

Article

The Combined Application of Biochar and Arbuscular Mycorrhizal Fungi (AMF) Enhanced the Physical and Chemical Properties of Soil and Rice Productivity in Indonesia

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Abstract: Plant productivity, soil quality, and nitrogen uptake can be increased via the combined application of biochar and arbuscular mycorrhizal fungi (AMF). Here, we evaluate the effects of the combination of four different rates of biochar (B) (B₀: 0 t ha⁻¹, B₁: 20 t ha⁻¹, B₂: 40 t ha⁻¹, and B₃: 60 t ha⁻¹) and four rates of AMF (M) (M₀: 0 g polybag⁻¹, M₁: 15 g polybag⁻¹, M₂: 30 g polybag⁻¹, and M₃: 45 g polybag⁻¹) on the rice (*Oryza sativa* L.) cultivar Trisakti, grown in polybags using a completely randomized design with three replications. Our results show that the combination of 60 t Biochar ha⁻¹ and 45 g AMF polybag⁻¹ (B₃M₃) was the best treatment for improving some parameters, such as soil porosity (with the highest values of 68.25 and 68.45%), BD (0.88 and 0.88 g cm⁻³), pH (6.77 and 6.76), SOM (3.05 and 3.02%), TN (0.48 and 0.47%), AP (31.04 and 31.15 ppm), AK (235.11 and 235.20 ppm), plant height (116.78 and 117 cm), SPAD chlorophyll at maturity stage (43.59 and 43.88), flag leaf area (15.12 and 15.33 cm²), root length (42.10 and 42.17 cm), root volume (53.79 and 53.08 cm³), and shoot dry matter (59.29 and 59.66 g), in the early and late season, respectively. However, the combination of 20 t Biochar ha⁻¹ and 45 g AMF polybag⁻¹ (B₁M₃) was the best treatment for enhancing the tiller number with the maximum values (52.67 and 53.22), flowering day (67 and 66 day), root dry matter (32.37 and 32.51 g), panicle number (34.67 and 35.21), panicle length (21.44 and 21.67 cm), 1000 grain weight (41.26 and 41.37 g), and nitrogen uptake (32.37 and 32.51 g polybag⁻¹), in the early and late season, respectively. These findings indicate that rice growth and productivity, the physical and chemical soil characteristics, and nitrogen uptake were better with the combined application of biochar and AMF treatments than sole biochar, sole AMF, or the control treatments.

Keywords: rice; biochar; AMF fertilizer; nitrogen uptake

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1. Introduction

More than half of the world's population depends on rice (*Oryza sativa* L.) as the primary source of food [1,2]. Indonesia is the largest producer of rice in Southeast Asia and was the fourth-largest producer of rice globally in 2022–2023, with an estimated production of 34.6 million t. Most of Indonesia's rice is produced in West Java (17%), East Java (17%), Central Java (14%), South Sulawesi (6%), and North Sumatra (5%). The amount of rice produced in 2022 was 57.66 thousand tons (0.22%), which is lower than compared to the amount of rice produced from January to September 2021 [3]. Indonesia has imported rice from several nations, including Thailand (13.7%), Pakistan (19.1%), and India (55%), to meet local rice demand [4]. Many factors affected Indonesia's decision to import rice, including population growth, limitations in rice production, rice consumption, gross domestic product, and the area dedicated to rice cultivation [5]. By 2030, rice production will need

to increase by 20% to meet domestic demand due to population growth [6]. On the other hand, the conversion of agricultural land into residential land reached 96,512 ha year⁻¹, which poses a major threat to Indonesia's food security [7].

Creating the ideal growing conditions for each stage of plant growth and development is key to enhancing rice productivity [8]. Previous research has shown that organic materials, such as biochar and arbuscular mycorrhizal fungi (AMF), can improve the physical and chemical characteristics of soil, promote soil microbial activity, and increase plant yield. Several studies have clarified that biochar addition increased plant growth and development. Other studies have indicated that root biomass, root morphology, root nutrient content, and root-associated microbes increase following the application of biochar [9–11]. Biochar addition also increases the availability and uptake of N in the soil, water-holding capacity, pH, and cation exchange capacity, decreases the bulk density (BD) of the soil, increases the abundance of beneficial microbes, and reduces the bioavailability of heavy metals; all of these effects are ultimately related to increases in the rate of plant photosynthesis [12,13].

AMF are an important component of the rhizosphere microflora in natural environments that provide nutrients to plants and promote plant growth [14–18]. AMF can form symbioses with the roots of plants and perform significant ecological and agronomic functions [19]. Moreover, mycorrhizae can promote the nutrient uptake of plants, especially the uptake of P and other macronutrients, such as N and K, hormone and growth regulator production, and resistance to drought, diseases that affect the roots, and heavy metals [20,21]. AMF and biochar have been proven to enhance the properties of soil and provide various agroecosystem services. The physical, chemical, and microbiological aspects of soil, as well as plant development, have been shown to be enhanced via the combined application of AMF and biochar [22–24]. The combined application of biochar and AMF can promote the development of plant roots, the mycorrhizal colonization rate, and extraradical mycelial length, which enhance the absorption of nutrients via the mycorrhizae [25]. Many studies have shown that the addition of biochar to soil promotes AMF root colonization and sporulation [22,23]. Moreover, the characteristics of biochar and other factors (e.g., rate of biochar application and type of biochar applied) contribute to the direct and indirect effects of biochar on soil [26–28].

Although many studies have explained that biochar and AMF improved the physical and chemical properties of soil, and increased nitrogen uptake and rice productivity, most of the studies applied solely biochar or solely AMF. Therefore, we conducted a research to explore the effect of combination of biochar and AMF on the physical and chemical properties of soil, nitrogen uptake, rice growth and productivity. We hypothesized that the combination of biochar and AMF could both enhance the physical and chemical properties of soil, and improve nitrogen uptake, rice growth, and rice productivity. The aim of this study was to determine the effects of a combined treatment with biochar and AMF, versus sole biochar and sole AMF application, on soil properties, the amount of chlorophyll in the leaves, rice growth and productivity, and nitrogen uptake. The results of our study have implications for improving rice production in Indonesia.

2. Materials and Methods

2.1. Experimental Site and Climate

Two experiments were conducted at the Experimental Farm, Senior Vocational School Sultan Daulat, Aceh, Indonesia (02°27'–03°00', 97°44'–98°10') during the early season (June–September) of 2022 and the late season (November–March) of 2023. The average annual precipitation at the tropical experimental site is 2,308 mm. The mean maximum and minimum temperature ranges for the early season are 30.9–32.8 °C and 23.2–24.1 °C, respectively, and the mean maximum and minimum temperature ranges for the late season are 31–33 °C and 23–23.9 °C. There was a total of 1154 mm of rainfall in both the early and late seasons. The early season relative humidity ranged from 61 to 93%, and the late-season relative humidity ranged from 63 to 93% (Table 1). The soil (Ultisol: 0–20 cm) was collected from Pulo Kedep Village; the soil was acidic (pH 5.28), soil BD was 1.32 g cm⁻³,

soil porosity (SP) was 41.76%, soil organic matter (SOM) was 1.52%, the total N (TN) was 0.05%, the available phosphorus (AP) was 21.22 ppm, and the available potassium (AK) was 223.11 ppm.

Table 1. Maximum and minimum temperatures, relative humidity, and total precipitation for both growing seasons.

Month	Temperature (°C)			Humidity (%)			Total Rainfall (mm)
	Max	Min	Average	Max	Min	Average	
June	32.8	24.1	28.45	91	66	78.5	49
July	32.9	23.7	28.3	92	64	78	74
August	33.4	23.7	28.55	91	61	76	171
September	31.7	23.5	27.6	93	69	81	173
October	30.9	23.2	27.05	93	74	83.5	687
November	31.5	23.8	27.65	92	72	82	336
December	31.5	23.9	27.7	92	69	80.5	272
January	31	23	27	93	70	81.5	231
February	32.4	23	27.7	92	62	77	142
March	33	23.5	28.25	90	63	76.5	173
Total Rainfall (mm)							2.308

2.2. Experimental Design and Crop Management

Three replications were performed for each treatment, and the experiment was conducted in a randomized factorial design. The experiments were conducted in 40 cm × 40 cm plastic pots (polybags). Before filling the polybags, topsoil from a depth of 0–20 cm was mixed with biochar; each polybag contained 10 kg of soil. A rapid pyrolysis process using rice husk was used to produce the biochar (temperature 500–550 °C, 20 min). Mycogrow is the name of the AMF fertilizer used in this study, which is produced by PT Agrofarm Nusa Raya, and contains zeolite grains, 33 spores g⁻¹, 300 propagules g⁻¹, five species of Endomycorrhizae, and organic nutrients. AMF fertilizer was applied one day before rice seeding. Drip irrigation was used to hydrate the polybags on a regular basis from transplanting until the rice plants reached physiological maturity. The “Trisakti” rice variety was planted in the early and late seasons using the direct seed rice method. Trisakti is a local rice variety that is suitable for dry land with a plant height of ±90 cm, a harvest time of ±75 days after transplanting, a production of ±7 t ha⁻¹, and resistance to *Pyricularia oryza* and *Nilaparvata lugens* [29]. The treatments consisted of four levels of AMF (M) (0 g, 15 g, 30 g, and 45 g polybag⁻¹) and four biochar (B) levels (0, 20, 40, and 60 t ha⁻¹, corresponding to 0 g, 252 g, 504 g, and 756 g polybag⁻¹, respectively). The biochar rates were determined based on previous research that improved the physical and chemical properties of soil, nitrogen uptake, and rice productivity [30]. Urea fertilizer was applied in three applications, with 360 kg N ha⁻¹ (4.54 g polybag⁻¹) at rates of 50% as a basal dose, 30% at the tillering stage, and 20% at the panicle initiation stage. KCl fertilizer was applied twice, with 240 kg K ha⁻¹ (3.02 g polybag⁻¹) at rates of 50% as a basal dose and 50% at the tillering stage. SP-36 fertilizer was applied at a rate of 240 kg P ha⁻¹ (3.02 g polybag⁻¹) as a basal dose in all treatments. The determination of fertilizer dosage was in accordance with the recommendation of the Indonesian Ministry of Agriculture [31]. Standard agronomic procedures, including the application of herbicides and pesticides, were applied to all the polybags during both seasons. The amount of biochar and AMF applied in the different treatments is presented in (Table 2).

Table 2. Various treatment combinations of biochar and AMF fertilizer applied to each polybag.

Treatments	Mix Ratio
B ₀ M ₀	0 g of Biochar and 0 g of AMF
B ₀ M ₁	0 g of Biochar and 15 g of AMF
B ₀ M ₂	0 g of Biochar and 30 g of AMF
B ₀ M ₃	0 g of Biochar and 45 g of AMF
B ₁ M ₀	252 g of Biochar and 0 g of AMF
B ₁ M ₁	252 g of Biochar and 15 g of AMF
B ₁ M ₂	252 g of Biochar and 30 g of AMF
B ₁ M ₃	252 g of Biochar and 45 g of AMF
B ₂ M ₀	504 g of Biochar and 0 g of AMF
B ₂ M ₁	504 g of Biochar and 15 g of AMF
B ₂ M ₂	504 g of Biochar and 30 g of AMF
B ₂ M ₃	504 g of Biochar and 45 g of AMF
B ₃ M ₀	756 g of Biochar and 0 g of AMF
B ₃ M ₁	756 g of Biochar and 15 g of AMF
B ₃ M ₂	756 g of Biochar and 30 g of AMF
B ₃ M ₃	756 g of Biochar and 45 g of AMF

2.3. Sampling and Analysis

2.3.1. Soil and Biochar

To measure its physical and chemical characteristics, soil was collected before and after the experiment from each treatment in both seasons. The pH (water), soil organic matter (SOM), total nitrogen (TN), available phosphorous (AP), available potassium (AK), and bulk density (BD) were analyzed using Soil Nutrient Analyzer equipment [32]. We used the following formula to determine the SP of the soil [33]:

$$\text{Soil Porosity} = (1 - (\text{Bulk Density} \div \text{Particle Density})) \times 100. \quad (1)$$

The rice husk biochar had the following characteristics: pH, 6.45; total C, 31.08%; TN, 0.06%; TP, 0.24%; total K, 0.07%; organic matter, 71.98%; C/N, 518; ash content, 27.18%; and water content, 4.23%.

2.3.2. Rice Growth

We took measurements of several growth variables at various time points during rice development, including plant height, tiller number, soil plant analysis development (SPAD) chlorophyll, flag leaf area, and flowering day. Plant height was measured using a ruler from the base of the stem to the tallest leaf at 3 weeks after planting to 8 weeks after planting. The tiller number was determined by counting the number of rice plants that emerged from the main plant internode at 3–8 weeks after planting. Measurements of the flag leaf area were taken on as many as four leaves on each plant. The flag leaf area was calculated using the following formula:

$$\text{Flag Leaf Area} = \text{length} \times \text{width} \times \text{constant} (0.7). \quad (2)$$

SPAD chlorophyll values were measured for both seasons using a SPAD meter following the method of Islam et al. [34]. Measurements were taken at three growth stages (tillering, heading, and maturity) in the polybags. The measurement times of the SPAD values were at (1) the tillering stage: 40 days after seeding (DAS) with a temperature of 30.2 °C; (2) heading stage: 75 days after seeding (DAS) with a temperature of 29.7 °C; and (3) maturity stage: 100 days after seeding (DAS) with a temperature of 28.2 °C.

2.3.3. Root Morphology

The root length (RL) was determined by measuring the distance from the base of the stem to the tip of the root using a ruler. To measure the root volume (RV), the roots of the rice plants were removed, cleaned, air-dried, and placed into a 1000 mL measuring cup containing 250 mL of water to determine the change in volume. RV was measured after harvest and was calculated using the following formula:

$$RV = \text{Volume}(2) - \text{Volume}(1). \quad (3)$$

2.3.4. Yield Components

The measurement of the yield components includes the panicle number, panicle length, and 1000-grain weight. The panicle number was determined by counting the number of productive tillers in the rice plants and the panicle length was measured using a ruler. The 1000-grain weight was measured by taking the weight of 1000 grains in each polybag.

2.3.5. Dry Matter and Nitrogen Uptake

The shoot dry weight and root dry weight are two components of dry matter. The roots and shoots were cleaned and dried in an oven at 70 °C for 48 h until a constant weight was achieved. Furthermore, the dried roots and shoots were weighed using an analytical balance [35]. To measure N accumulation, samples (root, stem, and leaves) were taken from each polybag at the maturity stage, then oven dried at 70 °C for 48 h. After that, the dried samples were chopped for the next process. The content of the total N was determined following the micro-Kjeldhal method [36].

2.4. Statistical Analysis

Data were input into Microsoft Excel (2013). All of the data experiments were analyzed using one-way analysis of variance (ANOVA) and two-way analysis of variance (ANOVA) for interaction analysis. SPSS 21 was used to analyze the data and sigma plot 14 software was used for plotting figures. The means of the treatments were compared using the least significant difference test with $p \leq 0.05$.

3. Results

3.1. Physical and Chemical Properties of Soil

Biochar and AMF fertilizer application had significant effects on the physical and chemical characteristics of the soil, including pH (water), soil organic matter (SOM), total nitrogen (TN), available phosphorous (AP), available potassium (AK), bulk density (BD), and soil porosity (SP) (Table 3). Results of the treatments in both seasons were similar; the soil quality attributes were higher for the biochar and AMF fertilizer treatments than for the control. The highest and lowest soil BD values were observed with the B₀M₀ and B₃M₃ treatments in both seasons, respectively. The highest SP value was observed with the B₃M₃ treatment, followed by the B₃M₂, B₂M₃, and B₃M₁ treatments. The lowest SP value was observed with the B₀M₀ treatment, followed by the B₀M₁, B₀M₂, and B₀M₃ (non-biochar) treatments in both seasons. All of the treatments with biochar addition into the soil increased the various physical (SP) and chemical (pH, SOM, TN, AP, and AK) variables of the soil compared to the non-biochar (control) treatments in both seasons. The combination of biochar and AMF (B₃M₃) had the best effect for increasing pH, SOM, TN, AP, AK, and SP, and decreasing the BD of the soil, compared to the other treatments.

Table 3. Variations in soil chemical and physical characteristics under different rates of biochar and AMF application.

Treatments	BD (g cm ⁻³)	SP (%)	pH	SOM (%)	TN (%)	AP (ppm)	AK (ppm)
Before	1.32	41.76	5.28	1.52	0.05	21.22	223.11
Early Season (S1)							
B ₀ M ₀	1.28 ± 0.005 i	41.83 ± 0.005 p	5.12 ± 0.01 l	1.57 ± 0.005 k	0.14 ± 0.005 k	22.10 ± 0.01 p	226.70 ± 0.02 k
B ₀ M ₁	1.22 ± 0.005 h	46.66 ± 0.005 o	5.89 ± 0.01 jk	1.93 ± 0.005 j	0.17 ± 0.005 j	25.20 ± 0.015 m	227.17 ± 0.045 l
B ₀ M ₂	1.20 ± 0.005 h	48.20 ± 0.01 n	5.96 ± 0.01 hijk	1.98 ± 0.005 i	0.19 ± 0.005 ij	25.77 ± 0.005 l	229.21 ± 0.025 h
B ₀ M ₃	1.18 ± 0.005 h	48.90 ± 0.005 m	5.99 ± 0.005 hi	1.99 ± 0.005 i	0.22 ± 0.005 h	26.19 ± 0.015 j	229.80 ± 0.015 g
B ₁ M ₀	1.12 ± 0.01 g	52.17 ± 0.01 l	5.89 ± 0.005 k	2.02 ± 0.01 h	0.21 ± 0.005 hi	22.74 ± 0.025 o	227.07 ± 0.015 j
B ₁ M ₁	1.09 ± 0.005 fg	56.80 ± 0.01 k	5.90 ± 0.01 ijk	2.23 ± 0.005 f	0.30 ± 0.01 f	26.36 ± 0.015 i	229.86 ± 0.01 g
B ₁ M ₂	1.04 ± 0.005 e	58.97 ± 0.01 j	6.10 ± 0.1 fg	2.35 ± 0.01 e	0.34 ± 0.015 e	26.78 ± 0.005 h	231.18 ± 0.02 e
B ₁ M ₃	1.03 ± 0.005 de	62.85 ± 0.02 g	6.14 ± 0.015 ef	2.57 ± 0.01 d	0.39 ± 0.005 d	27.92 ± 0.01 e	235.00 ± 0.02 b
B ₂ M ₀	1.05 ± 0.05 ef	60.87 ± 0.005 i	5.98 ± 0.005 hij	2.10 ± 0.01 g	0.23 ± 0.005 h	24.11 ± 0.02 n	229.19 ± 0.025 h
B ₂ M ₁	0.99 ± 0.005 cd	63.11 ± 0.01 f	6.07 ± 0.01 fg	2.23 ± 0.02 f	0.35 ± 0.01 e	26.88 ± 0.005 g	230.11 ± 0.02 f
B ₂ M ₂	0.98 ± 0.00 c	65.02 ± 0.015 d	6.22 ± 0.01 d	2.60 ± 0.015 d	0.40 ± 0.005 cd	26.93 ± 0.005 f	234.22 ± 0.01 d
B ₂ M ₃	0.96 ± 0.005 bc	66.13 ± 0.025 c	6.22 ± 0.005 de	2.78 ± 0.015 c	0.42 ± 0.005 bc	28.77 ± 0.01 c	235.14 ± 0.02 a
B ₃ M ₀	0.99 ± 0.00 cd	62.16 ± 0.025 h	6.03 ± 0.005 gh	2.34 ± 0.005 e	0.28 ± 0.005 g	26.03 ± 0.01 k	230.10 ± 0.015 f
B ₃ M ₁	0.92 ± 0.005 ab	64.08 ± 0.035 e	6.53 ± 0.005 c	2.79 ± 0.005 c	0.39 ± 0.005 d	28.67 ± 0.005 d	234.21 ± 0.025 d
B ₃ M ₂	0.89 ± 0.005 a	66.79 ± 0.01 b	6.63 ± 0.015 b	2.98 ± 0.005 b	0.44 ± 0.005 b	29.88 ± 0.01 b	234.79 ± 0.02 c
B ₃ M ₃	0.88 ± 0.005 a	68.25 ± 0.01 a	6.77 ± 0.02 a	3.05 ± 0.01 a	0.48 ± 0.005 a	31.04 ± 0.01 a	235.11 ± 0.025 a
Late Season (S2)							
B ₀ M ₀	1.27 ± 0.005 l	41.87 ± 0.01 p	5.15 ± 0.02 k	1.60 ± 0.015 k	0.15 ± 0.005 i	22.19 ± 0.005 o	225.68 ± 0.01 m
B ₀ M ₁	1.23 ± 0.00 kl	45.96 ± 0.01 o	5.74 ± 0.055 j	1.94 ± 0.005 j	0.18 ± 0.005 h	25.25 ± 0.015 l	227.20 ± 0.015 k
B ₀ M ₂	1.21 ± 0.005 jk	48.23 ± 0.01 n	5.83 ± 0.005 i	1.97 ± 0.005 i	0.19 ± 0.00 h	25.83 ± 0.01 k	229.19 ± 0.02 j
B ₀ M ₃	1.18 ± 0.01 j	48.86 ± 0.01 m	5.92 ± 0.01 h	1.98 ± 0.01 i	0.23 ± 0.005 g	26.22 ± 0.01 i	229.82 ± 0.005 h
B ₁ M ₀	1.13 ± 0.005 i	53.13 ± 0.01 l	5.80 ± 0.01 i	2.05 ± 0.01 h	0.21 ± 0.00 g	22.84 ± 0.01 n	227.18 ± 0.01 l
B ₁ M ₁	1.10 ± 0.005 hi	56.83 ± 0.01 k	5.92 ± 0.01 h	2.28 ± 0.01 g	0.33 ± 0.005 e	26.45 ± 0.005 h	229.88 ± 0.01 g
B ₁ M ₂	1.06 ± 0.005 gh	58.89 ± 0.01 j	6.02 ± 0.005 g	2.33 ± 0.005 ef	0.35 ± 0.005 d	26.82 ± 0.01 g	230.94 ± 0.015 d
B ₁ M ₃	1.04 ± 0.005 fg	62.21 ± 0.01 h	6.17 ± 0.015 e	2.57 ± 0.01 d	0.40 ± 0.005 c	27.88 ± 0.01 e	234.92 ± 0.01 b
B ₂ M ₀	1.07 ± 0.05 gh	61.07 ± 0.03 i	6.04 ± 0.015 g	2.08 ± 0.005 h	0.23 ± 0.005 g	24.13 ± 0.015 m	229.20 ± 0.015 i
B ₂ M ₁	1.01 ± 0.01 ef	63.25 ± 0.01 f	6.12 ± 0.01 ef	2.30 ± 0.01 fg	0.36 ± 0.05 d	26.80 ± 0.015 g	230.00 ± 0.015 f
B ₂ M ₂	0.98 ± 0.00 de	65.04 ± 0.015 d	6.24 ± 0.01 d	2.57 ± 0.01 d	0.42 ± 0.01 b	26.87 ± 0.01 f	234.26 ± 0.015 c
B ₂ M ₃	0.95 ± 0.005 cd	65.97 ± 0.05 c	6.24 ± 0.005 d	2.82 ± 0.01 c	0.43 ± 0.01 b	28.83 ± 0.005 c	235.23 ± 0.02 a
B ₃ M ₀	0.99 ± 0.015 cd	62.21 ± 0.01 g	6.08 ± 0.005 fg	2.35 ± 0.01 e	0.29 ± 0.005 f	26.06 ± 0.01 j	230.22 ± 0.025 e
B ₃ M ₁	0.93 ± 0.005 bc	64.53 ± 0.01 e	6.54 ± 0.03 c	2.82 ± 0.01 c	0.40 ± 0.01 c	28.60 ± 0.02 d	234.25 ± 0.025 c
B ₃ M ₂	0.89 ± 0.00 ab	66.81 ± 0.01 b	6.70 ± 0.025 b	2.98 ± 0.005 b	0.43 ± 0.01 b	29.91 ± 0.01 b	234.88 ± 0.015 b
B ₃ M ₃	0.88 ± 0.005 a	68.45 ± 0.005 a	6.76 ± 0.02 a	3.02 ± 0.01 a	0.47 ± 0.005 a	31.15 ± 0.03 a	235.20 ± 0.015 a
B	**	**	**	**	**	**	**
M	ns	**	ns	ns	*	**	**
B × M	**	**	**	*	*	**	**

Note: Bulk density (BD), soil porosity (SP), potential hydrogen (pH), soil organic matter (SOM), total nitrogen (TN), available phosphorous (AP), available potassium (AK). ± indicates the standard error among the replications. B: Biochar; M: Mycorrhizae; B × M: Interaction between Biochar and Mycorrhizae; B₀M₀: 0 t ha⁻¹ of Biochar and 0 g of AMF; B₀M₁: 0 t ha⁻¹ of Biochar and 15 g of AMF; B₀M₂: 0 t ha⁻¹ of Biochar and 30 g of AMF; B₀M₃: 0 t ha⁻¹ of Biochar and 45 g of AMF; B₁M₀: 20 t ha⁻¹ of Biochar and 0 g of AMF; B₁M₁: 20 t ha⁻¹ of Biochar and 15 g of AMF; B₁M₂: 20 t ha⁻¹ of Biochar and 30 g of AMF; B₁M₃: 20 t ha⁻¹ of Biochar and 45 g of AMF; B₂M₀: 40 t ha⁻¹ of Biochar and 0 g of AMF; B₂M₁: 40 t ha⁻¹ of Biochar and 15 g of AMF; B₂M₂: 40 t ha⁻¹ of Biochar and 30 g of AMF; B₂M₃: 40 t ha⁻¹ of Biochar and 45 g of AMF; B₃M₀: 60 t ha⁻¹ of Biochar and 0 g of AMF; B₃M₁: 60 t ha⁻¹ of Biochar and 15 g of AMF; B₃M₂: 60 t ha⁻¹ of Biochar and 30 g of AMF; B₃M₃: 60 t ha⁻¹ of Biochar and 45 g of AMF. Means with similar lowercase letters in the columns are not significantly different ($p > 0.05$) according to Duncan's test. ** indicates the significant difference, $p \geq 0.01$; * indicates $p = 0.01-0.05$; and ns (not significant) indicates $p \geq 0.05$.

3.2. Rice Growth

The effects of biochar and AMF fertilizer application on the weekly growth, including plant height, tiller number, chlorophyll content (SPAD), flag leaf area, and flowering day parameters are shown in Figures 1–5. At week 8 of observation, B₃M₃, B₃M₂, B₃M₁, and B₂M₃ were the best treatments for improving rice plant height, with plant height values of 116.78 cm, 116.56 cm, 116.33 cm, and 116 cm in the early season, and 117 cm, 116.67 cm, 116.56 cm, and 116.44 cm in the late season, respectively. The minimum plant height value was the B₀M₀ (control) treatment over the several weeks of the experiment. In all of the treatments, the tiller number increased until week 7 and then decreased in the maturity stage. The highest tiller number value observed was with the B₁M₃ treatment, with a tiller

number value of 40 at week 7 in both seasons. The minimum tiller number value was the B_0M_0 treatment from weeks 4 to 8. The combination of biochar and AMF fertilizer also had a significant effect on the chlorophyll content (SPAD) in all growth phases during both seasons. The highest chlorophyll content (SPAD) values in the tillering, heading, and maturity stages were observed with the B_3M_3 treatment, with values of 45.74, 47.92, and 43.88, respectively. The lowest chlorophyll content (SPAD) values were observed in the tillering, heading, and maturity stages with the B_0M_0 treatment, with values of 35.63, 37.72, and 31.38, respectively, in both seasons. The maximum flag leaf area value was observed with the B_3M_3 treatment (15.12 cm² and 15.33 cm² in the early and late season, respectively), followed by B_3M_2 (15.03 cm² and 14.95 cm² in the early and late season, respectively). The minimum flag leaf area value was observed with the B_0M_0 treatment (6.85 cm² and 6.88 cm² in the early and late season, respectively), followed by the B_0M_1 , B_0M_2 , and B_0M_3 treatments. The combined application of biochar and AMF fertilizer also had a significant effect on the flowering day. B_3M_3 was the best treatment for stimulating the flowering of the rice plant than other treatments.

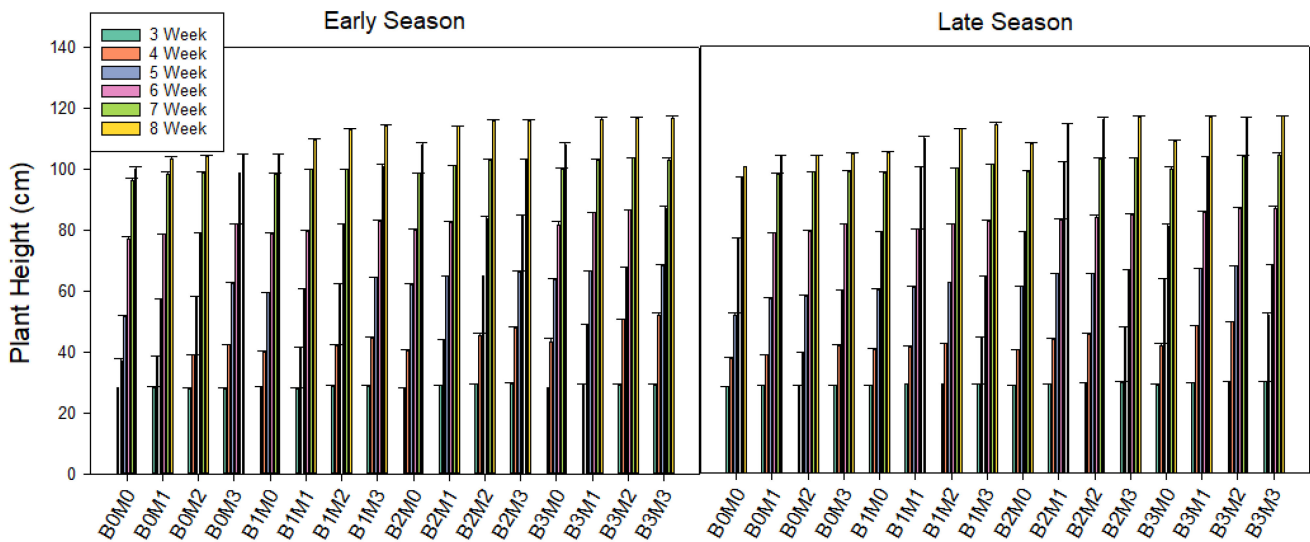


Figure 1. Effects of different rates of biochar and AMF fertilizer application on rice plant height (cm). Note: B_0M_0 : 0 t ha⁻¹ of Biochar and 0 g of AMF; B_0M_1 : 0 t ha⁻¹ of Biochar and 15 g of AMF; B_0M_2 : 0 t ha⁻¹ of Biochar and 30 g of AMF; B_0M_3 : 0 t ha⁻¹ of Biochar and 45 g of AMF; B_1M_0 : 20 t ha⁻¹ of Biochar and 0 g of AMF; B_1M_1 : 20 t ha⁻¹ of Biochar and 15 g of AMF; B_1M_2 : 20 t ha⁻¹ of Biochar and 30 g of AMF; B_1M_3 : 20 t ha⁻¹ of Biochar and 45 g of AMF; B_2M_0 : 40 t ha⁻¹ of Biochar and 0 g of AMF; B_2M_1 : 40 t ha⁻¹ of Biochar and 15 g of AMF; B_2M_2 : 40 t ha⁻¹ of Biochar and 30 g of AMF; B_2M_3 : 40 t ha⁻¹ of Biochar and 45 g of AMF; B_3M_0 : 60 t ha⁻¹ of Biochar and 0 g of AMF; B_3M_1 : 60 t ha⁻¹ of Biochar and 15 g of AMF; B_3M_2 : 60 t ha⁻¹ of Biochar and 30 g of AMF; B_3M_3 : 60 t ha⁻¹ of Biochar and 45 g of AMF. Vertical bars represent the standard error of the mean.

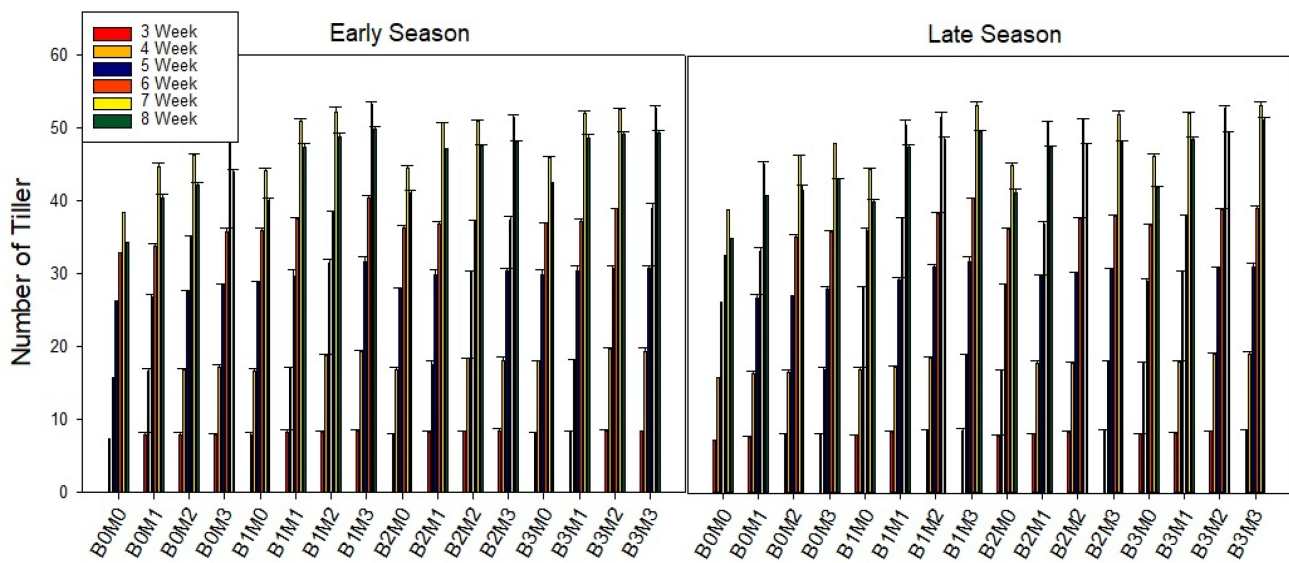


Figure 2. Effects of different rates of biochar and AMF fertilizer application on the number of tillers. Note: B₀M₀: 0 t ha⁻¹ of Biochar and 0 g of AMF; B₀M₁: 0 t ha⁻¹ of Biochar and 15 g of AMF; B₀M₂: 0 t ha⁻¹ of Biochar and 30 g of AMF; B₀M₃: 0 t ha⁻¹ of Biochar and 45 g of AMF; B₁M₀: 20 t ha⁻¹ of Biochar and 0 g of AMF; B₁M₁: 20 t ha⁻¹ of Biochar and 15 g of AMF; B₁M₂: 20 t ha⁻¹ of Biochar and 30 g of AMF; B₁M₃: 20 t ha⁻¹ of Biochar and 45 g of AMF; B₂M₀: 40 t ha⁻¹ of Biochar and 0 g of AMF; B₂M₁: 40 t ha⁻¹ of Biochar and 15 g of AMF; B₂M₂: 40 t ha⁻¹ of Biochar and 30 g of AMF; B₂M₃: 40 t ha⁻¹ of Biochar and 45 g of AMF; B₃M₀: 60 t ha⁻¹ of Biochar and 0 g of AMF; B₃M₁: 60 t ha⁻¹ of Biochar and 15 g of AMF; B₃M₂: 60 t ha⁻¹ of Biochar and 30 g of AMF; B₃M₃: 60 t ha⁻¹ of Biochar and 45 g of AMF. Vertical bars represent the standard error of the mean.

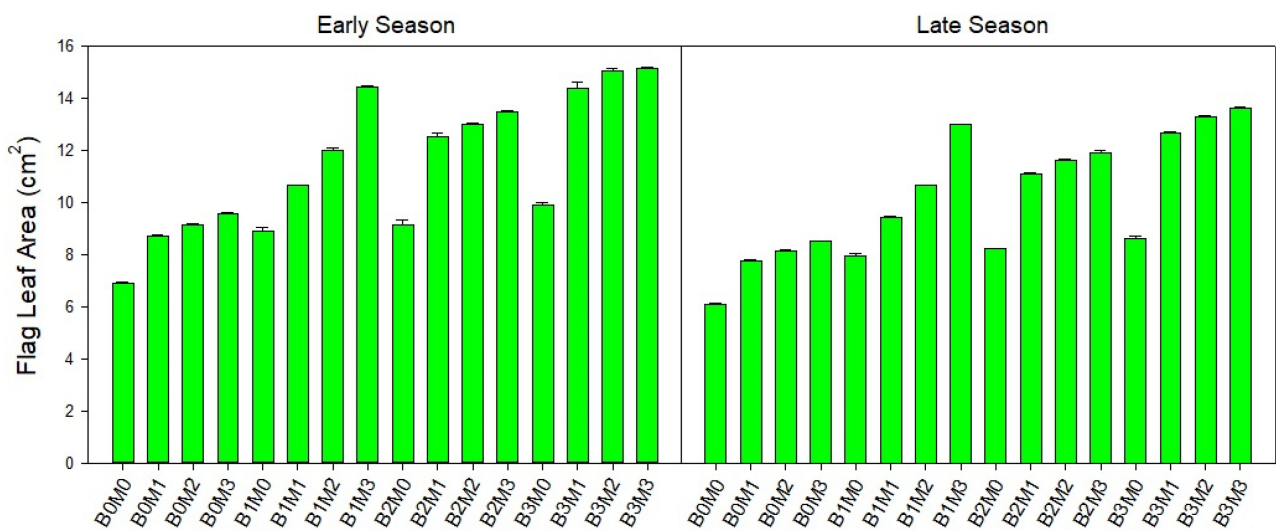


Figure 3. Effects of different rates of biochar and AMF fertilizer application on the rice flag leaf area (cm²). Note: B₀M₀: 0 t ha⁻¹ of Biochar and 0 g of AMF; B₀M₁: 0 t ha⁻¹ of Biochar and 15 g of AMF; B₀M₂: 0 t ha⁻¹ of Biochar and 30 g of AMF; B₀M₃: 0 t ha⁻¹ of Biochar and 45 g of AMF; B₁M₀: 20 t ha⁻¹ of Biochar and 0 g of AMF; B₁M₁: 20 t ha⁻¹ of Biochar and 15 g of AMF; B₁M₂: 20 t ha⁻¹ of Biochar and 30 g of AMF; B₁M₃: 20 t ha⁻¹ of Biochar and 45 g of AMF; B₂M₀: 40 t ha⁻¹ of Biochar and 0 g of AMF; B₂M₁: 40 t ha⁻¹ of Biochar and 15 g of AMF; B₂M₂: 40 t ha⁻¹ of Biochar and 30 g of AMF; B₂M₃: 40 t ha⁻¹ of Biochar and 45 g of AMF; B₃M₀: 60 t ha⁻¹ of Biochar and 0 g of AMF; B₃M₁: 60 t ha⁻¹ of Biochar and 15 g of AMF; B₃M₂: 60 t ha⁻¹ of Biochar and 30 g of AMF; B₃M₃: 60 t ha⁻¹ of Biochar and 45 g of AMF. Vertical bars represent the standard error of the mean.

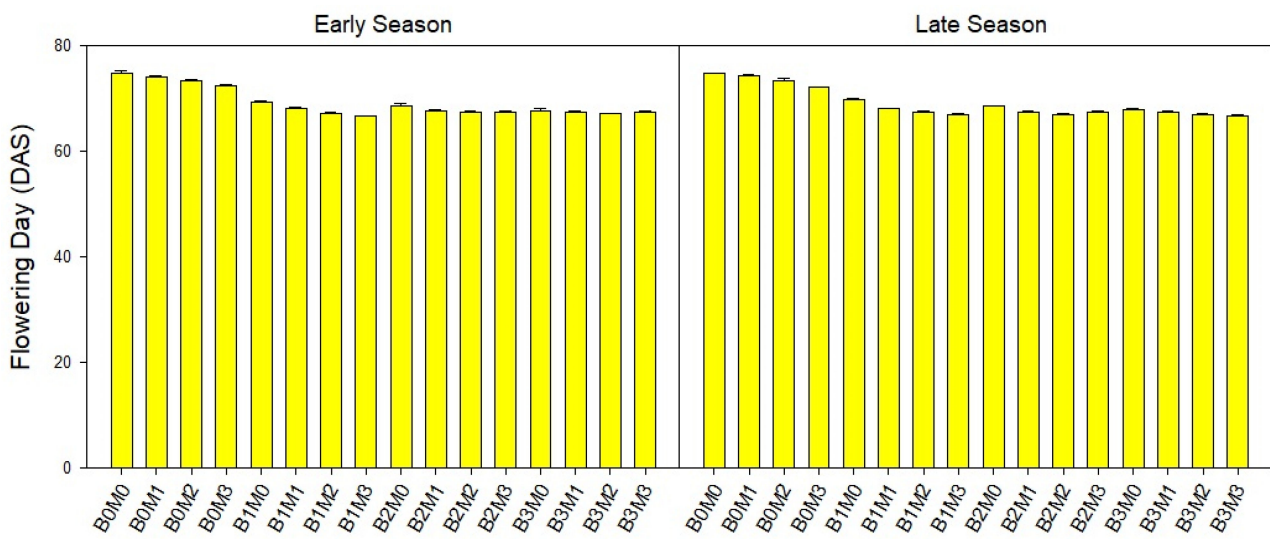


Figure 4. Effects of different rates of biochar and AMF fertilizer application on the rice flowering day (days after seeding). Note: B₀M₀: 0 t ha⁻¹ of Biochar and 0 g of AMF; B₀M₁: 0 t ha⁻¹ of Biochar and 15 g of AMF; B₀M₂: 0 t ha⁻¹ of Biochar and 30 g of AMF; B₀M₃: 0 t ha⁻¹ of Biochar and 45 g of AMF; B₁M₀: 20 t ha⁻¹ of Biochar and 0 g of AMF; B₁M₁: 20 t ha⁻¹ of Biochar and 15 g of AMF; B₁M₂: 20 t ha⁻¹ of Biochar and 30 g of AMF; B₁M₃: 20 t ha⁻¹ of Biochar and 45 g of AMF; B₂M₀: 40 t ha⁻¹ of Biochar and 0 g of AMF; B₂M₁: 40 t ha⁻¹ of Biochar and 15 g of AMF; B₂M₂: 40 t ha⁻¹ of Biochar and 30 g of AMF; B₂M₃: 40 t ha⁻¹ of Biochar and 45 g of AMF; B₃M₀: 60 t ha⁻¹ of Biochar and 0 g of AMF; B₃M₁: 60 t ha⁻¹ of Biochar and 15 g of AMF; B₃M₂: 60 t ha⁻¹ of Biochar and 30 g of AMF; B₃M₃: 60 t ha⁻¹ of Biochar and 45 g of AMF. Vertical bars represent the standard error of the mean.

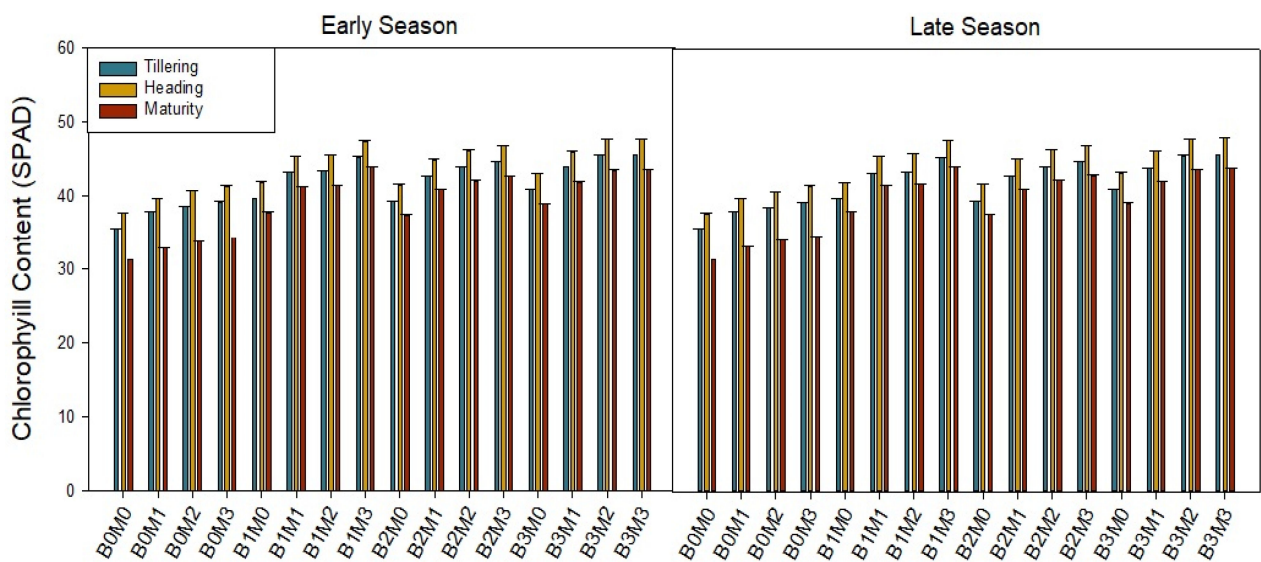


Figure 5. Effects of different rates of biochar and AMF fertilizer application on rice chlorophyll content (SPAD). Note: B₀M₀: 0 t ha⁻¹ of Biochar and 0 g of AMF; B₀M₁: 0 t ha⁻¹ of Biochar and 15 g of AMF; B₀M₂: 0 t ha⁻¹ of Biochar and 30 g of AMF; B₀M₃: 0 t ha⁻¹ of Biochar and 45 g of AMF; B₁M₀: 20 t ha⁻¹ of Biochar and 0 g of AMF; B₁M₁: 20 t ha⁻¹ of Biochar and 15 g of AMF; B₁M₂: 20 t ha⁻¹ of Biochar and 30 g of AMF; B₁M₃: 20 t ha⁻¹ of Biochar and 45 g of AMF; B₂M₀: 40 t ha⁻¹ of Biochar and 0 g of AMF; B₂M₁: 40 t ha⁻¹ of Biochar and 15 g of AMF; B₂M₂: 40 t ha⁻¹ of Biochar and 30 g of AMF; B₂M₃: 40 t ha⁻¹ of Biochar and 45 g of AMF; B₃M₀: 60 t ha⁻¹ of Biochar and 0 g of AMF; B₃M₁: 60 t ha⁻¹ of Biochar and 15 g of AMF; B₃M₂: 60 t ha⁻¹ of Biochar and 30 g of AMF; B₃M₃: 60 t ha⁻¹ of Biochar and 45 g of AMF. Vertical bars represent the standard error of the mean.

3.3. Root Morphology

Biochar and AMF fertilizer application had a significant effect on the root morphology of the rice plants, including root length (RL) and root volume (RV), during both seasons (Table 4). The highest value of RL was with the B₃M₃ treatment (42.10 cm and 42.17 cm in the early and late season, respectively), followed by the B₁M₃ treatment (41.56 cm and 41.69 cm in the early and late season, respectively) and B₂M₃ (41.02 cm and 41.13 cm in the early and late season, respectively). The lowest value of RL was with the B₀M₀ treatment (26.84 cm and 26.88 cm in the early and late season, respectively), followed by the B₁M₀ treatment (29.53 cm and 29.57 cm in the early and late season, respectively). There was no significant differences in the RL values among the B₁M₁, B₁M₂, B₀M₂, and B₀M₃ treatments. The combined application of biochar and AMF fertilizer resulted in a significant increase in the RV of the rice roots. B₃M₃ was the treatment with the greatest increase in RV with values of 53.79 cm³ and 53.08 cm³ in the early and late season, respectively, followed by the B₂M₃ treatment (50.15 cm³ and 50.20 cm³ in the early and late season, respectively) and B₁M₃ treatment (51.61 cm³ and 51.36 cm³ in the early and late season, respectively). There were no significant differences in the RV value among the B₀M₁, B₀M₂, and B₀M₃ treatments. The lowest value of RV was with the B₀M₀ treatment (27.37 cm³ and 27.66 cm³ in the early and late season, respectively), followed by the B₁M₀ treatment (30.13 cm³ and 30.16 cm³ in the early and late season, respectively). Both RV and RL increased markedly after the soil was amended with biochar and AMF fertilizer ($p < 0.05$).

Table 4. Effects of different levels of biochar and AMF fertilizer on the RL (cm) and RV (cm³) of the rice.

Treatments	Root Morphology			
	Early Season		Late Season	
	RL (cm)	RV (cm ³)	RL (cm)	RV (cm ³)
B ₀ M ₀	26.84 ± 0.34 i	27.37 ± 0.40 i	26.88 ± 0.19 m	27.66 ± 0.45 m
B ₀ M ₁	32.77 ± 1.10 f	35.57 ± 0.60 f	33.38 ± 0.23 i	35.84 ± 0.09 i
B ₀ M ₂	33.97 ± 0.03 e	36.29 ± 0.26 ef	33.88 ± 0.04 h	36.38 ± 0.17 i
B ₀ M ₃	33.98 ± 0.2 e	36.31 ± 0.19 ef	34.16 ± 0.07 h	36.94 ± 0.18 i
B ₁ M ₀	29.53 ± 0.10 h	30.13 ± 0.13 h	29.57 ± 0.15 l	30.16 ± 0.13 l
B ₁ M ₁	36.91 ± 0.17 d	38.03 ± 0.10 de	36.87 ± 0.11 g	38.29 ± 0.05 h
B ₁ M ₂	37.03 ± 0.12 d	39.07 ± 0.26 d	37.06 ± 0.13 g	39.34 ± 0.10 g
B ₁ M ₃	41.56 ± 0.09 a	51.61 ± 0.72 b	41.69 ± 0.20 b	51.36 ± 0.16 b
B ₂ M ₀	31.08 ± 0.09 g	32.56 ± 0.22 g	31.18 ± 0.03 k	32.49 ± 0.12 k
B ₂ M ₁	38.53 ± 0.06 c	39.33 ± 1 d	37.80 ± 0.06 f	39.84 ± 0.26 g
B ₂ M ₂	37.84 ± 0.17 cd	44.28 ± 1.70 c	38.08 ± 0.20 ef	44.13 ± 0.19 f
B ₂ M ₃	38.22 ± 0.13 c	45.67 ± 1.15 c	38.24 ± 0.13 e	45.32 ± 0.27 e
B ₃ M ₀	32.91 ± 0.18 f	33.11 ± 0.11 g	32.8 ± 0.13 j	33.82 ± 0.26 j
B ₃ M ₁	40.39 ± 0.56 b	46.39 ± 0.52 c	40.03 ± 0.10 d	46.60 ± 0.19 d
B ₃ M ₂	41.13 ± 0.22 ab	50.15 ± 0.51 b	41.02 ± 0.16 c	50.20 ± 0.11 c
B ₃ M ₃	42.10 ± 0.26 a	53.79 ± 0.58 a	42.17 ± 0.12 a	53.08 ± 0.45 a
B	**	**	**	**
M	**	**	**	**
B × M	**	**	**	**

Note: Root length (RL), root volume (RV). ± indicates the standard error among the replications. B: Biochar; M: Mycorrhizae; B × M: interaction between Biochar and Mycorrhizae; B₀M₀: 0 t ha⁻¹ of Biochar and 0 g of AMF; B₀M₁: 0 t ha⁻¹ of Biochar and 15 g of AMF; B₀M₂: 0 t ha⁻¹ of Biochar and 30 g of AMF; B₀M₃: 0 t ha⁻¹ of Biochar and 45 g of AMF; B₁M₀: 20 t ha⁻¹ of Biochar and 0 g of AMF; B₁M₁: 20 t ha⁻¹ of Biochar and 15 g of AMF; B₁M₂: 20 t ha⁻¹ of Biochar and 30 g of AMF; B₁M₃: 20 t ha⁻¹ of Biochar and 45 g of AMF; B₂M₀: 40 t ha⁻¹ of Biochar and 0 g of AMF; B₂M₁: 40 t ha⁻¹ of Biochar and 15 g of AMF; B₂M₂: 40 t ha⁻¹ of Biochar and 30 g of AMF; B₂M₃: 40 t ha⁻¹ of Biochar and 45 g of AMF; B₃M₀: 60 t ha⁻¹ of Biochar and 0 g of AMF; B₃M₁: 60 t ha⁻¹ of Biochar and 15 g of AMF; B₃M₂: 60 t ha⁻¹ of Biochar and 30 g of AMF; B₃M₃: 60 t ha⁻¹ of Biochar and 45 g of AMF. Means with similar lowercase letters in the columns are not significantly different ($p > 0.05$) according to Duncan's test. ** indicates the significant difference $p \geq 0.01$.

3.4. Yield Components

The combined application of biochar and AMF fertilizer had a significant effect on the yield components, including panicle number, panicle length, and 1000-grain weight, compared to the other treatments without biochar and AMF (B_0M_0 , B_1M_0 , B_2M_0 , B_3M_0 , B_0M_1 , B_0M_2 , and B_0M_3) in both seasons (Table 5). The maximum panicle number value was observed with the B_1M_3 treatment (34.67 and 35.21 in the early and late season, respectively), followed by B_3M_3 (33.89 and 35 in the early and late season, respectively). The minimum panicle number value was observed with the B_0M_0 treatment (18.87 and 19.44 in the early and late season, respectively), followed by the B_0M_1 and B_1M_0 treatments. The maximum panicle length value was observed with the B_1M_3 treatment (21.44 cm and 21.67 cm in the early and late season, respectively), followed by B_3M_2 (20.56 cm and 20.76 cm in the early and late season, respectively). The minimum panicle length value was observed with the B_0M_0 treatment (16.56 cm and 16.86 cm in the early and late season, respectively), followed by the B_1M_0 and B_2M_0 treatments. The 1000-grain weight was highest with the B_1M_3 treatment (41.26 and 41.27 g hill⁻¹ in the early and late season, respectively), followed by the B_3M_3 treatment (40.22 and 40.40 g in the early and late season, respectively). However, there was no significant difference between the B_3M_1 and B_3M_2 treatments in the 1000-grain weight.

Table 5. Effect of different rates of biochar and AMF fertilizer application on the panicle number (PN), panicle length (PL), and 1000-grain weight of rice.

Treatments	Yield Components					
	Early Season			Late Season		
	PN	PL (cm)	1000 Grain Weight (g)	PN	PL (cm)	1000 Grain Weight (g)
B_0M_0	18.87 ± 0.56 g	16.56 ± 0.44 f	28.12 ± 0.21 g	19.44 ± 0.56 h	16.89 ± 0.44 j	28.37 ± 0.15 k
B_0M_1	24.45 ± 0.40 f	18.67 ± 0.33 de	29.08 ± 0.03 g	24.89 ± 0.40 f	18.78 ± 0.33 i	29.19 ± 0.04 j
B_0M_2	26.56 ± 0.29 e	19.67 ± 0.38 bcd	30.33 ± 0.39 f	26.67 ± 0.29 e	19.89 ± 0.38 cdef	30.54 ± 0.09 i
B_0M_3	26.67 ± 0.59 e	19.67 ± 1.24 bcd	30.36 ± 0.18 f	28.11 ± 0.59 e	20.22 ± 0.40 defg	30.76 ± 0.05 hi
B_1M_0	23.78 ± 0.22 f	17.89 ± 0.22 e	30.76 ± 0.29 f	24.22 ± 0.22 fg	18.11 ± 0.22 hi	30.98 ± 0.14 h
B_1M_1	30.78 ± 0.59 d	20.22 ± 0.68 bc	36.76 ± 0.29 d	30.44 ± 0.59 d	20.11 ± 0.68 bcde	37.00 ± 0.13 e
B_1M_2	32.33 ± 0.51 c	20.33 ± 0.38 abc	39.61 ± 0.17 bc	32.33 ± 0.51 bc	20.78 ± 0.38 b	39.84 ± 0.14 cd
B_1M_3	34.67 ± 0.19 a	21.44 ± 0.11 a	41.26 ± 0.15 a	35.21 ± 0.19 a	21.67 ± 0.11 a	41.37 ± 0.13 a
B_2M_0	23.56 ± 0.11 f	18.22 ± 0.22 e	32.08 ± 0.30 e	23.78 ± 0.11 g	19 ± 0.22 gh	32.12 ± 0.18 g
B_2M_1	31.67 ± 0.51 cd	19.33 ± 0.38 cd	39.06 ± 0.75 c	31.89 ± 0.51 c	20.78 ± 0.38 b	39.42 ± 0.17 d
B_2M_2	32.11 ± 0.29 c	20.72 ± 0.45 ab	39.13 ± 0.48 bc	32.44 ± 0.29 bc	20.44 ± 0.45 bcd	39.82 ± 0.11 cd
B_2M_3	32.22 ± 0.22 c	20.11 ± 0.40 bc	39.41 ± 0.14 bc	33.11 ± 0.22 b	20.56 ± 0.40 bcd	39.54 ± 0.12 d
B_3M_0	23.89 ± 0.11 f	18.89 ± 0.11 de	32.99 ± 0.32 e	24.67 ± 0.11 fg	19.22 ± 0.11 fgh	33.18 ± 0.15 f
B_3M_1	33.56 ± 0.11 b	19.55 ± 0.22 bcd	39.27 ± 0.35 bc	34.11 ± 0.11 a	19.56 ± 0.22 efgh	39.98 ± 0.12 c
B_3M_2	33.78 ± 0.44 ab	20.56 ± 0.11 ab	39.79 ± 0.29 bc	34.56 ± 0.44 a	20.78 ± 0.11 b	39.98 ± 0.09 c
B_3M_3	33.89 ± 0.11 ab	20.50 ± 0.29 abc	40.22 ± 0.39 b	35.00 ± 0.11 a	20.67 ± 0.29 bc	40.40 ± 0.18 b
B	**	**	**	**	**	**
M	**	**	**	**	**	**
B × M	**	*	**	**	**	**

Note: Panicle length (PL), panicle number (PN). ± indicates the standard error among the replications. B: Biochar; M: Mycorrhizae; B × M: interaction between Biochar and Mycorrhizae; B_0M_0 : 0 t ha⁻¹ of Biochar and 0 g of AMF; B_0M_1 : 0 t ha⁻¹ of Biochar and 15 g of AMF; B_0M_2 : 0 t ha⁻¹ of Biochar and 30 g of AMF; B_0M_3 : 0 t ha⁻¹ of Biochar and 45 g of AMF; B_1M_0 : 20 t ha⁻¹ of Biochar and 0 g of AMF; B_1M_1 : 20 t ha⁻¹ of Biochar and 15 g of AMF; B_1M_2 : 20 t ha⁻¹ of Biochar and 30 g of AMF; B_1M_3 : 20 t ha⁻¹ of Biochar and 45 g of AMF; B_2M_0 : 40 t ha⁻¹ of Biochar and 0 g of AMF; B_2M_1 : 40 t ha⁻¹ of Biochar and 15 g of AMF; B_2M_2 : 40 t ha⁻¹ of Biochar and 30 g of AMF; B_2M_3 : 40 t ha⁻¹ of Biochar and 45 g of AMF; B_3M_0 : 60 t ha⁻¹ of Biochar and 0 g of AMF; B_3M_1 : 60 t ha⁻¹ of Biochar and 15 g of AMF; B_3M_2 : 60 t ha⁻¹ of Biochar and 30 g of AMF; B_3M_3 : 60 t ha⁻¹ of Biochar and 45 g of AMF. Means with similar lowercase letters in the columns are not significantly different ($p > 0.05$) according to Duncan's test. ** indicates the significant difference $p \geq 0.01$ and * indicates $p = 0.01-0.05$.

3.5. Root Dry Matter, Shoot Dry Matter, and Nitrogen Uptake

Nitrogen uptake, shoot dry matter, and root dry matter data are shown in (Table 6). The dry weight matter was significantly higher with the biochar treatments than with the non-biochar treatments (B_0M_0 , B_0M_1 , B_0M_2 , and B_0M_3). B_3M_3 had the highest shoot dry matter value (59.29 g and 59.66 g in the early and late season, respectively) and B_0M_0 had the lowest shoot dry matter value (40.80 g and 40.84 g in the early and late season,

respectively). There was no significant difference between the B₀M₂ and B₀M₃ treatments on the shoot dry weight matter in both seasons. The root dry matter value was highest with the B₁M₃ treatment (32.37 g and 32.51 g in the early and late season, respectively). The root dry matter value was lowest with the B₀M₀ treatment (19.80 g and 19.76 g in the early and late season, respectively). B₁M₃ was the best treatment for increasing nitrogen uptake, with a value of 9.12 and 9.14 g polybag⁻¹ in the early and late season, respectively, followed by the B₃M₃ treatment (8.95 and 8.97 g polybag⁻¹ in the early and late season, respectively) and the B₂M₃ treatment (8.89 and 8.95 g polybag⁻¹ in the early and late season, respectively).

Table 6. Effect of different rates of biochar and AMF fertilizer on the root dry matter (RDM), shoot dry matter (SDM), and nitrogen uptake (NU) of rice.

Treatments	Dry Matter and Nitrogen Uptake					
	Early Season			Late Season		
	RDM (g)	SDM (g)	NU (g Polybag ⁻¹)	RDM (g)	SDM (g)	NU (g Polybag ⁻¹)
B ₀ M ₀	19.80 ± 0.37 h	40.80 ± 0.37 i	3.21 ± 0.01 o	19.76 ± 0.15 m	40.84 ± 0.06 j	3.24 ± 0.01 o
B ₀ M ₁	20.77 ± 0.08 h	41.80 ± 0.23 h	4.50 ± 0.00 n	21.70 ± 0.15 l	42.78 ± 0.18 hi	4.52 ± 0.00 n
B ₀ M ₂	21.97 ± 0.03 g	42.97 ± 0.03 g	4.59 ± 0.01 m	21.93 ± 0.13 kl	42.99 ± 0.04 h	4.61 ± 0.00 m
B ₀ M ₃	21.98 ± 0.02 g	43.01 ± 0.04 g	4.72 ± 0.00 l	22.16 ± 0.08 k	43.30 ± 0.02 h	4.74 ± 0.00 l
B ₁ M ₀	23.53 ± 0.10 f	42.53 ± 0.10 gh	4.74 ± 0.00 k	23.54 ± 0.05 j	42.64 ± 0.06 i	4.76 ± 0.00 k
B ₁ M ₁	29.91 ± 0.17 d	51.91 ± 0.17 e	7.31 ± 0.01 h	26.93 ± 0.11 g	51.9 ± 0.02 f	7.33 ± 0.00 h
B ₁ M ₂	27.03 ± 0.12 d	54.03 ± 0.12 d	7.83 ± 0.00 g	27.31 ± 0.06 f	54.16 ± 0.18 e	7.84 ± 0.00 g
B ₁ M ₃	32.37 ± 0.04 a	59.37 ± 0.04 a	9.12 ± 0.01 a	32.51 ± 0.07 a	59.48 ± 0.08 ab	9.14 ± 0.01 a
B ₂ M ₀	24.08 ± 0.09 ef	44.08 ± 0.09 f	4.83 ± 0.00 j	24.36 ± 0.17 i	44.27 ± 0.09 g	4.84 ± 0.01 j
B ₂ M ₁	28.53 ± 0.06 c	57.53 ± 0.06 c	8.23 ± 0.00 f	28.64 ± 0.05 e	57.88 ± 0.07 d	8.24 ± 0.00 f
B ₂ M ₂	28.73 ± 0.11 c	57.73 ± 0.11 c	8.52 ± 0.00 e	28.99 ± 0.08 d	58.12 ± 0.07 cd	8.54 ± 0.00 e
B ₂ M ₃	29.11 ± 0.16 c	58.11 ± 0.16 bc	8.89 ± 0.01 c	29.24 ± 0.05 d	58.33 ± 0.14 c	8.95 ± 0.00 b
B ₃ M ₀	24.80 ± 0.08 e	44.40 ± 0.08 f	6.72 ± 0.00 i	24.92 ± 0.13 h	44.52 ± 0.06 g	6.73 ± 0.00 i
B ₃ M ₁	30.39 ± 0.56 b	58.39 ± 0.56 abc	8.64 ± 0.00 d	30.59 ± 0.14 c	58.41 ± 0.21 c	8.67 ± 0.01 d
B ₃ M ₂	31.13 ± 0.22 b	59.13 ± 0.22 ab	8.88 ± 0.00 c	31.34 ± 0.12 b	59.29 ± 0.12 c	8.90 ± 0.01 c
B ₃ M ₃	31.29 ± 0.16 b	59.29 ± 0.16 a	8.95 ± 0.00 b	31.42 ± 0.04 b	59.66 ± 0.03 a	8.97 ± 0.00 b
B	**	**	**	**	**	**
M	**	**	**	**	**	**
B × M	**	**	**	**	**	**

Note: Root dry matter (RDM), shoot dry matter (SDM), nitrogen uptake (NU). ± indicates the standard error among the replications. B: Biochar; M: Mycorrhizae; B × M: interaction between Biochar and Mycorrhizae; B₀M₀: 0 t ha⁻¹ of Biochar and 0 g of AMF; B₀M₁: 0 t ha⁻¹ of Biochar and 15 g of AMF; B₀M₂: 0 t ha⁻¹ of Biochar and 30 g of AMF; B₀M₃: 0 t ha⁻¹ of Biochar and 45 g of AMF; B₁M₀: 20 t ha⁻¹ of Biochar and 0 g of AMF; B₁M₁: 20 t ha⁻¹ of Biochar and 15 g of AMF; B₁M₂: 20 t ha⁻¹ of Biochar and 30 g of AMF; B₁M₃: 20 t ha⁻¹ of Biochar and 45 g of AMF; B₂M₀: 40 t ha⁻¹ of Biochar and 0 g of AMF; B₂M₁: 40 t ha⁻¹ of Biochar and 15 g of AMF; B₂M₂: 40 t ha⁻¹ of Biochar and 30 g of AMF; B₂M₃: 40 t ha⁻¹ of Biochar and 45 g of AMF; B₃M₀: 60 t ha⁻¹ of Biochar and 0 g of AMF; B₃M₁: 60 t ha⁻¹ of Biochar and 15 g of AMF; B₃M₂: 60 t ha⁻¹ of Biochar and 30 g of AMF; B₃M₃: 60 t ha⁻¹ of Biochar and 45 g of AMF. Means with similar lowercase letters in the columns are not significantly different ($p > 0.05$) according to Duncan's test. ** indicates the significant difference $p \geq 0.01$.

4. Discussion

4.1. Effect of Biochar and AMF Fertilizer on the Physical and Chemical Properties of Soil

The results of our study indicate that the B₃M₃ treatment significantly improved the properties of the soil, including pH, soil porosity (SP), soil organic matter (SOM), total nitrogen (TN), available potassium (AK), and available phosphorous (AP). Previous studies have indicated that the application of biochar alters the availability of soil nutrients by adsorption, desorption, and precipitation [37,38]. Ali et al. [30] indicated that the addition of 60 t ha⁻¹ biochar enhances soil quality under both low and high N treatments. We found that higher doses of biochar and AMF fertilizer have stronger effects on soil quality. Biochar had a positive effect on soil properties such as the cation exchange capacity and

surface area; AMF had a positive effect on the bacterial community in the soil and bacterial metabolites, which in turn increased the soil pH [39,40].

The unique structure and properties of biochar, including its nitrogen, phosphorous, and potassium content, can drive the activation process [13,41–43]. Additionally, the priming effects of biochar can improve the bioavailability of soil nutrients and thus increase their availability either directly or indirectly [44,45]. Biochar can also increase soil AP, extractable zinc (Zn), iron (Fe), copper (Cu), and manganese (Mn) [46]. We found that BD decreased when biochar was added. The BD of soil decreases when large doses of biochar (60 t ha⁻¹ t) are added [30]. Other studies have shown that the application of biochar at rates of 20 t ha⁻¹ and 100 t ha⁻¹ can significantly reduce the BD of clay loam, improve field capacity and water availability, and reduce compaction by up to 15%. Biochar can promote the water–air interaction between surface water, the atmosphere, and paddy soil, and this increases the likelihood that fertilized nutrients reach the root–soil layer [47].

4.2. Effect of Biochar and AMF Fertilizer on Rice Growth

Biochar and AMF treatment increased plant height, tiller number, SPAD chlorophyll, flag leaf area, and flowering day compared to the control treatment (B₀M₀), sole biochar treatments (B₁M₀, B₂M₀, and B₃M₀), and sole AMF (B₀M₁, B₀M₂, and B₀M₃) treatments. Many studies have shown that applying biochar can enhance plant growth, development, and yield in a variety of plants [48–52]. Several studies have suggested that AMF fertilizer application can enhance the development parameters of plants. Deniel et al. [53] found that biochar addition with rates of 10 t ha⁻¹ and 20 t ha⁻¹ has a significant effect in improving number of tillers and plant height. The addition of biochar (20 and 40 t ha⁻¹) promoted the number of tillers and plant height, and improved the grain number and productive panicle number, thus affecting rice yield [54].

We found that the addition of biochar at a high dose can better improve rice growth. Although our findings indicated that the application of biochar alone and AMF alone have positive effects on rice plants, the combined application of biochar and AMF had a much stronger positive effect on the plants compared to the sole biochar, sole AMF, and control treatments. This may be because biochar contains certain nutrients (e.g., P and K) which are used as nutrient sources for plants and AMF absorbs the nutrients in the soil, significantly increasing rice growth [55]. The positive effects of the combined treatment begin with biochar because it promotes and accelerates AMF colonization. The combined application of biochar and AMF also promotes nutrient availability and absorption, and increases nitrogen fixation and siderophore synthesis [56].

4.3. Effect of Biochar and AMF Fertilizer Application on Root Morphology and Dry Matter

Our results show that the combined application of biochar and AMF increased RV and RL, which is consistent with the results of previous studies [48,57,58]. AMF has good symbiosis with plants, enhancing the absorption of water and nutrients from the soil by the plants via the hyphae, which promotes plant growth and development [59]. Researchers have found that biochar and AMF improve growth performance, including shoot dry weight and root dry weight when compared to the control [60,61]. AMF can improve the physical, chemical, and biological characteristics of the soil, promote nutrient cycling, and increase the effectiveness of corrective measures and nutrients, which can increase root dry matter in compacted soils and promote decompression [57,62–64]. Wen et al. found that AMF of 20 gr pot⁻¹ significantly increased the root length and root volume of rice in the absence of biochar [65]. Ali et al. indicated that the amount of dry matter (DM) increased with an improvement in the biochar application, with the rate of biochar at 60 t ha⁻¹ [30]. RV, RL, and DM values were higher for plants treated with biochar and AMF than sole biochar, sole AMF, and the control. These findings are consistent with the results of previous study showing that the application of biochar and AMF significantly increases the height, diameter, shoot dry weight, root dry weight, and root and shoot biomass of plants [61,66,67].

4.4. Effects of Biochar and AMF Fertilizer Application on Yield Components and Nitrogen Uptake

The combined application of biochar and AMF fertilizer had a significant effect on yield components, including the panicle number, panicle length, and 1000-grain weight of the rice plants during both seasons. Yield component parameters were highest with the combined application of 20 t ha⁻¹ biochar and 45 g polybag⁻¹ AMF. This is consistent with the results of a previous study which showed that the application of biochar and AMF significantly affected the length, diameter, and weight of corn fruits [68]. Biochar increases the concentration of nutrients, such as nitrogen, phosphorus, and potassium, by promoting their absorption by the plants and enhancing rice productivity. AK in the soil is increased by biochar, and the interaction between biochar and AMF affects the development and structure of plant roots, which in turn affects the absorption of potassium by plants [69,70]. AMF increased the potassium content in the plants in our study, which is consistent with the results of a previous study which showed that AMF can dissolve P through phosphatase enzyme activity, thus enhancing the P available to plants [71].

In our study, biochar and AMF significantly increased nitrogen uptake. Rice can thrive in a suitable environment when biochar is applied, as biochar application improves nutrient-use efficiency [70,71]. Biochar has previously been shown to promote photosynthesis, N metabolism, and soil quality, and this in turn increases DM, N uptake, and grain yield [30]. Our findings are consistent with the results of previous studies showing that the addition of biochar to paddy soil enhances soil quality, increases rice yield, and increases nutrient uptake by plants [72–75]. The application of AMF has been shown to enhance plant nitrogen uptake and reduce the quantity of NH₄⁺-N in the soil. In the same study, the combined application of biochar and AMF was shown to enhance the soil nitrogen supply more than the sole application of biochar or AMF [76]. This may be because the mycelia of AMF can absorb inorganic nitrogen from the soil and transfer it to the host plants [77,78].

5. Conclusions

Biochar can have a positive effect on the symbiotic relationship between plants and AMF. The physical and chemical properties of the soil and the growth and productivity of the rice were significantly higher with a combined biochar and AMF treatment than with the sole biochar, sole AMF, and the control treatments. Plant height, flag leaf area, SPAD chlorophyll, flowering day, shoot dry matter, root length, root volume, and all soil parameters were higher with the combined 60 t ha⁻¹ biochar and 45 g polybag⁻¹ AMF (B₃M₃) treatment than with the other treatments. The combined application of 20 t ha⁻¹ biochar and 45 g polybag⁻¹ AMF (B₃M₃) treatment had the highest significant effect on tiller number, panicle number, panicle length, root dry matter, nitrogen uptake, and the 1000-grain weight of rice. We highly recommend farmers in our research area produce biochar from rice husk waste because, so far, rice husk has not been used properly, even though this area has the potential to produce 3 t of biochar in one harvest season. We hope that applying biochar to rice plants can enhance rice productivity, increase farmers' income, and support sustainable agriculture. In addition, we suggest that the study of the long-term effects of biochar and AMF on crop growth and soil nutrient cycling are needed.

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