Comparative effects of the combination of biofertilizer, NPK, and mycorrhizal application on maize production system

C Prayogo¹, B Prasetya¹ and N Arfarita²

¹ Soil Science Department, Faculty of Agriculture, University of Brawijaya, Jl.Veteran No.1 Malang, Indonesia
² Faculty of Agriculture, University of Islam Malang, Jl.MT Haryono 193, Malang, Indonesia

Corresponding author: arfarita@unisma.ac.id

Abstract. The use of uncontrolled an-organic fertilizer continuously will degrade soil fertility and nutrients balance. To minimize those impacts, biofertilizers and an-organic fertilizer are needed to maintain a sustainable maize production system. The study used a randomized complete block design with 3 replications with different levels of fertilizer application, conducted at University of Brawijaya experimental research station at Jatimulyo-Malang-East Java. Several parameters were measured to examine those effects on soil and crops. The treatments affect crop height, leaf chlorophyll indices, leaf area indices, maize yields, total number, and mycorrhizal infection. The best treatment was detected under the combination of 100% biofertilizer and 100% NPK, along with the addition of 100% microelements. The lowest was observed under mycorrhizal applications. There was a positive correlation between chlorophyll, crop height, leaf area, and maize yields.

1. Introduction
Maize (Zea mays L.) is one of the important food crops in Indonesia, and they are becoming a strategic commodity with high economic value. Nowadays, it has an important role in supporting the national economy and farmer income, given its multipurpose function, as food, cattle feed, fuel, and other industrial materials [1]. In 2018 imported maize to Indonesia increased by 42.46% to 737.2 thousand tons from 517.5 thousand tons in 2017. Regarding cattle feed production, in 2020, it is estimated that feed production will reach 21.53 million tons or grow about 5% compared to feed production in 2019 (20.5 million tons). The projected maize demand in 2020 for cattle feed mills is reached 8.5 million tons and for farmers 3.48 million tons [2]. Maize has a huge potential to be developed in the tropics and can yield up to 7.5 tons ha⁻¹ if plants are managed well. Unfortunately, maize yields are still generally below 5 tons ha⁻¹ [3]. Inorganic fertilizers strongly influence plant growth, development, and yield [4–6]. Maize can produce optimally under sufficient nutrient requirements, whereas maize cannot produce grains without adequate nutrition [5,7]. Inorganic fertilizers have a strong influence on plant growth, development, and yield [4]. The low yield of maize production is caused by several factors: the improper on crop cultivation and land management and the use of imbalanced fertilizers application [6,7].

The crop production that relies on inorganic fertilizers becoming less effective and efficient resulted in an unfavorable impact on soil conditions due to soil compaction and the disruptions of soil biota community and structure [8]. The declining soil quality was also caused by intensive soil tillage, which
could be decreasing soil organic matter content and reduced availability of microelements such as Fe, Cu, Zn, and Mg to be uptake by crop [9]. Excessive inorganic fertilization and those application are not suitable for maize nutrient needs and can lead to the loss of these nutrients through erosion or leaching [10]. The availability of those above microelements in the soil is needed. Even though they only existed in small quantities, it has an important role in supporting corn plants’ metabolic processes, especially during seed formation and development [11].

Balanced fertilizers between inorganic fertilizers and organic fertilizers in solid or liquid are necessary to provide optimal nutrients uptake. The NPK inorganic fertilizers can support crop macro nutrients requirements and be equipped with microelements [12]. The addition of the NPK 15-15-15 fertilizer in combination with organic fertilizer can stimulate maize growth and production [13]. On the other hand, organic fertilizers in solid or liquid form can contain various living soil organisms that mainly contain bacteria or fungi that can help regulate soil biochemical processes [14,15]. The use of microorganisms in organic fertilizers, better known as biological fertilizers or biofertilizers, can mobilize, facilitate, and increase the availability of soil nutrients [16]. The processes involve the activity of nitrogen fixing bacteria, or even the role of Phosphorus or Potassium solving bacteria, which could increase crop nutrients uptake and protect crop root pathogen infections [17]. Organic fertilizer containing mycorrhizal was able to replace ±50% of phosphate, 40% nitrogen, and 25% potassium [18]. Increasing nutrient uptake is due to the mycorrhizae, which could develop the number of root branches, elongating secondary roots and inducing the formation of quaternary roots, and increasing the number of lateral roots in maize [19]. Biofertilizers containing mycorrhiza could protect the crop from disease infection [20].

Unfortunately, the application of biofertilizer containing either bacteria or mycorrhizal fungi alone is not sufficient for fulfilling the nutrient needs of maize. Therefore, an in-depth evaluation of the impact of the composition of its use on maize cultivation is required. This study aims to see the impact of the combination treatment of biofertilizers under the combinations with NPK fertilizer to meet the need for a balance of maize nutrients to achieve optimum yields. The effect on improving soil quality was also examined in terms of how far those affect population and mycorrhizal infection and evaluated to what extent maize growth performance and its production.

2. Methodology
This research was carried out from June 2020 to August 2021 at the Jatimulyo experimental field, Faculty of Agriculture, Universitas Brawijaya-Malang-East Java. It is located geographically at the coordinates of 7°56'23"S 112°37'01"E, positioned at 500 m above sea level, with an average temperature of 22 to 25°C and an average of rainfall at about 2000 mm per year. Meanwhile, the soil and crop tissue analyses were conducted at Soil Science Laboratory, Faculty of Agriculture-University of Brawijaya. This study was conducted using a randomized block design, consisting of nine treatments with four repetitions. In one replication, the plots used were 4 meter × 3 meter wide, with a 20 cm × 75 cm spacing. There are 36 experimental units in this study (Table 1). The soil was developed and classified as an alluvial deposit from the nearby Brantas river. This site has a slope at about 5%, predominantly used as an agricultural field for cultivating various crops, including maize and rice.

Parameters observed in this study to determine how the effect of different doses of biological fertilizer and NPK fertilizer on the role of mycorrhizae and maize production. The assessment of the impact is carried out using the following parameters in Table 2.

Plant height was measured from the base of the stem on the soil surface to the limit of the last leaf segment of maize. This measurement is done using a ruler. The calculation of the number of leaves is done by counting the completely open leaves. Observation of the number of leaves was carried out simultaneously with plant height measurement until the plant reached the final vegetative phase.
Table 1. The treatment of the combination between organic and an organic fertilizer

| Code | Treatment |
|------|-----------|
| P0   | Control   |
| P1   | 100% NPK  |
| P2   | 100% Biofertilizer |
| P3   | NPK 25% + Biofertilizer 100% |
| P4   | 50% NPK + 100% Biofertilizer |
| P5   | NPK 75% + Biofertilizer 100% |
| P6   | 100% NPK + 100% Biofertilizer + 100% Micro Fertilizer |
| P7   | 50% NPK + 50% Biofertilizer + 50% Micro Fertilizer |
| P8   | Mycorrhizae fertilizer 100% |

Leaf area measurement can be done by picking the leaves sample collected randomly from the upper part of the crop. The leaves are then measured using a Leaf Area Meter (LAM), for large leaves need to be carefully arranged on the tool's surface and when measuring the condition of the leaves not closing and not folding. Leaf chlorophyll content was carried out using the SPAD-502 (Soil Plant Analysis Development) tool. Observation of chlorophyll was focused on the leaves by observing 3 leaves on each sample plant which was then continued by finding the average. The sample plants observed were the same as those used for plant growth observations (plant length, number of leaves). Leaf samples were positioned between two SPAD sensors that had been calibrated beforehand. The results of reading the data on the SPAD tool are then converted into chlorophyll values with units of g cm\(^{-3}\) using the formula \[ \text{Chlorophyll} = \left( \frac{117.1 \times \text{SPAD}_i}{148.84 - \text{SPAD}_i} \right) \]

Table 2. Parameters of maize based on the phase of observation

| Phase of observation | Parameter                  | Time of observation |
|----------------------|----------------------------|---------------------|
| Vegetative phase     | Plant height               | 2, 3, 4, 5, 6, 7, 8 WAP |
|                      | Number of leaves           | 2, 3, 4, 5, 6, 7, 8 WAP |
|                      | Leaf chlorophyll           | 8 WAP               |
|                      | Leaf area                  | 10 WAP              |
| Generative phase     | Cob weight (with husk)     | 14 WAP              |
|                      | Cob weight (without husk)  | 14 WAP              |
|                      | Cob dry weight             | 14 WAP              |
|                      | Cob length                 | 14 WAP              |
|                      | Total soil mycorrhizae spores | 14 WAP          |

Isolation of mycorrhizal fungal spores using a combination method between wet filter pouring technique [22] and the centrifugation technique [23], where the soil sample is weighed 100 g per sample, then dissolved in 1000 ml of distilled water and stirred until homogeneous. Soil samples were filtered through a graded sieve with a size of 2 mm. The remaining soil on a 45 sieve was transferred to a centrifuge tube, and 60% glucose was added in a ratio of 3: 1. The tube was centrifuged at 3000 rpm for 5 minutes. Then the clear liquid was taken and poured into a 45 m sieve, then washed with running water to remove the sugar. After washing, the spores were transferred to a petri dish and counted by a microscope.

\[ \text{Mycorrhizae spore density} = \frac{\text{No of spore}}{\text{Weight of soil being analyzed (g)}} \]

The analysis of the generative phase was carried out by weighing the maize cobs from plant samples, starting from the weight of wet weight maize using an analytical balance. Then peel the maize husks and weigh them again. The purpose of stripping the husks is to get the weight of maize without husks. Then the oven is done to get the dry weight of maize.
Observational data were analyzed by analysis of variance with the F-test at 5% significance level using Genstat 18th edition. If there is a significant effect between treatments, the analysis continues with the Least Significant Difference (LSD) test with a 5% confidential level. To detect each treatment's similarity pattern based on various parameters such as chlorophyll content, cob length, cob fresh and dry weight, including total mycorrhizae spore, we use Multidimensional Scaling (MDS) approaches. MDS is a multivariate statistical analysis that can be used as multiple variables to determine the position of an object based on similarity or dissimilarity. MDS is a data analysis technique that displays it in geometric images based on the similarity or the lack of resemblance, based on the euclidean distance [24]. The use of this analysis has been successfully determining the structure and community of soil biota [25,26].

3. Results and discussion

3.1. Plant height

The average height of maize for each treatment, starting from 2 WAP to 8 WAP, is presented in Table 1. The combination treatment of NPK fertilizer, biofertilizer, and mycorrhizae had a significant effect (P<0.05) on the average crop height. According to the LSD test, it was found that at 2 WAP the mean of maize height of treatment P2 was significantly different (P<0.05) from treatments of P7, P8, and P0, but not it was not significantly different from the treatments of P1, P3, P4, P5, and P6 which was the highest mean value of crop height. The crop height of P2 treatment was 33.3% higher than the maize height of P0 treatment. The average maize height at 3 WAP showed that treatment of P0 was significantly different (P<0.05) from P1 but not significantly different from P2, P3, P4, P5, P6, P7, and P8. The mean of maize height at 4 WAP in all treatments showed no significant difference amongst the treatments. At 5 WAP and 6 WAP, the treatment P0 was seen significantly different (P<0.05) from P2 but not significantly different from P1, P3, P4, P5, P6, P7, and P8. While at 7 MST, it was seen that treatment P0 was significantly different (P<0.05) from P2 but not significantly different from treatment P1, P3, P4, P5, P6, P7, and P8. The mean value of maize height of P6 treatment at 8 WAP was 26.5% higher than P0. P1 obtained the highest maize height at the age of 8 WAP to be about 99.95 cm, followed by the treatments of P5 to approximately 97.35 cm and P5 to about 95.55 cm. The lowest was detected at the treatment of P0 to about 74.80 cm or equivalent to 20% lower than those treatments (Table 3).

Table 3. The effect of combination treatment of biofertilizer, NPK, and mycorrhizal fertilizer to maize height at 2 to 8 WAP

| Treatments | 2 WAP | 3 WAP | 4 WAP | 5 WAP | 6 WAP | 7 WAP | 8 WAP |
|------------|-------|-------|-------|-------|-------|-------|-------|
| P0         | 4.96 a| 10.61 a| 16.30 | 21.95 a| 30.80 a| 47.65 a| 74.80 a|
| P1         | 6.81 bc| 12.07 ab| 17.21 | 22.70 ab| 33.91 ab| 64.79 cd| 99.95 c|
| P2         | 7.41 c| 12.98 b| 18.74 | 25.32 b| 37.00 b| 59.20 bcd| 93.75 bc|
| P3         | 6.79 bc| 11.72 ab| 16.52 | 23.02 ab| 31.69 ab| 55.45 abc| 90.82 abc|
| P4         | 6.37 bc| 11.66 ab| 16.03 | 22.85 ab| 31.54 ab| 53.27 ab| 85.93 abc|
| P5         | 6.74 bc| 12.41 ab| 15.40 | 23.05 ab| 33.62 ab| 59.53 bcd| 97.35 c|
| P6         | 6.72 bc| 11.84 ab| 16.98 | 24.50 ab| 35.58 ab| 65.22 d| 95.55 c|
| P7         | 5.90 ab| 11.13 ab| 16.17 | 22.39 ab| 32.62 ab| 56.92 bcd| 90.12 abc|
| P8         | 4.95 a| 11.47 ab| 18.70 | 23.62 ab| 32.74 ab| 60.92 bcd| 77.47 abc|

Note: P0=Control, P1=NPK 100%, P2=100% Biofertilizer, P3=25% NPK + 100% Biofertilizer, P4=50% NPK + 100% Biofertilizer, P5=75% NPK + 100 Biofertilizer %, P6=100% NPK + 100% Biofertilizer + 100% Micro Fertilizer, and P7=50% NPK + 50% Biofertilizer + 50% Micro Fertilizer, P8= 100% Mycorrhizal Fertilizer. Figures followed by the same notation show that they are not significantly different (P>0.05) according to the LSD test at the 5% confidential level.

Providing inputs to crop in the form of biological fertilizers, NPK fertilizers and mycorrhizae, affects plant growth, especially on improving crop height. The results of this study are in line with NPK fertilizer research [27]. Whereas fertilization N, P, and K have a significant effect on plant height, the height of plants ranges from 162.33 to 175.25 cm. This value is higher than plant height in this study, which reached about 74 to 95 at 8 WAP. In contrast, the treatment of the different type of fertilizer had
no significant effect on the height of plants observed at 15, 30, and 45 HST [28]. Likewise, another study used a dosage of NPK fertilizer at 200, 300, 400 kg ha⁻¹ showed no significant difference between treatments and it did not indicate any significant effect to all parameters being examined, including the height of plants [29]. Biofertilizers applied to plants can uptake nitrogen from the air through free bacteria absorption mechanism, dissolving phosphate, which is bound to soil minerals due to releasing organic acid or enzymatic processes by the microbe, breaking down complex organic compounds into simpler compounds [30]. Microorganisms such as Azotobacter in biological fertilizers can increase nitrogen levels up to 5 times compared to controls [31]. The biofertilizer used in this study also contained this type of bacteria at 2.3 x 10⁷ cfu ml⁻¹. Thus, the combination treatment of biofertilizer, NPK, and mycorrhizal fertilizer is an alternative way to fulfill nutrient demand.

3.2. Number of plant leaf

The results of ANOVA showed that the combination treatment of biofertilizer, NPK, and mycorrhizae had a significant effect (P<0.05) on the number of crop leaves, which can be seen in Table 4. Further analysis using LSD test showed that the number of leaves of 2 WAP was not significantly different from all treatments. At 3 WAP, treatment P8 was significantly different (P<0.05) from P0, P1, P2, P3, P4, P5, P6, and P7. The number of leaves 5 WAP in the P6 treatment was significantly different (P<0.05) from all treatments. Treatment of P3 was not significantly different from P0, P1, P2, P4, P5, P7, and P8 but significantly different (P<0.05) from P6. Almost the same pattern was seen in the previous observation at 4 WAP observations. The number of leaves at 6 WAP in P6 was not significantly different in each treatment. Table 4 shows that the number of leaves at 7 WAP treatment of P0 was significantly different (P<0.05) from P2, P5, P6 but not significantly different from treatments P1, P3, P4, P7, and P8. The treatment of P2 was not significantly different from all treatments but significantly different from treatment P0.

Table 4. The effect of combination treatments of biofertilizer, NPK, and mycorrhizal fertilizer to number of maize leaves at 2 to 8 WAP

| Treatments | 2 WAP | 3 WAP | 4 WAP | 5 WAP | 6 WAP | 7 WAP | 8 WAP |
|------------|-------|-------|-------|-------|-------|-------|-------|
| P0         | 2.90  | 3.8   | 4.10  | 4.80  | 5.35  | 5.90  | 8.20  |
| P1         | 2.75  | 3.75  | 4.20  | 4.60  | 5.60  | 6.40  | 8.15  |
| P2         | 3.05  | 3.75  | 4.20  | 4.65  | 5.50  | 6.60  | 8.40  |
| P3         | 3.15  | 3.70  | 4.80  | 4.45  | 5.35  | 6.25  | 8.25  |
| P4         | 2.80  | 3.70  | 4.30  | 4.70  | 5.35  | 6.55  | 8.25  |
| P5         | 3.30  | 3.85  | 4.35  | 4.70  | 5.50  | 6.65  | 8.25  |
| P6         | 3.05  | 3.70  | 4.35  | 4.95  | 5.40  | 6.70  | 8.85  |
| P7         | 3.10  | 3.95  | 4.35  | 4.60  | 5.50  | 6.20  | 8.1  |
| P8         | 2.50  | 2.95  | 3.95  | 4.65  | 5.35  | 6.25  | 6.65  |

Note: P0=Control, P1=NPK 100%, P2=100% Biofertilizer, P3=25% NPK + 100% Biofertilizer, P4=50% NPK + 100% Biofertilizer, P5=75% NPK + 100 Biofertilizer %, P6=100% NPK + 100% Biofertilizer + 100% Micro Fertilizer, and P7=50% NPK + 50% Biofertilizer + 50% Micro Fertilizer, P8=100% Mycorrhizal Fertilizer. Figures followed by the same notation show that they are not significantly different according to the LSD test at the 5% confidential level.

From Table 4, it can be seen, within the period of 2 to 8 WAP the highest number of crop leaves has been obtained from the treatment of P6 (100% NPK fertilizer + 100% biofertilizer + 100% micro fertilizer). The combination of organic fertilizers combined with inorganic fertilizers can create good soil physical, chemical, and biological properties and increase the efficiency of fertilizers [32]. The increase in the number of leaves is associated with the higher availability of nutrients in the soil [33]. For comparison, the number of leaves at 10 MST from the previous study has an average value of 10 leaves plant⁻¹ which was higher than the result of this study, which reached about 8 leaves plant⁻¹ [28]. [34] also showed that the addition of NPK fertilizer at the level of 25% lower than those recommended doses resulted in a decrease in leaf number and leaf area compared to the standard dosage of NPK.
3.3. Leaf area and chlorophyll content
The average crop leaf area is shown in Figure 1a, which shows that the significant effect (P<0.05) of the combination treatment of organic fertilizer, NPK, and mycorrhizae on the leaf area of maize plants was detected. The treatment of P0 was significantly different from treatment P5 and P6 but not significantly different from the treatment of P1, P2, P3, P4, P7, and P8, while the treatment of P5 was significantly different (P<0.05) from the treatment of P0, P4, P6 and P8, but it was not significantly different from the treatment of P1, P2, P3, and P7. The treatment of P7 was significantly different (P<0.05) from P6 but not significantly different from P0, P1, P2, P3, P4, P5, and P8. The P6 has the highest leaf area, and the P8 treatment was the lowest amongst all treatments at 10 WAP observations. The results showed that crop leaf area at the age of 10 WAP in treatment of P6 gave the highest leaf area yield almost 2 times compared to other treatments, with a value of 5000 cm². This shows that biological fertilizers and NPK fertilizers can increase leaf area to reduce the excessive dose of inorganic NPK fertilizers. Fertilizers containing nitrogen play an active role as one of the most important elements needed for plant growth, such as the number of leaves and leaf area [35]. The leaves area ranged between 2000 to 3000 cm² except for the leaf area of the treatment of P6. This is in line with the previous observation the leaf area ranges between 2156.70 to 3817.99 cm² under standard fertilization of NPK (15:15:15) [34].

The combination treatment of biofertilizers, NPK, and mycorrhizae had a significant (P<0.05) effect on leaf chlorophyll content (Figure 1b). P0 was not significantly different from treatments P2 and P8, but significantly different (P<0.05) from P1, P3, P4, P5, P6, and P7. These treatments had the lowest chlorophyll content among all other treatments. On the contrary, P6 was not significantly different from P7 but significantly different (P<0.05) to P0, P1, P2, P3, P4, P5, and P8. The treatment of P6 was the highest leaf chlorophyll concentration, and P2 had the lowest. Treatment of P7 had the second-highest leaf chlorophyll concentration following P6. P6 treatment resulted in a higher concentration of leaf chlorophyll content to about 46.86 µg cm⁻², which was greater than other treatments. P2 had the lowest chlorophyll concentration value of 26.87 µg cm⁻². Factors that influence chlorophyll formation include the adequacy of light, and macro elements such as NPK and micro elements such as Mg, Fe, which act as formers and catalysts in the synthesis of chlorophyll [36]. Potassium acts as an activator of various enzymes that are essential in the reactions of photosynthesis and respiration and enzymes that play a role in the synthesis of starch and protein. Through photosynthesis, plants obtain energy for plant physiological processes. Organic fertilizer contains nitrogen nutrients that are very useful for plants for growth and development, including making leaves fresher and containing lots of chlorophyll, which plays a vital role in the photosynthesis process and increases soil nutrient content [37].
3.4. Maize cob

Maize production can be calculated from the value of fresh weight of maize cob with husks, fresh weight of maize cobs without husks, and dry weight of maize cob without husks. The combination of biofertilizer, NPK, and mycorrhizae has a significant effect (P<0.05) on those above parameters. Figure 2 is the average result of the fresh weight of cob with and without husk in all treatments. The average fresh weight of maize cob with husk of P0 was significantly different from P6 and P8, but it was not significantly different from P1, P2, P3, P4, P5, and P7. P8 was significantly different (P<0.05) from the P1, P2, P3, P4, P5, P6, and P7, while the treatments of P6 was also significantly different (P<0.05) from the treatment of P8, P0, P2, and P7, but it was not significantly different from the treatment of P1, P3, P4 and P7 (Figure 2a).

The ANOVA showed a significant effect (P<0.05) on the average fresh weight of maize cob without husks. Figure 2b showed that the average fresh weight of cobs without husk in treatment of P0 was significantly different (P<0.05) from P6 and P8, but it was not significantly different from P1, P2, P3, P4, P5, and P7. Treatment of P8 was significantly different (P<0.05) from P1, P2, P3, P4, P5, P6, and P7. Meanwhile, the treatment of P6 was significantly different (P<0.05) from P8, P0, P2 treatments. Treatment of P7 was not significantly different from P1, P3, P4, and P5. Treatment of P8 had the lowest fresh weight of cob without husk (174 g plant⁻¹) compared to other treatments, while treatment of P6 had the highest value, about 412 g plant⁻¹.

The ANOVA showed a significant effect (P<0.05) on the average fresh weight of maize cob without husks. Figure 2b showed that the average fresh weight of cobs without husk in treatment of P0 was significantly different (P<0.05) from P6 and P8, but it was not significantly different from P1, P2, P3, P4, P5, and P7. Treatment of P8 was significantly different (P<0.05) from P1, P2, P3, P4, P5, P6, and P7. Meanwhile, the treatment of P6 was significantly different (P<0.05) from P8, P0, P2 treatments. Treatment of P7 was not significantly different from P1, P3, P4, and P5. Treatment of P8 had the lowest fresh weight of cob without husk (174 g plant⁻¹) compared to other treatments, while treatment of P6 had the highest value, about 412 g plant⁻¹.
The average dry weight value of cobs without husks is shown in Figure 3a. The average dry weight of cobs without husks in the treatment of P0 was significantly different (P<0.05) from P8, but it was not significantly different from P1, P2, P3, P4, P5, P6, and P7. The treatment of P8 was significantly different (P<0.05) to P0, P1, P3, P4, P5, P6, and P7, but it was not significantly different from the treatment of P2. The P8 treatment had the lowest mean dry weight of cobs without husk to about 120 g plant⁻¹ compared to the others, while the treatment of P6 had the highest weight to approximately 225 g per plant⁻¹, in which it was almost 2 times higher than the treatment of P8.

The results of the average measurement of cob length are shown in Figure 3b. The graph shows a significant effect (P<0.05) on the length of the cob in each treatment. The mean cob length in treatment P0 was significantly different from treatments P6 and P8 but not significantly different from treatments P1, P2, P3, P4, P5, and P7. The P8 treatment differed significantly from the P1, P2, P3, P4, P5, P6, and P7 treatments. The P0 treatment was not significantly different from the P2, P6, and P8 treatments, while the P6 treatment differed significantly from the P0, P2, P3, P7, and P8 treatments. Treatment P8 had the lowest mean cob length of 22.05 cm compared to other treatments. In comparison, treatment P6 had the highest value with an average cob length of 31.28 cm. Erselia et al. [34] stated that maize without fertilization or only organic fertilization showed lower cob length and diameter results compared to corn plants with standard NPK fertilizer treatment yielded a cob length to about 17.48 cm which was lower than those of average value of cob length in this study.

![Figure 4. The effect of combination treatment of biofertilizer, NPK, and mycorrhizae fertilizer to maize cob yield.](image)

In terms of fresh weight of cobs with or without husks and dry weight without husks, it had been shown that the treatment of P6 (100% NPK fertilizer + 100% biological fertilizer + 100% micro fertilizer) achieved a higher yield compared to other treatments. The lowest was detected at P8 (100% mycorrhizal fertilizer). NPK fertilization increased maize yield and nutrient uptake of N, P, K [38]. The dose of N resulted in differences in the cob weight with or without husk and cob length [28]. Based on the previous research results of Hawayanti et al. [39], it showed that the effects of the treatment of
biofertilizer + 50% fertilizer N, P, K yielded to about maize production to about 14.54 ton ha\(^{-1}\). The availability of nutrients is related to the seed filling process. Nutrients that are absorbed will be accumulated in the leaves into proteins that form seeds [40]. The accumulation of metabolic products in the formation of seeds will increase so that the seeds formed have a maximum size and weight. This occurs when the nutrient needs are met, which causes the metabolism to run optimally.

The visual maize cob in this study is presented in Figure 4. The best cob in terms of seed filling, length of cob, and their weight were obtained from the treatment of P6.

The graph of the relationship between leaf chlorophyll and corn cob yield is presented in Figure 5a. Leaf chlorophyll has a positive correlation with the dry weight of cobs without corn. The correlation value \(r\) \((P<0.05)\) between chlorophyll and dry weight of cob without shells was 0.57. The relationship between leaf chlorophyll and dry weight of cobs without cob is written with the equation \(y=2.0185x + 50.209\), where \(X\) is the chlorophyll content of the leaves and \(Y\) is the dry weight of the cob without the cob. The value of the coefficient of determination \((R^2)\) obtained is 0.27, which explains that the contribution value of the relationship between leaf chlorophyll and dry weight without husk is 27.32% dry weight of cobs without husk.

The fact that there is a positive relationship between chlorophyll and crop yields in this study was in accordance with previous findings, which stated that the leaf color is an indicator of the plant's nutritional status [40,41]. Plants that are fertile and well-nourished will look green on their leaves and indicate adequate nitrogen (N) content, and vice versa. If the nutrient content is adequately met, the plant productivity will also be higher [42]. According to the research of Putri et al. [43], it was shown that the chlorophyll value obtained from measuring the SPAD value in plants had a close relationship with plant health which was determined based on the yield of crop production.

The elements N and P are continuously absorbed by maize until they are near maturity, while K is mainly needed when silking. Most N and P are carried to the growing points, stems, leaves, and male flowers, where they are transferred to seeds. Adinata [5] stated that the available fertilizers, especially nitrogen fertilizers, will enhance the vegetative growth of plants. Plants that lack nitrogen face obstacles in the formation of green leaves for photosynthesis. Lacking nitrogen content disrupts the formation of carbohydrates that supply energy and the developing formation of cells. The plant growth becomes less, resulting in yellow plants and slow growth. The response of plants to N also depends on the supply of other nutrients. An increase in the dose of N fertilizer which is not accompanied by an increase in the dose of P and K, causes the non-optimal absorption of nutrients due to an imbalance between N, P, and K fertilizers [44].

Maize during the vegetative phase requires more nitrogen (N). Plants need this element to form their vegetative parts, especially in stems, roots, and leaves. Nitrogen during its generative growth is necessary for the formation of maize cobs [45]. The statement reinforces that nitrogen is the main nutrient in providing plant nutrition and is the main component in chlorophyll, protoplasm, and protein.
Nitrogen plays a role in many physiological processes, especially the vegetative growth phase, giving the leaves green. However, too much nitrogen can inhibit flowering and fruiting and even invite pests and diseases [46]. The use of mycorrhizae to improve crop production have been reported in previous research by the mechanism of enhancing crop nutrient uptake [6,25].

3.5. Mycorrhizae
The treatment significantly affects the total number of mycorrhizal spores (spores 100 g⁻¹), as shown in Figure 5b. The results of the LSD test indicated that the treatment of P0 was not significantly different from all other treatments, but it was significantly different from P6. P6 treatment was significantly different from P0, P1, P2, P3, P4, P5, but it was not significantly different from P7 and P8. The P6 was the treatment with the highest total soil spores, and the P0 was the lowest. The P8 was the second highest total value of mycorrhizal spores following the treatment of P6.

Figure 5b. showed that the highest total soil spores were found at the treatment of P6 (100% NPK + 100% Biofertilizer + 100% Micro-fertilizer) treatment with a total number of spore to about 50.52 spores 100 g⁻¹. The lowest total soil spore was found at the treatment of P0 (control), which yielded about 22.75 spores 100 g⁻¹. Meanwhile, P8 with an addition of 100% mycorrhizal fertilizer, the total soil spore was calculated at the value of 40.50 spores 100 g⁻¹, the second highest after P6. The combination of the application of biological fertilizers with inorganic fertilizers can increase the total soil mycorrhizal spores. Biofertilizers contain microbes that positively affect plants by increasing soil biological activity to improve crop nutrient uptake [47,48]. The addition of mycorrhizae improved mycorrhizal colonization and spore formation, which were influenced by each host plant root exudate. Another factor that also affects the formation of spores is the growth of the host plant. Population levels and species composition are very diverse influenced by plant characteristics and environmental factors such as temperature, soil pH, soil moisture, phosphorus, and nitrogen content [48]. Information that each ecosystem can contain endomycorrhizae of the same or different types because the diversity and distribution vary greatly due to varying environmental conditions [49]. Previous findings also detected Glomus sp is one of the dominant species in the maize cropping system [50].

This study only identified the mycorrhizae based on their visual characteristics, such as their color or the shape of the mycorrhizal spores. The characteristic spores of mycorrhizae of this study were round, and the color ranged from clear, yellow to light brown to dark brown, and some of the spores found were black. It can be concluded that the spores belong to Glomus sp family. Glomus sp has a fairly high level of adaptation to the environment compared to some spores of other mycorrhizae fungal genera [23,51,52]. The benefit of mycorrhizae to improve various crop yield have been reported from many researchers [53–58]. The results of the isolation and identification of mycorrhizae spores around the roots of maize were dominated by Glomus sp, which have a color mainly to be in yellow with a round shape.

3.6. Multivariate analysis
Multi Dimension Scale approaches presented in Figure 6 showed significantly different (P<0.05) amongst the treatments. The grouping of the treatments had been positioned to be separated from one to other treatments, particularly along with X-axis (MDS Score 1). A circle of confidence intervals indicated this at 95%, which were not overlapping and having a gap in between. Score 1 of MDS attached to the X axis contributed a percentage variation to about 76 %, which is higher than the Y axis of Score 2 MDS, which represents about 14% of the variation. Moreover, P6 was significantly separated from other treatments, positioned in far rights position along with X-axis (Score 1 MDS). The treatment of P0 only overlapped with the treatment of P2, and both were assumed to be similar. In addition to that, the plot of P1, P3, P4, P5, and P7 were separated from the treatment of P0 and P2, and they were on the top of one to each other. In term P8, it was also significantly placed in far up left position along with Score 2 MDS, separated to other treatments. In conclusion, The effects of using the combination of biofertilizers were successfully grouped by CVA multivariate analysis (Figure 6).
Figure 6. The MDS approaches for positioning and clustering the treatments based on various parameters.

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