



RESEARCH ARTICLE

Synthetic community improves crop performance and alters rhizosphere microbial communities

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Abstract

Introduction: Harnessing synthetic communities (SynCom) of plant growth-promoting (PGP) microorganisms is considered a promising approach to improve crop fitness and productivity. However, biotic mechanisms that underpin improved plant performance and the effects of delivery mode of synthetic community are poorly understood. These are critical knowledge gaps that constrain field efficacy of SynCom and hence large-scale adoption by the farming community.

Material & Methods: In this study, a SynCom of four PGP microbial species was constructed and applied to either as seed dressing (treatment T1, applied at the time of sowing) or to soil (treatment T2, applied in soil at true leaf stage) across five different cotton (*Gossypium hirsutum*) cultivars. The impact of SynCom on plant growth, rhizosphere microbiome and soil nutrient availability, and how this was modified by plant variety and mode of applications, was assessed.

Results: Results showed that the seed application of SynCom had the strongest positive impact on overall plant fitness, resulting in higher germination (14.3%), increased plant height (7.4%) and shoot biomass (5.4%). A significant increase in the number of flowers (10.4%) and yield (8.5%) was also observed in T1. The soil nitrate availability was enhanced by 28% and 55% under T1 and T2, respectively. Results further suggested that SynCom applications triggered enrichment of members from bacterial phyla Actinobacteria, Firmicutes and Cyanobacteria in the rhizosphere. A shift in fungal communities was also observed, with a significant increase in the relative abundance of fungi from phyla Chytridiomycota and Basidiomycota in SynCom treatments. A structural equation model suggested that SynCom directly increased crop productivity but also indirectly via impacting the alpha diversity of bacteria.

Conclusion: Overall, this study provides mechanistic evidence that SynCom applications can shift rhizosphere microbial communities and improve soil fertility, plant growth, and crop productivity, suggesting that their use could contribute toward sustainable increase in farm productivity.

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KEYWORDS

crop productivity, microbial community, seed dressings, synthetic community

1 | INTRODUCTION

Crop performance is strongly driven by biotic and abiotic environmental factors. For example, microbial pathogens can reduce plant fitness and productivity significantly. Likewise, the absence of key beneficial microbes can limit the bioavailability of key nutrients including nitrogen (N), phosphorus (P), and sulphur (S) which are essential for plant growth and productivity (de Souza et al., 2020; Oldroyd & Dixon, 2014; Singh & Trivedi, 2017). Harnessing beneficial microbes that promote plant fitness by increasing nutrient supply and use efficiencies is fast emerging as a complementary approach to traditional use of chemical fertilisers (Singh & Trivedi, 2017; Tabassum et al., 2017). Plant growth-promoting rhizobacteria (PGPR) applications can exert multiple plant-beneficial functions such as phytohormone provision, nutrient solubilisation (Mukherjee et al., 2020; Trivedi et al., 2011). PGPR-mediated plant performance and fitness often result in improved resistance to abiotic and biotic stresses (Batista & Singh, 2021; Mukherjee et al., 2020; Qiu et al., 2019; Trivedi et al., 2011).

Despite potential benefits, the adoption of PGPR tools in agriculture remains below expectation mainly due to inconsistent field efficacy (Batista & Singh, 2021; Qiu et al., 2019). In many cases, microbial inoculant products that contain single species either fail to colonise plant in field conditions or do not provide expected benefits (Dakora, 2003; Korir et al., 2017; Qiu et al., 2019). Conversely, it is increasingly recognised that the use of synthetic microbial communities (SynComs) can overcome some of these challenges (Trivedi et al., 2021). SynComs include multiple microbial species which can grow together and provide collective benefits to the plant host. Synergistic interactions between compatible but functionally diverse microbial strains can lead to better adaptation to new environments and simultaneously offer a broader range of plant growth-promoting functional traits to improve plant phenotypes and ultimately enhance crop productivity (Hays et al., 2015; Parnell et al., 2016; Pereg & McMillan, 2015; Singh & Trivedi, 2017). However, our understanding of the mechanisms that underpin these positive impacts on plant fitness remains poor, constraining the development and adoption of effective SynCom tools. Indeed, SynComs can affect plant performance directly by providing nutrients, phytohormones, and/or resistance against pathogens, or indirectly, by altering soil physicochemical status/modifying physiology and immune response of host plant, and composition of plant microbiome (Pereg & McMillan, 2015; Trivedi et al., 2021). For example, recent studies in pepper and tomato have highlighted positive impacts of PGPR on rhizosphere microbiome assembly and richness indices, with increases in the abundance of key plants beneficial microbes (Zhang et al., 2019). It is also reported that different plant

genotypes harbour different microbiomes, however, it is not known how genotype-linked plant microbes will respond to introduced SynCom inoculants and consequences for host functions (Hamonts et al., 2018; Qiao et al., 2017). However, the relative contribution of different processes (e.g., nutrient provision and recruitment of beneficial microbial taxa) that collectively underpin SynCom effects is poorly understood.

Timing and mode of application can also have impacts on microbial inoculant efficacy. For example, an early stage inoculation such as seed dressing could provide an advantage (i.e., priority effect) for beneficial microbial colonisation in the rhizosphere and root endosphere (Qiu et al., 2019). On the other hand, direct soil application has a logistic advantage as it can be integrated in irrigation or fertilisation systems, although, in late application, effective colonisation of plant roots by inoculants may be constrained by competition from indigenous microflora (Y. Liu et al., 2021; Qiu et al., 2019). However, experimental evidence on the impact of timing and mode of delivery on the efficacy of inoculants remain an important knowledge gap which constrains our ability to develop the best possible application approach (Backer et al., 2018; Trivedi et al., 2021).

Cotton (*Gossypium hirsutum*) is a major cash crop of the world (USDA, FAS, 2018) that requires high fertiliser inputs and irrigation to maintain productivity. However, excessive use of fertilisers can have significant negative consequences for environmental sustainability, particularly water pollution and nitrous oxide emissions (Berg, 2009; Pereg & McMillan, 2015). There is an increasing demand for a sustainable farming practice that reduces the usages of agrochemicals while maintaining/increasing farm productivity (Basu et al., 2021; Batista & Singh, 2021; Pereg & McMillan, 2015). Microbial-based biological solutions, especially in form of SynComs, are considered among the most promising approach to address these challenges but experimental evidence remains limited (Basu et al., 2021; Trivedi et al., 2021; Uzoh & Babalola, 2018).

In this study, we aimed to evaluate the ability of a bacterial SynCom (i.e., a combination of bacteria harbouring different plant-beneficial and synergistic traits) to promote cotton growth and productivity and to assess the SynCom effect on rhizosphere microbial community structure. Additionally, the effect of inoculation mode was assessed by comparing seed-dressing (i.e., SynCom application at the time of sowing) and soil treatment (i.e., SynCom application at 4-week growth stage) on plant yield-related parameters. We hypothesised that: (1) SynCom application would enhance plant growth and yield, with seed-dressing application having a stronger impact on yield compared to soil application due to priority effect; and (2) the impact of SynCom application on crop performance will be partially explained by a shift in rhizosphere microbial structure.

2 | MATERIAL AND METHODS

2.1 | Experiment setup, SynCom-treatment preparation, and delivery

Four PGPR (Table 1) were carefully selected from a laboratory library of microbes isolated from a cotton farm, based on their PGP traits (i.e., indole acetic acid [IAA] production, phosphorus [PO]-solubilisation, ammonium [NH₃] production, and *Fusarium oxysporum* f. sp. *vasinfectum* biocontrol properties; Table 1) and *in planta* growth-promoting effects. Before preparation of the SynCom treatment, the compatibility of the microbial candidates was confirmed using a synergistic assay following the method from a previous study (Berendsen et al., 2018), whereby the isolates were considered compatible when no growth inhibition was observed when grown on the same plate. For the inoculum preparation, each isolate was revived from the glycerol stocks on NA (Nutrient Agar) and transferred separately into 20 ml of nutrient broth by loop transfer then incubated at 28°C for 48 h at 100 rpm (RATEK, Rowe Scientific Pty Ltd.). Bacterial cells were harvested by centrifugation at 4000g for 10 min, then resuspended in 20 ml sterilised saline water (0.8% NaCl).

A pot experiment was set up at Western Sydney University, Richmond Australia (33°36'37.3"S 150°44'48.9"E) to examine the impacts of SynCom on plant growth, yield, soil nutrient availability and rhizosphere microbial communities. Three treatments were applied to five cotton cultivars in triplicates, and consisted of: (1) C-uninoculated control (2) T1-SynCom seed dressing at time of sowing, and (3) T2-SynCom-soil treatment at true leaf stage. Five cotton cultivars encompassing the most widely cultivated varieties of Australia were used in this study and included V1-CIM448, V2-Sikora, V3-CS50, V4-DP16, V5-Sicot BRF71 to examine cultivar-dependent response to SynCom treatments. Briefly, 60 seeds per cultivar were sterilised with 70% ethanol for 30 s and rinsed with sterile distilled water, followed by a 3% sodium hypochlorite solution treatment for 3 min, with regular shaking by hand (Yao et al., 2010). Seeds were then thoroughly washed with sterilised distilled water. For the SynCom seed dressing application (T1), seeds were soaked for 2 h in 20 ml of SynCom suspension (consisting of equal concentration of four bacteria, with an adjusted concentration of 10⁸ CFU per ml by checking OD at 600 nm on

Nanodrop™), whereas control seeds were soaked in sterilised saline water.

The experiment was conducted during the cotton growing season for 24 weeks (October 2018–April 2019) under outdoor conditions. Sandy loam soil (pH 6.9) was collected (0–10 cm) from an adjacent field that had a history of canola, cotton, and vegetable cultivation. Each pot was 15 cm in diameter and 40 cm deep and pots were kept 20 cm apart from each other. A common fertiliser (Yates "Thrive Soluble All Purpose Plant Food" with NPK ratio-25:5:8/kg) was applied at a rate of 80 kg/ha. Yates Lime Sulphur (200 g/L Sulphur) was applied at monthly intervals to control for mites/other pests as per manufacturer instructions. Before seed sowing, pots were irrigated to 60% field capacity and then daily manual watering was performed. Watering was withheld during rainy days. Seed sowing was performed by adding six seeds per pot at a depth of 3 cm, with an additional 5 ml of SynCom suspension added in T1 pots, while the control and T2 pots received the same amount of sterilised water.

Thinning was performed at the 12th day after sowing (DAS) with three plants maintained in each pot. Treatment T2-SynCom soil treatment was applied at the true-leaf stage (TL-stage; leaves developed from the cotyledons or up to 4-week stage), whereby the soil of each pot was drenched with 25 ml of the SynCom suspension (10⁸ CFU/ml adjusted by following the same protocol as seed dressing treatments was prepared) (Jetiyanon & Kloepper, 2002; Kumar & Gera, 2014; Myresiotis et al., 2012; Niknam & Dhawan, 2003), while the control and T1 pots received the same amount of sterilised saline water.

2.2 | Plant measures and soil nutrient parameters

Germination rate was recorded until the 12th DAS. Plant height (up to tip of main stem), root length at TL (4-week) and harvest stage (24-week) and fresh/dry biomass were measured at the TL stage, a number of flowers were counted, and cotton bolls were picked on maturation (when buds were fully opened) and seed cotton (lint + seed) weight was recorded as a measure of crop productivity.

For soil analyses, rhizosphere soil samples were collected at TL and flowering stages. At each sampling point, one cotton plant from each pot was carefully uprooted from the soil. Soil tightly attached to the roots (i.e., rhizosphere soil) was collected for DNA extraction in

SynCom PGPR candidates	PGP traits	FOV inhibition	Compatibility	Biofilm formation	Accession no. NCBI
<i>Arthrobacter</i> sp.	IAA	+	+	+	MH680887
<i>Enterobacter</i> sp.	IAA, PS, Ammonia	+	+	+	MT158576
<i>Brevibacterium</i> sp.	Ammonia	+	+	+	MH680885
<i>Plantibacter</i> sp.	PS	+	+	+	MH680891

TABLE 1 Detail of PGPR candidates of SynCom

Abbreviations: FOV, field of view; PGPR, plant growth-promoting rhizobacteria.

2 ml sterilised microcentrifuge tubes and immediately stored at -20°C until further analysis. Extractable nutrients ammonia $[\text{NH}_4^+]$, nitrate $[\text{NO}_3^-]$ and phosphorus $[\text{PO}_4^{3-}]$ in bulk soil samples were also determined at TL (4-week) and flowering (12th week) stage. These stages were considered as the most actively growing stages. Ammonia $[\text{NH}_4^+]$ and NO_3^- were measured following extraction from fresh soil with 2 M KCl (Keeney & Nelson, 1982) and determined on SEAL AQ2 (SEAL Analytical). Extractable soil phosphorus (P) was determined spectrophotometrically (880 nm) following extraction in 0.5 M sodium bicarbonate (Olsen et al., 1954) and PO_4 was determined on SEAL AQ2 Analyzer following acidification with 12 N sulphuric acid.

2.3 | DNA extraction from rhizosphere soils

Total genomic DNA was extracted from ~ 200 mg of rhizosphere soil using DNeasy PowerSoil Pro Kit (Qiagen), following the manufacturer's instructions. Extracted DNA was quality checked by NanoDrop 2000 (Thermo Fisher Scientific), and PCR checked to confirm the amplifiability. Amplicon sequencing was performed using 799F/1193R targeting 16S rRNA for bacteria and ITS2 region FITS7-ITS4R (Hamonts et al., 2018; H. Liu et al., 2019; Qiu et al., 2020) at the Next-Generation Sequencing Facility, Western Sydney University, Richmond, NSW Australia.

2.4 | Microbial community amplicon sequence analysis

Raw sequence data obtained were processed using Mothur standard operating procedure (Schloss et al., 2009). Briefly, forward and reverse sequences were merged into contigs. Sequences that contained unidentified bases or had greater than eight homopolymers were filtered out. Bacterial sequences were aligned against Silva 16S rRNA gene database version 132 (Pruesse et al., 2007). Aligned bacterial sequences and unaligned fungal sequences were pre-clustered at cutoff of $\text{diffs} = 1$ and 2, respectively, before chimera were identified and removed using UCHIME (Edgar et al., 2011). Additionally, singleton was removed to reduce read error (Reeder & Knight, 2009). Bacterial and fungal sequences were then taxonomically classified according to the Silva database version 132 and UNITE database version 8, respectively, with 60% cutoff confidence and sequences that match cotton mitochondria, chloroplast, archaea (bacteria), and host ITS regions were removed. The remaining sequences were clustered into Zero-radius Operational Taxonomic Units (ZOTUs) at 100% identity and taxonomy was assigned.

2.5 | Microbial community structure

Microbial community analysis was carried out at two stages: (1) TL-stage where we compared T1 and control treatment; and (2)

flowering stage, where we compared T1, T2, and control treatments. Bacterial and fungal OTU richness, Chao1, and Shannon indices were calculated with "Phyloseq" R package. Pairwise Bray-Curtis dissimilarity metrics of square-root-transformed bacterial/fungal community structure were calculated, and differences of bacterial and fungal community composition among cultivars and treatments were estimated using a PERMANOVA with 9999 permutations, using the "adonis" function in the R package "vegan" (Oksanen et al., 2012). Bacterial and fungal community structure were visualised using principal coordinate analysis (PCoA) based on the OTU feature tables using R packages "phyloseq" and "ggplot2" (McMurdie & Holmes, 2013). To identify the characteristics OTUs that were enriched by SynCom treatment, an indicator analysis combining both the abundance and occurrence of a given OTU across all treatments was used. The indicator values (IndVal) of each OTU were calculated using the "multipatt" function in the R package "indicpecies," with $i = 999$ random permutations (Cáceres & Legendre, 2009; De Cáceres et al., 2010).

2.6 | Statistical analyses

Two-way analysis of variance (ANOVA) and Tukey HSD test were used to evaluate the effect of treatments (cultivar and SynCom application) on plant and soil parameters at two stages: (1) TL-stage where we compared T1 and control treatment; and (2) flowering stage, where we compared T1, T2, and control. Data that did not meet the assumptions of the ANOVA were transformed before statistical analysis. Treatment-wise means were plotted as box plots and compared the overall effect of SynCom vs Control. For all statistical analyses, treatment effects were determined to be statistically different at $p < 0.05$. Two-way ANOVA and Tukey HSD test analysis were conducted by using "Agricolae" R package.

A structural equation model (SEM) was built to determine the casual pathways through which SynCom application influenced the crop productivity, where both direct and indirect (via changing microbial community and, nutrient availability) pathways were considered. The initial model also included cultivar identity as a controlling factor. We simplified the initial model by eliminating nonsignificant pathways and state variables based on regression weight estimates. Shannon index of bacterial and fungal communities was used as a measure of alpha diversity. We also performed principal coordinates analysis (PCoA) in the R package vegan (R version 4.0.2) (Dixon, 2003) based on the Bray-Curtis dissimilarities of square-root-transformed bacterial community operational taxonomic units (OTU) composition and PCoA scores of the first axis were used as proxies for changes in community composition in the SEM analysis. Before conducting SEM, soil phosphate and $[\text{NO}_3^-]/[\text{NH}_4^+]$ ratio was square-root-transformed to improve linearity. The model was then parameterised and its overall goodness of fit tested using the Chi-square test, the Comparative Fit Index (CFI), the Root Mean Square Error of approximation (RMSEA), and the (standardised)

Root Mean Square Residual (SRMR) (Kline, 2012). All SEM analyses were conducted using the R package "lavaan" (Rosseel, 2012).

3 | RESULTS

3.1 | Impact of SynCom seed dressing (T1) on germination and early plant performance

Germination rate was significantly (14.3%) higher in T1-SynCom seed dressing compared to control treatments at 12th DAS ($p < 0.05$) (Figure 1a, Table S1). Plant shoot height was significantly (7.4%) higher in T1 plants than control ($p < 0.05$). Cultivars and treatment \times cultivar also had a significant effect ($p < 0.01$ and 0.05 , respectively) on plant height (Figure 1b, Tables S1 and S2). Root length exhibited

significant effect of the cultivar and treatment \times cultivar ($p < 0.001$). T1 treatment increased root length by 9.27% compared with control plants although differences were marginally insignificant ($p = 0.053$) (Figure 1c, Tables S1 and S2). For plant biomass, shoot dry weight was significantly (5.14%) higher ($p < 0.01$) in T1 compared with control plants (Figure 1d, Table S1). However, root dry weight did not change after SynCom application, whereas cultivar exhibited a significant effect ($p < 0.001$) (Figure 1e; Tables S1 and S2).

3.2 | Impact of T1 and T2 treatments on plant growth at harvest stage

Plant shoot height (Figure 1f, Table S3) measured at the harvest stage was not affected by treatment or cultivar ($p = 0.335$ and 0.064 ,

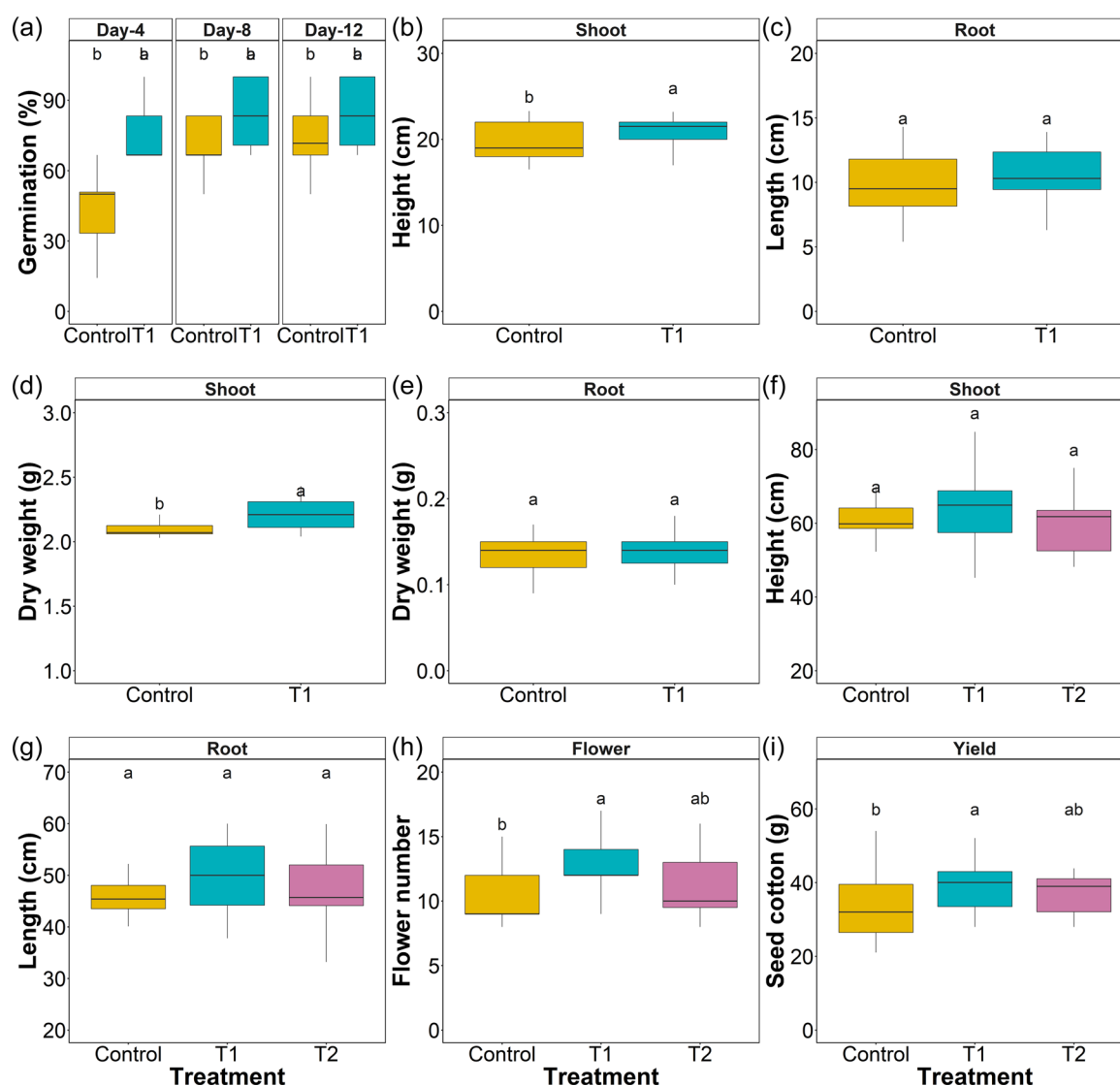


FIGURE 1 Treatment effect on early plant growth parameters (a) seed germination percentage (%) on 4th, 8th and 12th day after sowing (b and c) Shoot height and root length at TL (True leaf) stage (d and e) Shoot and root and dry weight at TL-stage, (f and g) Shoot height and root length at harvest stage, (h and i) Flower number and plant yield. Treatments-Control (C)-No-inoculation, T1-SynCom seed dressing and T2-SynCom-soil treatment. Different letters indicate significant difference among treatments (TukeyHSD $p < 0.05$).

respectively), although interaction of treatment \times cultivar was significant ($p < 0.05$). Root length (Figure 1g, Table S3 and S4) exhibited significant effect of treatment \times cultivar interaction ($p < 0.001$). However, no effects were observed for cultivar and treatments individually. Flower number per plant (Figure 1f, Table S3 and S4) was positively influenced by SynCom treatments ($p < 0.01$), where T1 elicited 10.44% and 7.36% higher flowers per plant compared to control and T2-SynCom soil treatment, respectively. For the plant productivity (Figure 1i, Table S3 and S4) measures taken as a combined weight of seed and lint, a significant effect of treatment ($p < 0.05$) and cultivar ($p < 0.001$) was observed. Among treatments, T1 and T2 showed 8.55% and 5.3% higher yield over control ($p < 0.018$ and 0.193, respectively). There was no significant interaction between treatment and cultivar suggesting that SynCom treatments have similar effect on productivity across all cultivars.

3.3 | Soil ammonium [NH₄⁺], nitrate [NO₃⁻] and phosphorus [PO₄³⁻] availability at TL stage and flowering stage

At the TL-stage, soil NH₄⁺ concentrations (Figure 2a, Table S1) were not significantly different between treatments or cultivar ($p > 0.05$). In general, 40.5% higher ammonium concentration was recorded in control over T1 soil samples ($C = 6.0 \pm 1.0$ SE and $T1 = 4.2 \pm 0.6$ mg/Kg). The interaction effect of treatment \times cultivar was also significant ($p < 0.05$). Soil extractable NO₃⁻ was 28.8% higher in T1 compared with control ($p < 0.01$) (Figure 2b, Table S1). Soil available P (Figures 2c, S1, Table S1) data suggested no independent impact of treatment and cultivar. A significant interaction between cultivar and T1 treatment ($p < 0.05$) was observed, with cultivar V1 and V4 showing higher soil available P than their controls.

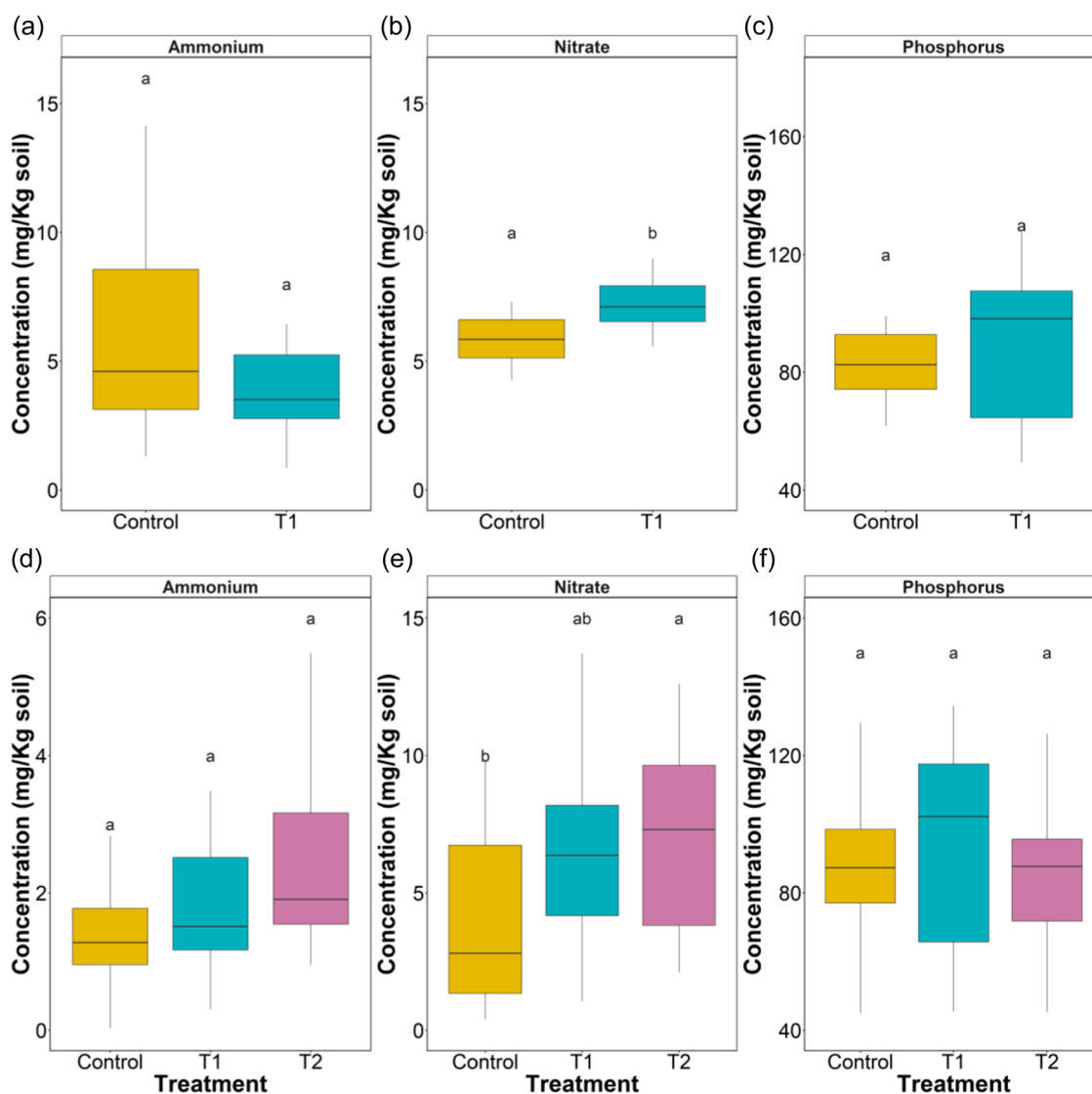


FIGURE 2 Treatment effect on soil extractable nutrients (a) Ammonia [NH₄⁺], (b) Nitrate [NO₃⁻], and (c) Phosphorus [PO₄³⁻] (mg/kg soil dry weight) at TL stage (true-leaf stage). (d) Ammonia [NH₄⁺] (e) Nitrate [NO₃⁻] and (f) Phosphorus [PO₄³⁻] (mg/kg soil dry weight) at Flowering stage. Treatments-Control (C)-No-inoculation, T1-SynCom seed dressing, and T2-SynCom soil treatment. Different letters are indicating significant difference among treatments (TukeyHSD $p < 0.05$).

At flowering stage, soil NH_4^+ concentrations were not significantly impacted by cultivars or treatments. Conversely, soil nitrate availability (Figure 2e, Table S3) was significantly different between treatments ($p < 0.01$) and cultivars ($p < 0.05$). Overall, NO_3^- was 55% higher in T2 compared with control soils ($p < 0.05$). Soil available P concentration was not affected by treatment or treatment \times cultivars (Figure 2f, Table S3).

3.4 | Microbial diversity

At TL stage, no significant difference ($p > 0.05$) was observed in bacterial OTU richness between treatments (Figure 3a, Table S1). However, the interaction impact of treatment \times cultivar was significant ($p < 0.05$). Fungal OTU richness showed no difference across treatments or cultivars (Figure 3b, Table S1). A higher value of bacterial Chao1 index in control versus T1 and a significant effect of cultivars and treatments independently ($p < 0.05$ and $p < 0.001$, respectively; Table S1) were observed. No trend was recorded in Chao1 index of fungal community (Figure 3d). Shannon index of bacteria changed by treatments where lower Shannon diversity was found in T1 compared with control ($p < 0.05$). The Shannon index of fungi did not respond to either treatment or cultivars (Figure S2a,b, Table S1).

At flowering stage, bacterial richness (Figure 3e, Table S3) did not differ between treatments, however fungal richness (Figure 3f, Table S2) showed strong treatment ($p < 0.001$), cultivar ($p < 0.01$),

and treatment \times cultivar effects ($p < 0.001$). For bacteria, the Chao1 index (Figure 3g, Table S2) showed significant treatment effect ($p < 0.001$), and both T1 and T2 had higher richness than control, whereas no effect of cultivar and treatment \times cultivar was observed. Similarly, the fungal Chao1 index (Figure 3h, Table S3) was higher in T1 and T2 treatments than control soil samples ($p < 0.05$). The fungal Chao1 index was significantly impacted by treatment ($p < 0.001$), cultivar ($p < 0.001$) and treatment \times cultivar ($p < 0.001$). Shannon index of bacteria and fungi were lower for T1 and T2 compared to the control treatment (Figure S2c,d). For bacteria, the Shannon Index was significantly affected by treatment and interaction of treatment \times cultivar ($p < 0.001$ and 0.01 , Table S3), whereas fungi Shannon index exhibited significant effects of treatment, cultivar, and treatment \times cultivar ($p < 0.001$, 0.05 , and 0.001) (Table S3).

3.5 | Community structure of bacterial and fungal communities

The PCoA analyses using Bray–Curtis distance matrices showed that bacterial communities were separated according to treatment on the first two coordinate axes that explain 46.7% and 20.7% of the variation, respectively (Figure S3a). This was further confirmed by PERMANOVA where significant differences in communities were observed due to treatment ($p < 0.05$, Table 2) and no effect observed due to cultivars ($p > 0.05$). Similarly, PCoA analysis of fungal communities at TL stage

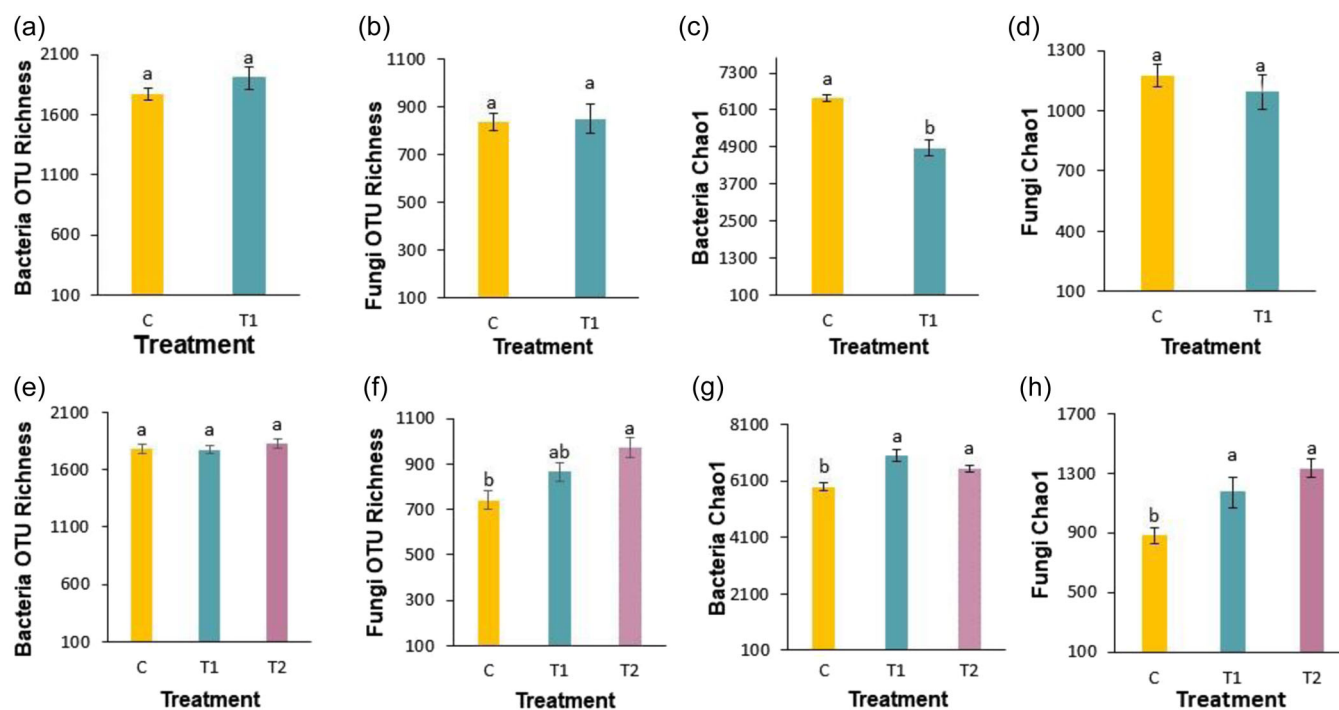


FIGURE 3 Treatment effect on bacterial and fungal community OUT richness (a) and (b), Chao1-bacteria (c), and fungi (d) at TL (true-leaf stage). Bacterial and fungal community richness (e) and (f), Chao1-bacteria (g) and fungi (h) at the flowering stage. Treatments-C-Control, T1-SynCom seed dressing, and T2-SynCom soil treatment. Different letters are indicating significant difference among treatments (TukeyHSD $p < 0.05$).

TABLE 2 Permanova analysis of bacterial and fungal communities of rhizosphere

	df	SumsOfSqs	MeanSqs	F.Model	R ²	Pr(>F)
Bacteria comparison 1: Control and T1 (True-leaf and flowering stage)						
Treatment	1	0.734	0.7348	3.749	0.053	0.035
Cultivar	4	0.563	0.140	0.719	0.041	0.819
Treatment:Cultivar	4	0.937	0.234	1.196	0.068	0.419
Residuals	50	11.505	0.230		0.837	
Total	59	13.740			1.000	
Bacteria comparison 2: Control, T1 & T2 (Flowering stage)						
Treatment	2	0.7907	0.39537	1.79217	0.07963	0.129
Cultivar	4	0.6751	0.16878	0.76505	0.06799	0.629
Treatment:Cultivar	8	1.8455	0.23069	1.04571	0.18586	0.4195
Residuals	30	6.6182	0.22061	0.66652		
Total	44	9.9296	1			
Fungi comparison 1: Control & T1 (True-leaf and Flowering stage)						
Treatment	1	0.425	0.425	1.530	0.025	0.039
Cultivar	4	1.152	0.288	1.035	0.069	0.350
Treatment:Cultivar	4	1.162	0.290	1.050	0.069	0.327
Residuals	50	11.505	0.230		0.835	
Total	59	13.740			1.000	
Fungi comparison 2: Control, T1, & T2 (Flowering stage)						
Treatment	2	2.2325	1.11623	6.6303	0.18465	1.00E-04
Cultivar	4	1.6905	0.42263	2.5104	0.13983	1.00E-04
Treatment:Cultivar	8	3.1164	0.38954	2.3139	0.25777	1.00E-04
Residuals	30	5.0506	0.16835	0.41775		
Total	44	12.0899	1			

(Figure S3b) showed separation of treatments at the first two coordinate axes (axis 1: 13.4% and axis 2: 11%). PERMANOVA showed significant effect of treatments ($p < 0.05$, Table 2). PCoA of bacterial at flowering stage (Figure S4a) showed that there was no significant difference between treatments and cultivar effects. In fungal communities at flowering stage, a clear separation of T2 from controls was observed (Figure S4b). Further PERMANOVA indicated that treatment and cultivars were significantly impacting on fungal community structure ($p < 0.001$ for all, and Table 2).

3.6 | Indicator species analysis

Indicator species analysis revealed that at TL-stage for bacteria, OTUs from phyla Proteobacteria (genera *Betaproteobacteriales_unclassified*, *Gammaproteobacteria_unclassified*, *Beijerinckiaceae_unclassified*, *Azospirillaceae_unclassified*, *Acidibacter*, *Rhizobiales_unclassified*, *Proteobacteria_unclassified*, *Alphaproteobacteria_unclassified*, and *Sphingomonadaceae_unclassified*), Acidobacteria (genus *Solibacteria*),

Actinobacteria (genera *Streptomyces*, *Nocardia*, *Blasococcus*, *Corynebacteria*, *Solirubrobacteracea_unclassified*, *Micrococcaceae_unclassified*, *Actinobacteria_unclassified*, *Gaiella*, and *Pseudonocardiaceae_unclassified*) and Cyanobacteria (genera *Nostocales_unclassified*, *Leptolyngbyaceae_unclassified* and *Oxyphotobacteria*) strongly associated with T1 as compared with control (Figure S5a, Table S5).

Fungal indicator species analysis at TL-stage identified OTUs from phyla Ascomycota (genera *Acremonium*, *Aspergillus*, *Chaetomium*, *Colletotrichum*, *Fusarium*, *Helicoma*, *Lecythophora*, *Microascus*, *Ochroconis*, *Penicillium*, *Preussia*, *Mycocleptodiscus*, *Ochroconis*, *Paecilomyces*), Basidiomycota (genera *Conocybe*, *Cryptococcus*, *Oliveonia*, and *Cryptococcus*), Chytridiomycota (*Kochiomyces*, *Rhizophlyctis*, and *Spizellomyces*) strongly associated with T1 treatment. Conversely, members of the phyla Glomeromycota (genera *Claroideoglossum*, *Paraglossum*, *Rhizophagus*, *Claroideoglossum*, and *Paraglossum*) and Zygomycota (genera *Mortierella* and *Rhizopus*) were highly and exclusively associated with control samples (Figure S5b, Table S6).

At flowering stage, in T2 there was an increased representation of bacterial indicator species related to phyla Actinobacteria (genera

Nocardioides, *Actinobacteria_unclassified*, *Pseudonocardiaceae_unclassified*, *Conexibacter*, *Microtrichales_unclassified*, *Solirubrobacterales_unclassified*, *Janibacter*, *Pseudarthrobacter*, *Frankiales_unclassified*, *Microtrichales_ge*, and Firmicutes (genera *Planococcaceae_unclassified*, *Bacillales_unclassified*, *Oceanirhabdus*, *Clostridiaceae_1_unclassified*, *Tumebacillus*, and *Periconia*). Cyanobacteria's OTUs were absent in control but present in T1 and T2. However, bacterial OTUs of phyla Proteobacteria (genera *Rhizobiales_unclassified*, *Acetobacteraceae_unclassified*, *Myxococcale_unclassified*, *Caulobacteraceae_unclassified*, *Deltaproteobacteria_unclassified*, and *Nitrobacter*) and Verrucomicrobia (genus *Verrucomicrobia*) showed a higher relative abundance in control compared to T1 and T2 (Figure 4a, Table S7).

The fungal indicator community in T2 exhibited a higher relative abundance of OTUs related to Ascomycota (genera *Exophiala*, *Claroideoglossum*, *Madurella*, *Funneliformis*, *Pyrenochaetopsis*, *Paecilomyces*, *Myceliophthora*, *Mortierella*, and *Gibberella* etc.), Basidiomycota (genera *Mortierella*, *Leptoxylum*, *Penicillium*, *Aspergillus*, and *Coniochaeta*) and Chytridiomycota (genus *Acremonium*). Interestingly, at TL-stage, members of Glomeromycota were absent in T1 and T2 but represented in control samples (Figure 4b, Table S8).

3.7 | Structural equation modelling

A rationale for the model is provided in the conceptual model (Figure 5a). SEM analysis showed that the SynCom application influenced cotton productivity (flower numbers). Among SynCom treatments T1 showed higher effects on the flowering and final yield as compared to T2, although both treatments positively induced higher flowering and yield. Plant flower number was impacted indirectly via changes in bacterial alpha diversity. SEM demonstrated that increase in flowering was positively linked with increase in final plant yield. The best SEM model also retained cultivar as the

additional explanatory variables for cotton productivity, while eliminating all other soil nutrient and plant growth measures (plant height, root length, nitrogen, and phosphorus concentrations) (Figure 5b).

4 | DISCUSSION

4.1 | Effect of SynCom application on plant growth

This study showed that the seed application of SynComs improved germination and early plant growth parameters. Enhanced germination percentage and rate in T1 treatments (SynCom-seed dressing) suggests that SynCom application helped to break seed dormancy, possibly by quickening metabolic processes (Garcia-Lemos et al., 2020; Rostamikia et al., 2016). Likewise, higher shoot growth parameters (7.4% higher shoot height and 5.1% higher shoot biomass) indicated that SynCom application at seedling stage can confer improved plant fitness at the early growth stage. The functional traits of the SynCom members used in this study included IAA and NH₄ production, and P-solubilisation, suggesting that these microbes were able to provide effective plant-growth-promoting phenotypes and hence increased plant performance. This finding is consistent with previous studies reporting that phytohormone production and higher acquisition of N and P could improve germination and early plant growth (Mukherjee et al., 2020; Pereg & McMillan, 2015; Trivedi et al., 2011). Increased performance of above-ground plant characteristics (plant height and biomass) at early growth stage supports our first hypothesis.

However, the early positive impacts of SynCom on plant height and root elongation were not apparent at the later stage of the development. It is possible that soil native microflora outcompeted the introduced SynCom (Pereg & McMillan, 2015). Alternatively, it is

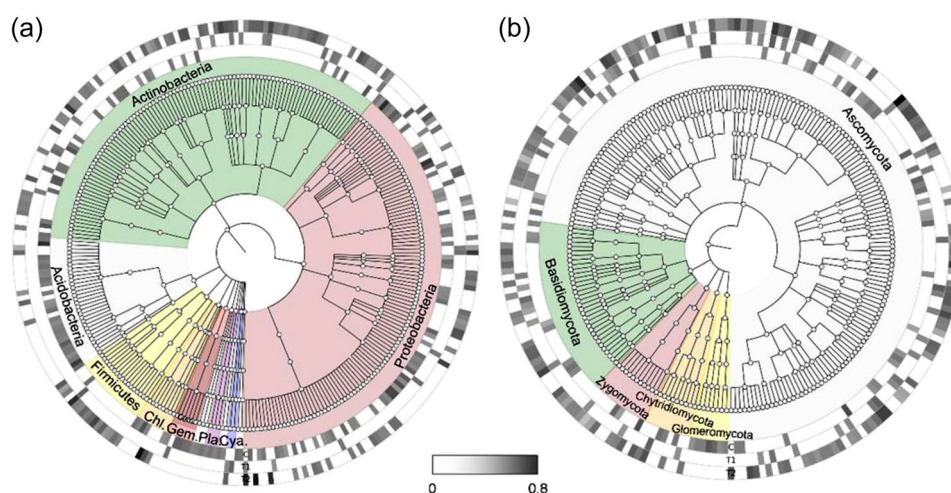


FIGURE 4 Lineage tree of the indicator species for different treatments. From inner to outer, the rings indicate Bacteria-(a) and Fungi-(b)-Treatments-C-Control of flowering stage, T1-T1-SynCom-seed dressing of flowering stage, T2-Syncom-soil treatment of flowering stage. The strength of the colour is proportional to the indicator robustness. Chl., Chloroflexi; Cya., Cyanobacteria; Fir., Firmicutes; Gem., Gemmatimonadetes; Pro., Proteobacteria; Plancto., Planctomycetes.

also possible that, while SynCom is still effective, plants growth parameter stabilised at later stage because plant invested heavily in reproductive structures (Berg et al., 2016; Paul et al., 2011; Pereg & McMillan, 2015) The second explanation is supported by increased

flower numbers and seed yields in this study. Our results are consistent with earlier findings (Gomathy et al., 2008; Liu et al., 2022; Narula et al., 2005), which reported the impact of PGPR on flowers and yield.

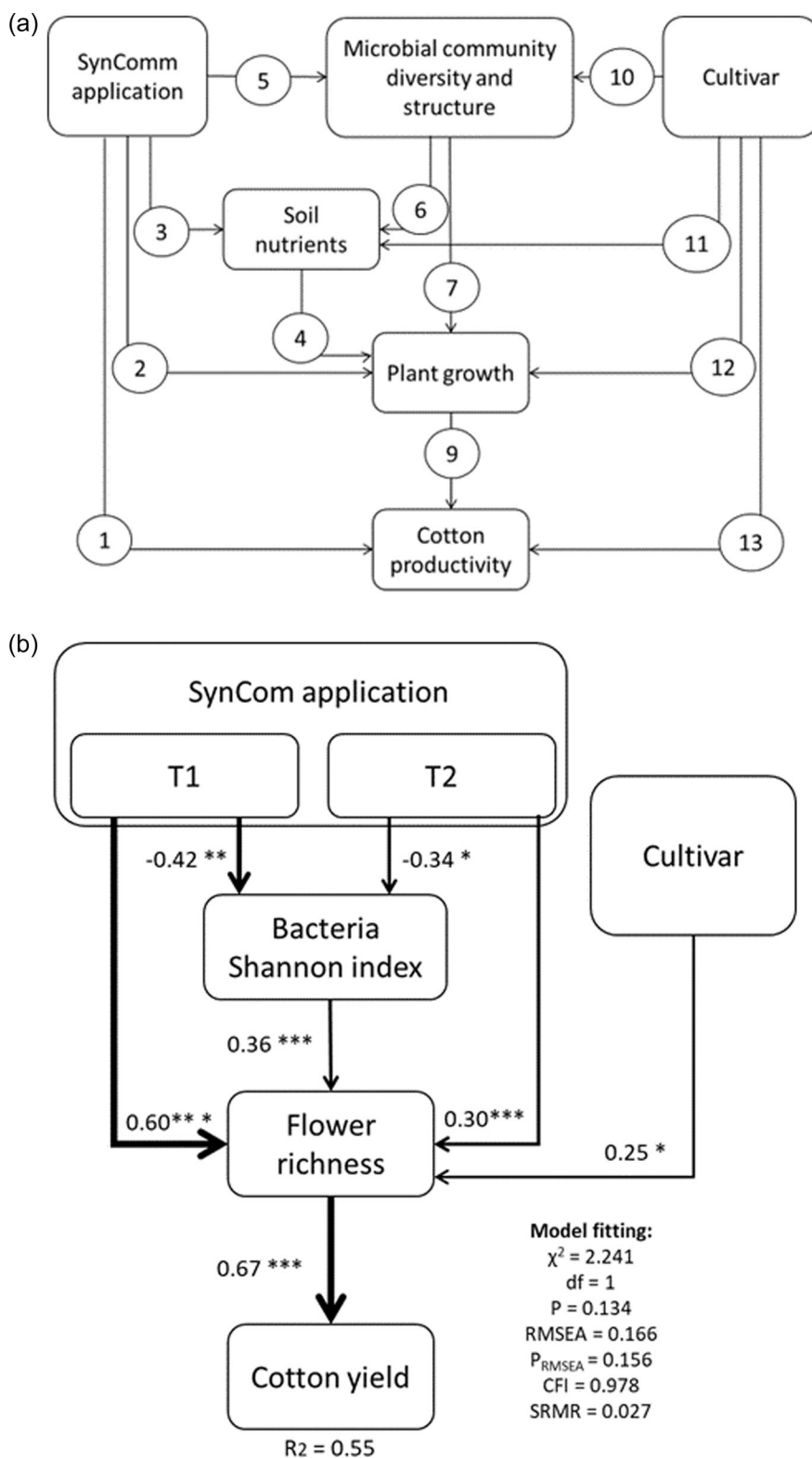


FIGURE 5 (See caption on next page)

Plant yield data showed that T1 and T2 elicited 10.4% and 7.4% higher yields than control plants. Interestingly, the early application of SynCom (T1) showed a higher yield than SynCom-soil treatment (T2), suggesting the role of priority effects on colonisation and host functions consequent to SynCom application. It further suggests that early plant fitness and microbial recruitment exert greater positive impacts on yield. Indeed, the SEM model demonstrated that alteration in bacterial alpha diversity and fungal beta diversity by the SynCom contributed to enhance flowering, which was positively associated with final cotton yield. Our findings are supported by recent studies that reported links between microbial community structure and plant productivity in different crops (Khan et al., 2019; Lebeis et al., 2015; Liu et al., 2022).

Most treatments showed significant effects on plant growth and productivity traits. However, in plant parameters such as plant height and root length, there were interactive effects of SynCom treatment cotton varieties. Particularly, at the true leaf stage, T1 promoted plant height in V1 and root length in V5 cultivar. In terms of flowering and plant productivity, V4 cultivar showed most positive response to SynCom treatment T1. This finding showed that PGPR as SynCom can also be adopted as potential a biological solution to achieve cultivar-specific gains in farming practices. Our findings are consistent with previous reports of interactions between PGPR consortia and crop genotypes to enhance specific plant growth and yield-related agronomic traits in crops for example chickpea and wheat (Imran et al., 2015; Pagnani et al., 2020; Tabassum et al., 2017).

4.2 | Effect of SynCom application on rhizosphere microbes

Alpha diversity (Chao1 and Shannon index) of bacteria declined with SynCom application. This indicates that the SynCom application has likely modulated the colonisation patterns by native microflora over time. Our findings are inconsistent with previous findings that reported a positive effect of PGPR on microbial diversity (Zhang et al., 2019). This contradiction can be explained by the mode of

application. Our seed dressing likely provided the introduced SynCom with a priority effect to colonise plant roots as soon as seed germinated (priority effect). Moreover, SynComs had been previously reported to help plants recruit beneficial microbes and thus indirectly promote growth (Nelson, 2018). SynCom seed dressing also affected bacterial community structure. Indicator species analysis showed that SynCom application was highly associated with higher recruitment of bacterial OTUs from Bacillales, Solibacterales, Nostocales, and Proteobacteria taxa. This suggests that SynCom PGPR are facilitating diverse groups of plant beneficial microbes to colonise plant roots. For example, a number of Proteobacterial taxa are known producers of plant hormones, N-fixation, and other PGPR activities (Nagel et al., 2018). Similarly, Bacillales and Actinobacteria members are known to provide pathogen protection as well as nutrient solubilisation (Suela, Silva et al., 2013). We also observed that our bacterial-based SynCom shifted fungal community structure in the rhizosphere. Particularly, SynCom seed dressing (T1) showed a strong effect at the TL-stage. It is possible that SynCom directly impacted fungal community. Alternatively, SynCom-induced shifts in bacterial communities could have indirectly elicited changes in the fungal community, as observed in previous research (Overbeek et al., 2021; Zhang et al., 2019). For example, enhanced recruitment of nonpathogenic fungal groups and *Bacillus* spp. abundance in the rhizosphere has been reported following PGP *Streptomyces* spp. inoculation (Yang et al., 2021). Further, the introduction of *Bacillus velezensis* NJAU-Z9 was reported to shift bacterial and fungal communities and increase yield (Zhang et al., 2019), which is consistent with our findings. Interestingly, we found that at TL-stage, a fungal OTU belonging to *Fusarium* sp. was significantly lower in SynCom-treated soils. A number of *Fusarium* species are well-known plant pathogens, suggesting that SynCom may provide biocontrol functions at early stage of plant growth, but this has yet to proven. Interestingly, the fungus *Gibberella* spp, which are known for production of gibberellins (Hedden & Sponsel, 2015), was found associated with T1 and T2 but was absent in the top 10 indicator OTUs of control samples. This supports our explanation that SynCom treatment can change fungal community structure to confer better

FIGURE 5 Structural Equation Modelling (SEM). (a) Conceptual model of the expected causal direct and indirect effects of SynCom application on cotton productivity. (A priori SEM model The direct SynCom effects in our study [Figure 5a, path 1 and 2 → 9] assume that SynComm application impacts the plant growth [number of flowers, plant height and root length] and productivity [yield] independently of SynComm-induced variations in soil biotic and abiotic properties via direct effects, that is, increase in plant growth and nutrient uptake via phytohormones like IAA, P-solubilisation and ammonia production [Mukherjee et al., 2020; Pereg & McMillan, 2015; Tabassum et al., 2017; Zhang et al., 2019]. The indirect effects of SynCom infer that microbial strain application impacts the plant variables through the changes in soil nitrogen and phosphorus concentration [Figure 5a, pathway 3 → 4] or soil microbiological properties [bacterial and fungal diversity and community structure; Figure 5a, path 5 → 7 → 9; Mukherjee et al., 2020; Pereg & McMillan, 2015; Tabassum et al., 2017; Yang et al., 2021]. The indirect effects of cultivar in our study assumes that cultivars affect soil microbiological and chemical properties [10 and 11, respectively], as well as plant growth and productivity [path 12 and 13, respectively; Hedden & Sponsel, 2015; Mukherjee et al., 2020; Pereg & McMillan 2015; Tabassum et al., 2017; Wei et al., 2019; Zhang et al., 2019]). (b) SEM showing the direct and indirect pathways through which SynComm application affected plant productivity (yield). Models were well supported by our data. Arrows thickness are proportional to the coefficient strength. CFI, Comparative Fit Index; RMSEA, Root Mean Square Error of Approximation; SRMR, Standardised Root Mean Square Residual. R^2 = variance in cotton yield explained by the model. Numbers associated with each arrow are standardised path coefficients with the following levels of significance: * $p \leq 0.1$; ** $p \leq 0.05$; *** $p \leq 0.01$.

plant fitness in some cases. This hypothesis is supported by our SEM analysis, which showed that a change in bacterial community with SynCom treatments was linked to higher number of flowers and thus directly associated with yield. This suggests that SynCom treatment has brought a change in plant performance and yield directly via providing nutrients and indirectly via changing rhizosphere microbial compositions and structure.

4.3 | Effect of SynCom application on soil nutrient availability

Among the soil parameters, enhanced availability of soil nitrate under SynCom treatments at TL- and flowering stages indicates the possible involvement of *Brevibacterium* sp., a nitrogen fixing bacterium included in the SynCom tested in this study. It is also possible that SynCom encouraged recruitment of microbial groups that are involved in the nitrification and nitrogen fixation. Increased nitrate availability in T1 and T2 soils might also be linked to higher recruitment of Cyanobacteria, an important nitrogen fixing microbial group (Babu et al., 2015), which was strongly associated with both SynCom treatments in this study. Overall, the PGPR-induced increase in NPK availability in soils is well documented (Cordero et al., 2018). At the flowering stage, T2 was found more effective than T1 in terms of nitrate availability suggesting higher survival of nitrogen-fixing inoculants in soil drenched with SynCom treatments. However, the increased nutrient availability did not confer benefit in terms of plant performance in this case. Collectively, our findings suggest that early vigorous plant growth is more important for yield than later development or nutrient availability, and thus plants received most benefit from SynCom when it was applied as a seed dressing (Fukami, 2015; Zhang et al., 2019).

5 | CONCLUSIONS

This study provides evidence for the positive impact of a SynCom on germination, plant shoot/root, and productivity. It also suggests that the seed dressing approach for SynCom application provides better outcomes than later stage soil treatment. The SynCom seed dressing can efficiently increase germination rate and early growth parameters. The cultivar and treatments both have differential growth responses and an impact on bacterial/fungal diversity. Notably, indicator species analysis showed different patterns, especially higher recruitment of Bacillales and Actinobacteriales by SynCom seed dressing. Overall, this study suggests that the use of SynCom can increase crop performance in cotton directly via provision of nutrients and hormones, and indirectly via manipulating microbial community structure in rhizosphere. If these findings can be replicated under field conditions, SynCom can provide an effective and complementary tool to conventional farming to increase farm productivity in an environmentally sustainable way.

AUTHOR CONTRIBUTIONS

Simranjit Kaur and Brajesh K. Singh designed the experiment. Simranjit Kaur performed the experiment and collected data and wrote the manuscript with significant inputs from all co-authors. Eleonora Egidi, Zhiguang Qiu, and Juntao Wang helped in microbial community analysis. Brajesh K. Singh, Catriona A. Macdonald, Eleonora Egidi, and Zhiguang Qiu provided guidance to undertake work. Pankaj Trivedi, Jay Prakash Verma, and Hongwei Liu provided with the microbial materials and preliminary data. All authors provided feedback on the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data will be publicly available.

ETHICS STATEMENT

The authors confirm that they have followed the ethical policies of the journal.

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REFERENCES

- Babu S, Bidiarani N, Chopra P, Monga D, Kumar R, Prasanna R, et al. Evaluating microbe-plant interactions and varietal differences for enhancing biocontrol efficacy in root rot disease challenged cotton crop. *Eur J Plant Pathol*. 2015;142(2):345–62.
- Backer R, Rokem JS, Ilangumaran G, Lamont J, Praslickova D, Ricci E, et al. Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Front Plant Sci*. 2018;9:1473.
- Basu A, Prasad P, Das SN, Kalam S, Sayyed RZ, Reddy MS, et al. Plant growth promoting rhizobacteria (PGPR) as green bioinoculants: recent developments, constraints, and prospects. *Sustainability*. 2021;13(3):1140.
- Batista BD, Singh BK. Realities and hopes in the application of microbial tools in agriculture. *Microb Biotechnol*. 2021;14(4):1258–68.
- Berendsen RL, Vismans G, Yu K, Song Y, de Jonge R, Burgman WP, et al. Disease-induced assemblage of a plant-beneficial bacterial consortium. *ISME J*. 2018;12(6):1496–507.
- Berg G. Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Appl Microbiol Biotechnol*. 2009;84(1):11–8.

- Berg G, Rybakova D, Grube M, Köberl M. The plant microbiome explored: implications for experimental botany. *J Exp Bot*. 2016;67(4):995–1002.
- Cáceres MD, Legendre P. Associations between species and groups of sites: indices and statistical inference. *Ecology*. 2009;90(12):3566–74.
- Cordero I, Balaguer L, Rincón A, Pueyo JJ. Inoculation of tomato plants with selected PGPR represents a feasible alternative to chemical fertilization under salt stress. *J Plant Nutr Soil Sci*. 2018;181(5):694–703.
- Dakora FD. Defining new roles for plant and rhizobial molecules in sole and mixed plant cultures involving symbiotic legumes. *New Phytol*. 2003;158(1):39–49.
- De Cáceres M, Legendre P, Moretti M. Improving indicator species analysis by combining groups of sites. *Oikos*. 2010;119(10):1674–84.
- de Souza RSC, Armanhi JSL, Arruda P. From microbiome to traits: designing synthetic microbial communities for improved crop resiliency. *Front Plant Sci*. 2020;11:1179.
- Dixon P. VEGAN, a package of R functions for community ecology. *J Veg Sci*. 2003;14(6):927–30.
- Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*. 2011;27(16):2194–200.
- Fukami T. Historical contingency in community assembly: integrating niches, species pools, and priority effects. *Ann Rev Ecol Evol Syst*. 2015;46:1–23.
- García-Lemos AM, Großkinsky DK, Saleem Akhtar S, Nicolaisen MH, Roitsch T, Nybroe O, et al. Identification of root-associated bacteria that influence plant physiology, increase seed germination, or promote growth of the Christmas tree species *Abies nordmanniana*. *Front Microbiol*. 2020;11:2933.
- Gomathy M, Sathya Prakash D, Thangaraju M, Sundaram SP, Manicka Sundaram P. Impact of biofertilization of Azophosmet on cotton yield under drip irrigation. *Res J Agric Biol Sci*. 2008;4(6):695.
- Hamonts K, Trivedi P, Garg A, Janitz C, Grinyer J, Holford P, et al. Field study reveals core plant microbiota and relative importance of their drivers. *Environ Microbiol*. 2018;20(1):124–40.
- Hays SG, Patrick WG, Ziesack M, Oxman N, Silver PA. Better together: engineering and application of microbial symbioses. *Curr Opin Biotechnol*. 2015;36:40–9.
- Hedden P, Sponsel V. A century of gibberellin research. *J Plant Growth Regul*. 2015;34(4):740–60.
- Imran A, Mirza MS, Shah TM, Malik KA, Hafeez FY. Differential response of kabuli and desi chickpea genotypes toward inoculation with PGPR in different soils. *Front Microbiol*. 2015;6:859.
- Jetiyanon K, Kloepper JW. Mixtures of plant growth-promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. *Biol Control*. 2002;24(3):285–91.
- Keeney DR, Nelson DW. Nitrogen—inorganic forms. In: Page AL, editor. *Methods of soil analysis. Part 2. Chemical and microbiological properties*. 2nd ed. Madison, WI: ASA and SSSA; 1983. p. 643–98.
- Khan N, Bano A, Babar MA. Metabolic and physiological changes induced by plant growth regulators and plant growth promoting rhizobacteria and their impact on drought tolerance in *Cicer arietinum* L. *PLoS One*. 2019;14(3):e0213040.
- Kline RB. Assumptions in structural equation modeling. 2012.
- Korir H, Mungai NW, Thuita M, Hamba Y, Masso C. Co-inoculation effect of rhizobia and plant growth promoting rhizobacteria on common bean growth in a low phosphorus soil. *Front Plant Sci*. 2017;8:141.
- Kumar V, Gera R. Isolation of a multi-trait plant growth promoting *Brevundimonas* sp. and its effect on the growth of Bt-cotton. *3 Biotech*. 2014;4(1):97–101.
- Lebeis SL, Paredes SH, Lundberg DS, Breakfield N, Gehring J, McDonald M, et al. Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science*. 2015;349(6250):860–64.
- Liu H, Khan MY, Carvalhais LC, Delgado-Baquerizo M, Yan L, Crawford M, et al. Soil amendments with ethylene precursor alleviate negative impacts of salinity on soil microbial properties and productivity. *Sci Rep*. 2019;9(1):1–13.
- Liu H, Qiu Z, Ye J, Verma JP, Li J, Singh BK. Effective colonisation by a bacterial synthetic community promotes plant growth and alter soil microbial community. *J Sustain Agric Environ*. 2022;1(1):30–42. <https://doi.org/10.1002/sae2.12008>
- Liu Y, Gao J, Bai Z, Wu S, Li X, Wang N, et al. Unraveling mechanisms and impact of microbial recruitment on oilseed rape (*Brassica napus* L.) and the rhizosphere mediated by plant growth-promoting rhizobacteria. *Microorganisms*. 2021;9(1):161.
- McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*. 2013;8(4):e61217.
- Mukherjee A, Singh BK, Verma JP. Harnessing chickpea (*Cicer arietinum* L.) seed endophytes for enhancing plant growth attributes and bio-controlling against *Fusarium* sp. *Microbiol Res*. 2020;237:126469.
- Myresiotis CK, Karaoglanidis GS, Vryzas Z, Papadopoulou-Mourkidou E. Evaluation of plant-growth-promoting rhizobacteria, acibenzolar-S-methyl and hymexazol for integrated control of *Fusarium* crown and root rot on tomato. *Pest Manag Sci*. 2012;68(3):404–11.
- Nagel R, Bieber JE, Schmidt-Dannert MG, Nett RS, Peters RJ. A third class: functional gibberellin biosynthetic operon in beta-proteobacteria. *Front Microbiol*. 2018;9:2916.
- Narula N, Saharan BS, Kumar V, Bhatia R, Bishnoi LK, Lather BPS, et al. Impact of the use of biofertilizers on cotton (*Gossypium hirsutum*) crop under irrigated agro-ecosystem. *Arch Agron Soil Sci*. 2005;51(1):69–77.
- Nelson EB. The seed microbiome: origins, interactions, and impacts. *Plant Soil*. 2018;422(1):7–34.
- Niknam GR, Dhawan SC. Effect of seed bacterization and methods of application of *Pseudomonas fluorescens* on the control of *Rotylenchulus reniformis* infecting tomato. *Nematol Mediterranea*. 2003.
- Oksanen J, Kindt R, Legendre P, O'Hara B, Simpson GL, Stevens MHH. *Vegan: community Ecology Package*. R Package version. 2012;2:0–3.
- Oldroyd GE, Dixon R. Biotechnological solutions to the nitrogen problem. *Curr Opin Biotechnol*. 2014;26:19–24.
- Olsen SR, Cole CV, Watanabe FS, Dean LA. Estimation of available phosphorus in soils by extraction with NaHCO₃. *USDA Cir.939*, U.S. Washington; 1954.
- Overbeek W, Jeanne T, Hogue R, Smith DL. Effects of microbial consortia, applied as fertilizer coating, on soil and rhizosphere microbial communities and potato yield. *Front Agron*. 2021;59.
- Pagnani G, Galieni A, Stagnari F, Pellegrini M, Del Gallo M, Pisante M. Open field inoculation with PGPR as a strategy to manage fertilization of ancient *Triticum* genotypes. *Biol Fertil Soils*. 2020;56(1):111–24.
- Parnell JJ, Berka R, Young HA, Sturino JM, Kang Y, Barnhart DM, et al. From the lab to the farm: an industrial perspective of plant beneficial microorganisms. *Front Plant Sci*. 2016;7:1110.
- Paul S, Singh Rathi M, Prakash Tyagi S. Interactive effect with AM fungi and *Azotobacter* inoculated seed on germination, plant growth and yield in cotton (*Gossypium hirsutum*). *Ind J Agric Sci*. 2011;81(11):1041.
- Pereg L, McMillan M. Scoping the potential uses of beneficial microorganisms for increasing productivity in cotton cropping systems. *Soil Biol Biochem*. 2015;80:349–58.
- Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J, et al. SILVA: a comprehensive online resource for quality checked and aligned

- ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res.* 2007;35(21):7188–96.
- Qiao Q, Wang F, Zhang J, Chen Y, Zhang C, Liu G, et al. The variation in the rhizosphere microbiome of cotton with soil type, genotype and developmental stage. *Sci Rep.* 2017;7(1):1–10.
- Qiu Z, Egidi E, Liu H, Kaur S, Singh BK. New frontiers in agriculture productivity: optimised microbial inoculants and in situ microbiome engineering. *Biotech Adv.* 2019;37(6):107371.
- Qiu Z, Wang J, Delgado-Baquerizo M, Trivedi P, Egidi E, Chen YM, et al. Plant microbiomes: do different preservation approaches and primer sets alter our capacity to assess microbial diversity and community composition. *Front Plant Sci.* 2020;11:993.
- Reeder J, Knight R. The 'rare biosphere': a reality check. *Nat Methods.* 2009;6(9):636–37.
- Rosseel Y. Lavaan: an R package for structural equation modeling and more. Version 0.5–12 (BETA). *J Stat Softw.* 2012;48(2): 1–36.
- Rostamikia Y, Kouchaksaraei MT, Asgharzadeh A, Rahmani A. Effect of plant growth promoting rhizobacteria (PGPR) and cold stratification on seed germination and early growth of *Corylus avellana* L. *Austrian J For Sci.* 2016;4:337–52.
- Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol.* 2009;75(23): 7537–41.
- Singh BK, Trivedi P. Microbiome and the future for food and nutrient security. *Microb Biotechnol.* 2017;10(1):50–3.
- Suela Silva M, Naves Sales A, Teixeira Magalhães-Guedes K, Ribeiro Dias D, Schwan RF. Brazilian Cerrado soil actinobacteria ecology. *BioMed Res Int.* 2013;2013:503805.
- Tabassum B, Khan A, Tariq M, Ramzan M, Khan MSI, Shahid N, et al. Bottlenecks in commercialisation and future prospects of PGPR. *Appl Soil Ecol.* 2017;121:102–17.
- Trivedi P, Mattupalli C, Eversole K, Leach JE. Enabling sustainable agriculture through understanding and enhancement of microbiomes. *New Phytol.* 2021;230(6):2129–47.
- Trivedi P, Spann T, Wang N. Isolation and characterization of beneficial bacteria associated with citrus roots in Florida. *Microb Ecol.* 2011;62(2):324–36.
- USDA, FAS. 2018. Public distribution. https://apps.fas.usda.gov/newgainapi/api/report/download_reportbyfilename?filename=Cotton/and/Products/Annual-Canberra_Australia_3-28-2018.pdf
- Uzoh IM, Babalola OO. Rhizosphere biodiversity as a premise for application in bio-economy. *Agric Ecosyst Environ.* 2018;265:524–34.
- Wei F, Zhao L, Xu X, Feng H, Shi Y, Deakin G, et al. Cultivar-dependent variation of the cotton rhizosphere and endosphere microbiome under field conditions. *Front Plant Sci.* 2019;10:1659.
- Yang D, Wang L, Wang T, Zhang Y, Zhang S, Luo Y. Plant growth-promoting rhizobacteria hñ6 induced the change and reorganization of fusarium microflora in the rhizosphere of banana seedlings to construct a healthy banana microflora. *Front Microbiol.* 2021;12.
- Yao L, Wu Z, Zheng Y, Kaleem I, Li C. Growth promotion and protection against salt stress by *Pseudomonas putida* Rs-198 on cotton. *Eur J Soil Biol.* 2010;46(1):49–54.
- Zhang Y, Gao X, Shen Z, Zhu C, Jiao Z, Li R, et al. Pre-colonization of PGPR triggers rhizosphere microbiota succession associated with crop yield enhancement. *Plant Soil.* 2019;439(1):553–67.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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