

COMPARABILITY OF MICROCOSM AND FIELD STUDIES IN ASSESSING NITROGEN-FIXING BACTERIA'S IMPACT ON CROP YIELD: A META-ANALYSIS

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ABSTRACT

Crop inoculation with nitrogen-fixing bacteria (NFB) is a promising strategy for sustainable agriculture, but its effectiveness has been assessed using both field and microcosm studies, raising questions about the translatability of results. To address this, we conducted a comprehensive weighted meta-analysis of 78 studies (410 observations) across five major crops—wheat (*Triticum aestivum* L.), rice (*Oryza sativa*), maize (*Zea mays* L.), chickpea (*Cicer arietinum* L.), and barley (*Hordeum vulgare*)—to compare yield outcomes from NFB inoculation in both study types. Our analysis revealed that NFB inoculation had a significantly positive effect on crop yield (Hedges' $g = 1.05$). While there was no significant difference in the mean effect size between the field and microcosm studies ($P = 0.326$), we found that soil inoculation provided a significantly larger yield enhancement than seed inoculation ($P = 0.011$). Crucially, a meta-regression of microcosm studies showed that experimental design parameters such as soil volume and plant density significantly influenced the magnitude of the NFB effect ($P \leq 0.006$). Although microcosm studies can produce results directly comparable to those from the field, they exhibit greater variability, and their outcomes are highly sensitive to experimental design. These findings underscore the utility of microcosms as models while highlighting the need for caution and careful design when extrapolating their results to field conditions.

Keywords: nitrogen-fixing bacteria, crop yield, biofertilizer, meta-analysis, microcosms.

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INTRODUCTION

The rising global demand for food production, along with growing concerns regarding the impact of synthetic fertilizers, has intensified the need for sustainable agriculture. Today, agriculture depends heavily on synthetic nitrogen fertilizers; however, they have important ecological effects, including greenhouse gas emissions, water pollution, and soil degradation (Martínez-Dalmau *et al.*, 2021). To mitigate these negative effects, inoculation of crops with nitrogen-fixing bacteria (NFB) has been studied as a strategy to enhance crop yield by reducing reliance on synthetic fertilizers (Mahapatra *et al.*, 2022). Numerous studies employing diverse methodologies have investigated the interactions between nitrogen-fixing bacteria (NFB) and various crop species.

The study of the effect of NFB on crop yield follows two primary approaches: field and microcosm

studies. Field studies conducted in agricultural settings provide insights into NFB performance under the influence of real-world environmental variability and agronomic practices. Although this provides high agricultural relevance, these trials can be expensive and subject to uncontrollable factors. In contrast, microcosm studies are a controlled and cost-effective way to investigate the effects of NFB on crop yield. These experiments make it easier to understand the underlying mechanisms. However, their main drawback is that they may not fully capture the complexity of the real-world agricultural systems.

The differences between microcosms and field studies create challenges regarding the translatability and comparability of their findings (Heinze *et al.*, 2016; Schittko *et al.*, 2016; Forero *et al.*, 2019). Several factors have been identified that can be affected by differences in soil volume limitation, simplification of microbial communities, and competence between plants in

microcosm studies. Therefore, a systematic, quantitative synthesis is needed to clarify this relationship and identify how these factors influence crop yield. Understanding this connection is crucial for optimizing NFB research strategies and ensuring that promising microcosm results can be reliably applied in agricultural practices.

The objective of this study was to compare the effects of NFB inoculation on crop yield in field and microcosm studies. To this end, we performed a meta-analysis of 410 observations from 78 research papers. We sought to determine whether the effect of NFB inoculation under these differing conditions was consistent, and whether it was moderated by crop type and bacterial genera. Additionally, we examined how different inoculation methods performed in field versus microcosm settings, and whether the choice of method influences the comparability of the results. For microcosm studies, we also investigated whether variations in experimental design parameters, such as soil volume per pot, number of plants per pot, and plant density per unit of soil, contribute to the variability in observed NFB inoculation effects.

MATERIALS AND METHODS

Data collection: We performed an extensive literature search using Google Scholar and Scopus for published papers on the effect of NFB inoculation on crop productivity. We used combinations of the keywords; “productivity”, “yield”, “growth”, “biofertilizer”, “inoculation”, “nitrogen-fixing bacteria”. The criteria used to include the studies were the use of only one bacterial species per experimental group, description of inoculation and growth methods, and inclusion of a control group for treatment. The exclusion criterion was the use of sterilized soil or substrate.

A meta-analysis requires independent datasets; thus, in cases where multiple sampling dates were reported in a study, only data from the most recent time point were employed. When several studies reported data from the same trial, the study with the most complete dataset was preferred. In all cases, we extracted the grain yield from the papers, as well as their associated variances. In studies that used microcosms as models, we extracted the volume and number of plants. Data from the papers were extracted from the text and tables when available, or from images using ImageJ (<https://imagej.nih.gov/ij/>). A total of 78 studies with 410 observations were included in the meta-analysis, and the detailed process is presented in a PRISMA flow diagram (Figure 1). References used for the meta-analysis can be found in the Zenodo repository (doi: 10.5281/zenodo.15743130).

Data Analysis: Data were primarily grouped by crop type into the following categories: wheat (*Triticum aestivum* L.), rice (*Oryza sativa*), maize (*Zea mays* L.), chickpea (*Cicer arietinum* L.), and barley (*Hordeum vulgare*), although the overall analysis considered all studies. We performed a weighted meta-analysis using a random-effects model, due to expected heterogeneity across studies, to calculate the effect size (ES) for each observation. The ES was measured in terms of Hedges' *g* (*g*), chosen because it provides a standardized mean difference corrected for a small sample size bias.

First, the pooled standard deviation (spooled) was calculated for each study.

$$S_{\text{pooled}} = \sqrt{\frac{(n_e - 1)s_e^2 + (n_c - 1)s_c^2}{n_e + n_c - 2}}$$

where n_e and n_c are the sample sizes and s_e and s_c are the standard deviations of the experimental (inoculated group) and control groups, respectively. The standardized mean difference (Cohen's *d*) was calculated as follows:

$$d = \frac{\bar{X}_e - \bar{X}_c}{S_{\text{pooled}}}$$

where \bar{X}_e and \bar{X}_c represent the mean values for the experimental and control groups, respectively. Hedges' *g* (*g*) was computed by applying a small sample correction factor (*J*), as follows:

$$J \approx 1 - \frac{3}{4(n_e + n_c - 2) - 1}$$

The approximate variance (*v_g*) for each Hedge's *g* value was estimated as follows:

$$v_g \approx \frac{n_e + n_c}{n_e n_c} + \frac{g^2}{2(n_e + n_c)}$$

The effect size was considered significant if the 95% CI value was not zero, and the effects were considered different when their respective CI did not overlap. The 95% confidence interval (95% CI) was calculated using the following equation:

$$95\% \text{ CI} = g_{\text{pooled}} \pm z_{0.975} \times SE_{g_{\text{pooled}}}$$

For studies that did not report SD, we estimated the SD to be 10% of the mean (Luo *et al.*, 2006). In order to explore the effects of NFB on individual crops and study the effects of the inoculation method on crop yield we conducted subgroup analyses. Finally, to explore the association between crop yield in microcosms studies and the volume, number of plants, and number of plants per kilogram of soil in the microcosm, we conducted a weighted meta-regression analysis (Greenland, 1987).

Publication Bias and Robustness Analysis: To check for publication bias, we conducted a visual examination of the funnel plot of effect sizes versus standard errors. This visual analysis was followed by Egger's regression test for detecting asymmetry in the funnel plot and Begg's rank correlation test. To assess the robustness of the overall findings and evaluate the influence of individual

studies, we conducted a leave-one-out sensitivity analysis. This process involved methodically omitting one study at a time and recalculating the overall pooled effect size to determine if any individual study had an excessive influence on the results.

RESULTS

Overall effect of nfb inoculation and heterogeneity:

The overall meta-analysis, encompassing 410 observations and employing a random-effects model, revealed a positive and statistically significant effect of nitrogen-fixing bacteria (NFB) inoculation on crop yield (Pooled Hedges' $g = 1.05$, 95% CI = 0.96–1.15, $P < 0.001$). A fixed-effects model also indicated a significant positive effect (pooled Hedges' $g = 0.87$, 95% CI = 0.82–0.93, $P < 0.001$). Significant heterogeneity was observed among the studies (Cochran's $Q = 1058.61$, $df = 409$, $P < 0.001$; $I^2 = 61.36\%$; $\tau^2 = 0.5116$), supporting the use of the random effects model for subsequent analyses.

Comparison of field vs. microcosm studies:

A subgroup analysis was conducted to compare the effects of NFB inoculation between field and microcosm conditions using a random-effects model (Figure 2). NFB inoculation resulted in a statistically significant increase in crop yield in field studies ($k = 328$; Pooled Hedges' $g = 1.02$, 95% CI = 0.93–1.12, $P < 0.001$; $I^2 = 56.62\%$; $\tau^2 = 0.3977$). Similarly, a statistically significant positive effect was observed in microcosm studies ($k = 82$; Pooled Hedges' $g = 1.25$, 95% CI = 0.97–1.54, $P < 0.001$; $I^2 = 73.35\%$; $\tau^2 = 1.1529$). The test for subgroup differences indicated that the difference in the pooled effect sizes between the field and microcosm conditions was not statistically significant ($QM = 0.96$, $df = 1$, $P = 0.326$).

Influence of crop type and study conditions:

Two-way subgroup analysis was performed to investigate the combined effects of crop type and study conditions. The test for differences between these combined subgroups was statistically significant ($QM = 22.34$, $df = 9$, $P = 0.008$). The detailed effect sizes for each crop in the field and microcosm settings are presented in Figure 3. Among these, wheat in microcosm studies showed a pooled Hedges' g of 2.07 ($k=34$; 95% CI = 1.47–2.67, $P < 0.001$; $I^2 = 79.34\%$). In field studies, maize yielded a pooled Hedges' g of 1.15 ($k=156$; 95% CI = 0.98–1.32, $P < 0.001$; $I^2 = 65.28\%$). Significant positive effects (all $P \leq 0.0002$) were also observed for barley, chickpea, rice, and wheat in field studies. In microcosm studies, barley, chickpea, maize, and rice also showed significant positive effects (all $P \leq 0.0405$).

Influence of inoculation method and study conditions:

NFB inoculation method was assessed as a moderator. Overall, soil inoculation yielded a pooled Hedges' g of

2.24 ($k=48$; 95% CI = 1.72–2.76, $P < 0.001$; $I^2 = 79.61\%$), while seed inoculation resulted in a pooled Hedges' g of 0.98 ($k=362$; 95% CI = 0.89–1.07, $P < 0.001$; $I^2 = 56.06\%$). The difference between these two methods was statistically significant ($QM = 6.46$, $df = 1$, $P = 0.011$). A two-way subgroup analysis of inoculation method and study conditions revealed significant differences among the combined subgroups ($QM = 17.48$, $df = 3$, $P = 0.00056$), with details presented in Figure 4. Soil inoculation in microcosm studies resulted in a pooled Hedges' g of 3.19 ($k=5$; 95% CI = 1.40–4.99, $P = 0.0005$; $I^2 = 81.28\%$). Soil inoculation in field studies yielded a pooled Hedges' g of 2.12 ($k=43$; 95% CI = 1.58–2.66, $P < 0.001$; $I^2 = 79.04\%$). Seed inoculation also produced significant positive effects under both field (Hedges' $g = 0.96$, $P < 0.001$) and microcosm conditions (Hedges' $g = 1.14$, $P < 0.001$).

Meta-regression analysis of microcosm study variables:

Mixed-effects meta-regression analyses were conducted on microcosm studies (k values vary per analysis, using overall τ^2 from the full dataset in weights) to investigate the associations between experimental design parameters and NFB effect sizes. A statistically significant negative association was found between the kilograms of soil per pot and effect size (coefficient = -0.319, 95% CI = -0.542 to -0.096, $P = 0.006$; $k=59$), as depicted in Figure 5b. This model had an R^2 of 12.6% and significant residual heterogeneity remained ($QE = 116.30$, $df=57$, $P < 0.001$). A statistically significant positive association was observed between the number of plants per microcosm and effect size (coefficient = 0.452, 95% CI = 0.302–0.602, $P < 0.001$; $k=59$; Figure 5c). This model had an R^2 value of 39.1%, and no significant residual heterogeneity was detected ($QE = 72.16$, $df=57$, $P = 0.085$). A statistically significant positive association was also found between the number of plants per kilogram of soil and the effect size (coefficient = 1.456, 95% CI = 0.789–2.123, $P < 0.001$; $k=57$; Figure 5a). This model had an R^2 of 25.8%, with significant residual heterogeneity ($QE = 87.81$, $df=55$, $P = 0.003$).

Publication bias and robustness:

Visual inspection of the funnel plot suggested some asymmetry. Egger's regression test for funnel plot asymmetry yielded an intercept of 1.42 (SE = 0.22, $P < 0.001$). Begg's rank correlation test indicated a Kendall's Tau of 0.57 ($P < 0.001$). Leave-one-out sensitivity analysis was also performed. The overall random-effects pooled estimate (1.05, 95% CI = 0.96–1.15) remained relatively stable upon removal of individual studies, with resulting pooled estimates ranging from 0.98 to 1.10. The removal of no single study altered the statistical significance of the overall positive effect of NFB inoculation.

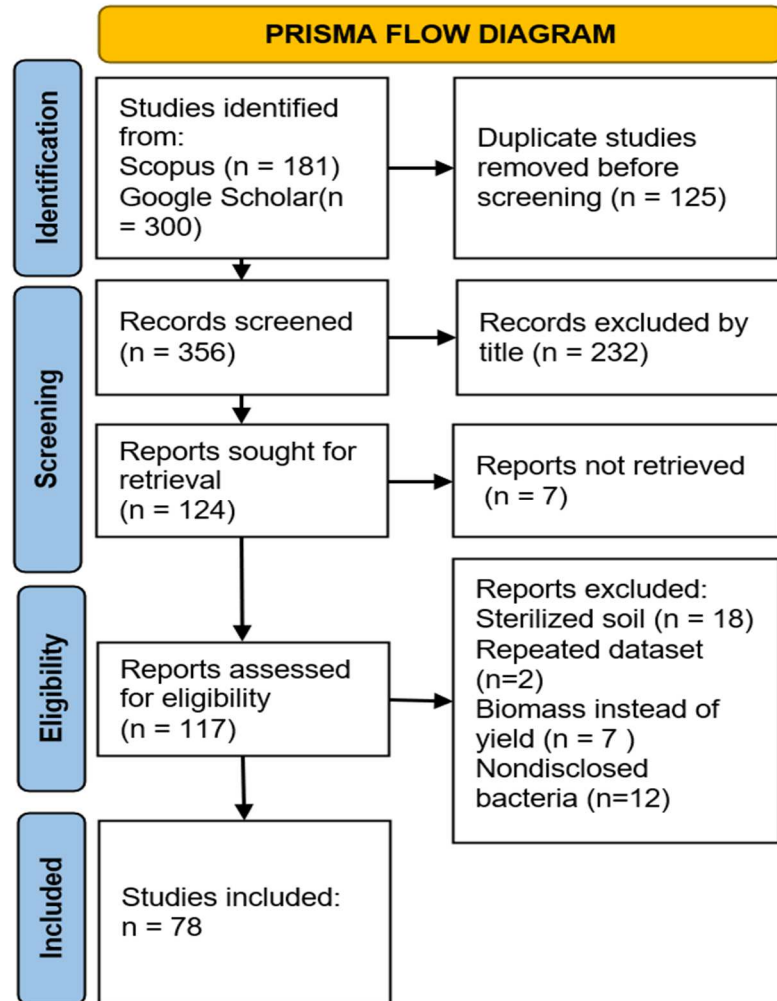


Figure 1. PRISMA flow diagram illustrating the literature search and study selection process. The initial search of Scopus (n=181) and Google Scholar (n=300) yielded 481 records. After removing duplicates and screening records based on title and eligibility criteria, 78 studies were ultimately included in the final meta-analysis.

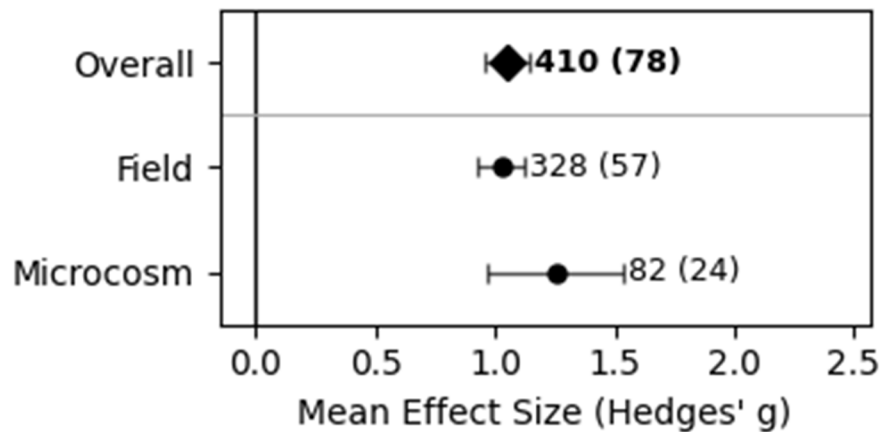


Figure 2. NFB inoculation significantly increased the overall crop yield, with comparable mean effect sizes in field (Hedges' g = 1.02) and microcosm (Hedges' g = 1.25) studies (P=0.326 for subgroup difference). Data points represent pooled Hedges' g with 95% CIs; Numbers accompanying data points indicate total observations, with the number of contributing studies in parentheses.

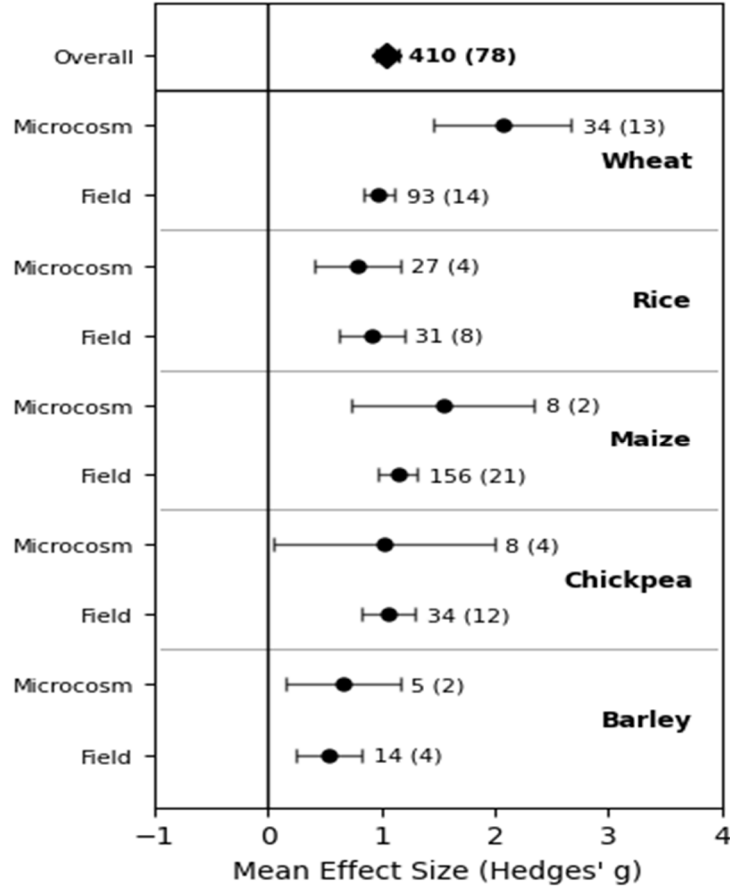


Figure 3. The beneficial effect of NFB inoculation on crop yield was significantly moderated by the interaction of crop type and study condition ($QM=22.34$, $P=0.008$), with wheat in microcosm studies exhibiting a notably large positive effect (Hedges' $g = 2.07$). Data points represent pooled Hedges' g with 95% CIs; Numbers accompanying data points indicate total observations, with the number of contributing studies in parentheses.

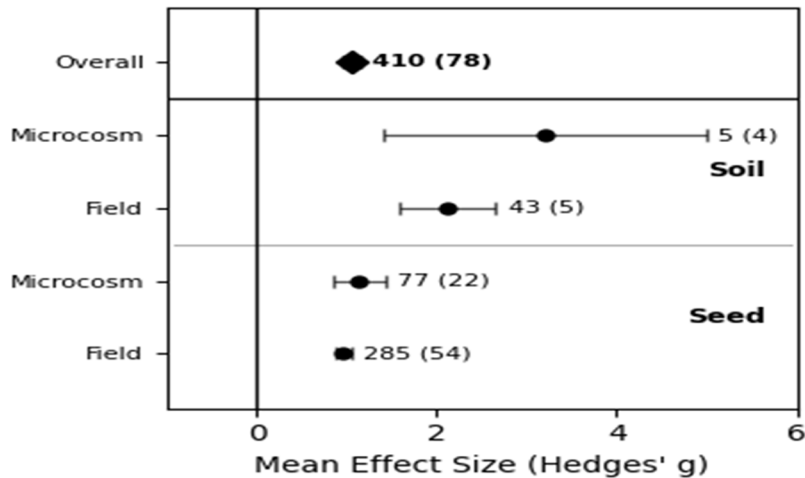


Figure 4. Soil inoculation (overall Hedges' $g = 2.24$) led to a significantly larger NFB-induced enhancement in crop yield than seed inoculation (overall Hedges' $g = 0.98$) ($P=0.011$ for method difference), and this effect was particularly pronounced in microcosm settings (soil inoculation Hedges' $g = 3.19$). Data points represent pooled Hedges' g with 95% CIs; Numbers accompanying data points indicate total observations, with the number of contributing studies in parentheses.

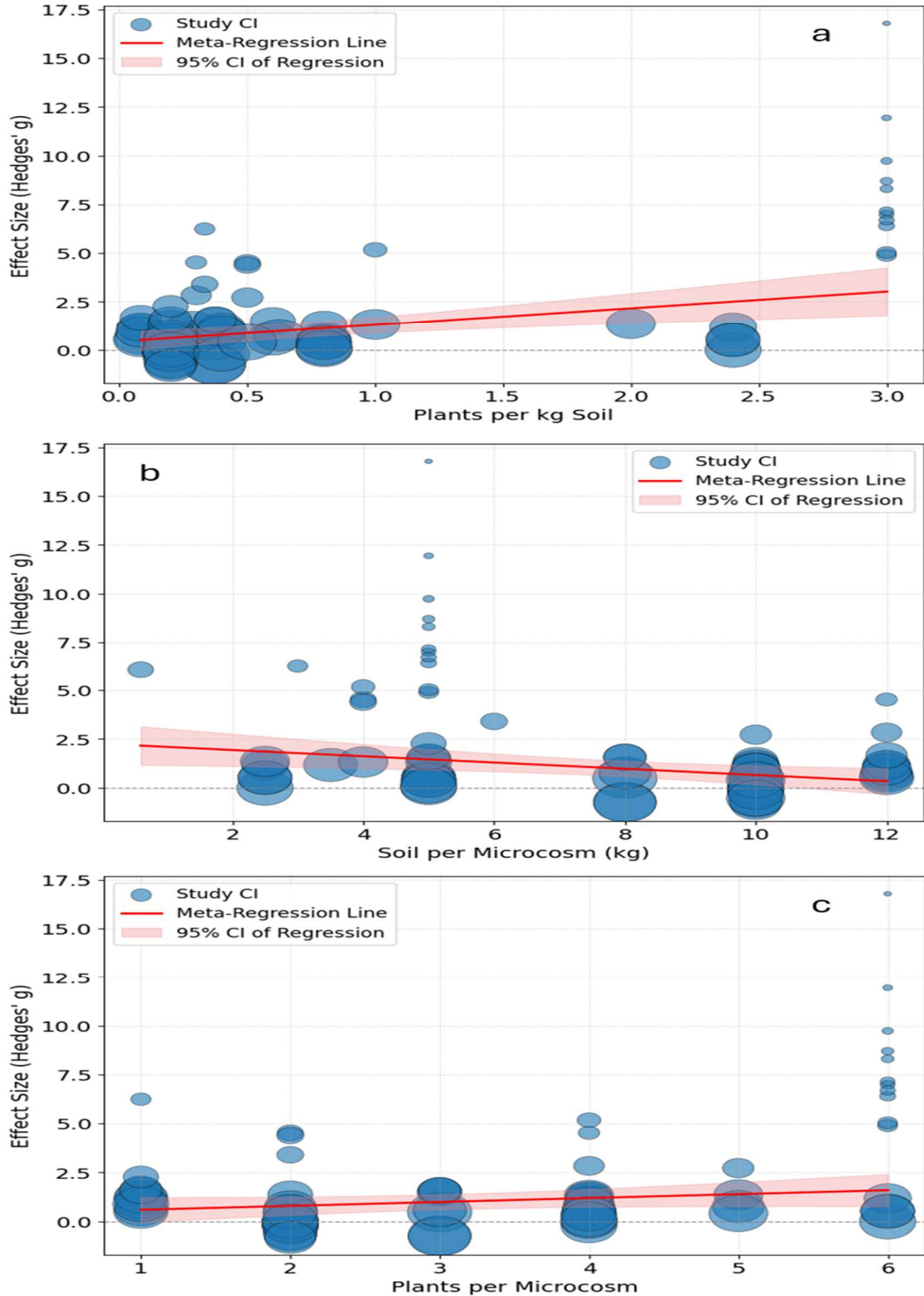


Figure 5. Meta-regression analysis of microcosm studies revealed significant associations between NFB effect size (Hedges' g) and the following experimental design parameters: (a) a significant positive relationship with the number of plants per kg of soil (coefficient = 1.456, $P < 0.001$), (b) a significant negative relationship with kilograms of soil per pot (coefficient = -0.319, $P = 0.006$), and (c) a significant positive relationship with the number of plants per pot (coefficient = 0.452, $P < 0.001$). Circles represent individual studies with their 95 % CIs.

DISCUSSION

The objective of this study was to evaluate the effectiveness of nitrogen-fixing bacteria (NFB) inoculation on crop yield in both field and microcosm environments and to identify the principal factors that influence these outcomes. The results enhance the understanding of the applicability of NFB research across varying experimental scales, and offers valuable insights for future research and practical applications.

The meta-analysis accounted for substantial inter-study heterogeneity ($I^2 = 61.36\%$), confirming a significant and positive overall effect of NFB inoculation on crop yield (Hedges' $g = 1.05$). This reinforces the potential of NFB as a valuable tool in sustainable agriculture to enhance productivity (Mahapatra *et al.*, 2022). A central objective was to compare the results from NFB inoculation between field and microcosm settings. Our results indicated that NFB inoculation significantly enhanced crop yield in both field (Hedges' $g = 1.02$) and microcosm studies (Hedges' $g = 1.25$), with no statistically significant difference in their average efficacy ($P = 0.326$). This suggests that, on average, microcosms can provide a relevant preliminary assessment of NFB performance, reflecting the general direction and broadly comparable magnitude of effects observed in the field. This aligns with the view that despite their inherent simplifications, microcosm studies can offer valuable initial insights (Kizilkaya, 2008; Zabihi *et al.*, 2011; Banik *et al.*, 2019; Sheirdil *et al.*, 2019). However, the numerically larger mean effect and wider confidence interval for the overall microcosm pooled estimate indicated that, while broadly comparable, results from microcosm studies might exhibit greater variability, a point that warrants consideration when extrapolating effect magnitudes.

The efficacy of NFB inoculation was significantly moderated by crop type when interacting with study settings ($QM = 22.34$, $P = 0.008$); however, wider confidence intervals were generally observed in microcosm studies. This could be attributed to several factors, including typically smaller numbers of studies within specific microcosm subgroups, a potentially higher underlying methodological heterogeneity among different microcosm experiments despite control *within* each (I^2 values often $>60\%$ for microcosm crop subgroups), or perhaps a greater diversity of NFB strains and experimental objectives in initial microcosm screenings.

While most crops (barley, chickpea, maize, and rice) exhibited broadly comparable mean effect sizes between field and microcosm conditions, wheat was a notable exception. For wheat, the beneficial effect of NFB inoculation was substantially greater in the microcosm studies (Hedges' $g = 2.07$) than in the field studies (Hedges' $g = 0.98$), with non-overlapping 95%

confidence intervals, indicating a significant difference in the response magnitude between the two environments, specifically for this crop.

We hypothesized that the significantly higher yield response of wheat to Nitrogen-Fixing Bacteria (NFB) inoculation under microcosm conditions is driven by a combination of several factors. First, wheat has lower water requirements than crops such as maize (Mehta and Pandey, 2015). The consistent and optimal water supply in microcosms removes the potential water stress that might occur in the field, which is a critical factor, especially during the seedling stage causing a decrease in yield (Song *et al.*, 2019). Second, microcosms are often conducted in controlled environments, which may have provided conditions favoring photoperiod and vernalization cues known to significantly influence wheat flowering and subsequent yield (Rodrigues *et al.*, 2014). With water and potentially optimized flowering conditions, the nitrogen supplied by NFB becomes the key driver of yield increases. The impact of NFB-supplied nitrogen is likely amplified in the microcosms for two main reasons. First, the contained systems prevent nitrogen loss through runoff and leaching, which can occur under field conditions. Second, wheat has a relatively low nitrogen requirement compared to crops such as maize (He *et al.*, 2013), which also leads to a higher contribution of N fertilizers to wheat yield (Liu *et al.*, 2010). Consequently, the additional nitrogen provided by NFB leads to a more pronounced effect on yield than that observed in more nitrogen-demanding crops. This underscores the importance of designing microcosm experiments to reflect the key resource limitations anticipated in the field and ensure that the findings can be more accurately translated to agricultural practice.

In addition, we found that the inoculation method significantly influenced NFB efficacy and its interaction with the study conditions ($QM = 17.48$, $P = 0.00056$). Soil inoculation demonstrated a markedly stronger effect (Hedges' $g = 2.24$) than seed inoculation (Hedges' $g = 0.98$), and this difference was most pronounced under microcosm conditions (Hedges' $g = 3.19$). This is consistent with previous studies where soil inoculation has been associated with higher yields (Schiffmann and Alper, 1968; Habish and Ishag, 1974). The higher effect in microcosms than in field conditions could be attributed to a reduction in mesofauna diversity, which was associated with an increase in available nitrogen in microcosms (Cole *et al.*, 2004), together with the controlled environmental parameters characteristic of microcosm settings, which stand in contrast to the greater ecological complexity and variability inherent to field studies (Dadzie *et al.*, 2024).

Our examination of the elements affecting NFB effectiveness in controlled environments showed that the size of the soil and the density of the plants played a significant role in altering the NFB effect sizes observed.

This finding is key for understanding translatability, as we identified a clear trend: smaller soil volumes and higher plant densities were consistently associated with larger NFB-induced yield enhancement. This strongly suggests that common laboratory setups, often prioritizing convenience or resource constraints, might inadvertently create conditions that inflate the perceived benefits of NFB inoculation compared with field scenarios. For instance, restricted soil volumes can lead to more rapid nutrient depletion (Salamanca *et al.*, 1998), thereby making the crop more reliant on, and responsive to, NFB-supplied nitrogen. Similarly, higher plant densities can heighten interplant competition for resources (Dukes, 2001), making the contribution of NFB more substantial. Additionally, small microcosm dimensions can simplify soil ecological dynamics (Spencer and Warren, 1996; Gustafson and Casper, 2004), leading to reduced ecological challenges and potentially amplifying the perceived NFB benefits relative to the field. These findings strongly imply that, to improve the translatability of microcosm research, experimental designs should aim to mimic field-relevant soil volumes and plant densities. Furthermore, detailed reporting of these parameters is essential for interpreting the results and improving cross-study comparisons (Heinze *et al.*, 2016; Schittko *et al.*, 2016; Forero *et al.*, 2019).

This meta-analysis has several limitations that warrant discussion. A formal quality assessment of individual studies was not performed, as standardized quantitative quality appraisal tools are less established and not yet widely adopted in agricultural research compared to other disciplines (Philibert *et al.*, 2012). Additionally, publication bias tests (Egger's $P < 0.001$; Begg's $P < 0.001$) indicated a significant potential for publication bias. This finding suggests an overrepresentation of studies reporting positive and significant effects, which may have inflated the overall pooled effect size. Although our leave-one-out sensitivity analysis shows that the statistical significance of the overall positive NFB effect is robust, the magnitude of this effect should be interpreted with this potential bias in mind. Second, despite the inclusion of numerous studies, considerable heterogeneity persisted, even within subgroups (I^2 values often $>50\%$). While we explored several key moderators, other unmeasured variables related to specific environmental conditions, soil types, NFB strains, or co-applied treatments likely contributed to this variability. The frequent lack of detailed reporting of such factors in primary studies limits the exhaustive exploration of the source of heterogeneity (Lin, 2018). Furthermore, the microcosm category itself encompasses a diverse range of experimental setups, and while our meta-regression addressed some design features, residual variability linked to unquantified aspects of microcosm design is probable.

Conclusion: The objective of this meta-analysis was to critically assess the effects of inoculation with nitrogen-fixing bacteria (NFB) on crop yield within both field and microcosm experimental frameworks. We found that NFB inoculation significantly enhanced crop yield in both settings, with an average magnitude of enhancement that was broadly comparable. However, nuanced distinctions are observed. The analysis confirmed the context dependency of NFB efficacy, demonstrating significant moderation by interactions between the experimental setting and key biological factors, whereby distinct crop types and bacterial genera exhibited differential response magnitudes across these environments. Furthermore, NFB inoculation method was found to be influential, with inoculation techniques interacting with study conditions, thereby affecting both perceived efficacy and congruence of outcomes between experimental scales. This meta-analysis identified that reduced soil volumes in microcosms and increased plant numbers per pot were significantly correlated with larger NFB-induced yield enhancements. This observation underscores the principal conclusion that such controlled experimental systems may inherently lead to an overestimation of NFB efficacy, thereby limiting the direct translatability of the findings to agricultural practice. Consequently, a salient implication of this research is that enhancing the predictive validity of microcosm-derived data for field agriculture necessitates rigorous consideration of these experimental design parameters to emulate field conditions more accurately, thereby fostering a more robust translation of NFB research into tangible sustainable agricultural applications.

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