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Responses of Legumes to Rhizobia and Arbuscular Mycorrhizal Fungi: A Global Meta-Analysis

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Abstract: Arbuscular mycorrhizal fungi (AMF) and rhizobia are crucial for plant productivity, microbial community structure, and soil quality, making them key components for sustainable ecosystem development. The symbiotic relationship between AMF and rhizobia is essential for effective biological nitrogen fixation and nutrient absorption, reducing reliance on chemical fertilizers and promoting sustainable agricultural practices. However, studies have shown that soil environment can hinder the symbiotic relationship between AMF and rhizobia. We conducted a meta-analysis of 158 articles from 1980 to 2022 to explore the synergistic interactions in legume-AMF-rhizobium systems and the potential mechanisms underlying synergism. Our findings revealed that the inoculation with AMF and/or rhizobia significantly ($P < 0.001$) increased legume plant nitrogen content, phosphorus content, shoot biomass, yield, AMF colonization rate, nodule number, and nodule weight compared to uninoculated controls (effect size $d > 0$). There was a substantial synergistic effect between AMF and rhizobia ($P < 0.001$). However, soil salt, drought and pH stress inhibited the positive effects of inoculation treatments, possibly due to the plant trade off strategies under adverse stress conditions. Under well-watered condition, the d values of plant yield in the rhizobia, AMF and co-inoculation treatments were 2.047, 1.661, and 3.412 ($Q_M = 246$, $P < 0.001$), respectively, which were higher than that of 1.196, 0.765, and 1.828 ($Q_M = 195$, $P < 0.001$) in the drought stress treatment. This research may potentially lead to new solutions for sustainable agricultural systems in the face of global climate change.

Keywords: arbuscular mycorrhizal fungus; rhizobia; synergism; soil salt; drought stress; soil pH

1. Introduction

In recent years, the combination of intensified human activities and the impact of climate change has resulted in elevated global temperatures, leading to increased evapotranspiration and persistent water scarcity (Hoegh-Guldberg et al., 2019). Consequently, this can induce plant drought, agricultural habitat destruction, reduced availability of water resources, soil salinization, and other associated problems that severely constrain plant growth (Li et al., 2021; Feng et al., 2023). Ecosystems often experience simultaneous impacts from multiple global change factors (von Storch, 2004), each with the potential to exert profound effects on the ecosystem (Qiu et al., 2020).

Drought and salinization are among the most severe climate change factors impacting ecosystems today. The stress caused by drought and salinization can significantly restrict the uptake of soil nutrients by plant roots, thereby severely constraining plant growth (Kahmen et al., 2015; Xu et al., 2023). Research has demonstrated that drought is detrimental to normal physiological activities, growth, and development of plants, and in extreme cases, it can even result in plant mortality (Dietz et al., 2021). Furthermore, soil salinization directly or indirectly influences vegetation-associated soil microbial communities, subsequently affecting ecosystem structure and productivity (Zörb et al., 2019; Melino et al., 2023).

Soil microorganisms play a pivotal role in regulating plant stress resistance and promoting sustainable development (Maestre et al., 2015). Numerous studies have demonstrated that beneficial soil microorganisms can mitigate the adverse impacts of abiotic stress on plants (Li et al., 2019). Arbuscular mycorrhizal fungi (AMF), as ubiquitous symbiotic fungi, establish mutualistic associations with over 90% of terrestrial flowering plant roots, forming arbuscular mycorrhizal structures that augment the host plant's capacity to assimilate soil moisture and nutrients (Smith and Read, 2008). AMF can enhance nutrient acquisition in host plants, modulate plant metabolism, increase crop yield, improve stress tolerance, and delay senescence (Chandrasekaran and Paramasivan, 2022). Additionally, AMF influences the antioxidant system of plants by mitigating the excessive accumulation of reactive oxygen species and safeguarding plant metabolism (Chandrasekaran and Paramasivan, 2022). Moreover, AMF triggers plant defense responses to bolster resistance against environmental stressors while maintaining redox balance and photosynthetic activity (Yilmaz et al., 2023; Duan et al., 2024a).

The symbiotic relationship between leguminous plants and rhizobia has evolved from the symbiotic relationship between plants and AMF (AMF) (Oldroyd and Downie, 2004; Riely et al., 2004). AMF and rhizobia are common symbiotic microorganisms abundantly present in the rhizosphere microbiota of leguminous plants, establishing a long-term symbiosis and evolution with leguminous plants. Through their interaction, AMF and rhizobia provide essential nutrients to host plants, enhance phosphorus and nitrogen absorption, promote plant growth, and improve stress resistance (Parihar et al., 2020; Duan et al., 2021; Duan et al., 2024). Dual inoculation of AMF and rhizobia exhibits greater benefits for host plant growth compared to single inoculation or no inoculation due to their synergistic effects on promoting plant development (Smith and Read, 2008). Upon infection by AMF, root nodules become enriched and grow further, thereby enhancing the host's ability for nitrogen fixation (Abd-Alla et al., 2014). Conversely, through biological nitrogen fixation, rhizobia provides sufficient nitrogen to support AMF colonization rates. The combined effect of dual inoculation with AMF and rhizobium primarily manifests in promoting N and phosphorus element uptake/utilization by plants while increasing biomass production as well as stress resistance capabilities (Pereira et al., 2019).

Maximizing the utilization of AMF rhizobia's nutritional absorption and stress resistance enhancement, in conjunction with plant symbiosis, holds significant importance for ensuring food security and promoting sustainable agricultural development. However, relevant experiments have also revealed negative effects, suggesting that initial inoculation with rhizobia or AMF can impede subsequent colonization by other symbionts (Ossler et al., 2015; Hao et al., 2019; Zhou et al., 2022). The interplay between AMF and rhizobia exhibits mutual constraints (Ossler et al., 2015), which may be influenced by host plant species and environmental conditions. A comprehensive analysis of plant-microorganism interactions under drought and salt-alkali stress could aid researchers in predicting and addressing the impacts of climate change and environmental factors on plants. In addition, analyzing the plant growth, productivity, and microbial responses of each symbiotic organism (number of mycorrhizal nodules/arbuscular mycorrhizal colonization) under different soil water, salt, and pH levels will help determine whether the synergistic results are related to soil nutrient levels, providing theoretical references for improving crop productivity and stress resistance, and offering new ideas for sustainable agricultural development.

To explore context dependence in synergistic outcomes in AMF-rhizobia-legume systems and the potential mechanisms underlying synergism, we conducted a meta-analysis. Specifically, we asked (1) whether host plants productivity differs in the benefits they derive from AMF and/or rhizobia under soil salt, drought and pH stress conditions; (2) whether the response of host plant tissue nitrogen and phosphorus content to the synergistic effect of AMF and rhizobia varies under different soil salt, drought and pH stress conditions; (3) whether co-inoculation enhances infection or

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nodulation in AMF or rhizobium, and whether this varies under different soil salt, drought and pH stress conditions?

2. Materials and Methods

2.1. Literature Survey and Data Compilation

We searched Web of Science (http://apps.webof knowl edge.com) for articles in English and CNKI (https://www.cnki.net) for Chinese articles that included 'mycorr* AND rhiz* AND nitrogen fix*' or 'arbuscular mycorrhizal fung* AND rhiz* AND nitrogen fix*' in the title, abstract and keywords. The use of the '*' character ensured that words such as mycorrhizae, mycorrhizas, mycorrhizal, rhizobia and rhizobium were included. All peer-reviewed literature published between 1980 and 2022 that investigated the effects of AMF and rhizobia inoculation integration on shoot biomass, yield, plant nitrogen and phosphorus content, nodulation (i.e., number of nodules and nodule weight), mycorrhizal total colonization (i.e., percent AMF colonization) of plants were selected and examined. We checked the references cited in the remaining articles and added studies not found by the initial search. Our criteria for inclusion of a paper in the meta-analysis was that the study (1) included a legume plant, a rhizobium and an AMF; (2) included an experiment that followed a full-factorial design, with a non-inoculated control, a rhizobium and AMF individual inoculum, and a combined treatment (AMF + rhizobia); and (3) reported mean biomass and/or N and P tissue concentration and sample size of plants grown under each experimental treatment level. The search was completed on 20 June 2022, and 158 studies fit our criteria for inclusion in the meta-analysis.

We recorded plant response as mean dry shoot biomass, grain yield, plant nitrogen, phosphorus content, nodulation, mycorrhizal colonization rate, and included sample size, mean effect, and standard deviation (SD)/error (we converted reported standard error to SD for analysis). For each data point in each study, we recorded the plant species examined, site of experiment. In total, the data included many AMF and rhizobia species (Supporting Information: Table S1). We extracted data (mean, sample size and SD/SE) from the text, tables and/or figures from each publication for the treatment with inoculation (i.e., inoculated with AMF and/or rhizobia) or without inoculation (CK) of legume nitrogen and phosphorus content, shoot biomass, grain yield, nodulation condition, mycorrhizal colonization rate. In cases where the data were only available from the figures, these were digitized, and means and variance were obtained using the software Getdata Graph Digitizer (http://www.getda ta-graph -digit izer.com/).

2.2. Data Analysis

For our analysis, we chose Hedges'*d* to calculate the effect size of AMF and/or rhizobia inoculation under salt, drought and pH stress treatment because it is less biased by small sample sizes (Newsham, 2011) and is commonly used in ecological meta-analyses (Koricheva et al., 2013; Kuppler and Kotowska, 2021; Nakagawa and Santos, 2012). The meta-analysis was performed using the random-effects model to estimate the effect size and 95% confidence intervals (CIs) per category and overall. In this study, if the 95% confidence interval values of the effect size for a variable did not overlap zero, the effect of the inoculation treatment on the variable was significant; otherwise, it did not represent a significant effect. When the effect size > 0, it indicated a positive effect of inoculation on plant index, and accordingly, the effect size < 0 showed an adverse effect.

For studies that used the severing approach, the treatment with inoculation (i.e., inoculated with AMF and/or rhizobia) was used as 'treated' and the treatment without inoculation as 'control'. We used Hedges'*d* as the effect size, which was calculated as follows:

$$d = \frac{\overline{x}_t \text{ - } \overline{x}_c}{S} \times J\#(1)$$

where $\bar{x}_t$ and $\bar{x}_c$ are the mean of the treated and control group, respectively. Negative Hedges'd values indicate that response variables were reduced in inoculation treatment, and vice versa. J is a weighting factor based on the replicates, calculated as:

$$J = 1 - \frac{3}{4 \times (n_t + n_c - 2) - 1} \tag{2}$$

and S is the pooled standard deviation based on the standard deviations, calculated as:

$$S = \sqrt{\frac{(n_t - 1) \times sd_t^2 + (n_c - 2\ ) \times sd_c^2}{n_t + n_c - 2}} \tag{3}$$

where n and sd are the sample size and standard deviation of the treated or control group, respectively. To account for inequalities in study variance, effect sizes were weighted using the inverse of the sampling variance, therefore the variance of each effect size (V_d) was calculated as (Koricheva et al., 2013):

$$V_d = \frac{n_t + n_c}{n_t \times n_c} + \frac{d^2}{2 \times (n_t \times n_c)} \tag{4}$$

We used the Q statistic to test the heterogeneity of effect sizes between groups and overall effect sizes. Between-group heterogeneity (Q_M) was considered significant if the P-value for moderators calculated in the random-effects model was < 0.05. The meta-analysis was performed using the Metan module (1.85) for Stata/MP 17.0 (Stata Corp., TX, USA). All the figures were prepared using OriginPro 2021 (OriginLab Corp., MA, USA).

3. Results

3.1. Plant Nitrogen and Phosphorus Content Responses to Symbionts

In this study, it was observed that regardless of soil stress, inoculation with AMF and/or rhizobia had significant positive effects ($d > 0$, $P < 0.001$) on the nitrogen and phosphorus content of the plants under different soil salt, water and pH levels (Table 1; Figure 1). Furthermore, the co-inoculation with AMF and rhizobia treatment showed a synergistic effect ($P < 0.001$; Table 1; Figure 1). In contrast, the soil stress of salt, drought and pH (acidic and alkaline) inhibited the promoting effect of inoculation treatments on plant nitrogen and phosphorus content, especially in co-inoculation with AMF and rhizobia treatment (Table 2; Figure 2).

Compared with the treatment without salt stress, the rhizobia, AMF, and co-inoculation treatments significantly inhibited the accumulation of nitrogen and phosphorus content in leguminous plants under salt stress. Specifically, in non-salt stress treatment, the rhizobia, AMF, and co-inoculation treatments showed higher d values of 2.572, 2.352, and 4.831 ($Q_M = 33.3$, $P < 0.05$; Table 2; Figure 2a) in plant nitrogen content, respectively, compared to the salt stress treatment with d values of 1.611, 0.898, and 3.297 ($Q_M = 23.6$, $P < 0.001$; Table 2; Figure 2a). Similarly, drought stress significantly suppressed the effect size of all inoculation treatments on plant nitrogen and phosphorus content (Table 2; Figure 2b,e). In non-water stress treatment, the rhizobia, AMF, and co-inoculation treatments showed higher d values of 2.842, 2.439, and 4.348 ($Q_M = 38.7$, $P < 0.001$; Table 2; Figure 2b) in plant nitrogen content, respectively, compared to the drought stress treatment with d values of 1.071, 1.372, and 2.228 ($Q_M = 37.0$, $P < 0.001$; Table 2; Figure 2b). On the other hand, high (alkaline soil) or low (acidic soil) soil pH stress inhibited plant nitrogen and phosphorus accumulation, but the effect was not significant ($P > 0.05$, Figure 2c,f).

3.2. Plant Yield and Shoot Biomass Responses to Symbionts

Figure 3 showed that inoculation with AMF and/or rhizobia significantly increased legumes yield and shoot biomass compared to uninoculated controls ($d > 0$, $P < 0.001$), but across all plants, these effects were not strictly additive when plants were grown with both symbionts, there was a synergistic effect between AMF and rhizobia (*P* < 0.001; Table 1; Figure 3). The promoting effect of inoculation on legume yield and shoot biomass was as follows: co-inoculation of AMF and rhizobia, rhizobia inoculation, and AMF inoculation (Yield Q_M = 1010, *P* < 0.001; Shoot biomass Q_M = 1479, *P* < 0.001; Table 1), where the mean *d* values of plant yield were 3.969, 2.263, and 1.696 in co-inoculation of AMF and rhizobia, rhizobia inoculation, and AMF inoculation treatment, respectively (Figure 3a–c).

The soil stress of salt, drought and pH (acidic and alkaline) inhibited the promoting effect of inoculation treatments on plant yield and shoot biomass, especially in co-inoculation with AMF and rhizobia treatment compared to non-stress treatment (Table 2; Figure 4). For example, under well-watered condition, the *d* values of plant yield in the rhizobia, AMF and co-inoculation treatments were 2.047, 1.661, and 3.412 (Q_M = 246, *P* < 0.001; Table 2; Figure 4b), respectively, which were higher than that in the drought stress treatment (the *d* values were 1.196, 0.765, and 1.828, Q_M = 195, *P* < 0.001; Table 2; Figure 4b). Compared with the control, the changes in plant shoot biomass and yield under soil stress conditions were consistent for all inoculation treatments.

3.3. Microbial Responses to Symbionts

Inoculation with AMF and/or rhizobia significantly (*P* < 0.001) increased legume nodule number, nodule weight and AMF colonization rate compared to uninoculated controls (Table 1; Figure 5). In most cases, these effects were enhanced when plants were grown with both symbionts regardless of soil stress treatment (Table 3; Figures 5 and 6), and there was a significant synergistic effect between AMF and rhizobia (Table 3; Figures 5 and 6).

For the nodulation of rhizobia, salt stress and drought stress all suppressed the promoting effect of co-inoculation with AMF and rhizobia treatment on the nodulation level of rhizobia by inhibiting the nodule weight and number of rhizobia compared to non-stress treatment (Figure 6a,b,d,e). In the non-salt stress treatment, the *d* values of nodule number in the rhizobia, AMF and co-inoculation treatments were 4.821, 0.856, and 5.762 (Q_M = 48.7, *P* < 0.001; Table 3; Figure 6a), which were higher than that of 3.846, 1.183 and 4.117 in the salt stress treatment (Figure 6a). Acidic and alkaline soil also inhibited the nodule weight and number of rhizobia in AMF and/or rhizobia treatments, which led to a decrease the synergistic effect of AMF and rhizobia (Figure 6c,f).

For the AMF colonization rate, soil salt stress, drought stress and pH stress all suppressed the promoting effect of co-inoculation with AMF and rhizobia treatment on the colonization level of AMF by inhibiting the AMF colonization rate of plant compared to non-stress treatment (Figure 6g–i). In the non-water stress treatment, the *d* values of AMF colonization rate in the rhizobia, AMF and co-inoculation treatments were 0.801, 2.538, and 3.879 (Q_M = 101, *P* < 0.001; Table 3; Figure 6h), which were higher than that of 0.727, 1.970 and 2.674 in the drought stress treatment (Figure 6h).

In addition, for the nodulation of rhizobia, the inoculation effect of rhizobia is greater than that of AMF, the mean *d* values of nodule number in the rhizobia inoculation treatment and AMF inoculation treatment were 2.661 and 0.954 (Q_M = 420, *P* < 0.001; Table 1; Figure 5a–c). In contrast, for the colonization level of AMF, the inoculation effect of AMF is greater than that of rhizobia, the mean *d* values of AMF colonization rate in the rhizobia, and AMF were 0.505 and 1.041 (Q_M = 677, *P* < 0.001; Table 1; Figure 5g–i).

4. Discussion

The interaction between plants and soil microorganisms is pivotal for the functioning of terrestrial ecosystems and their response to environmental and climate change (Cavicchioli et al., 2019). Plants contribute carbon sources to the soil through their litter, roots, and root exudates, serving as the primary energy source for the soil food web. Soil microorganisms can establish symbiotic relationships by colonizing roots (e.g., forming mycorrhizae and root nodules), modulating plant hormone production to enhance plant growth or mitigate plant stress signals, thereby engaging in direct interactions with plants (Coskun et al., 2017; Trivedi et al., 2020). The significance of rhizosphere microbial interactions for sustainable agricultural development has emerged as a major focus in soil microbial research (de Vries et al., 2020), wherein AMF and rhizobia are extensively employed in agricultural production due to their crucial ecological roles. AMF directly supplies nutrients such as nitrogen, phosphorus, zinc, copper, and water to plants in exchange for fatty acids from host plants' resources (Jiang et al., 2017; Smith and Smith, 2011). Rhizobia can convert atmospheric nitrogen gas into an accessible form essential for plant growth (Duan et al., 2024a).

In our meta-analyses, inoculations exhibited significant positive effects on plant nitrogen and phosphorus content. Furthermore, co-inoculation of AMF and rhizobia demonstrated a synergistic effect, consistent with the findings reported by Primieri et al. (2022). Consistent with previous studies conducted by Bai et al. (2017) and Leite et al. (2022), our study revealed that both AMF inoculation and co-inoculation of AMF and rhizobia significantly enhanced the phosphorus content in host plants under soil stress conditions. However, rhizobia inoculation alone did not exert a significant influence on plant phosphorus content. These results indicate that AMF plays a crucial role in facilitating host plants' acquisition of phosphorus.

Both AMF and rhizobia demonstrated a positive impact on shoot biomass and yield of leguminous plants, with an even more pronounced effect observed when AMF and rhizobia were co-inoculated. These findings highlight the synergistic relationship between AMF and rhizobia in promoting host plant biomass increase and yield accumulation, consistent with previous studies reporting enhanced growth, elevated nitrogen and phosphorus content, as well as improved biomass production and yield during co-inoculation of AMF and rhizobia (Bai et al., 2017; Leite et al., 2022; van der Heijden et al., 2016).

In the process of long-term evolution, a mutually beneficial symbiotic relationship gradually develops between AMF and rhizobia, which can be attributed to the following factors: firstly, AMF infection provides plants and rhizobia with essential phosphorus and other nutrients, thereby enhancing their nitrogen fixation ability (Ding et al., 2016). Secondly, by forming hyphal bridges and networks in the root system through extracellular hyphae, AMF enhances nodulation and increases the rate of nitrogen fixation in host plants. Thirdly, AMF infection of host plants stimulates higher rates of photosynthesis and respiration, resulting in increased production of photosynthetic products and enhanced transport of carbon to the roots. Ultimately, this improves the nitrogen fixation efficiency of rhizobia (Loo et al., 2022). Fourthly, AMF infection promotes plant growth and development by increasing root biomass and length while creating larger areas for rhizobial colonization (Tajini et al., 2012). Fifthly, AMF infection induces expression of nodule-related genes in host plants (such as Nod factor), leading to an increase in both the number and nitrogen fixation ability of rhizobia (van Rhijn et al., 1997). Sixthly, root nodules provide sufficient nitrogen sources for AMF growth through nitrogen fixation. This benefits spore germination and hyphal growth while increasing mycorrhizal infection rates in plant roots (Sakamoto et al., 2019). Last but not least important is that rhizobia promotes flavonoid excretion from host plant roots to induce colonization by AMF (Abdel-Lateif et al., 2012).

Recently conducted studies have provided confirmation that the infection of plants by AMF can enhance the growth and enrichment of root nodules, thereby leading to an improvement in the host's ability for nitrogen fixation (Abd-Alla et al., 2014). In reciprocation, rhizobia are capable of supplying sufficient nitrogen to AMF through biological nitrogen fixation, consequently increasing the rate of AMF colonization. The combined inoculation with AMF and/or rhizobia has been found to significantly augment legume colonization rates by AMF, as well as increase nodule number and weight when compared to uninoculated controls. Furthermore, a significant synergistic effect between AMF and rhizobia was observed. This synergism is likely attributed to the fact that rhizobia and AMF assist plants in acquiring complementary resources nitrogen and phosphorus respectively (Primieri et al., 2022).

However, the effects of co-inoculation are contingent upon various factors, including host plant species, strain type, and soil environment (Duan et al., 2024a). The interaction between AMF and rhizobia does not consistently exhibit a synergistic promoting effect; in fact, double inoculation of AMF and rhizobia may have no effect or even result in mutual restraint (Ossler et al., 2015). Furthermore, related experiments have also observed negative effects suggesting that prior

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inoculation of either rhizobia or AMF can inhibit subsequent colonization by other symbiotes (Catford et al., 2003; Hao et al., 2019; Tsimilli-Michael et al., 2000). For instance, Larimer et al. (2016) revealed that upon infection of *Amorpha fruticosa* by rhizobia, there was a reduction in AMF hyphal abundance, indicating an antagonistic relationship between AMF and rhizobia. However, the combined effect of these symbiotic associations ultimately resulted in enhanced host growth and development. Kaschuk et al. (2010) conducted a meta-analysis and determined that the single inoculation of AMF and rhizobia had a positive impact on host plant growth, while the double inoculation did not exhibit a significant synergistic effect on the host. Tsimilli-Michael et al. (2000) observed in a pot experiment that the single inoculation of AMF with Glomus fasciculatum enhanced photosystem II (PSII) activity in alfalfa, whereas double inoculation with both AMF and *Rhizobium meliloti* actually inhibited PSII activity. This inhibition can be attributed to competition between AMF and rhizobia, which hinders photosynthesis in the host plant and reduces carbon allocation to both symbionts. Additionally, studies have shown that high-density rhizobial inoculation in peas leads to reduced colonization of AMF hyphae in plant roots (Ossler et al., 2015). This finding suggests a negative correlation between rhizobial nodulation and AMF colonization under highly nodulated conditions.

In the case of limited soil nutrients, the mutualistic relationship between AMF and plants gradually transitions to parasitism, while a competitive relationship exists between AMF and rhizobia (Larimer et al., 2014). The symbiotic effect of dual inoculation with AMF and rhizobia is intricate, involving a “trade-off” of photosynthetic carbon among AMF, rhizobia, and plants. Depending on the accumulation, distribution, and competition for photosynthetic carbon resources, synergistic promotion effects, no effects or antagonistic effects can be observed. The interaction between AMF and rhizobia is influenced by various comprehensive factors. Therefore, when applying microorganisms to improve ecosystem environments or remediate polluted soils in agricultural applications, it is crucial to comprehensively consider the interactions among microorganisms in their natural environment as well as clarify the influencing factors.

A study demonstrated that under drought stress conditions, the dual inoculation of AMF and rhizobia had a certain impact on promoting the growth of alfalfa. Compared to the control group, this dual inoculation enhanced the growth and development of host plants while also improving their drought tolerance by increasing the activity of antioxidant enzymes in hosts (Goicoechea et al., 2005). Erman et al. (2011) found that double inoculation with AMF and *Mesorhizobium ciceri* had the most significant promoting effect on chickpeas under irrigation conditions. This could be attributed to sufficient soil moisture facilitating rhizobia nodulation, which requires an ample supply of phosphorus elements. Consequently, rhizobia promotes further infection by AMF, ultimately leading to increased yield accumulation in chickpeas. Interestingly, our study revealed that under soil salt, drought, and pH stress conditions compared to non-stress treatment, the promotion effects of inoculation on plant shoot biomass, yield, nitrogen and phosphorus content in plants as well as AMF colonization level and rhizobial nodulation were inhibited. This may be due to legume plants allocating resources towards adversity resistance when exposed to stressful soil environments resulting in reduced investment in mycorrhizal symbiosis and rhizobial nodulation thereby diminishing microbial synergy possibilities (Larimer et al., 2014; Duan et al., 2024a). Similarly recent studies have shown that double inoculation with AMF (a 1:1 mixture of *Fusarium graminearum* and *Fusarium oxysporum*) along with *Mesorhizobium tianshanense* has a more pronounced promoting effect on host plants compared to single inoculations under normal water supply conditions; however during drought stress periods this synergistic effect weakens significantly such that it is not as effective as single inoculations alone (Gao et al., 2022). This phenomenon can potentially be attributed to co-parasitism.

Liu et al. (2018) discovered that soil salinity concentration partially inhibits the colonization of AMF and rhizobia in alfalfa, thereby suppressing their interaction. However, dual inoculation of AMF and rhizobia significantly enhances the stress resistance of alfalfa, mitigating the damage caused by saline-alkali stress on host plants and providing a theoretical foundation for the rational utilization of saline-alkali land. Consistent with previous findings, our meta-analysis reveals that soil salt stress significantly reduces the effect size of inoculations on host nutrient absorption, yield accumulation, and microbial symbiosis. Ashrafi et al. (2014) observed a decrease in nodule number and AMF infection rate in alfalfa with increasing salt concentration. Salt stress hampers both AMF colonization and rhizobial nodulation due to increased energy allocation towards stress resistance rather than towards AMF and rhizobia support. Additionally, high salt ion concentration damages the cell structure of rhizobia while reducing their activity, thus inhibiting AMF and rhizobial colonization. Shafer et al. (1996) found that although double inoculation of AMF and *Rhizobium leguminosarum* promotes clover growth under low pH conditions, overall low pH impedes the interaction between AMF and Rhizobium possibly because it does not affect AMF infection but instead stimulates soil nutrient release promoting host plant growth without enriching harmful ions in soil. Therefore, low pH does not influence the effectiveness of AMF infection as suggested by recent research indicating that decreased soil pH directly suppresses AMF biomass leading to reduced community diversity (Wu et al., 2023). Soil pH affects mineral elements cycling through acidification pathways among others.

The symbiotic effect of co-inoculation between AMF and rhizobia is intricate, involving a trade-off among AMF, rhizobia, and plants in terms of photosynthetic carbon assimilation. Depending on the accumulation, distribution, and competition for photosynthetic carbon resources, synergistic promotion, no effect, or antagonistic effects can be observed. Various agronomic management practices such as aboveground litter input, cultivation techniques, soil type selection, and increased planting density can all influence the colonization of AMF (Liu and Chen 2007; Oehl et al., 2010; Duan et al., 2024a). These factors have the potential to regulate the interaction between AMF and rhizobia by affecting their infection process efficiency. Therefore, extensive hypothetical analyses and validation experiments are required in future research to establish a reliable foundation for the practical application of double inoculation with AMF and rhizobia. Furthermore, most current studies primarily focus on single-factor effects while neglecting multi-factorial impacts and their interactions on AMF-rhizobium diversity as well as ecosystem functioning, especially within the context of global climate change. Urgent attention should be given to future research endeavors in this area.

5. Conclusions

Microbial symbionts can exert a significant influence on plant growth and fitness; however, the outcomes of plant-microbial interactions are highly context-dependent. Our meta-analysis revealed that inoculation with AMF and/or rhizobia significantly enhanced AMF colonization rate, nodule number, nodule weight, plant nitrogen content, plant phosphorus content, shoot biomass, and yield in legumes compared to uninoculated controls. Furthermore, a synergistic effect was observed between AMF and rhizobia. The effects of AMF and/or rhizobia inoculation on plants were found to be contingent upon soil salt levels, soil moisture conditions, and soil pH values. Specifically, high levels of soil salt concentration as well as drought stress and acidic or alkaline pH hindered the symbiotic relationship between plants and beneficial microorganisms. Consequently, these stressors suppressed the promoting effects of inoculation treatments on host yield and nutrient absorption, particularly when co-inoculating with both AMF and rhizobia compared to non-stress conditions.

By integrating variations in host plant nitrogen content, phosphorus content, biomass, yield, root nodulation, and AMF colonization levels, we propose that the enhanced productivity of legumes facilitated by AMF may be attributed to their promotion of host nitrogen and phosphorus uptake. This enables plants to acquire sufficient nutrients for sustaining growth and ultimately increasing productivity. Simultaneously, rhizobia contributes to this process by providing adequate nitrogen through biological nitrogen fixation for both the host plant and AMF symbionts, ensuring shoot biomass accumulation and promoting overall plant productivity.

Supplementary Materials: The following supporting information can be downloaded at: Preprints.org.

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Open Research: All data contained in this article can be shared, and if detailed data analysis and data itself are required, we can provide it all during the review process.

Declaration of competing interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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