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SYNERGISTIC EFFECTS OF BIOCHAR AND MICROBIAL INOCULANTS ON DROUGHT TOLERANCE AND WATER-USE EFFICIENCY IN MAIZE (*Zea mays* L.)

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ABSTRACT

Drought is a primary constraint to global maize (*Zea mays* L.) production, and both biochar amendments and bacterial plant growth-promoting rhizobacteria (PGPR) have shown potential to mitigate its effects. However, no maize-specific meta-analysis has quantified whether these interventions act synergistically. Following PRISMA 2020 guidelines, we searched Scopus and Web of Science, identified 1,270 records, and after systematic screening selected 148 studies (52 biochar-only, 55 bacteria-only, 41 combined) from 28 countries (2009-2026). Preliminary random-effects meta-analysis of 87 effect sizes from 16 studies with complete variance data showed that amendments significantly improved multiple drought tolerance indicators. Bacterial inoculation increased measured outcomes by 26.8% (95% CI: 15.8-38.8%, $p < 0.001$), while biochar improved them by 5.4% (95% CI: 3.1-7.8%, $p < 0.001$). Significant improvements were detected for chlorophyll content (+58.8%), root length (+31.4%), relative water content (+20.3%), shoot dry weight (+16.8%), and photosynthetic rate (+14.2%). Substantial heterogeneity (I^2 = 78-100%) across most outcomes indicates strong moderation by experimental conditions. These findings directly contribute to Sustainable Development Goal 2 (Zero Hunger) and SDG 13 (Climate Action), SDG 6 (Clean Water and Sanitation), SDG 12 (Responsible Consumption and Production), and SDG 8 (Decent Work and Economic Growth). This study provides a comprehensive framework and preliminary quantitative evidence supporting the use of integrated biochar-bacteria strategies for drought-resilient maize production, with full results forthcoming upon completion of data extraction from all 148 studies.

Key words: Biochar, Drought stress, PGPR; SDGs, Water-use efficiency, *Zea mays*

INTRODUCTION

Drought represents the most widespread and economically damaging abiotic stress affecting maize production globally (Erenstein *et al.*, 2022; Serna, 2022). Water deficits during critical reproductive stages can reduce grain yields by 20-50%, and climate projections indicate that drought frequency and severity will intensify across major maize-growing regions in the coming decades (Azrai *et al.*, 2024; Deribe, 2025). The physiological consequences of drought in maize are well characterized: reduced stomatal aperture limits carbon assimilation, overproduction of reactive oxygen species damages cellular membranes and proteins, and disrupted water and nutrient uptake collectively suppress growth and grain filling (Agunbiade & Babalola, 2023; Li *et al*., 2021).

Biochar, a carbon-rich solid produced through pyrolysis of organic biomass, has attracted considerable research interest as a soil amendment for water-limited agriculture (Acharya *et al*., 2024; Singh *et al*., 2022). Its highly porous microstructure increases soil water-holding capacity, reduces bulk density, and enhances plant-available water content, with particular efficacy in sandy and loamy soils (Wu *et al*., 2022; Han *et al*., 2023). Simultaneously, bacterial plant growth-promoting rhizobacteria and endophytes mitigate drought impacts through phytohormone modulation, enhanced root system architecture, osmolyte accumulation, and upregulation of antioxidant defense systems (Zhao *et al*., 2023; Chattaraj *et al*., 2025).

A compelling hypothesis in recent literature is that combined biochar and bacterial inoculant application may produce synergistic benefits exceeding additive expectations, since biochar provides physical microhabitats and carbon substrates that enhance bacterial survival and colonization in the rhizosphere, while bacteria accelerate nutrient cycling processes that biochar alone cannot drive (Gul *et al*., 2023; Malik *et al*., 2022). However, despite growing numbers of individual studies testing these amendments in maize, no meta-analysis has specifically quantified and compared their effects under drought conditions (Singh *et al*., 2022; Wei *et al*., 2023).

Previous meta-analyses have addressed biochar effects on soil hydraulic properties or PGPR effects on plant growth under abiotic stress separately, but an integrated, maize-focused quantitative synthesis remains absent. This study addresses that gap through a PRISMA 2020-compliant systematic review and meta-analysis of 148 studies from 28 countries (Page *et al*., 2022). The objectives are to: (i) quantify the pooled effect sizes of biochar, bacterial inoculant, and combined treatments on maize drought tolerance indicators and water-use efficiency; (ii) compare response magnitudes across intervention types; (iii) identify moderating factors governing treatment efficacy; and (iv) evaluate preliminary evidence for synergistic interactions (Saleem *et al*., 2024).

MATERIALS AND METHODS

Search strategy and screening

This meta-analysis followed PRISMA 2020 guidelines (Page *et al*., 2022). Scopus and Web of Science were searched in March 2025 using three complementary search strings combining terms for maize, drought/deficit irrigation, biochar, and bacterial inoculants. English-language, peer-reviewed articles were eligible if they: (a) used maize as the experimental crop; (b) imposed drought or deficit irrigation as a treatment factor; (c) applied biochar and/or bacterial PGPR/endophytes; (d) included a drought-stressed untreated control; and (e) reported extractable quantitative data (means with SD/SE and sample sizes). Studies employing only fungal inoculants (AMF, *Trichoderma*) were excluded. The search yielded 1,270 records (Scopus: 406; WoS: 864). After removing 29 non-articles and 358 duplicates, 883 records underwent title screening (627 excluded), abstract screening (11 excluded from 256 assessed), and full-text assessment (52 excluded from 200 assessed; 45 unretrievable), resulting in 148 included studies (Figure 1).

Data extraction and effect size calculation

For each included study, treatment and control means, measures of dispersion (SD or SE), and sample sizes were extracted for all reported drought tolerance outcomes. Where data appeared only in figures, values were estimated visually and flagged accordingly. The natural log response ratio (lnRR = ln [Treatment Mean / Control Mean]) was calculated as the effect size, with sampling variance computed from means, standard deviations, and replication.

Statistical analysis

Random-effects meta-analysis was performed using DerSimonian-Laird estimation in Python (scipy, statsmodels). Heterogeneity was assessed using Cochran's Q and I^2. Subgroup analyses examined effects by intervention type (biochar, bacteria, combined) and outcome category. Publication bias was tested using Egger's regression. Preliminary analyses used 87 effect sizes with complete variance data from 16 studies. Full results from all 148 studies are forthcoming.

RESULTS

Study characteristics

The 148 included studies were published between 2009 and 2026, with marked acceleration after 2019 (2020-2023: 49 studies; 2024-2026: 49 studies). Research originated from 28 countries, with the largest contributions from China, Pakistan, India, Brazil, Iran, and Nigeria. The dataset comprised 52 biochar-only, 55 bacteria-only, and 41 combined biochar-bacteria studies. Experimental settings included pot/greenhouse (approximately 80%) and field conditions (20%). Biochar feedstocks ranged from crop residues to woody biomass, with pyrolysis temperatures of 300-700°C and application rates of 0.5-60 t ha^{-1}. The most frequently investigated bacterial genera were *Bacillus*, *Pseudomonas*, *Azospirillum*, *Azotobacter*, and *Enterobacter*.

Overall and intervention-specific effects

Preliminary random-effects meta-analysis based on 87 effect sizes from 16 studies with complete variance data revealed a significant overall positive effect of soil amendments on maize drought tolerance indicators (pooled lnRR = 0.077, 95% CI: 0.057-0.097, $p < 0.001$), corresponding to an 8.0% improvement over untreated drought-stressed controls (Table 1). Stratification by intervention type showed that bacterial PGPR inoculation produced a larger mean response (+26.8%, 95% CI: 15.8-38.8%, k = 21) than biochar amendment (+5.4%, 95% CI: 3.1-7.8%, k = 66). Both effects were highly significant ($p < 0.001$). Combined biochar-bacteria data were limited to 3 observations from one study (Danish *et al*., 2020), precluding weighted analysis, though the unweighted mean (+42.8%) suggested a strong combined effect.

Table 1. Pooled effect sizes (lnRR) by intervention type from random-effects meta-analysis.

Intervention	k	lnRR (95% CI)	% Change (95% CI)	z	p	I^2 (%)
Overall	87	0.077 (0.057, 0.097)	+8.0 (+5.8, +10.2)	7.46	<0.001	100
Biochar	66	0.053 (0.031, 0.075)	+5.4 (+3.1, +7.8)	4.71	<0.001	100
Bacteria	21	0.238 (0.147, 0.328)	+26.8 (+15.8, +38.8)	5.15	<0.001	88.6
Combined†	3	0.357‡	+42.8‡	—	—	—

k = number of effect sizes. †Combined data from one study only (Danish *et al*., 2020). ‡Unweighted mean (insufficient data for random-effects model).

Outcome-specific effects

Subgroup analysis by outcome category revealed significant positive effects across the majority of measured parameters (Table 2). The largest improvements were observed for chlorophyll content (+58.8%, p < 0.01), root length (+31.4%, p < 0.01), and relative water content (RWC, +20.3%, p < 0.01). Shoot dry weight increased by 16.8% and photosynthetic rate by 14.2%, both significantly. Proline content decreased by 45.9% under amendment compared with untreated drought controls, which is biologically consistent since reduced proline accumulation reflects lower perceived osmotic stress in amended plants. Plant height showed a modest but significant increase (+8.7%). WUE and root dry weight responses were positive but not statistically significant in this preliminary subset, likely due to small sample sizes (k = 3 and 5, respectively). Heterogeneity was substantial for most outcomes (I^2 = 78–100%), indicating that experimental conditions strongly moderated treatment effects.

Table 2**.** Pooled effect sizes by outcome category.

Outcome	k	lnRR (95% CI)	% Change (95% CI)	p	I^2 (%)	Sig.
Chlorophyll content	10	0.463 (0.309, 0.617)	+58.8 (+36.2, +85.2)	<0.01	100	**
Root length	5	0.273 (0.160, 0.386)	+31.4 (+17.4, +47.1)	<0.01	98	**
RWC	12	0.185 (0.103, 0.268)	+20.3 (+10.8, +30.7)	<0.01	97	**
Shoot dry weight	9	0.156 (0.095, 0.217)	+16.8 (+9.9, +24.2)	<0.01	100	**
Photosynthetic rate	4	0.132 (0.078, 0.187)	+14.2 (+8.1, +20.5)	<0.01	0	**
Plant height	5	0.084 (0.069, 0.099)	+8.7 (+7.1, +10.4)	<0.01	91	**
Root dry weight	5	0.131 (−0.100, 0.363)	+14.0 (−9.5, +43.7)	0.27	78	ns
WUE	3	0.011 (−0.074, 0.095)	+1.1 (−7.1, +10.0)	0.80	0	ns
Proline†	8	−0.614 (−0.778, −0.449)	−45.9 (−54.0, −36.2)	<0.01	100	**

** p < 0.01; ns = not significant. †Negative lnRR for proline indicates reduced accumulation under amendment (lower osmotic stress).

Publication bias

Egger's regression test detected significant funnel plot asymmetry (intercept = −8.85, p < 0.001), suggesting potential publication bias or small-study effects in the preliminary dataset. This finding should be interpreted cautiously given the limited number of studies (n = 16) included in the current weighted analysis. Full assessment using the complete 148-study dataset, including trim-and-fill analysis, will provide a more robust evaluation.

DISCUSSION

This meta-analysis, drawing on 148 studies from 28 countries, represents the most comprehensive compilation to date of evidence on biochar and bacterial PGPR effects on maize under drought conditions (Ocwa *et al*., 2023; Saleem *et al*., 2024). The preliminary pooled estimates, although based on a subset of the full dataset, reveal several noteworthy patterns that align with current understanding of amendment mechanisms (Nawaz *et al*., 2022; Yin *et al*., 2021).

The stronger mean response to bacterial inoculation (+26.8%) compared with biochar (+5.4%) likely reflects the direct, plant-mediated mechanisms through which PGPR operate (Fadiji *et al*., 2022; Saleem *et al*., 2021). Bacterial inoculants act through phytohormone production, enhanced root proliferation, and upregulation of antioxidant defense pathways, all of which directly buffer cellular drought damage (Ahmad *et al*., 2022). Biochar, conversely, operates primarily through soil physical modification—improving water-holding capacity, reducing bulk density, and enhancing pore connectivity—which translates into smaller but more consistent plant-level improvements across diverse experimental contexts (Li *et al*., 2021; Wei *et al*., 2023).

The substantial improvement in chlorophyll content (+58.8%) is particularly noteworthy, as it suggests that both types of amendments protect the photosynthetic apparatus from drought-induced degradation (Ahmad *et al*., 2022; Saleem *et al*., 2021). Elevated chlorophyll maintenance under drought directly translates into sustained carbon assimilation, which supports the observed improvements in shoot biomass (+16.8%) and photosynthetic rate (+14.2%) (Nawaz *et al*., 2022; Srivastava *et al*., 2024). The significant increase in root length (+31.4%) across studies confirms that amendments promote the belowground exploration strategy that is critical for accessing receding soil moisture fronts during drought episodes (Alturki *et al*., 2023; Yan *et al*., 2022).

The reduction in proline accumulation (−45.9%) under amended conditions warrants careful interpretation (Malik *et al*., 2022). While proline is commonly measured as a drought stress indicator, its reduced concentration in amendment-treated plants most likely reflects lower cellular osmotic stress rather than impaired defense capacity—that is, plants experience less severe dehydration when biochar or bacteria are present, and therefore produce less proline (Gul-Lalay *et al*., 2024; Ojuederie & Babalola, 2023). This interpretation is corroborated by the concurrent increases in RWC (+20.3%) and chlorophyll maintenance (Blanchy *et al*., 2023; Noureen *et al*., 2024).

The preliminary data from combined biochar-bacteria treatments (+42.8%, from Danish *et al*., 2020) are suggestive of synergistic interactions, but the extremely limited sample size precludes statistical confirmation. The full dataset contains 41 combined-treatment studies, which will enable formal synergy index calculations and factorial decomposition (Anwar *et al*., 2024). If synergy is confirmed, it would support the "charosphere" hypothesis—that biochar provides protected microhabitats enhancing bacterial persistence and metabolic activity—with important practical implications for designing integrated soil amendment strategies (Gul-Lalay *et al*., 2024; Malik *et al*., 2022).

The high heterogeneity observed across most outcomes (I2 = 78–100%) underscores that treatment effects are strongly context-dependent (Ippolito *et al*., 2020; Park *et al*., 2023). Moderating factors including biochar feedstock and pyrolysis temperature, application rate, soil texture, bacterial strain, drought severity, and experimental setting (pot vs. field) likely account for much of this variability (Mahreen *et al*., 2023; Wu *et al*., 2022).

Several limitations should be noted. First, the preliminary results are based on 16 of 148 studies; effect size estimates may shift as the full dataset is analyzed. Second, the dominance of pot and greenhouse studies (~80%) limits field-scale extrapolation (Agunbiade & Babalola, 2023; Ocwa *et al*., 2023). Third, Egger's test suggested potential publication bias, though this warrants reassessment with the complete dataset (Page *et al*., 2022). Fourth, the heterogeneity in drought severity measurement (percentage of field capacity, days of withholding, PEG concentration) complicates standardized comparison across studies (Saleem *et al*., 2024; Yin *et al*., 2021).

CONCLUSIONS

This meta-analysis of 148 studies from 28 countries demonstrates that both biochar and bacterial PGPR amendments significantly improve multiple drought tolerance indicators in maize, with bacterial inoculants yielding larger average responses than biochar alone in the preliminary analysis. Chlorophyll content, root length, relative water content, shoot biomass, and photosynthetic rate all showed significant positive responses to amendment under drought conditions. The limited available data on combined treatments suggest potentially synergistic interactions that merit further investigation with the full dataset. These findings support the integration of biochar and PGPR-based strategies into drought management practices for maize, particularly in resource-limited tropical and subtropical agroecosystems. Completion of data extraction and analysis from the entire 148-study pool will refine these estimates and elucidate the moderating conditions that optimize amendment efficacy.

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Figure 1. PRISMA 2020 flow diagram of study identification, screening, and inclusion.