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The effect of microbial inoculant origin on the rhizosphere bacterial community composition and plant growth-promotion

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Abstract

Aims Microbial inoculation has been proposed as a potential approach for rhizosphere engineering. However, it is still unclear to what extent successful plant growth-promoting effects are driven by the origin of the microbial inocula and which taxa are responsible for the plant-beneficial effects.

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Methods We conducted a microbial transplant experiment by using different microbial inocula (and nutrient controls) isolated from forest, soybean and tomato field soils and determined their effects on tomato plant biomass and nutrient assimilation in sterilized tomato soil. Rhizosphere bacterial communities were compared at the end of the experiment and correlative and machine learning analyses used to identify potential keystone taxa associated with the plant growth-promotion.

Results Microbial inoculants had a clear positive effect on plant growth compared to control nutrient inoculants. Specifically, positive effects on the plant biomass were significantly associated with microbial inoculants from the forest and soybean field soils, while microbial inoculants from the forest and tomato field soils had clear positive effects on the plant nutrient assimilation. Soil nutrients alone had relatively minor effects on rhizosphere bacterial communities. However, the origin of microbial inoculants had clear effects on the structure of bacterial community structure with tomato and soybean inoculants having positive effects on the diversity and abundance of bacterial communities, respectively. Specifically, *Streptomyces*, *Luteimonas* and *Enterobacter* were identified as the potential keystone genera affecting plant growth.

Conclusions The origin of soil microbiome inoculant can predictably influence plant growth and nutrient assimilation and that these effects are associated with certain key bacterial genera.

Keywords Microbial inoculation · Microbial transplants · Plant growth-promotion · Rhizosphere microbiota · Soil functioning · Diversity

Introduction

Plants have evolved an intimate association with their soil microbiome (Vezzani et al. 2018), which can confer various benefits to the plants such as suppression of bacterial and fungal diseases, promotion of plant growth and assimilation of important nutrient (Berendsen et al. 2012; Stringlis et al. 2018). In turn, plants invest a substantial part of their photosynthesized carbon into root exudates that attract and feed plant-beneficial and root-associated microbiota (Badri and Vivanco 2009; Bais et al. 2006). While interactions between the rhizosphere microbiome and plants play a key role in plant growth, it is still unclear how this process could be engineered to improve crop production and to restore microbial biodiversity in soils exposed to intensive agricultural practises. In this study, we experimentally tested to what extent bacterial plant-growth promotion is affected by the origin of microbial inoculant, and if certain taxa can be associated with the plant-beneficial effects.

Manipulation of the rhizosphere microbiome has great potential to increase agricultural production, which is vital for sustaining a steady supply of food in the face of a growing human population (Turner et al. 2013). Rhizospheric microbes can promote plant growth through a variety of mechanisms such as increasing the availability of nutrients, secreting phytohormones, suppressing pathogens or having positive effects on the plant metabolism (Perez-Montano et al. 2014; Zhou et al. 2015). While the introduction of single microbe or a synthetic mixture of few microbes can be effective for achieving desired microbiome functioning and plant growth-promoting effect (Lioussanne et al. 2010; Wei et al. 2015; Wubs et al. 2016), some studies have reported only limited success despite clear beneficial effects observed in simplified lab conditions (Kardol et al. 2008; Ryan and Graham 2018). This discrepancy could be due to various confounding factors. For example, some microbial inoculants might not be able to compete with the locally adapted native microbiome leading to low establishment success (Eisenhauer et al. 2013). It is also possible that some microbial inoculants could be too similar to the native microbiota being unable to

provide any new or complementary plant growth-promoting functions (Ambrosini et al. 2015). Lastly, some microbial inoculants may simply lack some important keystone species necessary for providing desired functions to the plant (Banerjee et al. 2018). Here we studied if it is possible to overcome these potential limitations by using highly diverse ‘microbiome’ transplants with different origins to improve plant growth and nutrient assimilation.

To this end, we isolated and created three different microbial inoculants originating from tomato and soybean field soils and subtropical forest soil ($N = 3$ for each soil type), and compared their effects on tomato (*Solanum lycopersicum*) growth relative to bacteria-free, nutrient control inoculants. Tomato plant (and soil) was chosen as a target transplant soil because continuous cropping of tomato often leads to reduced soil microbial diversity and changes in microbiome composition, which correlates with reduced crop yields (Pang et al. 2017). As a result, restoring tomato soil microbial diversity using microbial inoculants could offer an environmentally friendly strategy to engineer healthier plant rhizosphere microbiomes. Common sterilized rhizosphere soil of tomato was used for all microbial (and nutrient control) inoculations and differences in plant growth-promotion, bacterial abundances, bacterial community diversity and composition were measured at the end of the transplant experiment (5 weeks of tomato growth after inoculation). Correlative and machine learning analyses (Random forest analysis) were used to identify potential keystone taxa associated with tomato plant growth and nutrient assimilation. We hypothesized that the origin of microbial inoculant could have a considerable effect on the plant growth-promotion by potentially introducing different keystone taxa to the rhizosphere, while nutrient inoculants should have a relatively smaller effect. Furthermore, we expected that variation in plant growth-promotion should correlate with the abundance, diversity and community composition of rhizosphere bacteria potentially revealing important taxa associated with the plant-beneficial effects.

Materials and methods

Collection of soil samples for microbial inoculants

Three types of topsoils (0–20 cm) with different land-use histories (tomato or soybean field soil or subtropical

forest soil) were collected from different sites in Nanjing, China, for this study. Three independent soil samples were collected per site, and each independent soil sample (> 15 kg) consisted of 4 subsamples, which were pooled and homogenized for analyses to attain three independent replicates ($N = 3$) per sampling site. The physico-chemical properties of all soil samples were analyzed as described previously (Xun et al. 2015b), and results are reported in the Table S1. Before the extraction of microbial communities, all soil samples were wet-sieved (4 mm) to remove plant material, stored at 4 °C and used within one week of collection.

Preparation and application of microbial inoculants

Microbial inocula were prepared by mixing 350 g of soil (dry weight equivalent) from each independent sampling replicate with 700 mL of sterile Murashige and Skoog (MS) liquid medium (1% sucrose) on an orbital shaker at 120 rpm for 30 min at room temperature. After one hour of standing, microbes were separated into a supernatant fraction by centrifuging at $800\times g$ for 5 min. The resulting supernatants, that contained soil microbes and nutrients, were defined as the microbial inoculants (unfiltered inoculants). We also prepared filter-sterilized samples as controls to separate the effects mediated by microorganisms from the effects mediated by nutrients present in the soil filtrates (nutrient inoculant controls). To this end, microbial inoculants were further centrifuged at $10000\times g$ for 10 min and filtered (0.22 μm) to remove soil microbes. As a result, our experimental design consisted of microbial and nutrient inoculant controls from tomato field, soybean field and subtropical forest soils. We used sterilized gamma-irradiated (50 kGy) tomato field soil as a common target transplant soil (700 g of soil in pots with 11 cm diameter, 10 cm height), which was supplemented with 40 g of sterilized vermiculite to protect plant roots from mechanical damage when collecting rhizosphere soil samples at the end of the experiment. Tomato field soil was chosen as target transplant soil because it represents typical intensive monoculture farming in China with high fertilizer application rate, conventional management practices and two crop seasons per year as previously reported (Wei et al. 2019). Such soils often suffer from reduced microbial diversity, making them feasible targets for microbiome restoration using microbial inoculants (Moeskops et al. 2010; Tsiafouli et al. 2015). Target transplant soils were inoculated with 200 mL of all

microbial and nutrient inoculants, and additional negative control treatment was also established that received 200 mL of sterile MS liquid medium (1% sucrose). Each treatment consisted of three independent replicated pots resulting in a total of 21 pots. Inoculated pots were then let stand for 25 days at 25 °C in a plant growth chamber to allow the colonization and stabilization of microbiome (Vivant et al. 2013) before seeding with five germinated tomato seeds (*Solanum lycopersicum* cv. 'Hezuo 903') per pot that had been surface-sterilized and germinated as described previously (Gu et al. 2017). All pots were arranged in a randomized block design (re-arranged twice per week) and exposed to a light intensity of $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ with a 16:8 h light:dark photoperiod at 25 °C. All the pots were weighed and replenished with sterile deionized water three times per week to keep the soil moisture constant at 60% of the water holding capacity. Moreover, pots were fertilized with MS solution (50 mL) once per week. After five weeks of growth, five plants from each replicate pot were harvested to collect rhizosphere soil samples. The five-week time point was chosen based on previous studies, which have shown that soil bacteria can exert positive or negative effects on the plant growth at this stage of the tomato growth cycle (Gu et al. 2017; Nihorimbere et al. 2009; Tan et al. 2013). To collect rhizosphere soil samples, the excess soil was first removed from the plant roots by shaking and the remaining soil closely adhered to the roots (rhizosphere soil) was stored for further analyses. Rhizosphere soil samples collected from five plants per treatment within a replicate pot were pooled and stored at $-80 \text{ }^\circ\text{C}$ for DNA extraction and further sequence analyses.

Determining microbial inoculant effects on plant growth-promotion

The effects of microbial inoculants on the plant growth-promotion were determined at the end of the pot experiment as differences in plant biomass and nutrient concentration. The dry weight of plant roots and shoots was determined using a Sartorius BT25S balance (Sartorius, NY, USA) after heating for 30 min at 105 °C and drying at 60 °C. Plant biomass was assessed as the mean shoot and root dry weight for each treatment replicate pot. To determine plant P and K content, all subsamples per replicate pot (including shoot and root plant materials) were pooled, ground and passed through 0.5 mm mesh for a thorough homogenization. A 0.25–0.3 g replicate

pot subsample was then digested using $\text{H}_2\text{SO}_4\text{-H}_2\text{O}_2$ as follows. First, plant material was placed in a Kjeldahl flask and 5 mL concentrated H_2SO_4 was added. The contents were then digested on a block digester at about 365 °C for 1 h until the plant material had become dark brown. After a slight cooling (about 1 min), 10 drops of 30% (m/v) H_2O_2 were added. The contents were then heated again for 10–20 min, cooled and treated with H_2O_2 . This routine was repeated until the brown color disappeared and the solution became completely clear. The final solution was then dissolved to 100 mL of deionized water and allowed to stand for 3 h. The concentrations of P and K were measured directly in this solution. Plant P content was measured based on molybdenum-blue colorimetry (Thomas et al. 1967) and the whole plant K content was measured using atomic absorption spectroscopy (Varian Spectra AA 220 FS, Victoria, Australia). Plant trait data were then combined into a one quantitative index reflective of mean plant growth-promotion function as described previously (Bradford et al. 2014; Delgado-Baquerizo et al. 2016; Maestre et al. 2012). To this end, four measured plant growth traits (root biomass, shoot biomass, and plant K and P concentrations) were first standardized using the Z-score transformation and then averaged to obtain mean plant growth-promotion index.

Soil DNA extraction, 16S rRNA gene amplicon sequencing and data processing

Soil DNA was extracted from 0.25 g of rhizosphere soil subsamples using a PowerSoil extraction kit (MoBio Laboratories, CA, USA) following the manufacturer's protocol. The DNA extracts were quantified using a NanoDrop One/One C spectrophotometer (ThermoScientific, WI, USA). To analyze the composition and diversity of soil bacterial communities, the V4-V5 hypervariable regions of the 16S rRNA gene were amplified and sequenced using primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') (Beller et al. 2013) and PCR amplification was performed with adapters required for the Illumina sequencing platform, as described previously (Gu et al. 2016). The amplification products were purified using an AxyPrep PCR Clean-up Kit (Axygen, Hangzhou, China), quantified with a NanoDrop spectrophotometer and sequenced using Illumina MiSeq platform (Personal Biotechnology Co., Ltd., Shanghai, China).

Sequence data were processed using the UPARSE operating procedure (Edgar 2013). For each sample, sequence pairs were first merged and quality-filtered with a maximal expected error threshold of 1.0. After removing singletons, the sequence reads were clustered into operational taxonomic units (OTUs) based on 3% dissimilarity followed by filtration of chimeric reads using the UCHIME (Edgar et al. 2011). Samples were rarefied to the depth of the smallest sample (10,603 reads) and sequence reads and obtained OTU tables were analyzed using Mothur (Schloss et al. 2009). The representative sequences of each OTU were taxonomically classified using the Ribosomal Database Project classifier (Wang et al. 2007) using 80% confidence threshold. The bacterial community diversity of each sample was estimated using the non-parametric Shannon diversity index and bacterial community composition was clustered using the unweighted UniFrac distance metric (Lozupone et al. 2007). All raw sequence data have been deposited in the NCBI sequence read archive under the accession number PRJNA496246.

Quantitative PCR for determining bacterial abundances

Quantitative PCR (qPCR) was performed to compare differences in bacterial abundances at the end of the experiment based on the 16S rRNA gene abundances using primer pair 563F and 802R (Cardenas et al. 2010). All qPCR assays were performed in triplicate using the Applied Biosystems 7500 Real-Time PCR System (CA, USA) and the SYBR Premix Ex Taq Kit (Takara, Dalian, China). Each 20 μL reaction contained 10 μL SYBR Premix Ex Taq, 0.4 μL ROX Reference Dye II, 2 μL template DNA, 0.4 μL of each primer (10 μM) and 6.8 μL dH_2O . Thermocycling consisted of an initial denaturation at 95 °C for 30 S, followed by 40 cycles of 95 °C for 5 S and 60 °C for 34 S and eventually a dissociation stage (95 °C for 15 S, at 60 °C for 60 S and at 95 °C for 15 S). A standard for the 16S rRNA gene was generated by constructing a plasmid containing the 16S rRNA gene sequence from *Ralstonia solanacearum* strain QL-Rs1115, which is the dominant microorganism in tomato field soil used in this study (Wei et al. 2011).

Statistical analysis

We used Student's t test (SPSS v. 19) to compare differences between microbial inoculants treatments

and corresponding nutrient inoculants treatments. A factorial analysis of variance (ANOVA, Tukey's honestly significant difference test; SPSS v. 19) was used to compare the mean differences between inoculant treatments in relation with their origin and following dependent variables were analyzed in separate models: (1) bacterial community diversity, (2) total bacterial abundances, (3) root biomass, (4) shoot biomass and (5) plant P and (6) K concentrations. Due to non-normal data distribution of bacterial abundance data, log₁₀-transformed values were used. Statistically significant differences in microbiome composition were examined by analysis of molecular variance (AMOVA) (Schloss 2008) using Mothur. Multiple regression tree (MRT) analysis was conducted to determine the relative contribution of microbiome inoculation and the origin of the microbial inoculants on the composition of the rhizosphere bacterial community (R platform, mvpart and MVPARTwrap package). Mantel test (R platform, vegan package) and bivariate correlation (Pearson and Spearman correlation method; SPSS v. 19) were used to test relationships between plant growth traits, physico-chemical properties of donor soils and rhizosphere microbiome composition at the end of the pot experiment. Redundancy analysis (RDA) was performed using CANOCO (ETTEN 2005) to summarize correlations between plant growth traits (i.e., root and shoot biomasses and plant P and K concentrations), bacterial community characteristics (i.e., bacterial diversity, abundance, and the relative abundance of top 20 bacterial genera that accounted for 77.8% of the total bacterial sequences, that were considered as 'dominant' taxa) and the microbial inoculant treatments. Random forest (RF) analysis (Breiman 2001) was used to identify potential keystone taxa associated with plant growth-promotion using "randomForest" command in RF package (R platform). For this analysis, we used the relative abundances of top 10 bacterial genera, bacterial community diversity and total bacterial abundances to identify the main predictors for the mean plant growth-promotion (average Z-score based on all plant growth traits) between different microbial and nutrient inoculant treatments. The statistical significance and cross-validation of random forest model predictions (R^2 values) were tested using 1000 permutations of every given response variable (i.e., the relative abundance of top 10 bacterial genera, bacterial community diversity and total bacterial abundances) using "a3" command in A3 package (R platform).

Results

The effect of microbial inoculants on the plant growth and nutrient assimilation

Microbial inoculants originating from forest and soybean soil had clear positive effect on the root and shoot biomass of tomato relative to the MS medium control (Fig. 1a-b and Table S2) or corresponding nutrient inoculants controls (Fig. 1a-b and Table S2). In contrast, microbial inoculants originating from the tomato soil had no significant effect on the root and shoot biomass relative to the nutrient control treatments, mainly because the nutrient inoculant itself had a clear positive effect on tomato biomass (Fig. 1a-b and Table S2). Furthermore, relative to the nutrient control treatments, microbial inoculants from the forest and tomato soils had positive effects on the concentrations of P and K in tomato plant tissue. In contrast, microbial inoculant from the soybean soil had no significant effect (Fig. 1c-d and Table S2). Together these results suggest that the origin of microbial inoculant had a large positive impact on plant growth-promotion with forest topsoil having consistently positive effects on both tomato plant biomass and nutrient assimilation.

The effect of microbial inoculants origin on the rhizosphere bacterial community composition

The bacterial community composition of all target soils that received different microbial or nutrient inoculants formed seven distinct clusters ($p < 0.001$, AMOVA; Fig. 2a). Clear clustering was further observed based on the origin of microbial inoculants (Fig. 2a). Multiple regression tree analysis explained 65.9% of the total variability in rhizosphere bacterial community composition (Fig. 2b) and the composition of communities clearly differed depending if they had received microbial or nutrient inoculants ($p < 0.001$, AMOVA; Fig. 2a-b). The presence of microorganisms in the inoculant had the largest deterministic effect accounting for 47.66% of the total variation of rhizosphere microbiome assembly, while the origin of microbial inoculants explained 18.23% of the variation. Moreover, a significant positive correlation ($r = 0.90$, $p = 0.001$, Mantel test) was found between physico-chemical properties of the inoculant donor soils and the rhizosphere bacterial community composition of the target soils. All bacterial communities had higher diversity (Shannon diversity index)

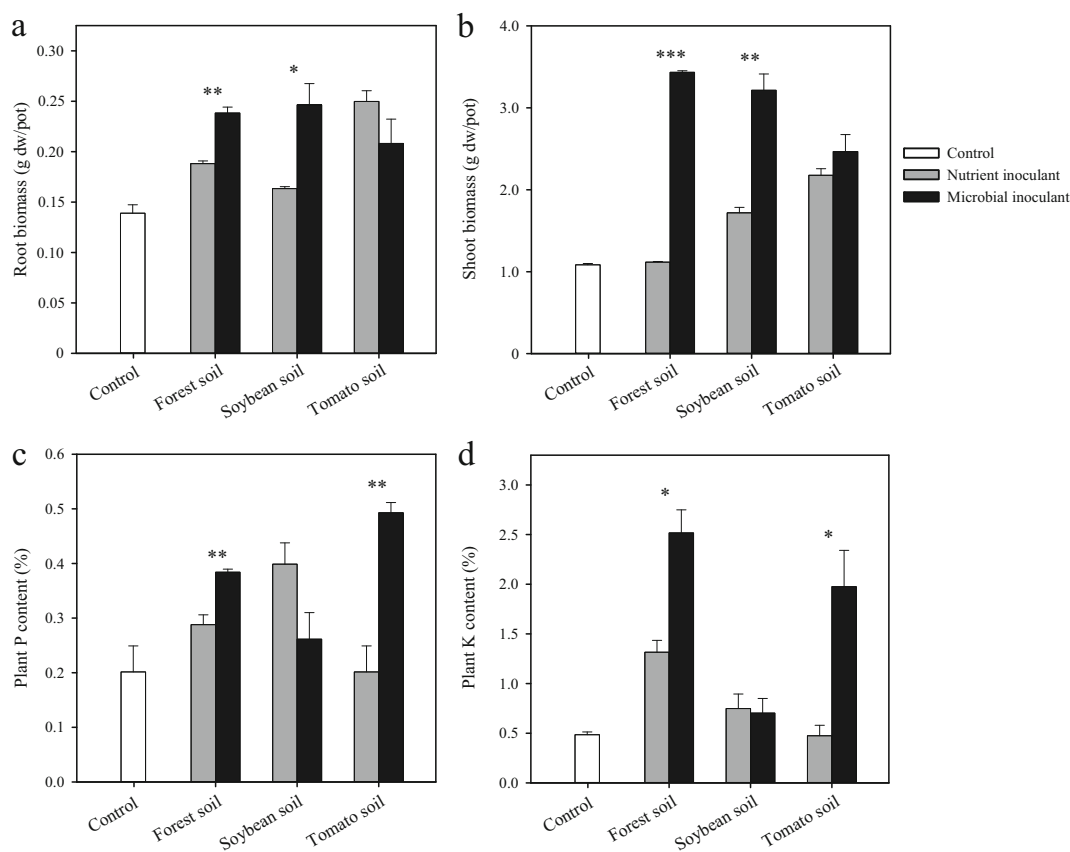


Fig. 1 Effects of microbial (unfiltered sample containing both soil nutrient and microbes; black bars) and nutrient inoculants (filtered sample containing only soil nutrient; dark grey bars) on plant growth-promotion of tomato (MS medium controls are shown on white). Different panels show the effect of microbial inoculants on

root biomass (a), shoot biomass (b), P content (c), and K content (d). In all panels, bars indicate means ± 1 standard error and asterisks above the bars indicate significant differences between microbial inoculant and corresponding nutrient inoculant treatments (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; Student's t test)

when they had received microbial inoculants compared to negative MS medium or nutrient inoculant controls (Fig. 3a and Table S3). Bacterial community diversity was the highest when target soils had received microbial inoculants from the tomato soil (Fig. 3a and Table S3). In contrast, only the microbial inoculant from soybean soil had a positive effect on total bacterial abundances relative to nutrient control treatment (Fig. 3b and Table S3). Furthermore, we found that 42.7% of all OTUs were shared between all microbial inoculant treatments and most of the shared OTUs belonged to Proteobacteria, Actinobacteria, Bacteroidetes and Gemmatimonadetes phyla, while 31.2% of OTUs were unique to each microbial inoculant treatment (Fig. S1). In contrast to the MS medium control and nutrient inoculants controls, all three microbial

inoculants treatments exhibited higher relative abundance of Deltaproteobacteria and other and unclassified bacteria at the end of the experiment (Fig. 3c and S2). Soils that received microbial inoculants from the soybean and tomato soils had lower relative abundances of Firmicutes relative to corresponding nutrient inoculants controls. In contrast, soils that received microbial inoculants from the forest and tomato soils had lower relative abundances of Gammaproteobacteria (Fig. 3c and S2), while soils that received microbial inoculants from the forest soil had higher relative abundance of Actinobacteria relative to the nutrient control treatment. Together these results suggest that the origin of microbial inoculant affected both the taxonomic composition and total bacterial abundances of target tomato rhizosphere soil bacterial communities.

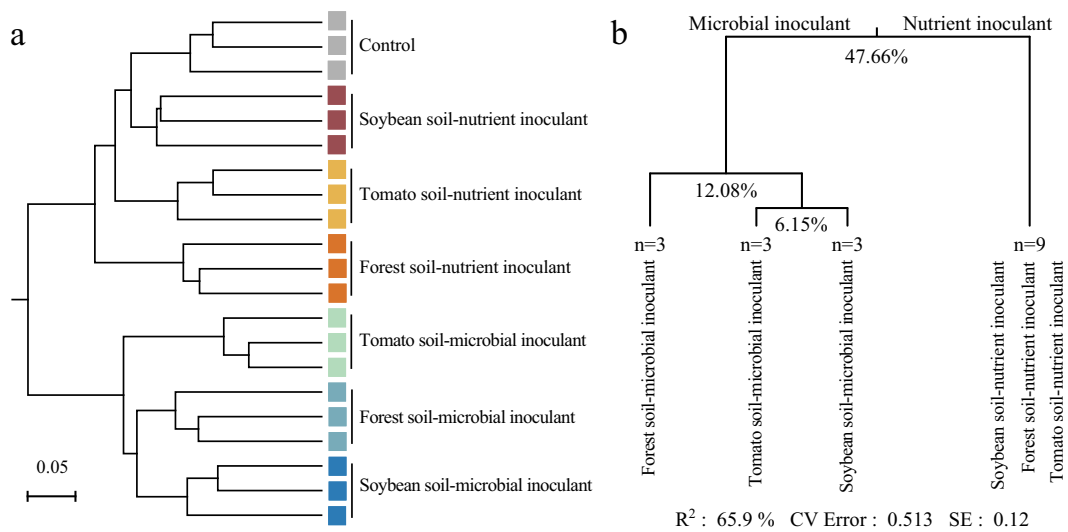


Fig. 2 Effects of microbial and nutrient inoculants on bacterial community composition in the tomato rhizosphere. (a) Clustering of variation in bacterial community composition dispersion by microbial and nutrient inoculants (scale bar denotes for 5% similarity) based on unweighted UniFrac metric. (b) Multivariate

regression tree analysis comparing the effects of nutrient and microbial inoculants originating from different origins on bacterial community composition. The percentage of total variation explained is denoted under the different branches and n denotes the number of independent replicates within each cluster

Identification of candidate bacterial taxa associated with plant growth-promotion

All plant characteristics linked with growth-promotion (shoot and root biomass and plant K and P concentrations) were significantly affected by the rhizosphere microbiome composition ($r = 0.72$, $p = 0.004$, Mantel test). To explore this in more detail, we used redundancy analysis to correlate microbial inoculant origin with the bacterial community composition and plant characteristics linked with growth-promotion. Microbial inoculants had positive effects on the plant growth by influencing the relative abundances of the top 20 bacterial genera that accounted for 77.8% of the total bacterial sequences (Fig. 4a). For example, an increase in plant shoot biomass was linked with high relative abundances of *Bordetella*, *Singulisphaera* and *Streptomyces* genera, which were more abundant in the forest and soybean microbial inoculants. In contrast, negative correlations were found between tomato shoot biomass and *Gemmatimonas*, *Sphingomonas*, *Gp3*, *Luteimonas* and *Rhizomicrobium* bacterial genera, which were more abundant in the tomato soil compared to other microbial inoculants. Similarly, high plant P and K concentrations were linked with high relative abundances of *Dyella*, *Burkholderia*, *Streptophyta*, *Enterobacter* and *Arachidicoccus* bacterial genera, which were relatively more abundant in the forest and tomato microbial

inoculant treatments. Additionally, both the bacterial abundances and community diversity were positively correlated with plant K and P concentrations in general ($p < 0.05$, Spearman correlation). To identify the most important bacterial taxa associated with plant growth-promotion in microbial inoculant treatments, we used random forest analysis based on the relationships between relative abundance of top 10 bacterial genera, total bacterial abundances, bacterial community diversity and average z-score summarizing all plant growth-promotion traits. Based on this analysis, we found that *Streptomyces* (Actinobacteria), *Luteimonas* (Gammaproteobacteria) and *Enterobacter* (Gammaproteobacteria) genera were most significantly associated with the plant growth (Fig. 4b) having positive ($r = 0.758$, $p = 0.018$, *Streptomyces*; $r = 0.802$, $p = 0.009$, *Enterobacter*) and negative ($r = -0.717$, $p = 0.03$, *Luteimonas*) effects. We also conducted another random forest analysis to compare the relative significance of nutrient and microbial inoculants treatments. We found that bacterial diversity was generally better at predicting plant growth-promotion instead of any specific taxa and that this effect was much clearer when the inoculants included microbes relative to nutrient control treatments (Fig. S3). This analysis confirms that the addition of nutrients played only a minor role in plant growth-promotion compared to microbial inoculants, and that both bacterial species identities and community

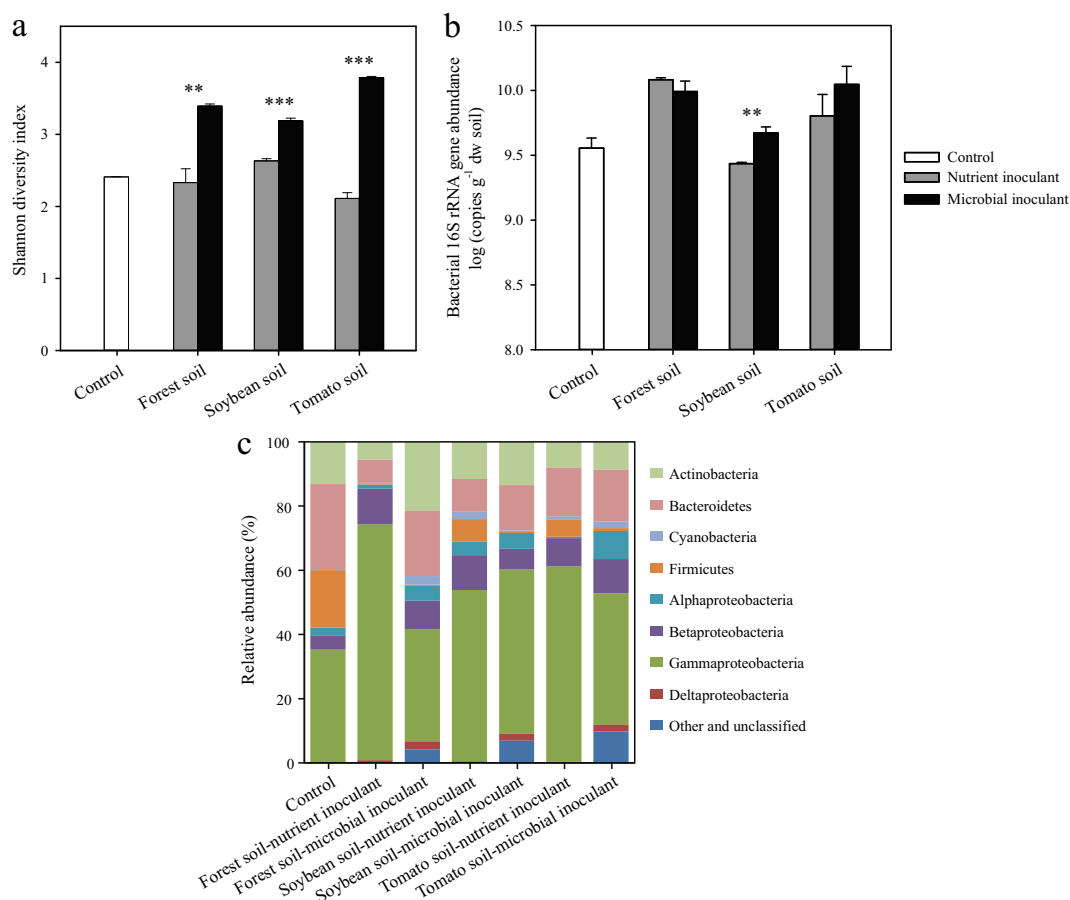


Fig. 3 Effects of microbial (black bars) and nutrient inoculants (dark grey bars) on Shannon diversity index (a), the total bacterial abundances (b) and relative abundances of the major bacterial phyla (c) in the tomato rhizosphere (MS medium controls are shown on white). In panels a and b, error bars indicate means \pm 1

standard error and asterisks above the bars indicate significant differences between microbial inoculant and corresponding nutrient inoculant treatments (**, $p < 0.01$; ***, $p < 0.001$; Student's t test)

diversity were important in explaining plant growth-promotion.

Discussion

Here we set out to study to what extent bacterial plant-growth promotion is affected by the origin of microbial inoculant and which taxa can be associated with the plant-beneficial effects. We found that community assembly was heavily affected by the origin of microbial inoculant, which suggests that plant selection was not strong enough to drive convergence in microbiome composition. Instead, the assembly of bacterial communities was clearly affected by the origin of microbial inoculants, which in turn affected the magnitude of plant

growth-promotion in terms of plant biomass and nutrient assimilation. Specifically, *Streptomyces*, *Luteimonas* and *Enterobacter* were identified as potential keystone taxa having both positive and negative effects on the plant growth. Together, these results suggest that microbial community inoculants can predictably promote plant growth and that this effect depends on the presence of specific bacterial taxa.

Plants can recruit or repress distinct microbial groups by producing and secreting root exudates thereby shaping the structure and function of rhizosphere microbial community (Mendes et al. 2014; Sasse et al. 2018; Zhahnina et al. 2018). However, this host-mediated filtering might also depend on the composition of the rhizosphere microbial community and the presence of certain plant-beneficial microbes. We found that the

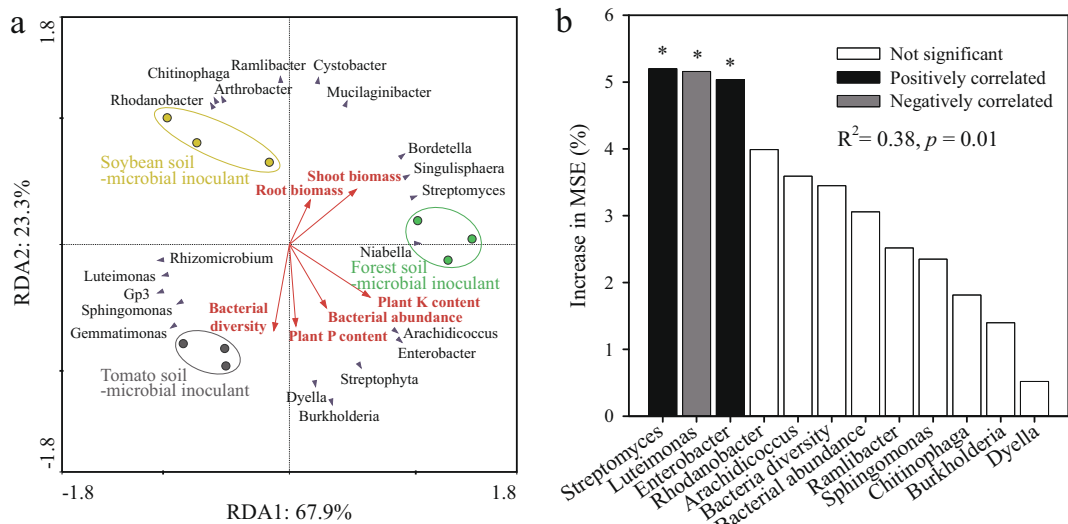


Fig. 4 Associations between plant growth-promoting traits and bacterial community composition of tomato rhizosphere soils amended with different microbial inoculants. (a) Redundancy analysis summarizing correlations between plant growth traits and the genera abundances between different inoculation treatments (top 20 genera were included in the redundancy analysis). The percentage of the total variation explained is indicated on the

axes and red arrows show the correlation (angle) and magnitude (length) of the effect on plant growth traits. (b) Random forest model results showing the mean predictor importance (percentage of increase in mean square error (MSE)) of bacterial taxa for plant growth. Different bar colors denote the direction of effects and asterisks above the bars indicate the significance levels (*, $p < 0.05$)

origin of microbial inoculant had clear effects on plant growth-promotion. Specifically, positive effects on the plant biomass were significantly associated with microbial inoculants originating from the forest and soybean field soils, while microbial inoculants from the forest and tomato field soils had clear positive effects on the plant nutrient assimilation. Soil nutrients alone had relatively minor effects on rhizosphere bacterial communities. Interestingly, the forest soil had the strongest positive effect on both plant biomass and nutrient assimilation. One explanation for this could be that bacteria habitating unmanaged forest soil are equipped with high enzymatic activity because the nutrient sources could be highly variable and hard to sequester compared to agricultural tomato and soybean soils that regularly receive chemical fertilization (Bandick and Dick 1999; Knight and Dick 2004). In support for this, it has previously been reported that forest soil microbiome can show a higher catabolic activity compared to arable soil microbiomes (Creamer et al. 2016). We also found that forest soil had relatively higher organic matter and carbon contents relative to the other soils (Table S1), which could favor microbes with

higher enzymatic and catabolic activity. Non-agricultural natural soils could thus be good potential sources for microbial inoculants.

All bacterial communities had higher bacterial community diversity when they had received microbial inoculants compared to negative MS medium or nutrient inoculant controls. Moreover, bacterial community diversity was the highest when target soils received inoculants from the tomato soil, while only the microbial inoculant from soybean soil had a positive effect on total bacterial abundances relative to nutrient control treatment. Together, these results suggest that plant-mediated recruitment of microbial communities depends on the diversity and composition of the communities they are initially surrounded with. Mechanistically, this could be for example be due to differences in bacterial ability to exploit the available niche space during the initial colonization (Xun et al. 2015a) and potentially prevent the colonization of late-arriving species via priority effects (Hiscox et al. 2015; Sprockett et al. 2018). Even though even the sterile MS medium control soils reached relatively high and diverse bacterial communities likely because of airborne bacterial migration from inoculant treatments or due to recovery from a dormant pool of spores (Barberán et al. 2015;

Delmont et al. 2014; Setlow 2007; Zarraonaindia et al. 2015), the presence of microbes in the inoculant accounted for the majority of the total variation in rhizosphere microbiome assembly.

To study the role of specific bacteria in more detail, we performed correlative and random forest analyses to statistically identify bacterial taxa that were significantly associated with for the plant growth-promotion. We found that *Streptomyces*, *Enterobacter* and *Luteimonas* were the major taxa affecting plant growth, which was positively correlated with the relative abundances of *Streptomyces* and *Enterobacter* and negatively correlated with the relative abundances of *Luteimonas*. Even though our study did not explore microbiome functioning at the level of metagenomes, culturable members of *Streptomyces* and *Enterobacter* have been shown to promote plant growth via various mechanisms including indoleacetic acid and siderophore production and phosphate solubilization (Palaniyandi et al. 2014; Sadeghi et al. 2012; Shoebitz et al. 2009; Taghavi et al. 2010). However, the role of *Luteimonas* in plant growth remains largely unexplored. Moreover, we found that *Dyella*, *Burkholderia*, and *Arachidococcus* genera were linked with high plant P and K contents, which is in line with previous studies where culturable members of these genera have been shown to be involved in K and P solubilization (Madhaiyan et al. 2015; Palaniappan et al. 2010; Zhang et al. 2011). Lastly, *Bordetella* and *Singulisphaera* genera were found to be linked with high shoot biomass. However, their role in plant growth-promotion is not known. Further studies are thus needed to unravel the specific mechanism by which rhizosphere microbiota alter the plant growth and to experimentally verify the causal role of these genera for the plant growth-promotion. This can hopefully be achieved by employing non-destructive repeated sampling of plants (Wei et al. 2019) and molecular approaches such as sequencing and stable isotope probing approaches combined with DNA and RNA biomarkers (Haichar et al. 2012; Vandenkoornhuys et al. 2007). It is also important to note that we only focused on bacterial community in this study and many other important soil microbes, such as arbuscular mycorrhizal fungi, are important for soil functioning and plant growth-promotion (Pellegrino et al. 2012; Van der Heijden et al. 1998). In the future, it would be interesting to identify plant growth-promoting microbes across multiple kingdoms, which could allow manipulation of the soil microbiomes at the level of multi-trophic communities (Banerjee et al. 2018).

Selection due to shared environmental or plant-mediated conditions has previously been reported to lead to convergence of microbial community structure (Kurtz et al. 1998; Langenheder and Székely 2011; Scheuerl et al. 2020). Unfortunately, we were unable to characterize the initial community composition of each microbial inoculant donor soil, which allowed us only to compare the changes between nutrient and microbial inoculants at the end of the experiment. As a result, further studies are needed to compare how the original composition of microbial inoculant affects the assembly of rhizosphere microbiome, and if the potential, plant-mediated, ‘host-filtering’ depends on the plant species or cultivar. Furthermore, more work is needed to understand to what extent existing native microbiota or physico-chemical soil properties affect establishment of inoculated microbes (Kardol et al. 2008). For example, we found a significant correlation between physico-chemical properties of the donor inoculant soils and the rhizosphere bacterial community composition of the target soils and clear differences in the contents of organic matter, nitrogen and carbon contents between different donor soils (Table S1). A systematic comparison of the roles of microbial community composition and the physico-chemical properties of the donor and target soils (Calderon et al. 2017; Xun et al. 2015a) would thus potentially help in improving the establishment success and efficacy of microbial inoculants.

Conclusions

Here, we show that the origin of microbial inoculants can have a significant effect on plant growth-promotion in terms of plant biomass and assimilation of key nutrients. Furthermore, our results suggest that these effects are driven by specific microbial taxa that could be important for soil functioning due to their keystone role. Specifically, microbial inoculants from the forest soil showed good potential for microbiome restoration by improving both tomato plant biomass and nutrient contents. In the future, it will be important to build a better picture about the underlying molecular mechanisms behind plant growth-promotion by microbial communities, and to better understand to what extent the microbiome assembly in the target soil is affected by the interaction between donor community inoculant composition, host plant type and variability between

soil physico-chemical properties between donor and target soils.

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Compliance with ethical standards

Conflict of interests All authors declare no conflict of interest.

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