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# *Rhizoglomus intraradices* Is More Prominent in Improving Soil Aggregate Distribution and Stability Than in Improving Plant Physiological Activities

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**Abstract:** Arbuscular mycorrhizal fungi (AMF) confer positive and negative effects on many plants, but it is unclear whether AMF has an effect on soil fertility, aggregate distribution, and stability. The aim of this study was to analyze the effects of *Rhizoglomus intraradices* on plant growth, root morphology, leaf chlorophyll and gas exchange, sugar concentrations, and soil nutrients, aggregate distribution, and stability in marigold (*Tagetes erecta* L.), maize (*Zea mays* L.), white clover (*Trifolium repens* L.), and vetch (*Vicia villosa* Roth.) plants. Twelve weeks after *R. intraradices* inoculation, maize presented the highest mycorrhizal development, while mycorrhizal dependence was shown to be the decreasing trend in marigold > white clover > vetch > maize. AMF inoculation significantly increased the chlorophyll index of marigold and white clover, the net photosynthetic rate of white clover, the stomatal conductance of maize and white clover, and the transpiration rate of maize. Fructose, glucose, and sucrose in the four plants were differentially affected by *R. intraradices*. *R. intraradices* significantly increased the soil organic carbon (SOC) of marigold, maize, and white clover, the Olsen-P of white clover, the available K content of marigold, the easily extractable glomalin-related soil protein (GRSP) of maize, and the difficultly extractable and total GRSP levels of marigold and vetch. In addition, *R. intraradices* significantly increased the stability of soil water-stable aggregates (WSAs) in all four plants, plus it increased WSA at 0.5–4 mm sizes. Root AMF colonization was significantly positively correlated with WSA stability, SOC, difficultly extractable GRSP, and total GRSP. It is concluded that AMF-triggered changes in plant growth, physiological activities, and soil fertility depended on plant species, but AMF-improved WSA distribution and stability were not dependent on plant species.

**Keywords:** aggregate stability; glomalin; maize; mycorrhizal fungi; soil organic carbon



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## 1. Introduction

Arbuscular mycorrhizal fungi (AMF) in the soil can colonize the roots of most terrestrial plants, thus establishing a reciprocal symbiosis with plants [1–3]. AMF are not strictly specific and can therefore form mycorrhizal structures with most plants [4]. An AMF strain can colonize various plants, and a plant can be colonized by a variety of AMF [5]. Nevertheless, AMF play an irreplaceable role in regulating plant interspecific competition and the soil nutrient cycle [6,7]. AMF can regulate the absorption of soil nutrients by plants, enhance stress resistance, stabilize soil structure, improve soil physical and chemical properties, and improve a plant's competitiveness and survival rate [8,9].

AMF have diverse effects on a host plant's growth, including improvement, inhibition, and no significant effect [10]. Jifon et al. [11] reported that *Rhizoglomus intraradices*

inoculation increased the growth of *Citrus aurantium* plants at suitable $CO_2$ concentrations, while an inhibition of plant growth under mycorrhization conditions was found at high $CO_2$ concentrations. The growth changes of tomatoes inoculated with *R. intraradices* depended on the tomato varieties, in which the fungus improved plant growth and enhanced antioxidant enzyme activities in the Momotaro variety but not the Rodeo variety [12]. In addition, different AMF species have diverse effects on the same host plant. For example, in *Vicia villosa* plants, *R. intraradices* showed a prominent positive effect on plant growth, root architecture, and sugar concentrations among *Diversispora spurca*, *Funneliformis mosseae*, and *R. intraradices* [13]. The competitive ratio contribution of *F. mosseae* to P was higher than that of *R. intraradices* in the maize–rape intercropping [14]. Yao et al. [15] also found that inoculation with *F. mosseae* and *Glomus versiforme* significantly improved plant growth in maize, soybean, and white clover but not wheat, along with the decreasing order of maize > soybean > white clover > wheat in terms of mycorrhizal dependence. Earlier studies revealed that AMF roles in maize are dependent on the soil conditions, fungal isolated environment, and tillage systems [16–18]. These results suggest that the responses between mycorrhizal fungi and plants are variable and complex.

Soil aggregates are an important component of soil structure, as they influence processes such as soil organic carbon (SOC) mineralization and microbial activity [19,20]. The formation of soil aggregates is influenced by various biotic and abiotic factors [21]. The distribution and stability of soil aggregates are commonly used to assess soil structure [22]. Macroaggregates (>0.25 mm) are formed and stabilized by soil organic matter, root exudates, and microorganisms [20]. AMF make important contributions to changing soil fertility, structure, and stability [23,24]. AMF enhance soil structure through the entanglement of their hyphae and the release of glomalin-related soil protein (GRSP), which, thus, promotes the formation and stability of soil aggregates [25]. In Lane Late navel orange, field inoculation with mycorrhizal fungi changed soil nutrient levels to some extent, dependent on the AMF species [26]. For example, *D. spurca* did not change soil nitrate nitrogen levels, but it inhibited soil available K levels, and increased soil Olsen-P and ammonium nitrogen levels; *D. versiformis* inhibited the levels of ammonium nitrogen, nitrate nitrogen, and available K levels, but increased the levels of Olsen-P in soil. These AMF inoculations, however, collectively improved soil aggregate stability in Lane Late navel orange, as compared with non-AMF control. In Newhall navel orange, *D. versiformis* and *D. spurca* increased soil Olsen-P levels and aggregate stability [27]. Inoculation with *F. mosseae*, *D. spurca*, and *R. intraradices* significantly reduced soil Olsen-P and available K levels in vetch [13]. As a result, AMF inoculation can change soil nutrient levels and soil aggregate stability and distribution, but whether this effect also depends on the host plant species has not been clarified.

Marigold (*Tagetes erecta* L.) is an ornamental plant used in medicine and cosmetics due to its rich lutein [28]. Maize (*Zea mays* L.) is an important food crop [29]. White clover (*Trifolium repens* L.) is an important perennial legume forage [30,31]. Vetch (*Vicia villosa* Roth.) is an annual herb in the legumes, which has the characteristics of increasing soil fertility and being a green fertilizer [13]. The present study assessed the responses of the four plants to a popular arbuscular mycorrhizal fungus (*Rhizoglomus intraradices*) in terms of plant growth, leaf gas exchange, sugar content, and soil fertility, aggregate distribution, and stability.

## 2. Materials and Methods

### *2.1. AMF Strains*

The arbuscular mycorrhizal fungal strain used here was *R. intraradices*, which was obtained from the Bank of Glomeromycota in China (BGC). This strain was collected from the Ecological Experiment Station of Red Soil, Chinese Academy of Sciences, Yujiang, China. After this strain was morphologically identified, single spore of this fungus was cultured at BGC using sorghum as the host [32]. The fungus was trapped by white clover for 10 weeks under potted conditions. After removal of the aboveground part of the plant, root

segments and potted substrates were collected as the mycorrhizal inoculum, in which spore density was determined by the wet sieving decantation–sucrose centrifugation method [33], achieving 125 spores/10 g.

### *2.2. Plant Culture and Experimental Design*

Seeds of marigold, maize, white clover, and vetch were provided by the Muyang Douyan Seed Industry Co., Ltd. (Muyang, China), Hefei Hefeng Seed Industry Co., Ltd. (Hefei, China), Muyang Huaxiang Seed Industry Co., Ltd. (Muyang, China), and Hubei Academy of Forestry (Wuhan, China), respectively. On May 19, 2022, these seeds were sown in plastic pots of 13.5 cm × 18.5 cm × 16.5 cm (height × top diameter × bottom diameter, respectively), each filled with 800 g of growth substrate plus 200 g inoculums of *R. intraradices*. Here, the growth substrate was a sand/soil mixture in a volume ratio of 1:1, and autoclaved at 121 °C for 2 h before use. The soil used here was Ferralsol (FAO system), whose characteristics were described by Liu et al. [23]. Plants inoculated without *R. intraradices* also received an equal amount of autoclaved mycorrhizal inoculums and 2 mL of filtered (25 μm) solution of the same dose of inoculums to establish the similar microbial community except for *R. intraradices*, as well as the same nutrient levels [34]. All plant materials were kept in a controlled incubator, the environmental conditions of which were described by Meng et al. [24]. The experiment ended on 11 August 2022.

The experiment was conducted using the two-factor experimental design, with the first factor being inoculation with *R. intraradices* or not, and the second factor being four plant species. Therefore, the experiment consisted of eight treatments, each of which was replicated four times, with a total of 32 pots arranged randomly.

### *2.3. Variable Determinations*

Before harvesting, leaf gas exchange parameters including transpiration rate, net photosynthetic rate, and stomatal conductance were measured using the Li-6400 photosynthetic apparatus (LI-COR Inc., Lincoln, NE, USA) during 9:00–11:00 a.m. on a sunny day by selecting the fourth mature leaf from the top.

Plant height, stem diameter, and leaf number were measured when plants were harvested. Subsequently, shoot and root biomass was dried at 80 °C for 48 h and then weighed. The harvested roots were scanned and analyzed for root morphological parameters using a WinRHIZO (Regent Instruments Inc., Quebec, QC, Canada).

Six 1 cm root segments of each plant were stained with 0.05% of trypan blue [35], and the rate of root AMF colonization was calculated as described by Liang et al. [36]. Hyphal length in the soil was calculated using the protocol of Ames and Bethlenfalvay [37]. Spores of air-dried growth substrate were separated using the wet sieving decantation–sucrose centrifugation method [33], and the spore number was counted under a stereomicroscope.

The chlorophyll index (Chi) was determined using a portable Plant Polyphenol–Chlorophyll Meter (Force-A, Orsay, France). Plant sugars were extracted using 50 mg of dried samples passed through a 1 mm sieve with 4 mL of 80% ethanol solution in a water bath at 80 °C for 40 min and then centrifuged at 2500× *g*/min for 6 min. The same method was used to extract once more and the two supernatants were combined for the determination of sucrose, fructose, and glucose. Leaf and root fructose, glucose, and sucrose concentrations were assayed using a colorimetric method [38]. For the sucrose assay, 150 μL of supernatant was reacted with 150 μL of 2 mol/L NaOH in a 100 °C water bath for 5 min, and 2.1 mL of 10 mol/L hydrochloric acid and 0.6 mL of 0.1% resorcinol were added at 80 °C for 10 min, whose absorbance value was measured at 480 nm. Fructose concentrations were determined by incubation of 150 μL of supernatant in 2.8 mL 10 mol/L HCl and 0.8 mL 0.1% resorcinol in a water bath at 80 °C for 10 min, and the absorbance value was measured at 480 nm. For the glucose assay, the supernatant and the enzyme preparation solution were reacted in a water bath at 30 °C for 5 min, and then 2 mL of 10 mol/L sulfuric acid was added and then colorimetrically determined at 460 nm.

Soil $NH_4^+$-N, $NO_3^-$-N, Olsen-P, and available K levels were measured using a Soil Nutrient Detector (Shandong Hengmei Electronic Technology Co. Ltd., Weifang, China). Easily extractable glomalin-related soil protein (EE-GRSP) and difficultly extractable glomalin-related soil protein (DE-GRSP) were extracted using the method of He et al. [13]. The protein concentration was measured using the assay of Bradford [39]. Total glomalin-related soil protein (T-GRSP) was the sum of EE-GRSP and DE-GRSP. SOC content was determined by the wet oxidation method [40].

The distribution of soil water-stable aggregates (WSAs) in the size of 2–4, 1–2, 0.5–1, and 0.25–0.5 mm was determined by the wet sieving method with the DM200-IV Soil Aggregate Analyzer (Shanghai Decode Information Technology Co., Ltd., Shanghai, China). WSA stability was performed by calculating mean weight diameter (MWD) described by Cheng et al. [41]. The formula was as follows:

$$\text{MWD} = \sum_{i=1}^{n} RiWi \quad (1)$$

where $n$ is the number of WSA size fractions, $Ri$ is the average diameter of the $i$ size, and $Wi$ is the proportion of WSA in the $i$ size.

### *2.4. Data Analysis*

Data were analyzed by the two-way analysis of variance using SAS software, and the Duncan's multiple range tests were used for significant comparisons between treatments ($p < 0.05$). SigmaPlot (v10) was used to make the figures.

## 3. Results

### *3.1. Effects on Plant Growth and Mycorrhizal Dependence*

Inoculation with *R. intraradices* affected plant growth parameters to different degrees (Figure 1; Table 1). In marigold, *R. intraradices* significantly increased plant height, stem diameter, leaf number, and root biomass by 94%, 109%, 70%, and 139%, respectively, compared with the non-AMF control. Nevertheless, *R. intraradices* only significantly increased stem diameter in maize by 30%, and even inhibited plant height by 22%. In white clover, *R. intraradices* significantly increased stem diameter and leaf number by 108% and 136%, respectively, compared with non-AMF treatment. The *R. intraradices*-inoculated vetch plants recorded 22%, 56%, and 56% higher plant height, stem diameter, and leaf number, respectively, than the non-AMF-inoculated plants. Among the four plants, their mycorrhizal dependence was listed as the trend of marigold > white clover > vetch > maize in decreasing order.

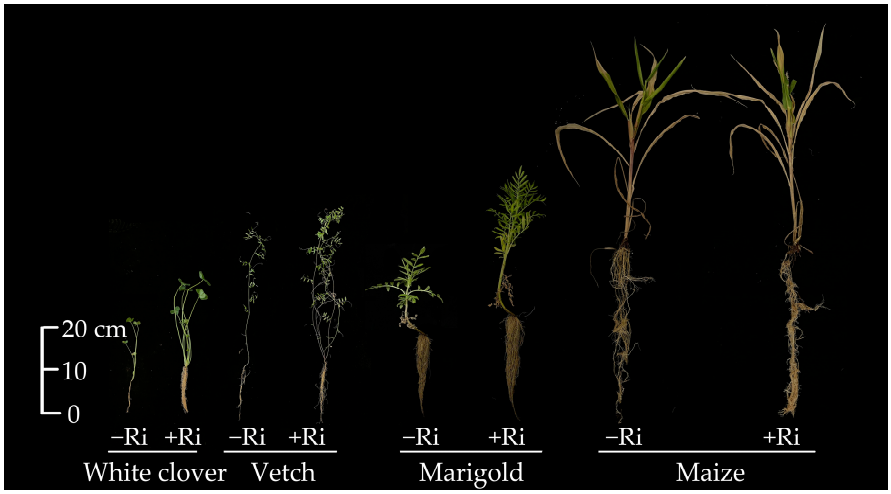


**Figure 1.** Plant growth behavior of white clover, vetch, maize, and marigold inoculated with and without *Rhizoglomus intraradices* for 12 weeks. See Table 1 for abbreviations.

**Table 1.** Effects of *Rhizoglomus intraradices* on growth and mycorrhizal dependence of four plants.

| Plants | AMF | Plant Height (cm) | Stem Diameter (mm) | Leaf Number (num./Plant) | Biomass (g/Plant) | | Mycorrhizal Dependence (%) |
|---|---|---|---|---|---|---|---|
| | | | | | Shoot | Root | |
| Marigold | +Ri | 35.43 ± 2.41 d | 5.04 ± 0.16 b | 15.8 ± 2.2 a | 0.331 ± 0.062 b | 1.288 ± 0.466 b | 88.87 ± 8.31 a |
| | −Ri | 18.30 ± 1.46 e | 2.41 ± 0.32 c | 9.3 ± 1.3 cd | 0.185 ± 0.047 bc | 0.540 ± 0.108 c | |
| Maize | +Ri | 48.75 ± 2.93 b | 6.13 ± 0.34 a | 10.8 ± 1.0 bc | 1.190 ± 0.322 a | 4.960 ± 0.277 a | −11.47 ± 2.88 d |
| | −Ri | 59.58 ± 4.43 a | 4.72 ± 0.28 b | 11.0 ± 0.8 bc | 1.140 ± 0.151 a | 4.798 ± 0.584 a | |
| White clover | +Ri | 17.98 ± 0.79 f | 1.04 ± 0.15 d | 8.3 ± 0.5 d | 0.047 ± 0.005 c | 0.106 ± 0.008 d | 48.19 ± 5.00 b |
| | −Ri | 14.45 ± 1.02 e | 0.50 ± 0.18 e | 3.5 ± 0.6 e | 0.018 ± 0.013 c | 0.039 ± 0.011 d | |
| Vetch | +Ri | 40.83 ± 1.12 c | 1.03 ± 0.21 d | 12.5 ± 0.6 b | 0.025 ± 0.010 c | 0.051 ± 0.009 d | 29.23 ± 6.22 c |
| | −Ri | 33.45 ± 2.73 d | 0.66 ± 0.10 e | 8.0 ± 1.6 d | 0.004 ± 0.001 c | 0.045 ± 0.019 d | |
| Significance | | | | | | | |
| Ri | | ** | ** | ** | ** | ** | ** |
| Plants | | ** | ** | ** | NS | * | ** |
| Interaction | | ** | ** | ** | NS | NS | ** |

Data (means ± SD, $n = 4$) followed by different letters among treatments indicate significant ($p < 0.05$) differences. NS, not significant ($p > 0.05$); +Ri, inoculation with *Rhizoglomus intraradices*; −Ri, inoculation without *R. intraradices*; *, $p < 0.05$; **, $p < 0.01$.

### 3.2. Effects on Spore Density, Root Mycorrhizal Colonization, and Soil Hyphal Length

In uninoculated marigold, maize, white clover, and vetch plants, root arbuscular mycorrhizal structure, soil spores, and soil hyphae were not seen (Figure 2a–c). The roots of four plants inoculated with *R. intraradices* had arbuscular mycorrhizal structures (Figure 3a–d), with root colonization rates ranging from 78.5 to 98.3% (Figure 2b), soil hyphal lengths ranging from 41.0 to 116.0 cm/g (Figure 2c), and spore densities of 35–223 spores/10 g (Figure 2a). Among them, spore density and soil hyphal length were the highest on maize, and the root AMF colonization rate was the highest on marigold, while inoculated vetch plants presented the lowest spore density, soil hyphal length, and root AMF colonization rate among all plants. Spore density, soil hyphal length, and the root AMF colonization rate were significantly interacted by *R. intraradices* inoculations and plant species.

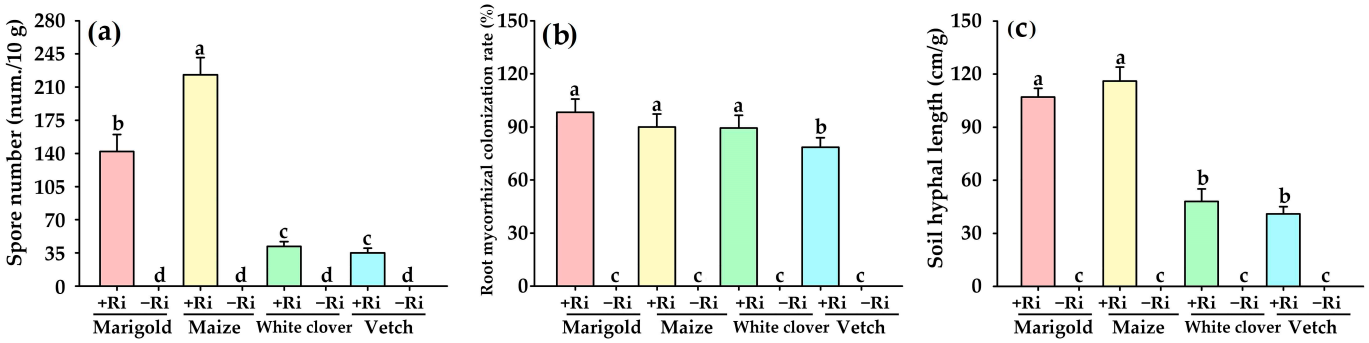


**Figure 2.** Effects of *Rhizoglomus intraradices* on spore density (**a**), root mycorrhizal colonization rate (**b**), and soil hyphal length (**c**) in four plants. Data (means ± SD, $n = 4$) followed by different letters above the bar indicate significant ($p < 0.05$) differences. See Table 1 for abbreviations.

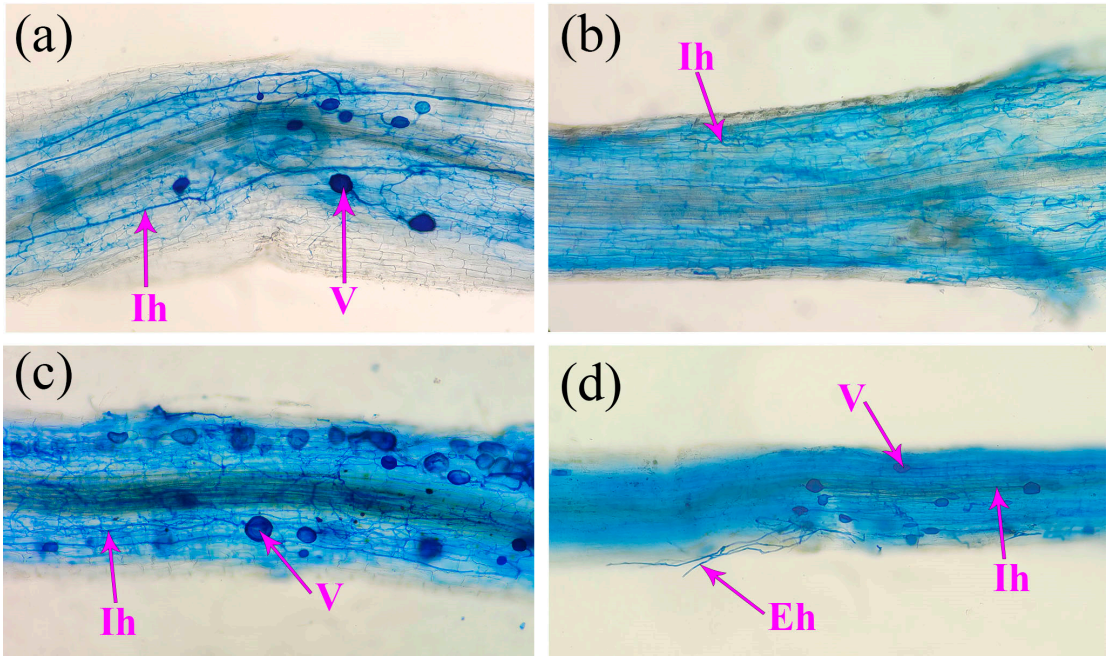


**Figure 3.** Arbuscular mycorrhizae in roots of marigold (**a**), maize (**b**), white clover (**c**), and vetch (**d**) after *Rhizoglomus intraradices* inoculation for 12 weeks. Abbreviations: Eh, extraradical hyphae; Ih, intraradical hyphae; V, vesicle.

### *3.3. Effects on Root Morphological Variables*

The root morphology of the four selected plants after inoculation with *R. intraradices* was superior to that of the uninoculated plants (Figure 1; Table 2). Root total length, projected area, surface area, diameter, and volume were significantly greater in *R. intraradices*-inoculated marigold plants than in uninoculated plants by 144%, 65%, 74%, 85%, and 158%, respectively. In the case of white clover, inoculation with *R. intraradices* significantly increased the root surface area and diameter by 52% and 69%, respectively, compared to the uninoculated treatment. In vetch, the root total length, projected area, and surface area were significantly increased by 96%, 54%, and 24%, respectively, after inoculation with *R. intraradices*. Inoculation with *R. intraradices* only significantly increased root diameter by 13%, compared with the uninoculated treatment. All root morphological variables were significantly interacted by plant species and inoculation with *R. intraradices*.

**Table 2.** Effects of *Rhizoglomus intraradices* on root morphological variables of four plants.

| Plants | AMF | Total Length (cm) | Project Area ($cm^2$) | Surface Area ($cm^2$) | Diameter (mm) | Volume ($cm^3$) |
|---|---|---|---|---|---|---|
| Marigold | +Ri | 197.28 ± 16.25 b | 10.85 ± 1.65 b | 18.00 ± 1.75 b | 0.76 ± 0.10 a | 2.795 ± 1.063 b |
| | −Ri | 80.97 ± 10.37 c | 6.56 ± 0.31 c | 10.37 ± 1.32 cd | 0.41 ± 0.04 e | 1.083 ± 0.215 c |
| Maize | +Ri | 270.54 ± 6.24 a | 12.92 ± 0.45 a | 20.99 ± 0.19 a | 0.69 ± 0.09 a | 4.044 ± 0.521 a |
| | −Ri | 268.93 ± 11.73 a | 12.87 ± 0.80 a | 20.92 ± 0.62 a | 0.61 ± 0.02 bc | 3.578 ± 0.710 a |
| White clover | +Ri | 30.81 ± 9.29 d | 2.94 ± 0.84 d | 10.43 ± 0.60 cd | 0.44 ± 0.07 de | 0.230 ± 0.071 d |
| | −Ri | 24.54 ± 7.69 d | 3.46 ± 0.76 d | 7.06 ± 1.43 e | 0.26 ± 0.03 f | 0.124 ± 0.018 d |
| Vetch | +Ri | 80.16 ± 18.85 c | 6.95 ± 1.87 c | 11.18 ± 2.41 c | 0.43 ± 0.05 de | 0.335 ± 0.192 d |
| | −Ri | 40.98 ± 6.76 d | 4.52 ± 0.59 d | 9.04 ± 0.43 d | 0.53 ± 0.09 cd | 0.281 ± 0.126 d |
| Significance | | | | | | |
| Ri | | ** | ** | ** | ** | ** |
| Plants | | ** | ** | ** | ** | ** |
| Interaction | | ** | ** | ** | ** | ** |

Data (means ± SD, $n = 4$) followed by different letters among treatments indicate significant ($p < 0.05$) differences. See Table 1 for abbreviations. **, $p < 0.01$.

### 3.4. Effects on Leaf Chlorophyll Index and Gas Exchange

Plants inoculated with *R. intraradices* showed relatively better leaf Chi and gas exchange variables than uninoculated plants (Table 3). Compared with non-inoculation with *R. intraradices*, inoculation with *R. intraradices* significantly increased Chi of marigold by 30%, Chi, the net photosynthetic rate, and stomatal conductance of white clover by 44%, 42%, and 57%, respectively, and the stomatal conductance and transpiration rate of maize by 69% and 44%, respectively. Significant interactions occurred in Chi, the net photosynthetic rate, and stomatal conductance.

**Table 3.** Effects of *Rhizoglomus intraradices* on leaf chlorophyll index and gas exchange of four plants.

| Plants | AMF | Chlorophyll Index | Net Photosynthetic Rate (μmol/m$^2$/s) | Stomatal Conductance (μmol/m$^2$/s) | Transpiration Rate (mmol/m$^2$/s) |
|---|---|---|---|---|---|
| Marigold | +Ri | 18.46 ± 2.44 b | 1.80 ± 0.15 d | 0.05 ± 0.03 c | 0.97 ± 0.44 c |
| | −Ri | 14.23 ± 1.89 c | 1.49 ± 0.51 d | 0.04 ± 0.01 c | 0.78 ± 0.17 c |
| Maize | +Ri | 15.38 ± 1.84 c | 8.26 ± 0.64 b | 0.27 ± 0.05 a | 5.14 ± 0.80 a |
| | −Ri | 12.78 ± 1.68 cd | 7.70 ± 1.27 bc | 0.16 ± 0.07 b | 3.57 ± 1.22 b |
| White clover | +Ri | 30.13 ± 2.05 a | 9.73 ± 1.22 a | 0.22 ± 0.06 a | 3.80 ± 1.16 b |
| | −Ri | 20.96 ± 2.81 b | 6.86 ± 0.51 c | 0.14 ± 0.04 b | 3.05 ± 0.64 b |
| Vetch | +Ri | 13.70 ± 2.14 cd | 2.35 ± 0.25 d | 0.05 ± 0.02 c | 1.01 ± 0.39 c |
| | −Ri | 10.90 ± 0.53 d | 2.13 ± 0.60 d | 0.04 ± 0.01 c | 0.99 ± 0.24 c |
| Significance | | | | | |
| Ri | | ** | ** | ** | ** |
| Plants | | ** | ** | ** | * |
| Interaction | | * | ** | * | NS |

Data (means ± SD, $n$ = 4) followed by different letters among treatments indicate significant ($p < 0.05$) differences. See Table 1 for abbreviations. NS, not significant ($p > 0.05$); *, $p < 0.05$; **, $p < 0.01$.

### 3.5. Effects on Leaf and Root Sugar Concentrations

Inoculation with *R. intraradices* affected leaf and root sugar concentrations to varying degrees (Table 4). In leaves, inoculation with *R. intraradices* significantly increased glucose concentrations of marigold, sucrose concentrations of maize, and sucrose and fructose concentrations of vetch by 46%, 39%, 57%, and 60%, respectively, compared with non-*R. intraradices* inoculation. Nevertheless, mycorrhizal colonization significantly reduced sucrose concentrations of white clover and glucose concentrations of vetch by 18% and 51%, respectively, as compared with non-mycorrhizal treatment. In roots, inoculation with *R. intraradices* significantly increased sucrose, fructose, and glucose concentrations of maize by 39%, 23%, and 19%, respectively, compared with no inoculation with *R. intraradices*. There was 55% and 48% significantly higher root sucrose in white clover and vetch, respectively, after inoculation with *R. intraradices*. There was a significant interaction in leaf sucrose, root sucrose, and leaf glucose between inoculation with *R. intraradices* and plant species.

### 3.6. Effects on Soil Nutrient Levels

Inoculation with *R. intraradices* significantly increased soil available K levels in marigold by 69% and soil Olsen-P levels in white clover by 20%, while it distinctly reduced soil available K levels in white clover by 68% and vetch by 26%, compared with the non-inoculation treatment (Table 5). In addition, inoculation with *R. intraradices* dramatically increased soil EE-GRSP levels in maize by 30%, soil DE-GRSP levels in marigold and vetch by 72% and 46%, respectively, and soil T-GRSP levels in marigold and vetch by 44% and 28%, respectively, compared with the non-inoculation treatment. Inoculation with *R. intraradices* did not affect soil $NH_4^+$-N and $NO_3^-$-N levels. The SOC contents of inoculated marigold, maize, and white clover plants were 24–54% higher than those of uninoculated plants. Compared to maize that was not inoculated with *R. intraradices*, its SOC content was reduced by 21% after inoculation with *R. intraradices*. Plant species and the inoculation of *R. intraradices*

significantly and interactively changed soil Olsen-P, and available K, EE-GRSP, DE-GRSP, T-GRSP, and SOC concentrations.

**Table 4.** Effects of *Rhizoglomus intraradices* on sucrose, fructose, and glucose of four plants.

| Plants | AMF | Sucrose (mg/g) | | Fructose (mg/g) | | Glucose (mg/g) | |
|---|---|---|---|---|---|---|---|
| | | Leaf | Root | Leaf | Root | Leaf | Root |
| Marigold | +Ri | 32.04 ± 1.27 d | 54.91 ± 8.08 e | 42.61 ± 9.23 b | 90.30 ± 5.45 bc | 39.79 ± 1.68 a | 16.12 ± 1.41 c |
| | −Ri | 33.59 ± 5.78 d | 46.33 ± 9.07 ef | 38.18 ± 1.60 b | 79.02 ± 4.83 c | 27.17 ± 4.95 b | 12.82 ± 2.57 c |
| Maize | +Ri | 56.11 ± 4.30 a | 141.17 ± 12.03 a | 63.30 ± 8.75 a | 124.88 ± 9.42 a | 22.19 ± 3.87 bc | 25.81 ± 3.37 a |
| | −Ri | 40.25 ± 5.13 c | 101.24 ± 4.21 b | 57.90 ± 11.48 a | 101.37 ± 18.15 b | 20.51 ± 3.94 c | 21.75 ± 3.30 b |
| White clover | +Ri | 49.46 ± 4.08 b | 62.60 ± 5.94 cd | 34.51 ± 4.28 bc | 30.50 ± 2.98 d | 22.55 ± 4.01 bc | 26.06 ± 0.70 a |
| | −Ri | 58.12 ± 2.63 a | 40.27 ± 3.91 f | 27.33 ± 0.76 cd | 23.86 ± 1.66 d | 24.54 ± 5.08 bc | 23.26 ± 3.47 ab |
| Vetch | +Ri | 59.92 ± 3.99 a | 67.60 ± 9.73 c | 35.25 ± 5.91 bc | 30.77 ± 4.14 d | 26.82 ± 5.29 bc | 15.11 ± 1.15 c |
| | −Ri | 38.12 ± 5.22 cd | 45.71 ± 8.17 ef | 21.98 ± 2.38 d | 26.28 ± 2.70 d | 40.41 ± 1.07 a | 15.64 ± 2.30 c |
| Significance | | | | | | | |
| Ri | | ** | ** | ** | ** | ** | ** |
| Plants | | ** | ** | ** | ** | NS | * |
| Interaction | | ** | ** | NS | NS | ** | NS |

Data (means ± SD, $n = 4$) followed by different letters among treatments indicate significant ($p < 0.05$) differences. See Table 1 for abbreviations. NS, not significant ($p > 0.05$); *, $p < 0.05$; **, $p < 0.01$.

**Table 5.** Effects of *Rhizoglomus intraradices* on soil nutrient levels of four plants.

| Plants | AMF | $NH_4^+$-N (mg/kg) | $NO_3^-$-N (mg/kg) | Olsen-P (mg/kg) | Available K (mg/kg) | EE-GRSP (mg/g) | DE-GRSP (mg/g) | T-GRSP (mg/g) | SOC (mg/g) |
|---|---|---|---|---|---|---|---|---|---|
| Marigold | +Ri | 64.9 ± 5.6 a | 115.6 ± 29.5 a | 91.1 ± 15.8 d | 134.6 ± 18.7 a | 2.12 ± 0.19 abc | 5.13 ± 0.67 a | 7.24 ± 0.76 a | 45.89 ± 5.15 a |
| | −Ri | 61.5 ± 3.9 a | 97.1 ± 17.5 ab | 66.3 ± 15.4 d | 79.5 ± 8.1 c | 2.06 ± 0.05 abc | 2.98 ± 1.26 c | 5.04 ± 1.26 d | 36.91 ± 3.82 bcd |
| Maize | +Ri | 61.8 ± 2.3 a | 111.4 ± 15.7 ab | 92.6 ± 9.0 d | 92.7 ± 3.6 c | 2.36 ± 0.47 a | 4.12 ± 0.15 b | 6.49 ± 0.35 ab | 43.89 ± 6.52 ab |
| | −Ri | 59.2 ± 3.3 a | 89.0 ± 14.9 b | 74.9 ± 19.5 d | 74.9 ± 10.9 c | 1.81 ± 0.03 c | 3.82 ± 0.21 b | 5.64 ± 0.21 bcd | 29.93 ± 5.15 edf |
| White clover | +Ri | 35.4 ± 3.7 c | 52.0 ± 5.1 c | 323.0 ± 22.9 b | 50.8 ± 5.9 d | 1.94 ± 0.05 bc | 4.01 ± 0.06 b | 5.95 ± 0.08 bc | 39.90 ± 6.52 abc |
| | −Ri | 35.8 ± 4.7 c | 50.4 ± 8.8 c | 269.9 ± 36.9 c | 85.3 ± 7.9 c | 2.01 ± 0.09 bc | 3.59 ± 0.07 bc | 5.61 ± 0.09 bcd | 25.94 ± 5.15 f |
| Vetch | +Ri | 44.1 ± 7.3 b | 52.4 ± 6.6 c | 371.8 ± 34.1 a | 90.5 ± 5.9 c | 2.19 ± 0.17 ab | 4.90 ± 0.32 a | 7.09 ± 0.41 a | 28.93 ± 3.82 ef |
| | −Ri | 41.9 ± 1.6 bc | 49.8 ± 2.0 c | 364.3 ± 17.6 a | 114.4 ± 17.8 b | 2.18 ± 0.03 ab | 3.34 ± 0.15 bc | 5.52 ± 0.17 cd | 34.92 ± 2.00 cde |
| Significance | | | | | | | | | |
| Ri | | ** | ** | ** | ** | NS | NS | NS | ** |
| Plants | | NS | * | NS | NS | NS | ** | ** | ** |
| Interaction | | NS | NS | * | ** | * | ** | * | ** |

Data (means ± SD, $n = 4$) followed by different letters among treatments indicate significant ($p < 0.05$) differences. See Table 1 for abbreviations. NS, not significant ($p > 0.05$); *, $p < 0.05$; **, $p < 0.01$.

### *3.7. Effects on Soil Aggregate Distribution and Stability*

*R. intraradices* significantly increased soil WSA content in marigold at 1–2 mm and 0.25–0.5 mm by 127% and 25%, respectively, compared to the control (Table 6). The inoculation also significantly elevated soil WSA content in the rhizosphere of maize at 2–4 mm and 1–2 mm by 66% and 53%, respectively, compared to the uninoculation control. It significantly increased soil WSA content in white clover at 2–4 mm, 0.5–1 mm, and 0.25–0.5 mm by 111%, 70%, and 51%, respectively, compared with the uninoculated control. In vetch, inoculation with *R. intraradices* significantly increased soil WSA content at 2–4 mm and 0.5–1 mm by 250% and 139%, respectively, compared with the uninoculated control. In addition, inoculated plants presented 33–109% relatively higher MWD, compared to uninoculated plants (Table 6).

**Table 6.** Effects of *Rhizoglomus intraradices* on distribution and stability of soil water-stable aggregates in four plants.

| Plants | AMF | WSA (%) | | | | MWD (mm) |
|---|---|---|---|---|---|---|
| | | 2–4 mm | 1–2 mm | 0.5–1 mm | 0.25–0.5 mm | |
| Marigold | +Ri | 5.00 ± 0.81 a | 4.16 ± 0.96 a | 5.41 ± 0.87 b | 16.25 ± 1.79 a | 31.41 ± 1.99 a |
| | −Ri | 3.33 ± 0.60 b | 1.83 ± 0.19 b | 3.75 ± 0.41 cd | 13.00 ± 0.66 bc | 20.44 ± 1.66 c |
| Maize | +Ri | 3.58 ± 0.50 b | 4.08 ± 0.73 a | 6.91 ± 0.31 a | 10.67 ± 0.72 cd | 26.06 ± 1.46 b |
| | −Ri | 2.16 ± 0.33 c | 2.66 ± 0.60 b | 5.41 ± 0.56 b | 13.42 ± 0.68 b | 19.60 ± 1.32 cd |
| White clover | +Ri | 4.75 ± 0.95 a | 4.08 ± 0.68 a | 6.66 ± 0.98 a | 14.00 ± 1.24 b | 30.63 ± 2.95 a |
| | −Ri | 2.25 ± 0.31 c | 2.58 ± 0.31 b | 3.91 ± 0.68 c | 9.25 ± 1.10 d | 17.03 ± 1.86 d |
| Vetch | +Ri | 4.66 ± 0.72 a | 3.91 ± 0.63 a | 6.58 ± 0.99 a | 12.08 ± 1.77 bc | 29.34 ± 2.92 a |
| | −Ri | 1.33 ± 0.27 c | 2.25 ± 0.31 b | 2.75 ± 0.56 d | 12.25 ± 2.87 bc | 14.03 ± 1.43 e |
| Significance | | | | | | |
| Ri | | ** | NS | ** | ** | ** |
| Plants | | ** | ** | ** | * | ** |
| Interaction | | * | NS | * | ** | ** |

Data (means ± SD, $n$ = 4) followed by different letters among treatments indicate significant ($p < 0.05$) differences. See Table 1 for abbreviations. NS, not significant ($p > 0.05$); *, $p < 0.05$; **, $p < 0.01$.

### 3.8. Correlation Analysis

The root AMF colonization rate was significantly positively correlated with soil hyphal length and soil spore density (Table 7). The root AMF colonization rate, soil hyphal length, and soil spore density were significantly and positively correlated with SOC, WSA in the sizes of 2–4 mm, 1–2 mm, and 0.5–1 mm, MWD, DE-GRSP, and T-GRSP (Table 7). In addition, the soil hyphal length and spore density were significantly positively correlated with soil $NH^{4+}$-N, $NO^{3-}$-N, and EE-GRSP levels, while the soil spore density was negatively correlated with soil Olsen-P. Soil SOC was significantly positively correlated with EE-GRSP.

**Table 7.** Correlation analysis between mycorrhizal status and soil properties.

| | | Root Colonization | Soil Hyphal Length | Soil Spore Density | SOC |
|---|---|---|---|---|---|
| Root colonization | | 1.00 | 0.82 ** | 0.72 ** | 0.54 ** |
| Soil $NH^{4+}$-N | | 0.12 | 0.35 * | 0.47 ** | 0.46 ** |
| Soil $NO^{3-}$-N | | 0.25 | 0.47 ** | 0.56 ** | 0.58 ** |
| Soil Olsen-P | | −0.07 | −0.31 | −0.40 * | −0.43 * |
| Soil available K | | 0.11 | 0.31 | 0.33 | 0.28 |
| SOC | | 0.54 ** | 0.63 ** | 0.63 ** | 1.00 |
| WSA | 2–4 mm | 0.82 ** | 0.58 ** | 0.42 * | 0.40 * |
| | 1–2 mm | 0.86 ** | 0.71 ** | 0.64 ** | 0.42 * |
| | 0.5–1 mm | 0.74 ** | 0.56 ** | 0.51 ** | 0.16 |
| | 0.25–0.5 mm | 0.30 | 0.21 | 0.06 | 0.30 |
| MWD | | 0.91 ** | 0.68 ** | 0.53 ** | 0.43 * |
| EE-GRSP | | 0.26 | 0.51 ** | 0.53 ** | 0.36 * |
| DE-GRSP | | 0.67 ** | 0.57 ** | 0.44 * | 0.23 |
| T-GRSP | | 0.69 ** | 0.67 ** | 0.55 ** | 0.30 |

*, $p < 0.05$; **, $p < 0.01$.

## 4. Discussion

AMF improve plant growth by promoting the uptake of nutrients and water from the soil and improving root growth in the host plant [13]. The degree of mycorrhizal dependence is an important factor in assessing the maximum plant growth of mycorrhizal plants in given soil conditions [42]. In this study, the growth performances of marigold, maize, clover, and vetch were improved to different degrees after inoculation with *R. intraradices*, with marigold showing the most pronounced effect. This indicates that AMF-improved plant growth is dependent on the host plant species. In addition, the root AMF colonization

rate, soil hyphal length, and soil spore density are important indicators to evaluate the symbiotic status between AMF and host plants [7,43]. Our results indicated that mycorrhizal development was variable among the four plant species, with marigold and maize showing better mycorrhizal development than white clover and vetch. This is consistent with the results of Du [44] inoculating *G. mosseae* on five greenery plants (*Ligustrum lucidum*, *Viburnum odoratissimum*, *Osmanthus fragrans*, *Galium ordoratum*, and *Paeonia lactiflora*), showing the selectivity between mycorrhizal fungi and hosts. Nevertheless, the growth-promoting effect was not significant in maize after inoculation with *R. intraradices*. This result may be due to the fact that maize is a field crop, which has limited root growth under potted conditions and thus affects the mycorrhizal function [45]. On the other hand, it is related to the number of host root hairs, such as maize, which has dense root hairs and is less dependent on mycorrhizae [46]. Soil nutrient levels and environmental conditions also influence AMF to improve plant growth [16,18].

Root morphology is a critical trait describing the variability of roots in response to environmental conditions [47], of which root length indicates the spatial range of root absorption, root surface area reflects the root-to-soil contact area, and root diameter affects water transfer efficiency [48]. This study showed that inoculation with *R. intraradices* improved to some extent root morphological variables, such as total length, area, diameter, and volume in marigold, root diameter in maize, root surface area and diameter in white clover, and root total length, surface area, and diameter in vetch. This is in agreement with the results of Qu et al. [49] on *Zenia insignis* plants colonized by *F. mosseae* and *R. intraradices*. In fact, earlier findings revealed that AMF improved root development in host plants by promoting root auxins, cytokinins, and polyamine levels [47,50]. Such better root morphology in mycorrhizal versus non-mycorrhizal plants would support the expansion of root nutrient uptake and improve water conduction efficiency.

This study demonstrated the variable effect of *R. intraradices* on the leaf gas exchange of host plants, with no significant changes in marigold and vetch, increased stomatal conductance and transpiration rate in maize, and increased net photosynthetic rate and stomatal conductance in white clover. Such results are in agreement with the findings of Frosi et al. [51] in woody plants and Sonal et al. [52] in maize. In a meta-analysis, the effect of AMF on leaf gas exchange variables of plants was regulated by various factors, including plant species, growth habits, soil types, experimental conditions, soil moisture, and salinity levels [53]. Goicoechea et al. [54] found that the effect of mycorrhizae on leaf gas exchange was associated with the balance between abscisic acid and cytokinins by AMF. However, whether such an association also appeared in the present study remains to be investigated.

In roots, the presence of mycorrhizal symbionts usually triggers the cleavage of sucrose in the host to glucose and fructose, which are then converted to other types of glycogen for mycorrhizal growth [13,55]. Approximately 4–20% of photosynthetic products are transferred from the host to the mycorrhiza [56]. In the present study, *R. intraradices* distinctly increased the Chi of marigold and white clover only. The root AMF colonization rate was significantly and positively correlated with the Chi ($r = 0.41$, $p < 0.05$), which may be due to the increase in the host's Fe and Mg acquisition under mycorrhization conditions [57]. Correspondingly, inoculated marigold presented significantly higher leaf glucose than uninoculated plants. *R. intraradices* inoculation also triggered elevated sucrose in the leaves and roots of maize, fructose and glucose concentrations in the roots, and sucrose concentrations in the leaves and roots of white clover and vetch. Thus, mycorrhizal plants could provide a carbon source into the roots for mycorrhizal development. In fact, higher chlorophyll levels of mycorrhizal versus non-mycorrhizal plants are associated with higher rates of photosynthesis and carbon sequestration, thus maintaining AMF–plant symbiosis [58].

Another important function of AMF is to improve the root microenvironment, including root secretions, soil fertility, and soil structure [13]. Although *R. intraradices* did not significantly alter rhizosphere $NH_4^+$-N and $NO_3^-$-N levels, it significantly increased soil available K levels of marigold and Olsen-P levels of white clover, while it decreased

available K levels of white clover and vetch. Meng et al. [55] also found that *F. mosseae* reduced soil available K levels of trifoliate orange but promoted soil Olsen-P levels. He et al. [13] also observed a decrease in soil available K and Olsen-P in vetch after *R. intraradices* colonization. As a consequence, the effect of *R. intraradices* on soil fertility is variable. The elevation of soil Olsen-P in white clover after AMF inoculation may imply that acid phosphatases of both mycorrhizal fungi and hosts are secreted into the soil to decompose organic P [58].

SOC is a marker of soil fertility, as well as a key component of biosphere stability and sustainability [19]. In most cases, AMF accelerate SOC formation and sequestration and also provide physical protection for SOC [59,60]. In our study, the SOC of marigold, maize, and white clover significantly increased after inoculation with *R. intraradices*, and there was no significant change in vetch. Additionally, there was a significantly positive correlation between root mycorrhizal colonization and SOC. The increase in SOC is derived from AMF secretion, mycorrhizal extraradical hyphal turnover (the dominant pathway), and carbon input from fine root turnover [61], which may be a key factor influencing SOC storage capacity after inoculation with *R. intraradices* [62].

The present study showed that a relatively high EE-GRSP level was observed in the rhizosphere of maize plants inoculated with *R. intraradices*, and higher DE-GRSP and T-GRSP levels in mycorrhizal marigold and vetch, varying by GRSP species and host plants. Holátko et al. [63] also concluded that there was not a direct relationship between GRSP levels and the abundance of AMF because GRSP is derived from proteins of both mycorrhizal and non-mycorrhizal fungal origin [64]. In our study, the root colonization rate, soil hyphal length, and spore density were significantly positively correlated with DE-GRSP and T-GRSP levels, plus there was a positive correlation between EE-GRSP and soil hyphal length and spore density. However, an important function of GRSP is to glue WSAs and stabilize WSAs [23], and mycorrhizal extraradical hyphae are also involved in the gluing of WSA for improved soil structure [25,65,66]. In this study, inoculation with *R. intraradices* significantly improved WSA distribution at a certain size level in the host plant, which in turn improved the stability of WSA in marigold, white clover, and vetch. This is in agreement with the results obtained by Xu et al. [67] in tomato and maize. Correlation analysis also revealed that the root AMF colonization rate was significantly positively correlated with WSA in 0.5–4 mm, plus there was a significantly positive correlation between MWD and root colonization, soil hyphal length, soil spore density, SOC, DE-GRSP, and T-GRSP. Better soil WSA distribution and stability in mycorrhizal versus non-mycorrhizal plants is the comprehensive result of mycorrhizae, GRSP, and SOC. How GRSP stabilizes WSA should be further deciphered in the future. In addition, soil WSA stability is important for maintaining soil structure, porosity, erosion resistance, and water-holding capacity [68]. Therefore, mycorrhizal plants have a great root microenvironment, which is particularly important for plant growth and stress tolerance [41].

## 5. Conclusions

The colonization of *R. intraradices* was able to positively regulate plant growth, leaf gas exchange, root morphology, sugar contents, and soil nutrients, structure, and stability of host plants, where the improvement in soil aggregate distribution and stability was not dependent on the plant species. This indicates that *R. intraradices* is more prominent in improving soil aggregate distribution and stability than in improving plant physiological activities. Overall, *R. intraradices* showed relatively prominent positive benefits on marigold. Maize plants showed relatively higher mycorrhizal development than other plants, with potential to be a host for trapped propagation of the fungus. Further work around the mycorrhizal function of *R. intraradices* on marigold is needed.

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