Microaggregate and Macroaggregate of Andisol Affected by Arbuscular Mycorrhizal Fungi and Rhizobacteria

C Hidayat¹, DH Arief², J Sauman², Anne Nurbaity²

¹Agrotechnology Dept, UIN Sunan Gunung Djati Bandung, Jl. AH. Nasution No. 105, Bandung 40614, Indonesia
²Faculty of Agriculture, Universitas Padjadjaran, Jl. Bandung-Sumedang Km.21, Sumedang 45363, Indonesia

E-mail: cephidayat62@uinsgd.ac.id

Abstract. The formation of soil microaggregate and macroaggregate involves different microbes. A Screenhouse experiment to determine the effect of Arbuscular Mycorrhizal Fungi (AMF) and rhizobacteria on soil microaggregate stability and soil macroaggregate stability (soil microaggregate and macroaggregate stability) had been carried out. The treatments included AMF (control and Glomus sp.) and rhizobacteria (Pseudomonas diminuta, P. diminuta + Bacillus alvei, P. diminuta + P. malei, P. malei). The results showed that inoculation Glomus sp. and rhizobacteria didn’t increase the stability of microaggregates (0.2 mm – 0.3 mm and < 0.2 mm). Inoculation Glomus sp and rhizobacteria increased water stable aggregate (2,83 mm – 4,8 mm, 0,50 mm – 1,00 mm, and 0,30 mm - 0,50 mm) and inoculation Glomus sp increased water stable aggregate (2,00 mm – 2,83 mm dan 1,00 mm – 2,00 mm).

1. Introduction

Aggregates are the basic unit of soil structure, consisting of primary particles, organic materials, and living organisms all bound together in clusters ranging in size less than 2 µm to greater than 2 mm [1]. Soil aggregates are essential components of soil structure that fundamentally affect soil quality, fertility, and sustainability [2]. For Andisol that has unstable sizes [3] and located in rugged terrain in the mountain, [4] soil aggregates become the determining factor of successful plant cultivation, moreover the aggregates stability processes are soil dependent [5].

Soil aggregation formation follows a hierarchical model where primary particles (<53 µm diameter) form microaggregates (<250 µm diameter) via physicochemical forces and persistent binding, whereas microaggregates are assembled into macroaggregates (>250 µm diameter) together with organic debris [2]. Soil microbiota plays important roles in the formation and the stability of soil aggregates [6]. Bacteria plays role in the formation and stabilization of microaggregate [7] through production of extracellular polymeric substances (EPS) that act as binding agent of soil particles [8]. AMF are involved in the formation of macroaggregates through external hyphae [9]. From the results of previous studies found that the rhizobacteria play a role in the formation of microaggregates and FMA involved in the formation of macro aggregates, in this study attempted to combine the microbes was to improve micro aggregates and macro aggregates simulant. The aim of the research was to find out the effect of arbuscular mycorrhizal fungi and different rhizobacteria on the formation of soil microaggregate and macroaggregate of Andisol.

2. Material and Method

The experiment was conducted at the Screen House of Kayu Ambon Lembang 1000 m above sea level using randomized block design (RBD) in factorial pattern with 2 factors and 3 replications. Factor 1:
AMF inoculation ($f_0 = \text{without Glomus sp}$ and $f_1 = \text{Glomus sp}$) and factor 2: rhizobacteria inoculation ($r_0 = \text{without MHB}, r_1 = \text{P. diminuta}, r_2 = \text{P. diminuta + B. alvei}, r_3 = \text{P. malei}, r_4 = \text{P. diminuta + P. malei}$). The parameters observed in this study were aggregate stability index, waterproof stable aggregate. Those observation parameters were analyzed by analysis of variance 5% using DSAASTAT statistical program.

Andisol from Lembang was mixed with fine goat manure with a dose of 20 ton ha$^{-1}$, then sterilized at temperature 100°C for 8 hours as much as 2 x with 24 hour interval. Once cooled the soil was put into polybag 30 cm x 20 cm as much as 6 kg. Seed of Granola ($G_3$) potato plant was planted in the middle. Microbial inoculation was done by placing 50 spores of Glomus sp. within 5 cm under potato seed and rhizobacteria inoculation using 80 ml (106 CFU ml$^{-1}$) dissolved in 800 ml of sterile aquades to spread throughout the media. Dosage of fertilizer used: N 300 kg ha$^{-1}$, 75 kg P$_2$O$_5$ ha$^{-1}$, 100 kg K$_2$O ha$^{-1}$. N fertilizer was used at half dose and the remainder at 30 days after planting. P and K fertilizers were given entirely at planting time. Maintenance consisted of irrigation carried out as needed, weed removal, and control of pests and diseases in the event of an attack using pesticides.

3. Result and Discussion

3.1 Microaggregate

Inoculation Glomus sp. and rhizobacteria either single or double had no interaction and main significant effect on micro aggregates (0.2 mm - 0.3 mm) (Table 1). Aggregate sizes of 0.2 mm - 0.3 mm and <0.2 mm are categorized as micro aggregates, inoculation Glomus sp. and rhizobacteria did not simultaneously increase the stability of micro aggregates. Both single and double rhizobacteria also did not significantly increase the value of micro aggregate stability. Similarly, the inoculation of Glomus sp. did not significantly improve the stability of micro aggregates. Based on the opinion of [8], the formation of micro aggregates is influenced by rhizobacteria. Rhizobacteria releases EPS that will glue the soil particles together [11].

Table 1. The Effect of AMF dan Rhizobacteria on Microaggregate (<0.2 mm and 0.2 mm – 0.3 mm) at last Vegetative Phase

| Microbe                  | < 0.2 mm$^*$ | 0.2 – 0.3 mm$^*$ |
|-------------------------|-------------|-----------------|
| Rhizobacteria inoculation : |             |                 |
| No rhizobacteria        | 8.42 a      | 20.30 a         |
| P. diminuta             | 8.88 a      | 23.22 a         |
| $P. \text{diminuta} + B. \text{Alvei}$ | 8.07 a      | 21.73 a         |
| $P. \text{malei}$       | 7.93 a      | 24.20 a         |
| $P. \text{diminuta} + P. \text{Malei}$ | 7.78 a      | 19.20 a         |
| AMF inoculation :        |             |                 |
| No Glomus sp.           | 8.11 a      | 21.25 a         |
| Glomus sp.              | 8.32 a      | 22.21 a         |

Means in the same column with the same letter are not significantly different (Duncan Multiple Range Test $\alpha = 0.05$).

From the data in Table 1, it can be seen that the rhizobacteria used in this experiment, $P. \text{diminuta}, P. \text{diminuta} + B. \text{alvei}, P. \text{malei}, P. \text{diminuta} + P. \text{malei}$ had no effect on micro aggregate formation. This is related with the dominance of the silt fraction (58.61%) on Andisol used in this experiment resulting low electrical charges (electrostatic/ electrochemical forces), thus less support for aggregate soil formation. EPS of rhizobacteria is an organic material rich with negative charges that will binds chemically with a rich allophane mineral clay with positive charge to form a stable aggregate. Such conditions do not occur on the experimental soil used due to the dominance of the silt fraction or slightly electrically charged.
3.2 Macroaggregates

Water stable aggregates (WSA) as an aggregate measurement which is not destroyed by wetting is an indicator that is used to measure macro aggregate stability. WSA are macro aggregates (> 0.25 mm) [11]. In this study, the WSA consist of aggregate size of 0.3 mm - 0.5 mm, 0.5 mm - 1 mm, 1 mm - 2 mm, 2 mm - 2.83 mm, and 2.83 mm - 4.8 mm.

The influence of inoculation *Glomus* sp. and rhizobacteria on WSA was divided into two groups. First, *Glomus* sp. and rhizobacteria gave interaction effect on water stable aggregate at aggregate sizes of 2.83 mm - 4.8 mm, 0.50 mm - 1.00 mm, and 0.30 mm - 0.50 mm. Second, independent effect of *Glomus* sp. on aggregate size 2.00 mm - 2.83 mm and 1.00 mm - 2.00 mm was occurred. Inoculation *Glomus* sp. and rhizobacteria showed interaction effect on the WSA 2.83 mm - 4.8 mm, 0.50 mm - 1.00 mm, and 0.30 mm - 0.50 mm in the last vegetative phase (Table 2).

Table 2. The Effect of AMF dan Rhizobacteria on WSA 2.83 mm – 4.8 mm, 0.50 mm -1.00 mm, and 0.30 mm – 0.50 mm at Last Vegetative Phase

|                | No *P. diminuta* | *P. diminuta* + B. alvei* | *P. malei* | *P. diminuta* + *P. malei* |
|----------------|-------------------|---------------------------|------------|-----------------------------|
| aggregate size 2.83 mm – 4.8 mm |                   |                           |            |                             |
| No *Glomus* sp. | 79.90 a           | 64.70 a                   | 69.27 a    | 56.73 a                     | 68.83 a         |
| B               | AB                | AB                        | A          | AB                          |
| *Glomus* sp.    | 69.57 a           | 89.03 b                   | 78.47 a    | 81.93 b                     | 67.60 a         |
| AB              | B                 | AB                        | AB         | A                           |
| aggregate size 0.50 mm - 1.00 mm |                   |                           |            |                             |
| No *Glomus* sp. | 8.57 a            | 14.60 a                   | 4.70 a     | 14.77 b                     | 17.47 a         |
| B               | C                 | A                         | C          | C                           |
| *Glomus* sp.    | 17.93 b           | 25.70 b                   | 17.90 b    | 2.97 a                      | 22.70 b         |
| B               | D                 | B                         | A          | C                           |
| aggregate size 0.30 mm - 0.50 mm |                   |                           |            |                             |
| No *Glomus* sp. | 25.27 a           | 27.13 a                   | 27.80 a    | 27.13 a                     | 24.93 b         |
| A               | A                 | A                         | A          | A                           |
| *Glomus* sp.    | 26.33 a           | 29.50 a                   | 27.27 a    | 25.93 a                     | 14.90 a         |
| B               | B                 | B                         | B          | A                           |

Means in the same column with the same letter are not significantly different (Duncan Multiple Range Test α = 0.05).

Data from Table 2 showed that rhizobacteria inoculation either single or double on aggregate size of 0.30 mm - 0.50 mm and 0.50 mm - 1.00 mm generally increased WSA compared with no microbial inoculation. However, at the largest aggregate size (2.83 mm - 4.8 mm) rhizobacterial inoculation actually lowered WSA compared with no microbial inoculation. Inoculation of rhizobacteria together with *Glomus* sp. non significantly increased the WSA value, except inoculation of *Glomus* sp. + *P. diminuta* on aggregate size 0.50 mm - 1.00 mm. These data reflect that *Glomus* sp plays a role in macro aggregate formation, in line with the results of [12] which stated that FMA plays a role in the formation of macro aggregates. The data in Table 2, further reinforces the belief that what increases the macro aggregate is *Glomus* sp, since it can increase WSA 1.00 mm - 2.00 mm and 2.00 mm - 2.83 mm which belongs to the macro aggregate category.

Inoculation *Glomus* sp. and rhizobacteria did not always show interaction effect on all sizes of WSA. Rhizobacterial inoculation had no significant effect on aggregate size 1.00 mm - 2.00 mm and
2.00 mm - 2.83 mm. While inoculation *Glomus* sp. gave significant effect (Table 3). Inoculation *Glomus* sp. increased WSA 1 mm - 2 mm by 15.20% and WSA 2 mm - 2,83 mm by 14.65% than no *Glomus* sp inoculation (Table 3).

**Table 3.** The Effect of AMF dan Rhizobacteria on WSA 1.00 mm – 2.00 mm and 2.00 mm – 2.83 mm at Last Vegetative Phase

| Treatments          | WSA 1.00-2.00mm | WSA 2.00-2.83 mm |
|---------------------|----------------|-----------------|
| FMA :               |               |                 |
| No *Glomus* sp.     | 71.23 a       | 68.56 a         |
| *Glomus* sp.        | 86.06 b       | 78.60 b         |
| Rhizobacteria:      |               |                 |
| No rhizobacteria    | 79.12 a       | 73.68 a         |
| *P. diminuta*       | 77.63 a       | 77.95 a         |
| *P. diminuta* + *B. Alvei* | 79.37 a     | 76.47 a         |
| *P. malei*          | 70.53 a       | 67.93 a         |

In macro aggregate formation, in this experiment > 0.3 mm, inoculation *Glomus* sp. significantly affected the WSA value. The increased in stability of macro aggregates as a result of *Glomus* sp inoculation due to the external hyphae possessed by the AMF species [13]. In this case *Glomus* sp. provides physically skeletal structures: 1) binds the soil particles together, and 2) the external hyphal acts as organic and inorganic binders in binding micro aggregates to macro aggregates.

From data of macro aggregate stability and stability of micro aggregate it can be concluded that *Glomus* sp. and rhizobacteria together do not play a role in the formation of macro aggregates, only *Glomus* sp. independently plays a role in the formation of macro aggregates. As for the formation of micro aggregates *Glomus* sp. and rhizobacteria either jointly or independently does not give effect. Specifically about rhizobacteria, according to [14], it plays an important role in the formation of micro aggregates, but in this experiment it was not so. The formation of micro aggregates determines the quality of soil aggregate stability because it lasts longer, so that if the micro aggregates are formed steadily, then the macro aggregates formed will also be stable. In this experiment, micro aggregate stability without microbial inoculation and inoculated microbial *Glomus* sp. and rhizobacteria was not significantly different. *Glomus* sp plays role in formation of the macro aggregate, although resulted unstable aggregate stability index. So it can be concluded, in order to produce a solid soil aggregate should start with the formation of a stable micro aggregate. Thus to improve the quality of agricultural soil to support maximum plant growth, formation of stable micro aggregate is the most important.

4. Conclusion
Rhizobacteria and *Glomus* sp jointly or independently had no effect on the formation of micro aggregates, while *Glomus* sp was involved in the formation of macro aggregates even resulting unstable aggregate.

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