Home-based microbial solution to boost crop growth in low-fertility soil

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Summary

- Soil microbial inoculants are expected to boost crop productivity under climate change and soil degradation. However, the efficiency of native versus commercialized microbial inoculants in soils with different fertility and impacts on resident microbial communities remain unclear.
- We investigated the differential plant growth responses to native synthetic microbial community (SynCom) and commercial plant growth-promoting rhizobacteria (PGPR). We quantified the microbial colonization and dynamic of niche structure to emphasize the home-field advantages for native microbial inoculants.
- A native SynCom of 21 bacterial strains, originating from three typical agricultural soils, conferred a special advantage in promoting plant growth under low-fertility conditions. The root:shoot ratio of fresh weight increased by 78-121% with SynCom but only 23-86% with PGPRs. This phenotype correlated with the potential robust colonization of SynCom and positive interactions with the resident community. Niche breadth analysis revealed that SynCom inoculation induced a neutral disturbance to the niche structure. However, even PGPRs failed to colonize the natural soil, they decreased niche breadth and increased niche overlap by 59.2-62.4%, exacerbating competition.
- These results suggest that the home-field advantage of native microbes may serve as a basis for engineering crop microbiomes to support food production in widely distributed poor soils.

Keywords: synthetic microbial community, home-field advantage, rhizosphere microbial community, metagenomic binning, niche structure, soil fertility

Introduction

Microbial products are considered an environmentally friendly agricultural strategy, potentially promoting soil biodiversity and increasing crop productivity (Chaparro *et al.*, 2012; Jez *et al.*, 2016). Developing environmentally compatible and efficient soil microbial inoculants is critical to boost crop production in a world with a growing human population as well as stresses from climate change and soil degradation (McCarty & Ledesma-Amaro, 2019; Singh *et al.*, 2020). Microbial inoculants generally include potentially beneficial microorganisms with target functional traits in facilitating crop resistance to biotic and abiotic stress (Berg *et al.*, 2017). For example, plant growth-promoting

rhizobacteria (PGPR) can promote plant tolerance to drought and salt stress (Sharma *et al.*, 2016; de Vries *et al.*, 2020). Beneficial plant–microbe interactions promote crop growth via associative nitrogen fixation, phosphorus solubilization, and phytohormone regulation (Richardson *et al.*, 2009; Lebeis *et al.*, 2015). However, the efficacy of commercial microbial inoculants remains unreliable due to a lack of assessment of environmental compatibility and in-field performance (Kaminsky *et al.*, 2019), since these commercial products are often applied in diverse environmental conditions to microbial taxa. Hence, one urgent concern is to evaluate the performance of these microbial consortia under varied environmental conditions, for example, soils with different fertility.

Microbial communities could optimize their performance and ecosystem functions at "home" sites compared with those at "foreign" sites, referred to as the "home-field advantage" (Ayres *et al.*, 2009; Li *et al.*, 2017). Since the plant microbiome is particular to the host and soil type (Berg & Smalla, 2009), the success of exotic microbes in establishing in soil and delivering the desired functions is very context-dependent (Hartmann & Six, 2022). Therefore, external inoculants with unstable performance are poorly expected compared to native microbial inoculants. Commercialized microbial inoculants are mostly oversimplistic, comprising a few microbes from nonnative soils. The expectation that these microbial consortia can carry out desired functions in field conditions often fails to consider metabolic plasticity, microbial interactions, and the fundamental importance of microbial diversity in promoting soil function (Delgado-Baquerizo *et al.*, 2017).

Alternatively, recent studies using synthetic microbial community (SynCom) approaches highlight the benefits of using indigenous microbes for increasing plant productivity and resilience against biotic and abiotic stress through various plant growth-promoting activities (Niu *et al.*, 2017; de Souza *et al.*, 2020). Having coevolved with the plant hosts in native soils under local environmental conditions, mutualistic microbial communities are expected to be highly efficient at supporting plant growth (Rua *et al.*, 2016). Native microbial communities can have long-lasting positive impacts (i.e., legacy effects) on soil functions and plant development (Crowther *et al.*, 2019), particularly in nutrient-limited conditions. However, this advantage may become counterbalanced—or even reversed—by host-specific pathogens in native microbial consortia (Fanin *et al.*, 2021). In particular, the introduction of exotic microbes may have a transient or persistent effect on resident microbes (Mallon *et al.*, 2018; Amor *et al.*, 2020), a phenomenon that remains unpredictable under varied soil types and

environmental conditions. Microbial inoculants developed from indigenous beneficial microbes would have a relatively more predictable influence on their original soil environment, thus leading to an agriculturally safer choice. However, few studies have compared the effectiveness and performance of commercial, exotic PGPRs versus native microbial consortia on promoting crop productivity in soils with different fertility.

Here, we evaluated the contribution of native and exotic microbial consortia in promoting maize growth in soils ranging from high to low fertility. We compared the performance of two microbial inoculants, including a 21-species SynCom containing indigenous strains from maize rhizospheres across three typical upland agricultural soils and a microbial inoculant using commercial PGPRs. We hypothesized that native SynCom could rapidly colonize the rhizosphere through adaptation to growing conditions and alleviate plant nutrient stress by providing essential nutrients and phytohormones, especially in low-fertility soils. The impact of PGPRs, however, was context dependent, with exaggerated nutrient competition in low-fertility soils. Our results indicated that home-field advantage drives the positive impact of native SynCom on crop growth in low-fertility soils, guiding SynCom design for further field applications.

Materials and methods

Research site and sample collection

The field experiment was set up at the Fengqiu National Agroecosystem Field Experiment Station of the Chinese Academy of Sciences (114°24′ E, 35°00′ N), which is at an altitude of 67.5 m, in Henan Province, China. The experimental site has a semihumid, semiarid warm, and monsoon climate with a mean annual temperature of 13.9 °C and precipitation of 605 mm (June to September). Three typical agricultural soils that have been maintained under long-term dryland farming, including Mollisol (derived from Hailun, Heilongjiang Province), Inceptisol (Fengqiu, Henan Province), and Ultisol (Yingtan, Jiangxi Province), were used to set up microplots 1.4 m in length × 1.2 m in width × 1.0 m in depth, which were randomly placed. Mollisol is the world's most fertile, organic carbon-rich, and productive soil type (Wang *et al.,* 2021). Inceptisol has moderate productivity but a low nutritional environment with low organic matter content and available nitrogen and phosphorus (Ge *et al.,* 2008). Ultisol has the lowest fertility among the three soil types, with high acidity, low productivity, and poor organic carbon (Xu *et al.,* 2003). We conducted an NMR analysis to understand the carbon structures

in the soil collected from the field trial (Methods S1). The relative contents of the various carbon chemical components were obtained by regional integration of spectral peak curves (Sun *et al.*, 2019). Each microplot was fenced by 20-cm cement mortar brick walls and underlaid by quartz sand (3 cm thick). Each soil had six biological replicates.

Zhengdan 958, a commercial maize hybrid with a large planting area in China, was planted in early June annually since 2006, and management measures were only taken for weeding by hand. Grain yield, aboveground biomass, and nutrient content, including total carbon, nitrogen, phosphorus, and potassium, in seed and straw were measured immediately after harvest. Rhizosphere soil samples were collected as follows: the loosely attached soil on the roots was removed with gentle shaking (shake-off method), and soils within approximately 1-4 mm of the root were collected as rhizosphere soils, sealed in a polyethylene wrapper, stored on ice, and transported to the laboratory. Rhizosphere soils for geochemical analyses, including soil organic matter, total nitrogen, nitrate and ammonium nitrogen, available nitrogen, total phosphorus, available phosphorus, total potassium, and available potassium, were stored at 4 °C (Methods S2), and those for DNA extraction were stored at −80 °C. These soil samples were then used to investigate the diversity and composition of bacterial communities associated with the maize rhizosphere in three types of soils.

Bacterial identification and synthetic microbial community preparation

Maize rhizosphere samples from the three agricultural soils were used for the isolation of culturable bacteria using the standard serial dilution culture method (Methods S3). Different gradients of soil suspensions were smeared onto four different types of nutrient media for isolation and culture (Table S1). After incubation, single colonies were picked based on different morphologies and were restreaked at least twice to ensure purity. Pure cultures of the strains were cultured overnight in 50 mL Luria-Bertani (LB) medium. A total of 5 mL of each bacterial suspension was used for complete 16S rRNA sequencing performed by Personalbio (Shanghai, China). Therefore, 47 bacterial strains were obtained. Single colonies were picked and preserved on LB plates at 4 °C. The bacterial 16S rRNA gene was amplified using primers 27F (5’ -AGAGTTTGATCCTGGCTCAG- 3’) and 1492R (5’ -CTACGGCTACCTTGTTACGA- 3’). These strains were identified by blasting against the EzBioCloud 16S database (Methods S4) (Yoon *et al.*, 2017). Finally, 21 unique indigenous species

that commonly existed across three soil types were used to design a native microbial inoculant, referred to as SynCom (Table S2).

When preparing SynCom, the OD_{600} value of each bacterial suspension was controlled between 0.6–0.8 during the exponential growth phase. The cells of the culture were then collected by centrifugation at 2,940 × g for 10 min at 4 °C and diluted to ~10^8 cells per milliliter. The cell suspension of each strain was mixed in 50 mL of 1× phosphate buffered saline (PBS) in an equal volume to prepare bacterial suspensions of the 21 different species.

Surface sterilization and germination of maize seeds

The surface sterilization and germination of maize seeds (Zhengdan 958) followed the standard protocols of Niu et al. (Niu *et al.*, 2017). Briefly, the seeds were immersed in 70% (vol/vol) ethanol for 3 min, then in 5% (vol/vol) sodium hypochlorite for 3 min, and finally rinsed with sterile distilled water three times. The surface sterilization surface-sterilized seeds were placed in a Petri dish (9 cm diameter) filled with 7 mL of sterile water and incubated at 30 °C in the dark for 50–55 hours until the seeds germinated. After incubation for 24 hours, 100 μl of water was taken from the Petri dish and spread onto tryptone soya agar (TSA) plates, which were then incubated at 30 °C to check for contamination.

Greenhouse experiment for maize plants with different microbial inoculants in soil

We conducted a greenhouse experiment under different fertility conditions to compare the effectiveness of native versus commercialized microbial inoculants in facilitating crop growth. The native microbial inoculant, SynCom, was derived from common species across Mollisol, Inceptisol, and Ultisol, while the commercial inoculant was composed of four model PGPRs from strain banks that have been reported to be applied in agricultural practice. They were selected with target traits, including *Rhizobium radiobacter* (J. M. Young), nitrogen fixation (Guo *et al.*, 2017); *Burkholderia cepacian* (Eiko Yabuuchi), phosphorus solubilization and antifungal activity (Zhao *et al.*, 2014); *Arthrobacter ilicis* (Collins M.D.), IAA production (Chou & Huang, 2005); and *Stenotrophomonas rhizophila* (Arite Wolf), antimicrobial compound production (Ryan *et al.*, 2009). The bacterial suspension of four PGPRs was prepared following the same steps as SynCom. SynCom+PGPRs were prepared with a 1:1 bacterial suspension of SynCom and PGPRs. Thus, four groups were included in the full-factor experimental design, including the SynCom, PGPRs, SynCom+PGPRs, and control

treatments (without microbial inoculation). Note that this study calls any species that enters a habitat when it is not a resident taxon an "exotic" species. This refers to the microbes selected based on available research that have been well studied and shown to have a variety of essential functions that contribute to the maintenance of plant health.

Surface-sterilized and germinated maize seeds with primary roots of 1-2 cm were transplanted into the soil. Microbial inoculation was carried out in the V3 growth period. The microbial inoculum (50 mL) was poured into the soil near the growing roots of each seedling. The greenhouse experiment was set up with four replications for each treatment under three soil types. No nitrogen or phosphorus fertilizers were applied except for regular watering. The greenhouse experiment lasted for 59 days with a natural light cycle. From day 29, the height and chlorophyll content of maize plants were measured every seven days, and photos were taken every 14 days. Plant tissues were removed from the soil at 59 days (V8 growth phase) after transplantation, and rhizosphere soil samples were harvested and stored at −80 °C for microbiome analysis. Subsequently, physiological indicators such as plant height, chlorophyll content, and root weight were measured. The indole-3-acetic acid (IAA) concentration of rhizosphere microbial communities was determined by means of the Salkowski reagent method (Methods S5) (Sarwar & Kremer, 1995).

Plant growth promotion test on axenic maize seedlings

To examine the plant growth-promoting effects of microbial inoculation in the absence of different soil matrixes, maize seedlings were grown in sterile 1/2 Murashige and Skoog (MS) agar in double-tube chambers (Niu *et al.*, 2017). Before the experiment, the rhizosphere soil suspension for inoculation was prepared by mixing 2 g of frozen rhizosphere soil in 20 mL of 1×PBS buffer, vortexing for 2 min, and then centrifuging for 6 min at 750 × g at 30 °C. All bacterial strains were propagated in 25 mL tryptic soy broth (TSB) medium for 2 days at 30 °C. Each bacterial fermentation broth was centrifuged at 4,000 × g for 8 min and resuspended in rhizosphere soil suspension with the OD_{600} adjusted to 0.5 (~10^8 cells/mL). Six surface-sterilized and germinated maize seeds were soaked in soil suspension with or without microbial inoculations for one hour. Sterile maize seedlings were used as bacteria-free controls. Thus, thirteen groups were designed for this experiment, including three soil suspensions with or without microbial inoculations (SynCom, PGPRs, and SynCom+PGPRs) and axenic control treatments. The maize seedlings were placed in a plant growth chamber under the

following conditions: 16 hours of light (day) and 8 hours of dark (night), 30 °C, and relative humidity of 54%. Plants were photographed every five days and harvested from each treatment on day 15. Maize growth was evaluated by measuring the length and fresh weight of shoots and roots, as well as the plant height and chlorophyll content of plants.

Additionally, since the 21-species SynCom (S21) was taxonomically redundant, we downsized the synthetic community to 12 species (S12) at the genus level and 4 species (S4) at the order level (Table S2). The other 4-species SynCom (SF4) was designed to be similar to PGPRs at both taxonomic and functional levels. For each treatment, four surface-sterilized maize seedlings were used, and the experiments were performed under the same conditions for 10 days.

Measurements of the colonization of the microbial inoculants

To investigate the successful colonization of SynCom members in the rhizosphere under greenhouse conditions, we intended to track individual SynCom members through 16S rRNA qPCR and correlation analysis. The abundance of SynCom members was measured by qPCR using rhizosphere soil samples of the control group (without microbial inoculation) and SynCom treatment. Bacterial DNA was extracted using the TIANamp Bacteria DNA Kit (TIANGEN, Beijing, China) according to the manufacturer's instructions. qPCR was performed on an ABI3730-XL (Applied Biosystems, USA) using TOROGreen ® qPCR Master Mix (TOROIVD, QST-100) with the following cycle conditions: 40 cycles of 95 °C for 15 s, 55 °C for 15 s, and 72 °C for 45 s. The specificity of the primers designed in this study and the quality of the PCR products were determined by gel analysis. All qPCRs were performed in triplicate. For the 16S rRNA correlation analysis, the representative ASVs of each species were identified from the microbiome sequencing data of the greenhouse by BLAST analysis. The matching ASVs displayed >98.7%, 99%, and 100% sequence identity with the sequence of the full length of the 16S rRNA gene of each strain were kept as measurable, highly matched, and best-matched ASVs, respectively. The abundance of SynCom members and PGPRs at the genus level counted all measurable ASVs.

Rhizosphere microbiome analyses

For rhizosphere samples collected from the field, a high-throughput absolute quantification sequencing method was employed to obtain an accurate and reliable absolute abundance of soil bacteria. Genomic DNA from 0.5 g of rhizosphere soil was extracted with the HiSeq Reagent Kit

(Illumina, USA) according to the manufacturer's instructions. The quality and quantity of the DNA were assessed by nanodrop and gel electrophoresis. The V4-V5 regions of the 16S rRNA gene were amplified using primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′). The spike-in sequences involved conserved regions identical to selected natural 16S rRNA genes and artificial variable regions, working as internal standards and allowing absolute quantification across samples (Mou *et al.*, 2020). The PCR procedure involved predenaturation at 94 °C for 2 min, denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and 72 °C for 60 s (a total of 25 cycles), and extension at 72 °C for 10 min. Sequencing was performed using Illumina NovaSeq 2 × 250 bp (Genesky Biotechnologies Inc., Shanghai, China) (Methods S6). TrimGalore (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) and FLASH2 were used to process the final V4-V5 tag sequences. The spike-in sequences were filtered out, and reads were counted. The standard curve of spike-in sequences was generated for each sample, and the sequenced microbial DNA was quantified and estimated in reference to the representative standard curve. Sequences were assigned to each sample based on its unique barcode.

For rhizosphere samples from the greenhouse experiment, the relative quantification sequencing method was employed (Methods S7). The V4-V5 region of the bacterial 16S rRNA was chosen for amplification with 515F and 907R and sequenced on the Illumina NovaSeq platform. The relative quantification of 16S rRNA was carried out as described above without spike-in sequences.

High-throughput sequencing and genome binning

Whole metagenomic shotgun sequencing was performed using the Illumina HiSeq platform and the 2×150 bp paired-end method (Methods S8). Clean reads were generated and assembled into scaffolds by SOAPdenovo (version 1.05) based on De-Brujin graph construction. Open reading frames (ORFs) were predicted and further functionally annotated by BLAST 2.2.28+ against KEGG (Kyoto Encyclopedia of Genes and Genomes). Assembled scaffolds were then grouped into metagenomic bins using MetaWRAP (Uritskiy *et al.*, 2018). Genome bins were assessed for estimated completeness and contamination markers by CheckM (Parks *et al.*, 2015). The completeness and contamination can be estimated by the number of single-copy genes that the genome of the bin's taxonomy is expected to have. Genome bins were filtered to > 50% completeness and < 10% contamination. Binned genomes were submitted to RAST for classification and annotation of nutrient metabolism, plant hormone

synthesis pathways, bacterial motility, and chemotaxis (Aziz *et al.*, 2008; Overbeek *et al.*, 2014; Brettin *et al.*, 2015). The phylogenetic tree of *rpoD* genes identified from each genome was constructed using MEGA X software.

Metabolic diversity of the microbial inoculants (Biolog EcoPlate^{TM})

Since the greenhouse experiment was set up under different soil fertility conditions, the capability of SynCom and PGPRs to utilize carbon sources was tested by Biolog EcoPlate^{TM}, containing 31 kinds of carbon sources. The carbon sources were defined as carbohydrates (n = 10), carboxylic acids (n = 7), amino acids (n = 6), polymers (n = 4), phenolic compounds (n = 2), and amines (n = 2) (Choi & Dobbs, 1999). For each carbon source, n = 3 replicates in Biolog plates. For each treatment, n = 4 replicates of Biolog plates. The mixed bacterial suspensions were prepared as described above and diluted to OD_{600} = 0.05. Then, 150 μL of the mixed bacterial suspensions were added to each microtiter well of the Biolog plates using an eight-channel pipette, and four replicates were set for each treatment. The plates were incubated at 30 °C for 96 hours, and the OD_{590} was measured every 12 hours during incubation. Biolog plates use a tetrazolium violet redox dye to monitor cell respiration, and oxidation of the nutrients will lead to respiration, resulting in the formation of a purple color in the well. Water without any carbon source served as a control in triplicate (Bochner, 2009).

The collected data were then used to analyze the average well color development (AWCD) and the preference of the bacterial community for various carbon sources. AWCD = Σ(Ci-R)/n where Ci is the difference between the absorbance values at 590 for each well, R is the absorbance value of the control well, and n is the total number of carbon sources (Garland, 1996). The AWCD value is an indicator of the ability of microorganisms to utilize carbon sources and the metabolic activity of microorganisms, as well as the growth rate of mixed bacterial cultures in wells. Principal component analysis (PCA) was used to characterize the functional structure of the bacterial community by community substrate utilization patterns. The microbial growth curve model based on the AWCD was constructed by Origin 2019b.

Data processing and statistical analysis

The molecular ecological networks (MENs) were constructed using a random matrix theory (RMT)-based approach (http://ieg4.rccc.ou.edu/mena/), where the abundance data obtained from absolute quantitative sequencing were selected unless they occurred in more than 80% of the samples.

The network was constructed using Pearson's correlation analysis. Network parameters such as R^2, average path length, average connectivity, average clustering coefficient, and modularity were obtained by the MENA method; the network was visualized using Gephi 0.9.2.

The analysis of soil physicochemical properties, microbiome statistical analyses, and the experimental data was performed using R 4.2.1, and plots were constructed using the "ggplot2" package. All data were tested for normal distribution before conducting statistical analyses. A nonparametric Mann–Whitney or paired Wilcoxon rank sum test was used for nonnormal data. Multiple comparisons were performed with nonparametric Nemenyi tests by the "PMCMRplus" packages (https://cran.r-project.org/web/packages/PMCMRplus/index.html). All samples were rarefied to 4,000 to 6,000 observations per sample. The alpha (Shannon and Chao1 indexes) and beta diversity of microbial community analyses were performed using the R package "vegan" (https://cran.r-project.org/web/packages/vegan/index.html). The beta-diversity analysis was conducted using Hellinger-transformed data. Analyses of niche breadth (Levins' niche breadth) and niche overlap (Levins' niche overlap) were performed by the "spaa" package (https://cran.r-project.org/web/packages/spaa/index.html).

Results

Distinct microbiota in maize rhizospheres across soil types of different fertility

The diversity and composition of field maize rhizosphere bacterial communities in three types of soils were characterized by gradient spike-in absolute quantification sequencing of 16S rRNA (Table S3). The rarefaction curves show good coverage of the diversity (Fig. S1a). The Shannon diversity index of Mollisol and Inceptisol was much higher than that of Ultisol ($P < 0.05$) (Fig. S1b), while the Chao1 index was the highest in the Inceptisol followed by Mollisol and Ultisol ($P < 0.05$) (Fig. S1c). Redundancy analysis indicated that the soil type explained most of the variation in the soil microbial groups (Fig. S1d). The dominant genera were *Gp6*, *Gaiella*, and *Gp4* in Mollisol; *Nitrososphaera*, *Gp4*, and *Gp6* in Inceptisol; and *Nocardioides*, *Gaiella*, and *Arthrobacter* in Ultisol (Fig. S2). In addition, Mollisol soil showed high soil fertility, while Inceptisol and Ultisol soils were low-fertility soils according to the molecular composition of soil organic matter and nutrient contents, such as total nitrogen, total phosphorus, available nitrogen, and available phosphorus (Fig. S3, Table S4).

Design of native SynCom and its growth-promoting effects.

There were 47 bacterial isolates obtained using four different nutrient media from the rhizosphere soil samples (Table S2). Twenty-one unique species commonly existed in the three soils belonging to the most abundant taxa at the order level (Fig. 1a, Fig. S4). These species were reported to have plant beneficial capacities except for *Sphingomonas desiccabilis,* which has been reported to be isolated from biological soil crusts (Reddy & Garcia-Pichel, 2007). Thus, we used these 21 culturable strains to construct a native SynCom.

In the greenhouse experiment with SynCom, we found that the plant promotion effect of SynCom varied among fertility conditions (Fig. 1b, c). Consistently, during the maize development stages during V4-V8 at days 35, 42, and 56, SynCom inoculation increased the chlorophyll content significantly by 24-35% ($P < 0.05$) in Ultisol, followed by Inceptisol (18-27%, $P < 0.05$) and Mollisol (1-3%, $P > 0.05$) compared to the uninoculated control. Plant height in Inceptisol showed an obvious increase with SynCom inoculation on days 35 (67%, $P < 0.001$) and 56 (36%, $P < 0.001$). Meanwhile, plant height increased by 23-38% ($P < 0.05$) in the Ultisol and 9-21% ($P > 0.05$) in the Mollisol.

Potential functional traits of the native microbial community

The metagenomes of rhizosphere soil samples were sequenced to assess the composition and function of the original soil microbial community at "home" sites (Table S5). The KEGG level 3 pathways were enriched in carbohydrate and amino acid metabolism in all soil samples (Fig. S5a). The metagenomes were then assembled to retrieve draft population genomes from the soil. The phylogeny of each scaffold was annotated at the family level, except for bin. 6 at the order level (Table S6). A total of 27 bins were obtained with completeness greater than 50%. Six genomes showed less than 5% contamination and were selected as high-quality genomes to annotate potential genomic functions (Fig. S5b). They belonged to Micrococcaceae, Conexibacteraceae, Rhizobiaceae, and Xanthomonadaceae, accounting for 9.5%, 4.2%, 6.8%, and 5.7% of the abundance at the family level, respectively. Burkholderiales accounted for 18.72% in abundance at the order level. The absolute abundances of Micrococcaceae and Rhizobiaceae were the highest in the Ultisol (Fig. 2a).

Subsequently, the high-quality assembled genomes were annotated by the RAST automated platform and were found to be related to nitrogen metabolism, the TCA cycle, cellular chemotaxis, oxidative stress, and biosynthesis of plant growth hormones, such as IAA production (Fig. 2b). Some of the genomes harbored pathways for assimilatory nitrogen reduction (*nirA*, *nirB*) and ammonification

(*ure*). The inorganic phosphorus transporter gene (*pst*) with high affinity under low phosphorus conditions was found in all genomes. Tryptophan, the major precursor for IAA biosynthesis, is biosynthesized via the tryptophan operon (*trp*), identified in all assembled genomes. The indole-3-pyruvate (IPyA) pathway of bacterial auxin biosynthesis was found in the complete metabolic pathway of the bin. 9 (Micrococcaceae), and bin. 9 could produce IAA through the tryptamine (TAM) pathway.

Putative rhizosphere microbial networks were investigated using random-matrix theory-based molecular ecological network analysis (Fig. S6a). The main modules showed close relationships with maize straw and grain weight (Fig. S6b, c, and d). The Inceptisol dominant module (Module #2) was positively and strongly correlated with seed and straw nitrogen content and seed phosphorus content. The Ultisol dominant module (Module #4) showed a similar correlation with seed carbon and phosphorus content, as well as biomass. Community assembly modules of resident taxa in low-fertility soils strongly correlated with crop yield, suggesting the natural advantage of resident microorganisms in promoting plant growth under stress conditions.

Fertility-dependent promotion efficiency of microbial inoculants

To further validate the relative effects of the native versus exotic microbial inoculants, we selected four commercial PGPRs that have similar plant growth-promoting properties as the native communities, including *Rhizobium radiobacter*, nitrogen fixation (Rasulov *et al.,* 2020); *Burkholderia cepacia*, phosphorus solubilization and antifungal activity (Zhao *et al.*, 2014); *Arthrobacter ilicis*, IAA production (Chou & Huang, 2005); and *Stenotrophomonas rhizophila*, antimicrobial compound production (Ryan *et al.,* 2009) (Fig. 3a). We compared the effects of inoculating native SynCom, PGPRs, and SynCom+PGPRs to CK to quantify the performance of microbial inoculants under different fertility conditions. The greenhouse experiments showed that the impact of microbial inoculants was fertility dependent. The plants with SynCom inoculation were consistently and significantly higher than those with PGPRs and SynCom+PGPRs inoculants in the low-fertility soils Inceptisol and Ultisol (Fig. S7). Conversely, the plant grew better with PGPR inoculation under high fertility conditions (Fig. 3b). Moreover, SynCom enhanced the secretion of IAA by rhizosphere microorganisms in low-fertility soils (Fig. 3c, Fig. S8), a phytohormone that stimulated plant growth and development (Keswani *et al.,* 2020).

We questioned whether the low diversity in PGPRs resulted in the low metabolic activity of the PGPRs, making them less effective than SynComs in low-fertility soils. Unexpectedly, fast-growing PGPRs showed higher carbon metabolic capacity and metabolized wider carbon sources, including carbohydrates, amino acids, amines, and polymers (Fig. S9a, b; Table S7). However, SynCom showed different metabolic preferences for a few amino acids, carboxylic acids, and carbohydrates compared with PGPRs (Fig. S9c, d). Furthermore, genomic information obtained from the NCBI database indicated a diverse metabolic potential in exotic PGPRs for amino sugars, oligosaccharides, organic acids, sugar alcohols, monosaccharides, and polysaccharides (Fig. S9e). These results suggested that in addition to providing essential nutrients and IAA for plants, there could be other crucial factors influencing the efficacy of SynCom in stimulating plant growth and health in low-fertility soils.

SynCom colonization and dynamics of niche structure

With the fully sequenced 16S rRNA gene, the SynCom strains could be matched to ASVs from the greenhouse rhizosphere community survey, which indicated strain presence and relative abundance. The ASVs with >98.7% sequence identity to any of the SynCom strains were considered targeted ASVs. The correlation analysis showed that there were 12 best-matched ASVs (100% sequence identity) and 3 highly matched ASVs (99% sequence identity) (Table S2). At the species level, the relative abundances of ASV127 (*Pseudomonas* spp.), ASV1479 (*Pseudomonas aeruginosa*), ASV176 (*Klebsiella* spp.), ASV30 (*Serratia marcescens*), and ASV60 (*Pseudomonas geniculata*) significantly increased in low-fertility soils (Fig. S10). The relative abundances of ASV127, ASV1479, ASV176, and ASV60 were significantly higher in high-fertility soils. The Shannon index of rhizosphere microbial communities with SynCom inoculation expressively increased in the Ultisol (Fig. 4a, Table S8). At the genus level, out of 11 relevant genera of SynCom, including all targeted ASVs, the richness of 6 genera was considerably increased in Ultisol (Fig. 4b). However, neither the alpha diversity of the rhizosphere microbial community nor the richness of relevant genera corresponding to the four PGPRs varied after PGPRs inoculation (Fig. 4a, c).

Although it is difficult to definitively distinguish the inoculated species from the natural microbiome through 16S rRNA gene surveys in nonsterile substrates, such as soils, we managed to track 16 individual members of the SynCom through 12 primer pairs (Fig. S11a, Table S9). A7, A8, A9, and C8 shared the same primers, which are not described below. In particular, the relative

abundances of *Acinetobacter pittii* (C15), *Bacillus cereus* (R5), *Enterobacter* spp. (R6 and C5), and *Sphingomonas desiccabilis* (X2) were significantly increased in the Ultisol (Fig. S11b). *Pseudomonas koreensis* (R9), *Chryseobacterium cucumeris* (R11), and *Sphingomonas desiccabilis* (X2) were notably enriched in Inceptisol, while *Enterobacter* spp. (R6 and C5), *Lysinibacillus macroides* (C6), and *Bacillus cereus* (R5) were enriched in Mollisol. Taken together, these results supported that some bacterial strains in SynCom were able to colonize the rhizosphere in low-fertility soils.

We found that the microbial inoculations had no influence on the soil microbial community (Table S10). Hence, we further explored the changes in niche breadth at the community level induced by microbial inoculation. The niche breadths of the microbial community with SynCom inoculation were consistently greater than those with PGPRs ($P < 0.001$, Fig. S12a, b, and c). In line with the community-level results, more ASVs (46.7-50.1%) increased their niches in low-fertility soils after SynCom inoculation than decreased their niches (40.1-43.7%) (Fig. S12b, c). This indicated that species with wider niche breadth became more competitive, especially under low resource availability. In contrast, after PGPRs inoculation, more ASVs decreased their niche breadth in all soils (Fig. 4d, Fig. S12a). Moreover, the niche overlap of the ASVs with significantly increasing niches ($P < 0.05$) was calculated. The proportions of ASV pairs with increased and decreased niche overlaps tended to be balanced after SynCom inoculation, which was consistent across all soils (Fig. S12d). However, 62.4% and 59.2% pairs of ASVs presented significantly higher niche overlap levels ($P < 0.05$) in the low-fertility soils Inceptisol and Ultisol after PGPR inoculation, respectively (Fig. 12e), which reflected a sharp competition in the rhizosphere community (Pianka, 1974).

The dependence of SynCom efficiency on resident microbiota from low-fertility soil

To further assess the fertility-dependent plant growth-promoting effects of microbial inoculation, we carried out a plant growth promotion test for the 21-species SynCom and the 4-species PGPRs in the absence of soil matrixes on axenic maize seedlings (Fig. 5a, b). We maintained a simplified but representative natural microbial community using a rhizosphere soil suspension. We found that native SynCom significantly promoted maize seedling growth at 15 days in low-fertility soil, as indicated by the increased chlorophyll content, plant height, root weight, and root:shoot ratio of fresh weight (Fig. 5c). In particular, the root:shoot ratio increased by 78-121% ($P < 0.001$) with SynCom and 23-86% ($P < 0.01$) with PGPRs compared to the noninoculated control. A higher root:shoot ratio is an important

morphological trait to support crop structure and enhance potential grain yield under nutrient-limited conditions, such as drought, low nitrogen and phosphorous availability (Anderson, 1988; Liu *et al.*, 2004; Chen *et al.*, 2022). Meanwhile, the differential effect sizes showed that the interaction of SynCom with resident microbial communities from low-fertility soils resulted in better growth promotion than PGPRs (Fig. S13). However, PGPRs interacting with the microbial community from high-fertility soil promoted better plant growth, such as root weight.

To test whether native SynCom performed better than PGPRs merely because of its higher microbial diversity, we prepared inoculants containing subsets of SynCom with only 12 and 4 species (S12, S4, and SF4, see **Materials and Methods**). With decreased taxonomic diversity from SynCom to S12 and S4, there was a decreasing trend but no significant difference in their effect on promoting root development at 10 days in low-fertility soil (Fig. S14). Notably, SF4, designed to be similar to PGPRs at both taxonomic and functional levels, including *Bacillus cereus* (phosphorus solubilization), *Lysinibacillus macrolides* (nitrogen fixation), *Stenotrophomonas maltophila* (IAA production and antimicrobial activity), and *Pseudomonas koreensis* (antifungal activity), did not outperform PGPRs in high-fertility soil or SynCom in low-fertility soil in promoting root length and root weight (Fig. S14). These findings demonstrated that the higher diversity of SynCom than PGPRs alone could not fully account for its success in promoting plant growth and health. The positive microbial interactions between SynCom and resident microbial communities in low-fertility soils could be critical to plant growth and health.

Discussion

Soil microbial inoculants for promoting crop productivity have been rapidly implemented since agricultural ecosystems are challenged by multiple environmental stresses associated with climate change and soil degradation (Singh, 2017). However, considerable challenges hinder the screening and development of microbial inoculants for the field (Kaminsky *et al.*, 2019). The efficacy of soil microbial inoculants remains unreliable and frequently depends on soil conditions (O'Callaghan, 2016; Hart *et al.*, 2018), with low performance in widespread low-fertility soils. Here, we constructed a native SynCom derived from common culturable species from maize rhizospheres in soils of varied fertility conditions. Native SynCom with agriculturally relevant traits, including nutrient facilitation, increased plant growth more effectively in low-fertility soil than in high-fertility soil. Specifically, compared

with commercial PGPRs, its home-field advantage potentially contributed to its success in field colonization, which ultimately enhanced soil biodiversity, enabled positive microbial interactions, and maintained a stable niche structure in low-fertility soil (Fig. 6).

Previous research on home-field advantages, particularly in the case of obligate symbiosis, has emphasized the role of varying soil conditions in mediating plant responses to arbuscular mycorrhizal fungi (Pankova *et al.,* 2014; Rua *et al.,* 2016). In conjunction with our results, this general importance of home-field advantage may be exemplified in the bacterial adaptation to the local soil environment. For example, some SynCom members, including *Pseudomonas* spp., *Enterobacter* spp., and *Chryseobacterium* sp., which were described as the core microbial taxa in the maize rhizosphere by host-mediated selection (Niu *et al.,* 2017), successfully colonized under nonsterile conditions. Subsequently, their colonization induced an increasing alpha diversity in a less diverse Ultisol. We speculated that the home advantage confers intrinsic environmental adaptability to reenter the soil they were isolated from. Thus, plants could receive unexpected bonuses with lower risks from these host-associated inhabitants, e.g., attracting beneficial microbes (Qiu *et al.,* 2019). Genetic features for successful plant colonization include functions related to carbon and nitrogen acquisition (de Souza *et al.,* 2019). Although the beneficial traits in native microbial communities were revealed by metagenomic analysis, more specific information targeting the SynCom species will be needed to confirm these beneficial traits. Further culture-independent single-cell techniques and metagenomics information will be conducive to rapidly deciphering the link between the microbial phenome and genome (Fierer *et al.,* 2014; Li *et al.,* 2022).

The primary obstacle for soil microbial inoculants is that resident soil communities compete with microbial inoculants for niches (Eisenhauer *et al.,* 2013), and nutrient resources (Yang *et al.,* 2017) and produce various antimicrobial metabolites (Chin-A-Woeng *et al.,* 2000). Niche breadth analysis provided more evidence for the home-field advantage of native SynCom. Specifically, SynCom inoculation increased the individual niche breadth and balanced the changes in niche overlap in low-fertility soil, implying a neutral disturbance to the resident community. Wider niches represent the metabolism of a broader range of resources, improving the efficiency of resource utilization in low-fertility soil (Xu *et al.,* 2022). Notably, applying multispecies consortia may result in more reliable survival than single strains across various environments (Gralka *et al.,* 2020). Our results showed that

reducing taxonomic and functional diversity did not cause an obvious loss in the efficacy of these native-sourced microbial assemblies. However, statistical analysis based on sequencing data limited the investigation into true interactions. Future experimental work is required to address how metabolic cross-feeding interactions introduced by the native SynCom-resident group continuum drive coexistence in complex environments.

Conversely, the generalized-type and fast-growing PGPRs encroached on the niche space of other species but were eventually eliminated and failed to colonize all soils. The short-lived failed invasions showed legacy effects on the niche stricture (Mallon *et al.,* 2018; Amor *et al.,* 2020), including shrinking niche breadths and increasing niche overlaps. These observations may be explained by the diversity resistance hypothesis that diverse communities are highly resistant to exotic microbial invasions due to complex interactions and intensified competition for niche space (van Elsas *et al.,* 2012). Meanwhile, the presence of protozoan predators or viruses controls the fast-growing species (Simek *et al.,* 1997). While nitrogen and phosphorus are typically abundant in conventional agricultural soils, the easily accessed carbon may not, and competed by microorganisms and roots, represents a cost to crops (Kaminsky *et al.,* 2019). In high-fertility soil, the legacy effect after inoculating PGPRs expanded the potential exploitation of noncompetitive resources to reduce potential competition (Pianka, 1974). However, in low-fertility soils, the limited nutrients and substrates are poorly matched for PGPRs, negatively impacting microbial establishment and growth.

An inevitable issue in developing effective inoculants for crops is the concern of human health risks. The market demand for PGPR is increasing annually on a global basis to reduce harmful chemical fertilizers and pesticides (Waltz, 2023). Nevertheless, since various bacterial genera have been used as commercial PGPRs, it is necessary to evaluate their potential pathogenicity before applying microbial products in agricultural practices (Keswani *et al.,* 2019). In addition, given the taxonomic and functional redundancy of native SynCom, future work will focus on maximizing the community-level functional outcomes with the simplest species combination. These noteworthy efforts will provide a fundamental understanding of the *in vitro* assembly of complex synthetic communities and targeted manipulation of crop microbiomes to achieve sustainable crop production (Gralka *et al.,* 2020; Maynard *et al.,* 2020).

In conclusion, our study showed that microbial inoculants composed of diverse species isolated from various soil types specifically boosted plant growth more than commercial PGPRs under nutrient-limited conditions. In addition to the beneficial traits, the home-field advantage critically contributes to the potentially robust colonization of the SynCom and the positive interactions between SynCom and the resident community. Commercial PGPRs, however, may have little beneficial or even reverse effects on the rhizosphere environment through legacy effects on niche structure and increasing potential competition with the resident community. Furthermore, field trial data beyond the vegetative growth stages of maize are needed to fully assess the benefits of native SynCom. Nonetheless, our findings highlight the home-field advantage of native microbes in synthetic biology and suggest avenues to effectively promote the sustainability of agriculture in the context of a changing world with increasing desertification and soil degradation.

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Author contributions

All authors contributed intellectual input and assistance to this study and manuscript. Y.L., D-B.M., and T.C. developed the original framework. M.J., J.D. and Y.L. contributed experiments and analysis. Y.L., M.J., D-B.M. and M.M.Y. wrote the manuscript with help from E.Y., J.Z. and T.C. All authors have reviewed and agreed with the manuscript.

Competing interests

None declared.

Data availability

The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive in BIG Data Center, Beijing Institute of Genomics (BIG), Chinese Academy of Sciences, under accession numbers CRA004483, CRA004483, and CRA004428, which are publicly accessible at http://bigd.big.ac.cn/gsa.

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Supporting Information

Figure S1-S14

Tables S1-S10

Methods S1 Solid-state ^{13}C nuclear magnetic resonance analysis

Methods S2 Soil physical and chemical properties

Methods S3 Bacterial cultivation and isolation

Methods S4 Identification of the isolated microbial strains

Methods S5 IAA concentration of rhizosphere microbial communities

Methods S6 Absolute quantification of 16S rRNA

Methods S7 Relative quantification of 16S rRNA

Methods S8 Whole metagenomic shotgun sequencing

Figure legends

Figure 1. Experimental greenhouse design and maize growth with SynCom inoculation. a Maize rhizosphere samples from the three agricultural soils were used for the isolation of culturable bacteria (①). Forty-seven bacterial isolates were obtained and twenty-one unique species were identified as common species across the three soil types, Mollisol, Inceptisol and Ultisol, as shown in the Venn diagram (②). Each strain was cultured independently and combined in equal proportions to create a native synthetic microbial community (SynCom). Maize seedlings at the V3 growth stage were transplanted into soils with SynCom inoculation, and each treatment had four independent biological replications. The experiment ended at the V8 growth stage. **b** Maize (Zhengdan 958) phenotype with microbial inoculations at 35 days (V4-V5 stage) and 56 days (V7-V8 stage) in Mollisol, Inceptisol and Ultisol. Scale bar, 10 cm. **c** Soil plant analysis development (SPAD) value of leaves and plant height between the SynCom treatment and CK at different developmental stages (35, 42, and 56 days). The symbol of Δ represents the differences between inoculated and inoculated treatments. The different letters in **c** indicate significant differences ($P < 0.05$) using multiple comparisons of nonparametric tests (Nemenyi test). n = 4 biological replicates of maize plants were evaluated. For the SPAD value, each plant was measured three times. In violin plots, the horizontal bars represent medians. The tops and bottoms of the boxes show the 75th and 25th percentiles, respectively.

Figure 2 High-quality genomes retrieved from soil metagenomes. a Absolute abundances of bin lineages across different soils. Data were obtained by absolute quantitative sequencing. The different letters in **c** indicate significant differences ($P < 0.05$) using multiple comparisons of nonparametric tests (Nemenyi test). Nonsignificant differences were not labeled. Data represent the means ± SEs. **b** High-quality genome bins of the native microbial community (completeness > 50%, contamination < 5%) were indicative of metabolic flexibility and the potential for phytohormone production. Selected metabolic pathways, including nitrogen metabolism and indole acetic acid (IAA) production, were represented by the name of the gene known to encode the protein enzyme by searching predicted

proteins against the KASS database. Other related cellular activities are listed. Pathways are displayed only if all or most genes of an operon involved in the same pathway/process are detected as present; if not, dotted lines are used. Colored circles alongside genes indicate that the bin assigned to that color (see legend below) encoded the gene. indCM, indole-3-acetamide; indPRY, indolepyruvate; indCH, indole-3-acetaldehyde; IAA, indoleacetate; SOD, superoxide dismutase.

Figure 3 a Experimental design and maize growth with PGPR inoculation. Four model plant growth-promoting rhizobacteria (PGPR) selected from strain banks were derived as commercial PGPRs. Green: *Rhizobium radiobacter*, red: *Stenotrophomonas rhizophila*, blue: *Burkholderia cepacia*, yellow: *Arthrobacter ilicis*. Each strain was cultured independently and combined in equal proportions. Maize seedlings at the V3 growth stage were transplanted into soils with different microbial inoculations, and each treatment had four independent biological replications. The experiment ended at the V8 growth stage. **b** Plant height of maize between the PGPR treatment and CK at different developmental stages (35, 42, and 56 days). n = 4 biological replicates of maize plants were evaluated. The symbol of Δ represents the differences between inoculated and inoculated treatments. **c** Secretion of IAA by rhizosphere microorganisms with different microbial inoculations. n = 3 rhizosphere soil samples were measured. The different letters in **b** and **c** indicate significant differences ($P < 0.05$) using multiple comparisons of nonparametric tests (Nemenyi test). In violin plots, the horizontal bars represent medians. The tops and bottoms of the boxes show the 75th and 25th percentiles, respectively.

Figure 4 Colonization of SynCom strains and dynamics of niche structure. a Shannon diversity of microbial communities in Mollisol, Inceptisol and Ultisol after inoculation with SynCom and PGPRs. n = 3 rhizosphere soil samples were measured. In box plots, the horizontal bars represent medians. The tops and bottoms of the boxes show the 75th and 25th percentiles, respectively. The different letters indicate significant differences ($P < 0.05$) using multiple comparisons of nonparametric tests (Nemenyi test). Nonsignificant differences were not labeled. **b** and **c** Relative abundance of ASVs highly matched to **b** SynCom and **c** PGPRs. The representative sequence of each ASV displays >98.7% sequence identity with the sequence of the full length of the 16S rRNA gene of each strain. The error bar represents the sd. Statistical analyses were performed by a paired Wilcoxon rank-sum test (* indicates $P < 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.001$). **d** Changes in niche breadths ($P < 0.05$)

with PGPR inoculation compared to CK. The phylogram was constructed using the NJ method and was colored at the phylum level. The relative abundance of bacterial taxa is shown in the 16S rRNA phylogenetic tree represented by the size of node.

Figure 5 Experimental design of the plant growth promotion test on axenic maize seedlings. a and **b** Sterile maize seedlings were grown in double-tube chambers for 15 days. S+P: SynCom+PGPRs. **c** Morphological traits of maize grown in double-tube chambers, including soil plant analysis development (SPAD) value, plant height, root weight, and root:shoot ratio of fresh weight. n = 6 biological replicates of maize plants were sampled, except for n = 2 and n = 5 for plants grown in Inceptisol and Ultisol with PGPR treatments, respectively. For the SPAD value, each plant was measured three times. The different letters in **c** indicate significant differences ($P < 0.05$) using multiple comparisons of nonparametric tests (Nemenyi test). In box plots, the horizontal bars represent medians. The tops and bottoms of the boxes show the 75th and 25th percentiles, respectively.

Figure 6 Differential effects of microbial inoculants on crop growth and resident microbial community under different fertility conditions. Left: Exotic microbial inoculants composed of commercial PGPRs convey the inherent conflicts in their efficiency and safety concerns. Various biotic and abiotic factors will influence the soil colonization of exotic inoculants, leading to failed colonization and undesired performance. However, the legacy effect will persistently influence the community's niche structure. In high-fertility soil with abundant labile carbon sources, PGPRs with stronger metabolic capacity expand the potential exploitation of noncompetitive resources (recalcitrant carbon) to reduce potential competition. **Right:** Native microbial inoculants composed of culturable species across varied fertility conditions possess a home-field advantage that specifically benefits plant growth in low-fertility soil. In the future, metagenomic sequencing combined with single-cell techniques will help to rapidly elucidate functional traits. Successful colonization contributes to positive microbial interactions, thus promoting plant growth through nutrient facilitation. On the other hand, PGPRs inoculated into low-fertility soil compete with the resident community for limited nutrients, thus increasing potential competition. The figure of root was downloaded from Figdraw (ID: TWTIYeedee).