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When a bacterial synthetic community effectively colonizes soil, it changes the microbial ecology and encourages plant development.

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Research Article

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Abstract

The use of plant-associated microorganisms as bioinoculants in agriculture is being investigated extensively. However, little is known about how quickly and effectively foreign microorganisms may colonize and interact with native soil microbiomes. Eight plant-growth-promoting bacteria that were isolated from the rhizosphere of wheat (*Triticum aestivum*) were used in this study to create a bacterial synthetic community (SynCom). These bacteria included three *Bacillus* species, two *Acinebacter* species, an *Enterobacter* species, a *Xanthomonas* species, and a *Burkholderia* species.

KEYWORDS

beneficial microorganisms, plant defense, microbial colonization, *Fusarium pseudograminearum*, and SynCom

1 | INTRODUCTION

A wide variety of microbiomes may be found in soil and the rhizosphere of plants. One of the key factors influencing the health of plants and soil is these bacteria and their interactions.¹ Numerous microorganisms have the ability to affect plant performance in a variety of direct and indirect ways. The synthesis of compounds that promote plant development, such as cytokinin, ammonia, and indole-3-acetic acid (IAA), as well as the solubilization of mineral phosphates (P), are examples of direct processes that lessen the need for nitrogen (N) and P fertilizers in soils.^{3,4} In addition to improving soil structure and organic matter content, microbes may stabilize soil aggregates.⁵ Indirect microbial techniques for promoting plant development include introducing antifungal or antibacterial substances (e.g., siderophores, cell wall-degrading enzymes) and reducing or mitigating the harmful effects of phytopathogens in soils via biofilm formation.^{6–8} In addition to the crucial host roles that microorganisms play in plant performance, recent research has shown that the composition, diversity, and metabolites of plant microbiomes, as well as their interactions with the environment, significantly impact the plant immune system, fitness, and growth. In order to maintain environmental sustainability and encourage healthy eating, pesticide usage must be drastically reduced in response to growing consumer expectations and new governmental frameworks (such as the European Green Deal).^{11–14} Microbial inoculants are becoming more widely recognized as a useful supplementary or substitute instrument for agricultural sustainability and production.^{7, 15} However, because of their uneven effectiveness in field settings, the use of microbial inoculants in agriculture has shown mixed outcomes. A portion of this can be attributed to a failure of colonization and intricate interactions between the microbial inoculants and the plant, soil macro/microorganisms (such as insects, bacteria, and fungi), and the environment (such as soil type, rainfall, and temperature). However, there aren't many mechanistic studies for the lack of colonization. Furthermore, the ecological process controlling the establishment and colonization of imported microbial inoculants is poorly understood.¹⁷ Such information is essential for controlling (e.g., maintaining ecosystem services), promoting (e.g., bioinoculants for increased agricultural output), and preventing (e.g., phytopathogens) the colonization of alien microbes (species not already part of resident microflora). In order to solve this problem, the priority effect—which describes how early arriving microbes may outcompete late comers due to numerical and previous adaptation advantages—has recently been suggested.^{18, 19} For instance, microbial competition in wheat roots showed that *Pantoea* agglomerans, a seed endophyte, had a considerable advantage over late-incoming colonizers. Twenty However, due to the

rich variety of native soil bacteria, assessing the persistence of microbial inoculants in natural soils is difficult.¹⁶ Gnotobiotic model systems with well defined communities provide an alternate strategy to reduce environmental complexity.²¹ It enables repeatable experimentation and the tracking and monitoring of changes in microbial inoculants in the environment.²¹ A helpful method for manipulating and comprehending natural communities is synthetic communities (SynCom).²² They have been used extensively to alter plant phenotypes, such as to improve disease resistance and agricultural productivity.^{23, 24} For instance, using a microbial product comprising *Trichoderma harzianum* and *Bacillus subtilis* to the soil prior to planted increased potato output and decreased the occurrence of common scab.²⁵ The treatments also increased the number of beneficial bacteria in the rhizosphere, including members of *Pseudomonadales* and *Burkholderiales*. Additionally, plant genetic variables influencing the formation of the leaf-associated microbial communities were identified using 25 SynCom.²⁶ However, the function of ecological interactions in the colonization of imported SynCom has not been well studied. Soil microbial biomass and diversity are periodically significantly reduced by human and environmental stressors. In agricultural systems, microorganisms may be purposefully eliminated from soils by fumigation, disinfestation, or solarization in order to manage diseases. These activities "reset" the soil microbial community to varying degrees, and it is essential to comprehend the recovery process that follows since the results of community assembly influence plant phenotypes and dictate soil function.^{30–32} However, owing to a dearth of empirical research, it is still unclear if characteristics of bioinoculants and their interactions with soil microorganisms contribute to the recovery of soil and plant microbial communities. Phytopathogens like *Fusarium pseudograminearum* (Fp) may seriously impair the productivity of wheat (*Triticum aestivum*), a significant crop farmed worldwide.^{33–35} In southern Australia alone, the pathogen's annual output losses are estimated to be \$79 million.^{34, 35} Even though Fp is becoming more common in Australia, there are currently no commercially accessible ways to successfully manage it.³⁶ There are several reasons for this, such as: (i) the pathogen has a broad host range and has been isolated from all major winter cereals; (ii) chemical control of the pathogen is frequently ineffective; (iii) there are no wheat cultivars that are 100% resistant to Fp; and (iv) the pathogen can survive and grow on organic residues in soils.³⁹ The bacterial and fungal communities in the wheat rhizosphere underwent substantial changes as a result of this pathogen's infection, according to our earlier research on wheat–Fp–plant microbiome interactions.⁴⁰ In this work, we examined the interaction and colonization of wheat-associated bacteria in soil as well as the efficacy of a beneficial bacterial SynCom. As a stand-in for fumigated soils, which are often used in agriculture, we used soil with limited microbial diversity. The SynCom represents the colonization of intentionally introduced beneficial microbial species, whereas the colonization of Fp in the plant and soil reflected the co-colonization of an invading pathogenic species. We hypothesized that: (i) both invasive and introduced species readily establish in disturbed soil, but their abundance decreases as native microflora recover from disturbance; and (ii) the SynCom inoculation modifies the structure and composition of the soil microbial community and enhances plant performance (tolerance to Fp infection and growth). Bacterial isolates were isolated from the wheat rhizosphere and examined for characteristics that promote plant development in vitro in order to test these theories. A beneficial SynCom was created using eight isolates with superior plant growth-promoting characteristics (PGPTs) and evaluated on wheat in a glasshouse experiment. A combination of high-throughput 16S ribosomal RNA (rRNA) gene amplicon sequencing and quantitative polymerase chain reaction (qPCR) analyzes was used to monitor plant growth and survival rates as well as changes of the SynCom and the pathogen in soil.

2 | MATERIALS AND METHODS

2.1 | Bacterial isolation from the rhizosphere

The favorable characteristics of the wheat rhizosphere-associated bacteria were examined for their ability to promote plant development and reduce disease. The bacterial isolation details were previously described. In short, a mature durum wheat plant from the Wellcamp experimental site (27°33'54.7"S, 151°51'52.0"E), Queensland, Australia, had its rhizosphere soil removed from its roots. Nutrient agar (NA), pikovaskaya agar, tryptophane soya agar, and King's B agar were used to isolate bacteria from 1.0 g of soil that had been pelleted by centrifugation (4000 rpm, 10 min). Using techniques outlined in the Supporting Information Materials, forty of the isolates were examined for IAA synthesis, phosphate solubilization, ammonia generation, and antifungal capacity. For the antifungal tests, three Fp strains—CS3427, CS3321, and CS3096—were used.

| Bacterial identification and whole-genome sequencing

The DNeasy Blood & Tissue Kits (Qiagen) were used to extract the genomic DNA of the bacterial isolates in accordance with the manufacturer's instructions. Using primers 27F and 1492R, 16S rRNA gene sequencing was used to characterize the isolates; Sanger sequencing was carried out at Western Sydney University. Bacterial sequences that have been trimmed are accessible under MT158540–MT158578. The Australian Center for Ecogenomics utilized the Illumina NextSeq 500 technology to sequence the whole genomes of the seven bacterial strains used to create SynCom. FastQC (0.11.8) was used to verify the quality of the sequencing, and SPAdes (v3.9.0) was used with default parameters to assemble genomes from scratch.⁴¹ The genomic assemblies may be found under project PRJNA745065 in the National Center for Biotechnology Information (NCBI) database. Noncoding genes of 23S and 16S rRNA were projected using

rRNAmmer1.2.42, whereas coding genes were annotated using databases NR, Swiss-Prot, COG, KEGG, and GO. An online program was used to determine the average nucleotide identity (ANI) of the bacterial genomes.⁴³

| **Glasshouse experiment to assess effects of the SynCom on wheat performance**

Using soil collected from the Wellcamp location (soil physiochemical parameters were previously documented), the effects of the SynCom on wheat performance were examined in a glasshouse experiment (20°C with natural light). Forty The soil, which had been in the glasshouse for five days prior to the studies, was autoclaved (121°C for 30 minutes) to deactivate Fp and lower soil microbial diversity. In many agricultural situations, autoclave-based sterilization is the most economical, practical, and efficient method. In comparison to natural settings (10,659 bacterial OTUs), the autoclaving method dramatically decreased soil microbial richness (478 bacterial operational taxonomic units [OTUs] discovered 12 days after autoclaving as evaluated by amplicon sequencing).⁴⁴ There is evidence that certain soil microbial taxa and spores can withstand autoclaving and may recover faster than other microorganisms.⁴⁴ Three treatments (Fp, SynCom, Fp + SynCom) and a control (no SynCom or Fp) were administered in pots (8 × 12 cm) throughout the same day in chronological sequence. Each treatment consisted of six duplicate pots with six wheat (variety: Jandaroï) seeds planted in them, covered with a thin layer of soil, and topped with 1.0 g of Fp/autoclaved Fp inoculant powder. Using a previously published technique, the Fp (strain Fp A11#04) inoculum on whole wheat grains was broken into powder and applied to the soil.⁴⁵ To provide a comparable chemical environment in Fp-excluded treatments, autoclaved Fp inoculants were used. Two loops (~2 µl) of each isolate were added to 35 ml of nutritional broth, agitated at 220 rpm, and kept at 37°C for 36 hours in order to subculture the eight bacterial strains. The cultures were combined to create the SynCom, which was then drenched in soil after being washed twice and resuspended in sterile water. The ratio of the strains was HW3 (*Priestia megaterium*): HW4 (*Bacillus* sp.): HW4Z (*Acinetobacter pittii*): HW15 (*Enterobacter asburiae*): HW16 (*Bacillus* sp.): HW32 (*A. pittii*): HWA1 (*Xanthomonas* sp.): HWK1 (*Burkholderia* sp.) = 1.0: 0.4: 0.3: 1.0: 0.5: 0.5: 0.25: 1.0.0. The natural growth rates of each strain in nutrient broth were represented by an unequal ratio. Additionally, we cultivated these isolates for a week on the same NA plate, and no discernible antagonistic interactions between isolates were seen. The soil of the SynCom-excluded treatments received the same volume of sterile water. All soils were watered to field capacity so that seeds could sprout, and they were then irrigated every day as required to keep the soil wet. For 22 days, the height of the seedlings was measured twice a day at 10 AM and 4 PM. Every day, the plant's survival rate and illness score were noted. In order to examine how Fp, SynCom, and both together affect the bulk and rhizosphere soil microbial populations, about 2.0 g of surface (~top 2 cm) bulk soil was collected on days 7, 14, and 21. Plant shoots were gathered, clipped, and weighed. After carefully separating the roots from the bulk soil, sterile water was used to remove the rhizosphere soil from the roots, which was then pelleted using centrifugation. After being properly cleaned with tap water, the roots were allowed to air dry at room temperature before being weighed.

| **DNA extraction from soil**

As directed by the manufacturer, microbial genomic DNA was isolated from 0.25 g of soil for each soil sample using the DNeasy PowerSoil™ DNA Isolation Kit (Qiagen). After determining the DNA concentration using a Qubit™ fluorometer and Quant-iT dsDNA HS Assay Kit (Invitrogen), the sample was kept at -20°C until amplicon sequencing and qPCR analyzes.

| **Fp quantification**

qPCR was used to quantify Fp in soil samples. In our earlier work, we detailed the qPCR system and temperature settings. The quantity of Fp in soil was estimated using the 40 Tri5 gene, which codes for the trichothecene of *Fusarium* species.⁴⁶ qPCR was carried out using a LightCycler 480 (Roche) detection system with 1× LightCycler480 SYBR. The gene's amplification specificity was assessed by melt curve analyzes, and the amount of Fp in soil was determined by comparing each soil sample's Ct value to a standard curve produced by qPCRs using serially diluted Tri5 amplicons.

| • **Relative electrolyte leakage** We assessed the relative electrolyte leakage (REL) of the infected and healthy tissues as a different way to estimate the damage induced by Fp on roots and shoots. Three root or shoot pieces were incubated for one hour at 25°C in 3.0 ml of Milli-Q water for the test. A ST300C conductivity meter (Ohaus) was then used to test the water's conductivity (EC1). After boiling the samples for one hour at 95°C and letting them cool to room temperature, the water's conductivity was once again measured (EC2). For every sample, REL was computed as $EC1/EC2 \times 100\%$, and six replicates were conducted for every treatment.

| **Leaf gas exchange measurement**

A Li-Cor Li-6400XT in-fared gas exchange system was used to measure leaf gas exchange on the upper leaves of the three-week-old plants in order to track the effects of various treatments on plant photosynthetic efficiency. Measurements were made at 400 µl L⁻¹ CO₂ concentrations and 1500 µmol m⁻² s⁻¹ PAR. Six replicates of the plants in the SyCom and control groups were measured between 10 AM and 2 PM on a sunny day.

Microbial community profiling and bacterial SynCom tracking in soil

The Western Sydney University Next Generation Sequencing Facility performed amplicon sequencing of the soil samples using primers 341F (CCT ACG GGN GGC WGC AG) and 805R (GAC TACHVG GGT ATC TAA TCC)⁴⁷ in accordance with conventional MiSeq sequencing protocols. QIIME2 (version 2020.2; <http://qiime2.org/>) was used to analyze the acquired FASTQ data.⁴⁸ In short, QIIME2 was used to input raw data and verify the overall quality of the sequencing. Using the default settings, DADA2⁴⁹ was used to eliminate primer sequences, fix mistakes, and eliminate chimera. Following primer trimming, forward and reverse sequences were combined and terminated at 260 bp (forward) and 240 bp (reverse) to preserve high-quality sequences. A RDP classifier pretrained on Silva 138 99% OTUs full-length sequences was used to summarize and assign taxonomy to the acquired feature sequences. Fifty By resampling the OTU table, the amount of reads was rarefied to 4,265 per sample. The feature-table filter-features command was used to exclude unassigned, chloroplast, and mitochondrial DNA-like sequences in order to preserve bacterial and archaeal sequences. For the numerous OTUs (>1.0%) in QIIME2, a phylogenetic tree was created and annotated using an Interactive Tree of Life (iTOL; <https://itol.embl.de/>). All 16S rRNA gene sequences from the amplicon sequencing were compared to the bacterial genome sequences using BLASTn (v2.7.1), permitting no nucleotide mismatch, in order to investigate persistent microorganisms from the injected SynCom.

Statistical analyses

R3.6.1 was used to do statistical analyzes. Using one-way analysis of variance (ANOVA) and Tukey post hoc tests, the effects of SynCom and Fp on plant root and shoot growth, REL, and Fp load in soil were investigated. Permutational multivariate analysis of variance on Hellinger transformed OTU abundances using the package vegan 2.5-6.⁵¹ was used to investigate the effects of Fp and SynCom on responses of the bulk soil and rhizosphere bacterial communities. ANOVA of the Tukey–Kramer test with Bonferroni

correction for multiple tests was used to analyze differences in OTUs between samples. Co-occurrence microbial networks were created using FastSpar⁵², which use the SparCC method for network analysis, in order to deduce microbial connections between OTUs for various treatments.⁵³ To calculate p values, Fastspar ran 50 iterations and 1000 bootstraps. Correlations that were statistically significant ($p < 0.05$) were retained for further analysis and network visualization. The Fruchterman Reingold layout approach was used to visualize network topology using Gephi (0.9.2).⁵⁴

RESULTS :

Bacterial isolation, in vitro plant growth promotion tests and construction of the bacterial SynCom

Forty bacterial strains were isolated from the wheat rhizosphere and examined for the generation of IAA and ammonia, P solubilization, and Fp suppression. Plant root growth, nutrient uptake, and disease resistance are all significantly impacted by these characteristics. The strains' P solubilization capability varied between 4.87 and 635.58 $\mu\text{g ml}^{-1}$ on the third day and 17.23 and 789.14 $\mu\text{g ml}^{-1}$ on the seventh day of incubation (Table 1, File S1). The range of IAA production was 1.38 to 51.15 $\mu\text{g ml}^{-1}$ at 24 hours, 1.41 to 67.13 $\mu\text{g ml}^{-1}$ at 48 hours, and 1.19 to 73.32 $\mu\text{g ml}^{-1}$ at 72 hours. At 48 hours and 72 hours of in-cubation, ammonia production varied from 9.46 to 78.86 $\mu\text{g ml}^{-1}$ and 18.91 to 157.72 $\mu\text{g ml}^{-1}$, respectively (Table 1, File S1). Eight isolates with outstanding PGPTs were chosen for the SynCom construction for plant testing based on the findings. To identify the strains, whole-genome sequencing (apart from HWK1) and Sanger sequencing were used. Table 1 lists the eight isolates as follows: HW3: *P. megaterium*, HW4: *Bacillus* sp., HW4Z: *A. pittii*, HW15: *E. asburiae*, HW16: *Bacillus pumilus*, HW32: *A. pittii*, HWA1: *Xanthomonas* sp., and HWK1: 3.2 The wheat plant's development was tracked for 22 days until it reached the tillering stage. In the absence of Fp-infection, the SynCom dramatically boosted plant biomass production aboveground (by +120%; $p < 0.01$) and belowground (by +157%; $p < 0.01$) (Figure 1a–c). In comparison to the control (Figure 1b), the SynCom inoculation increased root growth but had no effect on plant height (Figure 2a). Compared to plants that were not treated with Fp, the Fp-infected plants grew much shorter and weaker (Figures 1a and 2a). However, compared to the non-SynCom infected controls ($p < 0.001$; Figures 1a,b and 2a,b), the SynCom inoculation resulted in much greater plant survival rates ($p < 0.001$) and much taller and stronger plants. Similarly, when Fp was present in the soil, the biomass of the roots (+5.6-fold) and shoots (+17.6-fold) of the SynCom-treated plants was much larger than that of the non-SynCom-treated plants (Figure 1b,c). We used ion leakage measurement to quantify cell damage in the root and shoot tissues in order to indicate severity in connection to disease development. considerable increases in REL were seen in the roots of Fp-treated plants, but not in the shoots ($p < 0.01$), suggesting that the Fp infection caused considerable root cell rupture (Figure 2c,d). It's interesting to note that SynCom + Fp-treated plants had cell damage that was comparable to that of non-Fp-treated plants and less than that of Fp treatment (Figure 2c,d). Instantaneous leaf gas exchange measurements of photosynthesis revealed higher CO₂ assimilation rates in SynCom-treated plants at the two-week stage (Figure 2e). This is in line with the increases in biomass that have been reported (Figure 1c,d). Because of the narrow/dead leaves, the SynCom + Fp and Fp groups were not measured. Fp abundance in soil varied over time ($p = 0.001$) and across treatments ($p < 0.0001$; Figure S1), according to qPCR measurements of the Fp quantity in bulk soil at three time periods. Significantly, the SynCom inoculation reduced the quantity of Fp in the soil on days 14 and 21 (Figure S1), suggesting that the SynCom effectively inhibited the development of Fp. Compared to the 7th and 14th days, the Fp in the Fp-treated soil

significantly decreased by day 21 (Figure S1). 3.3| Variations in SynCom abundance Burkholderia sp.| Wheat root development, biomass output, and disease resistance were all enhanced by the addition of SynCom to the soil.

3.2 The wheat plant's development:

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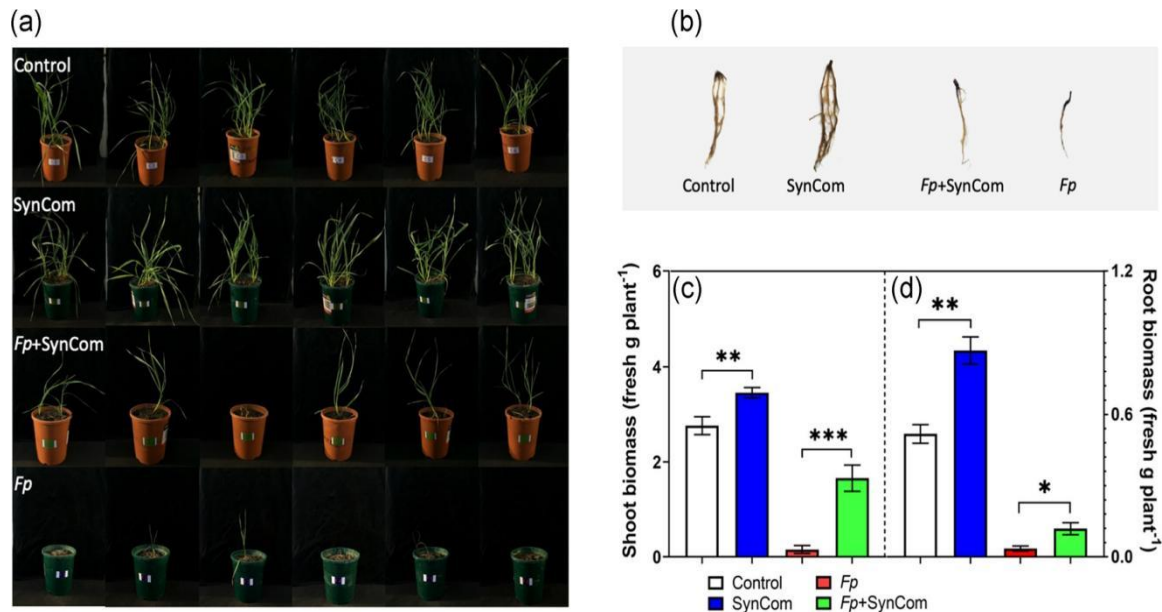


FIGURE 1 The performance of wheat in the sterile black Vetisol. Images documenting the development of wheat plants 22 days after seed planting (a). Three weeks after seeding, root morphology (b) and shoot and root biomass production (grams per fresh plant) were assessed (c, d). Significant changes between treatments are shown by stars in (c) and (d) (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Standard errors of the mean ($n = 6$) are shown by error bars. *Fusarium pseudograminearum*

(Fp);syntheticcommunity (SynCom)

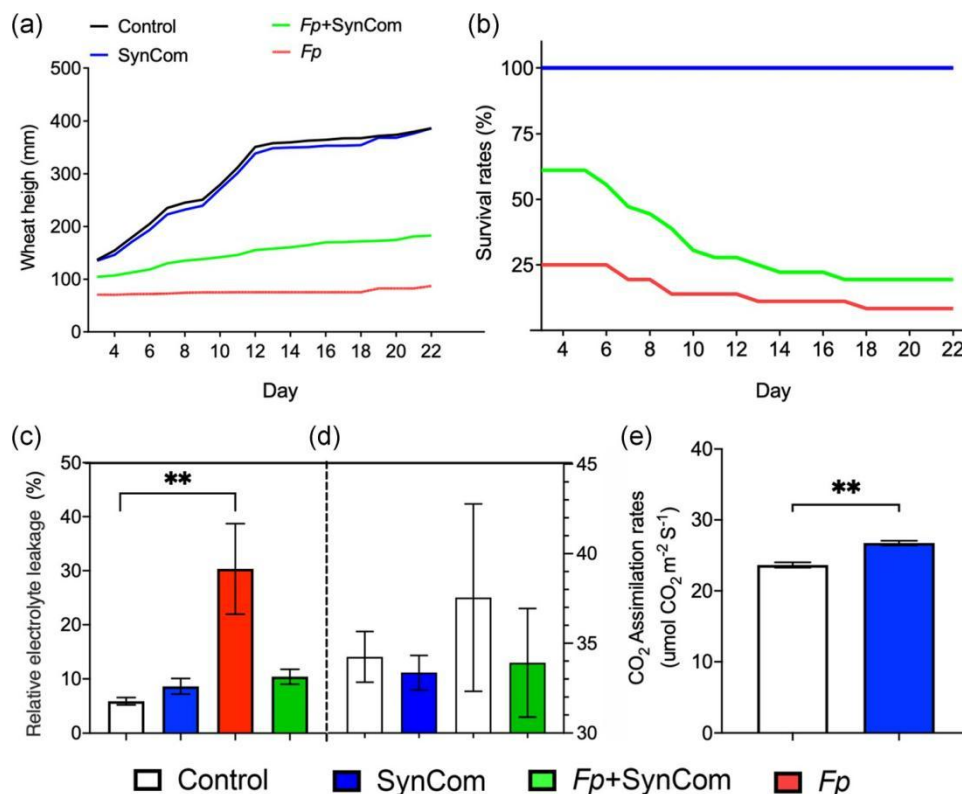


FIGURE 2 Plant growth and disease development.

Plant height (a) and survival rates (b) over time, root and shoot cell damage (c) as measured by relative electrolyte leakage, and leaf gas exchange rates (e). Significant changes between treatments are indicated by stars in (c) and (e) (**p < 0.01). Standard errors of the mean (n = 6) are shown by error bars. *Fusarium pseudograminearum* (Fp) and synthetic community treatments (SynCom) at every time point (Figure 3a). Using the most abundant OTUs (>1.0% in relative abundance), a phylogenetic tree of the bulk soil and rhizosphere samples was constructed, visualized, and annotated in iTOL. Proteobacteria, Bacteroidetes, Verrucomicrobia, Firmicutes, and Actinobacteria dominated the soil bacterial population (Figure 3b). Significantly, the composition of the soil microbial community varied over time (R² = 0.07, p < 0.0001) and was impacted by the SynCom treatment (R² = 0.08, p < 0.0001; Figure 4). Furthermore,

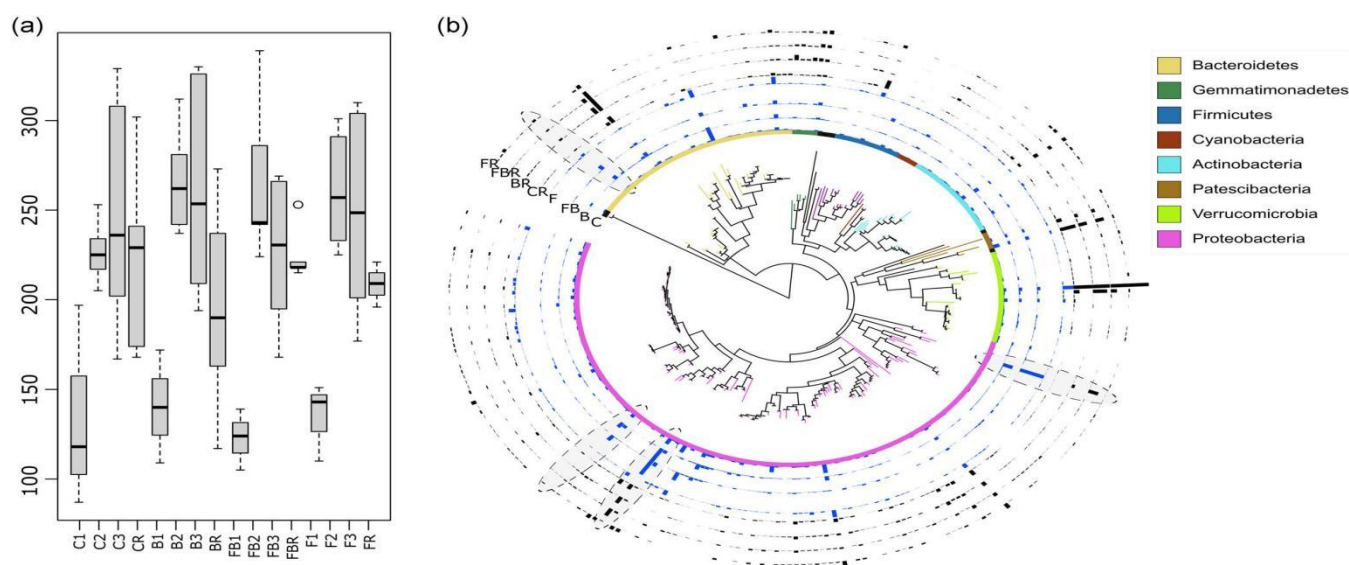


FIGURE 3: The relative abundance of major microbial taxa (b) and the bacterial operational taxonomic unit (OTU) richness (a) in soil. De novo multiple sequence alignment in QIIME2 was used to build the midpoint rooted tree, which

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was then annotated in iTOL. OTUs with higher relative abundance in SynCom-treated plants (0%–14.3%) were indicated by ellipses in the tree. R stands for rhizosphere, while the numerals 1, 2, and 3 stand for time points. Standard errors (n = 6) are shown by error bars. F, Fp, FB, Fp plus SynCom; B, SynCom; C, control

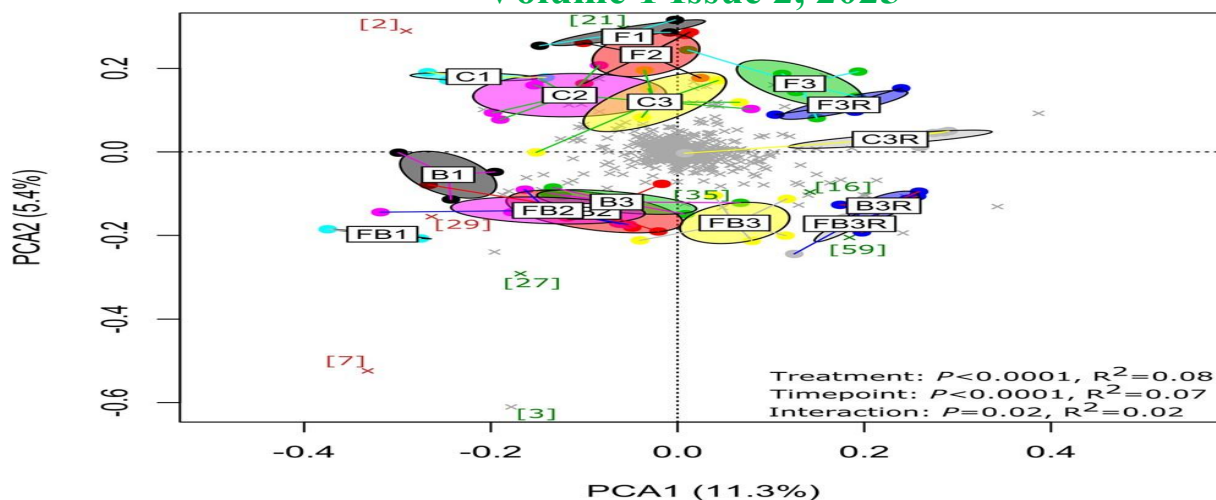


FIGURE 4:

Principal component analysis summarizing variations in the makeup of the bacterial communities among treatments. OTUs that varied between Fp and Fp + SynCom were labeled in green, while those that differed between control and SynCom were labeled in red. Standard errors at 95% confidence were shown by the ellipses. Different time points are represented by the digits 1, 2, and 3. There was an interaction effect between the time points and SynCom treatments ($R^2 = 0.02$, $p = 0.04$) in B, SynCom; C, control; F, Fp; FB, Fp + SynCom. This was probably caused by the soil microbiome's recovery and a decline in SynCom abundance over time (Figure 3b). OTU2 (an uncultured bacterium), OTU3 (*Bdellovibrio* sp. SKB1291214), OTU7 (*Pseudomonas* sp.), OTU16 (*Enterobacteriaceae*), OTU21 (*Pontibacter* sp. LX8), OTU27 (*Bacillus* sp.), OTU29 (an uncultured bacterium), and OTU35 (*Adhaeribacter* sp.) clearly separated the SynCom-treated (Control/Fp) groups for bulk soil samples. OTU3, OTU16, OTU21, OTU27, OTU35, and OTU59 varied between Fp and Fp + SynCom treatments, while OTU2, OTU7, and OTU29 substantially differed between the control and SynCom treatments (Figure 4). Similarly, the rhizo-sphere samples treated with SynCom and those not showed a distinct difference ($p < 0.001$, $R^2 = 0.26$; Figures 4 and S2). Additionally, OTUs that resembled SynCom were found in the rhizosphere, mostly *Enterobacter* and *Xanthomonas* species (Table 2). Finally, rhizosphere effects were significant, as predicted ($R^2 = 0.08$, $p < 0.0001$). A few OTUs were affected by the SynCom treatments when looking at each OTU separately, including OTU3 ($p < 0.001$), OTU7 ($p < 0.01$), OTU9 (*Pseudomonas* sp.; $p < 0.001$), OTU15 (*Xanthomonas* sp.; $p < 0.001$), OTU16 ($p = 0.053$), and OTU56.sp.), *Bacillus* sp. OTU491, and *Acinetobacter* sp. OTU1583 (Table 2). On the seventh day, the abundance of the possible SynCom OTUs decreased to low concentrations, and on the fourteenth and twenty-first days, it was much lower. On the twenty-first day after the SynCom inoculation, *Acinetobacter* sp. and *Bacillus* sp. decreased to very low quantities ($<0.2\%$ and $<0.1\%$, respectively) (Table 2). The bacterial SynCom reshaped the bulk and rhizosphere soil microbial network. The network analyses were carried out by combining samples of the same treatments at various time periods. The microbial networks' top linked nodes varied significantly across treatments (Figure 5). The SynCom-treated soil had more nodes and edges than the control, but there was no difference between the Fp and Fp + SynCom treatments (Figure 5 and Table 3). The top five nodes (OTUs) in the control were *s_Flavobacterium hauense*, *g_Azospirillum*, *g_Gemmatirosa*, *g_Phenylobacterium*, and *g_Microvirga*; *f_Micrococcaceae*, *o_Frankiales*, *g_Micrococcaceae*, *g_Haliangium*, and *g_Enterobacter*; five *g_Massilia* in the Fp treatment; and *g_Gemmatirosa*, *f_Enterobacteriaceae*, *g_Xanthomonas*, *f_Sphingomonadaceae*, and *c_Berkelbacteria* in the Fp + SynCom treatment. Additionally, certain SynCom member-like OTUs (such OTU16) were found among the network's most linked taxa. These findings suggested that the overall network structure of soil microbiomes had been altered by the SynCom and Fp treatments.

[DISCUSSION:

In this work, we examined the colonization of a bacterial SynCom in a soil with decreased microbial diversity and its impact on wheat plant development and disease prevention. Despite being observable, the relative abundance of SynCom members decreased to low levels after 22 days. The SynCom increased the relative abundances of a few *Pseudomonas* species and changed the bulk and rhizosphere soil microbial community. In addition to increasing plant survival rates from the Fp-infection, the SynCom also boosted root growth and biomass output. Our knowledge of how a beneficial SynCom colonizes a biodiversity-reduced soil (a stand-in for umigation or solarization techniques) and interacts with the plant under biotic The bacterial populations in the bulk soil were analyzed using co-occurrence networks. The 10 labeled nodes, which varied throughout subfigures, were (a) 1: *s_Flavobacterium hauense*, *g_Azospirillum*, 3: *g_Gemmatirosa*, 4: *g_Phenylobacterium*, 5: *g_Microvirga*, 6: *g_Massilia*, *g_Massilia*, 8: *g_Bacillus*, 9: *f_Micrococcaceae* and 10: *f_Enterobacteriaceae*; (b) 1: *f_Micrococcaceae*, *o_Frankiales*, 3: *f_Micrococcaceae*, 4: *g_Haliangium*, 5: *g_Enterobacter*, 6: *f_Microbacteriaceae*, 7: *g_Chthoniobacter*, 8: *g_Bacillus*, 9: *g_Microbacterium* and 10:

f_Enterobacteriaceae; (c) 1: g_Massilia, 2: g_Massilia, 3: g_Massilia, 4: g_Massilia, 5: g_Massilia, 6: g_Massilia, 7: g_Massilia, 8: g_Massilia, 9: g_Pseudomonas, and 10: g_Pseudomonas, and (d) 1: g_Gemmatirosa, 2: f_Enterobacteriaceae, 3: g_Xanthomonas, f_Sphingomonadaceae, 5: c_Berkeleyella, 6: f_Enterobacteriaceae, 7: o_Micavibrionales, g_Gemmatirosa, 9: s_Pontibacter sp. LX8, and 10: g_Bacillus. Different network modules are represented by different colors in (a–d) TABLE 3

Microbial network topology of different treatments

	Control	SynCom	<i>Fp</i> SynCom	+ <i>Fp</i>
Nodes	149	188	176	185
Edges	241	379	407	412
SynCom	3.235	4.032	4.625	4.454 (~ 4× 10 ⁶)
Pos. rate	61.83	60.16	63.14	55.58

A decrease in the use of *Streptomyces* to manage *Rhizoctonia solani* in wheat was also seen.⁵⁵ These findings might be explained by the following: (i) the niche environment (such as soil pH and nutrient content) in the soil at seed planting did not allow the continued high growth rates of a substantial proportion of the inoculated

| Colonisation of the SynCom in soil:

soil disruptions by significantly diminishing every aspect of the resident community, giving the colonists or new community a chance to settle. After two weeks, the amounts of SynCom in the soil significantly decreased, despite the fact that SynCom-inoculated plants showed improved plant performance and disease resistance. This implies that colonization's priority effects were transient. The quickly restored soil microflora outcompeted the injected SynCom for nutrients and niche, and it may have had antagonistic effects on the imported microorganisms (e.g., employing antimicrobial chemicals). A similar pattern of biocontrol agents has been reported.⁵⁶ Furthermore, in the wheat rhizosphere, *Bacillus* species, *Enterobacter* species, and *Acinetobacter* species were not predominant taxa. Forty For instance, slightly less than 1% of the bacterial community in the mature wheat rhizosphere was taken up by *Bacillus* and *Enterobacter* species, indicating a lack of favorable soil conditions for SynCom development. A diluting impact from the recovery of native microorganisms after autoclaving may also have contributed to the decline in relative abundances of SynCom in soil. We do recognize, however, that utilizing amplicon sequencing to monitor changes in the abundance of the imported isolates might be biased since it can be affected by shifts in the dominance of other soil bacterial members.

| Effects of the SynCom on plant performance-root development and biomass accumulation

We observed significant increases in wheat shoot biomass, which may be related to the advantageous characteristics of the SynCom members. It's interesting to note that plant shoots in the SynCom-treated group seemed stronger (higher stem size, for example) but not taller, indicating that the SynCom enabled the plant shoot to amass more biomass. This result was corroborated by the measurement of leaf gas exchange, which revealed that the plants treated with SynCom had greater rates of photosynthesis. The rhizosphere, which serves as an interface for the interaction between roots and soil bacteria, and the root system are crucial to a plant's aboveground performance.⁵⁷ We discovered that the SynCom inoculation significantly improved the wheat plant's root growth as measured by fresh weight. A microbial consortium made up of rhizobacteria that promote plant development and N-fixing bacteria (*Azospirillum* spp., *Azoarcus* spp., and *Azorhizobium* spp.) shown strong colonizations on the roots and leaves of common wheat (*Triticum aestivum* L.) in a prior research.⁵⁸ Additionally, the number of ramifications, root points, and volumetric root length density were all boosted by the SynCom.⁵⁸ The plant's resistance to *Fp*-infection in our research could have been influenced by the improvement in root growth and development. To varying degrees, the eight bacterial isolates were able to create IAA and ammonia, as well as perhaps additional phytohormones. Although it is difficult to identify the specific strains that were responsible for the soil microbial alterations in the present investigation, they together influenced the morphological and physiological characteristics of the roots. The SynCom-treated plants may have had

more root surface for nutrient uptake as a result of their larger roots. Enhancing the root system may also help plants anchor in the soil and utilize resources more effectively.⁵⁹ Overall, our SynCom's members' capacity to create IAA (which encourages root development), P-solubilization and ammonia production (which guarantees the availability of two key macronutrients), and *Fp*-inhibition (which eliminates soil-borne pathogens) explains the beneficial impacts on plant performance. These results are consistent with earlier research that found comparable outcomes for certain microbial strains.^{60–63}

| Colonisation of the SynCom altered the soil microbial community structure

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Because the SynCom enhanced the size and complexity of the soil microbial co-occurrence network, its colonization in the soil had an impact on the microbial composition of the soil and probably helped with soil microbial recovery. Previous research also shown that an introduced bacterium (*Escherichia coli*) altered the composition and niche structure of the soil microbial community, most likely as a result of the bacterium competing with the native bacteria for resources.⁶⁴ We also discovered that the SynCom treatment considerably increased the relative abundance of two common *Pseudomonas* species in the soil (16.3% and 15.2%, respectively). *Pseudomonas* microbial taxa are known to include a variety of helpful bacteria that either inhibit soil-borne diseases or stimulate plant development.⁶⁵ For instance, *Pseudomonas piscium*, which was isolated from wheat heads, suppressed the proliferation, pathogenicity, and mycotoxin production of *F. graminearum* and produced phenazine-1-carboxamide.^{Six} In line with our results, it was previously shown that accumulations of certain *Pseudomonas* species in soil were associated with the suppression of *F. oxysporum* by a bioorganic fertilizer (including *Bacillus* spp.).⁶⁶ But the biological effects of the *Pseudomonas* species that were generated. Future research is necessary since the processes causing the accumulations in the soil to the wheat plant are unknown. Our findings also imply that the SynCom had a significant impact on the soil microbiome's recovery. This may highlight a crucial function of the modified biocontrol agents in controlling the restoration of native soil microbiomes, which has to be taken into account when controlling soil microbial characteristics for increased crop disease resistance. A prior research also shown that the microbiota of maize plants was altered by the introduction of a SynCom.⁶⁷ These effects of SynCom on soil/plant microbiomes may be explained by the possibility that SynCom colonization changed the chemical microenvironment and nutrient availability in soil/plants.

Potential microbial interactions in soil microbiomes:

Plant performance and production are greatly impacted by microbial interactions, where central/hub microorganisms may be responsible for the assembly and activities of the microbiome.⁶⁹ In this research, the introduction of SynCom and Fp into the soil essentially increased the size and complexity of the microbial network. It's possible that the imported bacteria served as core microorganisms, enabling the establishment of both positive and negative microbial interactions in the soil that produced different patterns from those in the control. These results imply that SynCom treatments had a substantial impact on soil microbial co-occurrence, which may have consequences for soil microbial interactions and plant disease resistance.⁷⁰ It's interesting to note that in the network of Fp-treated soil, a few dominating *Massilia* spp. had the most connections. Although the implications for soil health are unknown, such a finding would point to a substantial interaction between *Massilia* spp. and the pathogen in soil.

3 | CONCLUSIONS

In agricultural production, soil fumigation and solarization are often used with the primary goal of lowering the pathogen load in the soil. However, both methods drastically lower soil microbial diversity and biomass, which has long-term effects on farm output. In this work, we show that plant growth and disease tolerance are enhanced by bacterial SynCom colonization in a soil with decreased microbial diversity. The native microorganisms may have outcompeted the imported bacteria as they recovered from disruptions, as shown by the fact that the introduced bacteria in the soil mostly survived albeit with lower relative abundances. In spite of this, the SynCom significantly improved plant performance and changed the relationships between soil microbiomes. By offering fresh proof of SynCom's impacts on plant health and the soil microbiome, these results advance the science. They also provide a foundation for further fieldwork to uncover strategies to improve bioinoculant colonization and effectiveness. Future research is necessary to determine if and how introduced SynCom's dominance and activities may be encouraged for long-term positive impacts on plant production and health.

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