FEMS Microbiology Ecology, 94, 2018, fiy094

doi: 10.1093/femsec/fiy094
Advance Access Publication Date: 19 May 2018
Research Article

RESEARCH ARTICLE

What is the agronomic potential of biofertilizers for maize? A meta-analysis

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One sentence summary: The authors used meta-analysis to examine whether greenhouse studies of plant-growth-promoting bacteria predict their performance in the field and the conditions under which these biofertilizers are most effective.

Editor: Wietse de Boer
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ABSTRACT

Biofertilizers are promoted as a strategy for sustainable intensification of agriculture, but their efficacy varies widely among published studies and it is unclear whether they deliver the promised benefits. Studies are commonly conducted under controlled conditions prior to deployment in the field, yet the predictive value of such studies for field-scale productivity has not been critically examined. A meta-analysis was conducted using a novel host crop-specific approach to evaluate the agronomic potential of bacterial biofertilizers for maize. Yield increases tended to be slightly higher and more variable in greenhouse studies using field soil than in the field, and greenhouse studies poorly predicted the influence of moderating climate, soil and taxonomic variables. We found greater efficacy of *Azospirillum* spp. and lower efficacy of *Bacillus* spp. and *Enterobacter* spp. under field conditions. Surprisingly, biofertilizer strains with confirmed plant-growth-promoting traits such as phosphorus solubilization, nitrogen fixation and phytohormone production *in vitro* were associated with lower yields in the field than strains not confirmed to possess these traits; only 1-aminocyclopropane-1-carboxylate deaminase synthesis increased yields. These results indicate the need for a novel biofertilizer development framework that integrates information from native soil microbial communities and prioritizes field validation of results.

Keywords: biofertilizer; ecological coherence; maize; meta-analysis; PGPR; rhizosphere

INTRODUCTION

Soil microorganisms are increasingly recognized as crucial elements of sustainable agricultural production. Whether introduced by inoculation or enhanced by management practices, plant-growth-promoting rhizobacteria (PGPR) have been shown to increase yields and stress tolerance without compromising environmental quality (Lugtenberg and Kamilova 2009; Coleman-Derr and Tringe 2014). Biofertilizers are defined as formulations of living microbial strains that are applied to seeds, plants or soil to colonize the rhizosphere. They can enhance the supply or availability of nutrients, and completely or partially replace agrochemicals, representing a lucrative and expanding product market. While both bacteria and fungi are key components of rhizosphere ecology and can be used in biofertilizer formulations, the scope of this meta-analysis is limited to bacteria. The statistical power of meta-analysis is increased by the higher number of studies and greater taxonomic breadth of bacterial biofertilizer strains, and the distinct metabolic preferences, physiological responses to environmental conditions and mechanisms of host colonization and plant growth promotion by beneficial fungi make fungal biofertilizers a topic best addressed separately.

Received: 17 May 2018; **Accepted:** 17 May 2018

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Although there is increased interest in harnessing rhizobacteria to improve crop productivity, critical questions remain: How large and consistent are yield gains due to inoculation? Under what conditions are bacterial biofertilizers most effective? Which taxa and combinations provide the greatest benefits? How effective are biofertilizers developed from *in vitro* and sterile potting media tests under field conditions?

Meta-analysis provides a powerful tool to improve the predictability and reliability of biofertilizers by quantifying the effects of moderating variables that influence the outcome of inoculation. Mechanisms of plant growth promotion possessed by potential biofertilizer strains are often tested *in vitro* and verified in sterile potting media, but the field relevance of these traits is not universally proven. Strains inoculated into field soil encounter heterogeneous resource availability and varied ecological interactions with native soil microbes, which mean that a plant-growth-promoting trait observed *in vitro* may not be expressed *in situ* (Laabas *et al.* 2017). Promising strains identified under controlled conditions have been shown to express plant-growth-promoting or stress-alleviating traits to a lesser extent in the field (Bacilio *et al.* 2017). Abiotic and biotic characteristics of the soil environment are primary regulators of microbial ecology and likely alter the persistence and efficacy of biofertilizers in the field (Vlassak and Vanderleyden 1997; Zengeni, Mpepereki and Giller 2006).

Climate conditions such as temperature and precipitation shape the soil environment and patterns of microbial biogeography (Pasternak *et al.* 2013). Soil type, fertility and management likewise determine resource availability, thus affecting microbial activity and community composition (Cenini *et al.* 2016; Francioli *et al.* 2016; Soman *et al.* 2017). Our understanding of interactions between inoculants and native soil microbial communities also remains limited (Trivedi *et al.* 2017).

Recognizing biofertilizers as living organisms rather than pseudo-chemicals raises questions about the extent to which plant-growth-promoting traits are phylogenetically conserved. This concept of ecological coherence (Philippot *et al.* 2010), where members of a given phylogenetic group share traits, has generally been used to describe patterns of microbial distribution according to habitat type (Hackl *et al.* 2004; Lozupone and Knight 2007), environmental variables (Fierer, Bradford and Jackson 2007), or even agricultural management practices (Philippot *et al.* 2009). Knowing whether metabolic capabilities and ecological traits that have been shown to promote plant growth in one strain are likely to be present in related strains as well could increase the efficiency of screening pipelines and aid in the prediction and identification of novel strains suited for biofertilizers.

Additional ecological complexity arises with biodiverse formulations that include not one, but multiple bacterial strains. These biofertilizers thus represent a form of synthetic microbial community (Großkopf and Soyer 2014) and may possess novel properties since bacteria behave differently in combination than in pure culture (De Roy *et al.* 2014). Compounds generated by one strain can serve as substrates in biosynthetic pathways of other strains, ultimately generating metabolites not created by either strain alone in a phenomenon termed 'emergent biosynthetic capacity' (Chiu, Levy and Borenstein 2014). Niche complementarity further enhances resource use efficiency and can increase rates of microbially mediated ecosystem functions (Goebel *et al.* 2014; Schnyder *et al.* 2018). Thus, interactions among microbial strains may determine the plant-growth-promoting mechanisms provided by a biofertilizer and the outcome for the host plant, but large-scale comparisons of single- and multiple-strain formulations are lacking.

Recent meta-analyses have sought to quantify the impact of PGPR under different environmental conditions (Rubin, Groenigen and Hungate 2017) or according to taxonomy of the biofertilizer strain (Chandrasekaran *et al.* 2016) but have not restricted their scope to a single plant species. Given the high degree of host specificity involved in recruitment of microbes to the rhizosphere and certain plant-growth-promoting mechanisms (Long, Schmidt and Baldwin 2008), focusing on individual crop species should reduce variability and increase the accuracy of effect size estimates. Maize (*Zea mays*) is an ideal host species candidate given its economic and nutritional importance globally and its frequent use as a model species and in inoculation studies.

A meta-analysis of bacterial biofertilizer studies on maize was conducted to evaluate: (i) the effectiveness of biofertilizers in field studies and pot studies using nonsterile field soil, (ii) variation in effect sizes by climate region and soil fertility, (iii) the influence of strain taxonomy and number and (iv) the predictive value of plant-growth-promoting mechanisms observed *in vitro*.

METHODS

Identification of studies

Publications testing the effect of biofertilizers in maize under field conditions and/or using field soil in greenhouse pot studies were identified by searching Web of Science, Agricola and Google Scholar databases. Keywords used included '(PGPR OR biofertilizer OR rhizobacteria) AND (maize OR corn) AND field'. Because the purpose of the meta-analysis was to evaluate biofertilizer performance in nonsterile field soil and to compare performance under field and greenhouse conditions for the same soils, pot studies were only included if the soil was not sterilized prior to planting and was not mixed with sand or other substrates. Studies were excluded if the experimental design was not randomized and replicated, no information was available on number of replicates, or if the inoculated and uninoculated treatments differed in parameters other than the addition of biofertilizer. Only studies that identified the biofertilizer to at least the phylum level were used. A total of 70 publications remained for inclusion in the meta-analysis, representing 576 unique treatment-control pairs (Table S1, Supporting Information). To ensure that studies were not biased by connections to the biofertilizer industry, author affiliations and funding sources were reviewed for each of these studies. Only four authors were identified with any connections to companies that have biofertilizer products, and no industry funding was reported for any study (Table S2, Supporting Information).

Moderator variables

Moderator variables that were included in the analysis included: Köppen climate region, soil N content prior to planting, fertilization, number of strains included in the biofertilizer and their taxonomy to the species level, and plant-growth-promoting mechanisms (ACC deaminase production, nitrogen fixation, phosphate solubilization, phytohormone production and/or siderophore production). Köppen climate regions were specified according to main climate region and precipitation level using latitude and longitude data with the *raster* and *rworldxtra* packages in R (Kottek *et al.* 2006; South 2012; Hijmans 2017; R Core Team 2017; Rubel *et al.* 2017). Names of climate regions were assigned in accordance with Kottek *et al.* (2006). Soil N levels were converted

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to a categorical low/high variable given the diversity of N forms and units reported in the selected studies. >25 ppm inorganic N was chosen as the threshold for high soil N, as 23–26 ppm nitrate has been shown to be a critical concentration for corn yields (Binford, Blackmer and Cerrato 1992) and additional pre-plant N is usually not recommended for maize above this level (Scharf 2001; Shapiro *et al.* 2008; Warncke 2010). The high N threshold was set at >0.1% based on a conversion of 25 ppm to % and using the median value (2.5%) of the 0%–5% range for inorganic N as a percentage of total N cited by Allison (1957). Plant-growth-promoting mechanisms were only used as a moderator if they were explicitly verified in the publication of interest. At each taxonomic level, only single-strain studies or studies in which all strains belonged to the same taxon were included.

Statistical methods

Analyses were carried out using the *metafor* and *boot* packages in R (Davison and Hinkley 1997; Viechtbauer 2010; Canty and Ripley 2017; R Core Team 2017).

Yield was used as the response variable where data were available (n = 49 studies), with biomass serving as a proxy for yield when yield was not reported (n = 21 studies). Only yield was used for treatments where both yield and biomass data were available and only the sampling date closest to maturity was used when multiple sampling dates were available to avoid bias associated with non-independence of correlated outcomes (Gurevitch and Hedges 2001; Harrison 2011). For each treatment-control pair, an effect size was calculated using the log response ratio: $\ln(X_t/X_c)$, where X_t represents the outcome (yield or biomass) of the treatment and X_c represents the outcome of the uninoculated control (Hedges, Gurevitch and Curtis 1999). To account for non-independence due to shared controls, effect sizes were weighted as given in the following equation: $w = n_{replicates}/2{*}n_{pairs}$ where w is the weight, $n_{replicates}$ is the number of replicates in the study, and n_{pairs} is the number of treatment-control pairs (Carrijo, Lundy and Linquist 2017).

Data were divided into two categories for all further analyses: field studies (48 publications and 442 observations) and pot studies under controlled conditions using field soil (21 publications and 134 observations). Nine publications included both field and pot studies and were represented in both categories. Meta-analyses were conducted within each category (field or pot) for each moderator variable (climate, soil inorganic N, fertilizer, biodiversity, taxonomy, and plant-growth-promoting mechanisms) by (i) subsetting the data by each level of the moderator, (ii) estimating a mean effect size and 95% confidence interval, and (iii) assessing the overlap of confidence intervals. Average effect sizes were calculated for each subset by taking the mean of the weighted effect sizes, and 95% confidence intervals were generated by bootstrapping with 4999 iterations.

Publication bias was evaluated in each subset via visual assessment for asymmetry of funnel plots and regression test (*regtest()* function of the *metafor* package) (Egger *et al.* 1997). Data without significant bias were evident when funnel plots of effect estimates against sample size were symmetrical and no significant regression was found (test returned $P > 0.05$). The 'trim and fill method' (*trimfill()* function of the *metafor* package) was used to estimate whether publication bias due to missing studies was present and its influence on the effect size (Duval and Tweedie 2000). If publication bias was evident, a per-study effect size was calculated by taking the mean of the log response ratios from each study. Studies with a mean effect size exceeding two standard deviations from the mean were removed from the dataset ($n \leq 2$) and the analysis was repeated (Carrijo, Lundy and Linquist 2017).

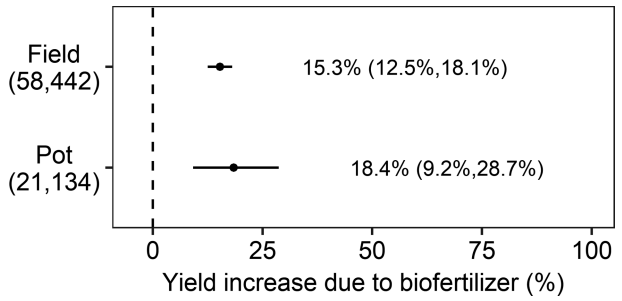

Figure 1. Biofertilizers increase maize yield by an average of 15.3% in the field and 18.4% in greenhouse studies using field soil. Data labels in parentheses represent the lower and upper bounds of the 95% confidence interval, and axis labels in parentheses indicate the number of studies and number of observations included in that category after correcting for publication bias. Effect sizes and confidence intervals were generated by bootstrapping with 4999 iterations.

For each analysis, the effect of biofertilizer use on yield/biomass was considered significant when the 95% confidence interval of the log response ratio did not overlap zero. Subcategories of each moderator variable were considered significantly different if the 95% confidence intervals did not overlap one another. Effect sizes and 95% confidence intervals were back-transformed and presented as % yield increase in the biofertilizer treatment relative to the control.

RESULTS

Effect of biofertilizers

Biofertilizers increased plant productivity in both pot and field studies by an average of 18.4% and 15.3%, respectively (Fig. 1). Inoculation had more variable effects in pot studies, with yield increases ranging from 9.2% to 28.7% as compared to 12.5% to 18.1% in field studies. Although productivity gains were 3% greater under controlled conditions than in the field, this difference was not statistically significant.

Climate

Climate region according to the Köppen classification system influenced biofertilizer efficacy in the field but not in greenhouse studies (Fig. 2). Biofertilizers were twice as effective in arid steppe climates (BS) as in equatorial climates (Aw) and three times as effective in arid steppe climates (BS) as in fully humid snow climates (Df). Mean effect sizes were consistent between field and pot studies in arid steppe and desert climates (BS and BW), but lower in the field in equatorial climates with dry winters (Aw). Effect sizes were more variable in pot studies for all climate regions.

Soil fertility

Starting soil N content influenced the outcome of inoculation under controlled conditions but not in the field (Fig. 3A). Inoculation in pot studies was more effective and widely variable when starting soil N was low (below 25 ppm inorganic N or 0.1% total N), increasing yields by 153% as compared to 17.1%, although only three studies were represented in the low-N dataset. Interestingly, fertilizer application within the growing season did not

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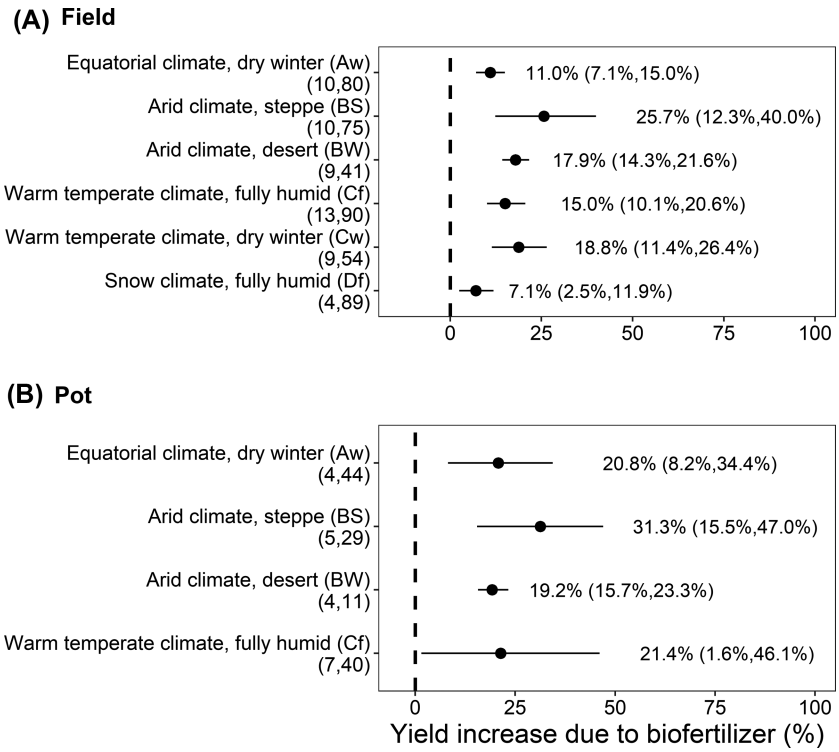

Figure 2. Climate affects biofertilizer performance only under field conditions, where biofertilizers are more effective in arid climates than snow climates. Climate classifications and names are according to the Köppen system, with temperature (T) and precipitation (P) criteria defined for each region as follows: Aw: $T_{min} \geq 18^{\circ}C$ and $P_{min} < 60$ mm in winter; BS: 5 $P_{th} < P_{ann} < 10\ P_{th}$; BW: $P_{ann} \leq 5\ P_{th}$; Cf: $-3^{\circ}C \leq T_{min} \leq +18^{\circ}C$ and not satisfying the criteria of Cs or Cw; Cw: $-3^{\circ}C \leq T_{min} \leq +18^{\circ}C$ and $P_{wmin} <$ Psmin and $P_{smax} > 10P_{wmin}$; Df: $T_{min} \leq -3^{\circ}C$ and not satisfying the criteria of Ds or Dw. P_{th} is a precipitation threshold defined based on mean annual temperature, P_{wmin} is minimum winter precipitation, P_{smin} is minimum summer precipitation and P_{smax} is maximum summer precipitation (Kottek *et al.* 2006). Data labels in parentheses represent the lower and upper bounds of the 95% confidence interval, and axis labels in parentheses indicate the number of studies and number of observations included in that category after correcting for publication bias. Effect sizes and confidence intervals were generated by bootstrapping with 4999 iterations.

alter the outcome of inoculation in a consistent manner in field and pot studies (Fig. 3B).

Taxonomy and ecological coherence

Meta-analyses within subcategories of phylum and genus suggest that ecological coherence may exist at both levels. Proteobacteria were more effective than Firmicutes in the field, while inoculation with strains belonging to the Bacteroidetes did not consistently improve maize yields (Fig. 4A). Under controlled conditions, yield increases due to Firmicutes and Proteobacteria were not significantly different.

Although relatively few genera included in the meta-analysis were represented by a minimum of three studies, the greatest taxonomic differences were observed at this level (Fig. 4B). Strains belonging to the genus *Azotobacter* increased yields by an average of 34.4% in the field, the largest increase of any genus. Biofertilizers composed of *Azospirillum* spp. and *Pseudomonas* spp. were also consistently effective, but *Burkholderia* spp. increased yields by only 5.0% on average. Inoculation with *Bacillus* spp., *Enterobacter* spp. or *Pantoea* spp. did not always increase yields, as shown by 95% confidence intervals that overlapped 0.

Pseudomonas spp. consistently increased yields in pot studies as well, with a 95% confidence interval of 11.7%–38.6%. The remaining three genera represented in both types of studies behaved differently under controlled conditions than in the field. Despite performing consistently well in the field, *Azospirillum* spp. were not significantly better than an uninoculated control in pot studies. In contrast, *Bacillus* spp. and *Enterobacter* spp. were not significantly more beneficial than uninoculated controls under field conditions, but increased yields by 23.0% and 18.6%,respectively, under controlled conditions.

Ecological complexity

Biodiverse consortia of two or more strains were equally as effective as single-strain formulations in both pot and field studies (Fig. 5). Single inoculant strains (95% CI 11.8%–17.9%) had more consistent effects in the field than multiple-strain biofertilizers (95% CI 10.4%–24.0%), but both types of biofertilizers were equally variable in pot studies (single-strain 95% CI 12.1%–34.8%, multiple-strain 95% CI 5.0%–25.4%).

Plant-growth-promoting mechanisms

Insufficient greenhouse studies were available to directly compare outcomes of field and pot studies. In the field, only ACC deaminase production affected the outcome of biofertilizer application out of the PGPR mechanisms investigated here (Fig. 6). Strains that produced ACC deaminase were more effective than those that were not proven to produce this compound, increasing yields by 18.5% as compared to 11.1%. Inoculation with strains possessing all other PGPR mechanisms, including P solubilization, N_2 fixation, phytohormone synthesis or

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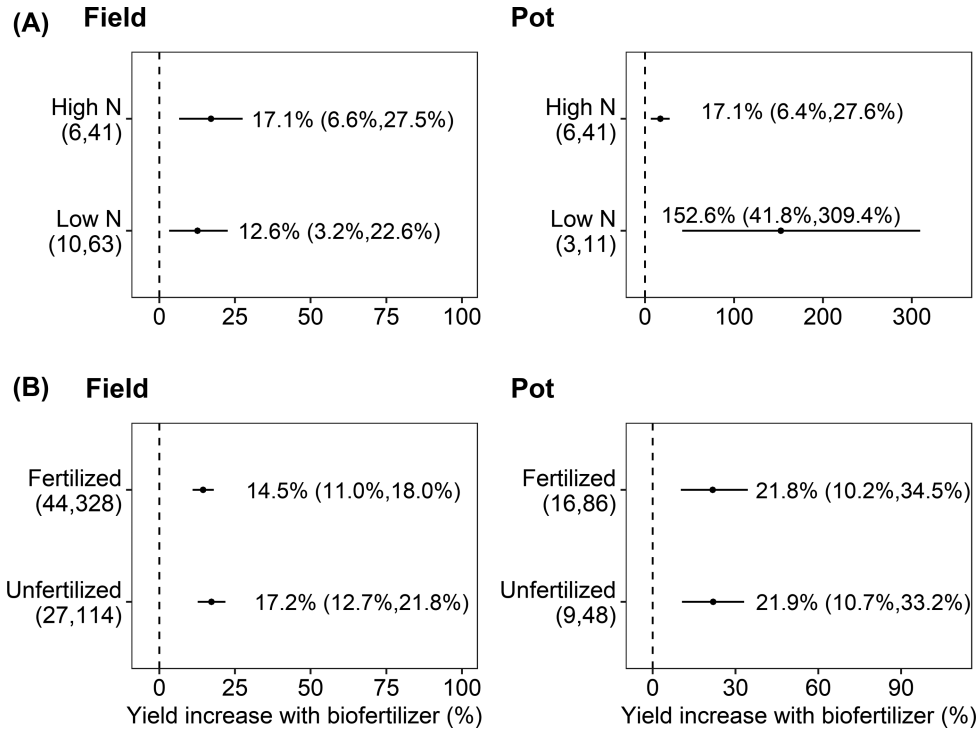

Figure 3. Pre-plant soil fertility affects biofertilizer performance only under controlled conditions. (**A**) Yield gains due to biofertilizer inoculation are largest when pre-plant soil nitrogen is below 25 ppm inorganic N or 0.1% total N in pot studies, but unaffected by pre-plant N in the field. (**B**) Fertilization during the growing season does not significantly influence biofertilizer efficacy. Data labels in parentheses represent the lower and upper bounds of the 95% confidence interval, and axis labels in parentheses indicate the number of studies and number of observations included in that category after correcting for publication bias. Effect sizes and confidence intervals were generated by bootstrapping with 4999 iterations.

siderophore production, was not more beneficial under field conditions than inoculation with strains not proven to possess these traits.

DISCUSSION

Biofertilizer effects on yields and/or biomass tended to be slightly higher and more variable in greenhouse pot studies using field soil than in the field (Fig. 1). Soil characteristics of the controlled environment that favor microbial growth and survival, such as higher temperature, more homogeneous moisture, altered bulk density and decreased investment in root biomass in pots as compared to the field, may have contributed to the trend towards greater productivity increases in pot studies. Alternatively, the trend towards higher mean effect size under controlled conditions could be a consequence of the product development pipeline, as successful *in vitro* and pot trials typically precede improvements in field-applied biofertilizers.

Even when restricting the scope of the meta-analysis to greenhouse trials that used unprocessed field soil rather than sterilized soil or potting media, studies under controlled conditions were relatively poor predictors of the influence of climate and soil variables on biofertilizer performance. Climate region significantly affected field study outcomes but not the outcomes of pot studies using field soil, showing that temperature and precipitation influence the effectiveness of biofertilizers separately from any climate-driven soil properties (Fig. 2). Our findings that mean effect size was higher in arid climates and lower in fully humid snow climates are consistent with a recent meta-analysis showing that PGPR may be more beneficial under water stressed conditions (Rubin, Groenigen and Hungate 2017). Variability according to climate is also consistent with theoretical understanding of context-dependent resource mutualisms (Hoeksema and Bruna 2015). It has also been argued elsewhere that plant-growth-promoting microorganisms provide greater benefit under stressful conditions (Nadeem *et al.* 2014). Downregulation of plant stress responses through bacterial production of ACC deaminase, which modulates ethylene signaling pathways and appears to consistently increase yields (Fig. 6), could ameliorate yield penalties due to drought and salinity stress in arid climates (Zafar-ul-Hye *et al.* 2014). However, the specific mechanisms underlying growth promotion at a given field site likely vary according to biofertilizer strain and environmental variables.

Soil N levels influenced the effectiveness of biofertilizers under greenhouse conditions but not in the field. Albeit highly variable, biofertilizers were significantly more beneficial in pot studies when pre-plant soil N was low (below 25 ppm inorganic N or 0.1% total N), and additional fertilization did not alter their effectiveness (Fig. 3). No single standard exists for 'high' soil N, with some sources recommending no additional pre-plant fertilization above 19 ppm NO_3-N (Schmitt and Randall 1994) and other fertilization guidelines declining to establish such a threshold (Geisseler 2011). When the analysis was repeated using a cutoff of 50 ppm, no effect of pre-plant soil N was found (data not shown), suggesting that there may be a critical threshold of pre-plant soil N above that biofertilizers are less effective. Unfortunately, given the paucity of data and inconsistency in forms of N reported, a regression to determine this threshold

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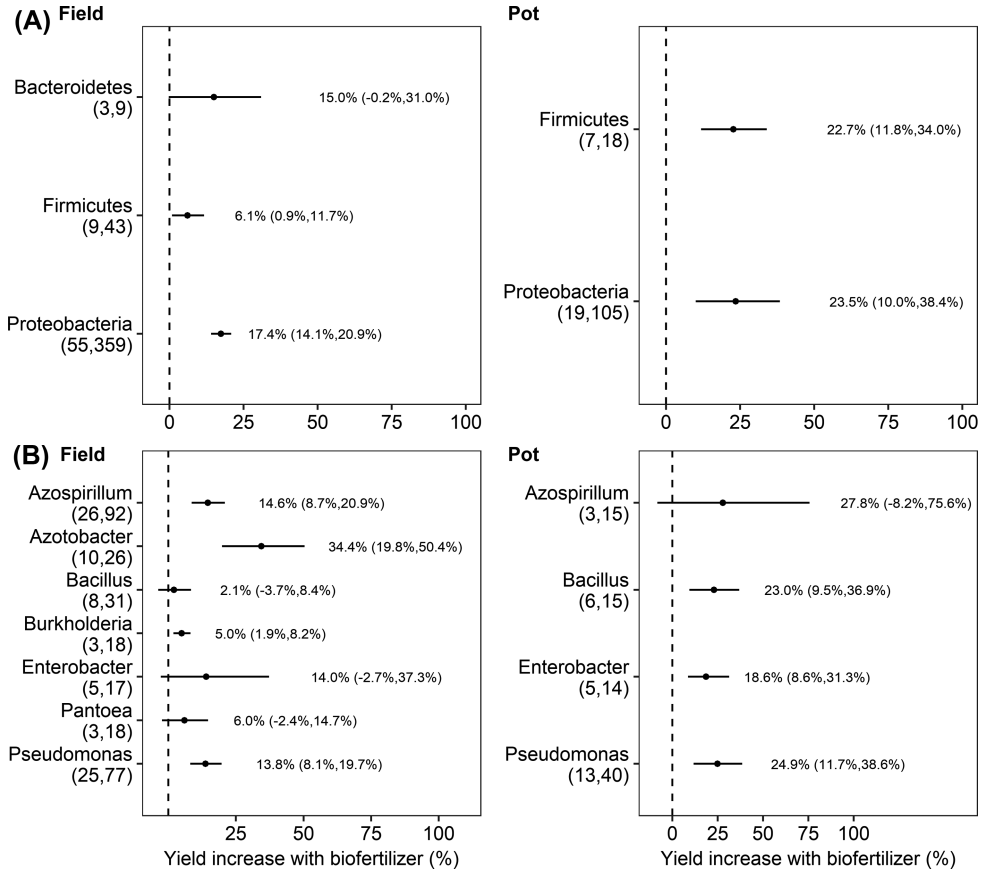

Figure 4. Taxonomy influences biofertilizer efficacy only in the field. (**A**) Strains belonging to the phylum Proteobacteria were more effective than those belonging to the Firmicutes in the field, and (**B**) inoculation with Azotobacter increased maize yields more than inoculation with Bacillus, Burkholderia, Pantoea or Pseudomonas. Data labels in parentheses represent the lower and upper bounds of the 95% confidence interval, and axis labels in parentheses indicate the number of studies and number of observations included in that category after correcting for publication bias. Effect sizes and confidence intervals were generated by bootstrapping with 4999 iterations.

could not be constructed from the present data set. The relationship between soil nitrogen, biofertilizers and yield is complex and likely not easily predicted by a single metric. Discrepancies between greenhouse and field results for soil fertility are unsurprising in light of poor greenhouse-to-field correlations reported for plant-soil feedbacks (Heinze *et al.* 2016) and nitrogen availability (Michrina, Fox and Piekielek 1981) but highlight the need for a novel approach to biofertilizer development and testing.

Understanding the extent of ecological coherence in biofertilizers could increase predictive power, facilitate targeted screening efforts, and help large -omics studies target bacterial taxa known to improve growth and productivity. At the phylum level, members of the Proteobacteria were more effective than Firmicutes in the field, but not under controlled conditions (Fig. 4). Proteobacteria are a large, metabolically diverse phylum of Gram-negative bacteria subdivided into five classes (Marin 2011), of which three were represented in this meta-analysis: Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria. Interestingly, the mean effect sizes of each class were identical, although the Gammaproteobacteria had lower variability (Fig. S1, Supporting Information). Observed patterns of ecological coherence at the phylum level do not guarantee that all members of a given taxon will promote plant growth. In fact, the extent of ecological coherence has been shown to vary among taxa (Koeppel and Wu 2012), which could explain the similar magnitude in variability within a single class of Proteobacteria and between the Proteobacteria and other phyla. It should also be noted that sampling bias could generate false positive findings of ecological coherence (Koeppel and Wu 2012), if researchers study only a few common or easy-to-culture members of any phylogenetic group. Genomic analysis could be used to confirm whether unstudied, related strains indeed share metabolic capabilities of interest.

Azotobacter, *Pseudomonas* and *Azospirillum* were the most consistently effective genera in the field (Fig. 4). Both *Azotobacter* and *Pseudomonas* are members of the Pseudomonodaceae, a family of Gammaproteobacteria, while *Azospirillum* belongs to the Alphaproteobacteria. Specific genes involved in plant growth promotion are conserved across members of these genera and other Proteobacteria, including genes controlling ACC deaminase production, N_2 fixation and P solubilization (Bruto *et al.* 2014). The poor performance in the field by *Bacillus* spp., spore-forming members of the Firmicutes, is particularly striking given the long history of plant growth promotion and biocontrol studies focusing on this genus (Pérez-García, Romero and de Vicente 2011; Shafi, Tian and Ji 2017). *Bacillus* spp., *Enterobacter* spp., and *Pseudomonas* spp. were all equally effective under controlled conditions.

Unfortunately, lack of data precluded meta-analysis at lower taxonomic levels, so it remains to be seen whether certain species are globally relevant for maize or whether biofertilizers must be tailored to a specific climatic and agronomic context. Philippot *et al.* (2009) observed that it is easier to identify an effect of ecological coherence when focusing on low taxonomic

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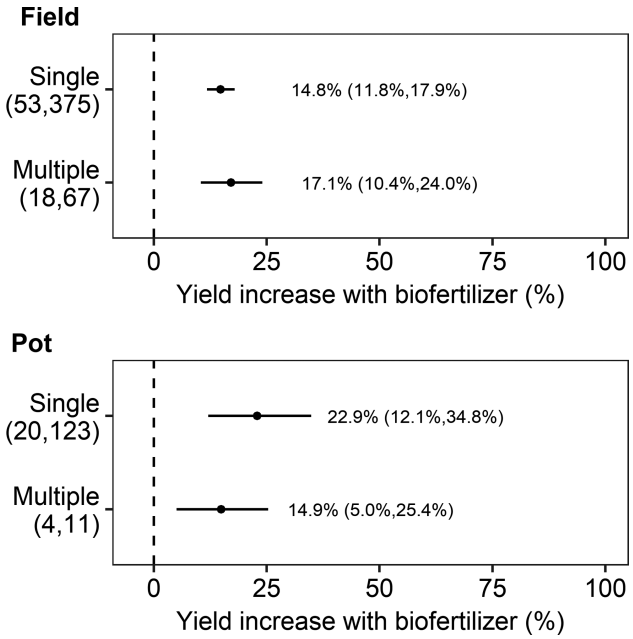

Figure 5. Multiple-strain biofertilizers tend to outperform single-strain formulations in the field, but not in the greenhouse. Data labels in parentheses represent the lower and upper bounds of the 95% confidence interval, and axis labels in parentheses indicate the number of studies and number of observations included in that category after correcting for publication bias. Effect sizes and confidence intervals were generated by bootstrapping with 4999 iterations.

ranks in the context of spatial distribution of microbes. However, the underlying rationale may pertain less to plant growth promotion than to habitat adaptation: shared physiological traits conferring tolerance to salinity, for example, may be more likely in closely related bacterial taxa (Lozupone and Knight 2007), whereas phylogenetically distant taxa may have evolved different biosynthetic pathways to produce the same plant-growth-promoting compound. Phylogenetic meta-analysis taking into account relatedness of the species being investigated could provide great insight into the question of ecological coherence were sufficient species-level data available (Lajeunesse 2009).

Multi-strain biofertilizer formulations represent an intermediate between single-strain inoculation and *in situ* enhancement of soil microbial communities, or rhizosphere engineering (Ryan *et al.* 2009; Dessaux, Grandclément and Faure 2016). We found that biodiverse consortia of two or more strains had more variable effects than single-strain formulations in the field, with yield increases of 10.4%–24.0% (Fig. 5). Single strains may be more predictable than multi-strain formulations, but the substantial yield increases at the upper end of the observed range suggest untapped potential for biodiverse consortia tailored to a specific environment. Successful multi-strain consortia would ideally include strains with complementary mechanisms of plant growth promotion and/or different preferences in edaphic and climatic conditions to provide multiple benefits under varied conditions. Emergent biosynthetic capacity is maximized at intermediate functional relatedness (Chiu, Levy and Borenstein 2014), suggesting that biofertilizers combining microbial strains from different ecological guilds could synthesize plant-growth-promoting compounds not seen in single-strain formulations. However, identification of strains that significantly increase plant growth individually and can also be combined into a shelf-stable product where mutualism and

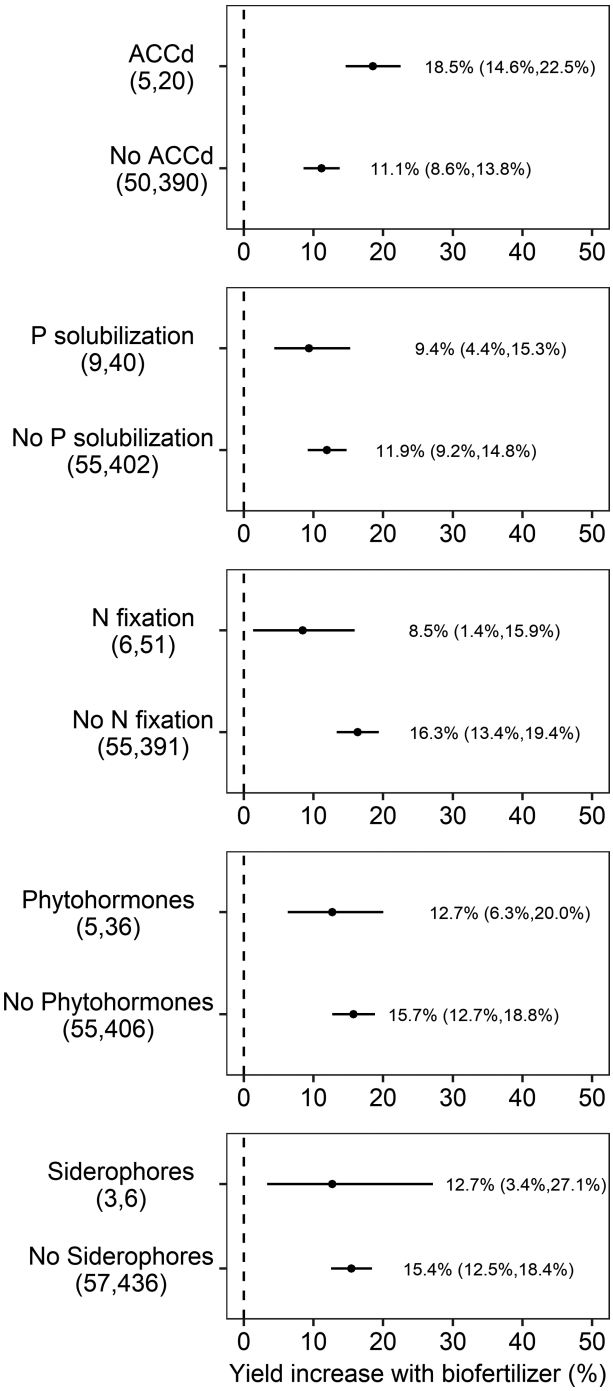

Figure 6. ACC deaminase production was the only plant-growth-promoting trait that increased biofertilizer performance in the field. *In vitro* proof of all other traits assessed did not make biofertilizer strains more effective than strains not possessing that trait. Data labels in parentheses represent the lower and upper bounds of the 95% confidence interval, and axis labels in parentheses indicate the number of studies and number of observations included in that category after correcting for publication bias. Effect sizes and confidence intervals were generated by bootstrapping with 4999 iterations.

commensalism are the dominant forms of microbe-microbe interaction poses substantial challenges.

Nonetheless, a shift towards increasing ecological complexity advances biofertilizers beyond a misplaced conception of microbial products as universally applicable agrochemicals to a

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more nuanced understanding of biofertilizer application as tools to manage soil microbial ecology. Soil microorganisms have evolved over millions of years to become individually adapted to a vast diversity of ecological niches, and the quest for a single microbial species that will improve plant growth under all conditions is unlikely to succeed. There is increasing understanding that complex microbiomes are more beneficial to plants, particularly under abiotic stresses such as salinity, than individual microbial partners (Qin *et al.* 2016). Microbial communities are extremely dynamic and adapt rapidly to alterations in environmental conditions such as moisture to confer adaptive traits to plant hosts (Lau and Lennon 2012). Isolation of indigenous microbial communities adapted to temperature or salinity extremes or nutrient-poor soils have been shown to improve plant growth in stressful environments (Kaplan *et al.* 2013), a phenomenon termed habitat-adapted symbiosis (Rodriguez *et al.* 2008, 2009). Microbial community-based approaches to inoculum development may thus represent the next advance to effective biofertilizers.

In vitro evidence of plant-growth-promoting traits does not appear to predict biofertilizer performance in the field (Fig. 6). Only ACC deaminase synthesis, a trait that has been previously identified as important in PGPR and described in detail elsewhere (Glick 2014), appears to increase biofertilizer efficacy in the field. Few studies explicitly measured mechanisms such as P solubilization, phytohormone production, or N fixation prior to application, but in every case, strains previously identified as possessing these traits tended to be *less* effective in the field. Furthermore, while *Bacillus* strains possessing multiple plant-growth-promoting traits have been shown to increase root length and weight, shoot biomass and grain yield of wheat more than single-trait strains (Baig *et al.* 2012), meta-analysis showed that multiple traits provided no advantage over single traits in field or pot studies (Fig. S2, Supporting Information). While traits such as P solubilization and siderophore production are advantageous in theory (Hayat *et al.* 2010) and under axenic conditions (Hussain *et al.* 2013), to the best of our knowledge, empirical evidence for their success in maize field studies is lacking. Energetically expensive symbiont traits such as N_2 fixation could represent a net cost to the host plant in a fertile field, but such a cost-benefit analysis likely does not apply to free-living strains and traits that require relatively little metabolic investment. This result may instead indicate the need to move from *in vitro* to *in situ* testing of plant-growth-promoting mechanisms, as strains positive for a given trait according to laboratory tests may fail to express the same trait in the field.

CONCLUSION

Biofertilizers present a realistic option for sustainable intensification of maize production with great potential to increase yields ~15%–18%. Nonetheless, the results of this meta-analysis indicate a critical need for pipelines to effectively tap into microbial communities and better understand factors that affect biofertilizer performance. Potential biofertilizer strains are frequently tested in laboratory and pot studies to predict efficacy in the field, but discrepancies with field outcomes for soil, taxonomic, and mechanistic moderating variables suggest the need to reconsider this approach. As proposed by Trivedi *et al.* (2017), biofertilizers should also be integrated with biocontrol goals, soil amendments and crop traits for different soil types. The complexity of tailoring this approach to diverse agroecosystems will require a much greater emphasis on location-specific abiotic and biotic factors than has previously been the case in biofertilizer development. Reversing the traditional lab-to-field pipeline may therefore hold better promise for developing effective biofertilizers in an agricultural context. Sequencing and network analysis of existing soil microbial communities that increase resilience to abiotic and biotic stressors could identify keystone taxa and interactions that are conserved across environments. While only the small percentage of microorganisms that can be cultured could be formulated into biofertilizers, such an analysis could identify many more candidate strains than are currently known. In the future, improved understanding of external variables that affect plant-microbe interactions and an emphasis on microbial ecology over reductionist methods may facilitate the development of widely relevant, ecologically complex biofertilizers.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

ACKNOWLEDGMENTS

The authors would like to acknowledge Caitlin Peterson, Leah Renwick, Vanessa Brisson, Emad Jahanzad, Meng Li and anonymous reviewers for their valuable feedback on this manuscript.

FUNDING

This work was partially funded by the Foundation for Food and Agriculture Research, the US Department of Agriculture (USDA) National Institute of Food and Agriculture, Agricultural Experiment Station Project CA-D-PLS-2332-H, to A.G. and by the UC Davis Department of Plant Sciences through a fellowship to J.S.

Conflicts of interest. None declared.

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