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Combined Application of Arbuscular Mycorrhizal Fungi (AMF) and Nitrogen Fertilizer Alters the Physicochemical Soil Properties, Nitrogen Uptake, and Rice Yield in a Polybag Experiment

Mulyadi [1] and Ligeng Jiang [1,2,3,*]

[1] College of Agriculture, Guangxi University, Nanning 530004, China; mulyadimanik14@gmail.com
[2] Key Laboratory of Crop Cultivation and Physiology, Education Department of Guangxi Zhuang Autonomous Region, Guangxi University, Nanning 530004, China
[3] Guangxi Key Laboratory of Agro-Environment and Agric-Products Safety, Guangxi University, Nanning 530004, China
* Correspondence: jiang@gxu.edu.cn

Abstract: Excessive application of nitrogen fertilizer during rice cultivation leads to progressive soil contamination in the long term and increases production costs. An alternative to reduce over fertilization is to partially replace the fertilizer with microbes that promote nutrition and growth, such as arbuscular mycorrhizal fungi (AMF). We investigated the combination of four different rates of AMF (M): (M_0: 0 g polybag^{-1}, M_1: 15 g polybag^{-1}, M_2: 30 g polybag^{-1}, and M_3: 45 g polybag^{-1}) and three rates of nitrogen (N) fertilizer: (N_0: 0 kg N ha^{-1}, N_1: 90 kg N ha^{-1}, N_2: 180 kg N ha^{-1}) on Trisakti rice cultivar cultivated in polybag. Our findings indicate that the combination of 45 g AMF polybag^{-1} and 180 kg N ha^{-1} decreased soil bulk density by 38.02% and 37.24%, increased soil pH by 14.81% and 14.95%, soil porosity by 60.68% and 61.09%, soil organic matter by 28.62% and 30.46%, total N by 92.59% and 89.66%, available phosphorus by 30.12% and 29.85%, available potassium by 3.75% and 4.01%, rice plant height by 19.19% and 19.79%, tiller number by 25.27% and 26.08%, SPAD by 20.71% and 20.62%, flag leaf area by 107.76% and 108.02%, panicle length by 49.72% and 52.31%, panicle number by 67.44% and 72.35%, 1000-grain weight by 30.70% and 32.44%, root dry matter by 54.34% and 53.69%, shoot dry matter by 26.08% and 28.26%, root length by 54.68% and 56.44%, root volume by 42.73% and 43.37%, and N uptake by 107.93% and 108.06% compared to control during the early and late seasons, respectively. Conclusively, the combined application of AMF and N fertilizer increased the physiochemical properties, rice growth, rice productivity, and N uptake compared to AMF alone, N fertilizer alone, and the control treatment.

Keywords: excessive N; rice growth; N uptake

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1. Introduction

Rice (*Oryza sativa* L.) is an essential food crop worldwide and a dietary staple for about 50% of the world's population [1,2]. Global rice consumption is estimated to increase from 480 million tons (mt) of milled rice in 2014 to nearly 550 mt by 2030, caused by population and economic growth [3]. Farmers have continuously increased their use of chemical fertilizers to enhance rice production [4]. Chemical fertilizers, especially nitrogen fertilizer, are an essential abiotic component of agricultural output [5,6]. Nitrogen (N) is a crucial macronutrient for plants, and its availability is a determinant of plant productivity [7]. Applying N fertilizer has become an important strategy to increase crop yield in intensive agricultural systems worldwide [8]. In order to increase production, rice farmers apply massive amounts of N fertilizer, yet only 20 to 50% of the N is actually absorbed by the crop since soil N availability often limits yield in the majority of agricultural cropping systems [9,10]. The N fertilizer applied in rice fields that escapes into the surrounding environment leads to reduced N use efficiency (NUE) and significant ecological issues [11,12].

NUE is a recognized metric used for assessing N management [13]. Improving NUE of agricultural crops has been used as a method to alleviate the effects of N fertilizer on surrounding water, air, and ecosystems, and to reduce costs associated with excessive fertilizer inputs, as well as to improve growth and productivity of crops [14,15]. Langholtz et al. discovered that increasing NUE by 20% can save USD 743 m yr^{-1} and reduce N loadings in freshwaters by 5.7%, and the N reductions are estimated to be worth USD 15.3 to 136.7 million yr^{-1} in the US [16]. Excessive use of N fertilizer causes some environmental problems, including soil acidification, groundwater contamination, greenhouse gas emission, and eutrophication of surface water [17–20]. New approaches to increase yield and decrease the quantity of N applied are required in order to achieve high crop production and high NUE under well-fertilized circumstances [21]. The creation of new fertilizers, enhancement of crop NUE, substitution of chemical fertilizers, and reduction in pollution are important directions for sustainable agricultural development [22]. Numerous N management strategies, such as deep placement and numerous split treatments, can increase rice production and NUE while lowering N losses [23–26]. However, these methods are either constrained by a lack of technology or demand more labor and expertise of N management than conventional methods [27].

On the other hand, there are microbes in the rhizosphere that interact with rice roots [28]. Rhizospheres play a significant role in the soil environment, plant growth and productivity, plant health, nutrient uptake, and heavy metal tolerance [29,30]. However, numerous factors, including the kind of fertilizer used, how it is applied, how much is used, and how frequently it is applied, frequently have an impact on the activities of soil bacterial communities [31,32]. In Xishuangbanna (China), Pang et al. identified bacteria and fungi from roots of rice. Based on study of the 16S rRNA and internal transcribed spacer (ITS) gene sequences, there are 462 endophytic and rhizospheric isolates (125 fungi and 337 bacteria), which were distributed among 43 genera [33]. The interaction between rhizosphere bacteria and fungi significantly increased rice production and decreased chemical fertilizers [34]. The contributing methods are: (1) creating siderophores and enzymes to increase the solubility of soil nutrients; (2) producing phytohormones; (3) regulating pathogens and reducing the negative impacts of stress; and (4) collaborating with other soil microorganisms [35–38].

Arbuscular mycorrhizal fungi (AMF) are essential fungi of soil microorganisms in the phylum Glomeromycota that form mutualistic symbioses with plant roots [39]. AMF have many functions in symbiotic systems, such as promoting plant growth, increasing yield, improving soil physicochemical properties, stimulating flowering, enhancing drought and disease resistance, boosting heavy-metal tolerance, improving the root physiology, and modifying microbial community structure and diversity in the rhizosphere [40–43]. Numerous studies have demonstrated that after inoculation with AMF, crops considerably improve their ability to absorb N, potassium (K), phosphorus (P), calcium (Ca), and magnesium (Mg) [44–47]. Applying AMF improves root development and the growth and productivity of wetland grasses, tomatoes, and rice [48–51]. Most studies have focused on the ability of AMF to absorb phosphate nutrients, so many researchers have combined AMF and phosphate fertilizer to examine the relationship between AMF, soil phosphate availability, and phosphate uptake by plants [52–54]. The purpose of this research was to investigate the impact of the combined application of AMF and N fertilizer on the physicochemical soil properties, rice growth, rice productivity, and N uptake. We hypothesized that (1) AMF would increase the availability of N in the soil and improve the physical and chemical properties of the soil, and (2) the combination of AMF and N fertilizer would improve the growth and productivity of rice.

2. Materials and Methods

2.1. Experimental Location and Conditions

Experiments were conducted at an agricultural field in Pulo Kedep, Subulussalam, Aceh, Indonesia (02° 27′–03°00′, 97°44′–98°10′) during July–November (early season) and December–April (late season) 2022/2023. The annual average precipitation was 2308 mm. The mean maximum and minimum temperature in the early season ranges were 30.9–32.9 °C and 23.2–23.7 °C, respectively, while the mean maximum and minimum temperature in the late season ranges were 31–34 °C and 23–24.9 °C. The total quantities of rainfall during the early and late seasons were 985 mm and 1468 mm, respectively [55]. In this research, we used unsterile soil collected from Pulo Kedep Village (Ultisol; 0–20 cm). Figure 1 presents the total temperature, rainfall, and relative humidity during both seasons.

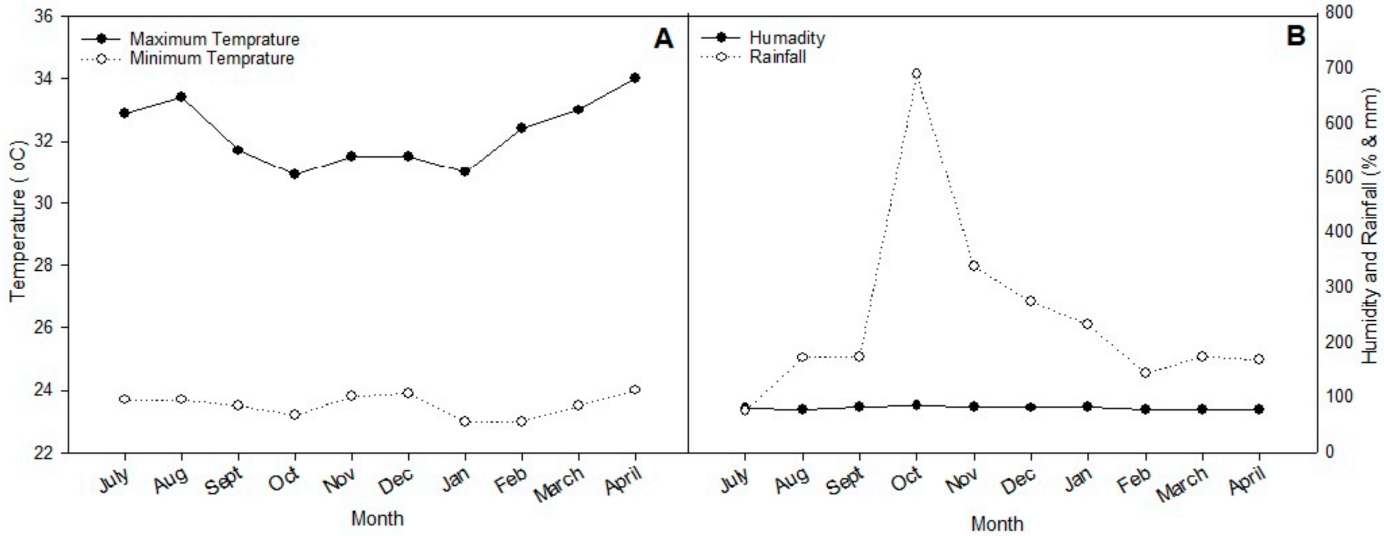

Figure 1. The mean average range of (**A**) temperature and (**B**) relative humidity and total rainfall during both seasons.

2.2. Experimental Design

Two experiments were performed with three replications and a complete randomized design. Rice was cultivated in polybags (40 cm × 40 cm) that contained 10 kg of soil with various doses of AMF and N fertilizer. We used Mycogrow as the AMF inoculum (produced by PT Agrofarm Nusa Raya), which contains five species of AMF (*Glomus claroideum, Glomus fasciculatum, Funneliformis mosseae, Glomus etunicatum, Aucolospora rogusa)*. The polybags were routinely hydrated using drip irrigation from transplantation until maturity stage. The 'Trisakti' rice variety was grown in nurseries for 20 days, then transplanted to the polybags. The characteristics of Trisakti are presented in [56]. AMF was applied 1 day before transplanting. Four levels of AMF (M) were used in the treatments (0, 15, 30, and 45 g polybag^{-1}) and three N fertilizer levels (0, 90, and 180 kg ha^{-1}). These treatments were referred to as M_0N_0: 0 g AMF + 0 kg N ha^{-1} (control), M_0N_1: 0 g AMF + 90 kg N ha^{-1}, M_0N_2: 0 g AMF + 180 kg N ha^{-1}, M_1N_0: 15 g AMF + 0 kg N ha^{-1}, M_1N_1: 15 g AMF + 90 kg N ha^{-1}, M_1N_2: 15 g AMF + 180 kg N ha^{-1}, M_2N_0: 30 g AMF + 0 kg N ha^{-1}, M_2N_1: 30 g AMF + 90 kg N ha^{-1}, M_2N_2: 30 g AMF + 180 kg N ha^{-1}, M_3N_0: 45 g AMF + 0 kg N ha^{-1}, M_3N_1: 45 g AMF + 90 kg N ha^{-1}, and M_3N_2: 45 g AMF + 180 kg N ha^{-1}. All treatments received a base dosage of urea fertilizer at a rate of 90 kg N ha^{-1} (1.13 g polybag^{-1}) and a subsequent dose of urea fertilizer at rates of 50% at the tillering stage and 50% at the panicle initiation stage. KCl (potassium chloride) fertilizer was applied twice: 50% as a basal dose and 50% at the tillering stage at a rate of 240 kg K ha^{-1} (3.02 g polybag^{-1}). SP-36 (super phosphate) fertilizer was treated once as a basal dose at a rate of 240 kg P ha^{-1} (3.02 g polybag^{-1}) in all treatments. The Indonesian Ministry of Agriculture's recommendations were used to determine the fertilizer dosages [57]. The harvest time in this research was 90 days after transplanting (DAT).

2.3. Analysis and Sampling

2.3.1. Biochar and Soil

Biochar that we applied was made from rice husk as described in [56]. To analyze physicochemical properties, soil samples were taken from each treatment throughout both seasons before and after the experiment. The physicochemical of soil properties including pH (potential hydrogen), soil organic matter (SOM), total nitrogen (TN), available phosphorus (AP), available potassium (AK), and soil bulk density (BD) were analyzed using soil nutrient analyzer equipment [58]. Determination the soil porosity (SP) was made with the following formula [59]:

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

2.3.2. Rice Growth

We measured a number of growth factors, including plant height, number of tillers, chlorophyll, flowering day, and flag leaf area (FLA). At 3 to 8 weeks after planting, plant height was measured using a ruler from the stem base to the tallest leaf. The tiller number was examined by counting the number of plants that emerged from the main plant internode at 3–8 weeks after planting. The SPAD meter procedure and method are described by Islam et al. [60]. The measurements were made in the polybags at three different growth stages (tillering, heading, and maturity). FLA measurements were taken on four leaves on each plant. FLA measurement was calculated at 70 DAT, using the following formula:

$$\text{FLA} = \text{length} \times \text{width} \times \text{constant}(0.7). \tag{2}$$

2.3.3. Root Morphology

Measurements were taken of the rice root morphology, such as root volume (RV) and root length (RL). RL was calculated by using a ruler to measure the distance between the stem base and the root tip. To measure RV, the rice plant roots were cut out and cleaned. To calculate the volume change, air-dried roots were put in a 1000 mL measuring cup with 250 mL of water. RV was computed using the formula below:

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

2.3.4. Yield Components

The number of productive tillers on a rice plant was counted to calculate the panicle number, and a ruler was used to calculate the panicle length. The 1000-grain weight was determined with an analytical balance.

2.3.5. Root Dry Matter, Shoot Dry Matter, and N Uptake

For the purpose of determining the shoot and root dry weight, the roots and shoots were washed and baked at 70 °C for 48 h to achieve a constant weight [61]. Plant samples (roots, stems, and leaves) at the maturity stage were oven-dried at 70 °C for 48 h, and then dried sub-samples were ground to a powder. The micro-Kjeldahl method was used to calculate the N uptake [62].

2.4. Statistics

All data were tested and checked for normality before statistical analysis. One-way analysis of variance (ANOVA) was used to analyze all data experiments, and two-way ANOVA was used to analyze the interaction between AMF and N fertilizer. IBM SPSS Statistics 21 software (SPSS Inc., Chicago, IL, USA) was used to analyze the data. Duncan's test was used to analyze the least significant differences ($p < 0.05$). Sigma Plot 14.0 was used to prepare the figures and graphs.

3. Results

3.1. Soil Properties

The combination of AMF and N fertilizer significantly increased soil physicochemical properties (Table 1). The combined application M_3N_2 reduced soil BD by 38.02% and 37.24% compared to control during the early and late seasons, respectively, followed by M_3N_1 35.90% and 34.50%. Similarly, M_3N_2 increased SP by 60.68% and 61.09% with values of 67.17% and 67.41% compared to control treatment during the early and late seasons, respectively, followed by M_3N_1 55.23% and 55.63%. The combined application of AMF and N fertilizer had significant effect on soil chemical properties, including TN, AP, and AK in the both seasons. The M_3N_2 treatment increased TN by 14.81% and 14.95% and AK by 3.75% and 4.01% during the early and late seasons, respectively. M_3N_0 increased soil pH by 17.69% and 18.12%, and SOM by 34.21% and 36.42% during both seasons, respectively. M_3N_1 increased AP content by 30.78% and 30.83% compared to control during the early and late seasons, respectively, followed by M_3N_2 30.12% and 29.84%. AK influenced by the combination of AMF and N fertilizer. M_3N_2 increased AK by 3.75% and 4.01% compared to control in the both seasons, respectively.

Table 1. Variations in soil physicochemical characteristics under different arbuscular mycorrhizal fungi and nitrogen application rates.

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)							
M_0N_0	1.33 ± 0.005 g	41.80 ± 0.01 l	5.20 ± 0.01 g	1.52 ± 0.01 d	0.14 ± 0.005 f	21.30 ± 0.005 i	223.45 ± 0.015 l
M_0N_1	1.27 ± 0.01 f	41.88 ± 0.01 k	5.22 ± 0.01 g	1.56 ± 0.005 d	0.18 ± 0.005 e	21.62 ± 0.015 h	225.69 ± 0.01 j
M_0N_2	1.27 ± 0.005 f	43.10 ± 0.02 j	5.29 ± 0.015 f	1.73 ± 0.005 c	0.18 ± 0.00 de	21.74 ± 0.005 g	225.46 ± 0.01 k
M_1N_0	1.22 ± 0.005 e	46.30 ± 0.02 i	5.87 ± 0.025 d	1.95 ± 0.01 b	0.17 ± 0.00 e	25.26 ± 0.015 f	227.16 ± 0.005 i
M_1N_1	1.17 ± 0.005 d	56.84 ± 0.03 f	5.76 ± 0.02 e	1.95 ± 0.005 b	0.20 ± 0.005 d	25.29 ± 0.005 f	227.83 ± 0.01 h
M_1N_2	1.25 ± 0.01 f	58.94 ± 0.03 e	5.31 ± 0.01 f	1.93 ± 0.005 b	0.22 ± 0.005 c	25.78 ± 0.011 e	228.3 ± 0.01 g
M_2N_0	1.19 ± 0.005 de	48.18 ± 0.01 h	5.97 ± 0.01 c	1.98 ± 0.005 b	0.18 ± 0.005 e	25.81 ± 0.04 de	229.25 ± 0.02 f
M_2N_1	1.01 ± 0.01 c	60.15 ± 0.025 d	5.87 ± 0.025 d	1.96 ± 0.00 b	0.23 ± 0.005 cd	25.92 ± 0.005 d	229.88 ± 0.015 d
M_2N_2	1.00 ± 0.025 bc	63.14 ± 0.045 c	5.33 ± 0.01 f	1.93 ± 0.005 b	0.24 ± 0.005 bc	25.84 ± 0.015 de	230.09 ± 0.005 c
M_3N_0	1.17 ± 0.005 d	48.92 ± 0.005 g	6.12 ± 0.01 a	2.04 ± 0.05 a	0.22 ± 0.005 c	26.38 ± 0.015 c	229.80 ± 0.01 e
M_3N_1	0.98 ± 0.005 ab	64.89 ± 0.035 b	6.02 ± 0.01 b	1.98 ± 0.005 b	0.25 ± 0.005 ab	27.85 ± 0.04 a	230.19 ± 0.005 b
M_3N_2	0.96 ± 0.01 a	67.17 ± 0.025 a	5.97 ± 0.00 c	1.96 ± 0.005 b	0.26 ± 0.01 a	27.71 ± 0.02 b	231.88 ± 0.01 a
Late Season (S2)							
M_0N_0	1.35 ± 0.005 h	41.85 ± 0.005 l	5.19 ± 0.005 i	1.51 ± 0.01 h	0.15 ± 0.005 f	21.28 ± 0.005 k	222.93 ± 0.055 l
M_0N_1	1.28 ± 0.005 g	41.92 ± 0.05 k	5.24 ± 0.005 h	1.55 ± 0.005 h	0.19 ± 0.005 e	21.66 ± 0.02 j	225.93 ± 0.04 j
M_0N_2	1.25 ± 0.005 f	43.14 ± 0.03 j	5.32 ± 0.03 g	1.65 ± 0.02 g	0.19 ± 0.005 e	21.77 ± 0.01 i	225.35 ± 0.025 k
M_1N_0	1.20 ± 0.005 e	46.62 ± 0.05 i	5.88 ± 0.01 d	1.93 ± 0.005 ef	0.18 ± 0.005 e	25.32 ± 0.005 h	227.35 ± 0.03 i
M_1N_1	1.15 ± 0.005 d	56.92 ± 0.015 f	5.95 ± 0.005 c	1.95 ± 0.005 cdef	0.21 ± 0.005 d	25.39 ± 0.005 g	227.70 ± 0.03 h
M_1N_2	1.25 ± 0.005 f	58.98 ± 0.005 e	5.74 ± 0.02 f	1.91 ± 0.01 f	0.22 ± 0.01 cd	25.76 ± 0.025 f	228.20 ± 0.015 g
M_2N_0	1.19 ± 0.005 e	48.28 ± 0.045 h	6.06 ± 0.03 b	1.99 ± 0.005 bc	0.18 ± 0.005 e	25.81 ± 0.015 f	125.78 ± 0.04 f
M_2N_1	1.04 ± 0.015 c	60.26 ± 0.01 d	5.98 ± 0.005 c	1.97 ± 0.005 bcde	0.24 ± 0.005 c	25.97 ± 0.01 d	229.77 ± 0.015 e
M_2N_2	1.01 ± 0.01 b	63.23 ± 0.02 c	5.82 ± 0.005 e	1.94 ± 0.00 def	0.26 ± 0.005 b	25.85 ± 0.02 e	230.12 ± 0.01 d
M_3N_0	1.15 ± 0.005 d	48.98 ± 0.005 g	6.13 ± 0.005 a	2.06 ± 0.03 a	0.21 ± 0.01 d	26.38 ± 0.01 c	229.88 ± 0.01 c
M_3N_1	1.00 ± 0.01 ab	65.13 ± 0.005 b	6.06 ± 0.02 b	1.99 ± 0.02 b	0.27 ± 0.005 ab	27.84 ± 0.005 a	230.22 ± 0.005 b
M_3N_2	0.98 ± 0.01 a	67.41 ± 0.02 a	5.96 ± 0.01 c	1.97 ± 0.00 bcd	0.28 ± 0.005 a	27.63 ± 0.005 b	231.88 ± 0.015 a
M	**	**	**	**	**	**	**
N	**	**	**	ns	**	**	**
M × N	**	**	**	**	ns	**	**
LSD	0.000	0.000	0.000	0.000	0.069	0.000	0.000

Note: Bulk density (BD), soil porosity (SP), potential hydrogen (pH), soil organic matter (SOM), total nitrogen (TN), available phosphorus (AP), and available potassium (AK), ± indicates the standard error among replications, LSD: least significant difference of M × N, M: mycorrhizae, N: nitrogen, M×N: interaction between mycorrhizae and nitrogen, M_0N_0: 0 g AMF + 0 kg N ha^{-1} (control), M_0N_1: 0 g AMF + 90 kg N ha^{-1}, M_0N_2: 0 g AMF + 180 kg N ha^{-1}, M_1N_0: 15 g AMF + 0 kg N ha^{-1}, M_1N_1: 15 g AMF + 90 kg N ha^{-1}, M_1N_2: 15 g AMF + 180 kg N ha^{-1}, M_2N_0: 30 g AMF + 0 kg N ha^{-1}, M_2N_1: 30 g AMF + 90 kg N ha^{-1}, M_2N_2: 30 g AMF + 180 kg N ha^{-1}, M_3N_0: 45 g AMF + 0 kg N ha^{-1}, M_3N_1: 45 g AMF + 90 kg N ha^{-1}, and M_3N_2: 45 g AMF + 180 kg N ha^{-1}. Means with the same lowercase letters in a column are not significantly different ($p > 0.05$) according to Duncan's test. ** indicates a significant difference ($p < 0.01$), and ns: no significant difference ($p > 0.05$).

3.2. Rice Growth

Based on the data analysis of two seasons of rice growth, we found that the combination of AMF and N fertilizer had substantial effects on plant height, number of tillers, FLA, flowering day, and chlorophyll content (SPAD). According to Figure 2, plant height was significantly affected by the combination of AMF and N, with the highest values presented in M_3N_2, M_3N_1, M_2N_2, and M_2N_1. M_3N_2 increased plant height by 19.19% and 19.79% compared to control during both seasons, respectively, followed by M_3N_1 17.49% and 18.17%, and M_2N_2 17.02% and 17.48%. The combined application of AMF and N also had a significant effect in enhancing the number of tillers (Figure 3); the higher the AMF dose, the higher the number of tillers. M_3N_2 improved the number of tillers by 25.27% and 26.08% compared to control during both seasons, respectively, followed by M_3N_1 23.38% and 23.62%. The flowering day data are presented in Figure 4. The combination of AMF and N fertilizer accelerated the day of flowering. M_3N_2 increased the day of flowering by 19.23% and 20.22% compared to control treatment during the both seasons, respectively, followed by M_2N_2 18.91% and 18.80%, and M_3N_1 17.90% and 19.14%. The M_3N_2, M_2N_2, and M_3N_1 treatments developed flowers 49 days after transplanting during both seasons. In our study, the higher the dose of AMF and N fertilizer, the faster the day of flowering. AMF at a rate of 45 g polybag^{-1} stimulated faster flowering than the 30 g polybag^{-1}, 15 g polybag^{-1}, or the control.

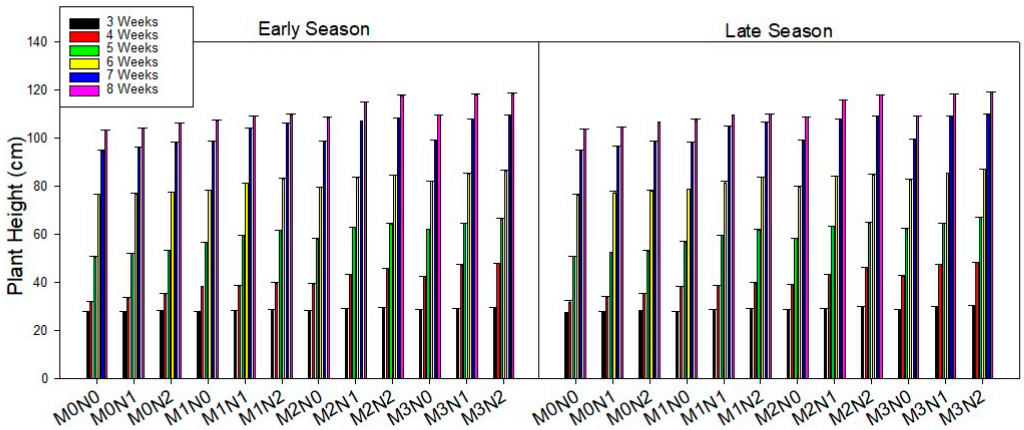

Figure 2. Impact of different levels of arbuscular mycorrhizal fungi and nitrogen fertilizer on plant height (cm). Note: The standard error of the mean is represented by vertical bars.

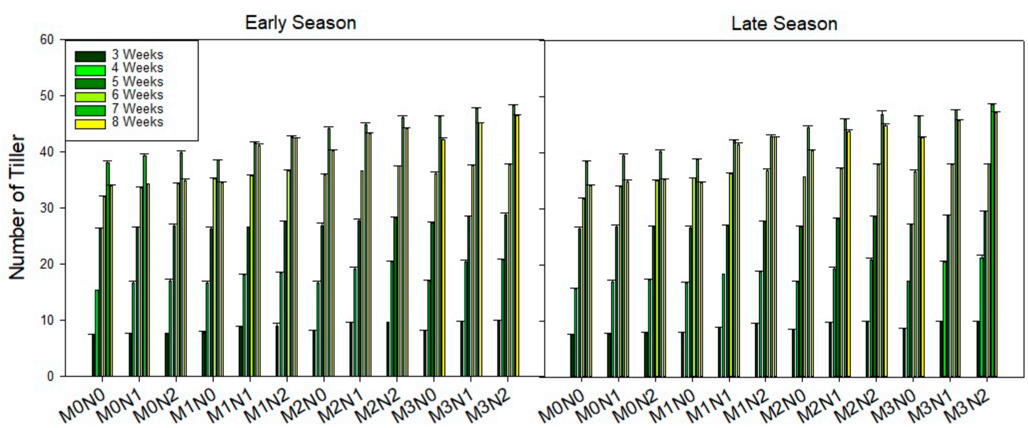

Figure 3. Impact of different levels of arbuscular mycorrhizal fungi and nitrogen fertilizer on tiller number. Note: The standard error of the mean is represented by vertical bars.

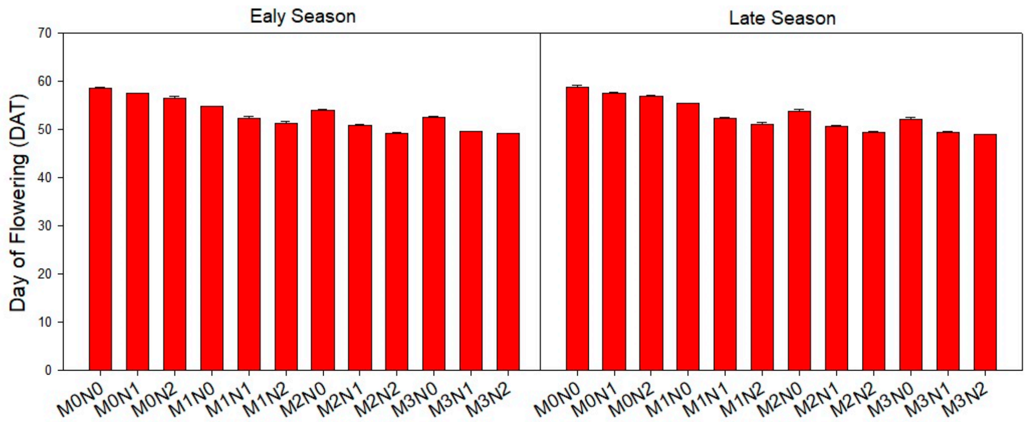

Figure 4. Impact of different levels of arbuscular mycorrhizal fungi and nitrogen fertilizer on flowering time days after transplanting (DAT). Note: The standard error of the mean is represented by vertical bars.

The effect of the combination of AMF and N fertilizer on FLA is presented in Figure 5. FLA was significantly affected by AMF and N fertilizer. M_3N_2 increased FLA by 107.76% and 108.02% compared to control during both seasons, respectively, followed by M_2N_2 103.67% and 103.68%, and M_3N_1 99.61% and 99.94%. The smallest FLA value occurred in M_0N_0, with 6.60 and 6.61 cm^2 during both seasons, respectively, followed by M_0N_1 and M_0N_2. The interaction between AMF and N was significant ($p < 0.05$) for chlorophyll content (SPAD) during all three growth stages (tillering, heading, and maturity) in both seasons (Figure 6). The results showed that the M_3N_2 treatment resulted in an average increased SPAD by 20.71% and 20.62% during both seasons. N fertilizer improved the chlorophyll content (SPAD) value. N fertilizer at a rate of 180 kg N ha^{-1} resulted in a higher SPAD value than a rate of 90 kg N ha^{-1}.

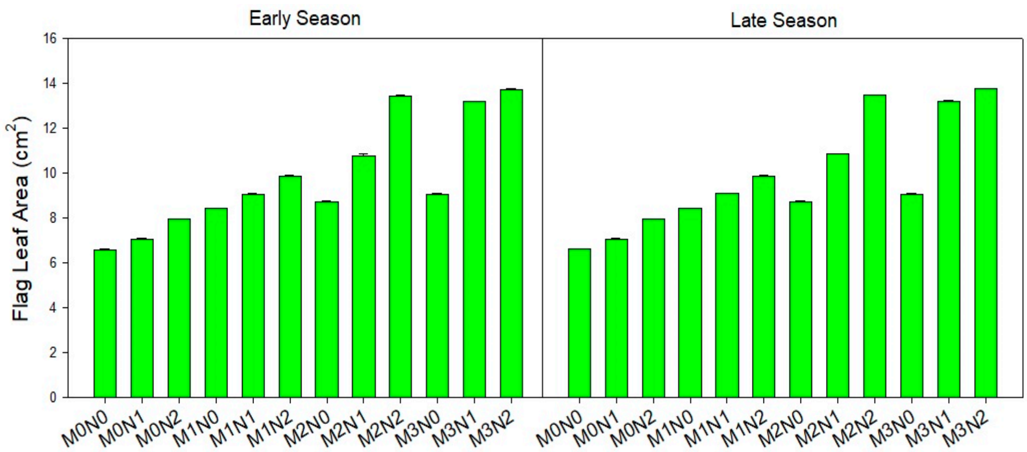

Figure 5. Impact of different levels of arbuscular mycorrhizal fungi and nitrogen fertilizer on flag leaf area (cm^2). Note: The standard error of the mean is represented by vertical bars.

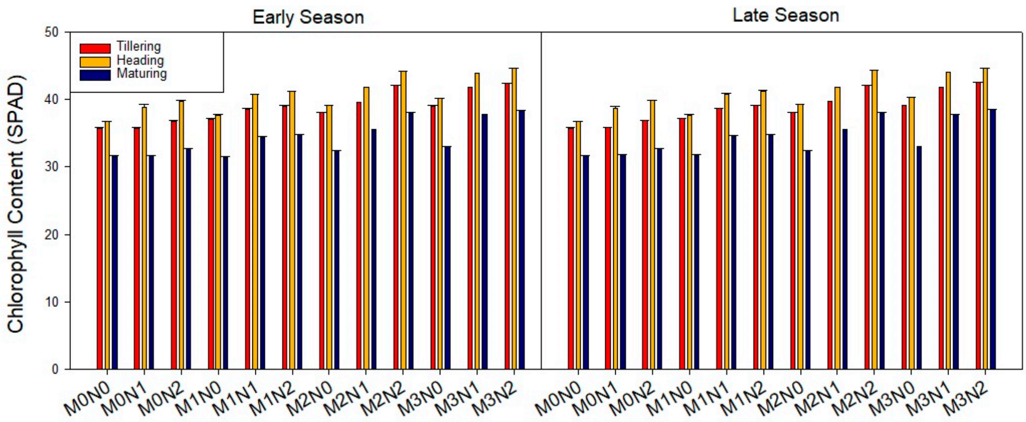

Figure 6. Impact of different levels of arbuscular mycorrhizal fungi and nitrogen fertilizer on SPAD chlorophyll content. Note: The standard error of the mean is represented by vertical bars.

3.3. Root Morphology

Rice RL and RV were significantly different among the combined AMF and N fertilizer treatments during the early and late seasons (Table 2). The M_3N_2 treatment increased rice RL by 54.68% and 56.44% compared to control during both seasons, respectively, followed by M_3N_1 52.04% and 51.65%, and M_2N_2 38.78% and 41.82%. The M_3N_2 treatment enhanced RV by 42.73% and 43.37% compared to control during both seasons, respectively. Our findings indicate that different combinations of AMF and N had different significant effects on improving root morphology. RL was lowest in the M_0N_0 treatment (26.09 cm and 26.22 cm during the early and late seasons, respectively), followed by the M_0N_1 treatment (28 cm and 28.19 cm during the early and late seasons, respectively). The M_0N_0 treatment exhibited the lowest RV of rice with values of 27.43 cm^3 and 27.72 cm^3 during the early and late seasons, respectively), followed by the M_0N_1 treatment (28.91 cm^3 and 29.26 cm^3 during the early and late seasons, respectively).

Table 2. Impact of different levels of arbuscular mycorrhizal fungi and nitrogen fertilizer on the root length (cm) and root volume (cm^3) of rice.

Treatments	Root Morphology			
	Early Season		Late Season	
	RL (cm)	RV (cm^3)	RL (cm)	RV (cm^3)
M_0N_0	26.09 ± 0.08 j	27.43 ± 0.02 k	26.22 ± 0.29 i	27.72 ± 0.06 j
M_0N_1	28.00 ± 0.08 i	28.91 ± 0.01 j	28.19 ± 0.11 h	29.26 ± 0.06 i
M_0N_2	28.21 ± 0.22 i	30.71 ± 0.22 i	28.27 ± 0.13 h	30.98 ± 0.13 h
M_1N_0	29.81 ± 0.30 h	33.04 ± 0.06 h	29.97 ± 0.30 g	33.34 ± 0.14 g
M_1N_1	32.01 ± 0.06 fg	34.33 ± 0.08 g	32.27 ± 0.12 f	34.88 ± 0.05 ef
M_1N_2	33.31 ± 0.07 e	35.61 ± 0.08 e	33.59 ± 0.07 e	36.24 ± 0.18 d
M_2N_0	31.78 ± 0.06 g	34.06 ± 0.18 g	32.00 ± 0.12 f	34.59 ± 0.11 f
M_2N_1	35.21 ± 0.11 d	35.97 ± 0.04 d	36.16 ± 0.05 d	36.54 ± 0.18 d
M_2N_2	36.47 ± 0.07 c	37.57 ± 0.13 b	37.19 ± 0.09 c	38.13 ± 0.20 b
M_3N_0	32.29 ± 0.11 f	34.77 ± 0.12 f	32.43 ± 0.02 f	35.28 ± 0.07 e
M_3N_1	39.67 ± 0.19 b	37.02 ± 0.08 c	39.77 ± 0.15 b	37.59 ± 0.17 c
M_3N_2	40.36 ± 0.11 a	39.16 ± 0.12 a	41.02 ± 0.06 a	39.74 ± 0.18 a
M	**	**	**	**
N	**	**	**	**
M × N	**	**	**	ns
LSD	0.000	0.000	0.000	0.014

Note: Root length (RL), root volume (RV), ± indicates the standard error among the replications, LSD: least significant difference of M × N, M: mycorrhizae, N: nitrogen, M×N: interaction between mycorrhizae and nitrogen, M_0N_0: 0 g AMF + 0 kg N ha^{-1} (control), M_0N_1: 0 g AMF + 90 kg N ha^{-1}, M_0N_2: 0 g AMF + 180 kg N ha^{-1}, M_1N_0: 15 g AMF + 0 kg N ha^{-1}, M_1N_1: 15 g AMF + 90 kg N ha^{-1}, M_1N_2: 15 g AMF + 180 kg N ha^{-1}, M_2N_0: 30 g AMF + 0 kg N ha^{-1}, M_2N_1: 30 g AMF + 90 kg N ha^{-1}, M_2N_2: 30 g AMF + 180 kg N ha^{-1}, M_3N_0: 45 g AMF + 0 kg N ha^{-1}, M_3N_1: 45 g AMF + 90 kg N ha^{-1}, and M_3N_2: 45 g AMF + 180 kg N ha^{-1}. Means with the same lowercase letters in a column are not significantly different ($p > 0.05$) according to Duncan's test. ** indicates a significant difference at $p < 0.01$, and ns: no significant difference ($p > 0.05$).

3.4. Yield Components

The combination of AMF and N fertilizer had a significant effect on the yield components of rice, including panicle number, panicle length, and 1000-grain weight during both seasons (Table 3). The combined application of 45 g AMF and 180 kg N ha^{-1} improved panicle number by 67.44% and 72.35%, and panicle length by 49.72% and 52.31% compared to control during the early and late season, respectively, followed by M_3N_1 and M_2N_2. The M_3N_2 treatment had a higher average 1000-grain weight than the other treatments across the seasons, whereas no significant difference was detected in 1000-grain weight between the M_3N_2 and M_3N_1 treatments. M_3N_2 improved 1000-grain weight by 30.70% and 32.44% compared to control during both seasons, respectively, followed by M_3N_2 29.71% and 31.11%. Our findings show that all of the combined treatments with AMF and N fertilizer improved the yield components compared to AMF or N alone, and the control treatment.

Table 3. Impact of different rates of arbuscular mycorrhizal fungi and nitrogen fertilizer on the panicle number, panicle length, 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)
M_0N_0	19.11 ± 0.11 h	16.42 ± 0.06 f	29.17 ± 0.06 h	18.89 ± 0.22 j	16.61 ± 0.15 g	29.46 ± 0.03 h
M_0N_1	20.22 ± 0.29 g	16.79 ± 0.17 f	29.71 ± 0.16 g	20.44 ± 0.11 i	17.00 ± 0.19 fg	29.91 ± 0.06 h
M_0N_2	21.00 ± 0.00 g	16.87 ± 0.17 f	31.31 ± 0.40 g	21.44 ± 0.11 h	17.38 ± 0.16 f	31.55 ± 0.17 g
M_1N_0	25.00 ± 0.33 f	19.18 ± 0.05 e	33.51 ± 0.24 f	25.33 ± 0.19 g	19.20 ± 0.20 e	33.79 ± 0.02 f
M_1N_1	25.67 ± 0.19 ef	20.16 ± 0.13 d	34.80 ± 0.31 e	26.11 ± 0.11 f	20.56 ± 0.22 d	35.06 ± 0.14 e
M_1N_2	26.00 ± 0.33 e	22.23 ± 0.23 c	36.08 ± 0.18 cd	26.56 ± 0.11 f	22.56 ± 0.11 c	36.43 ± 0.12 c
M_2N_0	27.22 ± 0.29 d	19.12 ± 0.06 e	34.52 ± 0.39 e	27.56 ± 0.11 e	19.67 ± 0.00 e	34.78 ± 0.22 e
M_2N_1	27.78 ± 0.40 cd	23.70 ± 0.32 b	36.43 ± 0.28 c	28.11 ± 0.29 d	23.79 ± 0.28 b	36.88 ± 0.14 c
M_2N_2	28.56 ± 0.11 c	23.99 ± 0.09 b	36.99 ± 0.32 bc	29.00 ± 0.19 c	24.04 ± 0.11 b	37.42 ± 0.12 b
M_3N_0	28.00 ± 0.33 cd	20.22 ± 0.29 d	35.23 ± 0.37 de	28.22 ± 0.11 d	20.78 ± 0.62 d	35.61 ± 0.14 d
M_3N_1	30.78 ± 0.40 b	24.03 ± 0.14 b	37.83 ± 0.24 ab	31.56 ± 0.11 b	24.49 ± 0.14 b	38.63 ± 0.24 a
M_3N_2	32.00 ± 0.19 a	24.59 ± 0.13 a	38.12 ± 0.27 a	32.56 ± 0.11 a	25.30 ± 0.09 a	39.02 ± 0.27 a
M	**	**	**	**	**	**
N	**	**	**	**	**	**
M × N	**	**	ns	**	**	**
LSD	0.000	0.000	0.021	0.000	0.000	0.000

Note: Panicle length (PL), panicle number (PN), ± indicates the standard error among the replications, LSD: least significant difference of M × N, M: mycorrhizae, N: nitrogen, M × N: interaction between mycorrhizae and nitrogen, M_0N_0: 0 g AMF + 0 kg N ha^{-1} (control), M_0N_1: 0 g AMF + 90 kg N ha^{-1}, M_0N_2: 0 g AMF + 180 kg N ha^{-1}, M_1N_0: 15 g AMF + 0 kg N ha^{-1}, M_1N_1: 15 g AMF + 90 kg N ha^{-1}, M_1N_2: 15 g AMF + 180 kg N ha^{-1}, M_2N_0: 30 g AMF + 0 kg N ha^{-1}, M_2N_1: 30 g AMF + 90 kg N ha^{-1}, M_2N_2: 30 g AMF + 180 kg N ha^{-1}, M_3N_0: 45 g AMF + 0 kg N ha^{-1}, M_3N_1: 45 g AMF + 90 kg N ha^{-1}, and M_3N_2: 45 g AMF + 180 kg N ha^{-1}. Means with the same lowercase letters in a column are not significantly different ($p > 0.05$) according to Duncan's test. ** indicates a significant difference at $p < 0.01$, and ns: no significant difference ($p > 0.05$).

3.5. Dry Matter and N Uptake

The results of N uptake and dry matter, including root dry matter and shoot dry matter, are presented in Table 4. Our results indicate that the combined application of AMF and N fertilizer significantly increased N uptake, root dry matter, and shoot dry matter. The M_3N_2 treatment improved N uptake by 107.93% and 108.06% compared to control during both seasons, respectively, followed by M_3N_1 97.50% and 96.35%. The combination of AMF with a 180 kg N ha^{-1} rate was more significant than the other treatments. Root dry matter and shoot dry matter showed that the combination of AMF and N significantly increased weight of root dry and shoot dry matter in rice. The M_3N_2 treatment increased root dry matter by 54.34% and 53.69% compared to control during both seasons, respectively, followed by M_3N_1 48.48% and 47.92%, and M_2N_2 46.41% and 46.61%. Shoot dry matter increased by 26.08% and 28.26% compared to control during both seasons, respectively, followed by

M_2N_2 22.64% and 23.11%, and M_3N_1 21.80% and 23.62%. The minimum values of root dry matter and shoot dry matter were detected in the M_0N_0 and M_0N_1 treatments during the early season and the late season, respectively.

Table 4. Impact of different rates of arbuscular mycorrhizal fungi and nitrogen fertilizer on the root dry matter, shoot dry matter, and nitrogen uptake of rice.

Treatments	Dry Matter and N Uptake					
	Early Season			Late Season		
	RDM (g)	SDM (g)	NU (g polybag^{-1})	RDM (g)	SDM (g)	NU (g polybag^{-1})
M_0N_0	19.34 ± 0.10 k	40.78 ± 0.17 i	3.19 ± 0.00 l	19.48 ± 0.13 i	40.97 ± 0.19 i	3.22 ± 0.00 l
M_0N_1	20.58 ± 0.17 j	41.06 ± 0.08 i	3.35 ± 0.00 k	20.71 ± 0.12 h	41.17 ± 0.07 i	3.37 ± 0.01 k
M_0N_2	21.74 ± 0.20 i	42.03 ± 0.10 h	5.39 ± 0.00 f	21.76 ± 0.06 g	42.23 ± 0.06 h	5.40 ± 0.00 f
M_1N_0	24.24 ± 0.06 h	44.21 ± 0.11 g	4.51 ± 0.00 j	24.64 ± 0.06 f	44.33 ± 0.06 g	4.52 ± 0.00 j
M_1N_1	25.53 ± 0.08 g	45.64 ± 0.06 ef	5.20 ± 0.01 g	25.70 ± 0.05 e	45.76 ± 0.05 e	5.23 ± 0.00 g
M_1N_2	26.81 ± 0.08 e	46.43 ± 0.39 d	5.84 ± 0.00 e	26.94 ± 0.13 d	46.54 ± 0.03 d	5.88 ± 0.01 e
M_2N_0	25.26 ± 0.18 g	45.10 ± 0.12 f	4.60 ± 0.00 i	25.52 ± 0.13 e	45.44 ± 0.04 f	4.61 ± 0.00 i
M_2N_1	27.66 ± 0.10 d	48.27 ± 0.25 c	5.96 ± 0.01 d	27.63 ± 0.07 c	48.51 ± 0.11 c	5.99 ± 0.02 d
M_2N_2	28.32 ± 0.06 c	50.01 ± 0.14 b	6.19 ± 0.00 c	28.56 ± 0.08 b	50.43 ± 0.02 b	6.22 ± 0.00 c
M_3N_0	25.97 ± 0.12 f	46.24 ± 0.37 de	4.76 ± 0.00 h	25.39 ± 0.09 e	46.50 ± 0.06 d	4.80 ± 0.00 h
M_3N_1	28.72 ± 0.16 b	49.67 ± 0.30 b	6.31 ± 0.01 b	28.81 ± 0.16 b	50.64 ± 0.11 b	6.33 ± 0.01 b
M_3N_2	29.86 ± 0.09 a	51.41 ± 0.19 a	6.64 ± 0.01 a	29.94 ± 0.09 a	52.54 ± 0.03 a	6.71 ± 0.00 a
M	**	**	**	**	**	**
N	**	**	**	**	**	**
M × N	**	**	**	**	**	**
LSD	0.000	0.000	0.000	0.000	0.000	0.000

Note: Root dry matter (RDM), shoot dry matter (SDM), nitrogen uptake (NU), ± indicates the standard error among the replications, LSD: least significant difference of M × N, M: mycorrhizae, N: nitrogen, M × N: interaction between mycorrhizae and nitrogen, M_0N_0: 0 g AMF + 0 kg N ha^{-1} (control), M_0N_1: 0 g AMF + 90 kg N ha^{-1}, M_0N_2: 0 g AMF + 180 kg N ha^{-1}, M_1N_0: 15 g AMF + 0 kg N ha^{-1}, M_1N_1: 15 g AMF + 90 kg N ha^{-1}, M_1N_2: 15 g AMF + 180 kg N ha^{-1}, M_2N_0: 30 g AMF + 0 kg N ha^{-1}, M_2N_1: 30 g AMF + 90 kg N ha^{-1}, M_2N_2: 30 g AMF + 180 kg N ha^{-1}, M_3N_0: 45 g AMF + 0 kg N ha^{-1}, M_3N_1: 45 g AMF + 90 kg N ha^{-1}, and M_3N_2: 45 g AMF + 180 kg N ha^{-1}. Means with the same lowercase letters in a column are not significantly different ($p > 0.05$) according to Duncan's test. ** indicates a significant difference ($p < 0.01$).

4. Discussion

4.1. Impact of the Combination of AMF and N Fertilizer on Physicochemical Properties of Soil

Soil is an essential component of the sustainable development of any crop. Based on our study, the combination of AMF and N fertilizer enhanced the soil physicochemical properties. The combined application of 45 g AMF polybag^{-1} and 180 kg N ha^{-1} had the greatest impact on improving soil properties. Adding AMF increased the soil pH, possibly because AMF improves the soil bacterial community and increases bacterial metabolites, which increase the soil pH [63,64]. Our findings indicate that a high N application decreased the pH of the soil. This was similar to other studies reporting that adding N decreases soil pH [65–68] because N fertilizer (urea) increases the nitrate concentration [69]. In this case, the continuous use of ammoniacal fertilizers tends to acidify the soil [70], particularly when applied at a high rate [71]. The combination of AMF and N fertilizer increased AP because AMF play a key role in improving P availability in the soil, and the metabolic activities of AMF produce alkaline phosphatases, which cleave substrates present in the soil and allow the phosphate to be accessible [64,69]. AMF colonization also contributes to P and K uptake by plants [72]. AMF improves SOM because many microbial components in the soil work synergistically with AMF, promoting growth and protecting the plants [71,72]. AMF communities affect the physicochemical environment of the rhizosphere and control various soil microbial interactions [73]. The combined application of AMF and N fertilizer increased total soil N because AMF increase microbial biomass N and plant biomass, thus reducing the availability of N substrates (NH_4^+ and NO_3^-) in the soil for N_2O producers and decreasing N_2O emissions [73–75].

4.2. Impact of the Combination of AMF and N Fertilizer on Rice Growth

The combination of AMF and N fertilizer significantly improved rice growth, including plant height, tiller number, FLA, chlorophyll SPAD, and day of flowering. Our results show that the combination of 45 g AMF polybag^{-1} and 180 kg N ha^{-1} increased plant height to the maximum. Similarly, previous studies found that AMF increased plant growth parameters of various crops [76–81]. This growth stimulation is linked to the fact that AMF extends the absorbing network beyond the nutrient depletion zones of the rhizosphere, which allows access to a larger volume of soil and AMF uptake of several major nutrients such as N and P [82–84], which improves the supply of nutrients [85–91]. This result also indicates that the high N level enhanced rice plant height. This was similar to a previous study reporting that increased plant height with a high N level is associated with greater availability of N in the soil and higher uptake by plants [92]. SPAD chlorophyll, tiller number, FLA, and day of flowering also increased under high N (180 kg ha^{-1}). This result follows previous research indicating that N is important for the growth and development of the aboveground and belowground structures of the rice plant [93]. SPAD units reflect relative crop N status and yield level. Research by Jabboravo et al. showed that AMF significantly enhanced the total chlorophyll content by 36.6%. AMF can improve water use efficiency [94], which affects plant growth parameters such as shoot fresh weight, dry weight, leaf number, leaf area, and plant height [95]. On the other hand, the application of N fertilizer affects the growth of aerial components, including leaf area, as well as the synthesis of pigments in leaves responsible for photosynthesis [96], and N stimulates the tiller number and flowering time of rice [92,93,96].

4.3. Impact of the Combination of AMF and N Fertilizer on Root Morphology and Dry Matter

Root development is intricately connected to environmental factors in the soil including water, oxygen, temperature, and nutrients [97,98]. In this study, we found that the higher doses of AMF and N fertilizer increased RL and RV the most. This was consistent with previous research indicating that AMF significantly increased RL and RV by 37% and 65%, respectively [99]. Moreover, AMF promotes the development of lateral roots, which can produce finer roots, thus increasing the uptake of water and nutrients from the soil [100,101]. The enhanced root absorption results from the larger root system due to AMF hyphae, which increase the area beyond the root zone, thereby increasing the available volume of the soil solution [82,99,102]. Our results indicate that the combination of 45 g AMF polybag^{-1} and 180 kg N ha^{-1} (2.27 g polybag^{-1}) increased the RL and RV of rice the most. This was consistent with previous research reporting that 4 g N pot^{-1} increases the number of roots, root diameter, RDM, and RV [103]. Root and shoot dry matter are also positively affected when AMF and N fertilizer are applied. Our results are similar to previous studies showing that the shoot and root biomass are significantly greater in AMF plants than in non-AMF plants [103–108]. Other researchers indicated that AMF enhanced the shoot and root development and noticeably elevated root colonization after 1 year of inoculation [109].

4.4. Impact of the Combination of AMF and N Fertilizer on Yield Components and N Uptake

The combination of AMF and N fertilizer increased the yield components, including panicle number, panicle length, and 1000-grain weight. These findings are similar to previous research showing that AMF had a significant effect on production and distribution of plant biomass including root biomass and shoot biomass [110], and another study reporting that AMF accelerates the translocation of nutrients from the shoot to the grain and increases the harvest index [111,112]. We found that the combination of 45 g AMF polybag^{-1} and 180 kg N ha^{-1} increased the yield components. Our findings indicate that N application increases the yield, which is consistent with a previous study reporting that the rate of N fertilizer applied is strongly related to crop yield, and N is important for rice yield because plant N status affects the development of the grain yield component [110,113]. The combination of AMF and N fertilizer also increased total N uptake by rice. This is consistent with previous studies showing that AMF significantly increases N uptake by 35% compared to non-AMF plants [114] and that AMF absorb and transfer N to host plants [115,116] because the AMF hyphae network extends more than 10 cm beyond the root surface, which helps obtain inorganic N from the soil more quickly and widely [117].

5. Conclusions

The combination of AMF and N fertilizer significantly affected soil physicochemical properties, rice growth and productivity, and N uptake. The combined application of 45 g AMF polybag^{-1} and 180 kg N ha^{-1} treatment significantly enhanced rice growth and productivity by improving root morphology (RL and RV) and N uptake. Overall, the combined application of AMF and N fertilizer provided an ideal environment to promote soil properties, rice growth, rice productivity, and nutrient uptake during agricultural production. To find out more significant effects of AMF, we suggest that other studies analyze the type of soil, AMF diversity in the soil, and crop cultivar before applying the AMF. It will also be necessary to further study the long-term effects of AMF and N fertilizer on crop growth and soil nutrient cycling. In conclusion, the combined application of AMF and N fertilizer is a good formula for enhancing rice growth and productivity by improving root growth and soil properties.

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