Increase production of *arachis pintoi* and *stenotaphrum secundatum* within coconut trees by using of indigenous *arbuscular mycorrhiza fungi* (AMF)

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Abstract. The study was done in order to determine the effect of *Arbuscular mycorrhizal fungi* (AMF) addition in the soil on plant height, fresh weight, dry matter yield and botanical composition of mixed plants of *A. pintoi* legume and *Stenotaphrum secundatum* grass within coconut trees. Completely Randomized Design (CRD) was used in this experiment, with 4 treatments as follows: T0 = 0 gr AMF, T1 = 5 gr AMF, T2 = 10 gr AMF and T3 = 15 gr AMF, 5 replications each treatment. So, there were 20 of experimental units. Measurements were taken after 30 days of planting. The level of AMFs showed a significantly effects (P<0.01) on all parameters among the treatments. It was found that the higher the AMF level, the taller the plant height, the heavier the fresh weight, the higher the dry matter yield and the more proportional the botanical composition of a mixture *A. pintoi* and *S. secundatum*. In short, the highest plant height, fresh weight and dry matter yield as well as the more proportional of botanical composition of *A. pintoi* and *S. secundatum* were found in the soil with application of 15 gr AMF.

1. Introduction
Forage is the largest portion of all feed consumed by ruminants, about 60-80%. Constraints faced in North Sulawesi province - Indonesia, especially in many of the Minahasa regencies, is un-sufficient land for forage development, because fertile land has been used for planting horticultural and agricultural crops, meanwhile the remaining land in general is marginal land, where its fertility is low, so that, when used for forage development the results are both low in quantity and quality. This is reflected in the low productivity of livestock. Furthermore, there is no any pasture in Minahasa regions.

North Sulawesi province is well known as the coconut land because it has a fairly large coconut plantation area. Based on Statistic Bureau Center of North Sulawesi province 2018 data, there was 267,812.35 ha of coconut plantation area in North Sulawesi province. However, in 1 ha sized of land only about 25% is used for planting the coconuts. Plants spacing of coconuts is generally ranged from 7-9 m². So, there are free land or space within the coconut trees which are still possible and quite potential for forage cultivation and development because there are several types of forages that have good tolerance to shade, among others, *A. pintoi* legume and *S. secundatum* grass. Utilization of land under coconut trees by
developing a forage-coconut integration model is an effort to increase land productivity and increase farmers' income [1].

*A. pintoi* was suitable to be developed in areas with low light intensity because it could be tolerant to shade up to 60% [2] and to moderate shade such as within coconut trees [3]. *Stenotaphrum secundatum* was tolerant to shade, with a moderate level of production [4], able to grow on infertile soils with moderate fertility with a pH of 5.0 to 8.5 and also had a good tolerant for high salinity levels. Both of *A. pintoi* legume and *S. secundatum* grass have good nutritional value [5,6]. The plant spacing of coconuts trees can be suitable for *A. pintoi* legume and *S. secundatum* grass. However, the availability of nutrient elements in the soil within coconut trees, can be decreased because those nutrients have been transported or absorbed by coconut trees for growth and development or harvests, so that, efforts is needed to be taken for land reconditioning.

As we know that forage production can be increased by fertilizing and there are inorganic fertilizer suffered in many agriculture shops. However, continuously using inorganic fertilizer was not only expensive but also given a negative impact on the environment, because it can be interfered the symbiosis mutualism of microorganisms in the soil, furthermore the soil was no longer responsive to fertilization and hardened [7]. So, eco-friendly fertilizer should be used in order to get the healthy ecosystem, in this case the spacing within the coconut trees or grazing field for livestock. To increase soil fertility, it is necessary to use alternative technologies that are environmentally friendly by using the *Arbuscular Mycorrhizal Fungi (AMF)* as a biological agent in plant species that can help increase the efficiency of nutrient absorption [8]. According to [9] most plants had a relationship with at least one type of *Mycorrhiza*. One type of *mycorrhiza* fungi that can be utilized in an effort to improve the quality of plant growth and development was *AMF* and it also could be survived on marginal lands [10]. Interaction between *AMF* and plants was an important component in the functioning of ecosystems [11].

*A. pintoi* and *S. secundatum* are types of forage that can be sought to optimize the area within the coconut trees, because they are shade resistant, have good nutritional value. The use of soil microbes such as *AMF* is the most likely to be implemented for these two kinds of forage. However, it is not yet known to what extent the effect of *AMF* on the growth and production of mixed *A. pintoi* and *S. secundatum* under coconut plants. In order to optimize the plant spacing in the coconut plantation area for forage and livestock production development a study was conducted to find out the effect of using *AMF* on height, fresh weight, dry matter yield and botanical composition of mixed *A. pintoi* and *S. secundatum* within coconuts trees.

2. Materials and Methods

This research was carried out within the approximately 75 years old of coconut trees at BALITPALMA (Palms Research station, before Coconut Research Station), in Kayuwatu Manado, North Sulawesi province (Figure 1); from February – July 2018. The materials used were *AMF* and mixed plants of *A. pintoi* legume and *S. secundatum* grass. *AMF* was taken from rhizosphere of *A. pintoi* within coconut trees in Palms Research Board/station (indigenous) by using the filter-pour method [12] followed by the centrifugation method [13] (Figure 2, Table 1). All materials were taken at Palms Research Station in Kayuwatu Manado. Equipment used were O’haus scales capacity of 2610 grams, rulers, a frame 1 x 1 m² sized, electric ovens, scissors, paper bags, and stationery.

Completely Randomized Design (CRD) [14] was used in this research; four treatments as follows: T0 = 0 grams AMF/plot, T1 = 5 grams AMF/plot, T2 = 10 grams AMF/plot and T3 = 15 grams AMF/plot; 5 replications each treatment so that, there were 20 experimental units. Measurements taken were plants height, fresh weight, dry matter yield and botanical composition.

The space within the coconut trees was about 9 mx 9 m = 81 m². The land space under coconut plants was first cleaned, measured for 1.5 x 1.5 m in size and then fenced, four plots within four coconut plants
and there were about 2 m distance from those 4 plots to the coconut trunks (Figure 3). Each plot was 2.25 m$^2$ sized or 11.25 m$^2$ each treatment and so, there were total of 45 m$^2$ spaces within the coconut plants had been used for this experiment.

The soil in all plots were dig of about 20 cm depth and mixed with sands as well as AMF, with the exception plots without AMF added (T0). After all the soil preparation done, plants of *A. pintoi* and *S. secundatum* which already cultivated before in poly beds were cut nearly the same height approximately ± 5 cm above the soil surface, then planted them alternately into the plots with 30 x 30 cm distance with 25 holes each plot (figure 2, kuning = yellow - *A. pintoi* and hijau = green - *S. secundatum*). Irrigation was not applied because it was raining after planting.

The height of *A. pintoi* and *S. secundatum* was measured from the base of those plants grew above the soil surface to the very top. Harvest was done in the same day, soon after the plants height measurement. A frame 1m x 1m sized was used when harvested mixed of *A. pintoi* and *S. secundatum*. All the plants fixed in the frame were cut of about 3 cm from the soil surface, and each kind of plant separated and weighed. After weighing all the plants samples, botanical composition was calculated. All the plants then, dried in the oven with temperature of 60°C until the samples weight become constant and finally weighed them again in order to determine the dry matter yield. Data taken were finally analyzed using Analysis of Variance (ANOVA) by means of MINITAB. F test was used to determine differences among treatment and then Tukey’s test was used to inspect treatment differences among group means.

*Figure 1.* Research location at “BALITPALMA” (Palms Research Board/station).
Figure 2. Few kinds of fungi based on the color, shape, thickness and texture of the wall of [15].

Figure 3. The map of experimental units.

Table 1. Morphological characteristics of *Arbuscular Mycorrhiza Fungi (AMF)* found in the rhizosphere of *A. pintoi* within coconut trees in Palms Research Board/station.

| Morphological Characteristics (100x enlargement) | Reaction with Melzer’s |
|------------------------------------------------|------------------------|
| The brownish-orange-round shape spore. Thick wall with relatively coarse surface look liked orange peel and escaped filter of 125 µm | Reacted with Melzer’s color. The color changed from yellow to orange brown |
| The yellow-round shape spore. Thick walled with relatively coarse surface look liked orange peel and escaped filter of 125 µm | Did not react with Melzer’s color |
| The brownish-yellow, round shape spore. Thick walled with smooth surface and escaped filter of 125 µm | Reacted with Melzer’s color. The inside pores changed to be brown, outside pores changed to be brown |
The brownish-yellow, round shape spore. Thick wall with smooth surface and escaped filter of 125 µm Reacted with Melzers color. The inside pores changed to be reddish-brown, outside pores changed to be yellowish

The yellow-orange, round shape spore. Thick wall with smooth surface and escaped filter of 125 µm Did not react with Melzers color

The yellow, round shape spore. Thick wall with rough surface and escaped filter of 125 µm Did not react with Melzers color

The blackish-brown, round shape spore. Thick wall with smooth surface and escaped filter of 125 µm Did not react with Melzers color

The blackish-brown, round shape spore. Thick wall with rough surface, no hyphal attached and escaped filter of 125 µm Did not react with Melzers color

The brownish-yellow, round shape spore. Thick wall with smooth surface, no hyphal attached and escaped filter of 125 µm Did not react with Melzers color

3. Results and Discussion

3.1. Mixed Plant Height, Fresh Weight and Dry Matter Yield

The experiment had been done in a rainy season in Palm Research Station. In three months mixed of A. pintoi legume and S. secundatum grass were nearly covered the soil, but we waited until the plants fully covered the soil or become so dense. The averages of plants height, fresh weight and dry matter yield of mixed A. pintoi legume and S. secundatum grass treated with different levels of AMF can be seen in table 2.

Table 2. Means of Plant Height (PH), Fresh Weight (FW) and Dry Matter (DM) of mixed A. pintoi and S. secundatum given different level of Arbuscular Mycorrhiza Fungi (AMF) in the coconut plantation area.

| Parameter      | T0 = 0 | T1 = 5 | T2 = 10 | T3 = 15 |
|----------------|--------|--------|---------|---------|
| PH (cm)        | 15.63a | 17.89b | 20.48c  | 22.69d  |
| FW (gr/m²)     | 887.99a| 1,049.56b | 1,121.35c | 1,259.53d |
| Dry Matter (gr/m²) | 170.38a | 199.19b | 202.21b | 227.30c |

Note: Different superscripts in the same row/line showed significant differences (P < 0.01)

As can be seen in table 2, the application of AMF was given asignificant effect (P <0.01) on the plants height of mixed A. pintoi and S. secundatum. It showed that the higher AMF level, the higher mixed plants of A. pintoi and S. secundatum grew. The highest average plants height was 22.69 cm found in mixed of A. pintoi legume and S. secundatum which infected with 15 grams of AMF (T3) and the lowest average plants height was 15.63 cm found in mixed of A. pintoi and S. secundatum without AMF application (T0). After nearly four months, the height of A. pintoi and S. secundatum was measured from the base of those plants grew above the soil surface to the very top. We were measured the height not the length of A. pintoi, even though the A. pintoi quickly grew creeping, it could be long until 50 cm. According to[16], one of the growth characteristics of plants can be seen from the size of plants, liked vertical accretion. Few research had been done to examine the AMF function for plants among others the AMF application until 30 grams to several types of grasses increased their growth and production because the plants ability to absorb the nutrients increased [17]: the application of AMF until 15 grams increased the growth of soybean due to the
absorption of phosphor increased [18]); the use of AMF until 10 grams increased mycorrhizal population in sorghum roots [19]. In addition, roots that had mycorrhiza can absorb nutrients which were not available to plants [20].

This was in line with [21] stated that the growth of the plants with the mycorrhiza were usually better than that of plants without mycorrhiza due to external hyphae able to accumulate and translocate nutrient in the soil [22] stated that AMF effectively increased the absorption of macro nutrients, especially phosphor. Furthermore [15] stated that the increased of plant height or growth can be due to the infected plants by AMF had more endomycorrhizal in the roots and triggered the roots development, as a result the more nutrient can be moved to plants through roots. In addition to AMF roles, [9] stated that AMF symbioses with plants would be significant shown in the marginal land. This experiment run in the coconut plantation area at Palm Research Station and the availability of nutrient elements in the soil within coconut trees could be decreased because had been used for growing and development or during harvest of the coconuts or even used for mixed crops research and so the AMF could be given a significant contribution to growth and development of mixed A. pintoi and S. secundatum. These were showed the important role of mycorrhiza in plant growth because it was able to absorb both macro and micro nutrients.

As can be seen in table 2, the application of AMF was given a significant effect (P <0.01) on the fresh weight of mixed A. pintoi and S. secundatum It showed that the higher AMF level, the higher fresh weight of mixed of A. pintoi and S. secundatum. The highest average fresh weight was 1,259.53 gram/m² found in mixed of A. pintoi and S. secundatum which infected with 15 grams of AMF (T3) and the lowest average fresh weight was 887.99 gram/m² found in mixed of A. pintoi and S. secundatum without AMF application (T0). The explanation was, when the A. pintoi and S. secundatum grew taller all their parts also increased growth and development. So, their shoot was increased as well, as a result the heavier the fresh weight yield. As mentioned before in few reports, when the AMF application level increased the growth and production of several types of grasses increased [17]; the production of sorghum seeds increased also [19]; and the growth rate of soybean also increased [18]. It could be said that, mycorrhiza or AMF were able to increase the roots surface area of A. pintoi and S. secundatum that used to absorb nutrients and also be able to dissolve phosphorus in a form not absorbed by plants to be absorbed or available to plants. So, if the nutrients availability increased, the plants grew and developed well, as a result the fresh weight also increased. Accordingly, [23] stated that the effect of mycorrhiza on plant’s growth showed the mutualistic symbiosis between the roots of plants with fungi in the soil, plants roots effectively absorbed water and nutrients. Moreover, it was often rain during the research, but there were still plenty of sunlight. The season is also one of environmental factors which affected the growth and developments of plants, as well as the forages yields [24].

As can be seen in table 2, the application of AMF had significant effect (P <0.01) on the dry matter of mixed A. pintoi and S. secundatum. It showed that the higher AMF level, the higher dry matter yield. The highest average dry matter yield was 227.30 gram/m² found in mixed of A. pintoi and S. secundatum which infected with 15 grams of AMF (T3) and the lowest average dry matter yield was 170.38 gram/m² found in mixed of A. pintoi and S. secundatum without AMF application (T0). [25] stated that plants which given AMF had higher productivity than plants without AMF.

There was a positive correlation between dry matter yield and fresh weight yield. If, fresh weight increased, the dry matter yield also increased. Dry matter yield of plants can be interpreted as the amount of assimilates (photosynthates) stored in plants part produced from photosynthesis process [26]. [27] reported that the dry matter production of S. secundatum in open conditions was reached 10.9 tons/ha/year, while at a shade level of 50% the dry matter production of S. secundatum was reached 8.8 tons/ha/year. While the dry matter production of A. pintoi could be reached 6-7 tons/ha/year [28]. If, we assumed that, we could cultivate mixed of A. pintoi and S. secundatum which infected with 15 grams of AMF (T3) on 25% of the land spacing in the coconut plantation area and there will be four times defoliation in a year, so,
in one ha sized (4 ha of coconut plantation area) the dry matter production of mixed of *A. pintoi* and *S. secundatum* will be 9,172 kg or about 9.2 tons/ha/year. So, our finding was still better in productivity both in quality and quantity compared to those results above, because the dry matter we got contains of mixed nutrient both from *A. pintoi* legume and *S. secundatum* grass. If, a 300 kg cow needs 3% of dry matter to be consumed, then, it will take 9 kg dry matter per day to be consumed. So, a cow needs about 3,285 kg dry matter per year. Then, our results on dry matter production of mixed of *A. pintoi* and *S. secundatum* can be given to 2 (two) until 3 (three) cows in a year. It could help to overcome the deficiency in forage quality as well as the forage scarcity for cattle development in North Sulawesi province.

3.2. Botanical Composition
The averages of botanical composition of mixed *A. pintoi* legume and *S. secundatum* grass treated with different levels of *AMF* can be seen in the table 3.

| AMF Level (gr) | Average of plants (%) | Weed         |
|---------------|-----------------------|--------------|
|               | *A. pintoi* | *S. secundatum* |               |
| T0 = 0        | 35.49       | 60.56         | 3.95          |
| T1 = 5        | 33.28       | 65.18         | 1.54          |
| T2 = 10       | 36.99       | 62.05         | 0.96          |
| T3 = 15       | 38.75       | 60.75         | 0.5           |

As can be seen on table 3, that the average of botanical composition of mixed *A. pintoi* and *S. secundatum* was ranged between 33.28 + 65.18% until 38.75 + 60.75%. It found in this experiment, that the higher the *AMF* level, the botanical composition was closer to the ideal botanical composition of a mixed crop and also, the lesser the weed. The ideal botanical composition was found in mixed *A. pintoi* and *S. secundatum* which infected with 15 grams of *AMF* (T3) where the *A. pintoi* legume was 38,75% and *S. secundatum* grass was 60,75%. According to [24], the ideal botanical composition for a grazing field was 40% legumes and 60% grasses, while [29] stated that the percentage of legumes in the grazing field supposed to be ranged between 30-40%. In this study, the grass grew dominantly than legumes, even though at the beginning of research, when planted *A. pintoi* and *S. secundatum*, their composition was nearly the same (figure 3). This may be due to *S. secundatum* grass was sprouting, and well developed with rhizomes or stolon that easily formed the roots or additional roots, so that the surface of the soil can be quickly covered by grass. According to [30] that *S. secundatum* grass was a fast-growing plant, had dense rhizomes and stolon, strong roots, strong ability to compete with weeds, so that, it could suppress weed growth and was resistant to heavy grazing. Its height was about 30-50 cm and able to sustain continuous vegetative growth, while *A. pintoi* legume was easily cover the soil by creeping, but the height of *A. pintoi* was lower than *S. secundatum*. Its height was about 10-20 cm. Furthermore, besides of *mycorrhizae*, it is well known that in most of legumes roots live also rhizobium bacteria. This bacteria in *A. pintoi* nodules could be able to supply N free from the air and could then be a good N contributor to *S. secundatum* and the coconut plants as well through *AMF* roles. So, if the nodules increased by *AMF* activity, so it was not only increased P but also N as well. These two kinds of plant could be planted and developed together under coconut plants because they were tolerant to shade properties, and even though *A. pintoi* grew creeping but it did not wrap around the *S. secundatum* and Further through *AMF* application they could have a symbiose mutualism because the production of mixed *A. pintoi* and *S. secundatum* increased with
the increased of AMF level (Table 3). AMF had a symbiotic mutualism with the roots of tall plants, because it improved physical, chemical and biological properties of the soil and environment [20].

4. Conclusion
In short, the highest plant height, fresh weight and dry matter yield as well as the more proportional of botanical composition of A. pintoi and S. secondatum were found in the soil with application of 15 gr AMF.

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