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RESEARCH ARTICLE

Exploring the potential of seed inoculation with microbial consortia to mitigate drought stress in maize plants under greenhouse conditions

Bunlong Yim · Marie Amelie Heider · Elke Bloem · Doris Vetterlein · Jan Helge Behr · Doreen Babin · Kornelia Smalla

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Abstract

Background and aims Plant-beneficial microbes may attenuate climate change-induced stresses on plants such as drought. We investigated the potential of beneficial microbial consortia (BMc) on plant growth and rhizosphere bacterial/archaeal community under drought.

Methods Seeds of *Zea mays* B73 were inoculated with six plant-beneficial bacterial isolates either alone or combined in two three-member consortia (BMc1, BMc2) before sowing in loamy or sandy substrates in the greenhouse. A known effective consortium (BMc3) was included as positive control. Drought treatment was established with the BMc treatments by omitting watering in the last of the five weeks growth period. The maize growth in single and BMc treatments was determined. Colony-forming units (CFUs) of inoculants were evaluated by selective plating, and effects of BMc treatments on the native rhizosphere bacterial/archaeal community were assessed using 16S rRNA gene amplicon sequencing of basal root and root tip rhizosphere of plants grown in loam.

Results In both substrates and water conditions, CFUs of single and BMc inoculations were higher at rhizosphere basal roots than root tips. Under well-watered conditions, seed inoculation with a single bacterial isolate had no effect on maize growth in both substrates. BMc treatment resulted in higher shoot (but not root) growth compared to non-inoculated controls in both water conditions in loam. The root zone was the most important driver for bacterial/archaeal beta-diversity, followed by water conditions, while BMc treatments showed no effect.

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B. Yim (✉) · M. A. Heider · D. Babin · K. Smalla
Institute for Epidemiology and Pathogen Diagnostics, Julius Kühn Institute (JKI) - Federal Research Centre for Cultivated Plants, 38104 Braunschweig, Germany
e-mail: bunlong.yim@julius-kuehn.de

E. Bloem
Institute for Crop and Soil Science, Julius Kühn Institute (JKI) - Federal Research Centre for Cultivated Plants, 38116 Braunschweig, Germany

D. Vetterlein
Helmholtz-Centre for Environmental Research - UFZ, 06120 Halle/Saale, Germany

D. Vetterlein
Martin-Luther-University Halle-Wittenberg, 06120 Halle, Germany

J. H. Behr
Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Plant-Microbe Systems, 14979 Großbeeren, Germany

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Conclusion Our study suggests that BMc seed inoculation has the potential to attenuate drought stress during maize growth.

Keywords Amplicon sequencing · Bacterial inoculant · Beneficial microbial consortium · Drought · Seed inoculation · *Zea mays* B73

Introduction

Drought is one of the major threats exacerbated by climate change, which is negatively affecting cereal crop production worldwide as revealed by a significant reduction in shoot and root growth and yield of plants (Ray et al. 2018; Orimoloye 2022; Renard et al. 2023). The negative impact of drought on plant growth and yield is a product of drought-induced changes in plant metabolic processes in leaves as plants attempt to reduce transpiration without sacrificing assimilation (Yasmin et al. 2020; Nephali et al. 2021). As a consequence, alterations in the quantity and quality of root exudates (Tiziani et al. 2022), modifications in root architecture (Yan et al. 2022) and male sterility due to lack of carbohydrates in crops (Armanhi et al. 2021) can be observed. Drought is affecting the plant-associated microbiome that coevolved and interacts with plants as a holobiont (Hassani et al. 2018; Chun et al. 2022; Li et al. 2023). Particularly, it can affect the diversity, abundance and function of rhizosphere microbes as they experience significant changes in carbon availability, pH and O_2 availability due to drought (Jaeger et al. 2023; Akinola et al. 2023). Gram-positive bacteria (e.g. Actinobacteriota and Firmicutes) are more tolerant to drought than Gram-negative (e.g. Proteobacteria) because of their thick peptidoglycan cell walls and sporulation ability (Xu et al. 2018). It has been shown that drought-induced plant growth reduction can be mitigated by the single or combined (consortium) inoculation of drought-tolerant microbes with potential plant-beneficial functions (Armanhi et al. 2021; Azizi et al. 2022; Ojuederie and Babalola 2023). Plant inoculation with a beneficial microbial consortium (BMc) has been shown to be more effective under challenging environmental conditions like drought than single beneficial microbes due to their diverse functions and different ecological niches occupied by BMc members (Bradáčová et al. 2019; Compant et al. 2019). Compensation of crop growth reduction under drought stress was accompanied by an increased polyphenol content in leaves because polyphenols are known to play a significant role in stress reduction due to deactivation of reactive oxygen species (Singh et al. 2020).

Although, a potential reduction of maize growth by drought and its compensation by the inoculation with BMc was investigated (Armanhi et al. 2021; Nephali et al. 2021; Lephatsi et al. 2022), the beneficial traits of individual members and functions, and their compatibility in the consortium were not evaluated. Siderophores produced by microbes provide soluble iron (Fe^{2+}) for plant uptake, which is especially relevant under drought conditions. Iron is required for plant metabolic processes like synthesis of chlorophyll for photosynthesis and phytohormones (ethylene or abscisic acid) for environmental adaptation (Rout and Sahoo 2015; Khasheii et al. 2021). Furthermore, some microbes are able to convert the amino acid tryptophan that is exuded by plant roots into indole-3-acetic acid, IAA (Passari et al. 2016; Spaepen and Vanderleyden 2011), which stimulates root length, seminal roots and root hair growth of plants (Edelmann 2022; Ma et al. 2022). The 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity of microbes (Gamalero et al. 2023) is known to reduce the level of ethylene produced by plants upon stress such as drought (Fatma et al. 2022). Therefore, assembling a BMc including members with those diverse traits may be beneficial for plants to cope with drought.

Several approaches were reported for microbial inoculation, including soil mixing or drenching (Abideen et al. 2022; Behr et al. 2023), root dipping (Singh et al. 2018; Hauschild et al. 2024), foliar spray (Efthimiadou et al. 2020; Fu et al. 2020) and seed coating followed by drenching (Schreiter et al. 2018; Eltlbany et al. 2019; Elsayed et al. 2020) with single bacterial isolates or BMc. Only a few studies applied seed inoculation alone (Raja et al. 2019; Chai et al. 2022; Arnault et al. 2024). Seed inoculation significantly reduces not only the amount of inoculum required, but also time and costs, and enhances inoculation success due to priority effects (Arnault et al. 2024), especially in cereal crops like maize. As maize is cultivated from seeds sown directly into the soil, root dipping is not a recommended application. Furthermore, microbial assembly begins with seeds

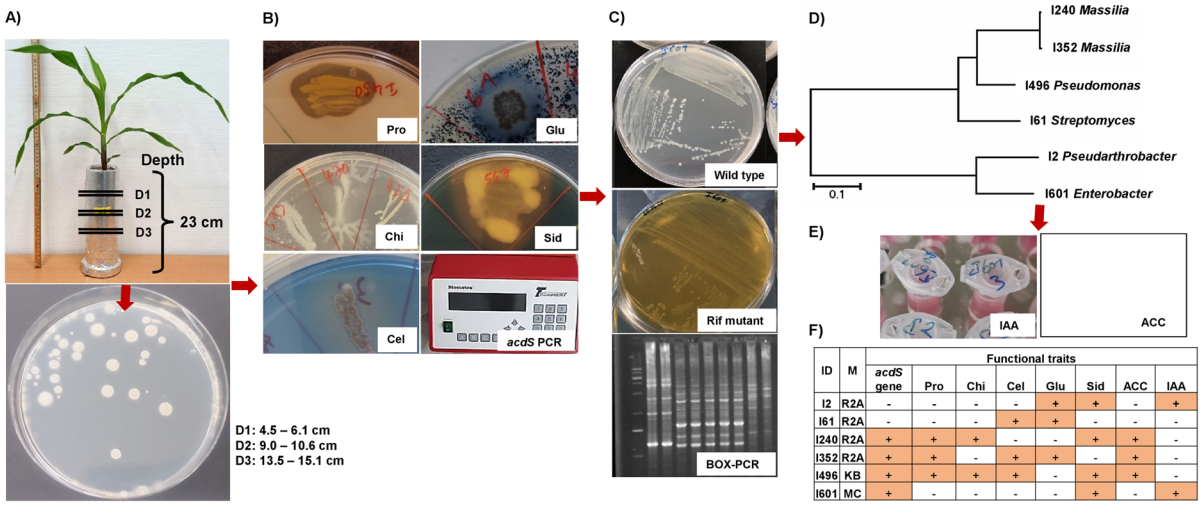
(Berg and Raaijmakers 2018), and is distinct along the rhizosphere of growing roots (root zones or rooting depths) (Rüger et al. 2021; Yim et al. 2022) due to different rhizodeposits (Vetterlein et al. 2020) and depth-dependent microbial diversity and composition (Bonkowski et al. 2021). Because previous studies focused on the entire root system, the spatial resolution of whether the inoculants are capable of moving along the growing roots to the root tips and contribute to microbial assembly was not investigated. Thus it is noteworthy to broaden the investigation on the efficiency and efficacy of seed inoculation.

Our study aims at investigating whether six beneficial bacterial isolates, obtained from maize (rhizosphere or rhizoplane), and selected due to their potential drought-mitigating traits (siderophores, IAA or ACC deaminase), on the one hand are rhizocompetent and show plant growth promoting traits, when applied as single inoculant on maize seeds under well-watered conditions. On the other hand, we aim to study whether the isolates, when applied as BMc on maize seeds, can mitigate drought stress and modulate the native rhizosphere microbiome since Berg et al. (2021) reported that inoculation causes transient shifts in the native soil microbiome. A greenhouse experiment was conducted to test three hypotheses: 1) Selected bacterial isolates applied as seed inoculation either as single bacterial isolates or BMc are rhizocompetent in different root zones, 2) BMc inoculation mitigates drought stress in maize independent of substrate, and 3) the BMc alters the bacterial/archaeal diversity and composition of the maize rhizosphere.

Materials and methods

Selection of bacterial isolates for inoculation experiments

In total 510 bacterial isolates were obtained from rhizosphere and rhizoplane of maize grown in soil columns (Fig. 1A; Yim et al. 2022; Supplementary information 1 and 2). The isolates were taxonomically identified based on Sanger sequencing (Table S1; Supplementary information 1), and tested for the production of hydrolytic enzymes such as protease, cellulase, chitinase and glucanase that are important for soil fertility (Xin et al. 2016)



ID	M	Functional traits							
		acdS gene	Pro	Chi	Cel	Glu	Sid	ACC	IAA
I2	R2A	-	-	-	-	+	+	-	+
I61	R2A	-	-	-	+	+	-	-	-
I240	R2A	+	+	+	-	-	+	+	-
I352	R2A	+	+	-	+	+	-	+	-
I496	KB	+	+	+	+	-	+	+	-
I601	MC	+	-	-	-	-	+	-	+

Fig. 1 Workflow of six selected beneficial bacterial isolates for inoculation experiments. **A**, Isolation of bacterial isolates from rhizosphere/rhizoplane of maize at three different rooting depths grown in a central soil column experiment (see Yim et al. 2022); **B** and **E**, in vitro assays for characterization of phenotypic traits of isolates; **C**, mutant generation of bacterial isolates resistant to the antibiotic rifampicin (Rif): morphological comparisons and BOX-PCR fingerprints to verify whether the wild type and corresponding mutant strains are identical; **D**, Phylogeny of six chosen bacterial isolates based on maximum likelihood and **F**, their functional traits of chosen isolates for inoculation experiments. M, isolated medium: MC, MacConkey agar; KB, King's B agar and R2A, Reasoner's 2A agar. Pro, protease; Chi, chitinase; Cel, cellulase; Glu, glucanase; Sid, siderophores; IAA, indole-3-acetic acid; ACC, 1-aminocyclopropane-1-carboxylate deaminase; *acdS* gene, gene coding for ACC-deaminase; +, positive and -, negative

and fungal phytopathogen suppression (Budi et al. 2000; Smitha et al. 2014). Furthermore, all isolates were tested for traits relevant to plant drought stress tolerance, such as ACC deaminase activity (PCR-based detection of the *acdS* gene) and siderophores (Fig. 1B; Supplementary information 3). Subsequently, a subset of eleven isolates were chosen because they carry *acdS* gene (I2, I222, I240, I352, I496, I542, I598 and I601), produce siderophores (I33 and I343) or cellulase (I61), and belong based on their taxonomy (Table S1) to the previously reported most dominant taxa in the maize rhizosphere (Yim et al. 2022; He et al. 2024). From the selected isolates, spontaneous mutants resistant to the antibiotic rifampicin (Rif) at 75 µg mL^{-1} were generated by plating overnight bacterial cultures onto Reasoner's 2A (R2A agar; Merck KGaA, Darmstadt, Germany) supplemented with Rif (Fig. 1C; Supplementary information 4). Rif mutant isolates allowed us to track their establishment along plant roots using selective plating because only Rif-resistant bacteria are able to grow on Rif-supplemented media. Finally, only six isolates were successfully resistant to Rif, and were used in greenhouse inoculation experiments (Fig. 1D), and tested for IAA and ACC deaminase production in vitro (Fig. 1E).

Single bacterial inoculation experiments

The six chosen bacterial isolates I2, I61, I240, I352, I496, and I601 with Rif resistance were evaluated for their competence to colonize different root zones and for plant growth promotion in pot experiments under well-watered greenhouse conditions (Fig. 2). To prepare for inoculation, a single colony of the corresponding isolate was picked from a 24 h old R2A agar plate supplemented with Rif 75 mg L^{-1}, and transferred to an overnight culture in Luria–Bertani (LB) broth (Carl Roth GmbH, Karlsruhe, Germany) for I2, I61, I496 and I601, or R2A broth (HiMedia Laboratories Pvt. Ltd., Mumbai, India) for I240 and I352, all supplemented with Rif 75 mg L^{-1}. The cell pellets of the overnight bacterial cell suspension (OD_{600} = 0.8–1.2) were washed three times with sterile 0.3% NaCl via resuspension and centrifugation at

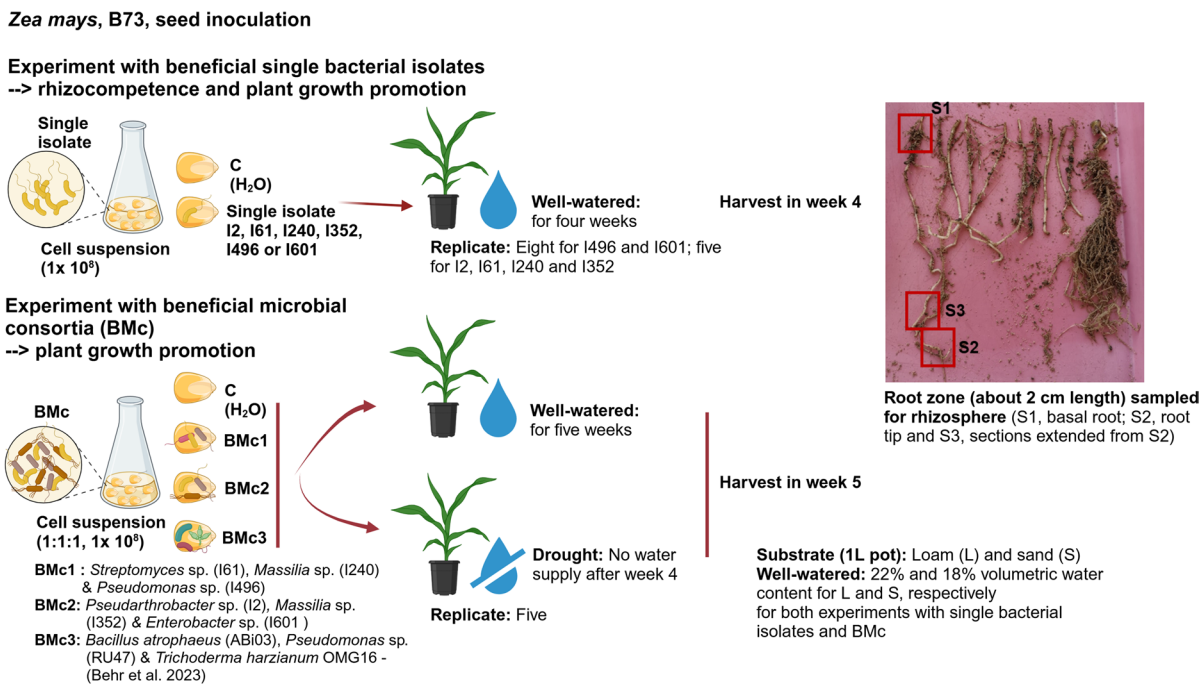


Fig. 2 Experimental set-up for experiments with beneficial single bacterial isolates and microbial consortia inoculated onto *Zea mays* B73 seeds. C, non-inoculated control and BMc, beneficial microbial consortium. Substrates (≤2 mm mesh size through sieving) were filled into pot with the bulk density of 1.26 cm^3 g^{-1} and 1.47 cm^3 g^{-1} soil for loam and sand, respectively. The figure was generated using Biorender

5,000 × *g* for 10 min at room temperature. Then, the bacterial cell density was adjusted to 1×10^8 CFUs mL^{-1} ($OD_{600} = 1.0$) using sterile distilled H_2O.

Before inoculation, wild type *Zea mays* B73 seeds were surface-sterilized (using 10% H_2O_2 for 10 min, followed by three rinses and a final incubation for 5 min using sterile distilled H_2O, followed by incubation in $CaSO_4$ 2.65 g L^{-1} for three hours to support growth at seedling stage (Yim et al. 2022). Then, the maize seeds were incubated in the bacterial cell suspension for 30 min at 150 rpm (at room temperature), and sterile distilled H_2O was used for non-inoculated control. One maize seed (with or without inoculation) was sown per pot (1L) filled with fertilized substrate (mesh size ≤ 2 mm through sieving) loam (1.26 g per cm^3 soil) or sand (1.47 g per cm^3 soil) at approximately 2 cm depth. The substrate texture and fertilization was described previously (Ganther et al. 2020; Vetterlein et al. 2021). Five replicates were performed per bacterial isolate (five seeds), except for the experiments with I496 and I601, where eight replicates were prepared because of additional analyses of polyphenols in maize shoot (see Supplementary information 5), respiration and dehydrogenase activity in bulk soil (see Supplementary information 6).

The rhizocompetence experiments of the six different bacterial isolates in the greenhouse were set up independently (from July to August 2022 for I496 and I601 and from February to March 2023 for I2, I61, I240 and I352), each with its own non-inoculated control. Throughout the experimental duration of four weeks, the volumetric water content of loam and sand was maintained at 22% and 18%, respectively (Ganther et al. 2020; Yim et al. 2022), and the growth conditions were set at 22/18 °C, day (16 h)/night (8 h) with an additional light from Master Son-T PIA Plus 400W E E40 (Philips, Belgium) as needed (to maintain day/night cycle).

At harvest, the aboveground length, fresh and dry biomasses of maize shoots were measured. The rhizosphere was sampled from a composite of root segments (approximately 2 cm in length) taken at three zones/depths: basal roots or S1 (about 2 cm distance from seed), root tips (S2), and extended root tips (S3, 4 cm from root tips) for experiments with I496 and I601 (Fig. 2). For experiments with I2, I61, I240 and I352, only two root zones S1 and S2 were sampled. After shaking off soil loosely adhering to roots, the corresponding root zone (S1, S2 or S3) was weighted (≤ 1 g root fresh mass), and transferred into sterile 50 mL falcon tubes filled with 0.3% sterile NaCl. Vortexing was applied for 30 s to detach soil tightly adhering to the roots. This process was repeated twice, and the supernatants (rhizosphere cell suspension) obtained from vortexing were combined (for 1:10, w:v NaCl, Yim et al. 2022).

In order to evaluate the CFU counts of the inoculant at different root zones, an aliquot of 100 µL from the corresponding rhizosphere cell suspension was used for a serial dilution (10^{-2}, 10^{-3} and 10^{-4}) plating onto R2A agar plates supplemented with Rif 75 mg L^{-1} and cycloheximide (Cyclo) 100 mg L^{-1}, using ColiRollers™ plating beads (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). The remaining rhizosphere cell suspension was centrifuged at 5,000 × *g* for 15 min to obtain the rhizosphere pellet, which was then stored at −20 °C until total community (TC-) DNA extraction. After the rhizosphere was sampled, the remaining maize roots were washed with tap water, and dried in an air-dry oven at 40 °C for one week to determine the root dry mass.

Soil left over from root sorting (for rhizosphere sampling) was used as bulk soil for respiration and dehydrogenase activity analyses (in the I496 and I601 experiments, see Supplementary information 6).

Beneficial microbial consortia (BMc) inoculation experiments

Besides seed inoculation with single bacterial isolates, experiments with BMc were performed to test their plant growth-promoting ability as a consortium, not only under well-watered but also drought conditions (Fig. 2). Based on the mutual compatibility on R2A plates (following the protocol described in Hettiarachchi et al. (2017)), the six chosen bacterial isolates were combined into two small consortia, i.e. BMc1 (I61, I240 & I496) and BMc2 (I2, I352 & I601) for the experiments. Additionally, BMc3 which consisted of *Bacillus atrophaeus* ABi03 (DSM 32285, provided by ABiTEP, Berlin, Germany), *Pseudomonas* sp. RU47 (DSM 117411) and *Trichoderma harzianum* OMG16 (DSM 32722, provided by Anhalt University of Applied Sciences, Bernburg, Germany) was included because the consortium showed positive effects on growth of winter rye under field conditions (Behr et al. 2023).

The greenhouse experiment for all BMc was conducted similarly to the pot experiments with single bacterial isolates (substrate preparation, seed surface sterilization and $CaSO_4$ incubation), except for the seed inoculation using BMc1, BMc2 or BMc3 (five replicates), five week duration and additional drought stress. For the inoculation, the corresponding BMc cell suspension was prepared in an equi-molar ratio of each corresponding isolate of the consortium (1:1:1/v:v:v, 1×10^8 CFUs mL^{-1}; spore suspensions were used for ABi03 and OMG16), while the non-inoculated control (C) treatment received only H_2O.

Throughout the entire experiment, the 1L potted plants were maintained at a volumetric water content of 22% for loam (from April to May 2023) and 18% for sand (from June to July 2023) for well-watered conditions (and watering was applied every two days). For the drought treatment, watering stopped after week four. At harvest (week five), plant height, fresh and dry biomass of maize shoots were determined. Two root zones S1 and S2 were sampled for the rhizosphere (see above). The remaining roots were washed using tap water, and stored in a clean plastic bag at 4 °C overnight before being scanned (using Epson WinRHIZO™ LA2400 Scanner, Regent Instruments Inc., Canada) for total length, surface area and diameter. The roots after scanning were dried in an air-dry oven at 40 °C for seven days for dry mass determination. To evaluate the CFU counts of BMc1, BMc2 or BMc3 (as a consortium) at S1 and S2, serial dilutions of the rhizosphere cell suspension were plated onto R2A agar plates supplemented with Rif 75 mg L^{-1} and Cyclo 100 mg L^{-1}, except for the OMG16 of the BMc3 (BMc3-Tri), which was cultured on potato dextrose agar (PDA) supplemented with 100 mg L^{-1} penicillin, 50 mg L^{-1} streptomycin and 10 mg L^{-1} tetracycline (Carl Roth, Karlsruhe, Germany) to suppress growth of most other fungal species. Plating was also performed with non-inoculated control samples, serving as a negative control.

Amplicon sequencing of the 16S rRNA gene fragments

Because of the fact that significant effects of the BMc treatment on maize growth were only detected in loam (Fig. 3), in addition the rhizosphere bacterial/archaeal diversity and community composition was analysed in these samples based on metabarcoding

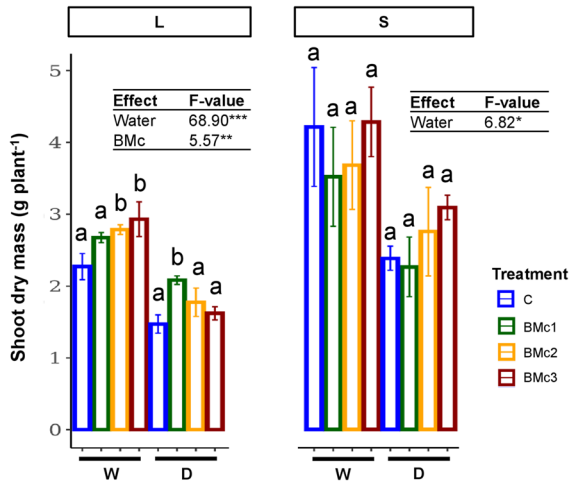


Fig. 3 Drought significantly reduced shoot dry mass, and treatment with beneficial bacterial consortium (BMc) in loam retained a relatively higher maize biomass compared to non-inoculated control plants. L, loam; S, sand; W, well-watered; D, drought; C, non-inoculated control and Water, W vs. D. Asterisks *, ** and *** indicate $p < 0.05, < 0.01$ and < 0.001, respectively (two-way ANOVA, Water x BMc), and letters indicate significant differences between C vs. BMc1, BMc2 or BMc3 under W or D and in L or S (Dunnett's test for $n = 5$)

of the 16S rRNA gene fragments amplified from TC-DNAs (well-watered and drought) to check whether maize seed inoculation affected the native rhizosphere bacterial/archaeal diversity and community composition.

The TC- DNAs of the S1 or S2 rhizosphere samples ($n = 5$) from loam were extracted using the FastDNA Spin Kit and the GeneClean Spin Kit for soil (MP Biomedicals, Heidelberg, Germany). Sequencing libraries of the 16S rRNA gene fragments, sequencing and analyses were performed as previously described (Ganther et al. 2020; Yim et al. 2022). Briefly, primers Uni341F, 5'-CCTAYG GGRBGCASCAG-3' and Uni806R, 5'-GGACTA CNNGGGTATCTAAT-3' (Sundberg et al. 2013) were used to target the V3-V4 of the 16S rRNA gene fragments. Novogene (Cambridge, UK) performed the amplicon libraries and sequencing using NovaSeq PE250, 30 K tag per sample. The amplicon sequence variants (ASVs, 100% identity) were generated following the DADA2 pipeline (Callahan et al. 2016), and the SILVA SSU rel. 138.1 (Quast et al. 2013) database was used to obtain the corresponding taxonomic information. The ASV abundance and

taxonomy table were imported using the phyloseq package (McMurdie and Holmes 2013), and ASVs associated with chloroplast, mitochondria and singletons were removed for further analyses.

Statistical analyses

Prior to performing ANOVA or Tukey tests, data for the analysis of CFUs, plant growth parameters, soil respiration and dehydrogenase activity were checked for variance homogeneity (Levene's test) and normal distribution (Shapiro–Wilk test). The two-way ANOVA (substrate x with/without seed inoculation) was applied in experiments with single bacterial isolates to evaluate the effects of inoculants on maize growth and performance (length, fresh and dry mass of shoot, root dry mass and polyphenols), soil respiration and dehydrogenase activity. When ANOVA revealed significant differences ($p < 0.05$), Tukey and Dunnett's test followed. Likewise, the two-way ANOVA (BMc x Root-zone) was also performed to test the effects of root-zone and BMc treatments on CFU abundances in well-watered or drought treatment in loam or sand. After two-way ANOVA, the Tukey test (with Bonferroni p-value adjustment at $p < 0.05$) was used. The drought effect on the CFU abundances of the respective BMc treatments within the root-zone and substrate was tested using Student's t-test ($p < 0.05$).

For amplicon sequencing of the 16S rRNA gene fragments amplified from the rhizosphere TC-DNAs in loam, the vegan package (Oksanen et al. 2020) was used to rarefy the number of sequences per sample to 23,581 (smallest library size) for downstream analysis. The bacterial/archaeal beta-diversity was visualized using a principal coordinate analysis based on the Bray–Curtis dissimilarity. The Permutational Analysis of Variance (PERMANOVA) test (with 10,000 permutations of the vegan package) was used to compare the differences in the beta-diversity between root zones (root tips vs. basal roots), water availability (well-watered vs. drought) and BMc (non-inoculated control vs. BMc) treatments. The Kruskal–Wallis, followed by Dunn's tests were carried out to determine the significant effects of the treatments on the alpha-diversity (Shannon index), relative abundance of bacterial and archaeal community composition at the phylum level, and of the bacteria at genus and ASV levels that were taxonomically affiliated to the BMc members. The Wald test from Deseq2 was used to check significantly different relative abundance of bacterial and archaeal genera or ASVs in different treatments. Significant effects for pairwise comparisons were based on log2 fold change with adjusted $p\text{-}value < 0.05$ based on Benjamini-Hochberg (e.g. effects of root zone under well-watered or drought conditions, of water at basal roots or root tips and effects of BMc1, BMc2 or BMc3 at basal roots or root tips under well-watered or drought conditions).

To determine whether any ASV sequences from the 16S rRNA gene amplicons were closely related to our inoculant sequences (from Sanger sequencing), phylogeny using MEGA7 was performed (sequence alignment with Muscle and phylogeny with maximum likelihood, Tamura and Nei 1993; Kumar et al. 2016).

All data analyses were carried out using the statistical software R 4.3.2 (R Core Team 2023).

Results

BMc treatments improved plant growth in contrast to single inoculation

Overall, inoculating maize seeds with a corresponding single bacterial isolate had no significant effect on plant growth (shoot length, shoot fresh and dry mass and root dry mass, Table 1) in loam or sand under well-watered conditions (non-inoculated vs. inoculated treatments). Inoculation using BMc resulted in significantly increased shoot fresh and dry mass in loam (F-value = 5.57, $p < 0.01$, Fig. 3). When compared to non-inoculated treatments, maize shoot dry mass significantly increased in BMc2 (22%) and BMc3 (29%) under well-watered and in BMc1 (41%) under drought conditions (Fig. 3). Because of the high variability within the treatment, no BMc effects on maize growth were observed in sand (Fig. 3). Inoculation had no effect on polyphenols in maize shoots under either single bacterial isolate or BMc treatments (Tables S2 and S4A).

At harvest, all drought-treated plants wilted (data not shown), indicating drought stress when compared to well-water treatment. Drought significantly decreased maize shoot dry mass in both loam (F-value = 68.90, $p < 0.001$) and sand (F-value = 6.82, $p < 0.05$) (Fig. 3). Similarly, other plant growth parameters such as shoot length, root surface area

Table 1 Inoculation with single bacterial isolates had no significant effect on the growth of maize grown under well-watered conditions

Data is presented as mean ± standard error of mean. Letters indicate significant differences between treatments (of SL, SFM, SDM or RDM of the corresponding inoculant experiment) using the two-way ANOVA (Substrate x with/without Inoculation), followed by Tukey test at $p < 0.05$, $n = 8$ for I496 & I601 and $n = 5$ for I2, I61, I240 and I352. SL, SFM, SDM and RDM denote aboveground shoot length, shoot fresh mass, shoot dry mass and root dry mass, respectively. *L* loam, *S* sand, *C* non-inoculated and *I* inoculated treatment. As the experiment was conducted independently, comparisons of plant growth data between inoculated experiments are not applicable

Isolate	Substrate	Treatment	SL (cm)	SFM (g plant^{-1})	SDM (g plant^{-1})	RDM (g plant^{-1})
I496	**L**	**C**	58.50 ± 0.91 a	8.02 ± 0.46 a	0.57 ± 0.06 a	0.34 ± 0.03 a
		I	58.69 ± 0.93 a	8.56 ± 0.62 a	0.63 ± 0.06 a	0.31 ± 0.02 a
	S	**C**	57.42 ± 1.27 a	7.61 ± 0.68 a	0.71 ± 0.07 a	0.23 ± 0.05 b
		I	59.79 ± 0.71 a	8.43 ± 0.56 a	0.72 ± 0.06 a	0.27 ± 0.03 b
I601	**L**	**C**	59.75 ± 0.83 a	6.91 ± 0.40 a	0.45 ± 0.05 a	0.19 ± 0.02 a
		I	58.21 ± 0.51 a	6.46 ± 0.46 a	0.40 ± 0.06 a	0.16 ± 0.01 a
	S	**C**	57.43 ± 1.04 a	6.23 ± 0.39 a	0.55 ± 0.05 b	0.20 ± 0.02 a
		I	57.29 ± 0.84 a	6.71 ± 0.44 a	0.60 ± 0.03 b	0.21 ± 0.02 a
I2	**L**	**C**	40.10 ± 1.14 ab	3.30 ± 0.17 a	0.27 ± 0.04 a	0.30 ± 0.04 a
		I	41.90 ± 0.93 a	3.65 ± 0.21 a	0.36 ± 0.05 a	0.27 ± 0.02 a
	S	**C**	36.50 ± 0.63 b	3.95 ± 0.56 a	0.26 ± 0.05 a	0.18 ± 0.01 b
		I	40.50 ± 1.50 ab	4.17 ± 0.21 a	0.34 ± 0.03 a	0.17 ± 0.02 b
I61	**L**	**C**	36.00 ± 1.27 a	2.84 ± 0.24 a	0.23 ± 0.02 a	0.17 ± 0.02 a
		I	36.00 ± 0.47 a	2.90 ± 0.21 a	0.20 ± 0.03 a	0.17 ± 0.02 a
	S	**C**	34.67 ± 1.30 a	3.07 ± 0.47 a	0.15 ± 0.07 a	0.27 ± 0.06 a
		I	34.00 ± 1.84 a	2.58 ± 0.24 a	0.15 ± 0.05 a	0.16 ± 0.03 a
I240	**L**	**C**	39.60 ± 0.29 a	3.49 ± 0.29 a	0.35 ± 0.05 ab	0.20 ± 0.01 a
		I	38.70 ± 1.33 ab	3.11 ± 0.39 a	0.38 ± 0.05 a	0.20 ± 0.01 a
	S	**C**	35.30 ± 0.90 b	2.77 ± 0.29 a	0.20 ± 0.04 ab	0.23 ± 0.02 a
		I	38.50 ± 0.00 ab	3.66 ± 0.13 a	0.40 ± 0.06 a	0.19 ± 0.01 a
I352	**L**	**C**	36.40 ± 0.60 a	3.11 ± 0.22 a	0.41 ± 0.06 a	0.18 ± 0.03 a
		I	34.70 ± 1.02 a	3.02 ± 0.23 a	0.39 ± 0.04 a	0.19 ± 0.02 a
	S	**C**	31.00 ± 1.50 a	2.32 ± 0.51 a	0.13 ± 0.05 b	0.15 ± 0.06 a
		I	33.75 ± 1.98 a	2.73 ± 0.24 a	0.16 ± 0.03 b	0.20 ± 0.04 a

and root diameter were also negatively affected by drought (Table S4A and B).

Single inoculation had no effect on soil dehydrogenase activity (in I496 and I601 experiments), but the activity was significantly higher in loam than sand (Table S2). Soil respiration did not differ between loam and sand, but I496 treatment significantly increased respiration compared to non-inoculated treatments (Table S2).

Inoculant CFU counts were higher in rhizosphere basal roots than root tips

Plate counts of serial dilutions of rhizosphere samples from both experiments with single bacterial isolates and BMc consistently revealed that inoculant CFU counts were 2 to 3 $\log_{10}$ CFUs higher at basal roots than at root tips shown in both substrates loam and sand (Table S3; Fig. 4).

The BMc1 (about 7 $\log_{10}$ CFUs) showed the highest CFU counts in the rhizosphere basal roots in both substrates compared to the other two consortia BMc2 and BMc3, that were one to two order of magnitudes lower, under both well-watered and drought conditions (Fig. 4). We found no significant differences in inoculant CFU counts between well-watered and drought conditions at rhizosphere basal roots or root tips.

Seed inoculation with BMc had a minor effect on the rhizosphere bacterial/archaeal community compared to the effects of root-zone and drought

The metabarcoding using high-throughput amplicon sequencing of 16S rRNA gene fragments was performed using TC-DNAs extracted from maize rhizosphere in loam only, as significant effects of BMc on maize growth were only detected in loam, not in sand (Fig. 3).

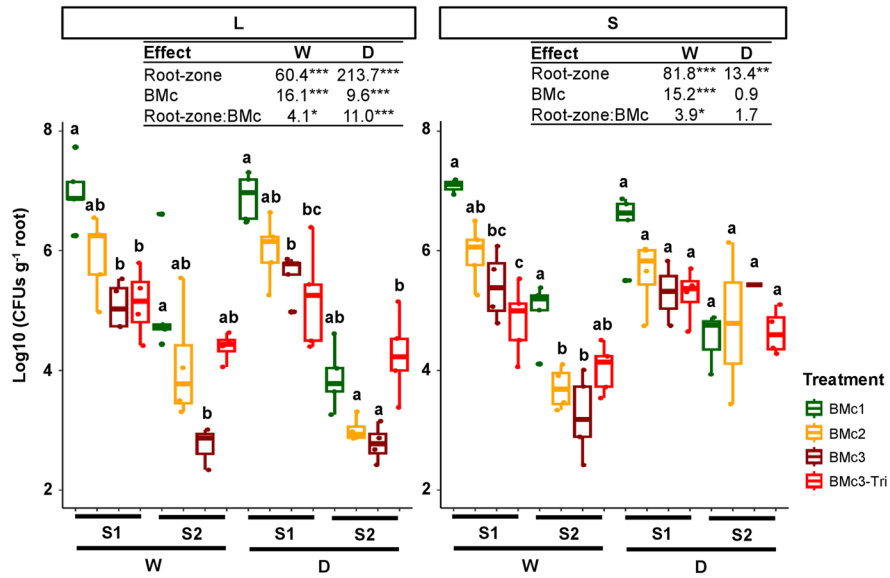


Fig. 4 All beneficial bacterial consortia (BMc) were most abundant (based on colony forming units, CFUs) at basal roots. The abundance of BMc1 and BMc2 consisted of three inoculant isolates, while BMc3 included two bacterial inoculants because the BMc3-Tri (*Trichoderma*) was grown on a different medium. Two-way ANOVA (BMc x Root-zone) was performed to test the effects of root-zone and BMc treatments on CFU abundances in well-watered (W) or drought (D) treatment in loam (L) or sand (S). After two-way ANOVA, the Tukey test (with Bonferroni p-value adjustment at $p < 0.05$) was used. Letters indicate significant differences between CFU abundances within the basal root (S1) or root tip (S2) rhizosphere under W or D treatment, and in L or S. The drought effect on the CFU abundances of the respective BMc treatments within the root-zone S1 or S2 and in L or S was tested using Student's t-test. Drought significantly decreased only BMc1 CFU abundance at rhizosphere S2 and S1 in L and S, respectively (t-test at $p < 0.05$). Asterisks *, ** and *** indicate $p < 0.05, < 0.01$ and < 0.001, respectively.

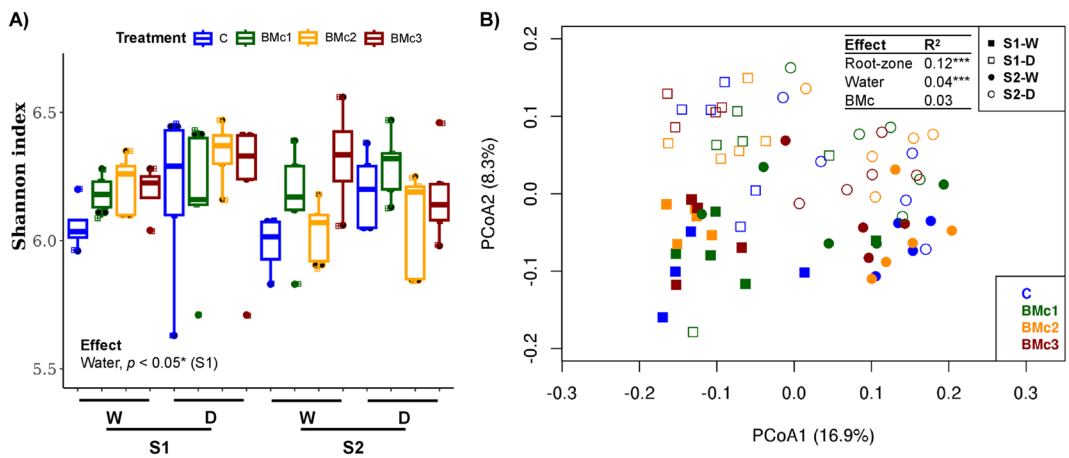


Fig. 5 Seed inoculation with beneficial bacterial consortia (BMc) had no effect on the native bacterial and archaeal alpha- (**A**) and beta- (**B**) diversity of maize rhizosphere in loam. PCoA, the principal coordinate analysis based on Bray–Curtis dissimilarity; W, well-watered; D, drought; C, non-inoculated control; BMc, beneficial bacterial consortia; S1, basal root; S2, root tip and Water, W vs. D. The Kruskal–Wallis test, followed by Dunn's test, was used to analyse the Shannon index, whereas the PERMANOVA was applied to analyse the beta-diversity ($n = 5$). Asterisks *, ** and *** indicate $p < 0.05, < 0.01$ and < 0.001, respectively. The PERMANOVA did not show any significant interaction of Root-zone:Water, Root-zone:BMc or Water:BMc

Overall, drought had a significant impact on the bacterial/archaeal beta-diversity, accounting for 4% of the variance (PERMANOVA test, Fig. 5B). Drought significantly increased bacterial/archaeal alpha-diversity (Shannon index) in the rhizosphere basal roots, independent of BMc treatment (Fig. 5A). Drought also had a significant impact on bacterial/archaeal relative abundance at the taxonomic level (Figs. S1 & S2). For example, in the basal root rhizosphere, the relative abundance of ASVs belonging to the bacterial genera *Massilia* and *Sphingomonas* (both Proteobacteria), respectively, was significantly decreased under drought, whereas the relative abundance of ASV76_*Bacillus* (Firmicutes) increased.

The root zone had a significant and stronger impact on bacterial/archaeal beta-diversity than drought, as evidenced by 12% explained variation (Fig. 5B) and an increase in the number of bacterial genera and ASVs (phyla Actinobacteriota, Verrucomicrobiota and Patescibacteria) that significantly differed in relative abundance (Figs. S1 & S2). The root zone effect was dependent on the water treatment (well-watered or drought).

The bacterial/archaeal beta-diversity in the BMc treatments differed slightly (3% explained variance) from the non-inoculated treatments, but this was not significant (Fig. 5B). There was a trend towards increased bacterial/archaeal alpha-diversity (Shannon index) due to BMc treatments under well-watered conditions, for both basal root and root tip rhizospheres, but the effect was not significant (Fig. 5A).

The effects of BMc treatments on the relative abundance of bacterial/archaeal phyla were dependent on root zone and water (well-watered or drought) treatments (Fig. S1). BMc1 significantly reduced the relative abundance of Verrucomicrobiota (mean relative abundance of 1.0) and Bacteroidota (mean relative abundance of 1.7) at basal roots under well-watered and at root tips under drought conditions, respectively when compared to non-inoculated controls (mean relative abundance 2.4 and 2.8, respectively). The relative abundance of Bacteroidota was also significantly reduced at root tips of BMc2 (mean relative abundance of 1.5) treatment under drought and BMc3 (mean relative abundance of 1.9) treatment under well-watered conditions, when compared to non-inoculated controls (mean relative abundance 2.8 and 3.2, respectively). Only the relative abundance of Firmicutes in BMc3 at basal roots increased significantly under well-watered conditions.

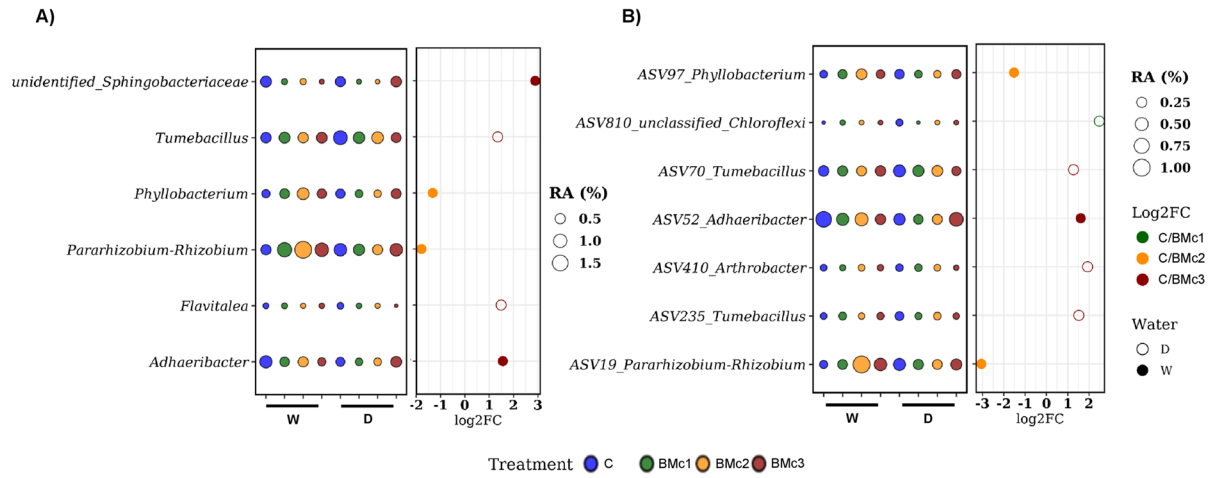


Fig. 6 Inoculation with beneficial bacterial consortia (BMc) significantly affected the relative abundance of several bacterial genera (**A**) and ASVs (**B**) in maize rhizosphere of root tips in loam. No significant effect of BMc on the relative abundance (RA) of bacterial genera and ASVs at rhizosphere basal roots. The Wald test from Deseq2 was used to determine whether there were significant differences in the RA of bacterial and archaeal genera or ASVs between non-inoculated (**C**) and inoculated (BMc1, BMc2 or BMc3) treatments under well-watered (W) or drought (**D**) conditions. Significant effects for pairwise comparisons were based on log2 fold change (log2FC) with adjusted *p-value* < 0.05 ($n = 5$) based on Benjamini-Hochberg. A positive log2FC (right panels of **A** and **B**) indicates significantly higher RA (left panels of **A** and **B**) of bacterial genera or ASVs in non-inoculated than BMc treatments

At genus and ASV levels, BMc had a significant effect on the relative abundance of several ASVs in rhizosphere root tips only (Fig. 6). Two ASVs of the Proteobacteria belonging to *Rhizobium* (ASV19) and *Phyllobacterium* (ASV97) had a significantly higher relative abundance in BMc2 than the non-inoculated treatments under well-watered conditions. ASVs from Firmicutes (*Tumebacillus*, ASV70 and ASV235 under drought), Actinobacteriota (*Arthrobacter*, ASV410 under drought) and Bacteroidota (*Adhaeribacter*, ASV 52 under well-watered conditions) had significantly lower relative abundances in the BMc3 treatments compared to the non-inoculated control. In comparison to the effects of root zone and drought, the BMc treatments resulted in fewer bacterial responders on genus and ASV levels (Figs. 6 & S2).

We found only four ASV sequences that were identical to BMc members (Fig. 7A): ASV144 was identical to the inoculant I2 (*Pseudarthrobacter* for BMc2), ASV265 and ASV277 were identical to I601 (*Enterobacter* for BMc2), and ASV22 was identical to RU47 (*Pseudomonas* for BMc3). Only ASV265 and ASV277 (affiliated to *Enterobacter*) had significantly higher relative abundance in BMc2 treatment in rhizosphere basal roots under well-watered (mean relative abundance of 0.23 and 0.22, respectively) and drought (mean relative abundance of 0.19 and 0.17, respectively) conditions compared to non-inoculated control (the relative abundance of both ASVs was below the detection limit under both water conditions) (Fig. 7B). Similarly, at the genus level, the relative abundance of *Enterobacter* was significantly higher in BMc2 than non-inoculated treatments in the rhizosphere basal roots under both well-watered and drought conditions (Fig. S3).

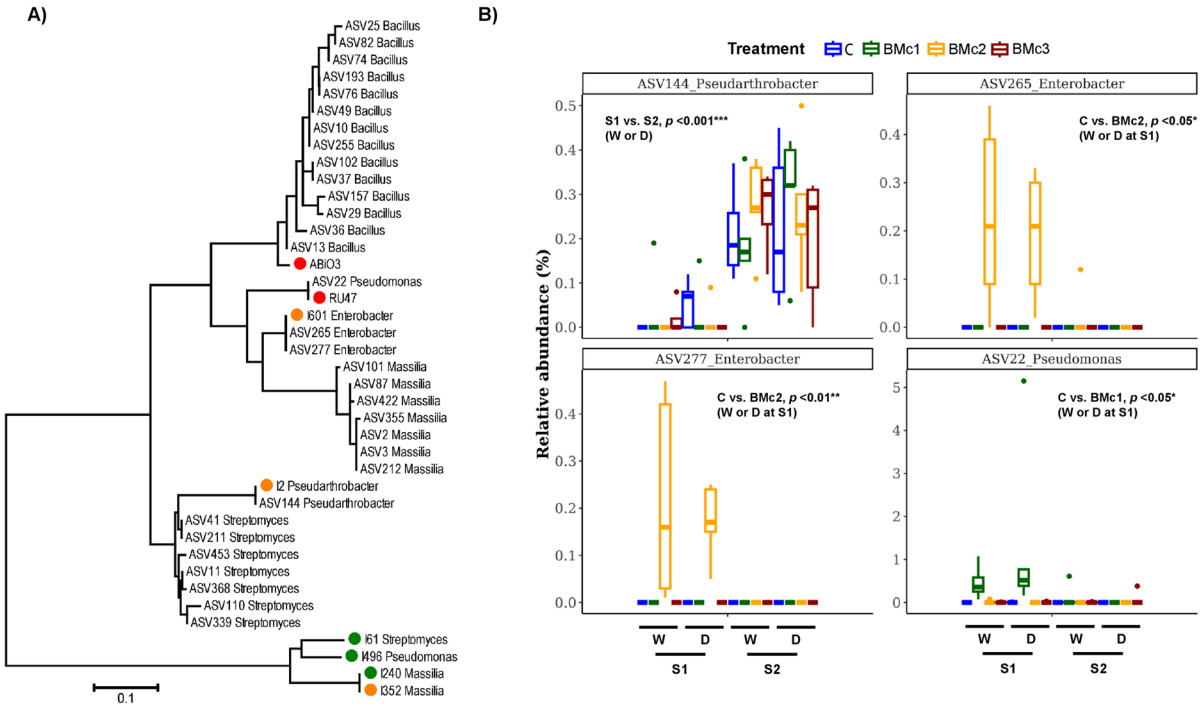


Fig. 7 Maximum likelihood phylogeny of inoculants and ASVs from six bacterial genera affiliated to BMc taxonomy (**A**) and the ASV relative abundances in different treatments (**B**).The phylogeny was constructed using only sequences of ASVs with the relative abundance greater than 0.1% with the exception of ASVs associated to *Enterobacter* (<0.1% due to low relative abundance). Asterisks *, ** and *** indicate $p < 0.05, < 0.01$ and < 0.001, respectively (Kruskal–Wallis test, followed by Dunn's test for $n = 5$). NS, no significant differences; C, non-inoculated control; BMc, beneficial microbial consortium; W, well-watered; D, drought; S1, basal root and S2, root tip

Discussion

Rhizocompetence of bacterial isolates and their colonization pattern

The CFU counts of six bacterial isolates in rhizosphere samples from both single and BMc inoculated experiments in loam and sand at harvest (higher at basal roots than root tips) confirmed their rhizocompetence, and supported our first hypothesis.

Several factors were reported to influence successful and efficient colonization of bacterial inoculants in the rhizosphere, including colonization ability of an individual isolate (Zhang et al. 2022), inoculation approaches (Fiuza et al. 2022), the density of inoculants (Arnault et al. 2024), antimicrobial compounds exuded by host plants (Thoenen et al. 2023) and competition with low or high microbial diversity of native soil (Mawarda et al. 2022). Using the same substrates, Yim et al. (2022) found that loam had more bacterial diversity than sand. In contrast to Mawarda et al. (2022), comparable CFU counts of BMc1, BMc2, BMc3, or BMc3-Tri in the rhizosphere loam and sand (Fig. 4, at basal roots or root tips) revealed that the native soil bacterial diversity of the two substrates had no effect on inoculant colonization. The similar abundances of the BMc in the rhizosphere of both substrates may be attributed to the seed inoculation approach, which introduced only a small number of bacterial cells occupying maize seeds to the rhizosphere. One inoculated maize seed (per pot) was colonized by 6.6, 7.0, 6.6 and 5.0 $\log_{10}$ CFUs for BMc1 (all three isolates), BMc2 (all three isolates), BM3 (ABi03 and RU47) and BMc3-Tri (*Trichoderma*), respectively, before sowing ($n=4$). Furthermore, our inoculants originated from maize, and they were most likely already recruited and shaped by complex maize exudates containing antimicrobial compounds. For example, benzoxazinoids like 6-methoxybenzoxazolin-2-one (MBOA) are metabolite compounds with a wide range of bioactivities against the soil microbiome (Thoenen et al. 2023). Thus, our inoculants may tolerate these compounds and colonize the developing roots.

In general, root exudation in root tips or immature roots (the primary site of root exudation) is often higher than in basal or mature roots (Proctor and He 2017; Wang et al. 2022; Tiziani et al. 2022). This implies more favourable conditions for growth of beneficial microbes in the rhizosphere root tips. In contrast, our findings showed that the CFU inoculant abundance was significantly higher at basal roots than at root tips (Fig. 4; Table S3), but we cannot exclude that their metabolic activity and competition with native microbial community was higher in the vicinity of the root tips. Furthermore, because of seed inoculation, inoculants may be filtered by the soil matrix, resulting in a lower abundance at the root tips compared to the basal roots.

Effects of single bacterial isolate or BMc treatments on maize plant performance

Treatments with single bacterial isolates (I2, I61, I240, I352, I496 or I601) did not promote plant growth under well-watered conditions in both substrates. Single inoculants may have a positive effect on maize if the inoculated plants were grown under nutrient deficient, abiotic or biotic stress conditions (Eltlbany et al. 2019; Elsayed et al. 2020; Chen et al. 2021; Abideen et al. 2022). As reported by Chen et al. (2021), maize growth and grain yield were significantly higher in seed inoculated treatments with the plant growth promoting strains *Sinorhizobium* sp. A15, *Bacillus* sp. A28, *Sphingomonas* sp. A55 or *Enterobacter* sp. P24 than in non-inoculated treatments when grown in substrates with 50% less N and no P supply. Similarly, inoculation with *Pseudomonas* or *Pantoea* strains significantly increased barley plant biomass when cultivated under drought conditions (Abideen et al. 2022).

Drought reduced maize growth significantly in both loam and sand substrates, as expected and consistent with previous studies (Armanhi et al. 2021; Lephatsi et al. 2022; Guevara-Hernandez et al. 2024).

The positive effects of BMc1, BMc2 and BMc3 seed inoculation were only observed in loam under both well-watered and drought conditions, which partially supports our second hypothesis. The plant growth promoting effect of BMc in loam could be attributed to the synergistic effect of isolates from the corresponding consortium (Jarak et al. 2012), as single inoculants did not reveal the effects on plant growth (under well-watered conditions). Furthermore, in addition to Behr et al. (2023), this study revealed the rhizocompetence as well as growth promotion of BMc3 in maize grown under greenhouse conditions in loam.

A previous study (Bradáčová et al. 2019) found that the growth of maize inoculated with BMc depended on soil type. They discovered that inoculating maize plants in clay-loam with high organic matter and a more active microflora significantly increased biomass, while sandy-loam with low organic matter and microbial activity did not. This observation may also partially support our current findings that BMc in sand had no effect on maize growth (Fig. 3; Table S4) due to a lower microbial activity, as indicated by low dehydrogenase activity (Table S2). Yim et al. (2022) also found that soil potential microbial extracellular enzyme activities ß-glucosidase, acid phosphatase and chitinase were significantly lower in sand than loam. Furthermore, because of the difference in texture, the substrate sand with higher pore connectivity and lower organic matter content (see Ganther et al. 2020; Vetterlein et al. 2021 who used the same substrates) may support different microbial activity (due to differences in O_2, nutrient and water availability) than loam (Hemkemeyer et al. 2018; Yim et al. 2022).

The plant growth data were collected one week after stopping watering for seven days. The effects of all BMc treatments on maize growth may have been greater if the measurements would have been taken at a later stage of plant development, particularly in sand with higher variation within the treatment (Fig. 3). Calvo et al. (2017) found that BMc treatment of maize had a stronger effect on shoot dry mass in 43- and 72-day old plants than in 14- or 27-day old plants.

Both single and BMc seed inoculation had no effect on total phenols in maize shoots under well-watered or drought conditions (Tables S2 & S4), indicating that the plant defense reaction was not activated. Differences in shoot total phenols may be observed in maize plants that were affected by pathogens (Rahaman et al. 2020) or cultivated in a reduced microbial community or sterilized substrate (Cappellari et al. 2017; Singh et al. 2020), indicating that plants lacked support from native microbiome, which had a significant impact on plant health.

BMc seed inoculation had only minor effects on the native rhizosphere bacterial/archaeal community

Sequencing of 16S rRNA gene fragments amplified from TC-DNAs was performed only for loam samples, and the effects of the BMc on bacterial/archaeal alpha- and beta-diversity were not significant (Fig. 5), thus our findings did not fully support the third hypothesis.

Significant differences in bacterial/archaeal diversity (Fig. 5) and relative abundance of several bacterial taxa (at the genus and ASV levels) between rhizosphere basal roots and root tips (Fig. S2), may be attributed to differences in root exudation quality and quantity at the respective root zones (Tiziani et al. 2022).

Significantly decreased relative abundance of *Sphingomonas* (ASV30, ASV 409; Fig. S2B) by drought compared to well-water treatments was also reported by Tiziani et al. (2022), which was linked to differences in the root exudate p-coumaric acid ethyl ester and L-serine.

Berg et al. (2021) reported that one of the six mechanisms of microbial inoculation is the modulation of the native soil microbiome. The fact that no significant change in the bacterial diversity and composition in response to the inoculant in the rhizosphere of maize was found in the present study could be attributed to drought duration (Guevara-Hernandez et al. 2024) and to lower numbers of inoculant cells reaching the rhizosphere via seed inoculation (see above). As reported by Arnault et al. (2024) changes in the microbial community composition in seven-day-old common bean rhizosphere was positively correlated with the abundance of BMc inoculants. The modulation of native soil microbiome in winter rye by BMc3 observed by Behr et al. (2023) was possibly due to a large volume of 50 mL cell suspension used for inoculation by drenching.

Although, the relative abundance of two *Enterobacter* associated ASVs (ASV265 and ASV277) increased significantly in BMc2 treatment compared to the non-inoculated control, we would be cautious to conclude that these ASVs represent the inoculant due to partial sequencing of the 16S rRNA gene fragments (Fig. 7B). Mapping ASV sequences to inoculant whole genome sequences may result in higher resolution. Thus, whole genome sequencing of all inoculants needs to be performed.

Future studies should address mechanistic interactions among inoculants of the corresponding BMc, as well as transcriptomic analyses to better understand the BMc effect on the plant. Furthermore, different sampling time points could be considered to determine whether the effect occurred earlier or later in maize growth.

Conclusion

The CFU counts of inoculants demonstrated their rhizocompetence independent of substrate and in different root zones. The plant growth-promoting effects were only revealed with BMc seed inoculation, both under well-watered and drought conditions (in loam), indicating synergistic effects of consortium members and improved drought resistance. Seed inoculation had no effect on the composition of the native rhizosphere bacterial/archaeal community. The significant increase in maize growth in loam could be attributed to the direct interaction of the BMc with germinating and growing plants.

These findings emphasize that seed inoculation with BMc facilitate the reduction of drought stress in maize in loam without affecting the native rhizosphere bacterial/archaeal diversity and composition.

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Author contributions BY, MAH and KS designed and carried out experiments. MAH performed Rif mutant of isolates, BOX-PCR and helped with the sampling of the greenhouse-experiment. BY collected the samples, prepared rhizosphere DNA, and carried out amplicon sequencing analyses of 16S rRNA gene fragments. MAH and EB analysed polyphenols in maize shoots, soil CO_2 respiration and dehydrogenase activity. BY and DB did a serial diluted plating and JHB provided OMG16 spores and protocol for inoculation. BY, MAH, EB, DV, JHB, DB and KS wrote the manuscript.

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Data availability All raw sequences of the 16S rRNA gene fragments from NovaSeq and Sanger sequencing for the 510 bacterial isolates are accessible at the National Centre for Biotechnology Information (NCBI) under project numbers PRJNA1132941 and PRJNA1133225, respectively.

Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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