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RESEARCH ARTICLE



# Effects of six compost products on soil organic carbon and nitrogen levels in alfisol, rice yields, and diazotrophic endophytic populations

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## Abstract
The decrease in nutrient content in the soil occurs because of an imbalance between nutrient intake and loss. This study aimed to evaluate the effect of applying six types of compost products, with different compost materials and bioactivators, on the soil organic carbon (SOC) and total nitrogen (TN) levels in Alfisol, on rice yields, and diazotrophic endophytic populations. The pot experiment was arranged in a Completely Randomized Design with a single factor consisting of 10 levels and 3 replications. The treatments compared control (R0), 6 types of compost (R1-R6), 50% chemical fertilizer (CF) (R7), 100% CF (R8), and a set combination (R9). Rice with a variety of Mentik Wangi was grown and harvested on 118 DAP. The results showed that the treatment R5 (compost C4 of a mixture of leaf litter, cow dung, peanut plant residue, and rock phosphate with bioactivator RMC) gave the highest increases of SOC and TN by 200% and 228.57%, and the highest plant dry weight and total seed weight by 247.55% and 171.16% compared to the control, respectively. Three treatments of R4 (compost C3), R6 (compost C5), and R9 (50% dosage of compost C3 in combination with mycorrhiza, zeolite, Azolla, and rock phosphate) yielded a similar effect at the second-highest levels in increasing SOC, TN, and rice yields. There was no significant effect of treatments on the population of diazotrophic bacterial endophytes in rice leaves. The present study revealed that compost enrichment with an effective bioactivator contributed significantly higher effects on soil fertility, plant growth, and yield compared to 100% chemical fertilizer (R8).

Keywords: bioactivator; chemical fertilizer; enriched compost; leaf bacterial endophytes; set combination.

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## 1. Introduction
Rice is one of the main foods for more than half of the world's population. Rice is cultivated under the widest range of agroecosystems and soil characteristics, including flood and drought-prone environments (Ahmadi et al., 2014), with various soil types, such as Alfisols, Inceptisols, Vertisols, Entisols (Jadhav et al., 2023), Ultisols (Ojobor & Egbuchua, 2020), Oxisols (Nkongolo et al., 2025), Mollisols (Guo et al., 2022), Histosols (Amgain et al., 2022), Andisols (Kamewada & Ooshima, 2024), Aridisols (Wasim et al., 2021), Spodosols (Manickam et al., 2015), Gelisols (Fujii et al., 2019).

Alfisol occupied approximately 10.1 percent of the global ice-free land area (Hatfield et al., 2017) showed high potential for the extension and intensification of rice production. Alfisol are characterized as acid mineral soil with low pH, low organic matter content, low nutrient N and P, and high exchangeable Al, Fe, and Mn (Soil Survey Staff, 2014). To improve rice production, many treatments have been applied to increase the soil fertility of Alfisols, including compost, biochar, zeolite, liming, and chemical fertilizer. According to (Sadegh-Zadeh et al., 2018), the application of compost and biochar of rice straw improved total N, available P concentration, and rice grain yield. Fertilization with liming increased soil pH, dehydrogenase activity (DHA), and bacterial population by 15.7%, 38%, and 145% respectively, than NPK (Vishwanath et al., 2022). (Behera et al., 2020) proved that lime and farmyard manure (FYM) were one of the options for the amelioration of acid soil, as liming resulted in the maximum maize biomass yield, and also the addition of lime and FYM improved soil extractable Zn and maize Zn concentration.

The application of composted organic materials provides substantial agronomic and environmental benefits. Hefner et al. (2024) demonstrated that compost derived from diverse green wastes increased soil bacterial and fungal abundance, potential nitrogen (N) mineralization, and soil enzyme activities compared with untreated soil. Gil-Martínez et al. (2025) reported that compost composed of olive mill waste (alperujo), pruning residues, and manure (2:1:1, 50 t/ha) significantly increased total C, N, P, and K, as well as microbial biomass and diversity, with these improvements becoming more pronounced after 12 months. This treatment also enhanced plant dry biomass by 24% and increased N concentration in plant tissue compared with unamended pots. Gewaily (2019) found that rice straw compost at 5 t/ha significantly increased filled grains per panicle and tended to increase both grain and straw yields compared with rice straw (uncomposted) applied at the same dosage. Sharma & Dhaliwal (2019) observed that rice straw compost (10 t/ha) combined with 60 kg N/ha (urea) produced the highest rice and wheat yields, soil C, and available N–P–K among all treatments. For micronutrients, this treatment performed better than the control but lower than sewage sludge + 60 kg N/ha. Jiao et al. (2021) reported that composting reduced the phytotoxic allelochemicals of *Ageratina adenophora*, resulting in a compost product that significantly enhanced ryegrass seed germination, seedling and plant growth, nutrient uptake, soil nutrient availability, enzyme activities, and microbial biomass and diversity compared with the uncomposted material.

In contrast, incorporating fresh or uncomposted organic materials can have adverse effects. Returning rice straw to flooded soils stimulates reductive reactions, increases soluble $Fe^{2+}$ concentrations, and accelerates soil acidification, thereby inhibiting plant growth (Li et al., 2025). After 35 years of continuous application, rice and maize straws had limited ameliorative effects and were correlated with acidification in the 0 – 10 cm and 10 – 20 cm layers of paddy soils (Dong et al., 2025). C-rich straw incorporation in wet paddy soils reduced cellulase activity and induced N deficiency, redirecting microbial enzyme production from C to N acquisition, as shown by the negative correlation between cellulase and ammonia-oxidase activities (Guo et al., 2025).

Besides effects on soil fertility, plant growth and plant production, the existence of plant endophytes is also important to be observed and analyzed to examine the effect of the application of compost or fertilizer, or an ameliorant. Endophytes are microorganisms that inhabit the inner plant tissues. They can have beneficial (Wu et al., 2021), detrimental (Erlacher et al., 2014), or no apparent/neutral (Hardoim et al., 2015) effects on the host. However, the use of the term 'endophyte' remains controversial, with the definition often being altered to meet the needs of the user (Sikora et al., 2007).

Nitrogen-fixing bacteria are one type of endophytic microbes that have been reported in some previous studies. Shabanamol et al. (2018) found endophytic nitrogen-fixing bacteria isolated from leaf, stem, and root samples of various rice cultivars and their ability to play a role as a biocontrol potential against *Rhizoctonia solani*. Bianco et al. (2021) found endophytic nitrogen-fixing bacteria isolated from African rice (*Oryza glaberrima* L.) with plant growth-promoting activities such as IAA and ethylene production. Cahyani et al. (2024c) successfully isolated nitrogen-fixing endophytic bacteria from leaf and root tissues of rice on YEMA+BTB medium, with multifunctional abilities in phosphate and potassium solubilization. Ma et al. (2021) showed that different long-term fertilization affected the endophytic bacterial community in the wheat endosphere. Although many studies reported the application of compost on Alfisol for rice (Attanayake et al., 2022; Dada et al., 2014) it is still limited information about the comparative effects of various compost products made from different compositions and bio-activators on soil fertility and rice yield on Alfisols. Previously, the composting of five different compost material compositions, four of which used rice straw as the main material, was investigated, with each formulation activated by either aerobic or fermentative bioactivators (Cahyani et al., 2024b). The effects of these five compost products, which differ in both material composition and type of bioactivator, on soil fertility and plant yield still need to be evaluated. Furthermore, including the commonly used rice straw compost produced by local farmers as a comparison is essential for a comprehensive evaluation.

The present study, building upon previous research on compost formulation (Cahyani et al., 2024b), aimed to evaluate the effects of five compost products resulting from that study and one commonly used by local farmers on soil carbon and total nitrogen levels, rice yield, and the abundance of leaf-associated symbiotic and non-symbiotic diazotrophic bacterial endophytes.

## 2. Methodology

### 2.1 Soil sampling

Soil samples for the greenhouse pot experiment were taken from a non-rhizospheric area of Alfisol soil taken from Jumantono, Karanganyar, Central Java, Indonesia (07º37'47" S and 110º56'51" E) in September 2021. Soil samples were taken at the topsoil horizon (0-30 cm), the samples were air-dried, then homogenized, and sieved using a 2 mm mesh sieve. Soil samples were distributed into plastic pots (d = 28 cm, t = 15 cm) with 6 kg of soil per pot. The chemical characteristics of the initial soil samples were pH $H_2O$ 6.45 ± 0.21, total organic carbon (TOC) 0.36%, organic matter (OM) 0.62%, total nitrogen (TN) 0.03%, C/N ratio 12, and available phosphate 0.81 ppm.

### 2.2 Compost used for the experiment

Six compost products were used in the present study. Five of them (designated as C0 to C4) were derived from a previous study and prepared between July and September 2021, with a composting period of 77 days (Cahyani et al., 2024b). The sixth compost product, referred to as C5, was obtained from the "Sri Rejeki II" Farmers Group in Kuto Village, Kerjo District, Karanganyar Regency, Central Java, Indonesia. The composition and the product characteristics of each compost product are shown in Table 1.

### Additional materials for pot experiment treatments

Some additional materials were used for the pot experiment: chemical fertilizers (CF) (Urea, SP36, and KCl), rock phosphate, Azolla, zeolite, and mycorrhizal culture using zeolite media which spores originated from Andisol from Tengaran, Semarang, Indonesia.

### 2.3 Pot experimental design

The pot experiment was carried out from October 2021 to February 2022 at the Green House, Faculty of Agriculture, Sebelas Maret University, Surakarta, Central Java, Indonesia. This study applied a single factor of organic fertilizer treatment (R) using a Completely Randomized Design (CRD) that consisted of 10 levels of treatments with 3 replications. The treatments were R0 = control (no fertilizer), R1 = application compost C0, R2 = application compost C1, R3 = application compost C2, R4 = application compost C3, R5 = application compost C4, R6 = application compost C5, R7 = application 50% chemical fertilizers (CF), R8 = application 100% CF, and R9 = application a set combination (a mixture of 50% compost C3, mycorrhiza, zeolite, Azolla and rock phosphate). The experimental design and research steps are illustrated in Figure 1.

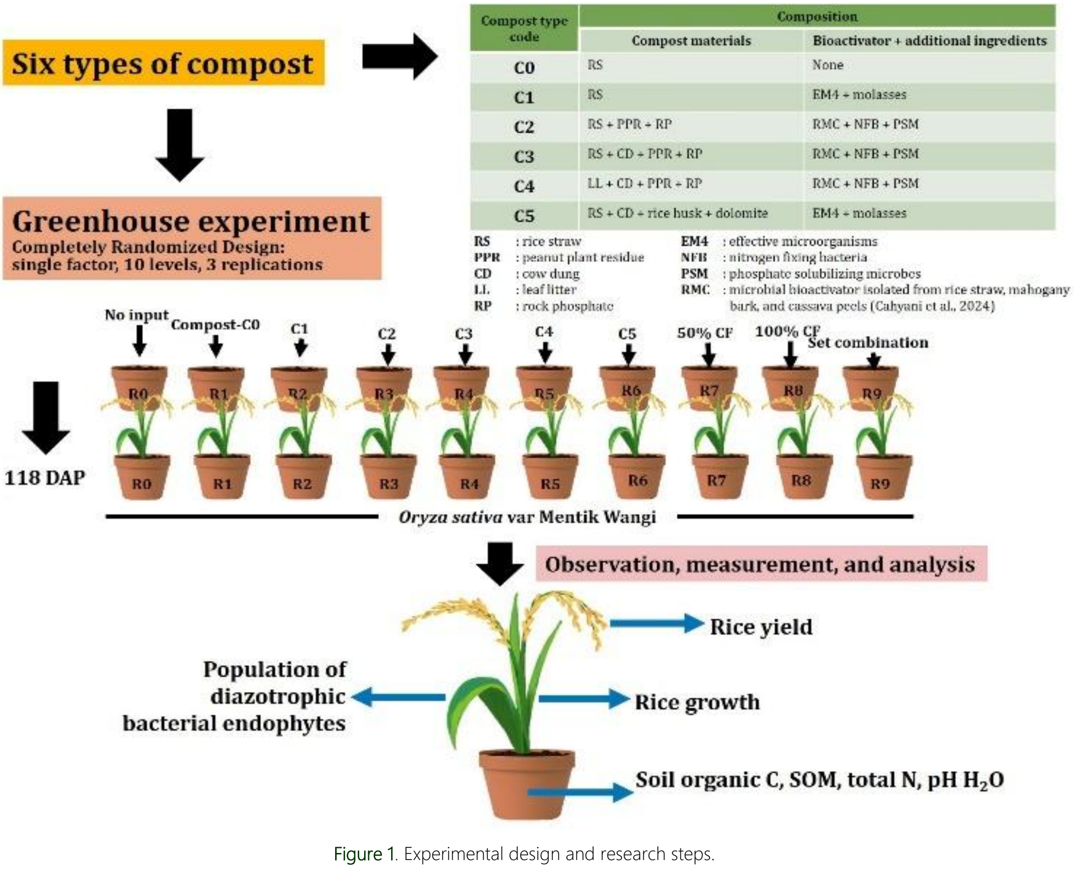


Figure 1. Experimental design and research steps.

Table 1
Characteristics of the 6 types of compost used for the experiment

| Compost Type Code | Composition | | Organic Carbon (OC) (%) | Total N (TN) (%) | C/N Ratio | Total P (mg/100g) | pH ($H_2O$) |
|---|---|---|---|---|---|---|---|
| | Compost materials | Bioactivator + additional ingredients | | | | | |
| C0 | RS | None | 40.25±0.69 | 1.34±0.02 | 30±0.09 | 0.79±0.02 | 7.45±0.28 |
| C1 | RS | EM4 + Molasses | 38.00±1.02 | 1.52±0.04 | 25±0.07 | 0.93±0.02 | 7.60±0.23 |
| C2 | RS + PPR + RP | RMC+NFB+PSM | 32.92±0.78 | 1.61±0.01 | 20±0.03 | 0.94±0.03 | 7.62±0.27 |
| C3 | RS + CD + PPR + RP | RMC+NFB+PSM | 34.18±0.77 | 1.66±0.03 | 21±0.06 | 1.90±0.06 | 7.32±0.23 |
| C4 | LL + CD + PPR + RP | RMC+NFB+PSM | 32.37±1.58 | 1.67±0.03 | 19±0.08 | 1.67±0.03 | 7.30±0.31 |
| C5 | RS + CD + Rice Husk + Dolomite + | EM4 + Molasses | 37.20±1.33 | 1.55±0.02 | 24±0.06 | 1.42±0.02 | 7.50±0.25 |

Remarks: RS = Rice Straw, LL= Leaf Litter, CD = Cow Dung, PPR = Peanut Plant Residue, RP = Rock Phosphate, EM4=Effective microorganism 4 (a commercial bioactivator product), RMC=microbial bioactivator isolated from rice straw, mahogany bark, and cassava peels (Cahyani et al., 2024b), NFB = Nitrogen Fixing Bacteria, PSM = Phosphate Solubilizing Microbes.

## 2.4 Application of organic and chemical fertilizer treatments

Pot experiments were prepared and treated according to their respective code of treatment. Compost application for each treatment of R1, R2, R3, R4, R5, and R6 was at a dosage of 40 t/ha, respectively, except for treatment R9 was given at a dosage of 20 t/ha. The treatment of R9 was also supplemented by the mycorrhizal inoculum at a dosage of 10g per plant, zeolite at a dosage of 10 t/ha (30 g/pot), rock phosphate at a dose of 75 kg/ha (0.225 g/pot), and fresh Azolla at a dose of 5 t/ha (15 g/pot). After compost application, the soil was incubated for 3 days. The treatment of R8 applied 100% CF with the recommended doses from the Indonesian Ministry of Agriculture: Urea 200 kg/ha, SP36 100 kg/ha, and KCl 100 kg/ha. The treatment of R7 applied 50% CF consists of urea 100 kg/ha, SP3650 kg/ha, and KCl 50 kg/ha. Chemical fertilizers were given one day before planting. During incubation, the soil was moistened at field capacity.

## 2.5 Preparation of rice seedlings, mycorrhizal inoculation, and planting

Rice seeds with a variety of Mentik Wangi were soaked in a saltwater solution for the pithy test then rinsed in clean water and soaked for 24 hours to stimulate germination. Seeds were sown on the seedling tray using Alfisol soil as media at a depth of 3 cm with an addition of a layer of mycorrhizal inoculum (Cahyani et al., 2024a). After 2 weeks, the rice seedling was planted in the experimental pots with 2 seedlings in each pot. Standing water was maintained at around 3 cm depth for 3 months until maximal vegetative and then dried to the level around 70-80% field capacity. Harvesting rice was done after the rice was fully ripe, 118 days after planting (DAP).

## 2.6 Observations, measurements, and analyses of plant growth and yield, populations of diazotrophic bacterial endophytes, and soil characteristics

The plant growth parameters were observed at the vegetative maximal phase on 45 days after planting (DAP), including plant height (cm), total tillers per pot, chlorophyll content, and N concentration in plant tissue. A small piece of fresh rice leaf was cut (around 1 g) for chlorophyll analysis, and some was dried and ground for N tissue analysis. Chlorophyll content in the maximum vegetative phase of rice plants was analyzed using the acetone extraction method (Chand et al., 2022). N concentration in plant tissue was analyzed using the Basal Ashing method with $H_2SO_4$ (Yadav et al., 2022). In addition, at the vegetative maximal phase, the population density of diazotrophic (Nitrogen-fixing) bacterial endophytes in rice leaves was analyzed after surface sterilization (Cahyani et al., 2024c) using the plate count method on Yeast Extract Mannitol Agar (YEMA) medium for symbiotic bacteria and Jensen medium for non-symbiotic bacteria (Aroh & Udensi, 2021).

At the generative phase, the number of panicles per clump and the panicle length (cm) were observed. Rice plants were harvested on 118 DAP. The rice shoots and roots were weighed for the plant fresh weight, then air-dried and oven-dried for further observation of the plant dry weight, the number of seeds per panicle, and the weight of 100 grains (g) was calculated.

The soil characteristics after harvest time were analyzed for soil organic carbon (SOC), soil organic matter (SOM), Total N (TN), and pH $H_2O$. Total organic C analysis was treated with the Walkley and Black method (Indonesia Soil Research Institute 2023). Total N analysis was conducted with the Kjeldahl method (Indonesia Soil Research Institute

2023). Soil pH was measured using a digital pH meter (soil:solution = 1:2.5) (Indonesia Soil Research Institute 2023).

### 2.7 Data analysis

The data obtained were analyzed statistically for Analysis of Variance (ANOVA). If the results of the F test for the treatment in variance show a significant difference, then to find out the best treatment, the test is continued with the average difference test using Duncan Multiple Range Test (DMRT) at a 5% significance level. The correlations among variables were tested using Pearson correlation analysis. Statistical analysis was performed using IBM SPSS Statistics 25.

## 3. Results and discussion

### 3.1 The effects of treatment on soil chemical properties

The treatment of fertilizer types yielded a statistically significant effect on the SOC, SOM, and Total N ($p < 0.01$), however, resulted in no significant effect on pH $H_2O$ and C/N ratio ($p > 0.05$) (Table 2).
The treatments of compost application (R1-R6) significantly increased SOC and SOM compared to the control (R0). The highest SOC and SOM content among all compost application treatments (R1-R6) were indicated by the treatments R4 and R5, with the increase of SOC by 180% - 200%, and the increase of SOM by 176% - 201% compared to the control (R0), respectively. The treatment of R7 (50% chemical fertilizer), showed no significant effect on SOC content and SOM content compared with the control (R0). Statistical analysis showed that the treatments of R2, R3, R6, and R8 gave the same level of increase of SOC and SOM to the range 1.1 – 1.2 and 1.84 – 2.05, respectively. The treatment of R9 as the Set Combination (mixture of 50% compost C3 (RS+CD with bioactivator RMC), mycorrhiza, zeolite, Azolla, and rock phosphate) showed the same level effect on the SOC and SOM compared to R4 and R5. Thus, the treatment of R9 proved that using a 50% dosage of compost (20 t/ha) with a combination of mycorrhiza, zeolite, Azolla, and rock phosphate resulted in a similar effect with a 100% dosage of compost in the treatment R4 and R5. The present study proved that the addition of compost contributed significantly to the increase of SOC and SOM. The measurement of SOC and SOM after harvest time was considered to cover the total carbon in soil not only from the input of organic matter but also from the soil biomass from the effect of plant growth and soil microbial activities.
Morra et al. (2021) reported that the application of biowaste compost amendment at 30 t/ha on a dry matter basis during the first three years, and 15 t/ha of dry matter from the fourth year onward on vegetables and fruits, increased SOC by 38.90% after seven years compared to the control. This demonstrated that an intensive and appropriate plant management system could increase the amount of stable organic matter (Morra et al., 2021). This statement was supported by Xie et al. (2022), who stated that the addition of tomato plant residue compost at a dose of 15,000 kg/h on Burmese ginger increased SOM by 17.34% and TN by 18.75% compared to the control. Byeon et al. (2023) reported that application of rice straw compost 30 t/ha with NPK increased SOC by 121% and SOM by 116%. Compared to those three studies by Byeon et al. (2023), by Morra et al. (2021), and by Xie et al. (2022) the present study resulted in the higher increase of SOC and SOM by the treatment of compost application of rice straw based materials, which might be affected by several factors such as the differences of soil type and soil characteristics and the period of application until the measurement of soil variables. It is important to note in Byeon et al. (2023) research that the treatment of combination of NPK and compost 30 t/ha increased the SOM level from the initial level of 1.9 to 3.9.

Table 2
The effect of fertilizer type on soil chemical properties after harvest

| Treatment Code | pH $H_20$ | SOC (%) | SOM (%) | TN (%) | C/N Ratio |
|---|---|---|---|---|---|
| R0 | 6.55 ± 0.33 | 0.5 ± 0.22 a | 0.87 ± 0.37 a | 0.07 ± 0.03 a | 7.3 ± 1.8 |
| R1 | 6.69 ± 0.26 | 0.8 ± 0.15 ab | 1.30 ± 0.27 ab | 0.09 ± 0.02 a | 8.8 ± 2.9 |
| R2 | 6.75 ± 0.19 | 1.1 ± 0.30 bc | 1.90 ± 0.51 bc | 0.13 ± 0.06 ab | 10.8 ± 1.7 |
| R3 | 6.86 ± 0.10 | 1.1 ± 0.09 bc | 1.93 ± 0.16 bc | 0.13 ± 0.05 ab | 10.2 ± 2.1 |
| R4 | 6.66 ± 0.05 | 1.4 ± 0.33 c | 2.40 ± 0.58 c | 0.12 ± 0.04 ab | 12.0 ± 1.9 |
| R5 | 6.53 ± 0.70 | 1.5 ± 0.36 c | 2.62 ± 0.62 c | 0.23 ± 0.03 c | 6.7 ± 3.1 |
| R6 | 6.73 ± 0.05 | 1.2 ± 0.14 bc | 2.05 ± 0.23 bc | 0.14 ± 0.02 ab | 9.2 ± 2.5 |
| R7 | 6.65 ± 0.25 | 0.6 ± 0.11 a | 1.11 ± 0.19 a | 0.10 ± 0.05 a | 7.9 ± 3.0 |
| R8 | 6.37 ± 0.33 | 1.1 ± 0.29 bc | 1.84 ± 0.50 bc | 0.11 ± 0.04 a | 11.5 ± 2.3 |
| R9 | 6.55 ± 0.16 | 1.3 ± 0.18 c | 2.20 ± 0.31 c | 0.18 ± 0.02 bc | 7.0 ± 2.1 |
| ANOVA | ns | ** | ** | ** | ns |

Remarks: ** = p<0.01; * = p < 0.05; ns = non-significant = p > 0.05. Means with the same letter (s) in the same column are not significantly different at 5% level of DMRT. SOC=soil organic carbon, SOM=soil organic matter, TN=total nitrogen.

In the present study, the increase in carbon was quite high, but the highest carbon level only reached a level of 1.5, which was lower than Byeon's research that reached 3.9. In addition, Meena et al. (2016), reported the effect of the application of rice straw compost (14 t/ha) in the second year resulted in an increase in SOC to the levels 4.0 and 4.1 g/kg, whereas, the application of municipal waste compost (16 t/ha) resulted to the level 4.4 and 4.5 g/kg after mustard and pearl millet harvest, respectively.

In the treatments of compost application (R1-R6), the TN content increased significantly compared to the control by the range of 0.07% - 0.23%, with the highest TN content indicated by R5 at 0.23%. The treatment of R5 which used compost C4 (LL + CD + RMC + PP + NFB + PSM + RF) indicated the highest TN and the lowest C/N ratio compared to the other compost product (Table 1). This treatment proved a consistent quality and gave the highest effectiveness in increasing TN of Alfisol in the present study. Although the chemical fertilizer treatment (R7-R8) showed a higher level of TN content compared with R0 and R1; however, the statistical DMRT analysis indicated the same level as the R0 and R1. The TN content of the set combination treatment (R9) was statistically lower than R5 but higher than the R2, R3, R4, and R6 treatments.

In the other study, Meena et al. (2016) applied compost of the mixture of rice straw and fresh cow dung with *Trichoderma viride* inoculation at the dosage of 14 t/ha increased TN of 11.5% and 39.7% compared to the control on sandy loam soil after the harvest of mustard and pearl millet. The result of the treatment R4 in the present study showed a higher increase of TN by 71.4% compared to the control (R0). Thus, compared with the study of Meena et al. (2016), the higher increase in TN in the present study was considered mainly by the higher dosage of applied compost (40 t/ha).

### 3.2 The effects of treatment on rice growth and yield

ANOVA showed a significant effect of the treatments of the application of a variety of compost, chemical fertilizers, and set-combination on plant height (PH) ($p < 0.01$), chlorophyll content ($p < 0.05$), leaf N concentration ($p < 0.01$), plant fresh weight (PFW) and plant dry weight (PDW) ($p < 0.05$), number of panicles (NoP) ($p < 0.01$), number of seeds (NoS) ($p < 0.05$), 100-seed weight ($p < 0.01$), total seed weight ($p < 0.01$), and but showed no significant effect ($p > 0.05$) on number of rice tillers (RT), panicle length (PL), (Tables 3 and 4).

Tables 3 and 4 show that the control treatment (R0) exhibited the lowest level of plant growth and yield, indicating that all applications of various compost products (R1-R6), chemical fertilizers (R7-R8), and a set combination (R9) resulted in the enhancement of rice growth and yield. Comparing the 3 treatments of R1, R2, and R3, which consisted of rice straw as the main compost material, R3 showed the highest effect on plant growth, as significantly indicated by leaf N concentration, PFW, and PDW. In the meanwhile, comparing the treatments of R3, R4, and R5, which used different compositions of materials with the same bioactivator RMC and the same additional supplement of peanut plant residue, nitrogen-fixing bacteria, phosphate-solubilizing microorganism, and rock phosphate, it was found that R5 showed the highest effect on PFW and PDW, followed by R4 and then R3. This fact was supported by the significantly higher levels of chlorophyll content and leaf N concentration yielded by the treatments R5 and R4 compared to R3 (Table 3). This finding revealed that among these 3 treatments, which used the same bioactivator of RMC and the same additional supplement, the treatment R5, which applied compost C4 consisting of leaf litter and cow dung, gave a higher effect on plant growth compared to the compost that consisted of RS only (R3) and RS with CD (R4).

Table 3
Effects of the application of fertilizers on rice growth

| | PH (cm) | RT | Chlorophyll content (mg/g) | Leaf N Concentration (%) | PFW (g) | PDW (g) |
|---|---|---|---|---|---|---|
| R0 | 67.83±2.93a | 9.67±0.58 | 0.26 ± 0.02a | 2.21 ± 0.19a | 12.10±1.16a | 3.68±0.21a |
| R1 | 80.17±7.08b | 11.67±2.89 | 0.28±0.09ab | 3.22 ± 0.38ab | 30.01±0.07b | 7.27±0.21b |
| R2 | 81.50±2.60b | 13.67±1.53 | 0.37±0.17abc | 4.08 ± 0.85bc | 30.87±0.07bc | 7.86±0.06bc |
| R3 | 83.50±6.87b | 14.67±2.08 | 0.41±0.05bc | 4.53 ± 0.65cd | 33.83±2.95c | 10.07±0.49d |
| R4 | 88.00±3.61b | 15.33±2.31 | 0.44 ± 0.06c | 6.04 ± 0.29e | 43.72±2.49e | 11.30±0.41e |
| R5 | 88.83±2.75b | 16.67±2.00 | 0.44 ± 0.07c | 6.55 ± 0.52e | 54.98±0.42f | 12.79±1.06f |
| R6 | 84.33±3.21b | 15.00±2.08 | 0.43 ± 0.02c | 5.65 ± 1.24de | 40.17±3.08de | 10.97±0.71e |
| R7 | 79.83±2.93b | 13.67±2.47 | 0.28 ±0.02ab | 3.33 ± 0.60ab | 31.11±0.14bc | 8.15±0.32c |
| R8 | 85.00±5.00b | 14.67±3.06 | 0.42 ±0.06bc | 4.73 ± 0.60cd | 37.31±3.02d | 10.02±0.15d |
| R9 | 87.00±6.08b | 15.33±1.00 | 0.43 ± 0.05c | 5.91 ± 0.41e | 40.43±2.22de | 11.27±0.1e |
| ANOVA | ** | ns | * | * | ** | ** |

Remarks: ** = $p < 0.01$; * = $p < 0.05$; ns = non-significant = $p > 0.05$. Means with the same letter (s) in the same column are not significantly different at 5% level of DMRT. PH= plant height, RT= rice tiller, PFW= plant fresh weight, and PDW= plant dry weight.

Table 3 shows that the 3 treatments of R4 (compost C3), R6 (compost C5), and R9 (a set combination) demonstrated similar effects on PFW and PDW at the second-highest levels after R5 (compost C4). Kausar et al. (2014) reported that compost made from rice straw and chicken manure, inoculated with ligninolytic and cellulolytic microbes and applied at 15 Mg/ha, increased PDW by 350% compared with 150% for commercial rice straw compost, relative to the control. The present study showed similar results, in which treatment R4 (application of compost C3 [RS + CD + PPR + RP with bioactivator RMC]) increased PDW by 207.1%, compared with 113.6% for treatment R2, relative to the control. The greater PDW increase reported by Kausar et al. (2014) compared with the present study might be due to the application of basal chemical fertilizers (40 kg/ha urea, 60 kg/ha TSP, and 30 kg/ha MP) before planting.

Among all the treatments, R5 yielded the highest effect on plant growth. The treatment R5 (application of compost C4) significantly increased plant height by 30.96%, plant fresh weight (PFW) by 354.38%, plant dry weight (PDW) by 247.55%, compared to the control (Table 3). The combination of leaf litter, cow dung, peanut plant residue, and rock phosphate as the main compost materials with bioactivator RMC and the supplemented ingredients of nitrogen-fixing bacteria and phosphate-solubilizing microbes resulted in the high quality of compost product (C4) (Cahyani et al., 2024b). The present evaluation in the greenhouse assessment confirmed that the application of compost C4 in the treatment R5 was more effective in promoting rice growth compared to other compost treatments. Similar trends were observed in the study by Omara et al. (2022), who reported that in the 2021 season, rice plants irrigated every 9 days and treated with foliar spray of compost tea at a dose of 140 L $ha^{-1}$ in combination with PGPR (*Pseudomonas koreensis* + *Bacillus coagulans*, 1:1 ratio) increased plant height by 8.11% and the number of panicles by 31.33%, 1000-grain weight 11.74%, straw yield 33.75%, and grain yield 7.01% compared to the control. On all irrigation treatments, the treatment T4 (combination of compost tea and PGPR) resulted in the highest population of total count bacteria.

The treatment of R9 (combination of application of compost C3 (50% dosage), mycorrhiza, zeolite, azolla, and rock phosphate) showed an increase in plant height by 28.26%, chlorophyll content 65.38%, leaf N concentration 167.42%, and PDW 206.25%, compared to the control (Table 3). Research by Seleiman et al. (2022) demonstrated that the combination of 50% NPK and 50% Azolla compost (treatment T6) during the 2020 growing season increased plant height by 33.58%, the number of panicles by 77.11%, and dry matter production by 147.82% compared to the control. The treatment T6 yielded the highest values of panicle weight (4.15 g), 1000-grain weight (30.94 g), and grain yield (11.32 ton/ha) compared to all other treatments. Thus, the study by Seleiman et al. (2022) reported similar trends of the effects of a mixture of compost tea with PGPR in increasing plant growth and yield, with the results of the present study.

The significant effects of the treatments on leaf N concentration and PDW indicated significant and positive correlations with SOC ($r = 0.678$, $p < 0.01$; $r = 0.522$, $p < 0.01$), SOM ($r = 0.678$, $p < 0.01$; $r = 0.522$, $p < 0.01$), and TN in soil ($r = 6.77$, $p < 0.01$; $r = 0.640$, $p < 0.01$). Thus, the increasing SOC, SOM, and TN in soil or increasing soil fertility status by the application of the fertilizer treatments significantly affected plant growth, as indicated by the higher plant biomass and N-nutrient content compared to the untreated pot.

Table 4
Effects of the application of fertilizers on rice yield

| Treatments Code | NoP | PL (cm) | NoS | 100-Seed Weight (g) | Total Seed Weight (g) |
|---|---|---|---|---|---|
| R0 | 2±0.00a | 21.67±0.38 | 173±6.0a | 1.13±0.02a | 8.67±0.02a |
| R1 | 3±0.57b | 23.04±2.55 | 183±7.0a | 2.11±0.1b | 11.99±0.11b |
| R2 | 3±0.57b | 24.08±1.57 | 261±17.0bc | 2.35±0.03c | 13.51±0.13c |
| R3 | 4±1.00b | 24.25±0.66 | 263±16.0bc | 2.47±0.02d | 15.59±0.23d |
| R4 | 4±0.57b | 25.06±0.82 | 306±11.0de | 3.03±0.02i | 22.67±0.03i |
| R5 | 4±0.57b | 25.89±1.64 | 330±13.0e | 3.15±0.03j | 23.51±0.01j |
| R6 | 4±0.00b | 24.72±0.62 | 292±24.0d | 2.83±0.03g | 21.06±0.02g |
| R7 | 3±0.57b | 24.02±0.81 | 254±13.0b | 2.56±0.04e | 16.03±0.03e |
| R8 | 4±1.00b | 24.31±1.89 | 287±11.0cd | 2.73±0.02f | 18.15±0.02f |
| R9 | 4±0.57b | 24.79±1.25 | 306±21.0de | 2.93±0.05h | 21.38±0.02h |
| ANOVA | * | ns | ** | ** | ** |

Remarks: ** = $p < 0.01$; * = $p < 0.05$; ns = non-significant = $p > 0.05$. Means with the same letter (s) in the same column are not significantly different at 5% level of DMRT. NoP = number of panicles, PL = panicle length, NoS= number of seeds.

Based on the statistical analysis, there was a significant effect of compost, chemical fertilizer, and set combination applied to the number of panicles (NoP), number of seeds (NoS), total seed weight, and 100-seed weight of rice, but there was no significant effect on panicle length (PL) (Table 4). Among all the treatments, R5 (compost of LL + CD (1:1) with bioactivator RMC at the dosage of 40 t/ha) significantly demonstrated the highest rice yield. Anwar et al. (2017) reported that the application of co-composted cow manure with poplar leaf litter (1:1) with the dosage of 20 t/ha enhanced spinach biomass by 62% in sandy loam soil and 39% in silt loam soil compared to the respective soil control (soil applied with manure composted without plant litter). Compared with the report by Anwar et al. (2017), the present study indicated that the treatment R5 had higher effects as demonstrated by an increase of rice PDW by 247% compared to the R0 (no compost application), and by 13.2% compared to R4 (compost of RS + CD with bioactivator RMC).

Table 4 showed the effect of the application of fertilizers, in which the treatment R5 gave the highest effect on plant yield, as indicated by an increase in number of seeds (NoS) (90.75%), 100-seed weight (178.76%), and total seed weight (171.16%), compared to the control, respectively.

The effect of treatment with 100% chemical fertilizer (R8) significantly showed higher rice growth as indicated by PFW, PDW, chlorophyll content, and leaf N concentration (Table 3), and higher rice yield as indicated by NoS, 100-seed weight, total seed weight (Table 4), than the treatment of 50% chemical fertilizer (R7). The treatment of compost application of R4 (compost C3), R5 (compost C4), R6 (compost C5), and R9 (a set combination) yielded higher rice growth (Table 3: PFW, PDW, chlorophyll content, and leaf N concentration) and rice yield (Table 4: NoS, 100-seed weight, total seed weight) than treatment R8 (100% CF). These findings demonstrated that compost enrichment either in the material compositions which mainly composed of RS or LL with CD (ratio 50:50), in the microbial composition for bioactivator, or in the supplemented ingredients that were applied in producing compost C3, C4, and C5 (Cahyani et al., 2024b) contributed to higher effects in increasing plant growth and yield compared to the treatment 100% CF. In the meanwhile, the effects of compost products which are composed of only RS as the dominant material with different bioactivators (compost C0, C1, and C2 for the treatments R1, R2, and R3) on plant yield were lower compared to the treatments of 100% CF, but lower or similar levels to those treatments for PFW and PDW.

The application of compost yielded higher plant growth than chemical fertilizer as the nutritional supply is more complete than chemical fertilizer, because it also contains compounds that support plant growth, micronutrients (Sharma & Dhaliwal, 2019), and increases soil microbes. Tahiri et al. (2022) reported that treatment of compost (produced from the green waste) could produce good plant growth, shoot dry weight, root dry weight, and yield measured by 160%, 252%, and 176% compared to the control treatment on tomato, respectively. In alignment with study by Phares & Akaba (2022) showed that application of compost (from poultry manure and elephant grass) combined with coconut husk biochar on rice plants for 3 consecutive years gave higher results on rice grain yield than NPK (15-15-15) and urea (46-0-0) chemical fertilizers, but compost alone without biochar still had lower results than chemical fertilizers. The result of the present finding was also supported by Naeem et al. (2017), who proved that the application of CF gave a higher effect than the compost of wheat straw, in detail the application of 60 mg/kg Urea, 30 mg/kg $(NH_4)H_2PO_4$, and 25 mg/kg $K_2SO_4$ resulted in 13.9% and 17.7% the increase of plant height and fresh shoot on maize, whereas, the compost of wheat straw (1.5% w/w on 15kg soil media) application gave an increase of plant height and fresh shoot by 11.5% and 9.2% than control, respectively.

The treatment combination (R9) showed similar effects as treatments R4 and R6, and slightly lower effects than R5, on rice plant growth (Table 3) and plant yield (Table 4). This phenomenon indicated that R9 which applied 50% dosage of compost (consisting of RS and CD with bioactivator RMC, and additional supplement) in combination with Azolla, mycorrhiza, zeolite, and rock phosphate showed that the efficiency of organic input (50% dosage) resulted in similar effect closely with the application of 100% dosage of high quality of compost (R4, R5, and R6) by combining with biofertilizer (Azolla and mycorrhiza) and slow release mineral fertilizer (zeolite and rock phosphate). The treatment R9 increased plant height by 28.26%, PDW by 206.25%, and 100-seed weight by 159.29% compared to the control. The present results showed a similar trend compared to the study combining the same proportion of chemical fertilizer and organic fertilizer by Sultana et al. (2021) who reported that the combination of 50% chemical fertilizer with 50% municipal solid waste, 20% mustard oil cake, and 30% sugarcane press mud increased plant height by 38.51%, tillers by 56.63%, panicle length by 26.17%, grains per panicle by 42.71%, and 1000-grain weight by 17.67% compared to the control.

Hindersah et al. (2022) showed that compost of a mixture of materials (made from cow manure mixed with sawdust, fodder grass residue, and decayed cajuput (*Melaleuca cajuputi*) leaves with biofertilizer (composed of $N_2$-fixing bacteria and P-solubilizing microbes such as *Azotobacter chroococcum*, *Azotobacter vinelandii, Azospirillum* sp., *Acinetobacter* sp., *Pseudomonas* sp., and *Penicillium* sp.) and chemical fertilizer (NPK + urea) treatments gave higher results on root length and plant dry weight compared to the treatment of chemical fertilizer. Moreover, this treatment of combination compost with biofertilizer and chemical fertilizer produced higher yield by 31% and 29.4% compared to chemical fertilizer in transplanting and broadcasting methods, respectively.

Ouyabe et al. (2019) reported that the application of cow manure as a nitrogen source with a dosage of 2000 kg.10a$^{-1}$ resulted in higher N content and N uptake in 2 accession numbers of Water Yam (A-18 and A-133) compared to the application of urea with the dosage of 30 kg.10a$^{-1}$, on the contrary, Water Yam with accession number A-19 showed higher N content and N uptake in the treatment of urea compared to the treatment of cow manure on limestone soil. Thus, the type of the N source, the level of dosage, the type of plant, and also the type of soil/media determine the level of N content and N uptake in plant tissue.

### 3.3 Nitrogen-Fixing Endophytes Population

ANOVA revealed that the treatment of compost, chemical fertilizer, and the set combination had no significant effect on the population of diazotrophic (nitrogen-fixing) bacterial endophytes in the rice leaves. However, the present study found that the population density of nitrogen-fixing endophytic bacteria tended to be higher in the treatment of compost application of R4, R5, R6, and a set combination of R9 compared to control and chemical fertilizer application. Figure 2 shows that the population of symbiotic and non-symbiotic nitrogen-fixing endophytes was in the range of $10^5$ – $10^6$ CFU/g fresh rice leaves. Although there was no significant effect, the treatment R4 (compost of RS+CD with bioactivator RMC) tended to give the highest symbiotic and non-symbiotic nitrogen-fixing endophytic bacteria compared to other treatments (Figure 2).

The application of goat manure liquid organic fertilizer gave higher total endophytic bacteria on the potato and black nightshade compared to the no application treatment. Moreover, the treatment of liquid fertilizer significantly increases the endophytic bacteria on the connected stem of grafted potato and black nightshade compared to the treatment without liquid organic fertilizer. According to Yang et al. (2016), the application of chicken manure and commercial organic fertilizer gave higher total bacteria, total endophytic bacteria, and antibiotic-resistant endophytic bacteria on pakchoi. By using Denaturing Gradient Gel Electrophoresis fingerprint, Wemheuer et al. (2016) reported that fertilizer application significantly altered the endophytic community structure of *L. perenne* and *F. rubra* but not in *D. glomerata*. Compared with the study by Piromyou et al. (2015), the population of endophytic nitrogen-fixing bacteria in rice leaf tissues in the present study was higher than the endophytic bradyrhizobia in the fresh rice roots of three cultivars that fluctuated at the range $10^2$ – $10^5$ log CFU/g among the strains and cultivars during the period of growth.

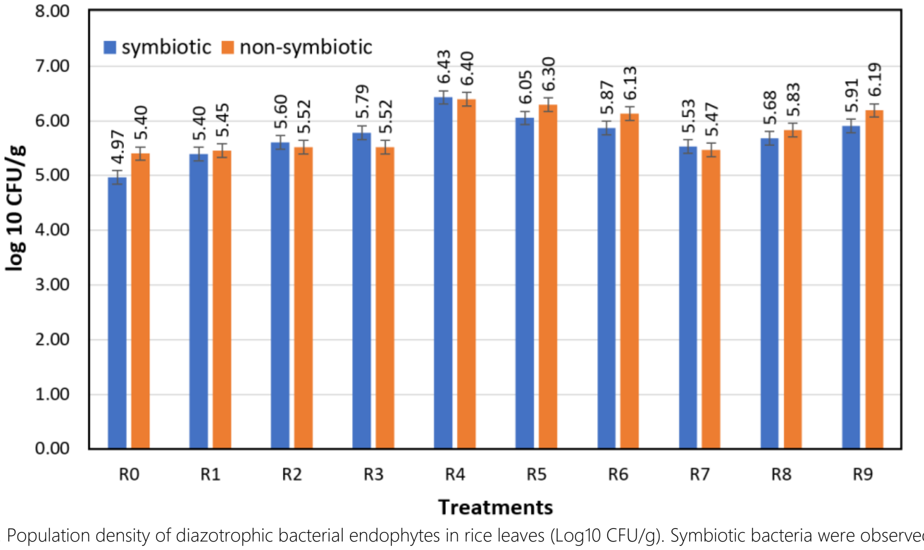


Figure 2. Population density of diazotrophic bacterial endophytes in rice leaves (Log10 CFU/g). Symbiotic bacteria were observed from the grown bacteria on YEMA medium, while non-symbiotic bacteria were observed from Jensen medium.

## 4. Conclusions

The application of 6 compost types increased SOC, SOM, and TN by 20% – 200%, 27.59% – 201.15%, and 28.57% – 228.57% in soil compared to the control, respectively. Overall, the treatment R5 (the application of a compost of a mixture of leaf litter and cow dung with the bioactivator RMC) gave the highest effect to improve soil nutrient status and gave the highest plant growth and yield, followed by the three treatments of R4, R6, and R9, which are at a similar level.

The treatment set-combination of R9 demonstrated the efficient input of compost (50% dosage of compost C3) in combination with mycorrhiza, Azolla, zeolite, and rock phosphate resulted in the high plant growth and plant yield at the same level with treatment R4 (100% dosage of compost C3) and treatment R6 (100% dosage of compost C5). Moreover, four treatments of R4, R6, R9, and R5 yielded higher effects than treatment R8 (100% chemical fertilizer), which indicated by higher SOC 9.09% – 36.36%, TN 9.09% – 109.09%, and higher plant growth and yield as represented by higher PDW 9.48% – 27.64% and total seed weight 16.03% – 29.53%. Although compost treatments showed higher populations of diazotrophic endophytic bacteria than control, statistical analysis revealed that the differences were not statistically significant. Further study is needed to explore the other potential sources for compost enrichment and to evaluate the effects in natural field assessments.


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### Author contribution statement (CRediT)

V. R. Cahyani: Conceptualization, Methodology, Investigation, Supervision, Writing-review and editing, Funding acquisition. R. A. Z. Wakak Megow: Investigation, Data analysis, Data presentation, Writing-review, and editing. A. T. Sakya: Methodology, Data analysis, Supervision, Data presentation, Review, and editing. Sri Hartati: Methodology, Investigation, Data analysis, Review, and editing. S. Minardi: Methodology, Investigation.

### Conflict of interests

The authors declare that they have no conflict of interest.


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