*Bulgarian Journal of Agricultural Science, 23 (No 6) 2017, 994–1003*
*Agricultural Academy*

# THE EFFECT OF MANURE AND MYCORRHIZA APPLICATION TO THE SOIL MICROBES BIODIVERSITY IN TERMS OF INCREASING SOYBEAN YIELD IN MARGINAL LAND IN INDONESIA

SAMANHUDI[1*]; BAMBANG PUJIASMANTO[1]; AHMAD YUNUS[1]; SUPYANI[1]; SUNTORO[2]; HERY WIDIJANTO[2]; SHALAHUDDIN MUKTI PRABOWO[3]
[1] *Sebelas Maret University, Faculty of Agriculture, Study Program of Agrotechnology, Surakarta 57126, Indonesia*
[2] *Sebelas Maret University, Faculty of Agriculture, Study Program of Soil Science, Surakarta 57126, Indonesia*
[3] *Sebelas Maret University, Graduate Program of Agronomy, Surakarta 57126, Indonesia*

## Abstract

Samanhudi, B. Pujiasmanto, A. Yunus, Supyani, Suntoro, H. Widijanto and S. M. Prabowo, 2017. The effect of manure and mycorrhiza application to the soil microbes biodiversity in terms of increasing soybean yield in marginal land in Indonesia. *Bulg. J. Agric. Sci.*, 23 (6): 994–1003

The extensive of marginal land in Indonesia continues to increase for every year. These is can be solved using an alternative technology such as the utilization of microorganisms and organic matter. This research aimed to study the effect of various types of manure (cow, goat, quail) and mycorrhiza in soil microbial biodiversity in terms of soybean yield in marginal lands. The research used Randomized Complete Block Design (RCBD) which consists of two factors, the mycorrhiza treatment with two levels and types of manure treatment with five levels. So we got 10 treatment combinations, each of which was repeated three times. The results showed that there was significant differences between without and with mycorrhiza treatment as biodiversity of microbial populations were higher in the treatment with mycorrhiza than without mycorrhiza. There were 22 types of bacteria in all samples, while fungi were identified in four genera which are *Aspergillus sp, Penicillium sp, Rhizopus sp*, and *Mucor sp*. Treatments with mycorrhiza provide higher yields than without mycorrhiza but the result did not show significant result.

*Key words:* marginal land; mycorrhiza; soil microbial biodiversity; soybean
*Abbreviations: AM* – Arbuscular Mycorrhizal, NA – Nutrient Agar, PDA – Potato Dextrose Agar

## Introduction

Land resources is one of the factors that determine the success of a farming system, because almost all agricultural enterprise based on land resources. Land is an area that cover all the character embedded in the atmosphere, soil, geology, generation, hydrology and plant and animal populations, also steady and recyclable, as well as human activities on it. So, the land has a natural and cultural characteristics.

In terms of supporting bioenergy production and increasing food security, marginal land now gets high interest in global in subject of limited arable land resources (Koonin, 2006; Tilman et al., 2006; Food and Agriculture Organization/FAO, 2008; Robertson et al., 2008; Milbrandt and Overend, 2009; Vuichard et al., 2009). Marginal land has some limitations for agriculture purpose as it has low productivity and economic return. Marginal lands also have high environmental risk (Barbier, 1989; Wiegmann et al., 2008) and it becomes concerns besides environmental impacts, ecosystem services, and sustainability of marginal lands such as erosion, land degradation, biodiversity, and climate change mitigation (O'Connor et al., 2005; Intergovernmental Panel on Climate Change/IPCC, 2007; Searchinger et al., 2008; Fischer et al., 2009). The marginal land use has been associ-

**Corresponding author*: samanhudiuns@gmail.com

ated with the trilemma of land use (food security, bioenergy, and environmental concerns) and became a critical issue aat the moment (Lal, 2008; Tilman et al., 2009). This issue of marginal land use has triggered a discussion not only for agriculture use but also for future land use management in many disciplines (Kang et al., 2013). The planning of using marginal lands can be use in management practices with multiple goals which is in concerns of environment, ecosystem service and sustainability under framework of landinformatics.

Millions hectares of marginal land are spread across several islands, good prospect for the development of agriculture, but now these have not been well managed. Dry land use need to be expanded and provided important aspect, especially for the development of food crops as supporting a variety of community life, while maintaining its role as the stabilization and improvement of ecosystem functions. To support the success in using marginal land, it is necessary to arrange an alternative technology that can be done such as the utilization of microorganisms and the application of organic matter in terms to support the growth of soybean plants.

Arbuscular mycorrhiza (AM) fungus is a type of oligotrophic microorganism that can promote the plant growth by making a symbiotic relationship through root celss infection. AM fungi makes widely infection range with hostspecific dynamic (Eisenhauer et al., 2009; Mummey et al., 2009; Whiteside et al., 2012a). AM fungi has some advantages in the rhizosphere soil: (1) improve the absorption and utilization efficiency of inorganic nutrient elements in plants (Vaz et al., 2012); (2) strengthen disease resistance of host plants (Asghari, 2012; Whiteside et al., 2012b) ; (3) increasing the good environment condition for host plants growth; (4) elevate the community succession; (5) acts as a biofertilizer which is good for ecosystem stability (Porras-Soriano et al., 2009; Fedderman, 2010). The some recent studies have concerated on the AM application to crops (Golubski, 2011; Tan et al., 2011; Hart and Forsythe, 2012) as one of them is the effects of AM fungi on leguminous crops experiments which is done in sterilized pot experiments and by studying plant growth under ecologically damaged conditions (Meghvansi et al., 2008; Miransari, 2011). The result studies show that AM fungi can elevate the soybean to absorb available nutrition in the soil by increasing both the nitrogen-fixing ability of Rhizobium and the colonization structure in the rhizosphere niche, and it resulted high yields and economic efficiency (Tian et al., 2013). The results by Jie (2013) showed addiotional informations in terms of AM fungi community structures in the rhizosphere soils which were significantly different among different soybean cultivars at the branching stage.

This research aims to study the effect of the use of sorts manure (cow, goat, quail) and mycorrhiza on soil microbial biodiversity, as well as its influence on soybean yields in marginal lands.

## Materials and Methods

The analysist was done by isolate the rhizosphere soil directly from rhizosphere of some soybean plants. The identification was conducted at Soil Biology Laboratory, Faculty of Agriculture, Sebelas Maret University. Research used Complete Randomized Block Design consist of two factors, the treatment of mycorrhiza treatment with two levels (M0: without mycorrhiza inoculation and M1: mycorrhiza inoculation) and the type of manure treatment with five levels (P0: without manure, P1: 10 t cowshed fertilizer $Ha^{-1}$, P2: 10 t goat manure $Ha^{-1}$, P3: 10 t quail manure $Ha^{-1}$, P4: 5 t straw $Ha^{-1}$) to obtain 10 combinations of treatments, each of which was repeated 3 times.

***Microbial isolation***

Soil samples were taken ± 200 g for each treatment plot near soybean roots and repeated 3 times. The soil samples were taken 10 g and then inserted into 90 ml physiological saline, and shaked until homogeneous ($10^{-1}$ dilution). After that 1 ml solution of $10^{-1}$ were transferred into 9 ml physiological saline, then shaken until homogeneous (dilution $10^{-2}$) and repeated same thing until the dilution became $10^{-5}$. As much as 0.1 ml solution of $10^{-5}$ was transferred into NA medium for bacteria and PDA media for mushrooms, then spread over the media, did the same thing to all of dilution. The isolates were incubated at room temperature with petridish in inverted position, stored at room temperature, isolated after purification performed 4-7 days old. Isolates were identified morphologically after the age of 3 days, then bacteria and fungi colonies were counted.

***The degree of mycorrhizal infection***

The degree of mycorrhiza infection was done using root staining technique adopted from Phillip and Hayman (1970). Root samples were washed and cut into pieces, and then put in a film canister, after that added with 2.5% KOH, stayed for 24 hours. After that, the roots were washed and added with 2% HCl, soaked for 24 hours, then replaced with a staining solution, stayed for 24 hours and kept on film canisters. To calculate the root infection, root samples (5 pieces per treatment) with a length of ± 1 cm were placed in a preparation glass and closed with cover glass then counted using microscope. The positive sign is when there is arbuscular mycorrhizal structure (hyphae, vesicles, arbuscular) every visibil-

ity distance. Every 1 cm root gave visibility 7-10. When the infected roots could not been calculated yet, better to store in the refrigerator. The percentage of infected root is calculated with the following formula:

$$\text{The Degree of Mycorrhizal Infection} = \frac{\sum \text{field of view(+)}}{\sum \text{field of view(+) and (–)}} \times 100\%$$

***Diversity indeks***

Calculating the index of species diversity of soil microbial populations that have been identified, by using the formula Shannon-Weaver diversity index as follows:

$$H' = \sum_{i=1}^{n} pi \ln pi,$$

where:

$H'$ = diversity index

$pi$ = number of individuals of species $i$ / total number of sample

Data analysis using descriptive analysis and F test, if significant continued with DMRT.

## Results and Discussion

Marginal land that was used in this study had poor nutrition and low organic C (See Table 1), so if it is used for agricultural purposes, the result possibly will be low. A research which was conducted by Soepraptohardjo (1983) stated that if the N total between 0.1-0.2%, $P_2O_5$ <10 ppm, and $K_2O$ <10 me% is categorized in low level.

Marginal land is a nutrient-poor soils, water availability and limited rainfall, thin of solum soil and hilly topography and the low productivity as well. Dry land is included as a marginal land which needs to be managed in order to be used for agricultural activities. Organic materials such as manure and mycorrhiza are expected to improve the condition of the soil, became fertile and have more soil microbial diversity. Table 2 shows that there are five types of bacteria, whereas Table 3 reveals that there are two types of fungus. So the diversity of microbes on marginal land is low. These happens because of marginal land has low organic matter and nutrient poor, where the organic material is the energy source for microbes.

Microbes were isolated from the rhizosphere of soybean using dilution method. Soil samples which were taken for each treatment plot were analyzed in the laboratory. The observation of soil microbial diversity after being given treatment is presented in Table 4 and Table 5. Table 4 shows the diversity of fungi which has been identified. It was found 12 isolates from 4 genera *Aspergillus sp, Penicillium sp, Rhizopus sp,* and *Mucor sp*. These four genera are classified as decomposers in the soil as decomposers play an important role in the process of soil fertility. Results of the former states that *Penicillium sp* and *Aspergillus sp* act as decomposers in the soil. The other research concluded that the five species of fungus are known as dominant decomposers from coffee plantations "Sumber Jaya", Lampung Barat, namely *Fusarium sp, Aspergillus ochraceus, Rube monascus, Aspergillus niger* and *Trichoderma sp*. While Table 5 shows that the morphological diversity of bacteria identified were 22 isolates. The results show that microbial diversity is higher after treatment than before treatment. So the treatment in the form of adding mycorrhiza and manure gives a positive effect to soil microbial diversity.

**Table 1**
**Soil analysis before treament**

| Number | Parameters | Marginal land |
|---|---|---|
| 1 | N total | 0.13 % |
| 2 | $P_2O_5$ | 9.41 ppm |
| 3 | $K_2O$ | 0.23 me% |
| 4 | C organic | 1.50 % |
| 5 | BO | 2.59 % |
| 6 | C/N ratio | 11.54 |

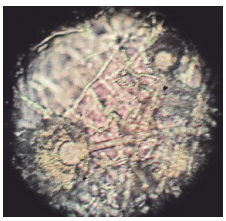
*Source*: Laboratory of Soil Biology of Sebelas Maret University (2015)

**Table 2**
**The diversity of bacteria before treatment**

| Isolate | Size | Shape | Elevation | Margin | Colour |
|---|---|---|---|---|---|
| Isolate 1 | small | circular | raised | entire | clear |
| Isolate 2 | average | circular | raised | lobate | clear |
| Isolate 3 | average | circular | umbunate | undulate | clear |
| Isolate 4 | small | circular | flat | entire | yellow |
| Isolate 5 | big | irregular | flat | lobate | turbid |

*Note:* The isolated bacteria were identified morphologically using a magnifying glass. Morphology observed that the size, shape, elevation, ledges and colors. The identification results showed there were 5 isolates of bacteria with a variety of different morphological traits

**Table 3**
**The diversity of fungi before treatment**

| Isolate | Colour | Colony reverse | Colony surface | Microscope | Genus |
|---|---|---|---|---|---|
| Isolate 1 | green | white | smooth |  | *Penicillium sp* |
| Isolate 2 | white | white | like velvet |  | *Aspergillus sp* |

*Note:* The isolated fungi were identified morphologically using a magnifying glass and a microscope to look at the spores. Morphological variable which were observed are color, colony reverse, and the surface shape of the colony. The identification results showed there were 2 isolates fungus with different morphological characters

**Table 4**
**The diversity of fungi after treatment**

| Isolate | Colour | Colony reverse | Colony surface | Microscope | Genus |
|---|---|---|---|---|---|
| 1 | green | white | smooth |  | *Penicillium sp* |
| 2 | green white | white | smooth | | *Penicillium sp* |
| 3 | green | green | fiber | | *Penicillium sp* |
| 4 | green blue | white | like velvet | | *Penicillium sp* |
| 5 | black brown | black brown | like straight pin |  | *Rhizopus sp* |
| 6 | orange | white | rough |  | *Aspergillus sp* |
| 7 | green rough | green rough | rough fiber | | *Aspergillus sp* |
| 8 | white | white | like velvet | | *Aspergillus sp* |
| 9 | rough | white | rough fiber | | *Aspergillus sp* |
| 10 | white | rough | like velvet |  | *Mucor sp* |
| 11 | black white | black | fiber | | *Mucor sp* |
| 12 | white | white | like yarn | | *Mucor sp* |

*Note:* The isolated fungi were identified morphologically using a magnifying glass and a microscope to look at the spores. Morphological variable which were observed are color, colony reverse, and the surface shape of the colony. The identification results showed there were 2 isolates fungus with different morphological characters

**Table 5**
**The diversity of bacteria after treatment**

| Isolate | Size | Shape | Elevation | Margin | Colour |
|---|---|---|---|---|---|
| Isolate 1 | small | circular | raised | entire | clear |
| Isolate 2 | average | circular | raised | lobate | clear |
| Isolate 3 | average | circular | umbunate | undulate | clear |
| Isolate 4 | small | circular | flat | entire | yellow |
| Isolate 5 | big | irregular | flat | lobate | turbid |
| Isolate 6 | spot | circular | flat | entire | turbid |
| Isolate 7 | average | circular | flat | serate | turbid |
| Isolate 8 | big | irregular | flat | undulate | turbid |
| Isolate 9 | small | filament | flat | serate | turbid |
| Isolate 10 | big | circular | flat | lobate | turbid |
| Isolate 11 | average | circular | convex | entire | turbid |
| Isolate 12 | spot | circular | convex | entire | yellow |
| Isolate 13 | big | filament | flat | entire | clear |
| Isolate 14 | average | irregular | umbunate | undulate | clear |
| Isolate 15 | average | circular | raised | undulate | turbid |
| Isolate 16 | average | irregular | raised | lobate | turbid |
| Isolate 17 | average | irregular | flat | serate | turbid |
| Isolate 18 | big | irregular | flat | serate | turbid |
| Isolate 19 | average | circular | umbunate | lobate | turbid |
| Isolate 20 | average | irregular | raised | undulate | clear |
| Isolate 21 | average | circular | raised | entire | turbid |
| Isolate 22 | average | circular | umbunate | entire | turbid |

*Note:* The isolated bacteria were identified morphologically using a magnifying glass. Morphology observed that the size, shape, elevation, ledges and colors. The identification results showed there were 22 isolates of bacteria with a variety of different morphological traits

Then Fulthorpe et al. (2008) has analyzed number of 139 819 bacterial strains from the four regions with different geographical location. The analysis showed that the most abundant species of bacteria in the rhizosphere is *Chitinobacteria sp, Acidobacterium sp,* and *Acidovorax sp* with an abundance of between 13-20%. Then Buée et al. (2009) stated that the microbes which inhabit the rhizosphere are generally divided into groups of bacteria, archaea and fungi. However, all these three groups when grown on artificial media in laboratory quantities can only be grown in a few numbers. The results of other studies indicate that there are 10 genera which are always found in various rhizospheres, the sequence of the most abundant is *Bacillus, Flavobacterium, Pseudomonas, Proteobacteria, Bacteroidetes, Acidobacteria, Firmicutes* and *Gemmatimonades* (Kielak et al., 2009). Not only observing the diversity of soil microbes but also observed soil microbial populations which are presented in Table 6.

The observations in Table 6 show that mycorrhizal inoculation treatments + without fertilizer has the highest microbial populations among the treatments. Overall, treatment of mycorrhizal inoculation has higher microbial populations than treatment without mycorrhiza. Based on this study, the presence of mycorrhizae affects other microbial diversity.

The role of mycorrhizae in this study affects the existence of soil microbes. This has been proven by Curl and

**Table 6**
**Microbial population**

| Treatments | Fungi (x $10^3$ Spores/ml) | Bacteria (x $10^5$ CFU) |
|---|---|---|
| Without mycorrhiza + without manure | 4.5 | 14 |
| Without mycorrhiza + cow manure | 10 | 9.6 |
| Without mycorrhiza + goat manure | 7.5 | 17 |
| Without mycorrhiza + quail manure | 2.5 | 18 |
| Without mycorrhiza + straw | 5.5 | 10 |
| inoculation mycorrhiza + without manure | 23 | 18 |
| inoculation mycorrhiza + cow manure | 7.5 | 5.6 |
| inoculation mycorrhiza + goat manure | 7.5 | 14 |
| inoculation mycorrhiza + quail manure | 14 | 3.6 |
| inoculation mycorrhiza + straw | 8.5 | 4.2 |

*Note:* The number of population of microbes (fungi and bacteria) is calculated using a hand colony counter. The calculation is performed by observing the number of colonies that grow on the medium

Bryan (1985), which explained that the plants which are inoculated with mycorrhizal and bacterial N fixation have higher microbial populations in the rhizosphere than the plants without inoculation.

Microbes that exist in phyllosphere and rhizosphere can be endophytic, epiphytes, or association (Koopman et al., 2010). The existence of microbes in the rhizosphere is highly influenced by root exudates in the nearest land (Bais et al., 2006). In other cases, microbes can act as a protectant against pathogens (Innerebner et al., 2011), increase plants growth through the production of phytohormones (Ali et al., 2009), help plants resist heat, salt (Zhang et al., 2008). Plants have a function to fertilize microbiome by adjusting the pH of the soil, thus it can reduce the competition for beneficial microbes, and provide most of the energy source in the form of carbon-rich rhizodeposits (Bais et al., 2006).

Microbial community structure in the phyllosphere and rhizosphere often differ across plant species (Kuske et al., 2002), as well as among genotypes within a single species (Aira et al., 2010; Bouffaud et al., 2012). The number of rhizospheres from inbreds maize is less but significant proportion of heritable variation in total bacterial diversity across fields, and substantially more heritable variation between replicates of the inbreds within each field (Peiffer et al., 2013). Recent work with model systems (*Arabidopsis thaliana* cultivated under controlled conditions in natural soils) indicated that the host genotype has a small but measurable effect on the microbes inhabiting the endophyte compartment of the root (Bulgarelli et al., 2012; Lundberg et al., 2012).

The other research concluded that microorganisms that inhabit the rhizosphere play a very important role in helping the growth and improving the ecological health of its host plant, either directly or indirectly. Directly microbes in the rhizosphere produce a variety of vitamins, antibiotics, plant hormones and other molecules are certainly favorable for plant growth. Kent and Triplett (2002) stated that indirectly, some microbes release substances that can against harmful microbes pathogenic, so that it can protect plants from disease. Due to the interaction of microbes that produce anti-pathogenic substances, it can control the parasite in the rhizosphere microbial population.

In the present study, rhizosphere soils are rich in microbial population, which leads to increasing microbial activity compared to nonrhizosphere soils. The increased number can be attributed to enriching effect of root exudates, sloughed off cells leading to increased microbial activity that is characteristic to rhizosphere zone (Hindumathi and Reddy, 2011). It is well known that root exudates have a direct influence on the microbial population in the rhizosphere (Namdas et al., 2009). Then to find out how the diversity of species on soil microbes, it is presented in Table 7.

**Table 7**
**The diversity index**

| Treatments | H' of Bakteria | H' of Fungi |
|---|---|---|
| Without mycorrhiza + without manure | 1.9 | 1.5 |
| Without mycorrhiza + cow manure | 2.2 | 1.9 |
| Without mycorrhiza + goat manure | 2.1 | 2.2 |
| Without mycorrhiza + quail manure | 2.3 | 1.4 |
| Without mycorrhiza + straw | 2.2 | 1.8 |
| inoculation mycorrhiza + without manure | 2.1 | 1.5 |
| inoculation mycorrhiza + cow manure | 1.6 | 1.5 |
| inoculation mycorrhiza + goat manure | 1.6 | 1.7 |
| inoculation mycorrhiza + quail manure | 1.9 | 1.3 |
| inoculation mycorrhiza + straw | 1.4 | 1.7 |

*Note:* Diversity index calculated using the formula which is adopted from Shannon-Weaver. The value of the diversity index is low if H'<1.5, the diversity index is moderate if the value H' is in the range from 1.5 to 3.5, and the diversity is high if H'>3.5

Table 7 shows the stability of species diversity which was identified from soil samples. Overall, the value of H', or diversity index, values from 1.5 to 3.5. The species diversity index was categorized as moderate level. This condition indicates that species diversity is quite stable. According to the research which was conducted by Odum (1996) stated that the higher the value of H', the more stable the diversity of species in the community. Conversely the lower is the value of H', then the lower is the stability of species diversity in the community.

Species diversity index is the value or level of diversity found among different types of living creatures in an ecosystem. In this study, the diversity of a community is affected by the magnitude of the density, the large number of types and prevalence of each type.

Then to find out how effective is mycorrhiza in infecting soybean is shown in Figure 1. The observation of the degree of mycorrhizal infection in Figure 1 shows that mycorrhizal inoculation treatment + cow manure has the highest level of mycorrhizal infection (56%) among other treatments, while the treatment without mycorrhizal + without fertilizer was the lowest (16%). Previous research that has been done by Harinikumar et al. (1990) suggested that the accumulation of organic matter can increase the diversity of AM spores and the endurance and ability to grow the fungal spores in the soil.

The success of AM symbiotic depends on the condition of the soil, plants, and fungi forming associations (Sieverding et al., 1991). Results of the research which was conducted by

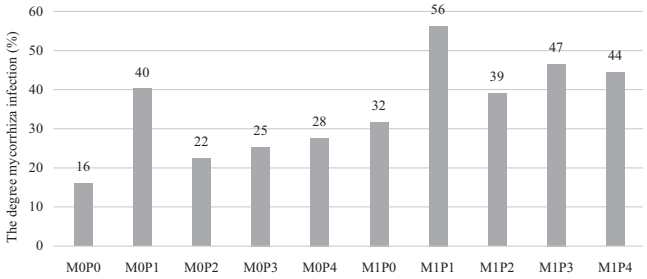



**Fig. 1. The result of measurement of the degree mycorrhiza infection**

*Note*: M0P0: Without mycorrhiza + without manure; M0P1: Without mycorrhiza + cow manure; M0P2: Without mycorrhiza + goat manure; M0P3: Without mycorrhiza + quail manure; M0P4: Without mycorrhiza + straw; M1P0: inoculation mycorrhiza + without manure; M1P1: inoculation mycorrhiza + cow manure; M1P2: inoculation mycorrhiza + goat manure; M1P3: inoculation mycorrhiza + quail manure; M1P4: inoculation mycorrhiza + straw

Hayman (1975) showed that the use of chemical fertilizers in long term can reduce the population and diversity of AM spores. However, organic fertilizer can increase the diversity of AM spores in the soil as well (Harinikumar et al., 1990).

Warner (1984) also stated that the role of organic matter in the soil is very important for durability and proliferation of fungi in the soil AM. The AM colonization levels of soybean in Japan are largely influenced by the chemical soil properties (Isobe et al., 2008). The AM colonization levels are positively correlated with the density of mycorrhizal spores, while negatively with soil phosphorus available. The AM spore density is also assumed to be lower in acid and alkaline soil (Isobe et al., 2007).

Based on the observations of microbial diversity (See Table 4, Table 5, and Table 7) and microbial populations (See Table 6), then correlated with the level of mycorrhiza infection (See Figure 1) could be assumed that mycorrhiza inoculation affects the diversity and microbial populations in the rhizosphere. This was confirmed by Hindumathi and Reddy (2011) that AM populations and microbial populations are influenced by factors such as soil nutrient status, season, soil temperature, pH, water content, organic content and the type of host plant.

In the decomposition process occur interactions among bacteria, actinomycetes and fungi. Fungi are the main actor in the process of organic materials decomposition which is solid and recalcitrant. The bacteria continue to degrade organic materials into simple and easy form to dissolve which is already initiated by fungi and become nutrients that are ready to be absorbed by plants (Van der Wal et al., 2007). While actinomycetes have specialties in polymer degradation, such as solid cellulose into simple compounds such as sugars. Fungi will rapidly degrade organic matter when there is an addition of sugar supply in its environment (Kirby, 2005; Van der Wal et al., 2006).

Rhizosphere microbes are critical for the plant sustainability life. Although its scope is very small compared to the whole soil ecosystem but billions of microbes are inhibiting rhizosphere to form a complex community. Each microbe has its own role, it interacts with another to build network and react to the environmental changes. Therefore, maintaining the health and the ecological balance in the rhizosphere is very important to help increasing the productivity of plants and stand, especially on marginal land.

In addition to know the microbial diversity, the study also looked at the effect of treatment in terms of crop yields which are shown in Figure 2 and Figure 3.

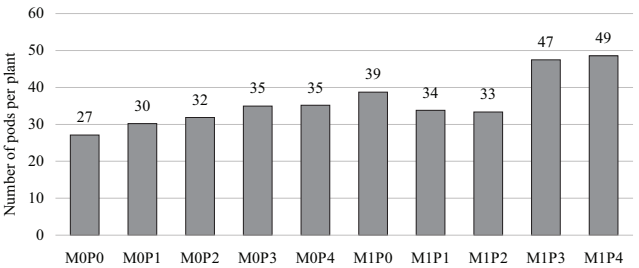



**Fig. 2. The average number of pods per plant**

*Note*: M0P0: Without mycorrhiza + without manure; M0P1: Without mycorrhiza + cow manure; M0P2: Without mycorrhiza + goat manure; M0P3: Without mycorrhiza + quail manure; M0P4: Without mycorrhiza + straw; M1P0: inoculation mycorrhiza + without manure; M1P1: inoculation mycorrhiza + cow manure; M1P2: inoculation mycorrhiza + goat manure; M1P3: inoculation mycorrhiza + quail manure; M1P4: inoculation mycorrhiza + straw

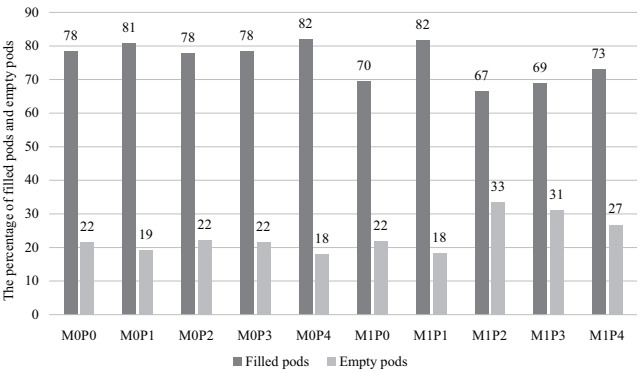



**Fig. 3. The percentage of filled pods and empty pods**

*Note*: M0P0: Without mycorrhiza + without manure; M0P1: Without mycorrhiza + cow manure; M0P2: Without mycorrhiza + goat manure; M0P3: Without mycorrhiza + quail manure; M0P4: Without mycorrhiza + straw; M1P0: inoculation mycorrhiza + without manure; M1P1: inoculation mycorrhiza + cow manure; M1P2: inoculation mycorrhiza + goat manure; M1P3: inoculation mycorrhiza + quail manure; M1P4: inoculation mycorrhiza + straw

The test results showed that the treatments have no significant effect to the total number of pods per plant; the percentage of filled pods; and the percentage of empty pods. The treatment of inoculation mycorrhiza + straw gave the highest number of pods per plant (See Figure 2). While the treatment without mycorrhiza + straw gave the highest result in the percentage of filled pods (See Figure 3). The treatment of inoculation mycorrhiza + goat manure resulted the highest percentage of empty pods (See Figure 2) but with no significant result. The mycorrhiza inoculation treatments showed higher results than the treatment without mycorrhiza.

Parameter of yields components are the number of pods per plant; the percentage of empty pods; and the percentage of filled pods. Figure 2 shows the number of pods per plant is good enough, but in Figure 3 the percentage of empty pods is high above 15% especially in the treatment of inoculation mycorrhiza + goat manure that reached 33%. This is presumably due to the influence of environmental factors when soybeans entered the generative phase.

Charging pods require maximum sunlight and enough water, but when too much water came into pods when filling process, it would be disrupted. The less radiation during pod filling will reduce the number and weight of pods and will increase the number of empty pods.

Mycorrhizal inoculation provides benefits that increase yields. Research conducted by Hindumathi and Reddy (2011) states that increasing spore density and root colonization with increase in age of the crop plant offers the possibility of using AM fungi as a potential biofertilizer for enhancement of crop growth as well as productivity.

In this study, treatment with mycorrhizal inoculation have higher yields than treatments without mycorrhizal, but nevertheless the percentage of empty pods is quite high as due to environmental factors. The other research conducted that not all the pods that form filled by seed, it can be caused by a variety of disorders including unfavorable climatic conditions on the generative phase (flowering) and the presence of pests and diseases. The provision of mycorrhiza on soybean helps improve nutrient uptake that impact on soybean growth and yield improvements. So clearly the function of mycorrhiza can improve soybean yield.

## Conclusions

Although the F test results are not significant, but there are differences between mycorrhiza and without mycorrhiza inoculation treatments. The treatments of mycorrhiza inoculation resulted higher microbial diversity than the treatments without mycorrhiza inoculation. The number of bacteria in all samples was 22 isolates, whereas the fungi were 12 isolates from four genera *Aspergillus sp, Penicillium sp, Rhizopus sp*, and *Mucor sp*. The treatments of mycorrhiza inoculation tend to provide higher yields than treatments without mycorrhiza inoculation. However, a fairly high percentage of empty pods are caused by environmental factors.

***Acknowledgements***

The authors are grateful to the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia that have funded this research through the scheme of Masterplan Percepatan dan Perluasan Pembangunan Ekonomi Indonesia (MP3EI) for fiscal year 2015.

## References

**Aira, M., M. Gómez-Brandón, C. Lazcano, E. Bååth and J. Domínguez,** 2010. Plant genotype strongly modifies the structure and growth of maize rhizosphere microbial communities. *Soil Biol Biochem*. **42**: 2276-2281.

**Ali, B., A. N. Sabri, K. Ljung, and S. Hasnain,** 2009. Auxin production by plant associated bacteria: impact on endogenous IAA content and growth of *Triticum aestivum* L. *Lett in Appl Microbiol*. **48**: 542-547.

**Asghari, H. R. and T. R. Cavagnaro,** 2012. Arbuscular mycorrhizas reduce nitrogen loss via leaching. *PLoS ONE*. **7**(1): e29825.

**Bais, H. P., T. L. Weir, L. G. Perry, S. Gilroy, and J. M. Vivanco,** 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu Rev Plant Biol*. **57**: 233-266.

**Barbier, E. B.,** 1989. Sustaining agriculture on marginal land. *Environment*. **31**: 12-40.

**Bouffaud, M. L., M. Kyselková, B. Gouesnard, G. Grundmann, D. Muller, and Y. Moënne-Loccoz,** 2012. Is diversification history of maize influencing selection of soil bacteria by roots?. *Mol Ecol*. **21**(1): 195-206.

**Buée, M., W. de Boer, F. Martin, L. van Overbeek, and E. Jurkevitch,** 2009. The rhizosphere zoo: an overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and of some of their structuring factors. *Plant and Soil*. **321**(1): 189-212.

**Bulgarelli, D., M. Rott, K. Schlaeppi, E. V. L. van Themaat, N. Ahmadinejad, F. Assenza, P. Rauf, B. Huettel, R. Reinhardt, E. Schmelzer, J. Peplies, F. O. Gloeckner, R. Amann, T. Eickhorst, and P. Schulze-Lefert,** 2012. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature*. **488**(7409): 91-95.

**Curl, E. and T. Bryan,** 1985. The Rhizosphere. Springer-Verlag. Berlin Heidelberg New York, Tokyo. 290 pp.

**Eisenhauer, N., S. König, A. C. W. Sabais, C. Renker, F. Buscot, and S. Scheu,** 2009. Impacts of earthworms and arbuscular mycorrhizal fungi (*Glomus intraradices*) on plant performance are not interrelated. *Soil Biol & Biochem*. **41**(3): 561-567.

**Feddermann, N., R. Finlay, T. Boller, and M. Elfstrand,** 2010. Functional diversity in arbuscular mycorrhiza – the role of gene

expression, phosphorous nutrition and symbiotic efficiency. *Fungal Ecol*. **3**: 1-8.

**Fischer, G., E. Hizsnyik, S. Prieler, M. Shah, and H. T. van Velthuizen,** 2009. Biofuels and food security: implication of an accelerated biofuels production. Summary of the OFID study prepared by IIASA, Vienna, Austria.

**Food and Agricultural Organization of United Nations (FAO).** 2008. A framework for bioenergy environmental impact analysis. Öko-Institut, Ifeu-Institute, Copernicus Institute financed by the Food and Agriculture Organization (FAO), Rome.

**Fulthorpe, R. R., L. F. W. Roesch, A. Riva, and E. W. Triplett,** 2008. Distantly sampled soils carry few species in common. *The ISME Journal*. **2**: 901-910.

**Golubski, A. J.,** 2011. Dual plant host effects on two arbuscular mycorrhizal fungi. *Pedobiologia*. 5**4**(4): 209-216.

**Harinikumar, K. M., D. J. Bagyaraj, and B. Mallesha,** 1990. Effect of intercropping and organic soil amendments on native VA mycorrhizal fungi in an oxisol. *Arid Soil Res. and Rehabil.* **4**(3): 193-197.

**Hart, M. M. and J. A. Forsythe,** 2012. Using arbuscular mycorrhizal fungi to improve the nutrient quality of crops; nutritional benefits in addition to phosphorus. *Sci Hort*. **148**: 206-214.

**Hayman, D. S.,** 1975. The occurrence of mycorrhiza in crops as affected by soil fertility In: F. Sanders, B. Mosse, and P. Tinker. (Eds.). Endomycorrhizas. Academic Press. London, pp. 495-509.

**Hindumathi, A. and B. N. Reddy,** 2011. Occurrence and distribution of arbuscular mycorrhizal fungi and microbial flora in the rhizosphere soils of mungbean [*Vigna radiata* (L.) wilczek] and soybean [G*lycine max* (L.) Merr.] from Adilabad, Nizamabad and Karimnagar districts of Andhra Pradesh state, India. *Adv. in Biosci. Biotechnol.* **2**: 275-286.

**Innerebner, G., C. Knief, and J. A. Vorholt,** 2011. Protection of *Arabidopsis thaliana* against leaf-pathogenic *Pseudomonas syringae* by *Sphingomonas* strains in a controlled model system. *Appl and Environ Microbiol.* **77**(10): 3202-3210.

**Intergovernmental Panel on Climate Change (IPCC),** 2007. Chapter 5: Food, fiber, and forest products. IPCC fourth Assessment Report (AR4) – Climate Change 2007.

**Isobe, K., E. Aizawa, Y. Iguchi, and R. Ishii,** 2007. Distribution of arbuscular mycorrhizal fungi in upland field soil of Japan 1. Relationship between spore density and the soil environmental factor. *Plant Prod Sci.* **10**(1): 122-128.

**Isobe, K., H. Sugimura, T. Maeshima, and R. Ishii,** 2008. Distribution of arbuscular mycorrhizal fungi in upland field soil of Japan 2. Spore density of arbusclar mycorrhizal fungi and infection ratio in soybean and maize fields. *Plant Prod Sci*. **11**(2): 171-177.

**Jie, W., X. Liu, and B. Cai,** 2013. Diversity of rhizosphere soil arbuscular mycorrhizal fungi in various soybean cultivars under different continuous cropping regimes. *PLoS ONE*. **8**(8): e72898.

**Kang, S., W. M. Post, J. A. Nichols, D. Wang, T. O. West, V. Bandaru, and R. C. Izaurralde,** 2013. Marginal lands: concept, assessment and management. *Journal of Agricultural Science*. **5**(5): 129-139.

**Kent, A. D. and E. W. Triplett,** 2002. Microbial communities and their interaction in soil and rhizozphere ecosystem. *Annu. Rev. of Microbiol*. **56**: 211- 236.

**Kielak, A., A. S. Pijl, J. A. van Veen, and G. A. Kowalchuk,** 2009. Phylogenetic diversity of *Acidobacteria* in a former agricultural soil. *The ISME J*. **3**: 378-382.

**Kirby, R.,** 2005. Actinomycetes and lignin degradation. *Adv in Appl Microbiol*. **58**: 125-168.

**Koonin, S. E.,** 2006. Getting serious about biofuels. *Science*. **311**(5760): pp 435.

**Koopman, M. M., D. M. Fuselier, S. Hird, and B. C. Carstens,** 2010. The carnivorous pale pitcher plant harbors diverse, distinct, and time-dependent bacterial communities. *Appl and Environ Microbiol*. **76**(6): 1851-1860.

**Kuske, C. R., L. O. Ticknor, M. E. Miller, J. M. Dunbar, J. A. Davis, S. M. Barns, and J. Belnap,** 2002. Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. *Appl and Environ Microbiol.* **68**(4): 1854-1863.

**Lal R.,** 2008. Soil and world food security. *Soil & Till. Res.* **102**(1): 1-12.

**Lundberg, D. S., S. L. Lebeis, S. H. Paredes, S. Yourstone, J. Gehring, S. Malfatti, J. Tremblay, A. Engelbrektson, V. Kunin, T. G. del Rio, R. C. Edgar, T. Eickhorst, R. E. Ley, P. Hugenholtz, S. G. Tringe, and J. L. Dangl,** 2012. Defining the core *Arabidopsis thaliana* root microbiome. *Nature*. **488**(7409): 86-90.

**Meghvansi, M. K., K. Prasad, D. Harwani, and S. K. Mahna,** 2008. Response of soybean cultivars toward inoculation with three arbuscular mycorrhizal fungi and *Bradyrhizobium japonicum* in the alluvial soil. *Eur J of Soil Biol*. **44**: 316-323.

**Milbrandt, A. and R. P. Overend,** 2009. Assessment of biomass resources from marginal lands in APEC economics. The APEC Energy Working Group under EWG 11/2008A.

**Miransari, M.,** 2011. Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals. *Biotechnol Adv*. **29**: 645-653.

**Mummey, D. L., P. M. Antunes, and M. C. Rillig,** 2009. Arbuscular mycorrhizal fungi pre-inoculant identity determines community composition in roots. *Soil Biol & Biochem*. **41**(6): 1173-1179.

**Namdas, D. D., A. M. Bhosale, and C. J. Khilare,** 2009. Rhizosphere and soil mycoflora of sorghum and tomato growing at Ahmednagar. *Bioinfolet*. **6**(3): 244-245.

**O'Connor, G. A., H. A. Elliott, N. T. Basta, R. K. Bastian, G. M. Pierzynski, R. C. Sims, and J. E. Smith,** 2005. Sustainable land application: an overview. *J Environ Qual*, **34**: 7-17.

**Odum, H. T.,** 1996. Environmental Accounting: Emergy and Environmental Decision Making. John Wiley, New York. 370 p.

**Peiffer, J. A., A. Spor, O. Koren, Z. Jin, S. G. Tringe, J. L. Dangl, E. S. Buckler, and R. E. Ley,** 2013. Diversity and heritability of the maize rhizosphere microbiome under field conditions. *PNAS.* **110**(16): 6548-6553.

**Phillips, J. M. and D. S. Hayman,** 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Soc*. **55**(1): 158-161.

**Porras-Soriano, A., M. L. Soriano-Martin, A. Porras-Piedra, and R. Azcon,** 2009. Arbuscular mycorrhizal fungi increased

growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions. *J of Plant Physiol*. **166**(13): 1350-1359.

**Robertson, G. P., V. H. Dale, O. C. Doering, S. P. Hamburg, J. M. Melillo, M. M. Wander, W. J. Parton, P. R. Adler, J. N. Barney, R. M. Cruse, C. S. Duke, P. M. Fearnside, R. F. Follett, H. K. Gibbs, J. Goldemberg, D. J. Mladenoff, D. Ojima, M. W. Palmer, A. Sharpley, L. Wallace, K. C. Weathers, J. A. Wiens, and W. W. Wilhelm,** 2008. Sustainable biofuels redux. *Science.* **322**(5898): 49-50.

**Searchinger, T., R. Heimlich, R. A. Houghton, F. Dong, A. Elobeid, J. Fabiosa, S. Togoz, D. Hayes, and T. H. Yu,** 2008. Use of U.S. croplands for biofuels increases greenhouse gases through emissions from land use change. *Science*. **319**(5867): 1238-1240.

**Sieverding, E., J. Friedrichsen, W. Suden,** 1991. *Vesicular-Arbuscular Mycorrhiza Management in Tropical Agrosystems*. Schriftenreihe der GTZ, Eschborn, Germany.

**Soepraptohardjo**, 1983. *Survei Kapabilitas Tanah. Lembaga Pusat Penelitian Tanah*. Bogor, Indonesia.

**Tan, F., J. Wang, Z. Chen, Y. Feng, G. Chi, and S. U. Rehman,** 2011. Assessment of the arbuscular mycorrhizal fungal community in roots and rhizosphere soils of Bt corn and their non-Bt isolines. *Soil Biol & Biochem.* **43**: 2473-2479.

**Tian, H., R. A. Drijber, J. L. Zhang, X. L. Li,** 2013. Impact of long-term nitrogen fertilization and rotation with soybean on the diversity and phosphorus metabolism of indigenous arbuscular mycorrhizal fungi within the roots of maize (*Zea mays* L.). *Agric Ecosyst and Environ*. **164**: 53-61.

**Tilman, D., J. Hill, and C. Lehman,** 2006. Carbon-negative biofuels from low-input high-diversity grassland biomass. *Science*. **314**: 1598-1600.

**Tilman, D., R. Socolow, J. A. Foley, J. Hill, E. Larson, L. Lynd, S. Pacala, J. Reilly, T. Searchinger, C. Somerville, and R. Williams,** 2009. Beneficial biofuels – the food, energy, and environment trilemma. *Science.* **325**: 270-271.

**Van der Wal, A., W. de Boer, W. Smant, and J. A. van Veen,** 2007. Initial decay of woody fragments in soil is influenced by size, vertical position, nitrogen availability and soil origin. *Plant Soil*. **301**: 189-201.

**Van der Wal, A., J. A. van Veen, A. S. Pijl, R. C. Summerbell, and W. de Boer,** 2006. Constraints on development of fungal biomass and decomposition processes during restoration of arable sandy soils. *Soil Biol & Biochem.* **38**: 2890-2902.

**Vaz, A. B. M., I. Sampedro, and J. A. Siles, J. A. Vasquez, I. Garcia-Romera, H. Vierheilig, C. A. Rosa, and J. A. Ocampo,** 2012. Arbuscular mycorrhizal colonization of *Sorghum vulgare* in presence of root endophytic fungi of *Myrtus communis*. *Appl Soil Ecol*. **61**: 288-294.

**Vuichard, N., P. Ciais, and A. Wolf,** 2009. Soil carbon sequestration or biofuel production: new land-use opportunities for mitigating climate over abandoned Soviet farmlands. *Environ Sci & Technol*. **43**(22): 8678-8683.

**Warner, A.,** 1984. Colonization of organic matter by vesicular-arbuscular mycorrhizal fungi. *Trans. of the British Mycol. Soc.* **82**(2): 352-354.

**Whiteside, M. D., M. A. Digman, E. Gratton, and K. K. Treseder,** 2012a Organic nitrogen uptake by arbuscular mycorrhizal fungi in a boreal forest. *Soil Biol Biochem*. **55**: 7-13.

**Whiteside, M. D., M. O. Garcia, and K. K. Treseder,** 2012b. Amino acid uptake in arbuscular mycorrhizal plants. *PLoS ONE.* **7**(10): e47643.

**Wiegmann, K., K. J. Hennenberg, and U. R. Fritsche,** 2008. Degraded land and sustainable bioenergy feedstock production. Oko-Institut, Darmstadt Office. Joint International Workshop on High Nature Value Criteria and Potential for Sustainable Use of Degraded Lands, Paris, France.

**Zhang, H., M. S. Kim, Y. Sun, S. E. Dowd, H. Shi, and P. W. Paré,** 2008. Soil bacteria confer plant salt tolerance by tissue-specific regulation of the sodium transporter *HKT1*. *Mol Plant-Microbe Interact*. **21**(6): 737-744.

*Received July, 6, 2017; accepted for printing November, 8, 2017*