Environmental Microbiome

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Identifying rhizosphere bacteria and potential mechanisms linked to compost suppressiveness towards *Fusarium oxysporum*

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Abstract

Background Soilborne fungal phytopathogens pose a significant threat to global food security. While chemical control remains an effective method for managing these pathogens, increasing regulations due to health and environmental concerns, along with rising fungicide resistance, have restricted their use, underscoring the urgent need for sustainable alternatives. The use of compost to enhance soil fertility and suppress plant diseases is well documented. Several studies have underlined the role of microorganisms in disease suppression, but the mechanisms facilitating this disease suppression remain unclear. We evaluated the impact of compost amendment on the composition and functional capacity of the rhizosphere microbiome in cucumber plants (*Cucumis sativus*) inoculated with *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (FORC) under controlled greenhouse conditions using amplicon sequencing, shotgun metagenomic and culture-based techniques.

Results Compost amendment significantly reduced FORC-induced disease in cucumber relative to non-amended treatments. While FORC inoculation resulted in significant shifts in microbial (bacterial and fungal) community composition in the rhizosphere of non-amended plants, this phenomenon was substantially less pronounced in the rhizosphere of compost-amended plants. Specifically, compost amendment sustained the presence of *Actinomycetota* (*Streptomyces*, *Actinomadura*, *Saccharomonospora*, *Pseudonocardia*, *Glycomyces*, *Thermobifida*) and *Bacillota* (*Planifilum*, *Novibacillus*) in FORC inoculated plants, that diminished significantly in inoculated plants without compost. These taxa contained a myriad of non-ribosomal peptides and polyketides synthetases biosynthetic gene clusters (BGCs) with putative antimicrobial and iron-chelating functions. We successfully isolated two *Streptomyces* strains from FORC-suppressing compost amended rhizospheres that were almost identical to the *Streptomyces* bin2 (99% ortho ANI) metagenome assembled genome identified in the shotgun metagenome analysis. These strains produced extracellular metabolites that inhibited growth of FORC in-vitro and contained BGCs that encode for compounds with potential antimicrobial capacity.

Conclusions Based on results presented in this study, we demonstrate that compost alleviates FORC-induced dysbiosis of the rhizosphere microbiome, maintaining abundance of specific bacterial taxa. These bacterial groups may contribute to disease suppression through a myriad of mechanisms including iron chelation and production of fungal antagonizing secondary metabolites.

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Keywords Rhizosphere, Microbiome, FORC: *Fusarium oxysporum* f. sp. *radicis-cucumerinum*, Compost, Suppressiveness, BGCs: Biosynthetic gene clusters, NRPS: Non-ribosomal peptide synthetase, PKS: Polyketide synthase, Amplicon sequencing, Shotgun metagenomics.

Background

Soilborne fungal phytopathogens pose a significant threat to global food security because they infect a wide range of crops and are difficult to control [1, 2] especially since most of them can survive in soil in the absence of host plants [3, 4]. The *Fusarium oxysporum* species complex includes several strains that cause wilt or root and crown rot in a variety of commercially significant crops [5]. These pathogens are particularly challenging for plant protection [6] due to growing resistance of some Fusarium species to commonly used fungicides, such as azoles [7]. Chemical fungicides are often the most effective way to control soil borne fungal pathogens [4], but increased regulation due to human and environmental health ramifications has strongly restricted use of several conventionally applied compounds [8]. This phenomenon, coupled to increasing global demand for food production and the predicted geographic expansion of phytopathogens due to climate change, has created a dire need for sustainable alternatives [9].

Soil amendments, including animal and green manure, organic wastes [1, 10, 11], compost, [12, 13] and more recently, biochar [14, 15] are considered to be beneficial strategies for controlling soilborne pathogens. The capacity of compost to effectively suppress soilborne fungal pathogens has been previously demonstrated in a diverse array of studies [1, 12, 16, 17] and while abiotic factors such as the presence of compost-derived fungitoxic chemicals have been reported, collective evaluation of current research suggests that the primary mechanisms behind compost disease suppressiveness are linked to shifts in the overall soil microbial community composition, diversity, activity and functioning [17, 18], that facilitate enrichment of specific groups of bacteria and fungi with antagonistic activity against soilborne pathogens [19, 20]. Compost is a reservoir of microbial diversity, including bacteria and fungi capable of colonizing the rhizosphere. Upon compost amendment, plant roots selectively recruit specific microbes, shaping a rhizosphere community distinct from that of the compost itself [21]. This newly assembled rhizosphere community acts as the first line of defense against soilborne pathogens [22] by suppressing the pathogens directly through antibiotic production and secretion of lytic enzymes, or indirectly through competition for space and nutrients [23–25]. Compost-derived bacterial communities were found to be typically dominated by the phyla *Bacillota* and *Actinomycetota*, regardless of compost origin or maturity [26]. Within these groups, notably, *Bacillus* (*Bacillota*) and *Streptomyces* (*Actinomycetota*) strains isolated from compost have shown antagonistic activity against phytopathogens such as *Pythium*, *Fusarium*, and *Phytophthora* [27].

Rhizosphere bacteria especially *Streptomyces* [28] produce diverse secondary metabolites (SMs) that play vital ecological roles in interactions between the microbiome, plant roots and soilborne pathogens, and subsequently impact plant growth and health [29, 30]. These SMs include iron scavenging siderophores, stress protectant pigments, signaling molecules, and antimicrobials, such as antifungals which contribute to disease suppression through antibiosis [31]. Non-ribosomal peptides (NRPs) and polyketides (PKS) constitute the majority of bacteria-produced SMs [32]. NRPs and PKS are synthesized by multimodular enzymes, non-ribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs) respectively that are encoded by large biosynthetic gene clusters (BGCs) [33, 34]. The modularity and ability of NRPS and PKS to incorporate a wide array of substrates enable the synthesis of chemically diverse, bioactive compounds [35, 36], making them a promising source of novel antifungal agents for agricultural use.

While numerous studies have demonstrated compost-facilitated disease suppressiveness in various pathosystems and identified bacterial taxa that are stimulated by compost amendment [19, 37], few have deliberated the mechanisms responsible for disease suppression by compost-induced bacteria [27]. We hypothesize that compost enhances disease suppression by stimulating rhizosphere bacteria that antagonize pathogens at least partially through the production of antimicrobial secondary metabolites.

This study aimed to identify microbial populations and associated secondary metabolite encoding genes involved in compost-induced soilborne pathogen suppression, using a model comprised of greenhouse grown cucumber plants inoculated with *F. oxysporum f. sp. radicis-cucumerinum* (FORC). Initially, we evaluated the impact of compost amendment on the microbial community composition of cucumber rhizospheres

(with and without FORC inoculation) using bacterial 16S rRNA gene and fungal ITS (internal transcribed spacer) amplicon sequencing, and then identified bacterial populations potentially associated with the observed compost-induced suppressiveness. Subsequently, we identified functional genes possibly linked to compost facilitated suppressiveness using shotgun metagenomics, specifically focusing on genes encoding for secondary metabolites from compost stimulated bacterial taxa. Using culturomics, we isolated representative bacteria associated with compost-suppressiveness and tested their capacity to secrete FORC-antagonizing extracellular metabolites in-vitro.

Materials and methods

Greenhouse experiment

For compost amended treatments, soil, commercial perlite (Perlite 212 Agrekal, Israel), and compost (Azur nurseries, Israel) were mixed in a 2:2:1 (v/v) ratio, whereas in non-amended treatments, soil was mixed with perlite in a 1:1 (v/v) ration. These soil mixtures were watered every other day for a week before transplanting seedlings (see below). The study used a clay-rich soil sourced from Ahituv, Israel (32.389988, 34.990965), commonly used for greenhouse cucumber cultivation. It was air-dried at room temperature, ground, and sieved through a 2 mm mesh to ensure uniform particle size.

Cucumber (*Cucumis sativus*) seeds (Manny- Genesis seeds, lot # 10614C) were sown in seedling trays filled with perlite:vermiculite mixture (1:3, respectively) and the trays were incubated in a temperature-controlled growth chamber (28–30 °C) for 7 days and were watered every other day with tap water to facilitate germination. Germinated seedlings were then transplanted into 2.5-inch square black nursery pots (one plant per pot) containing the potting mixtures described above, producing the four following treatments: (i) non-inoculated, non-amended (5 plants), (ii) non-inoculated, compost-amended (5 plants), (iii) FORC-inoculated, non-amended (6 plants), and (iv) FORC-inoculated, compost-amended (6 plants). The inoculated treatments contained 1% FORC (w/w), prepared according to the protocol previously described by [38]. In brief, a mycelial plug taken from 5 days old FORC cultivated on PDA (potato dextrose agar) was subsequently grown on pearl millet seeds (*Pennisetum glaucum*) that had been soaked in water overnight and sterilized by autoclaving twice on successive days, 24 h apart. After 12 days of incubation, FORC-colonized seeds were incorporated into the soil mixtures at a ratio of 1% (w:w). Non-colonized seeds treated identically served as the control. All plants were fertilized with 500X diluted commercial greenhouse liquid fertilizer for 21 days.

Rhizosphere samples were collected by gently shaking off excess soil from the roots and retaining the soil that remained tightly adhered to the root surface. Five grams of rhizosphere soil from each sample were stored in sterile 50 ml polypropylene tubes at −80 °C for DNA extraction. An additional five grams from each remaining rhizosphere sample were transferred into 50 ml Falcon conical centrifuge tubes containing 5 mm glass beads, suspended in 15 ml of sterile 0.85% NaCl solution, vortexed at maximum speed for 2 min, and centrifuged at 300 rpm for 5 min at room temperature. The resulting supernatant was transferred into a clean tube, and 500 µl of the supernatant were mixed with 500 µl of sterile 50% glycerol, and stored at −80 °C in triplicates for subsequent strain isolation, as described below.

Disease phenotyping

To evaluate the capacity of compost to induce disease suppression, disease incidence and plant growth parameters including shoot dry weight and root fresh weight were measured to assess overall plant performance. Disease incidence was recorded daily following the method described by Madden et al. [39] by calculating the percentage of wilted plants in each treatment until no further wilting was observed (21 days after inoculation). At the end of the experiment (21 days post-inoculation), plants were photographed. Shoots were excised, dried overnight at 100 °C, and weighed to determine shoot dry weight. After collecting rhizosphere soil samples as described above, root fresh weight was measured by gently washing off excess soil, patting the roots dry with tissue paper, and weighing them using an analytical scale.

Rhizosphere sampling, DNA extraction, and sequencing

DNA was extracted from 0.25 g of rhizosphere material of all plants (N = 22) using the DNeasy PowerSoil Kit (Qiagen, USA), according to the manufacturer's protocol. For characterization of bacterial and fungal communities, we PCR-amplified the V3-V4 region of the 16S rRNA gene (bacteria) and the nuclear ribosomal internal transcribed spacer (ITS, fungi), using the 341F/806R [40] and ITS1-F/ITS2R [41] primer sets, respectively. The primers were appended with CS1(forward), and CS2 (reverse) Illumina sequencing adapters and sequencing was conducted using a paired-end 2×300 bp cycle run on an Illumina MiSeq sequencing system using MiseqV3 chemistry, at Rush University Genomics and Microbiome Core Facility (Chicago, USA).

Shotgun metagenomic sequencing was conducted on 12 DNA samples from the FORC-inoculated treatments (6 non-amended and 6 compost-amended) using an Illumina NovaSeq S4 platform with 2×150 bp

paired-end reads, employing Illumina dsDNA shotgun library preparation. The sequencing was performed at Rush University Genomics Microbiome Core Facility. All datasets have been deposited in the NCBI Short Read Archive under Bioproject PRJNA1169419.

Bioinformatic analysis of generated amplicon sequences

The paired-end, demultiplexed 16S and ITS reads were processed independently using QIIME2 (v2019.07) [42]. Adapter and primer sequences were removed with the Cutadapt [43] plugin, and the trimmed reads were subsequently denoised using the DADA2 pipeline [44] to generate amplicon sequence variants (ASVs) (Table S1a and b, Additional file 2). The resulting feature tables were then filtered to exclude ASVs with fewer than 10 counts across all samples.

Taxonomic assignment of the 16S and ITS ASVs was performed using a Naive Bayes classifier trained on the SILVA (v132) [45] and UNITE (v8) [46] databases, respectively. For the 16S dataset, ASVs that were classified as chloroplasts, were removed. For the ITS dataset, the most abundant ASV, classified as *Fusarium* and likely representing the FORC inoculum, was removed prior to downstream analyses as it accounted for over 90% of reads in inoculated samples but was nearly absent in the non-inoculated samples. A mid-point rooted phylogenetic tree for the calculation of diversity matrices was created with fasttree and mafft alignment (QIIME2) using the generated ASVs. Raw count tables, ASV taxonomy, and the rooted phylogenetic tree generated in QIIME2 were exported for downstream analyses and visualization in R (version 4.3.1).

Quality control and assembly of shotgun metagenomic reads and characterization of assembled contigs

The quality of raw shotgun metagenomic reads was assessed using FASTQC, and low-quality reads and adapters were removed using Trimmomatic [47] with default parameters for adapter trimming, and CROP:145 HEADCROP:15 SLIDINGWINDOW:4:20 MINLEN:100 parameters for low quality trimming. Subsequently, FORC sequences were removed by aligning the trimmed reads against the FORC reference genome (GenBank GCA_001702695.2) using Bowtie2, -very-sensitive-local flag [48] and FORC reads were removed using SAMtools [49]. Removal of these reads prevented FORC dominance, enabling clearer assessment of microbial community responses specifically associated with FORC introduction. High quality FORC-filtered reads from all samples were pooled and assembled using MEGAHIT [50] (Table S2, Additional file 2). The assembled contigs were filtered to retain contigs > 2000 bp, and these were used as input for prediction of open reading frames (ORFs) using Prodigal [51], in sensitive mode. The predicted ORFs were aligned to the NCBI-nr protein database using DIAMOND [52], and MEGAN V6 Ultimate Edition [53] was then used for taxonomic assignment of these ORFs based on the Lowest common ancestor (LCA).

Detection and annotation of biosynthetic gene clusters (BGCs) from assembled shotgun metagenomic contigs

Assembled contigs from compost-amended and non-amended FORC-inoculated treatments were filtered to maintain contigs that were > 5000 bp length, which were used for identification and annotation of secondary metabolite encoding BGCs with antiSMASH v6 [54], using the following parameters: –cb-general –cb-subclusters –pfam2go –strictness relaxed –genefinding-tool prodigal-m mode. A biom table of reads mapping to BGC core regions was generated using BiG-MAP [55], and differential abundance analysis between compost amended and nonamended groups was performed using MaAsLin2 [56] on a Total Sum Scaling (TSS) normalized table. The taxonomy of the identified BGCs was assigned by aligning contigs to the NCBI nonredundant database using DIAMOND and LCA classification with MEGAN [53]. NRPS and PKS BGCs were aligned against the MiBiG 3.0 [57] database to predict their putative functions using DIAMOND [52] (cutoffs: e-value < 10^{-40} and identity > 50%), as previously described [14].

MAG binning and annotation

Contigs were binned using metaWRAP v1.3.2 [58] with a minimum completeness of 75% and a maximum contamination of 5%. Binning was performed using MaxBin2 [59], metaBAT2 [60], and CONCOCT [61]. The resulting bins were dereplicated using dRep [62] v3.4.2 with default parameters. Taxonomic classification was achieved using the GTDB-Toolkit [63] v2.3.2 (GTDB-Tk) classify workflow.

Statistical analysis

Statistical differences in shoot dry weight and root fresh weight between treatments were evaluated using one-way ANOVA and multiple comparisons of means using the Tukey–Kramer (HSD) test ($\alpha = 0.05$) in JMP software (JMP®, Version 17. SAS Institute Inc., Cary, NC, 1989–2023).

Alpha diversity indices as well as beta diversity based on Bray–Curtis dissimilarity, were calculated using the vegan package in R. The adonis function, a permutation-based multivariate analysis of variance (PERMANOVA), was used to assess the statistical significance of

differences in microbial community composition based on amendment and inoculation factors. Differentially abundant taxa were detected using MaAsLin2 [56] and LEfSe [64] (false discovery rate < 0.05).

Bacteria isolation, identification and whole-genome sequencing

Selected bacteria were isolated from glycerol stocks of compost-induced disease suppressed rhizosphere samples using cucumber plant-extract media prepared according to [65] with several modifications. Briefly, 20 g of cucumber shoots were washed in tap water to remove any soil particles, cut into small pieces, and blended with minimum amount of water in a waring blender. The resulting extract was filtered through a triple layered gauze and stored at 4 °C as a stock solution. Agar plates were prepared by diluting the cucumber extract with distilled water in a ratio of 1:10 (v/v) and addition of 1.5% (w/v) Bacto agar (Difco Laboratories, Detroit). The medium was sterilized by autoclaving at 121 °C for 30 min and 50 mg/l of cycloheximide was added to preclude the growth of fungi. Cultures were incubated for 14 days at 30 °C, and the isolated bacteria were taxonomically identified based on 16S rRNA gene amplicon sequencing using primers; 11F (GTTTGATCC TGGCTCAG) and 1392R (ACGGGCGGTGTGTRC) [66]. The PCR products were sequenced by Macrogen Inc. (Seoul, Korea) and aligned against the NCBI nr database using BLASTn with a sequence similarity threshold of over 95% for genus-level identification.

High molecular weight genomic DNA was isolated from *Streptomyces* CC6 and CC27 isolates using MagAttract HMW DNA (Qiagen, USA) extraction Kit following the manufacturer's protocol. Hybrid whole genome sequencing and assembly were performed by Plasmidsaurus Inc. (USA) service using Oxford Nanopore and Illumina Sequencing Technologies.

Screening for in-vitro antifungal activity

Green fluorescent protein-labeled (gfp) FORC [67] was exposed to cell-free supernatants (CFS) from *Streptomyces* CC6 and CC27 to assess potential presence of inhibitory secreted metabolites, using the methodology developed by [68]. Briefly, CFS were prepared from bacterial spent media as follows; one milliliter of 3 days old starter culture was used to inoculate 50 ml of Tryptic Soy Broth (TSB, BD Bacto™ Soybean-Casein Digest Medium) in 250 ml Erlenmeyer flasks. The flasks were incubated for 7 days at 30 °C and 170 rpm. The bacterial spent media was then centrifuged (5000 g for 10 min) and the supernatant was filtered using a 0.22 µm Millex-HV syringe filter (Sigma-Aldrich, Israel).

Stock spore solution was prepared by inoculating a plug of gfp-labeled FORC mycelia taken from a 5 days old PDA (potato dextrose agar) plate in 25 ml of fresh PDB (potato dextrose broth) media in 250 ml Erlenmeyer flasks and flasks were incubated for 7 days 25 °C and shaking at 100 rpm. FORC culture was filtered through 40 µm filters, and concentration was determined by counting spores using a Hemocytometer. In-vitro antifungal assays were carried out in 96-microwell plates by adding 100 µl of bacterial cell-free supernatant (CFS) into 100 µl of gfp-labelled FORC spores (10^5 spores/ml). CFS-treated FORC spores were incubated at 25 °C for 24 h, and growth changes were monitored by measuring fluorescence and OD using the multimode Microplate Reader (Synergy H1, BioTek, USA).

For dual culture plate assay, *Streptomyces* CC6 and CC27 starter cultures were grown on LB medium (Luria–Bertani) for 3 days. Ten microliters of the bacteria culture were spot inoculated in quadruplets on half strength LB agar plates, 2cm from the center of the plate. Plates were incubated at 30 °C for 5 days and thereafter a plug of 7 days old FORC mycelium was placed in the center of plate. The plates co-inoculated with bacteria and fungi were incubated at 28 °C and visually monitored for a period of 7 days.

Results

Compost amendment alleviates FORC-induced disease in cucumbers

Disease incidence in FORC-inoculated cucumber plants was determined by counting the number of wilted plants in soil with and without compost at 21 days post inoculation. No mortality was recorded for inoculated plants grown in compost-amended soil, while 50% mortality (N = 6) was observed in inoculated plants grown in non-amended soil (Fig. 1). Additionally, FORC inoculation significantly reduced shoot dry weight and root fresh weight of cucumber plants grown in compost-free soil (Tukey HSD test, $P < 0.05$), whereas in compost-amended soil, these values were comparable to control (pathogen free) plants with and without compost amendment (Tukey HSD test, $P > 0.05$) (Fig. 1).

Compost amendment reduces the relative abundance of Fusarium, and alleviates FORC-induced reduction in rhizosphere microbial diversity and shifts in community composition

We evaluated the impact of compost amendment on the rhizosphere bacterial and fungal diversity and community composition in FORC-inoculated and non-inoculated plants using fungal ITS and bacterial 16S rRNA gene amplicon sequencing.

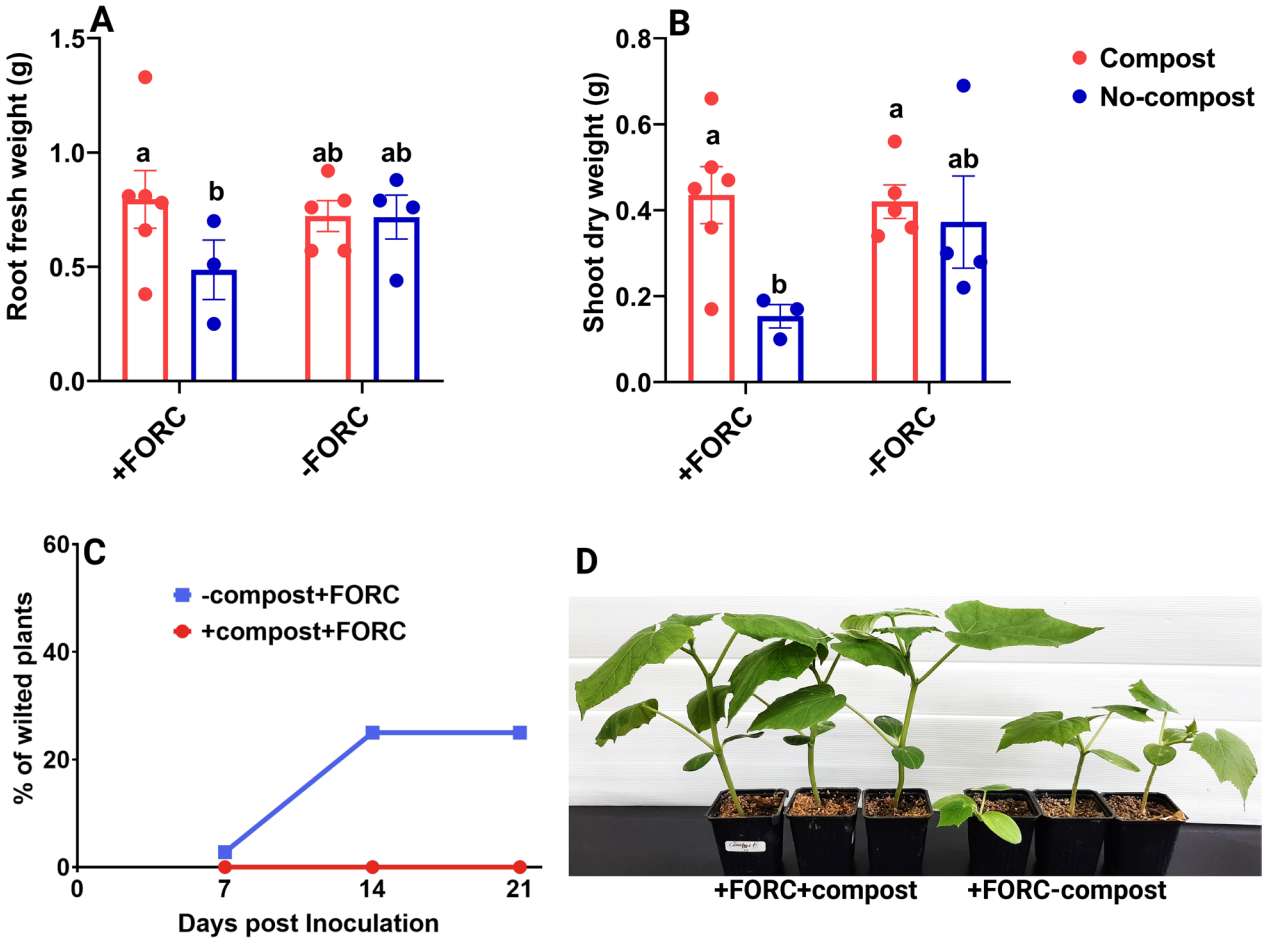

Fig. 1 Impact of compost amendment on disease incidence and plant growth parameters in FORC inoculated treatments. Root fresh weight (**A**) and shoot dry weight (**B**) of compost-amended and non-amended cucumber plants with and without pathogen inoculation. Different letters indicate statistically significant differences obtained by multiple comparison of all pairs of means (Tukey HSD test, $P < 0.05$). Disease incidence (**C**) and photographs (**D**) of compost amended and non-amended FORC-inoculated cucumber plants. Disease incidence was determined by calculating the percentage of wilted plants in each soil amendment at 21 days post inoculation. N = 6 and 5 for FORC-inoculated and non-inoculated respectively

Bacterial community richness and phylogenetic diversity did not differ significantly across treatments ($P > 0.05$, Tukey HSD; Figure S1A and S1B, Additional file 1). However, FORC inoculation significantly reduced bacterial evenness by 2.7% compared to non-inoculated plants without compost ($P < 0.05$), while the 1.56% reduction observed in compost-amended, FORC-inoculated plants was not statistically significant ($P > 0.05$; Fig. 2A, Figure S1C, Additional file 1).

Without FORC, compost amendment slightly reduced fungal diversity in the rhizosphere. However, without compost, FORC inoculation facilitated a 32% ($P < 0.05$, Tukey HSD) decrease in fungal diversity, whereas in compost amended samples the decrease was only 13% and was not significant compared to the compost-amended samples not inoculated with FORC (Fig. 2B, and Figure S2A, Additional file 1).

Distinct bacterial and fungal communities were observed in compost amended *vs.* non-amended cucumber plants (Bray–Curtis Dissimilarity matrix), and the amendment group explained 59% and 53.6% (Adonis test—amendment group $R^2 = 0.59$ and 0.536, bacterial 16S rRNA and fungal ITS respectively, $P < 0.05$) of the differences in bacterial and fungal communities respectively (Fig. 2C and 2D). In the absence of compost, bacterial and fungal communities were significantly affected by FORC inoculation, as opposed to the compost amended communities in which FORC-induced shifts were considerably less pronounced. Estimation of the relative abundance of FORC-associated ITS amplicon sequence variants (ASV) and of FORC-characterized reads from shotgun metagenomic analyses, indicated that compost amendment significantly reduced the relative

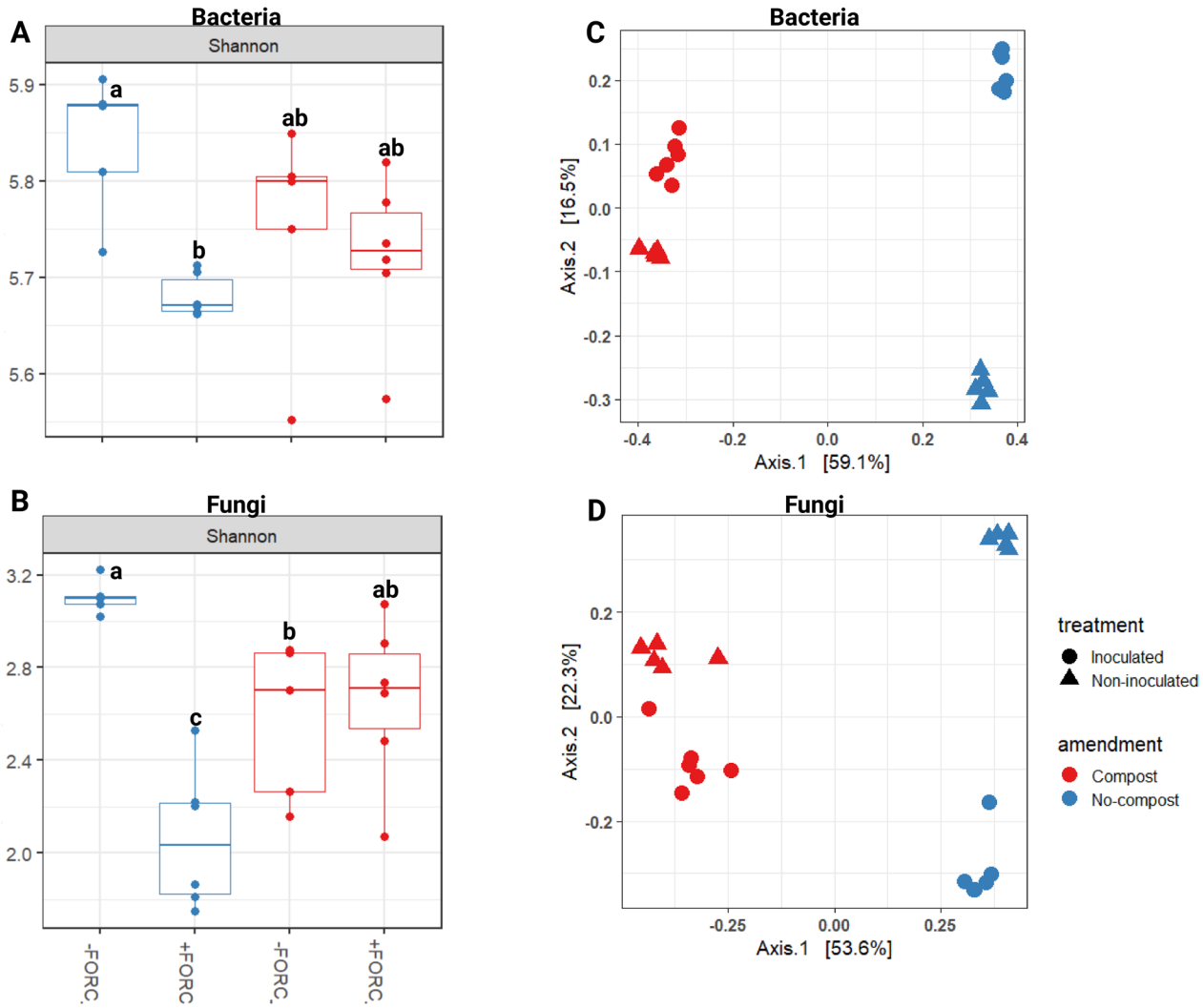

Fig. 2 Diversity (Shannon diversity index) of bacterial (**A**) and fungal (**B**) communities in the rhizosphere of compost amended and non-amended plants with and without FORC inoculation. Different letters indicate statistically significant differences obtained by multiple comparison of all pairs of means (Tukey HSD test, $P < 0.05$). PCoA ordination based on Bray–Curtis dissimilarity matrices of bacterial 16S rRNA gene (**C**) and fungal ITS gene (**D**) amplicons. The fungal ASV that was classified as *Fusarium* only present in FORC-inoculated samples, was excluded from this analysis. Compost amendment explained 59.1% and 53.6% of the differences in bacterial and fungal communities respectively (Adonis test—amendment group $R^2 = 0.59$ and 0.54 respectively, $P < 0.05$)

abundance of FORC in the rhizosphere (Figure S2B, S2C and S2D, Additional file 1).

Taxonomic analysis of fungal ITS ASVs showed that over 60% of fungi were unclassified, particularly in compost-amended samples (Figure S2B, Additional file 1). In non-compost treatments, 19 fungal genera differed significantly between inoculated and non-inoculated groups (LEfSe analysis, LDA score > 2.0 and FDR-adjusted $P < 0.1$), with only *Fusarium* and *Cutaneotrichosporon* enriched in FORC-inoculated samples (Figure S2E, Additional file 1). In summary, compost significantly reduced fungal diversity but did not have notable impact on bacterial diversity. It significantly altered the composition of both bacterial and fungal communities in addition to mitigating the negative impact of FORC inoculation by reducing FORC abundance and minimizing microbial community shifts in the rhizospheres of FORC inoculated cucumber plants.

In depth investigation focused on bacterial community composition and functions due to the limited taxonomic resolution of the fungal community, that was especially pronounced in the compost-amended samples. This decision is supported by

previous research highlighting the role of bacteria in disease suppression and their abundant capacity to produce antifungal metabolites within the rhizosphere.

Compost amendment mitigates FORC-induced reduction in the relative abundance of the *Actinomycetota* and *Bacillota*

To identify bacterial populations potentially involved in facilitating compost suppressiveness, we taxonomically annotated the rhizosphere communities in all treatments and performed differential abundance analysis on the 16S rRNA gene amplicon sequencing data using LEfSe.

FORC inoculation significantly reduced the relative abundance of *Bacillota* and *Actinomycetota* in the absence of compost amendment, but this reduction was not significant in the compost-amended treatments. Specifically, in FORC inoculated cucumber plants, the relative abundance of the *Bacillota Thermoactinomycetaceae*, *Clostridiales* Family_XI, and *Peptococcaceae*, as well as *Actinomycetota* families *Streptomycetaceae*, *Thermomonosporaceae*, *Glycomycetaceae*, *Propionibacteriaceae*, *Nocardiopsaceae*, *Intrasporangiaceae*, *Pseudonorcadiaceae* and unclassified *Micrococcales* families was significantly higher in the rhizosphere of the compost-amended treatments than in the plants that were not amended with compost (Fig. 3, Table S1c, Additional file 2).

To validate 16S rRNA gene amplicon results and achieve high-resolution taxonomic classification, we performed shotgun metagenomics on FORC-inoculated samples (with and without compost), binning 116 medium- to high-quality dereplicated MAGs (> 70% completion, < 5% contamination) using the metaWRAP pipeline. Taxonomic classification (using the GTDB_tk classify workflow) revealed that MAGs from *Actinomycetota* and *Bacillota* were significantly associated with compost amendment (Table S5, Additional file 2 and Figure S3, Additional file 1). These included genera such as *Streptomyces*, *Actinomadura*, *Saccharomonospora*, *Pseudonocardia*, *Glycomyces*, *Thermobifida*, *Planifilum*, and *Novibacillus*, (Figure S4, Additional file 1), consistent with the compost-associated families identified in the 16S rRNA gene

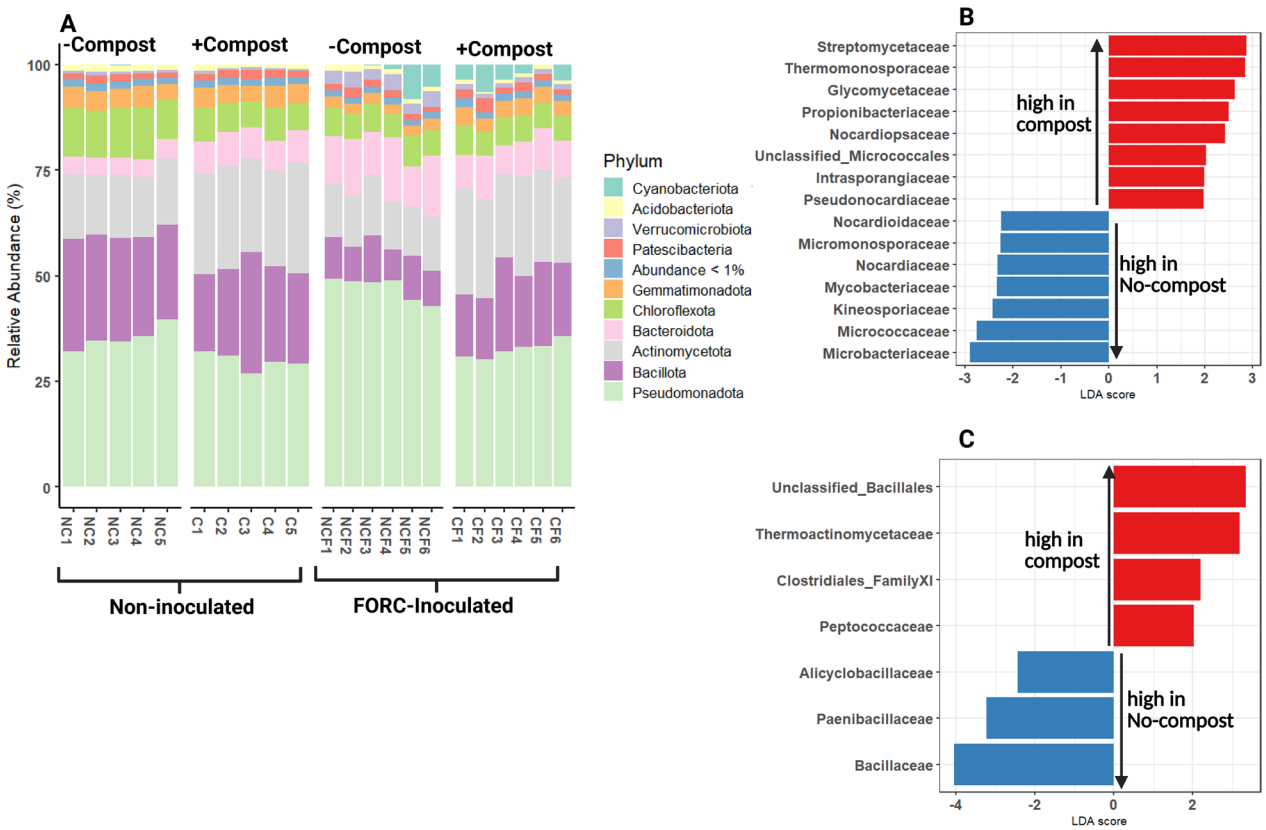

Fig. 3 Relative abundances of bacterial phyla with significant differential abundance in rhizospheres of FORC-inoculated and non-inoculated and compost-amended and non-amended treatments (based on 16S rRNA gene ASVs) (**A**). LDA scores of *Actinomycetota* (**B**) and *Bacillota* (**C**) families showing statistically significant differences between compost-amended (red) and nonamended (blue) FORC-inoculated cucumber plants, determined by LEfSe analysis. LDA score > 2.0 and FDR-adjusted $P < 0.1$

amplicon analysis. Collectively, these results suggest that compost amendment mitigated FORC-induced dysbiosis of the rhizosphere microbiome, maintaining high relative abundance of specific *Bacillota* and *Actinomycetota* genera.

Actinomycetota and *Bacillota* associated SM-encoding gene clusters are highly abundant in disease suppressed compost-amended cucumber rhizospheres

We postulated that the compost-facilitated suppressiveness and alleviation of FORC-induced dysbiosis, was at least partially linked to bacterial secondary metabolites that antagonize FORC. To identify secondary metabolite-encoding gene clusters, contigs larger than 5000 bp were pooled and used as input for antiSMASH v6, and BiG-MAP tool was used to generate gene cluster abundance table, focusing only on the core genes of a BGC.

In total, 1237 BGCs were identified in FORC inoculated samples with and without compost (Table S3, Additional file 2). While no differences in the BGCs diversity (Shannon index: Figure S5A, Additional file 1) were observed between compost amended and non-amended samples, there was a significant difference in the BGC composition between these two groups (Fig. 4A). BGCs phylogenetically linked to *Actinomycetota* and *Bacillota* were significantly more abundant in disease suppressed compost amended cucumber rhizospheres, while *Pseudomonadota* and *Bacteroidota* BGCs were more prevalent in non-amended FORC-inoculated rhizospheres (Figure S5B, Additional file 1). Compost-enriched BGCs were mainly taxonomically associated with the *Actinomycetota* families *Streptomycetaceae, Thermomonosporaceae, Pseudonorcadiaceae, Glycomycetaceae,* and *Nocardiopsaceae*, and the *Bacillota* family *Thermoactinomycetaceae,* corresponding

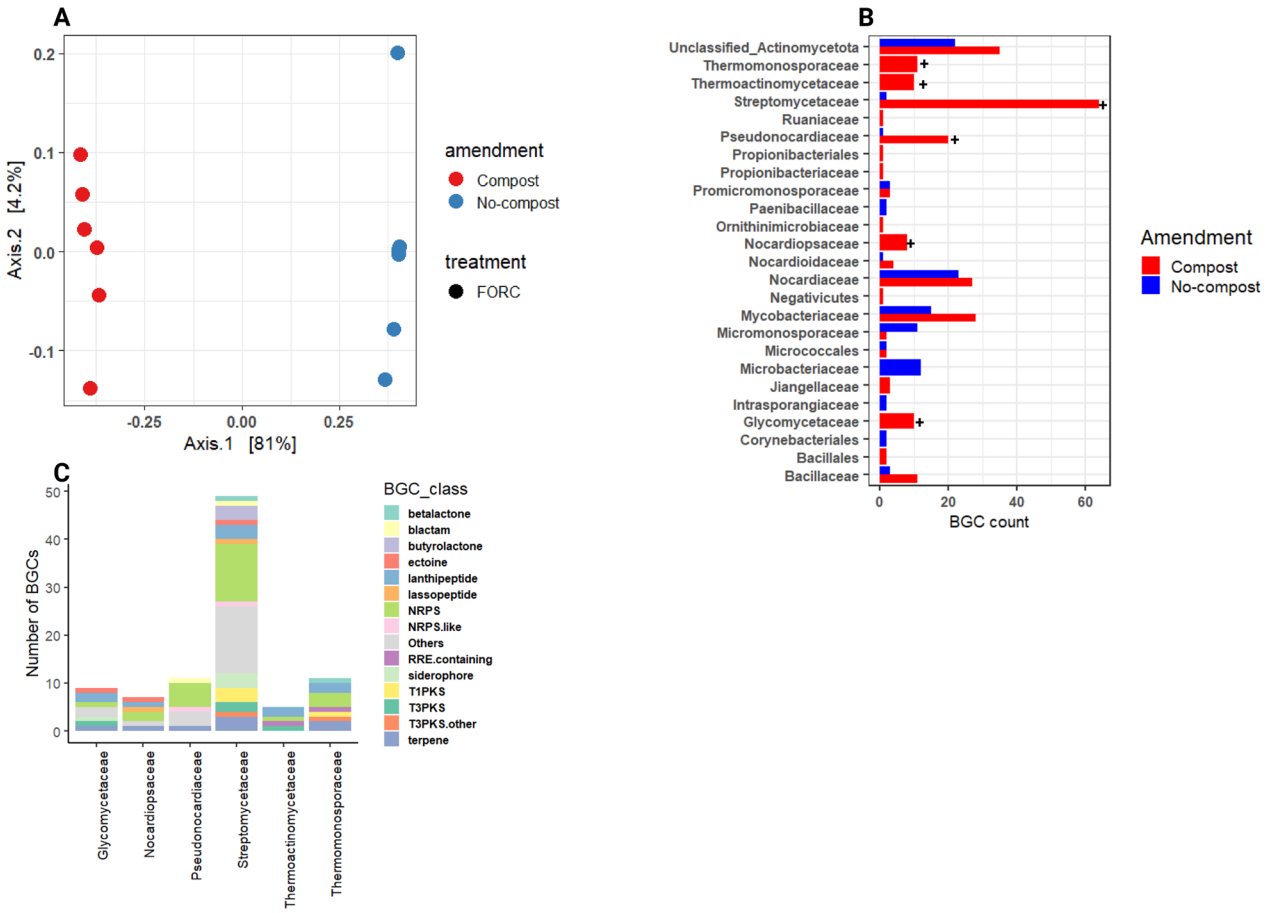

Fig. 4 **A** PCoA ordination of the identified BGCs based on Bray–Curtis dissimilarity matrices. The amendment factor explained 81% of the differences observed in BGC composition between compost amended and non-amended pathogen-inoculated plants (Adonis test: pairwise permanova—amendment group $R^2 = 0.81$, $P < 0.05$). **B** Family level taxonomic classification of *Actinomycetota* and *Bacillota* BGCs, highly abundant in the rhizosphere of compost amended FORC inoculated plants (MaAsLin2, coef > 2, *P* adj < 0.05). + indicates compost stimulated families that were also identified in 16S rRNA gene amplicon sequencing analysis. **C** BGC classes of compost protected bacterial families that were more abundant in compost-amended samples. Singleton BGCs were grouped as others

to the results of 16S rRNA gene amplicon analysis (Fig. 4B). The compost "protected" bacterial families harbor diverse classes of SM-encoding BGCs including NRPS, PKS (T1, T2 and T3), siderophores, betalactones, lanthipeptides (classes i-iii), ectoine and terpenes, with *Streptomycetaceae* harboring the most abundant and diverse collection of SMs (Fig. 4C). In summary, although the diversity of BGCs was similar in compost-amended and non-amended FORC-inoculated rhizospheres, their composition differed significantly. The rhizospheres of disease-suppressed, compost-amended plants were enriched in a variety of BGCs from the *Actinomycetota* and *Bacillota* families, including *Streptomycetaceae*, *Thermomonosporaceae*, *Pseudonocardiaceae*, *Glycomycetaceae*, *Nocardiopsaceae*, and *Thermoactinomycetaceae*, consistent with the results of 16S rRNA gene amplicon analysis.

Compost stabilized bacteria possess NRPS and PKS BGCs encoding for antimicrobial compounds and siderophores

NPRS and PKS secondary metabolites are known to play important ecological roles in microbe-microbe and microbe-plant interactions in soil and roots microbiomes. To shed light on the potential functions of the identified NRPS and PKS gene clusters in compost amended, FORC suppressive rhizospheres, we focused on NRPS and PKS BGCs from the *Actinomycetota* and *Bacillota* taxa, due to their profuseness in these samples. A total of 160 NRPS- and PKS-encoding BGCs were aligned against the MIBiG database using Diamond blastx and function was assigned if a BGC had an E-value $< 10^{-40}$ and > 50% similarity to a documented secondary metabolite producing cluster [14]. In total, 118 out of the 160 identified NRPS/PKS BGCs displayed statistically significant differences (MaAsLin2, P adj < 0.05) in abundance between the compost-amended and non-amended treatments (Table S4, Additional file 2), 47% (56/118) of whom were functionally categorized. The functionally annotated NRPS and PKS BGCs were primarily phylogenetically associated with the *Actinomycetota* phylum, in contrast to those affiliated with *Bacillota* phylum (particularly; *Thermoactinomycetaceae,* unclassified *Negativicutes* and *Bacillales*) that could not be assigned a functional role according to our cutoff criteria. The annotated NRPS and PKS BGCs were primarily linked to production of antimicrobial compounds and siderophores (Fig. 5A and B). The antimicrobial encoding NRPS and PKS BGCs in disease suppressed, compost amended samples were predominantly associated with the *Streptomyces* genus. These included NRPS BGCs encoding for atratumycin, enduracidin, cadaside A, and maduralactomycin A antimicrobials; and PKS BGCs encoding for concanamycin A, azalomycin F3a, A-47934, and diazepinomicin antimicrobials (Fig. 5A

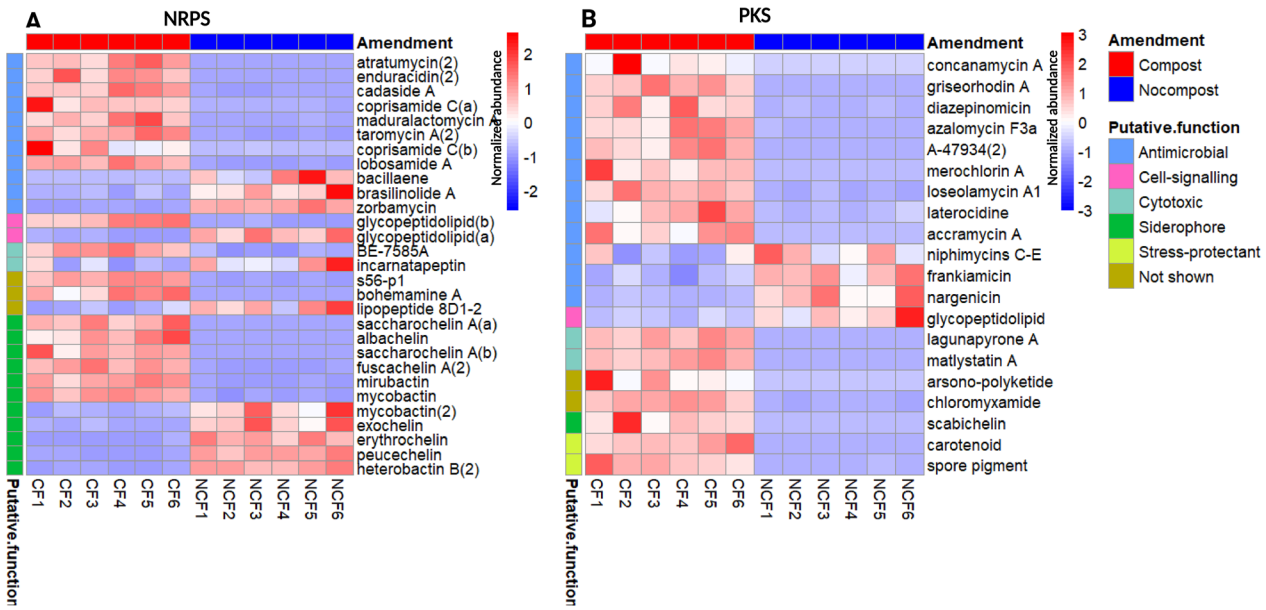

Fig. 5 Differential abundance (normalized by z-score scaling of rows) of functionally characterized NRPS (**A**), and PKS (**B**) encoding BGCs in compost-amended vs. non-amended cucumber rhizospheres. Differential abundance analysis was conducted using MaASlin2, and functional annotation was based on > 50% amino acid identity and E value of $< 10^{-40}$ of the identified NRPS/PKS BGCs to BGC sequences in MIBiG database. Taxonomic classification of BGCs was achieved using MEGAN V6 Ultimate Edition based on the Lowest common ancestor (LCA). The numbers in parentheses show the count of that specific BGC within the same genus, while the letters indicate that the BGC is taxonomically associated with different genera

and B). Other notable antimicrobial encoding BGCs include: *Actinomadura*-associated NRPS and PKS BGCs encoding for coprisamide C and merochlorin A, respectively; the NRPS taromycin A gene cluster from *Saccharomonospora*; PKS loseolamycin A1; and griseorhodin A from *Glycomyces* and *Thermobifida*. Siderophores were mainly encoded by NRPS BGCs (Fig. 5A). Additionally, NRPS gene clusters encoding for siderophore production were found in compost-stimulated *Saccharomonospora* (mirubactin), *Thermobifida* (fuscachelin A), *Pseudonocardiaceae* (saccharochelin A and albachelin), and *Actinomadura* (saccharochelin A) families, but not in *Streptomyces*. Overall, NRPS and PKS BGCs enriched in compost-amended, FORC-inoculated rhizospheres were primarily associated with antimicrobial and siderophore production. Most antimicrobial BGCs were associated with the *Streptomyces* genus, and the majority of siderophore encoding BGCs belonged to the *Saccharomonospora*, *Thermobifida*, and *Actinomadura* genera.

Compost stimulated *Streptomyces* excrete FORC-antagonizing metabolites

The contig-based functional annotation of NRPS and PKS BGCs highlighted the *Streptomycetaceae* family as a potential producer of antimicrobial compounds in the compost-amended FORC inoculated samples. Using plant-based medium, we isolated two *Streptomyces* strains (CC6 and CC27) from compost induced, disease suppressed rhizosphere samples. 16S rRNA gene analysis confirmed that these strains clustered with the *Streptomyces* ASV_2 (Fig. 6A), whose abundance was slightly augmented by compost in the presence of FORC, in contrast to plants not amended with compost where it was significantly lower in FORC inoculated samples (Figure S6A, Additional file 1). Additionally, the genomes of these two isolates were over 99% identical to the *Streptomyces* bin2 MAG (ortho ANI) (Table S6, Additional file 2 and Figure S6B, Additional file 1), confirming isolation of these key strains.

The genomes of CC6, CC27, and bin2 share 11 unique BGCs not found in MIBiG, along with a transAT-PKS-NRPS cluster sharing 93% of its genes with the weishanmycin BGC and a HR-T2PKS-NRPS hybrid cluster sharing 55% of its genes with the ishigamide BGC (Figure S7, Additional file 1). *Streptomyces* CC6 and CC27 extracts significantly inhibited growth (in-vitro) of FORC (Fig. 6B). The fungal inhibitory phenotype was validated using a bacteria-fungal dual culture plate assay (Fig. 6C).

Discussion

The ability of compost to suppress soilborne pathogens in various pathosystems is well established [1, 12, 16]. Evidence indicates that while physicochemical properties of compost contribute to its suppressiveness, sterilization leads to a loss of this effect, underscoring the crucial role of microorganisms [13, 17, 69]. While various processes such as altering soil physicochemical characteristics to promote microbial growth and activity, and augmenting the native rhizosphere microbiome by introducing new microbes [11, 70, 71] have been associated with compost-induced suppressiveness, the specific microbial populations and mechanisms driving compost induced disease suppression remain unclear. This study aimed to identify key rhizosphere bacterial communities and genes related to secondary metabolite production, potentially involved in compost-mediated suppression of FORC in greenhouse grown cucumbers.

Greenhouse experiments validated that compost amendment mitigated FORC-induced disease, and microbial analyses revealed that this phenomenon was linked to a significant reduction in the relative abundance of FORC in the rhizosphere. While compost amendment altered the rhizosphere microbial community composition when compared to non-amended samples, our findings indicate that it also "stabilized" the rhizosphere microbiome in the presence of FORC, mitigating pathogen-induced reduction of microbial diversity and alleviating shifts in community composition. Compost may stabilize the rhizosphere microbiome in the presence of a pathogen by altering the soil physicochemical characteristics such as pH and organic carbon to promote proliferation of the native microbiome or augmentation of the native microbial communities by introducing in new species [11, 13, 18, 27, 71].

Table S1c shows the relative abundance of *Actinomycetota* and *Bacillota* ASVs that were enriched in FORC-inoculated, compost-amended plants. We find both ASVs initially present in the native soil that showed increased abundance following compost amendment, along with ASVs that were exclusive to compost-amended samples that proliferated in the presence of FORC. This indicates that the suppressive microbiome is composed of both beneficial taxa introduced by the compost, and by native soil microorganisms that are induced by compost, as revealed in previous studies [19, 72].

We propose that compost may facilitate disease suppression by alleviating pathogen-induced dysbiosis of the rhizosphere microbiome, maintaining robustness of specific bacterial populations. Our findings are supported by previous studies that found that compost amendment led to sustained alterations in the

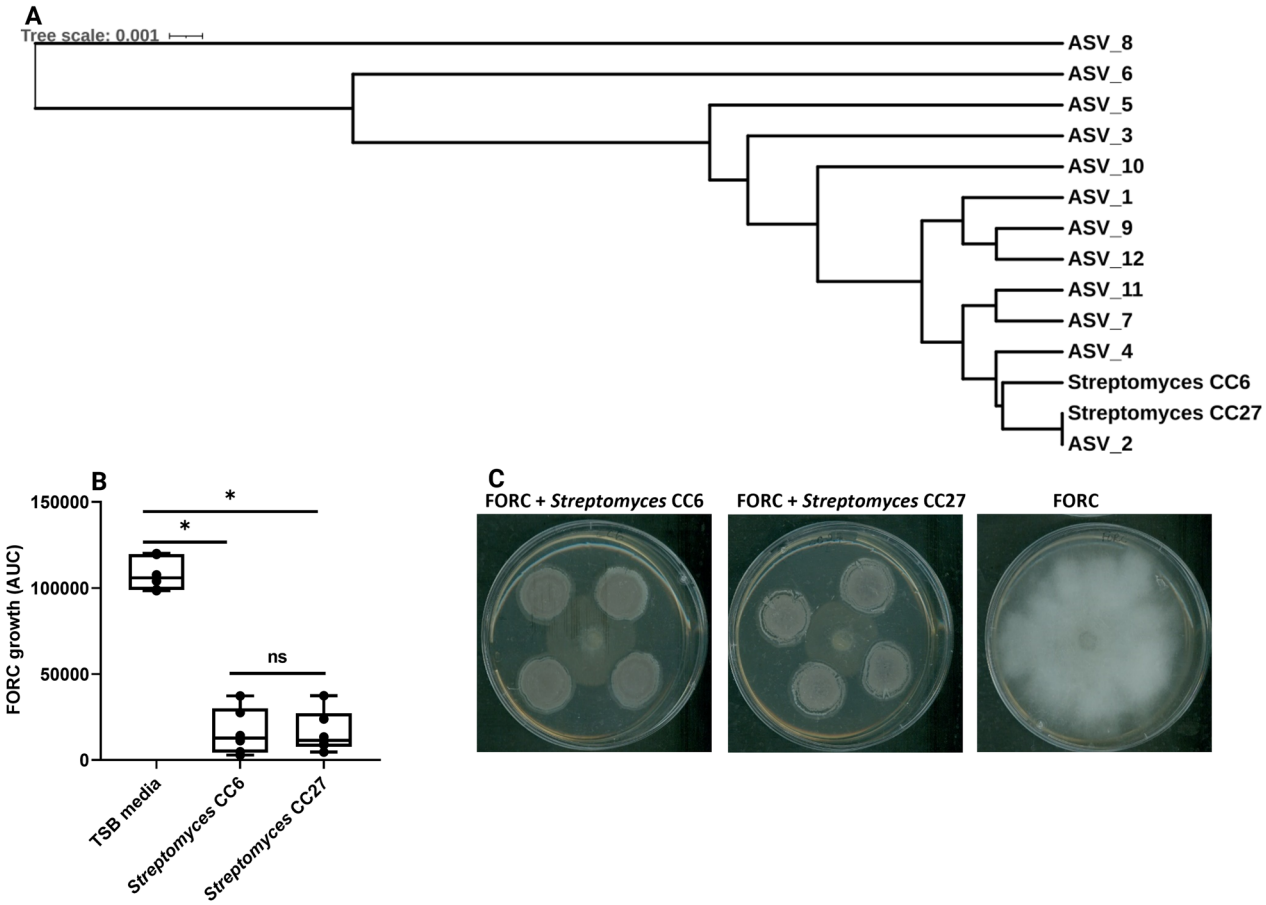

Fig. 6 **A** Phylogenetic analysis of *Streptomyces* 16S rRNA gene amplicon ASVs and the isolates CC6 and CC27 *Streptomyces* 16S rRNA gene. *Streptomyces* CC6 and CC27 cluster together with *Streptomyces* ASV_2. MAFFT v6.864 online version was used for Multiple sequence alignment and construction of UPGMA (Unweighted Pair Group Method with Arithmetic Mean) phylogenetic tree and the tree and visualized using iTOL. **B** In-vitro screening for FORC growth inhibition with cell-free supernatants of compost-induced *Streptomyces* isolates CC6 and CC27. FORC treated with bacterial media (TSB) was used as the control. Growth was quantified by the change in GFP fluorescence (arbitrary units) monitored over 24 h of incubation and normalized to the initial fluorescence measurements before estimation of the area under the growth curve (AUC). Statistical analysis was conducted using Dunnett's multiple comparison test to compare the growth of FORC treated with *Streptomyces* CC6 and CC27 extracts against TSB media control. * denotes $P < 0.05$ and ns denotes $P > 0.05$ (6 technical replicates). **C** Dual culture plate assay of isolates CC6 and CC27 with FORC. Images were taken 7 days after inoculation with FORC

structure of soil microbial communities [12, 70, 71], however these were all conducted without addition of pathogens. The compost stimulated eubiosis of the FORC-amended rhizosphere microbiome was most prominent in the case of selected *Bacillota* and *Actinomycetota* genera that were highly abundant in FORC inoculated plants with compost, but almost completely absent in the compost-free inoculated treatments. This is supported by past studies that found positive correlations between *Actinomycetota* and *Bacillota* abundance, and inhibition of soilborne pathogens such as *Fusarium oxysporum* [73, 74] and *Ralstonia solanacearum* [75]. Interestingly, reduction of these phyla in tomato rhizosphere was associated with increased disease incidence [75].

Delving into the 16S rRNA and MAG binning of shotgun metagenomic data revealed significantly higher relative abundance of *Streptomyces*, *Actinomadura*, *Saccharomonospora*, *Pseudonocardia*, *Glycomyces* and *Thermobifida* (*Actinomycetota*) and of *Planifilum* and *Novibacillus* (*Bacillota*) in FORC-inoculated rhizosphere samples amended with compost, compared to inoculated plants without compost. A metabarcoding study of 116 composts from 16 composting companies across China revealed that these taxa were present in over 90% of the composts, regardless of the starting materials or composting process, indicating that they

are key members of the compost core microbiome [26]. However, their enrichment in the rhizosphere despite pathogen pressure suggests they are robust colonizers and potentially contribute to disease suppression through competition or antagonism.

These genera were enriched with genes encoding production of diverse secondary metabolites including NRPS and PKS genes associated with production of antimicrobial and siderophore compounds, suggesting that they may directly antagonize soilborne pathogens through excreted antimicrobial and iron scavenging compounds. Previous studies indicated that *Actinomycetota* and *Bacillota* strains can directly antagonize Fusarium and other pathogens through production of antibiotics and lytic enzymes, and indirectly by competition such as production of siderophores [76, 77].

Secondary metabolites produced by non-ribosomal peptide synthetases (NRPS) and polyketide synthases (PKS) are known to play crucial roles in antagonizing fungal pathogens in the rhizosphere [14, 29]. *Streptomyces* are known to produce a wide range of antimicrobial compounds and includes strains that have been shown to antagonize Fusarium wilts in several *in-planta* studies [74, 78]. We found that *Streptomyces* spp. were associated with the majority of NRPS and PKS gene clusters predicted to produce antimicrobial compounds in the compost-amended FORC inoculated samples. We successfully isolated two *Streptomyces* strains from the compost-induced FORC-suppressing cucumber rhizospheres that secrete FORC-inhibiting extracellular metabolites in-vitro. Their genomes contained NRPS-transAT-PKS and HR-T2PKS-NRPS hybrid clusters that encoded for potential congeners of weishanmycin and ishigamide, respectively. Weishanmycin is a *Streptomyces*-produced congener of the well-known, highly potent antitumor drug leinamycin [79]. Nonetheless, it may also have antimicrobial attributes, however this along with its native ecological role is currently unknown. Ishigamide is a *Streptomyces* derived amide-containing polyene whose function is unknown [80]. Polyenes are broad-spectrum antifungal agents produced by *Streptomyces* that act by increasing the permeability of the fungal cell membrane through targeting ergosterol, resulting in fungal cell death [81]. We posit that these, and potentially additional *Streptomyces*-produced compounds could play a crucial role in compost-induced disease suppression through direct fungal antagonism.

It is imperative to emphasize that our findings are based on a single compost type within a defined cucumber–*Fusarium* pathosystem, and should be regarded as context-specific rather than broadly generalizable. Compost is a heterogeneous material whose composition varies with feedstocks, processing methods, and maturity, affecting its physicochemical properties and microbial communities, and leading to variable disease-suppressive outcomes [19, 27]. This variability also extends to rhizosphere microbial communities, which are shaped by the combined influences of compost type, plant species, and pathogen presence [19, 21]. While the present study focused on the contribution of bacterial community to disease suppression, compost amendments also impact soil chemistry and may influence other microbial groups, such as fungi and protists, as well as abiotic factors not assessed here. The observed disease suppression is likely the outcome of multiple interacting mechanisms, and attributing it to a single factor may oversimplify the system. It could be argued that the study would have benefited from a larger number of replicates and a more detailed analysis of other microbial communities, particularly fungi that may also contribute to disease suppressiveness. However, considering the complexity of compost, we believe that future research should target specific microbial taxonomic and secondary metabolite encoding genetic indicators of suppressiveness (such as those identified in this study) in different pathosystems using both greenhouse and field experiments. Identifying such taxonomic markers could improve the predictability and application of compost-based disease management strategies. Additionally, designing consortia of strains maintained in eubiosis by compost could help determine their ability to promote disease suppression under disease-conducive conditions. Beyond the production of antimicrobial secondary metabolites, other compost suppressive mechanisms, such as the production of fungal lytic enzymes and activation of plant-induced systemic resistance (ISR), also merit attention.

Conclusions

This study underlines a link between compost, eubiosis of the rhizosphere microbiome, and mitigation of soilborne pathogen facilitated plant disease, which appears to be analogous to prebiotic-facilitated eubiosis of the gut microbiome that prevents disease in mammals. We posit that compost facilitates a buffering effect that protects the rhizosphere microbiome from pathogen-induced dysbiosis, sustaining *Actinomycetota* and *Bacillota* associated strains that potentially antagonize Fusarium through a range of mechanisms including the production of antimicrobial secondary metabolites and siderophores. These findings can be exploited to promote sustainable crop protection strategies that preserve beneficial rhizosphere microbiomes, and to isolate

pathogen-antagonizing strains (and their metabolites) that can be as sustainable alternatives to chemical pesticides.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40793-025-00710-9.

Additional file 1.

Additional file 2.

Acknowledgements
We thank Elad Cohen for his support with computational resources, and Dr. Omer Frenkel for valuable advice and guidance regarding greenhouse experiments.

Author contributions
H.A and E.C conceptualized and designed the experiments. H.A performed the experiments and data analyses. A.D.F performed shotgun metagenome assembly. H.A, J.F and E.C wrote the manuscript, J.K.C provided technical assistance during experimental setup and sample preparation. All authors contributed to the article and approved the submitted version.

Funding
This study was funded by Israel Ministry of Agriculture and Rural Development Chief Scientist grant 20-03-0061.

Availability of data and materials
Sequence data that support the findings of this study have been deposited in the NCBI Sequence Read Archive (SRA) database under Bioproject PRJNA1169419.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 28 October 2024 Accepted: 18 April 2025
Published online: 16 May 2025

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