The potential of arbuscular mycorrhizal fungi application on aggregate stability in alfisol soil

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Abstract. The aim of this study was to determine the soil aggregate stability and its relationship with another variable in alfisol. The research used completely randomized design with four treatments: two sterilization levels (no sterilization and with sterilization) and two levels of mycorrhizal inoculation (no mycorrhizal and with mycorrhizal). Mycorrhizal (5 grams/pot) was inoculated before planting rice seeds. The soil aggregate stability was measured by wet-sieving and turbidimetric measurements. The results showed that soil aggregate stability was higher in mycorrhizal inoculated than non-mycorrhizal inoculated treatment, by 5% in sterilization soil and 3.2% in non-sterilization soil. The correlation analysis indicated that soil aggregate stability has a tight relationship with spore population, total glomalin, available glomalin, dry weight, tiller number of plant, and soil organic C. Inoculation of mycorrhizal contributed to stabilize soil aggregates in alfisol.

1. Introduction
Soil structure is crucial to crop management and sustainable agricultural ecosystems [1] and is usually expressed by the degree of aggregate stability [2]. Poor arrangement of aggregates will induce serious problems, such as soil compaction, which can easily occur in poorly drained clay soils and detrimental to plant cultivation due to reduce the permeability to air, water, and roots [3], and causes nutrient stress and poor emergence of seeds. Appropriate measures for the alleviation of soil compaction were established through agriculture practices. Studies concerning the ecological role of arbuscular mycorrhizal fungi (AMF) put the emphasis on the benefits to host plants. For instance, AMF are widely known to enhance plant growth and improve nutrient uptake [4], and protect the host plant from drought, temperature, and toxic metal stress [5]. Recently, some studies have focused on the effects of AMF on soil structure [6]. The AMF have been found to provide great benefits in improving soil structure by the combined function of hyphae [7], which can physically entangle soil particles over an extended region and release an immune-reactive glycoprotein called glomalin, which is highly correlated with soil aggregate stability [8].

The AMF are the form of mutualism symbiotic association between plant roots and fungal mycelium [9], with more than 70% of plant families [10]. The other studies showed that the growth of plants with mycorrhizal inoculation was higher than without mycorrhizal inoculation due to more provided nutrients to the host plant [11], increase the resistance of drought [12] and protect the plants from fungal pathogens [11, 13, 14], and nematodes. In addition, mycorrhizal can improve the soil structure by forming stable soil aggregates through the external hyphae tissue that it produces. Application of AMF is one of the ways to increase the crops production in upland that is cheap, easy
and environmental friendly. Inoculation of mycorrhizal is expected to assist in rehabilitating critical land. It can be regarded as bio-fertilization, both for food crops, plantations, forestry and greening crops.

Soil organisms are known to play a crucial role in soil ecological processes such as organic matter turnover, nutrient cycling and engineering of the soil physical properties. Therefore, they are essential to soil fertility and nutrient uptake by plants [15, 16]. The soil physical habitat is widely assumed to be of prime importance in determining and regulating biological activities [17]. As a result, interactions between soil physics and the biological and chemical processes are key determinants of ecosystem health [18], but are still largely to be deciphered.

Plant roots, microorganisms (bacterial and fungal mycelium) and soil fauna are considered to be major cause of soil aggregation and porosity [2, 19, 20, 21]. The AMF influence soil physical processes with soil particles entanglement and glue substance secretion such as glomalin, but the respective effects of roots, but there is still controversy due to the symbiotic relationship between plants and AMF [22, 23, 24].

Glomalin is one of the deposits C in the soil, because it has a long-life time in the soil and contains soil organic C as the main binding material that will stabilize the soil [21], thus, soil aggregate stability increases. The working principle of mycorrhizal is to infect the root system of host plants and produce hyphae tissue intensively so it will be able to increase the capacity of nutrients absorption. Glomalin is one of the deposits C in the soil, because it has a long-life time in the soil and contains soil organic C as the main binding material that will stabilize the soil [21], so that soil aggregate stability increases. The working principle of mycorrhizal is to infect the root system of host plants and produce hyphae tissue intensively leads to increase the capacity of nutrients absorption.

The AMF can contribute to soil aggregate stability directly by a physical effect of a network around soil particles, and indirectly by the hyphal exudation of an iron-containing, heat stable glycoprotein named glomalin as an aggregate binding agent [25, 26]. Glomalin has been operationally defined as glomalin-related soil protein (GRSP) by extraction and detection conditions from soil, and it is detected in large amounts in diverse ecosystems [27]. The sticky GRSP acts as biological glue, helping to bind soil tiny particles into small aggregates of different sizes [28]. Well-aggregated soil is stable enough to resist wind and water erosion, and has better air and water infiltration rates favorable for plant and microbial growth [29]. The present study was conducted to determine the soil aggregate stability and its relationship with another variable in alfisol due to soil sterilization and inoculation of mycorrhizal.

2. Methods
The alfisol was sampled from the top layer (0-20 cm) at the Jumantono experimental site of the Universitas Sebelas Maret. The experiment was arranged in pot experiment used completely randomized design with four treatments: two sterilization levels, no sterilization (T0) and with sterilization (T1) and two levels of mycorrhizal inoculation, no mycorrhizal (M0) and with mycorrhizal (M1). Each treatment had six replicates, which made a total of 24 pots. Soil sterilization used autoclave, 7 kilograms of alfisol passed through 5 mm sieve was sterilized using autoclave at 0.11 MPa and 121 °C for 2 h. The AMF were provided by Soil Biology Laboratorium of Agriculture Faculty, Gadjah Mada University. Mycorrhizal at 5 grams pot⁻¹ was inoculated before planting rice seeds. The base fertilizer was used 100 kg Nitrogen Ha⁻¹, 40 kg P₂O₅ ha⁻¹ and 50 kg K₂O ha⁻¹. Portions of soil samples were dried by spreading them out on paper in ambient air and at room temperature for two weeks, and then were homogenized, grounded in an agate mortar and sieved to 0.2 mm and 0.5 mm. for. Soil pH was determined in a suspension with deionized water (soil/water, 1:2.5) using digital pH meter electrode. Soil organic matter (SOM) was measured by the wet combustion method using a mixture of potassium dichromate and sulfuric acid under heating. Total nitrogen (TN) concentration was determined according to the semi-micro Kjeldhal method. Available phosphorus concentration was measured colorimetrical. Exchangeable cations were extracted with ammonium acetate at pH 7.8 and measured by Atomic Absorption Spectrophotometry (Shimadzu
AA). The blank reagent and standard reference soil were assayed for quality assurance and quality control.

The soil parameter that measured was soil aggregate stability, C organic, total glomalin, available glomalin, spore population. Total glomalin related-soil protein was analyzed with 8 ml 50 mM sodium citrate (pH = 8), and then mixed using autoclave for 90 minutes. The supernatant was determined as a glomalin related-soil protein with standard bovine serum albumin [9]. The plant growth parameters included plant height, tiller number, and dry weight. Spearman correlation coefficients between soil aggregate stability and other variables were calculated using Proc-Corr procedure in program SAS version 9.13 portable. Correlation test was used to determine the relationship between variables.

3. Results and Discussion

3.1. Soil chemical and physical properties

Soil chemical and physical property of sample are presented in Table 1. Soil pH was acid (5.93), total C organic concentration was low (0.95%), total Nitrogen was low (0.11%), C/N ratio was low (8.74), CEC was moderate (20.00 cmol\textsuperscript{+} kg\textsuperscript{-1}), available phosphate was low (6.90 ppm), exchangeable potassium and calcium were low (0.31 and 3.24 cmol\textsuperscript{+} kg\textsuperscript{-1}), exchangeable magnesium and sodium were moderate (2.95 and 0.77 cmol\textsuperscript{+} kg\textsuperscript{-1}), base saturation was moderate (36.55%), and the texture was clay. So, based on the chemical and physical properties, the soil sample that used in this research have a low fertility.

| Soil Properties          | Value | Level* |
|--------------------------|-------|--------|
| pH H\textsubscript{2}O (1:2.5) | 5.93  | Acid   |
| C organic (%)            | 0.95  | Low    |
| Total Nitrogen (%)       | 0.11  | Low    |
| C/N Ratio                | 8.74  | Low    |
| P\textsubscript{2}O\textsubscript{5} Bray (ppm) | 6.90  | Low    |
| Exchangeable K (cmol\textsuperscript{+} kg\textsuperscript{-1}) | 0.31  | Low    |
| Exchangeable Ca (cmol\textsuperscript{+} kg\textsuperscript{-1}) | 3.24  | Low    |
| Exchangeable Mg (cmol\textsuperscript{+} kg\textsuperscript{-1}) | 2.95  | Moderate |
| Exchangeable Na (cmol\textsuperscript{+} kg\textsuperscript{-1}) | 0.77  | Moderate |
| CEC (cmol\textsuperscript{+} kg\textsuperscript{-1}) | 20.00 | Moderate |
| Exchangeable Al (cmol\textsuperscript{+} kg\textsuperscript{-1}) | 2.50  |        |
| Exchangeable H-(cmol\textsuperscript{+} kg\textsuperscript{-1}) | 0.70  |        |
| Base saturation (%)      | 36.55 | Moderate |

3.2. Effects of sterilization soil and mycorrhizal inoculation on soil parameters and aggregate stability

Soil sterilization and inoculation of mycorrhizal had a high correlation with soil aggregate stability from the highest to the lowest; C organic, total glomalin, available glomalin, and spore population (Table 2). Inoculation of mycorrhizal contribute to heat stable glycoprotein named glomalin as an aggregate binding agent. Some previous studies indicated that SOC affected the growth of AMF [30, 31] and was positively correlated with hyphal length density [32], which is sufficient to positively affect soil aggregation in the absence of other soil biota [7]. The effects of SOC on AMF were induced both by compounds released during the decomposition process and by secondary metabolites produced by microorganisms during organic matter decomposition [33]. Conversely, fungal inoculation enhanced the decomposition of organic matter in soil [34] and carbon could be lost from mycorrhizal
roots into soil and external hyphae [35], which might be the main reasons for the increase in SOC when inoculating with AMF.

Table 2. Spearman correlation coefficients between soil aggregate stability to sterilization soil and mycorrhizal inoculation on several soil parameters

| Soil Parameter          | Spearman Correlation Coefficients |
|-------------------------|-----------------------------------|
| Total glomalin          | 0.1856                            |
| Available glomalin      | 0.304                             |
| C-organic               | 0.111                             |
| Spore population        | 0.9853                            |

3.2.1. Effects of sterilization soil and mycorrhizal inoculation on soil aggregate stability and total glomalin A significant positive correlation between total glomalin and soil aggregate stability was observed in this study (Figure 1). Mycorrhizal increased the aggregates stability. This suggested that mycorrhizal contributed to the improvement of soil aggregates stability partly via the function of glomalin, which bound soil primary particles into larger aggregates.

3.2.2. Effects of sterilization soil and mycorrhizal inoculation on soil aggregate stability with available glomaline and C-organic A significant positive correlation between soil aggregate stability with available glomalin and C-organic was observed in this study (Figure 2 and 3). The significant positive relationships between SOC and >1-mm aggregates in a five-year tillage experiment, and a significant positive correlation between C-organic was found. Mycorrhizal reduced the proportion of
<0.25-mm aggregates while there was an increase in the proportion of >1-mm aggregates [36]. These results suggested that mycorrhizal contributed to the improvement of soil aggregate stability via the function of soil organic carbon, which bound soil primary particles into larger aggregates.

3.2.3. Effects of sterilization soil and mycorrhizal inoculation on soil aggregate stability with spore population.
Correlation test showed that there was a positive correlation between spore population and soil aggregate stability because of sterilization soil and inoculation of mycorrhizal (Figure. 4). Inoculation of mycorrhizal increased spore population up to 21%. Inoculation of mycorrhizal increase spore population up to 6% in unsterilized soil and up to 43% at sterilized soil (without inoculation of mycorrhizal). Partly, inoculation of mycorrhizal significant increased soil aggregates stability. This result showed that soil microorganism did not influence the forming of spore in soil.

3.3. Effects of sterilization soil and mycorrhizal inoculation on plant growth parameters
Soil sterilization and inoculation of mycorrhizal increased plant height, dry weight and tiller number of rice. A positive correlation between soil aggregate stability with plant growth showed at Table 3. The highest correlation, from the high to the weak are tiller number, dry weight, and plant height of rice.

Table 3. Spearman correlation coefficients between soil aggregate stability with several plant growth parameters

|                         | Spearman Correlation Coefficient |
|-------------------------|----------------------------------|
| Plant height            | 0.5455                           |
| Dry Weight              | 0.4931                           |
| Tiller number           | 0.3978                           |

There were positive correlation between sterilization soil and inoculation of mycorrhizal and plant growth parameters. Sterilization soil and inoculation of mycorrhizal increase plant height, dry weight, and tiller number. Tiller number increased up to 38% than unsterilized soil without inoculation of mycorrhizal. Unsterilized soil, inoculation of mycorrhizal improved tiller number up to 33 % than unsterilized soil without inoculation of mycorrhizal (Table 3).

4. Conclusion
There was a correlation between soil aggregate stability with the other variables. Soil sterilization and inoculation of mycorrhizal increased total glomalin, available glomalin, C organic, spore population, plant height, tiller number, dry weight of rice. Inoculation of mycorrhizal increased total glomalin up to 16 %, available glomalin 20%; C organic 11.44%; spore population up to 43% in sterilized soil. Inoculation of mycorrhizal increased plant height up to 9% at sterilized soil and 4% at unsterilized soil, tiller number up to 21% at sterilized soil and 16% at unsterilized soil, dry weight of rice up to 16% at sterilized soil and 34% at unsterilized soil.

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