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Systematic Review

Not a Universal Solution: A Systematic Review of Context-Dependent AMF and AMF–PGPR Effects in Maize Production

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Abstract

The intensive use of chemical fertilisers has reduced nutrient uptake efficiency in plants, mainly due to salinisation. This effect has prompted the investigation of alternative forms of plant nutrient management. One of the promising solutions to this is the use of microbial inoculants, specifically arbuscular mycorrhizal fungi and the plant growth-promoting rhizobacteria (PGPR). This systematic review, conducted in accordance with PRISMA 2020 guidelines, analysed 36 peer-reviewed studies published between 2008 and 2025 across 18 countries to assess the effects of inoculation with arbuscular mycorrhizal fungi (AMF), both alone and in combination with plant growth-promoting rhizobacteria (PGPR), on maize growth, physiology, and biochemical responses. The main finding was that soil P availability was the primary factor determining whether inoculation was successful. This was especially noted in soils with soil-available P of less than 17 mg P kg^{-1} of soil. Soil texture and pH also played key roles in both single AMF inoculation and dual AMF-PGPR inoculation. Several studies showed that dual inoculation improved maize stress tolerance by coordinating antioxidant activity, osmotic balance, and hormonal signalling, rather than through simple additive growth effects. Overall, the findings suggest that AMF-based inoculation is most effective when applied strategically in reduced-input, phosphorus-limited, or drought-prone maize systems, rather than as a universal solution. These results support targeting inoculation toward resource-constrained systems where returns are most likely, especially P-deficient soils.

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1. Introduction

The use of chemical fertiliser, specifically NPK (nitrogen, phosphorous and potassium) has increased by almost 5.8 times since 1961 to 2023 [1] (Our World in Data is a continuously-updated live dataset). This increase was attributed to the industrialization period which followed the development of hybrid crops that could intake more nutrients leading to higher quantity and quality of produce. Subsequently, the yield gains between 1961 and 2023 were nearly 300% as a result of conventional fertilisation schemes. However, a critical aspect of these numbers represents a critical message: "we are reaching the maximum threshold for plant fertilizer absorption". The data clearly shows that chemical fertilisation application has been constantly increasing with some depression over the years due to policy changes and climate change. The yield increase has primarily been slowing down since the late 2000s, indicating a saturation point is getting closer [2,3]—a point at which the plant can no longer absorb more fertiliser supplied, reducing nutrient uptake efficiency and leading to leaching of nutrients such as nitrogen to the soil and water bodies, leading to both soil degradation and water pollution, further enhancing climate change effects [3,4]. Climate change itself has been a driving factor in reducing nutrient uptake efficiency, especially due to the mistiming of fertiliser application caused by sudden weather changes. Previously, farmers applied fertilisers at specific time frames to meet plant needs depending on growth stages; however, this timing has become more challenging with climate change, leading to more leached nutrients due to missed timing [5,6].

Integrated nutrient management techniques and organic fertilisation have emerged as a silver lining in this crisis, offering farmers a chance to obtain similar yields or higher with a more sustainable approach [7]. One concept in this field is the use of naturally available systems of nutrient circulation such as microbial inoculants [8]. Microbial inoculants are fungi or bacteria introduced into an environment to perform a specific function, such as biocontrol or plant growth promotion [9]. Specifically, the group of root-obligate mycorrhizal fungi most commonly associated with agricultural inoculation, all classified within the single phylum *Glomeromycota*, are namely arbuscular mycorrhizal fungi (AMF) [8].

AMF are a member of mycorrhiza, which consists of five groups based on their morphological characteristics: arbuscular, arbutoid, ecotoericoid, monoptropoid and orchid. AMF are the most common in this group and are found in 80% of terrestrial plants, forming beneficial symbiotic relationships with the host [10,11]. Arbuscules, highly branched/differentiated structures formed within root cortical cells of host plants by the hyphae of the fungi, serve as the site of nutrient transfer between the plants and the fungi, forming crucial points for AMF symbiosis with the host plant [12].

As reported in multiple studies, closely associated microbial partners working in close cooperation with AMF are so-called plant growth promoting rhizobacteria (PGPR) [13]. The majority of PGPR belong to the genera *Frankia*, *Acinetobacter*, *Arthrobacter*, *Azotobacter*, *Azospirillum Streptomyces* spp., *Bacillus*, *Enterobacter*, *Burkholderia*, *Bradyrhizobium*, *Rhizobium*, *Serratia*, *Thiobacillus*, *and Pseudomonas* [14]. The combination of these inoculants has been reported to have various benefits to the plant host. AMF, for instance, have been reported in multiple studies for their benefits in terms of increasing nutrient uptake efficiency and acquisition, especially in nutrient deficient soils, through their extensive hyphal networks. PGPR, on the other hand, have been reported to improve abiotic stress resistance and act as biological nitrogen fixers (BNFs), producing plant growth regulators like auxins, cytokinins,

gibberellic acids, and indole-3-acetic acids (IAA) [14,15]. They have also been reported to solubilize and mineralize inaccessible nutrients for plants in cooperation with AMF, especially phosphate nutrients.

In maize cultivation, it is crucial to understand how the integration of these inoculants, especially AMF, leads to yield increases alongside environmental sustainability. Their integration has led to multiple research projects in connection with this topic. For instance, a meta-analysis study conducted on published studies from 1950–2021 found that, on average, AMF inoculation led to a 23% increase in crop yields and enhanced stress tolerance across 13 popular crops under rainfed conditions [16]. Similarly, a review of manuscripts published from 2010–2024 on the assessment of microbial inoculant benefits to maize reported plant benefits in terms of plant height, growth increase in plant vegetative stages and grain yield benefits, especially in plants inoculated with PGPR and AMF of the *Glomus* genus [17]. Additionally, some studies provide a structured and detailed introduction to AMF functions in nutrient uptake, from the genes and chemical signals involved to the process of AMF colonization of the plant, giving readers interested in this topic comprehensive starter information [18]. Similarly, a meta-analysis of 168 studies on seven important cereals crops and the effects of AMF inoculation on yields revealed that an overall grain yield of 21% was realised post-AMF inoculation [19]. While these studies offer meaningful contributions to the understanding of this topic, systematic review studies focusing on single crop types—for instance, maize—alongside their inoculation with AMF in combination with PGPR are still lacking. To address this gap, we conducted a systematic review on the effectiveness of AMF inoculation in the presence and absence of PGPR alongside nutritional supplementation in the form of chemical fertilisers such as NPK and organic materials with respect to *Zea mays*.

Our aim in this systematic review was to evaluate the role of arbuscular mycorrhizal fungi (AMF) in improving maize performance and their potential to enhance nutrient use efficiency as an alternative to conventional fertiliser-intensive systems. Specifically, we investigated:

- The effects of AMF on maize morphological traits, including plant height, stem diameter, leaf number, and leaf area.
- The impact of AMF on yield-related parameters, such as biomass, silage yield, grain yield, and grain characteristics (e.g., kernel number and weight).
- The influence of AMF on physiological performance, particularly plant nutrient uptake (N, P, K) and chlorophyll content.
- The role of AMF in modulating stress-related biomarkers, especially under drought and salinity conditions.
- The potential synergistic effects of co-inoculation with plant growth-promoting rhizobacteria (PGPR) on maize growth and productivity.

Through this, we aim to assess whether AMF-based strategies can maintain or improve maize productivity while reducing reliance on chemical fertilisers.

2. Materials and Methods

This research conducted a systematic literature review according to Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines 2020, to allow for the transparency and repeatability of our methodology and to reduce biases in the findings [20]. The completed PRISMA 2020 checklist is available in the Supplementary Material (PRISMA_2020_checklist). Prior to study screening and data extraction a review protocol was developed internally following discussions. The protocol defined the review questions, search strategy, eligibility criteria, and data extraction framework. The protocol was consequently registered on the Open Science framework registry for systematic reviews.

The keyword selection and search strategy utilised the PICO framework, which stands for Population, Intervention, Comparison/Control, and Outcome. To ensure the studies included or excluded in this review were justified, the following eligibility criteria were decided upon.

The inclusion criteria outline the types of scientific papers that were deemed eligible for integration into this review. The following are the inclusion criteria:

- Types of studies included: peer-reviewed articles or research papers, conference papers, and studies obtained from the reference sections of included papers.
- There were no restrictions regarding the publication dates of the studies. This was applied to ensure a comprehensive historical capture of the literature.
- Systematic reviews, meta-analyses, and literature reviews were excluded; however, they were used as benchmarking tools for our methodology and findings.
- Only studies published in English were considered.
- Studies conducted in the field, laboratory, greenhouses, or using pot trials were eligible, with no restrictions on the environmental setting.
- To be included, studies must explicitly mention the application of arbuscular mycorrhizal fungi (AMF) or AMF inoculation in soil or on plants. The application methodology must be clearly described, and results must be comprehensively elaborated.
- Studies involving dual inoculation with bacterial strains such as *Bacillus mojavensis*, *Bacillus thuringiensis*, *Streptomyces*, and *Bacillus subtilis* were preferred over single AMF inoculation studies. Nonetheless, all plant growth-promoting rhizobacteria (PGPR) were considered, depending on their methodology.
- The primary focus of the review was on maize (*Zea mays*). Therefore, studies that investigated AMF or dual inoculation were included only if they examined this crop or researched its connection to it.
- Clear identification of the AMF or PGPR species was a mandatory inclusion criterion. Exceptions were only considered if a study provided crucial information amidst a scarcity of literature.
- Selected studies must evaluate at least one relevant outcome, such as effects on plant nutrient levels, biomass, growth, or yield, as well as changes in soil properties or agricultural practices.
- Each study should clearly describe the methods used to apply AMF or AMF inoculants to soil or plants, provide detailed application procedures, and explicitly present the results.
- Preference was given to studies reporting on soil properties, particularly soil available NPK content, soil pH, and soil texture.
- Studies are expected to present their findings clearly, emphasizing significance levels, preferably including means of yields or other relevant data.
- The study should clearly present a control treatment that was used to compare the inoculated to the non-inoculated plant. Controls were considered in the context of the study. For instance, some studies utilized a baseline chemical fertilisation on all treatments; in this case, this was considered a control.
- There were no restrictions regarding studies that used fungicide applications; however, such studies were given less preference.
- Similarly, no limitations were imposed based on soil sterilization status. All studies were included regardless of whether soil sterilisation via autoclaving was performed.

2.1. Source of Information

The studies were sourced from two main platforms; i.e., Web of Science and Scopus. In some cases, the studies were obtained from the Reference section of the included studies. The database searching was conducted from 28 August 2025 to 17 November 2025.

2.2. The Search Strategy

The search strategy was initially drafted and refined along the course of the selection process. The main framework utilised to frame our research question into keywords was the PICO framework, which categorised the keywords into 4 main groups:

- Population: This is the subject or plant in focus in the study, which is maize. It was described using three different keywords: "Maize", "Corn", "*Zea mays*".
- Intervention: The intervention in this review was regarded as inoculation with a bio stimulant, which included the inoculation of AMF or PGPR or both dual inoculations. This was addressed in the keywords in the form of "AMF inoculation", "PGPR inoculation", ("Arbuscular mycorrhizal fungi" AND "inoculation"), "AMF inoculation" AND "*Bacillus* sp.".
- Comparator: This described the baseline comparison to identify whether inoculation was successfully effective or not. Although no keyword clearly fit in this category, it was useful in formulating the research question and the inclusion criteria. However, in this review, it was regarded as the control test that did not receive inoculation; whether or not it received another form of fertilisation, it was regarded as the control so long as it did not receive inoculation.
- Outcome: These keywords described the effect of AMF in terms of physiological and morphological gains. They included: "plant nutrition", "plant yield", "yield", "nutrition", "plant growth".

The keywords were arranged in a table and the combination was chosen at random within the 3 main categories: Population, Intervention and Outcome. The combinations were then tested on Scopus and Web of Science until a hit was returned. This approach was carried out since, as previously noted, some keyword combinations can have no hits due to misarrangement or wrong placement of keywords. This process resulted in a baseline combination, to which other keywords were added, based on the search needs:

- The baseline keyword combination: "arbuscular mycorrhizal fungi" OR "AMF inoculation " OR "AMF") AND ("plant nutrition" OR "plant yield" OR "yield" OR "nutrition") AND ("inoculation" OR "application methods ");
- Added keywords: "*Zea mays*", "corn", "Maize", "*Bacillus*", "PGPR", "plant growth promoting rhizobacteria", "*Streptomyces*", "*Trichoderma*".

The available automatic filters on Scopus, the "exclude" button and the "AND NOT" option on Web of Science were utilised to further enhance the study selection process.

2.3. The Selection Process

The initial process started on Scopus and Web of Science, where the baseline keyword combination resulted in 1814 and 2152 hits, respectively. Upon the addition of the keyword "*Zea mays*", the process resulted in an exclusion of 1440 studies on Scopus and 2107 studies on Web of Science. The records that were excluded due to duplication and for other reasons were 37 in total. The final number of studies in the identification process was 382 studies eligible for inclusion.

These studies were then subjected to title screening or title relevance, where the study titles were checked to identify whether the topic was AMF related or not. These resulted in the exclusion of 278 studies. The remaining 104 studies were exported in RIS format

and imported to the reference management tool, Zotero (version 9.0.6, 64-bit; Corporation for Digital Scholar, Vienna, VA, USA), for abstract screening to check for relevance to the research questions; this resulted in an exclusion of 42 studies. The remaining studies underwent full-text screening, while 3 other studies were excluded since full-text manuscripts were not available. The full-text evaluation followed the specified exclusion and inclusion criteria specified in the previous section; the specific details are covered in the registered protocol. Following this, the included studies were limited to 36 after 23 other studies were excluded due a reason mentioned in Figure 1.

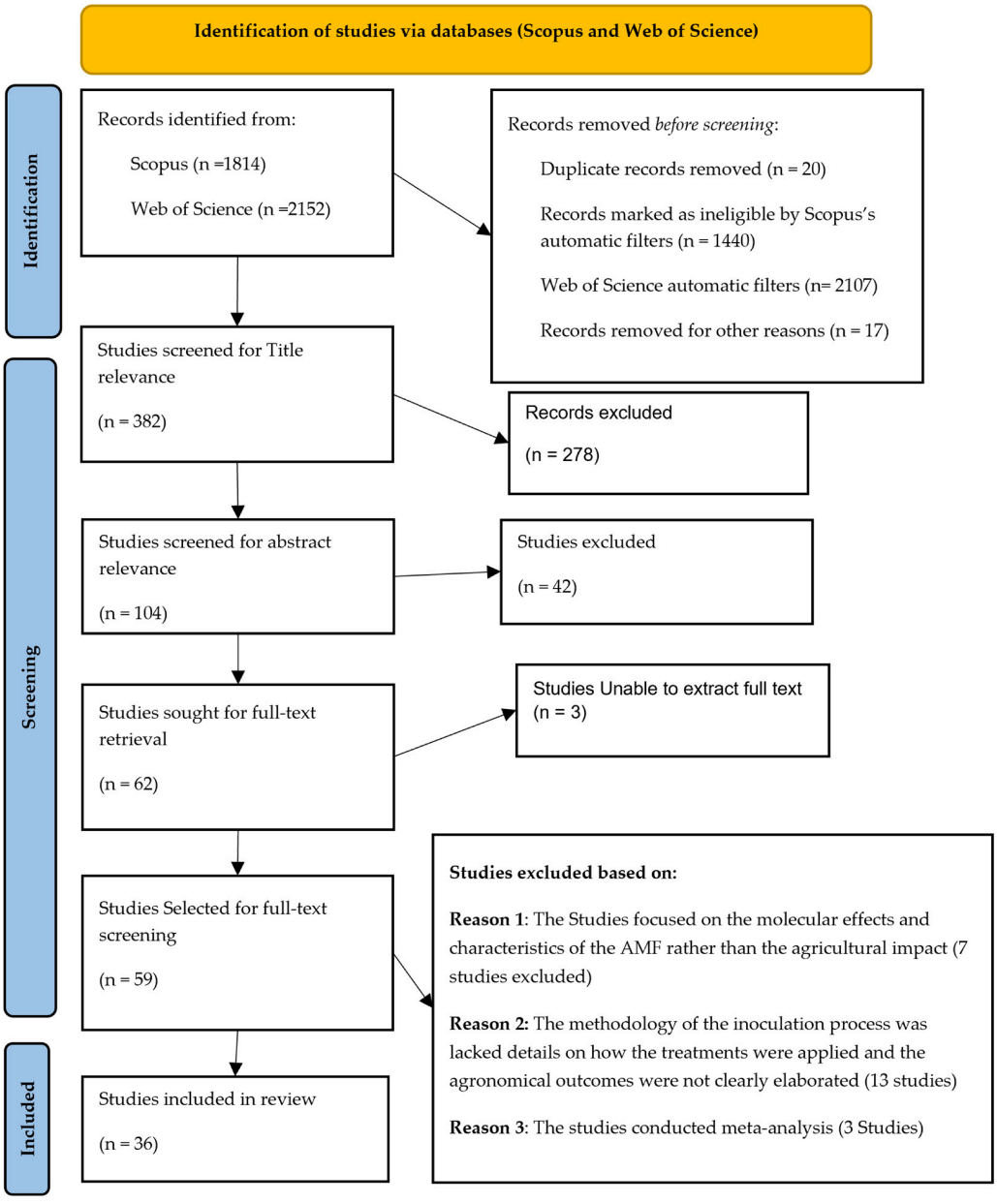

Figure 1. Adapted Prisma flow diagram showing the selection process for the included studies.

2.4. Risk and Bias Assessment

A structured quality assessment adapted from the core principles of established frameworks (such as ROBINS-I and SYRCLE) was applied to evaluate each included study. This approach was chosen over direct application of ROBINS-I or SYRCLE since both methods were developed for study contexts distinct from those presented in this review (ROBINS-I: clinical or exposure-based interventions; SYRCLE: randomised animal experiments) and

neither mapped directly onto the heterogeneous mix of pot, greenhouse, and field-based agronomic trials included here, which vary widely in randomisation practice. Nevertheless, this framework assessed both methodological quality and reporting completeness across five criteria: (1) use of an appropriate uninoculated control; (2) adequate replication (at least three replicates per treatment) within a randomized design; (3) reporting of a recognized statistical test with significance values; (4) complete baseline soil physicochemical characterization; and (5) clear identification of the inoculant to at least the genus level, together with the application method.

Studies were independently evaluated by two reviewers, with a third reviewer acting as a moderator to resolve any discrepancies. Following this procedure, studies were categorized into three categories:

- Low risk: Met all five criteria and was clearly reported in the methodology.
- Medium risk: Met all core experimental criteria (1–3) but omitted secondary reporting details, specifically failing on baseline soil data (4) and/or exact inoculant details (5). Medium-risk studies were not excluded, particularly where a study's contribution to a specific finding was disproportionate relative to the number of studies supporting that finding.
- High risk: Failed to meet one or more of the core experimental criteria (1–3) and was completely excluded from the review.

3. Data Extraction and Qualitative Analysis

Following the study selection phase, data were extracted systematically from the 36 included studies using a predefined and structured extraction framework. Data extraction was conducted to ensure consistency, transparency, and reproducibility across all included studies. The formal data extraction process was carried out starting on 25 November 2025 and lasting until the start of 16 March 2026. This process was a collaboration between four reviewers, three of whom worked independently extracting data, while one of the reviewers acted as a moderator solving disagreements raised in the extraction process. The data extracted during this process were classified as:

- Bibliographic information: This entails year of publication, journal, author names and publisher.
- Geographical information: Country and continent.
- Study characteristics: Study design and experimental setting (greenhouse, field, laboratory, microcosm).
- Inoculation strategy: Grouped into single-inoculation trials (AMF only) and dual-inoculation trials (AMF + PGPR). This was further classified into AMF and PGPR strains used in the studies.
- Supplementary treatments: Classified into inorganic fertilisers (Note: single inorganic fertilisers were also considered), organic fertilisers and studies without supplementary nutrient inputs.
- Soil characteristics: Soil texture, soil macro- and micronutrient concentrations, and soil pH.
- Morphological parameters: Included plant height, stem diameter, biomass, grain yield, leaf number and area.
- Physiological parameters: Included nutrient concentration and uptake (specifically macronutrients, including nitrogen (N), phosphorous (P) and potassium (K)), chlorophyll content, and bio-stress markers (for instance, hydrogen peroxide concentration in plant tissues).

Formal heterogeneity statistics (e.g., I^2, Cochran's Q) could not be computed because the variance data (standard deviations or standard errors) required for such calculations

were inconsistently reported across included studies. Beyond this reporting gap, included studies also differed systematically along several dimensions that would confound a pooled quantitative estimate: experimental setting (pot, greenhouse, or field trials); AMF and PGPR species and strain identity; baseline soil phosphorus status and texture; abiotic stress conditions (drought, salinity, or none); plant growth stage at measurement; and the specific tissue or metric basis of the outcome reported (e.g., tissue concentration versus whole-plant accumulation). Additionally, this heterogeneity was further enhanced by the number of included studies in this review. This heterogeneity was the primary basis for adopting a qualitative, pattern-based synthesis rather than a formal meta-analysis.

Initially, bibliographic information, including year of publication, was extracted to enable grouping of studies by publication period and to assess the temporal distribution of the literature. Subsequently, geographical information, including the country in which each study was conducted, was recorded to examine the spatial distribution of the selected studies. These data were used to generate the distribution map presented in Figure 2.

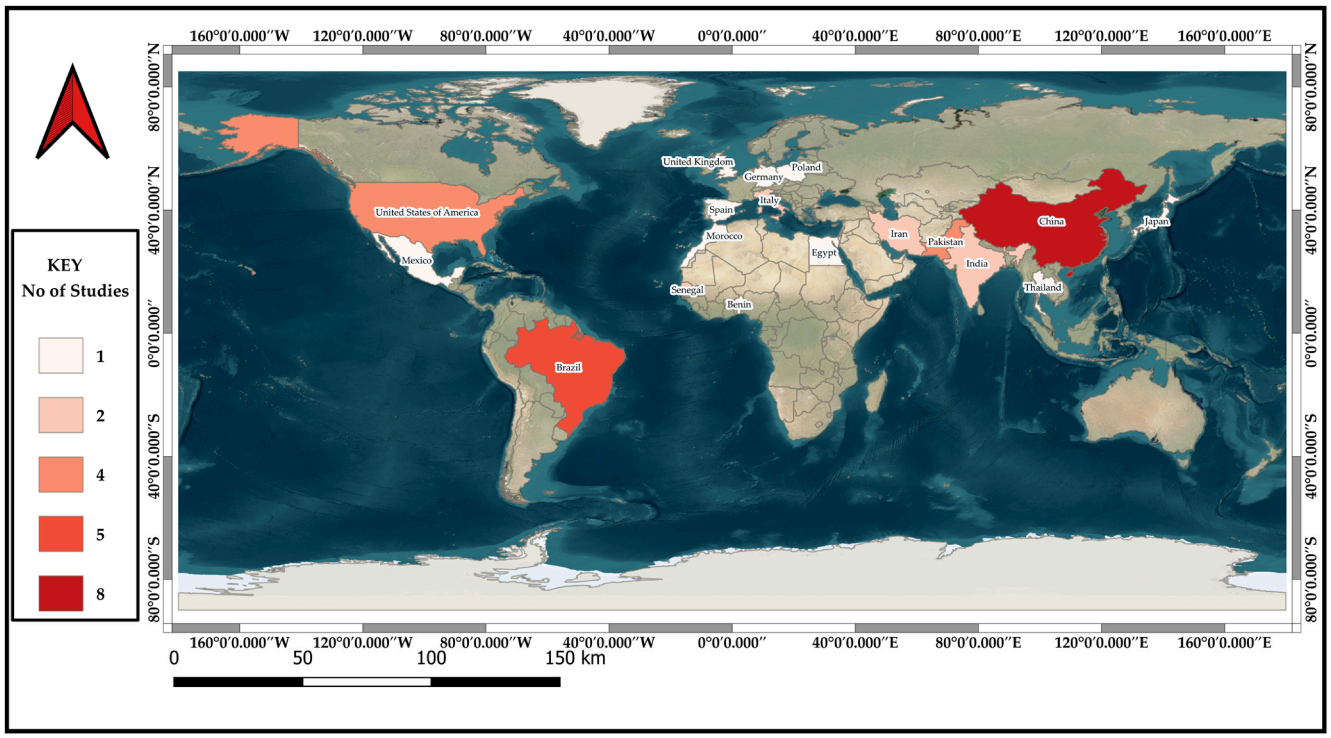

Figure 2. Geographical distribution of the studies included in this review (n = 36), showing spatial distribution.

The included studies were then classified into thematic categories based on study characteristics relevant to the objectives of this review. These categories encompassed study design, plant parameters assessed, type of microbial inoculant, and experimental setting, including greenhouse, field, laboratory, or microcosm conditions. This classification was guided by a conceptual framework derived from the search strategy and was designed to facilitate systematic synthesis in relation to the research questions addressed in this review.

Study design was categorised according to the inoculation strategy employed, distinguishing between single inoculation (AMF only) and dual inoculation (combined AMF and PGPR). Information on the species or strains of AMF and PGPR used in each study was extracted. Additional treatments, such as the application of chemical or organic fertilisers, were recorded and analysed separately from studies that relied solely on microbial inoculation without supplementary nutrient inputs.

Where reported, soil characteristics, particularly soil texture, were extracted to evaluate their potential influence on inoculation outcomes. To synthesise study outcomes, reported results were grouped into two primary categories: morphological parameters (including plant height, stem diameter, biomass accumulation, leaf number, leaf area, and root biomass) and physiological parameters (including chlorophyll content, stress indicators, and nutrient uptake, particularly nitrogen, phosphorus, and potassium).

Information on sampling methods and sampling periods was also extracted, as these factors were considered critical for interpreting variability and heterogeneity among study outcomes. All extracted data were compiled into a shared spreadsheet to facilitate collaborative review and consistency checking. The characteristics of the sample size of the included studies is presented in Table A1.

For qualitative synthesis, study outcomes were further classified based on whether inoculation resulted in a statistically significant effect or no significant effect on the measured parameters. These outcomes were mapped using a binary significance-based classification (significant vs. non-significant outcomes) approach, enabling identification of recurring patterns and trends across studies. This structured approach allowed individual studies to be systematically linked to specific components of the research questions and supported a transparent qualitative synthesis of the evidence.

4. Results

This systematic review consolidates the results of 36 studies conducted across five continents: Africa, Asia, Europe, North America, and South America. These studies were conducted between 2008 and 2025. The research encompasses 18 countries, including Benin, Brazil, China (includes one study from Hong Kong), Egypt, Germany, India, Iran, Italy, Japan, Mexico, Morocco, Pakistan, Poland, Senegal, Spain, Thailand, the United Kingdom, and the United States. Most studies originate from Asia, with over 18 investigations, most of which are from China, which accounts for approximately one-third of this number. Europe accounts for the second-largest share, comprising seven studies, two of which are from Germany. South America ranks third, representing five studies, all conducted in Brazil. This geographical distribution highlights the global scope and diversity of research included in the systematic review (Figures 2 and A1).

4.1. Study Characteristics

The experimental designs varied considerably across studies. Both field trials and controlled experiments were represented, including greenhouse experiments, pot trials, microcosm systems, and large-scale field evaluations. Several studies evaluated microbial inoculation under abiotic stress conditions such as drought stress, salinity stress or both stresses, while others investigated microbial effects under standard agronomic conditions. The designs also ranged from one-factor experiment—i.e., the effect of AMF or AMF-PGPR dual inoculation—to a three-factor experiment investigating tillage, inoculation and inorganic fertiliser concentrations such as zinc or phosphorus or nitrogen or NPK (nitrogen, phosphorous and potassium) fertilisers.

The studies evaluated maize response to these factors using parameters such as morphological growth parameters (plant height, stem diameter, leaf area and leaf number), biomass production, yield components, nutrient uptake, photosynthetic indicators and biochemical stress markers. These measured outcomes formed the overall theme of the results section of this review. These outcomes are discussed in detail in the following sections. It is important to note that the interpretations presented in the following sections are based on the 36 studies included in this review. Consequently, some outcomes may

be over- or underrepresented due to the available evidence base. The findings should therefore be interpreted with this limitation in mind.

4.2. Microbial Diversity and Inoculation Strategies

Across these studies, 32 distinct species of AMF were identified in the literature. These species belonged primarily to the genera *Glomus*, *Rhizophagus*, *Acaulospora*, *Gigaspora*, *Diversispora*, *Septoglomus*, *Claroideoglomuse*, *Cetraspora*, *Dentiscutata*, and *Scutellaspora*. Notably, some investigations referred to the same species using different or outdated nomenclature. This is particularly evident within the genus *Glomus*, where the name *Glomus intraradices* was historically applied to what molecular data have since shown to be more than one distinct species. Strains matching the original species concept are now classified as *Rhizophagus intraradices*, while other widely studied strains were found to represent a separate, closely related species, transiently named *Rhizoglomus irregulare* before being formally established as *Rhizophagus irregularis*. Similarly, revisions have occurred for other species, such as *Funneliformis mosseae* which was previously referred to as *Glomus mosseae* [21].

In addition to AMF, studies also investigated plant growth-promoting rhizobacteria (PGPR) either independently or in combination with AMF. The most frequently reported bacterial genera included *Bacillus*, *Azospirillum*, *Azotobacter*, *Pseudomonas*, *Streptomyces*, *Rahnella*, *Micrococcus* and *Burkholderia*. Some studies also evaluated additional beneficial microorganisms such as *Trichoderma* species, a plant beneficial fungus, that is not considered an AMF [22].

Two primary inoculation strategies for AMF inoculation were identified across the reviewed studies:

- Single AMF inoculation, where only mycorrhizal fungi were introduced to maize roots.
- Dual-inoculation systems, combining AMF and PGPR to evaluate potential synergistic effects on plant growth and stress tolerance.

Most of the recent studies focused on dual-inoculation strategies, reflecting increasing interest in microbial consortia capable of improving nutrient acquisition, enhancing plant physiological performance, and mitigating abiotic stress conditions such as drought or salinity. These inoculation approaches formed the basis for comparing microbial effects on maize growth and productivity throughout the studies included in this review.

4.3. Morphological Assessment of AMF Inoculation and PGPR on Zea mays

4.3.1. Plant Height

Fifteen studies reported on plant height in this review. These studies focused on both single AMF inoculation and dual inoculation (AMF-PGPR). The effects of AMF inoculation on plant height varied among studies. Two greenhouse studies in Senegal and the USA found no significant change in plant height post single AMF inoculation, attributing differences in height between treatments mainly to maize variety [23,24]. Similarly, field experiments in Italy reported no significant changes in plant height after AMF inoculation [25].

Synthesis from the studies revealed that the impact of AMF on plant height was heavily dependent on concurrent nutrient supplementation protocols. Across multiple studies, standalone AMF inoculation yielded marginal height growth increments, exemplified by a 4.8% increase in shoot length using *Acaulospora laevis* (AMF) [26] and a 30% plant height [27] increase in China and Mexico, respectively. Conversely, co-inoculation with reduced-dosage inorganic fertiliser consistently maximized height parameters, especially at doses of 50% of the recommended dosage of inorganic fertiliser, achieving results comparable to 100% NPK fertilisation [28]. In Mexico, AMF inoculation with reduced dosage of NPK fertiliser resulted in a 12% increase in plant height as compared to standalone

AMF inoculation. Furthermore, inoculating AMF under varied zinc and phosphorus levels resulted in elevated plant heights, especially in inoculations at 20 mg Zn kg^{-1} and 2.8–3.8 mg P kg^{-1} of soil fertiliser supplementation in Pakistan [29,30]. These trends were mirrored in organic fertiliser supplementation studies, where poultry litter application alongside AMF inoculation resulted in the highest plant heights, outperforming inorganic fertilisation (NH_4) in both Hong Kong and the USA [31,32]. These findings indicate that without nutritional supplementation (fertiliser), AMF benefits in terms of plant height could be diminished.

Dual inoculation of AMF with PGPR also showed a similar pattern to single AMF inoculation, indicating that the synergetic effect was most pronounced in cases where inoculation was combined with fertilisation. For instance, a field study in the USA noted a substantial increase in plant height post-dual inoculation of AMF and PGPR. This was enhanced with organic fertilisation (poultry litter (PL)), leading to a plant height increase of 17–21% as compared to the control [31]. Similar observations were made in studies of *G. intraradices* combined with *Azotobacter* and *Bacillus* spp., alongside organic matter fertilisation [32]. Inorganic fertilisation with either NH_4 or 50% recommended TSP in the form of K_2SO_4 also resulted in an enhanced synergetic effect after inoculation with AMF and PGPR, with height increases of ~11–18% reported [31,33]. Additionally, dual inoculation with 50% recommended NPK supplementation resulted in ~61% increase in plant height across a 2-year period [34]. These studies indicate, similar to single AMF inoculation, that dual inoculation with PGPR could result in plant height gains, especially when combined with nutritional supplements at reduced dosages or with organic fertilisation.

Furthermore, water stress conditions were noted to have an effect on the success of dual inoculation. Several studies reported highest increases in plant height in drought conditions especially at R-stages, reporting increases of ~85% in some of the cases [31,35]. Similarly, root length increase in drought conditions was highest with dual inoculation, leading to a 17% increase [36]. On the contrary, Ref. [26] reported under severe water stress, with no synergetic effect observed despite dual inoculation. Instead, all three inoculation treatments—i.e., PGPR-AMF, PGPR and AMF inoculation—resulted in similar plant height gains, and the treatments saliently outperformed the control [26]. On the other hand, a 2-year organic field study in Germany found that plant height was not significantly affected by either single AMF or dual inoculation with PGPR in the second year. In the first year, PGPR inoculation alone significantly increased plant height 7.6% at anthesis. However, dual inoculation, predominantly with AMF, showed no significant difference in plant height in both years [37]. Similarly, a mesocosm study in the UK reported that PGPR inoculation was more effective at increasing plant height than AMF inoculation, particularly under severe water stress. Additionally, it was observed that PGPR protected AMF under such stressful conditions [38]. Overall, the literature demonstrates that dual inoculation effectively mitigates drought stress in plants, actively promoting increased plant height and vegetative growth under water-limiting conditions.

To sum up, plant height responses to AMF and AMF-PGPR inoculation were strongly dependent on nutrient availability, environmental conditions (water availability, soil properties) and inoculation strategy, with the most consistent and pronounced increases observed under combined microbial inoculation with supplemented nutrients (Table 1).

Table 1. Summary of plant height investigating studies.

Study	Country	AMF	PGPR	Supplements	Settings	Findings
[23]	USA	*Glomus mosseae*	___	___	Greenhouse,	No significance, plant height affected by maize cultivar
[39]	Senegal	*Rhizophagus irregularis*	___	___	Greenhouse	No significance; however, local cultivars showed potential with AMF
[25]	Italy	*Glomus mosseae*, *G. intraradices*	___	Urea (N)	Field	No significant change ($p > 0.05$); AMF (314 cm) vs. control (299 cm)
[28]	Benin	*Glomus caledonius*, *R. intraradices*, *Funneliformis geosporum*, *Acaulospora capsicula*, *A. dilatata*, *Diversispora globifera*	___	50% NPK and urea	Field	Measurable increase with supplements ($p \leq 0.001$); glomus + 50% NPK yielded 146.35 cm. No significance without supplements. AMF species varied in performance
[37]	Pakistan	*Glomus* spp.	___	Zinc ($ZnSO_4$)	Pot experiment	Significant increase in height in both treatments with or without supplements. Best results in Zn (20 mg kg^{-1} of soil) + AMF
[29]	Pakistan	*Glomus*, *Gigaspora*	___	Zinc ($ZnSO_4$)	Pot experiment	Substantial increase with supplements ($p \leq 0.001$); AMF (160 cm) vs. control (147 cm). Max height (184 cm) at 20 mg Zn/kg. AMF without supplements not significant
[31]	USA	*Glomus intraradices*	*Bacillus* spp.	Poultry litter (PL), NH_4	Field	Significant in both AMF and PGPR inoculation; combined inoculation had a notable impact. PL improved inoculation results; 165–182 cm vs. 140–150 cm control
[37]	Germany, Italy	*A. morrowiae*, *G. gigantea*, *G. rosea*, *G. mosseae Rhizophagus Irregularis*, *Scutellaspora pellucida*, *Glomus mosseae*, *Glomus coronatum*, *Glomus caledonium*, *Septoglomus viscosum*	*Bacillus*, *Azotobacter*, *Pseudomonas*, *Rahnella*, *Burkholderia*, *Azospirillum*	Potato protein liquid (PPL)	Organically managed fields	Significant increase in PGPR alone in Year 1 (224 vs. 208 cm, $p \leq 0.05$) at stem elongation only, with no significant effects in Year 2. AMF and dual treatments were not significant in both years; AMF reduced height at ripening

Table 1. *Cont.*

Study	Country	AMF	PGPR	Supplements	Settings	Findings
[40]	China	*Rhizophagus irregularis*	*Bacillus velezensis*	Hoagland solution containing N and P	Greenhouse	Significant effect: Monoculture: PGPR increased height by 40%, AMF co-inoculation by 72%. Intercropping: dual inoculation highest (199%), followed by AMF (101%) and PGPR (90%)
[26]	China	*Acaulospora laevis*	*Bacillus subtilis*	——	Pot experiment	Well-watered: dual inoculation highest (11.8%), followed by PGPR (6.9%) and AMF (4.9%). Medium stress: only dual significant (7.4%). Severe stress: Only dual and PGPR significant
[38]	UK	*Rhizophagus clarus*	*Bacillus* spp.	Hoagland solution	Microcosms in growth chambers	Significant effects on height noted in moderate water stress with PGPR inoculation only
[32]	Hong Kong (China)	*Glomus mosseae. G. intraradices*	*Bacillus megaterium*, *B. mucilaginous*, *Azotobacter chroococcum*	Biofertiliser (OM): poultry litter + organic rock phosphate	Greenhouse pot experiment	Significant synergistic effect ($p \leq 0.05$); PGPR + AMF + OM reached ~110 cm vs. Control ~40 cm and CF ~70 cm.
[33]	Iran	*Funneliformis mosseae*	*Pseudomonas flourescens*	Triple super-phosphate (TSP)	Field experiments (transplanting)	Tassel height sig. increased by AMF (45 cm vs. 40 cm, $p \leq 0.05$); (synergistic effect) dual inoculation exceeded 50 cm. *Pseudomonas* alone was non-significant
[27]	Mexico	*A. morrowiae*, *A. scrobiculata*, *A. spinosa*, *F. geosporum*, *F. mosseae*, *Gigaspora decipiens*, *G. rosea*, *Glomus aggregatum*, *Gl. macrocarpum*, *C. pellucida and Claroideoglomus etunicatum*	*Azospirillum brasilense*	50% chem. fertiliser (simple super-phosphate calcium, simple super-phosphate urea	Field experiments (no tillage)	AMF: 112.5 cm (b); PGPR: 112.57 cm (b); AMF + 50% chem and PGPR + 50% chem: 123.59 (b) and 144.9 cm (a) respectively. Highest in dual inoculation + 50% chem: 151.93 (a); while control: 86.52 cm (a). Letters show significant difference

Table 1. *Cont.*

Study	Country	AMF	PGPR	Supplements	Settings	Findings
[36]	Spain	*Septoglomus constrictum*, *Diversispora aurantia*, *Archaespora trappei*, *Glomus versiforme*, *Paraglomus occultum*,	*Bacillus thuringiensis*	——	Greenhouse (pot experiment)	Focused on root length; dual inoculation was highest significantly in both drought regimes; PGPR and AMF, not significant. In drought, PGPR was lowest significantly, while AMF and control showed no difference (411 cm and 410 cm respectively)
[34]	Egypt	*Glomus clarum*, *G. mosseae*, *Gigaspora margarita*	*Azotobacter chroococcum*, *Bacillus circulans*	NPK fertiliser (urea, calcium superphosphate P_2O_5, potassium sulphate), organic fertiliser (rabbit manure), biogas slurry	Field experiment	Dual inoculation significant across 2 years. 2019: Bio (PGPR-AMF) + 50%NPK (300.1 cm) vs. 100% NPK control (179.5 cm)

4.3.2. Stem Diameter

Stem diameter was reported across six studies, with the majority investigating dual-inoculation effects with PGPR.

Studies found stem diameter increased significantly with inoculation of AMF, especially with supplementations of fertiliser at reduced levels [28,29]. For instance, inoculation of *Glomus* and *Gigaspora* species (AMF) resulted in a 16% increase in stem diameter as compared to the control. However, this effect was even stronger with supplementation of Zn and P, which resulted in a 35.8% increase in stem diameter as compared to non-inoculated plants at a double dosage of Zn and P fertilisation [29]. Furthermore, similar observations were made by a similar study, where inoculation of *Glomus* + 50% NPK and urea yielded results comparable to 100% NPK and urea fertilisation in non-inoculated plants [28]. Moreover, AMF species performed differently in stem diameter, with the lowest improvements noted in plants inoculated with the *Acaulospora* species, especially at the R1-R2 stage [28]. Another study observed that stem diameter was strongly correlated with the intensity and frequency of root colonization [39]. In contrast, some studies reported no significant changes. For instance, a pot experiment in Pakistan reported that diameter was significantly increased only when AMF were inoculated with zinc at the rate of 20 mg kg^{-1} [30]. Similarly, in Mexico, inoculation of AMF with 50% recommended NPK resulted in measurable improvement in stem diameter; however, without supplementation, it was not significant [27]. Cumulatively, the findings indicate that single AMF inoculation could only affect stem diameter if supplemented with nutrients in the form of fertiliser.

Dual-inoculation studies reported heterogenous findings. For instance, in Mexico, AMF and PGPR (*Azospirillum brasilense*) inoculation resulted in significant increases, especially when accompanied by application of 50% of the recommended NPK dosage, resulting in a 30% increase in stem diameter. Additionally, without supplementation, stem diameter was still statistically similar to the treatment receiving 100% recommended NPK fertilisation in the non-inoculated plants [27].

On the contrary, AMF inoculation was reported to have no significant effect on stem diameter [37], while in another case it was reported to have yielded lower measurements in diameter as compared to other treatments [38], especially in cases where inoculation was carried out in drought conditions.

Overall, stem diameter responses to inoculation were less consistent than other morphological parameters, with single-AMF effects generally requiring nutrient supplementation to reach significance, while dual-inoculation outcomes varied depending on fertilisation regime and water availability.

4.3.3. Leaf Number and Leaf Area

Leaf number was reported in three cases among the studies included in this review, while leaf area was reported in six studies, including those that conducted both dual and single AMF inoculation.

Single AMF inoculation in several studies was reported to have no to minimal effects regarding leaf number, especially when inoculated without supplements. A greenhouse experiment measured leaf number at three key developmental stages of maize post-inoculation with AMF and found no effect across all stages, which was supported by another greenhouse study in Senegal [39]. However, notable improvements were observed in studies combining AMF with nutritional supplements like zinc. In zinc-deficient soils in Pakistan, the highest leaf number increase was seen with 20 mg kg^{-1} of zinc plus AMF, resulting in 12–13 leaves compared to 8–9 leaves in the non-inoculated plants (control) with similar zinc levels—an over 40% increase due to inoculation. Furthermore, even without zinc supplementation, AMF treatments outperformed controls in leaf number [29]. Similarly, application of 50% NPK alongside dual inoculation led to a 90% increase in leaf number. Adding humic acid with NPK and AMF further enhanced these effects, though this was only observed during the first year [34]. An Italian study involving maize seed inoculation with *G. intraradices* and *G. mosseae*, using a green adhesive powder and a solution of vegetal amino acids and peptides mixed with the seeds resulted in a 9.6% increase in leaf number [25].

In the leaf area measurements, as with the previous traits, contrasting outcomes regarding inoculation effects were observed. For instance, leaf area was reported to be unaffected by inoculation, with reported effects mainly due to maize cultivar used [39]. Similarly, in drought conditions, AMF was associated with lower leaf area than the control, with the lowest values observed in treatments inoculated with compost and AMF [41]. However, another study in Benin reported that AMF (*Glomus*) combined with 0% NPK resulted in a 32% increase in leaf area, but upon inoculation with 50% NPK fertilisation the AMF resulted in a lower leaf area as compared to the non-inoculated control [28]. Furthermore, a 7.8% increase in leaf area was reported in the Italian study following AMF inoculation [25].

Likewise, dual inoculation studies [33], also observed higher leaf area as compared to the control treatments, especially with dual inoculation (AMF-PGPR) combined with 50% TSP (37.5 kg/ha), exceeding 0.15 m^2. Leaf area increased significantly, especially in maize plots treated with PGPR alone, which showed a 27% increase, followed by chemical fertiliser at 21.5%, then CoMyBa (Compost + Dual inoculation) at 17%, under well-watered conditions. Under drought conditions, the highest leaf area was observed in plants treated with chemical fertiliser or CoMyBa [41]. Consistent with this, an Egyptian field experiment on AMF strains (*Glomus* and *Gigaspora*) alongside PGPR (*Azotobacter* and *Bacillus*) and organic and inorganic supplements reported that leaf area was significantly increased after dual inoculation alongside biogas slurry and 50% NPK dosage, resulting in increases of 61% and 28% in the 1st and 2nd year of the experiment, respectively, as compared

to the non-inoculated controls at 100% NPK fertilisation [34]. These studies support the conclusion that leaf area and leaf number can be significantly increased with inoculation. However, most of the studies indicate that supplementary additives are required to achieve this effect (Table 2).

Table 2. Leaf number and leaf area summary.

Study	AMF	PGPR	Supplement	Growth Stage	Leaf Number (LN)	Leaf Area (LA)
[30]	*Glomus* sp.	_____	Zinc ($ZnSO_4$)	Harvest (R6)	Peak LN recorded with Zn_2O + AMF; 51.8% Increase over non-mycorrhizal control in ZnO	N/A
[33]	*Funneliformis mosseae*	*Pseudomonas fluorescens*	TSP (phosphorus), urea (N)	Seed filling (R stages)	No effect	AMF inoculation led to a 32% increase in leaf area. Dual inoculation led to 42% (control: 0.19 m^2, dual: 0.27 m^2)
[41]	*Glomus* (60%), *Acaulospora* (40%)	*Bacillus subtilus*, *Bacillus* sp.	Compost	At harvest (R6)	N/A	Highest increase in PGPR at 27%, then dual inoculation at 17% (no water stress). Under drought: Dual inoculation + compost was most effective at a 16.8% increase
[34]	*G. intraradices*, *G. mosseae*, *G. claroideum*, *G. etunicatum*, *Gigaspora margarita*	*Bacillus licheniformis*, *B. azitofixans*, *B. megaterium*, *B. subtilis*	Biogas slurry, humic Acid, NPK (urea, calcium superphosphate (15.5% P_2O_5), potassium sulphate (48% K_2O)	Harvest (R6)	Dual inoculation: 90% increase in leaf number compared to the control.	Two-fold increase (approx. 200%) when combined with 50% NPK. Especially after addition of humic acid
[39]	*Rhizophagus irregularis*	_____	_____	Four days prior to	No effect.	N/A
[28]	*G. caledonius*, *R. intraradices*, *F. geosporum*, *A. capsicula*, *A. dilatata*, *D. globifera*	_____	NPK	At R1 (silking stage)	N/A	Not significant at 50% NPK, control yielded higher leaf area. At 0%NPK inoculation was significant leading to 32% increase in leaf area

4.3.4. Agronomic Gains (Biomass and Grain Yield)

Across included studies, biomass and grain yield response to microbial inoculation were heterogeneous, with both significant gains and non-significant responses reported depending on inoculant strain, maize genotype, nutrient availability, plant tissue and environmental conditions.

Response magnitude in both biomass and grain yield was noted to track soil nutrient status. For instance, a five-state Brazilian field experiment on *Rhizophagus intraradices* inoculation alongside varied phosphorus dosages reported average increases of 48% (biomass) and 54% (grain yield). The highest gains were observed in soils with low to medium P content, reporting gains of up to 122% and 138% in both biomass and grain yield, respectively [24]. A comparable pattern was reported for native AMF strains, which had

no significant effect alone, but, combined with urea + 50% NPK, significantly increased shoot biomass (+6.44%) as well as grain yield (+62.5%) [28]. Similarly, field experiment in Germany found that the inoculation effect on biomass was only significant in the organic fertilised fields [37].

Microbial strains, consortia, and maize genotypes exhibited distinct variations in their capacity to modulate biomass and grain yield. A Senegalese field trial reported biomass increases were only noted in mycorrhizal-dependent maize varieties, after inoculating *Rhizophagus irregularis* in six maize varieties [39]. One study found shoot biomass increased significantly after *Glomus mosseae* inoculation, while root biomass did not [23]. Another, separate field trial found no significant shoot biomass effect across three AMF regimes; *Funneliformis mosseae* (also known as *Glomus mosseae*), *Rhizophagus irregularis* and mixed species [42]. This was also reciprocated in Japan [43], where *A. morrowiae* produced the highest biomass numerically but not significantly. Environmental stress modulated single-AMF response direction in a three-factor (AMF, saline stress and water stress) Chinese pot trial: shoot biomass increased significantly by 47% in moderate stress, but showed no significant effect under drought or saline conditions, with saline stress lowering AMF-treated biomass and drought raising it (non-significantly) relative to the control [44]. Similarly, the inoculation of AMF strains *Glomus*, *Acaulospora*, *Entrophospora*, and *Scutellaspora* resulted a significant increase in stubble dry weight under both AMF and phosphorus treatment in Thailand [45], while field-grown biomass rose by 16.2% following AMF inoculation in Italy [25].

Dual inoculation and cropping system further shaped biomass outcomes, Co-inoculation of *Rhizophagus irregularis* and *Bacillus velezensis* raised dry weight by 190% versus 48% for AMF alone, partly attributable to an intercropping system in which co-inoculated intercropped maize outweighed monoculture by 50% [40]. Under water stress, shoot dry weight was significantly higher in AMF and AMF-PGPR relative to the control [26], resulting in increases in shoot dry weight of ~6% and ~21% in AMF and dual (AMF-PGPR), respectively, under medium water stress conditions, and increases of 9.5% and 12.8% in severe water stress, respectfully. Additionally, biological yield increased by 10.25%, 11.03%, and 13.78%, in AMF, PGPR and dual inoculation respectively, while in drought conditions, biological yield increased by 12.02%, 7.89% and 19.81% respectfully [26]. A similar trial found a *Glomus mosseae*-PGPR combination produced the highest biomass increases (+300%) in a multi-strain experiment [32]. Similarly, a study in Senegal utilising a similar commercial inoculant (M2) containing *Glomus* species and PGPR species observed that locally identified inoculum (M1) outperformed both the control (+30%) and a commercial product (+10%) [46]. Single AMF inoculation significantly increased leaf dry weight, stem dry weight, silk dry weight, and tassel dry weight by 31.3%, 4.9%, 23.1%, and 4.7% in normal irrigation and by 17.4%, 16.6%, 18.2%, and 21.2% under water deficit conditions, respectively. With dual inoculation (AMF-PGPR) these traits were significantly increased by 38.4%, 9.21%, 28.6%, and 14.6% in normal irrigation and by 18.9%, 21.7%, 28%, and 25.2% under water deficit conditions, respectively [33]. Under combined stress (salinity and drought) in Iran [22], AMF species and *Trichoderma* inoculation treatments on maize significantly increased total biomass by 139%, while in saline and drought conditions, AMF yielded a 125% and 156.7% increase in biomass respectively. However, under both drought and saline stress, biomass increase was lower (+67%); additionally, the synergistic effect between AMF and *Trichoderma* was not observed [22]. In another dual-inoculation study under water-stress conditions, it was reported that the total dry weight of the plant was significantly highest in *Bacillus* + AMF inoculation, while single AMF inoculation treatments were not significant in their effects on total dry weigh; however, *Azospirillum* species yielded the highest root dry weights [47]. A two-year Egyptian trial [34] found that

dual inoculation combined with 50% chemical fertiliser (NPK) significantly increased both leaf and root dry weights, yielding increases of 83% (leaf dry weight) and 98% (root dry weight). In another study [35], dual inoculation of AMF and PGPR increased shoot dry weight from a range of 345.7–590.4 g in the control to 670.7–1419 g/shoot in AMF-PGPR treatments, especially in the 30–48-day-old treated plants.

Similarly, ref. [28] found that native AMF strains significantly affected maize productivity. Specifically, co-inoculation of *Glomus caledonius*, *Acaulospora* (*A. dilatate* and *A. capsicula*), and *Diversispora globifera* combined with 50% NPK induced a 62.5% increase in grain yield compared to the uninoculated control with 0% NPK, while *Glomus* treatments combined with 50% NPK matched the uninoculated control with 100% NPK fertilisation, yielding an average of 4.21 t/ha [28]. Similarly, ref. [25] reported that cob weight increased by 17.9% post-inoculation with *G. intraradices* and *G. mosseae* mycorrhizal treatment [25]. Reintroducing a native *Glomus* strain promoted grain yield in maize specifically when planted after a fallow period [45]. Under 0% phosphorus fertilisation following fallow, inoculated plants yielded 107.1 g/plant compared to 99.0 g/plant for the control. The highest grain yields were noted in the no-disturbance-to-soil practice that was followed by a fallow period, especially in the 92 kg/ha phosphorous (P3) application, yielding 115.4 g/plant in the uninoculated plants, while in the inoculated plants, it yielded 119 g/plant at the same dosage of phosphorous. AMF treatment in P 2 (33 kg/ha) yielded similar results to the control in the P3 dosage (AMF + P2: 114 g/plant) [45], indicating AMF inoculation could reduce usage of chemical fertilisers, especially P, and still offer similar outcomes as conventional dosages. Finally, in the field component [42], maize under rotary tillage (which increased the dominance of *Gigaspora*) achieved a significantly higher yield compared to no-tillage systems, suggesting that the shift in AMF community structure directly influenced final grain weight.

In contrast, some studies reported no significant increase in grain yields post-inoculation. In [42], no significant differences in fresh or dry ear yield were observed after AMF treatments. The authors noted that natural AMF colonization across all plots likely provided a functional baseline that masked the effects of supplemental inoculation [42]. In [45], while yield increased after a fallow period, inoculation had no significant effect on the grain yield of maize grown in continuous rotation or following a non-AMF, host-like cabbage. Additionally, the controlled pot experiment in [43] showed that aboveground biomass (a proxy for yield) did not change with any of the four tested AMF inoculants compared to the control.

Among dual-inoculation trials (AMF-PGPR), a field experiment in the USA [31] reported that AMF (*Glomus intraradices*), PGPR (*Bacillus* sp.), and their combination (AMF-PGPR) produced a statistically significant increase in grain yields of 34.8% (AMF), 27.7% (PGPR) and 26.81% (AMF-PGPR) compared to the control; however, no synergetic effect was observed. Similarly, ref. [33] found that AMF (*Funneliformis mosseae*) resulted in higher grain yields than PGPR (AMF: 8.0 t/ha; PGPR: 7.3 t/ha). Dual treatment (AMF-PGPR) achieved the highest significant grain yields of 8.8 t/ha, especially in drought conditions, indicating a possible synergetic effect on grain yield. Additionally, ref. [41] reported similar synergetic effects under drought conditions after the combination of compost-AMF-PGPR (CoMyBa) resulted in a yield (10.65 t/ha) increase of +175% over the untreated control (3.8 t/ha). This yield was also higher than the plants treated with NPK (8.4 t/ha), but not significantly so. In well-watered (100% etc.) conditions the single AMF (composed of 60% *Glomus* sp. and 40% *Acaulospora* sp.) inoculation treatment yielded the highest grain yield of 13.8 t/ha as compared to the control at 6.6 t/ha. However, in drought conditions, PGPR (Bacillus sp.) outperformed the single AMF inoculation (AMF: 7.6 t/ha, PGPR: 9.1 t/ha), but these results were statistically similar. Similar findings were also reflected in [26],

where dual inoculation (AMF-PGPR) significantly mitigated severe water stress (35% field capacity), producing a yield gain of 26.7% relative to the control. Similarly, they observed that the PGPR grain yield (78.48 g/plant) was higher than the AMF (75.55 g/plant) but was not statistically significant. In well-watered conditions, the highest grain yield ranked AMF-PGPR > PGPR (Bacillus subtilus) > AMF (*Acaulospora laevis*) > control, resulting in 120.19 g/plant, 115.56 g/plant, 108.33 g/plant and 102.84 g/plant respectively [26]. This suggests that single-AMF-inoculation success is affected in drought conditions, while dual inoculation could offer a solution in such conditions.

Nutrient supplementation using both chemical fertilisers and organic supplements shaped yields further. In [48], the combined application of rock phosphate (RP) and AMF + *Bacillus* nearly doubled productivity, resulting in a +190% increase in yield compared to the control, while single AMF treatments resulted in grain yield increases of +118%. Interestingly, dual inoculation (AMF-PGPR) without the RP still produced yield gains comparable to single superphosphate (SSP) treatments (SSP: +196%, AMF-PGPR without RP: +174%). In [27], the *Azospirillum* (Ab) + 50% inorganic fertiliser (IF) treatment promoted a 74.21% increase in grain weight, while the AMF + 50% IF led to an increase of 47.42% as compared to traditional management. A synergetic effect was also noted in cases where inorganic fertiliser was not applied together with each treatment of inoculant (AMF, PGPR and AMF-PGPR; $p \leq 0.05$). Ref. [34] further demonstrated that the biogas–biofertiliser–50% NPK treatment significantly enhanced yield attributes, achieving a cob weight of 227.56 g and a grain weight of 35.4 g/100 grain in 2018, compared to only 61.5 g and 25.8 g/100 grain, respectively, in the 100% NPK control ($p \leq 0.05$). The biofertiliser in this particular study was composed of AMF (*Glomus* and *Gigaspora*) and PGPR (*Azotobacter* and *Bacillus*) species. Similarly, a field study investigating co-inoculation of AMF and earthworms, demonstrated a significant increase in yields ($p \leq 0.05$), with the combined treatment reaching 11.90 t/ha in 2010 versus the control's 9.97 t/ha [49]. The AMF treatment also led to significant increases in yields (11.23 t/ha, $p \leq 0.05$), while the earthworm inoculation also increased yields (10.83 t/ha) moderately as compared to the control.

A field experiment conducted in Poland during dry and extremely hot seasons investigated AMF inoculation, PGPR inoculation and algae inoculation with respect to their effects on yield alongside biochar treatments [50]. The biochar + AMF (BWM) treatment was the most effective, yielding 8.83 t/ha, with a harvest moisture content of 18%, which was 1.89 t/ha higher and 4.8% lower than the control, respectively ($p \leq 0.05$). The combined synergistic effect of the AMF and algae inoculation (BWM + algae: 8.02 t/ha) resulted in significant yield increases when compared to the control, while it did not exceed the single AMF treatment with biochar [50]. Another study [51] experimented with the effects of PGPR species together with earthworms on the root colonization rate of AMF. They noted that dual inoculation (PGPR + earthworms) increased the colonization rate significantly ($p \leq 0.5$). Consequently, the combination of PGPR and earthworms (BE) significantly increased average grain yields by 11.2% relative to the control ($p \leq 0.05$). Similarly, ref. [30] found that a balanced application of Zn20 + AM significantly increased biological yield by 42.2% over sole AM inoculation. Finally, in 2021, ref. [37] reported a significant 10% increase in grain yield specifically for the MC_C_AMF consortium, a dual inoculant containing both AMF and PGPR (13.5 t/ha vs. 12.3 t/ha control). Ref. [40] also noted that co-inoculation significantly increased the dry weight of intercropped maize by 190% relative to non-inoculated controls, suggesting a substantial boost to final productivity.

Several studies also identified treatments that did not impact yield. In [37], the novel consortium MC_C (constituted mainly of PGPR) failed to significantly impact maize productivity in 2021, and the commercial consortium Micosat F (AMF) also showed no

significant difference compared to the non-inoculated control in either 2020 or 2021. In [51], inoculation with PR bacteria (B) alone or earthworms (E) alone had no significant effect on grain yield ($p > 0.05$). Similarly, in [46], high data variability resulted in no statistically significant differences in total biomass yielded by microbial treatments compared to the control. In [50], the application of biochar (BW) without additives had no significant effect on seed yield, which the authors attributed to the low application dose. Finally, ref. [31] noted that while all inoculants were better than the control, the co-inoculation of PGPR and AMF did not produce a synergistic effect, as its yield was not significantly different from the yields of single inoculations. Additionally, it could be noted that in terms of experimental environment mycorrhizal responsiveness was observed to be higher in the field experiments (Figure 3).

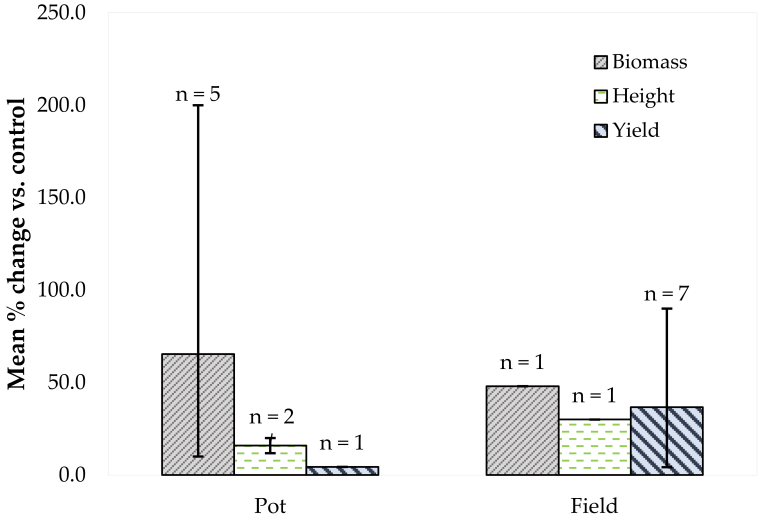

Figure 3. Variation in mycorrhizal responsiveness (%) between pot and field environments, as measured by plant height, biomass accumulation, and grain yield across the studies.

4.4. Physiological Assessment of AMF and PGPR on Zea mays

4.4.1. Nutritional Uptake or Concentration in the Plant (N, P and K)

Studies in this review, demonstrated that microbial inoculation, particularly AMF, was highly effective at enhancing the accumulation of nitrogen (N), phosphorus (P), potassium (K), and various micronutrients in the treated plants.

In [52], researchers found that mycorrhizal symbiosis significantly increased the total accumulation of N, P, and K in maize tissues. The result showed that the accumulation of the nutrients depended on AMF species, growth stage and plant tissue. Furthermore, the impact of AMF inoculation was most pronounced during the grain filling stages, especially after inoculation with a mixture of three AMF species. Moreover, at V12 stage, the highest accumulation of P and N leaf content was observed in plants inoculated with *Rhizophagus aggreatus* and *Funneliformis mosseae*, respectively [52]. Similarly, AMF symbiosis increased both shoot and root uptake of P and zinc (Zn), without supplementation of inorganic fertiliser, resulting in 80–126% increase in P accumulation, while zinc was noted to have increased by 20–22% in both shoot and root tissue [29].

A multi-state evaluation study in Brazil also observed that AMF-inoculated plants achieved an average increase of 80% in P accumulation across various states. The highest response was noted in states with the least soil P and the lowest P supplementation. Treatments that received 100% recommended P supplementation resulted in higher P content. However, the P increment as compared to treatments at lower P supplementation—i.e., 50% and 0%—was reduced, indicating diminishing returns with increases in P [24]. Additionally, ref. [45] confirmed that reintroducing native *Glomus* strains promoted the effectiveness of P

fertilisers and enhanced both P and Zn uptake following fallow periods. Similarly, ref. [44] reported a significant increase in P concentration in the stem tissues in both well-watered and drought conditions in AMF-inoculated plants. Specific strains, such as *Dentiscutata cerradensis* (TK-1), *Cetraspora pellucida* (SZ-3), *G. intraradices* and *R. irregularis*, were significantly more effective at increasing P concentration in maize than other strains tested [32,42,43].

Researchers observed that inoculation did not always provide evident nutritional benefits because the rich native AMF community already provided a functional baseline for nutrient uptake [53]. Furthering this, ref. [43] found that two specific strains (*Acaulospora morrowiae* and *A. longula*) failed to change P concentrations relative to the control, while *A. morrowiae* did successfully improve P uptake. Contrasting observations were made in plants inoculated with *Dentiscutata cerradensis*, which significantly increased P concentration while failing to increase P uptake [43]. This highlights strain-specific functionality in nutrient concentration and uptake.

Temporal variations significantly influenced the efficacy of dual inoculation on grain quality. In a two-year study, dual inoculation paired with fertilisers (ammonium nitrate and poultry litter) significantly increased grain N content per gram during the 1st year, though this effect was minimal in the 2nd year [32]. Conversely, a similar study evaluating a multi-strain biofertiliser (*Glomus* and *Gigaspora* spp. paired with *Azotobacter*, and *Bacillus* spp.) reported minimal nutritional benefits in the 1st year but substantial gains in root N, P, and K concentration during the 2nd year, indicating a possible residual effect [34].

Nutrient supplementation levels also heavily modulated the inoculation outcome. For instance, the efficacy of dual inoculants was suppressed when combined with either biogas slurry (rabbit manure) [34] or 50% inorganic fertiliser [32]. The combination with biogas slurry actively depleted plant nutrient levels, reducing leaf and root nutrient content during the first year, with the sole exception of potassium [34]. Other studies reported that co-inoculation of *G. intraradices* with organic matter resulted in N concentrations and accumulation in the plant similar to the dual inoculation [32].

Furthermore, AMF inoculation was more effective in increasing the uptake of the nutrients (N, P, K and magnesium [Mg]) as compared to the other treatments [32]. Surprisingly, *G. intraradices* inoculation resulted in higher N uptake than PGPR plant tissues, while dual inoculation yielded the highest returns [31]. Other studies reported that PGPR was more effective in terms of N concentration under drought stress [36]. In addition, ref. [47] noted that *Azospirillum* (PGPR) inoculation significantly influenced nitrate reductase activity, indicating enhanced capacity for nitrogen assimilation.

Dual inoculation generally matched or exceeded single AMF inoculation for macronutrient responses, with the advantage most apparent under water-deficit conditions, where co-inoculated plants showed larger relative gains in both N and P than under well-watered conditions [33,38]. This benefit was not universal: in at least one study, dual inoculation failed to significantly improve shoot P concentrations despite significant gains in N and K under the same treatment [26]. A combination of earthworms with AMF and PGPR was also effective in increasing concentration of the NPK content, especially in individual P acquisition and K content [49,51].

Microbial inoculation was also effective in micronutrient acquisition. Under drought conditions, dual inoculation of a multi-strain AMF complex with *B. thuringiensis* (PGPR) significantly increased shoot carbon accumulation by 28%, while *B. thuringiensis* provided the highest concentration of N, K, Mg, Ca, B, Fe, Zn and Cu nutrients, as AMF provided higher concentrations of P and carbon contents in the shoot [36]. Additionally, ref. [30] highlighted that AMF inoculation effectively regulated the uptake of Zn. Furthermore, ref. [54] reported that application of AMF inoculation was also effective in uptake of cerium post-supplementation.

Conversely, several studies identified conditions where inoculation had no statistically significant impact. In [46], although general trends showed higher mineral contents (N, P, K, Mg, Ca) in inoculated leaves, the differences were was not statistically significant compared to the control, while K levels in the leaves were reported to have declined post-inoculation [46]. At physiological maturity [31], while per-plot nutrient removal was significant, the effect of inoculants on phosphorus uptake per gram of plant tissue was not significant. Data anomalies also complicated results; ref. [50] found no significant differences between the various biofertiliser treatments and the control. However, the mean values presented in its data tables for these parameters were identical, introducing reporting errors that influence its credibility. Finally, ref. [55] reported that both starter fertilisation and microbial inoculation had a very limited effect on the concentrations of plant-available nutrients in the soil at the sampling dates measured.

Overall, nutrient uptake responses were most consistent for phosphorus, particularly under P-limited conditions, while nitrogen, potassium, and micronutrient responses were more strain-, tissue-, and condition-dependent, with several studies reporting no measurable benefit where a functional native microbial community was already established (Figure 4).

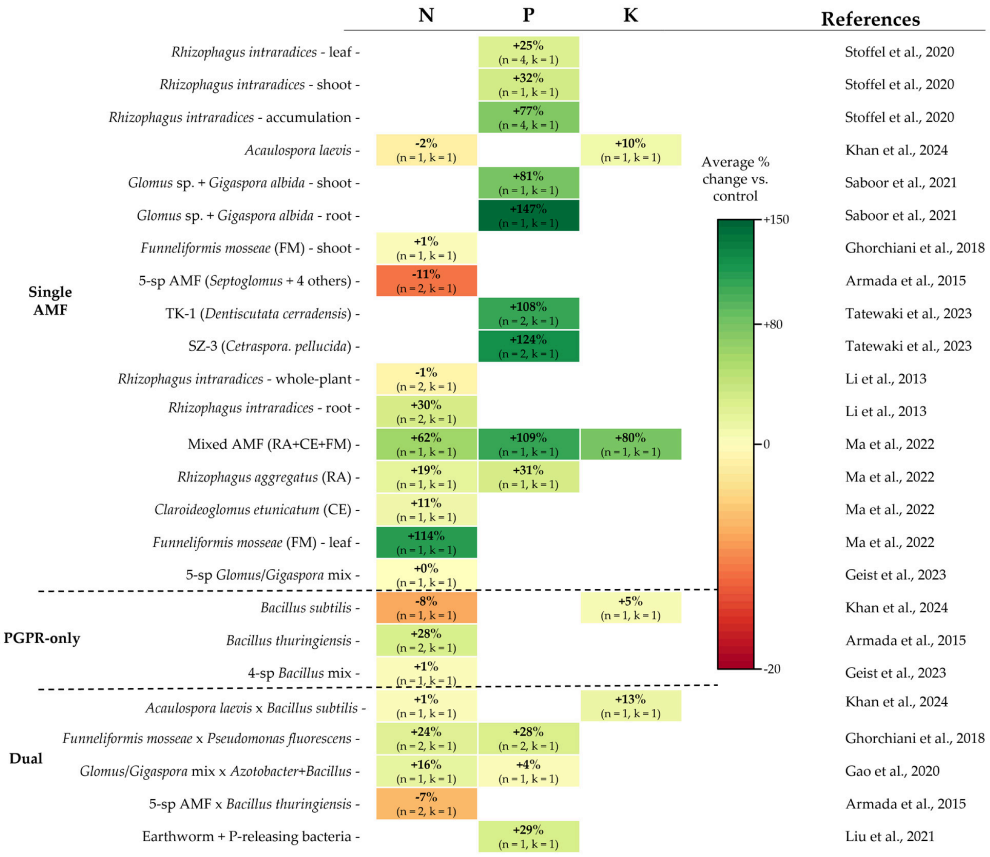

Figure 4. The reported effects of AMF, PGPR, and AMF–PGPR co-inoculation on maize N, P, and K relative to uninoculated controls. Values represent the mean reported percentage change (n = number of comparisons; k = number of studies). Responses for *Rhizophagus intraradices*, *Funneliformis mosseae*, and *Glomus* sp. + *Gigaspora albida* are presented separately where measurement bases differed. References: Stoffel et al., 2020 [24]; Khan et al., 2024 [26]; Saboor et al., 2021 [29]; Ghorchiani et al., 2018 [33]; Gao et al., 2020 [34]; Armada et al., 2015 [36]; Tatewaki et al., 2023 [43]; Li et al., 2013 [49]; Liu et al., 2021 [51]; Ma et al., 2022 [52]; Geist et al., 2023 [55]. Further details are provided in Table S1.

4.4.2. Chlorophyll/SPAD

Several studies investigated the effects of arbuscular mycorrhizal fungi (AMF) inoculation alone on chlorophyll content in maize. Inoculation with *Glomus* sp. and *Gigaspora albida* led to a statistically significant increase in chlorophyll concentrations compared to uninoculated plants [29]. The researchers observed that while zinc (Zn) deficiency reduced chlorophyll synthesis, an application of 20 mg kg^{-1} Zn increased total chlorophyll by approximately 9% over the control. The most pronounced response was found in AMF plants receiving 20 mg kg^{-1} Zn and 29 kg/ac (~71.66 kg/ha) phosphorus (P), which achieved a 62% increase in total chlorophyll content compared to the control. Furthermore, it was also noted that AMF helped protect chlorophyll pigments from oxidative damage caused by high toxic zinc concentrations. Similarly, another study reported that inoculation with *R. irregularis* significantly increased SPAD values in three of the six maize varieties tested, specifically Bambou, Ecavel and Mudishi-3 [39]. Correlation analyses within this study revealed a strong, positive relationship between mycorrhization parameters (frequency and intensity) and SPAD values for the Bambou variety. For the Ecavel variety, SPAD values were also positively and significantly correlated with the presence of AMF. However, this effect was not same across the other varieties; for instance, ZM627 showed a negative correlation between mycorrhizal dependence and mycorrhization rate, which was the same for the SPAD values.

In contrast, some of the studies reported no significant increase in chlorophyll content after inoculation. Ref. [23] found that higher levels of AMF colonization did not increase chlorophyll content, after conducting chlorophyll content analysis using the solvent extraction (80% aqueous acetone) method. In addition, plants without AMF inoculum were observed to have significantly higher leaf chlorophyll levels at the end of the experiment compared to those inoculated with *G. mosseae*. They highlighted that fertiliser levels were the primary driver of chlorophyll, with the "high" fertiliser treatment consistently resulting in the greatest chlorophyll content (>400 μmol m^{-2}) regardless of inoculation status. Furthermore, in the "No fertiliser, 0 spore" treatment, Bt 11 maize cultivars showed higher chlorophyll than the Providence (P) parental line, but this variety-specific difference was no longer detectable once the plants were inoculated at the 40- or 80-spore level. The summary of the findings is provided below (Table 3).

The variability of response was also evident in [39], where the remaining three varieties (Suan-1, ZM625, and ZM627) showed no significant differences in SPAD values between the inoculated and non-inoculated treatments. In the ZM627 variety specifically, there was no significant correlation between SPAD values and mycorrhizal colonization, indicating AMF effectiveness with respect to chlorophyll content could be affected by maize cultivars or genotypes.

In [33], researchers recorded the highest chlorophyll content via a dual-inoculation treatment, AMF (*Funneliformis mosseae*) and *Pseudomonas fluorescens*, reaching SPAD values exceeding 45 in well-watered conditions and approximately 40 under drought stress, representing the most substantial increase among all treatments within these conditions. This synergistic effect was corroborated by [41], who observed that drought stress typically leads to degradation of photosynthetic pigments. However, dual inoculation alongside compost significantly improved chlorophyll a, b, and total chlorophyll content by 77%, 95%, and 83%, respectively. Improvements also varied with water stress conditions, where PGPR yielded the highest total chlorophyll content in well-watered conditions, whereas in drought conditions, dual inoculation produced the most significant increase. Similarly, ref. [34] showed that the combination of biogas slurry with a biofertiliser mixture (AMF-PGPR) achieved the highest total chlorophyll, outperforming chemical fertilisers in both years of the experiment.

Table 3. Summary of the chlorophyll results from the included studies.

Study	Inoculant	Parameters	Outcome	Result Details
[29]	*Glomus* spp., *Gigaspora* spp.	Chl a, b, Total	Significant (Higher)	Synergistic effect observed when co-inoculated with specific zinc (Zn) and phosphorus (P) concentration gradients.
[39]	*Rhizophagus*	SPAD	Variable	Inoculation efficacy was highly genotype-dependent; significant SPAD increases in some varieties, whereas commercial hybrids showed no response.
[23]	*Glomus*	Chl a, b, Total	Significant (Lower)	Non-inoculated controls exhibited significantly higher chlorophyll levels than inoculated hybrid varieties.
[33]	*Funneliformis* + *Pseudomonas*	SPAD	Significant (Higher)	SPAD values were significantly elevated at 60 days post-inoculation compared to the control group.
[41]	*Glomus*, *Acaulospora* + *Bacillus*	Chl a, b, Total	Significant (Higher)	Dual inoculation combined with organic compost yielded a much higher significance than inoculation alone.
[34]	*Glomus*, *Gigaspora* + *Azotobacter*, *Bacillus*	SPAD	Significant (Higher)	Optimal chlorophyll performance achieved through dual inoculation integrated with biogas slurry and 50% NPK fertilisation.
[26]	*Acaulospora* + *Bacillus*	Chl a, b	Significant (Higher)	Peak outcomes for Chl a and b were consistently recorded in dual-inoculation treatments across jointing, silking (R1), and maturity (R6) stages.
[54]	*Gigaspora*, *Acaulospora*, *Scutellospora*	Chl	Significant (Higher)	Enhanced chlorophyll concentrations were noted specifically when co-inoculated with rare earth metals (e.g., cerium).
[47]	Mixed AMF + *Bacillus*, *Azospirillum*	Fluorescence	Variable	Single AMF inoculation showed no effect; *Azospirillum* was effective, but *Bacillus* alone produced the highest overall concentration.
[46]	*Glomus* + *Pseudomonas*, *Bacillus*, *Halomonas*	Chl Index	Not Significant	No significant difference during initial phases; however, commercial AMF inoculants showed enhanced SPAD recovery post-irrigation.

The stress-mitigating benefits of microbial inoculation were further validated in [26], where dual inoculation with AMF and PGPR increased chlorophyll a by 10.53% and chlorophyll b by 13.12% in severe drought stress. Additionally, inoculation with AMF alone enhanced chlorophyll a by 9.04% relative to controls. In [54], both cerium (Ce) application and AMF inoculation increased chlorophyll index values, with AMF-treated plants reaching 19.49 compared to 16.02 in non-mycorrhizal plants. Similar effects were observed with lanthanum (La) treatments. On the other hand, ref. [47] indicated that the *B. velezensis* ZK strain significantly increased the maximum quantum yield of photosystem II (F_v/F_m),

reflecting enhanced light-use efficiency and physiological health. Furthermore, dual inoculation of AMF with *Azospirillum brasilense* resulted in significantly higher chlorophyll content compared to single inoculations and controls.

These studies collectively indicate that while microbial inoculation is a robust tool for boosting chlorophyll under stress, its effectiveness can be masked in environments where the photosynthetic apparatus remains stable. Despite this, dual inoculation stands out as an effective strategy to increase chlorophyll in water stress.

4.4.3. Stress Markers in the Maize Plant

Several studies reported on critical indicators for stress in the maize plant, with most of the studies experimenting on both AMF and PGPR. The parameters that were measured included electrolyte leakage, malondialdehyde (MDA), superoxide dismutase (SOD), Catalase (CAT), proline, hydrogen peroxide (H_2O_2), and ACC (1-aminocyclopropane-1-carboxylic acid). Research on superoxide dismutase (SOD) and peroxidase (POD) was conducted in several studies included in this review. In [26], researchers observed that severe water stress decreased SOD activity. However, dual inoculation with arbuscular mycorrhizal fungi (AMF) and plant growth-promoting bacteria (PGPR) significantly increased both SOD and POD activities relative to non-inoculated plants. Notably, the effects of inoculation varied with water availability; under well-watered conditions (80% field capacity), the highest SOD and POD activities were observed in dual-inoculation treatments, whereas under severe water stress (35% field capacity), the highest POD activities appeared in AMF-inoculated plants. Supporting these results, ref. [22] demonstrated that plants subjected to combined salinity and drought stress showed peak SOD activity when treated with M1T1 (AMF + *Trichoderma*), reaching nearly 100% relative activity compared to approximately 47% in unstressed controls. Additionally, while AMF inoculation increased SOD activities, inoculation with *Trichoderma* resulted in significantly higher SOD activity under stress conditions [22].

During severe drought, hydrogen peroxide (H_2O_2) levels were observed to increase in several studies, especially in the uninoculated plants. However, inoculation with either AMF or PGPR was reported to mitigate this effect. For instance, inoculation with AMF + PGPR significantly reduced H_2O_2 levels (0.57 µmol/g FW) compared to the control (0.79 µmol/g FW). Additionally, AMF led to better reductions in H_2O_2 levels than PGPR treatments [26]. Similarly, ref. [41] reported that treatments with AMF, NPK fertiliser, and BaCo (PGPR + compost) reduced H_2O_2 level accumulation by 27%, 51%, and 29%, respectively. Moreover, ref. [36] reported that the H_2O_2 concentrations under drought conditions were significantly decreased by 58% in the AMF-inoculated plants, reporting that H_2O_2 levels were higher in root tissues than in shoots. For instance, in the drought conditions in the control plants, the shoots had a H_2O_2 concentration of 395.4 mmol/g DW, while in the roots, the concentration was at 560.9 mmol/g DW. This concentration was reduced with inoculation, with the highest reduction in shoots noted in the PGPR treatments, while AMF treatments reduced root concentration significantly. Consequently, dual inoculation allowed for a synergetic effect in both of the plant tissues, reducing the concentration significantly.

Another key biomarker for stress in plants is glutathione. Ref. [36] was the primary study evaluating glutathione, finding that its accumulation in roots was significantly affected by the presence of microbes. Under drought stress, glutathione levels were considerably lower in inoculated treatments; specifically, dual AMF + Bt inoculation reduced glutathione in roots by 56%. However, during the shoot, *Bacillus thuringiensis* yielded the lowest glutathione concentration (132.1 mmol/g DW) compared to the control (179.6 mmol/g DW). The authors interpreted lower concentration in the inoculated

plants as an indication that the inoculation successfully mitigated the severity of the stress, reducing the plant's need to produce high concentrations of chemical defences.

In [41], drought stress caused a significant increase in catalase (CAT) activity as the plant attempted to clean up excess H_2O_2. The highest CAT activity was observed in untreated stressed plants (337 $\pm$ 46.6 UE/min/mg protein) or those with single inoculation, either in My (AMF) (165.8 $\pm$ 66.0 UE/min/mg protein) or in PGPR (Ba) (282.4 $\pm$ 47.7 UE/min/mg protein), whereas the CoMyBa inoculation showed significantly lower CAT activity at 37.8 $\pm$ 12.7 UE/min/mg protein. Additionally, with dual inoculation of AMF and PGPR, MyBa also resulted in lower CAT activities (34.8 $\pm$ 11.7 UE/min/mg protein). The researchers concluded that the tripartite bio stimulant combination improved the plant's overall physiology enough to neutralize reactive oxygen species more efficiently, resulting in lower "emergency" enzymatic cleanup requirements.

Microbial inoculation frequently enhances the accumulation of proline to help plants maintain water balance. In [41], significant proline accumulation ($p \leq 0.05$) was observed in plants treated with N-P-K (Ctr+), reaching an increase of 92.90%. Plants exposed to bio stimulants alone exhibited elevated levels as well (PGPR (Ba): 64.32% and AMF (My): 57.30%), while combined treatments resulted in increases of 54.27% for PGPR + compost (BaCo) and 42.95% for AMF + compost (MyCo). These findings were measured under severe irrigation conditions (DS) and compared to untreated controls. Study 17 also highlighted that AMF and *Trichoderma*, a plant beneficial fungus, played a crucial role in enhancing adaptive metabolism by increasing cellular concentrations of proline. Conversely, study 27 reported that in roots, mycorrhizal plants had significantly lower proline (200.1 mmol/g DW) than control plants (1754.7 mmol/g DW), a reduction of 86% due to inoculation. They explained that the inoculation applied alleviated the oxidative stress generated in the plant by water limitation, causing a decrease in malondialdehyde (MDA; a biomarker for lipid peroxidation or plant tissue damage) and H_2O_2 levels.

MDA serves as a key indicator of lipid peroxidation and membrane damage. Under high drought stress MDA levels have been reported to increase if no treatments or mitigations are applied [26,41]. In [26], AMF + PGPR reduced MDA levels by 21%, while the highest reduction was noted in plants treated with inorganic fertiliser, reducing it by 24.89% ($p \leq 0.001$). Additionally, single AMF and PGPR inoculation also reduced MDA levels compared to the control by 21.04% and 15.61%, respectively. This highlights that AMF (*Acaulospora*) play a more crucial role in MDA regulation as compared to PGPR (*Bacillus subtilus*). In [41], plants treated with BaCo, MyCo, Ba, Co, MyBa and CoMyBa were able to decrease MDA levels by 46.91%, 46.64%, 45%, 34%, 28% and 15%, respectively, relative to the control ($p \leq 0.05$). However, the single inoculation of AMF resulted in the highest concentration of MDA, contrary to the previous study [26]. This indicates that species-specific AMF inoculation can affect efficiency of MDA level reduction, since the strain used in [26] was *Acaulospora*, while in [41] they used 60% *Glomus* sp and 40% *Acaulospora*. Further evidence for MDA level reduction was reported by [36], where AMF association decreased shoot MDA by 40%. Notably, MDA levels in the roots were lowest in the AMF-inoculated plants as compared to all the other treatments of PGPR and the control, further supporting the finding of [26]. Interestingly, ref. [47] found that while ZK (PGPR) and AMF treatments alone increased lipid peroxidation, their combination (ZK + AMF) significantly decreased it to around 90 nmol/g DW, which was lower than the control. The PGPR utilised in this study were Bacillus velezensis, and for the AMF it was a mixture of *Gigaspora*, *Acaulospora*, *Rhizophagus* and *Claroideoglomuse*. MDA and H_2O_2, if not controlled in higher concentrations, lead to cell membrane damage, which leads to the leakage of electrolytes from the cells. This aspect can be measured as electrolyte leakage.

While drought stress increased electrolyte leakage in non-inoculated plants (8.46%), AMF colonization significantly reduced this value to 3.57%. The authors concluded that the reduction in electrolyte leakage, alongside the decrease in MDA, demonstrated that microbial inoculation effectively maintained membrane integrity and function under severe water limitation [36].

Overall, biochemical stress-marker responses consistently indicated that microbial inoculation—particularly dual inoculation—mitigated oxidative and osmotic stress under moderate water deficit, although the specific enzymatic pathway responsible shifted toward the bacterial partner's ACC deaminase activity under more severe stress.

5. Discussion

This review explored the effects of AMF inoculation, alone and in dual inoculation with PGPR or fungi like *Trichoderma*, on maize (*Zea mays*). Effects investigated were morphological (plant height, stem diameter, leaf number and area, biomass, grain yield), physiological (N, P, K content and chlorophyll) and biochemical stress marker response. Among the AMF species investigated, *Rhizophagus* species (*R. irregularis*, *R. intraradices*) was most common, followed by the *Glomus* species (including *G. caledonium*, *G. albida* and *G. mosseae* revised to *Funneliformis mosseae*). The *Acaulospora* and *Gigaspora* species were also investigated. AMF inoculation effectiveness proved highly context-specific, depending on nutrient availability, inoculation conditions and species, stress factor, plant genotype compatibility and environmental conditions.

Nutrient supplementation level was a consistent determinant of single-AMF success across phosphorus, zinc, and general NPK dosing. For phosphorus, AMF effectiveness was strongest in P-deficient soils and declined progressively as supplementation increased, becoming negligible above approximately 140 kg/ha, or 16 mg kg^{-1} soil P [24,28,52], indicating that AMF's contribution substitutes for, rather than adds to, adequate soil P. A similar pattern held for zinc: AMF inoculation at 50% of the recommended Zn dosage matched or exceeded full-dosage non-inoculated outcomes, with the most effective supplementation identified at approximately 20 mg kg^{-1} in Zn-poor soils [24,48]; higher doses offered no additional benefit once this threshold was reached. This pattern generalised across NPK fertilisation overall: AMF inoculation combined with approximately 50% of the recommended nutrient dose consistently matched the performance of full-dose fertilisation without inoculation, producing gains of over 30% in plant height and 25% in stem diameter, alongside measurable improvements in grain and dry matter yield [26,30,31]. Full-dose fertilisation alongside inoculation rarely improved on this outcome further, suggesting that once a nutrient sufficiency threshold is crossed, additional fertiliser input provides diminishing returns regardless of inoculation status, in agreement with [56]. Taken together, these findings indicate that AMF inoculation is most valuable as a fertiliser-sparing tool: its benefit is concentrated in the gap between a deficient and a fully nutrient-sufficient soil, with an approximate optimum around 50% of the conventional recommended dose across P, Zn, and general NPK regimes.

Beyond inorganic nutrient dosing, organic amendments similarly improved AMF performance, including organic material such as poultry litter, potato protein liquid (PPL), compost and biogas slurry [22,31,32,34,37,41,50,55]. Poultry litter resulted in gains in plant height, grain yield, nitrogen, phosphorus, and potassium content through inoculant-fertiliser interactions [31,32], whereas Biogas slurry, when used alongside AMF, did not demonstrate synergistic effects; its impact was comparable to either AMF inoculation or biogas slurry application alone [34,55]. These findings suggested that although organic materials, when co-inoculated with AMF, could lead to synergistic effects, the outcomes depended on the type of organic material used.

Soil texture also played a crucial role in AMF efficacy. Studies noted that higher clay-content soils exhibited significant improvements in morphological parameters and yield gains [24,25,45,52]. For example, maize biomass increased by an average of 16–48% in clay-textured soils during field experiments, regardless of differences in phosphorus availability; one study reported available P levels above 30 mg kg^{-1}, while another observed levels below 16 mg kg^{-1} [24,25]. Additionally, grain yield and nutrient uptake were enhanced following inoculation [24,45,52]. This trend aligns with findings of [57], who observed that mycorrhizal responsiveness in sorghum correlated positively with clay content up to a threshold of approximately 43%, beyond which symbiotic efficiency declines. While increased aeration in sand-textured soils can promote AMF spore proliferation, this benefit appears to have limits. Ref. [58] investigated three AMF spore concentrations and found that concentrations exceeding 12.35 spores per gram often resulted in a parasitic relationship between the fungi and the plant, impairing plant performance. Conversely, [56] observed that although clay soils are inherently more fertile than sandy soils, they may restrict AMF development due to suberin deposition in the root epidermis. Together these findings suggest that an optimal soil texture exists; balancing clay and sand proportions to restrict AMF development just enough to benefit the plant while preventing parasitic interactions is crucial.

Co-inoculating AMF and plant growth-promoting rhizobacteria (PGPR) represents a specialized "functionally complementary interaction between AMF and PGPR" within the maize rhizosphere. PGPR function as biological architects, deploying phytohormones like auxins to provide the stimulation for root elongation, mineral nutrient solubilization and structural development, while AMF act as the "logistics network", providing the hyphal network for the nutrients acquisition and transport. However, this synergetic effect is not always a guaranteed outcome and is governed by multiple factors.

The most consistent predictor of synergistic outcome was plant-functional P deficiency at the time of inoculation. Co-inoculation of *Funneliformis mosseae* with *Pseudomonas flourescens* led to a synergetic increase in grain yield of 31% in water stress conditions and under P-deficient conditions. *P. fluorescens* functioned explicitly as a mycorrhization helper bacterium by enhancing AMF colonization rates under water deficit conditions [33]. Similarly, in Pakistan [43], the combination of a six-species AMF consortium and *Bacillus* sp. PIS7 applied alongside rock phosphate in a silty clay soil in P-deficient conditions and at slightly alkaline pH resulted in a two-fold increase in grain yield. This was an effect of the P-solubilizing capacity of the PGPR unlocking soluble phosphate that AMF hyphae could then access and translocate [43]. In contrast, a long-term managed field trial [31] found no synergistic effect from AMF-PGPR co-inoculation, with single AMF outperforming the dual treatment in two of three years. The authors attributed this to an established native microbial community occupying the ecological niches that introduced inoculants would otherwise fill [31].

The functional mechanism of the PGPR partner was the second critical determinant of synergy. An ACC deaminase producing *Bacillus stubtilis* strain reduced ACC accumulation in dual-inoculated maize roots in both moderate and severe water-stressed conditions [26], while simultaneously increasing both POD and SOD activities under moderate drought. AMF (*Acaulospora laevis*) increased P acquisition through hyphal extension. This functional division—the bacterium managing ethylene-mediated stress signalling while the fungus managed nutrient acquisition—produced the most consistent synergistic outcomes in the dataset [26]. A categorically different outcome emerged when indigenous AMF was paired with *Trichoderma harzianum*, a plant beneficial fungus. Single AMF inoculation outperformed co-inoculation for total biomass across both experimental settings. This

could be attributed to the fact that *Trichoderma harzianum* is not considered as part of the AMF, thus it may have been in competition with the inoculated AMF [22].

Soil pH also shaped synergetic outcomes. Observed in multiple successful synergetic studies, the pH level of the soil was above 7.0. For instance, inoculation of AMF and *B. subtilis* at a soil pH level of 8.3 resulted in a grain yield increase of 12.4%, while ACC levels decreased by 64% [26]. Grain yield increases were also reported at soil pH levels of 8.1, 8.9, and 7.83 upon dual inoculation of AMF with *P. flourescens*, *Azotobacter chroococcum*, *B. circulans*, *B. thuringiensis* and *Bacillus* sp. PIS7, respectively [33,34,36,48]. Ref. [59] reported that *Bacillus* could modulate soil pH to enable optimum conditions for growing, which was reported to be around 6.5. Below this range (pH 5.46–5.8), synergy became conditional on nutrient supplementation. For instance, inoculation of AMF and *Azotobacter* at a pH of 5.46 was only effective upon supplementation with 50% NPK. Additionally, the authors reported to have used poultry manure, which could have reduced the soil pH even further, crippling the efforts of the bacteria to raise the pH [32]. This implies that soil pH is a crucial factor in the success of synergetic effects.

A fourth driver of synergy was stress severity as a modulating relay between the two organisms. Under moderate drought, single AMF inoculation produced the highest 33P uptake (2.1-fold above uninoculated control), while under severe drought (30% WHC), PGPR or dual inoculation produced the highest 33P uptake (2.4-fold above control), despite dual-inoculated plants showing significantly lower root colonisation than single AMF plants under severe stress. The authors attributed this pattern to AMF investing heavily in hyphal, vesicle, and spore production under severe water limitation, creating a carbon drain from the host rather than a nutrient return [38]. A Moroccan field trial with high soil P (98.2 mg kg^{-1}) in severe water stress showed a similar pattern: under well-watered conditions, AMF alone outperformed co-inoculation, but under drought conditions, dual treatment became superior, indicating that severe water stress effectively replicated functional P deficiency by preventing P diffusion to roots, thereby temporarily activating the conditions under which co-inoculation is most valuable [41]. Collectively, these findings indicate that synergy between AMF and PGPR in maize is ecologically real but conditioned, most reliably achieved when plant-functional P deficiency is present, the PGPR partner provides a mechanistically complementary rather than overlapping function, and stress severity has reached the threshold at which root-based P diffusion becomes physically limited.

A subset of studies extended their investigation to measure biochemical stress markers, demonstrating that microbial inoculation modulates oxidative stress responses, osmotic adjustment, hormonal regulation, and hydraulic functioning of the plants. The specific contribution of each inoculant to each system differed consistently across studies, supporting the interpretation that synergistic biochemical effects reflect a specialized functioning partnership between AMF and PGPR.

Oxidative stress markers (SOD, POD, CAT, MDA, and H_2O_2) were the most comprehensively characterized biochemical pathway. Under moderate water stress, dual inoculation increased POD activity and SOD activity while simultaneously reducing MDA and H_2O_2, reducing oxidative damage [26]. Critically, the hierarchy of treatment effects differed between the two drought intensities: under moderate drought, the pattern was AMF + PGPR > AMF > PGPR > control for both POD and SOD, indicating that the combined treatment maintained the highest antioxidant defence. Under severe drought, however, the PGPR treatment alone exceeded the dual treatment for POD activity (46.10% vs. 33.09% above the control), suggesting that under extreme water limitation the bacterium's ACC deaminase activity became the dominant stress-regulatory mechanism rather than the fungal symbiosis [26]. Contrasting but similar findings were reported in Morocco: plants

inoculated with the full AMF-PGPR-compost combination (CoMyBa) showed lower CAT activity than control plants under drought conditions, which was interpreted as evidence of improved baseline cellular physiology, effectively managing stress through upstream mechanisms and reducing the need for emergency antioxidant response [26], indicating the plants were not stressed from the dual inoculation. This finding illustrates that single biochemical markers cannot be interpreted in isolation and the directionality of enzyme response must be read in conjunction with lipid damage and ROS data from the same study.

The osmotic adjustment response, measured through proline accumulation, produced the most contentious pattern across studies. One study reported drought stress increased proline accumulation in all inoculated treatments compared to unstressed controls, interpreting elevated proline as active osmotic protection during water deficit [41]. In contrast, mycorrhizal inoculation reduced proline in both shoot and root tissues under drought conditions compared to uninoculated stressed plants, with dual inoculation reducing root proline by 55% and *Bacillus thuringiensis* single inoculation reducing it by 79%. Reductions in proline occurred simultaneously with reductions in MDA (by 71% for AMF alone), H_2O_2 (by 58%), and electrolyte leakage, a convergence indicator of stress alleviation rather than active response, since the plant no longer required high concentrations of Osmo protectants once physiological stress was mitigated upstream [36]. These two studies differed in soil P concentrations, partly explaining their differences in proline concentrations.

The hormonal regulatory pathway, mediated through ACC deaminase activity in PGPR strains, emerged as the primary mechanism by which bacteria extend the plant's functional stress window. Dual inoculation of AMF and *Bacillus* subtilis significantly reduced ACC accumulation during jointing under moderate water stress by 63.68% and under severe water stress by 23.23%, with reductions persisting through silking and maturity stages [26]. ACC is the direct precursor to ethylene, a phytohormone that inhibits root elongation, reduces nutrient uptake capacity, and accelerates senescence during stressful conditions. By enzymatically degrading ACC before it is converted to ethylene, ACC deaminase-producing PGPR effectively extends the plant functional window and allows for continuous AMF symbiosis under water stress. Ref. [33] characterised *Pseudomonas fluorescens* in this role, describing it as a functional mycorrhizal helper, a PGPR that facilitates AMF colonisation and function by reducing the ethylene-mediated suppression of hyphal development that occurs under drought and nutrient stress conditions. These findings indicate that the biochemical response to co-inoculation is coordinated: the bacterium manages the hormonal and oxidative signalling environment in which the fungus operates, and the fungus manages the nutrient and water acquisition infrastructure that reduces the severity of the stresses to which the bacterium responds.

Taken together, these findings translate into a practical decision framework for AMF-PGPR inoculation in maize. Under phosphorus-limited conditions (soil available P below approximately 16–17 mg kg^{-1}), single AMF inoculation alone is likely to produce the strongest and most cost-effective response, with additional Zn supplementation (~20 mg kg^{-1}) recommended in Zn-poor soils. Where soil nutrient status is closer to sufficiency, partial supplementation (approximately 50% of the recommended NPK dose) alongside inoculation offers a more sustainable alternative to full fertilisation without sacrificing yield. Dual AMF-PGPR inoculation is best reserved for soils at neutral-to-alkaline pH (above 7.0) and for production systems where moderate-to-severe water stress is expected, provided the PGPR partner is functionally complementary (e.g., an ACC deaminase producer) rather than a competing organism; under the most severe stress conditions, the bacterial partner's contribution becomes proportionally more important than the fungal one. Conversely, where a well-established native microbial community is already present, or where inoculant partners compete for the same ecological niche, additional inoculation

is unlikely to provide a measurable benefit. It is also worth noting that these interpretations may be over- or underrepresented given the number of studies included in this review (n = 36) and their uneven geographic distribution, which was concentrated in a small number of countries, particularly China, Brazil, and parts of Europe, while many major maize-producing regions, including much of sub-Saharan Africa, South Asia, and Southeast Asia, remain underrepresented, and that the absence of a fully comprehensive PGPR-only analysis limits confirmation of true synergistic effects. These conditions collectively define the circumstances under which AMF-based inoculation, alone or in combination with PGPR, is most likely to deliver a measurable return.

6. Conclusions

This systematic review summarized the findings of 36 studies examining the effects of single AMF inoculation and dual inoculation using plant-growth-promoting rhizobacteria (PGPR) on maize (*Zea mays*). Across the reviewed evidence, AMF and PGPR inoculation significantly improved biomass, plant height, grain yield, nutrient uptake and abiotic stress resistance, but this effect was not universal: the success of inoculation was consistently linked to soil texture, P availability, maize genotype, stress conditions, soil pH and the specific AMF and PGPR species utilised.

Dual inoculation was effective under specific, identifiable conditions. Biochemical evidence indicated that a combined inoculant system operates through coordinated rather than additive mechanisms: with a bacterial partner regulating hormonal and oxidative signalling, while the fungal partner manages the nutrient and water acquisition, together producing reductions in membrane damage markers, reactive oxygen species, and osmotic stress markers that neither organism achieves independently at the same magnitude. Dual inoculation also promoted grain yield increases in multiple studies [33,41]; however, this synergy was not guaranteed. By contrast, pairing AMF with *Trichoderma harzianum*, a beneficial fungus that occupies the same root niche, produced active interference rather than synergy [22].

The success of dual inoculation depended on the specific combination of inoculant strains, soil pH, and abiotic stress conditions present. However, the precise mechanisms underlying these interactions are still yet to be fully understood. Future research should prioritise standardised reporting of soil P thresholds, direct comparisons of PGPR-only treatments alongside AMF and dual inoculation, and investigation of AMF-PGPR pairing effects across a wider range of soil pH and texture conditions. Taken together, these findings support AMF-based inoculation as a targeted tool for resource-constrained, phosphorous-limited, or drought-prone maize systems, rather than as a universal input recommendation.

7. Limitations

This review has notable limitations that should be considered during interpretation and application of its findings. First, soil phosphorus thresholds were derived from varied studies without standardising testing methods. Available P was mostly reported as Olsen-P, but reporting differed by country and lab, with many studies lacking methodological details. Thus, the 10–17 mg kg^{-1} threshold should be viewed as a range based on heterogeneous protocols. Users should consider their local extraction methods before comparison. Future research adopting a standardised protocol across soil types would enhance reliability. Second, the inclusion of studies using sterilised or artificial substrates may overestimate inoculation effects, as sterilisation eliminates native microbes. Although sterilisation did not appear to majorly influence outcomes, further studies are needed to compare sterilised and natural soils explicitly. Third, limiting data sources to Scopus and Web of Science and restricting to English reduced the pool of studies, a limitation acknowledged here.

Future efforts should expand scope to include additional databases and languages. Lastly, heterogeneity in measures, assessment timings, and durations prevented meta-analysis, relying instead on qualitative synthesis. Few studies reported multi-season or economic outcomes, limiting confidence in applying results to long-term or economic decision-making. In addition, the relatively small number of included studies (n = 36), combined with their uneven geographic distribution, restricts the extent to which conclusions can be generalised across all maize-producing regions; this should be borne in mind when applying the findings beyond the specific soil, climate, and production contexts represented in the included literature.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture16151635/s1. Table S1: NPK concentrations across studies depending on the inoculant used. PRISMA_2020_checklist: PRISMA 2020 Checklist.

Author Contributions: Conceptualization, J.A.A., S.Z., K.P.T. and I.M.K.; Methodology, J.A.A., M.A., B.M.T. and I.M.K.; software, J.A.A., Á.L., M.M.V., J.B.K. and I.M.K.; validation, S.Z., B.H., J.A.A., Z.D. and I.M.K.; formal analysis, S.Z., J.A.A., I.M.K. and G.B.; investigation, D.S., G.H., J.A.A., I.M.K. and V.B.; resources, I.M.K.; data curation, J.A.A., H.A., I.M.K. and M.A.; writing—original draft preparation, J.A.A., R.K. and I.M.K.; writing—review and editing, S.Z., J.A.A., V.B., J.M.-K. and I.M.K.; visualization, D.S., G.H., L.B. and I.M.K.; supervision, I.M.K. and S.Z.; project administration, I.M.K., S.Z. and K.P.T.; funding acquisition, I.M.K. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Not applicable. This study is a systematic literature review and did not involve humans or animals.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding author.

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Conflicts of Interest: Author Zoltán Drucskó, and Veronika Bedő, were employed by the company IKR Agrár Ltd., Bábolna, Hungary and Agrofil-SZMI Ltd., Püski, Hungary, respectively. The remaining authors declare that the research was conducted in the absence of any commercial of financial relationships that could be interpreted as conflict of interest.

Appendix A

Table A1. Characteristics of included studies with sample size details.

Reference	Experimental Design	Sample Size (Exact, Where Computable)	Effect Measures/Traits Evaluated
[31]	Split-split plot, randomized complete block	64 subplots (two tillage × two fertiliser × four inoculation × four replications)	Plant Height, Yield, Nutrient Uptake

Table A1. *Cont.*

Reference	Experimental Design	Sample Size (Exact, Where Computable)	Effect Measures/Traits Evaluated
[37]	Split-plot design	48 plots (four MC consortium levels × three organic fertiliser levels × four replications)	Plant Height, Stem Diameter, Biomass, Yield, Nutrient Uptake
[40]	3 × 4 factorial (microcosm)	48 microcosms (three cropping systems × four inoculation levels × four replications)	Biomass, Yield, Nutrient Uptake
[60]	Not specified	165 experimental units (11 microbial levels × three salinity levels × five replications)—explicitly stated in source	Plant Height, Stem Diameter, Biomass, Chlorophyll, Stress Markers
[55]	Randomized field plots	30 plots (six treatments × 5-fold repetition)	Biomass, Yield, Nutrient Uptake
[26]	Two-factor factorial (inoculation × water stress)	144 units (four inoculation levels × three water regimes × 12 replications)	Plant Height, Biomass, Yield, Nutrient Uptake, Chlorophyll, Stress Markers
[30]	Completely randomized design (CRD)	14 treatment combinations (two AMF status × seven Zn levels); replication count not stated in extraction	Plant Height, Stem Diameter, Biomass, Yield, Nutrient Uptake, Chlorophyll
[38]	Two-factor CRD (4 × 3, inoculation × water stress)	36 units (four inoculation levels × three water regimes × three replicates)	Plant Height, Stem Diameter, Biomass, Nutrient Uptake
[51]	Randomized design, four treatments	Greenhouse/field arm: 16 pots + 12 field units (four treatments × four replications pot/×three replications field). Microcosm arm: eight treatments, replication not stated	Biomass, Yield, Nutrient Uptake
[32]	Randomized design	36 units (nine treatments × four replicates)	Plant Height, Biomass, Yield, Nutrient Uptake
[46]	Two-inoculum comparison	Three inoculant levels; replication/design explicitly noted as not reported in source	Biomass, Nutrient Uptake, Chlorophyll
[33]	Randomized block, split-split plot (2 × 4, irrigation × inoculation)	72 units (two irrigation × four bio-treatment × three P-source × three replications) if fully crossed	Plant Height, Stem Diameter, Leaf Area, Biomass, Yield, Nutrient Uptake, Chlorophyll
[41]	Split-split plot, two-block design, 18 treatments	54 units (18 treatments × three replications)	Plant Height, Stem Diameter, Leaf Area, Biomass, Yield, Nutrient Uptake, Chlorophyll, Stress Markers
[48]	Complete randomized block, 10 treatments	30 units (10 treatments × three replications)	Plant Height, Biomass, Yield, Nutrient Uptake
[43]	Pot trial, three seeds/pot	Pot experiment: 12 pots (four AMF species × three replications). Field experiment treatment/replicate count not separable from extraction	Biomass, Yield, Nutrient Uptake
[22]	Factorial randomized block (2 × 4, stress × microbe)	64 units (four microbe treatments × four stress levels × four replications)	Biomass, Nutrient Uptake, Stress Markers
[42]	Randomized split-split plot (two tillage systems)	32 units (two tillage × four AMF species × four replications)	Yield, Nutrient Uptake

Table A1. *Cont.*

Reference	Experimental Design	Sample Size (Exact, Where Computable)	Effect Measures/Traits Evaluated
[27]	Randomized block, seven treatments	28 units (seven treatments × four replications)	Plant Height, Stem Diameter, Leaf Area, Yield
[52]	Five-treatment pot trial	150 pots total (five treatments; ~30 pots/treatment)	Nutrient Uptake
[29]	Three-factorial (Zn ×7, P ×4, mycorrhiza ×2)	56 treatment combinations (two mycorrhiza status × two P levels × seven Zn levels)	Plant Height, Stem Diameter, Biomass, Nutrient Uptake, Chlorophyll
[47]	6 × 1 completely randomized design, six treatments	Six treatments; replication count not stated in extraction	Biomass, Chlorophyll, Stress Markers
[50]	Randomized block, seven treatments	21 plots (seven treatments × three replicates, 30 m^2 plots)	Biomass, Yield, Nutrient Uptake, Stress Markers
[23]	Three-factorial randomized block (genotype × AMF × fertiliser)	18 treatment combinations (2 = two genotypes × three AMF doses × three fertiliser levels)	Plant Height, Biomass, Nutrient Uptake, Chlorophyll
[34]	Randomized block, nine treatments	27 units (nine treatments × three replications)	Plant Height, Leaf Area, Biomass, Yield, Nutrient Uptake, Chlorophyll, Stress Markers
[35]	Pot experiment, eight treatments	24 pots (eight treatments × three replications)	Plant Height, Biomass
[36]	3 × 2 factorial, four treatments	40 units (four bio-treatments × 10 replications) or 80 if water regime (two levels)	Biomass, Nutrient Uptake, Chlorophyll, Stress Markers
[24]	2 × 3 factorial randomized block (+ split-plot subset)	Six treatment combinations (two inoculation × three P levels)	Biomass, Yield, Nutrient Uptake
[39]	6 × 2 × 6 factorial design	12 combinations (six genotypes × two inoculation levels)	Plant Height, Stem Diameter, Leaf Area, Biomass, Chlorophyll
[54]	6 × 2 random factorial design	48 units (six REE dose levels × two microbe status × four replications)	Biomass, Nutrient Uptake, Chlorophyll
[44]	2 × 2 × 2 factorial (salt × water × AMF)	24 units (two microbe × two Ca level × two water level × three replications)	Biomass, Nutrient Uptake
[28]	Two-factor split-split randomized block, nine treatments	Nine named treatments (uneven factorial—not a clean grid)	Plant Height, Stem Diameter, Leaf Area, Biomass, Yield, Nutrient Uptake
[49]	Two-factor split-split plot, randomized block, four treatments	Four treatment combinations (two earthworm status × two AMF status)	Biomass, Yield, Nutrient Uptake
[53]	Split-split plot randomized block, eight treatments	20 combinations (five P doses × four AMF doses)	Yield, Nutrient Uptake
[45]	Randomized complete block, two sub-experiments	144 pots total (Subexp.1: 120 = five treatments × four blocks × six pots; Subexp.2: 24 = six combos × four blocks × one pot)	Yield, Nutrient Uptake
[25]	Randomized block design	Four units (two treatments × two replications)	Plant Height, Leaf Area, Yield

Table A1. *Cont.*

Reference	Experimental Design	Sample Size (Exact, Where Computable)	Effect Measures/Traits Evaluated
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[40]	3 × 4 factorial (microcosm)	48 microcosms (3 cropping systems × 4 inoculation levels × 4 replications)	Biomass, Yield, Nutrient Uptake
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[22]	Factorial randomized block (2 × 4, stress × microbe)	64 units (4 microbe treatments × 4 stress levels × 4 replications)	Biomass, Nutrient Uptake, Stress Markers
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Table A1. *Cont.*

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[34]	Randomized block, 9 treatments	27 units (9 treatments × 3 replications)	Plant Height, Leaf Area, Biomass, Yield, Nutrient Uptake, Chlorophyll, Stress Markers
[35]	Pot experiment, 8 treatments	24 pots (8 treatments × 3 replications)	Plant Height, Biomass
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[54]	6 × 2 random factorial design	48 units (6 REE dose levels × 2 microbe status × 4 replications)	Biomass, Nutrient Uptake, Chlorophyll
[44]	2 × 2 × 2 factorial (salt × water × AMF)	24 units (2 microbe × 2 Ca level × 2 water level × 3 replications)	Biomass, Nutrient Uptake
[28]	2-factor split-split randomized block, 9 treatments	9 named treatments (uneven factorial—not a clean grid)	Plant Height, Stem Diameter, Leaf Area, Biomass, Yield, Nutrient Uptake
[49]	2-factor split-split plot, randomized block, 4 treatments	4 treatment combinations (2 earthworm status × 2 AMF status)	Biomass, Yield, Nutrient Uptake
[53]	Split-split plot randomized block, 8 treatments	20 combinations (5 P doses × 4 AMF doses)	Yield, Nutrient Uptake
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[25]	Randomized block design	4 units (2 treatments × 2 replications)	Plant Height, Leaf Area, Yield

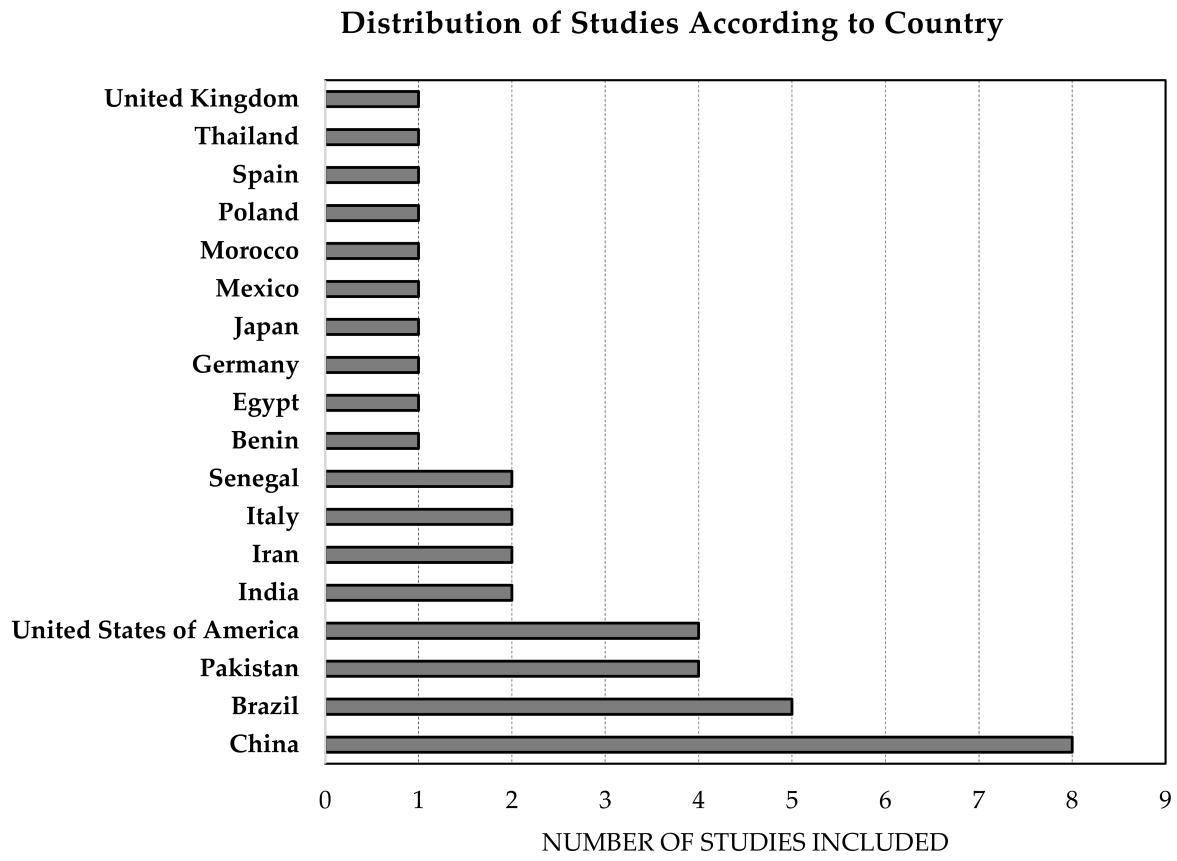

Figure A1. Distribution of studies included in this review based on country.

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