect of the depth is not significant, the microbial population is higher at a depth of 0-5 cm compared to a depth of 5-20 cm. This is related to the presence of higher organic matter at 0-5 cm depth (Table 1). Most of the microbes in the soil are heterotrophic, so they need organic material as an energy source and carbon source. The existence of microbes in the rhizosphere will affect the growth of plants on it. Otherwise, the presence of plants will also affect microbial populations in the rhizosphere. Plants will release root exudates that contain various compounds such as organic acids, vitamins, and nutrients needed by microbes for their survival [5,19].

5. Conclusion

The microbial population in the rhizosphere area of the raru tree is higher at a depth of 0-5 cm compared to a depth of 5-20 cm. The presence of microbes in the rhizosphere affects plant growth on it.

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