Microbiological Research 302 (2026) 128346

Contents lists available at ScienceDirect

Microbiological Research

journal homepage: www.elsevier.com/locate/micres

Citrus fruit microbiome changes under copper-based and biological alternative treatments and its biocontrol potential

M.F. Lombardo [a,*], A. Abdelfattah [b], V. Catara [a], N. Wang [c], G. Cirvilleri [a,*]

[a] Department of Agriculture, Food and Environment (Di3A), University of Catania, Catania, Italy
[b] Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Potsdam, Germany
[c] Citrus Research and Education Center, Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences (IFAS), University of Florida, Lake Alfred, FL, United States

ARTICLE INFO

Keywords:
Carposphere
Disease management
Amplicon sequencing
Beneficial microorganisms
Copper fungicides
Chitosan
Sweet orange essential oil

ABSTRACT

Citrus, a globally significant fruit crop, harbours a distinctive microbial community crucial for maintaining citrus health and enhancing disease resistance. While the structure and shaping factors, including phytosanitary treatments, of citrus root and leaf microbiomes are well documented, the carposphere (fruit surface) microbiome and its response to phytosanitary inputs remain poorly understood. In the present study, we combined culture independent (amplicon sequencing) and culture dependent techniques to analyse the citrus carposphere microbiome across three citrus hosts and its response to field-applied phytosanitary treatments (biologicals and copper-antimicrobials). Despite host-specific variation in the relative abundance of dominant taxa such as Proteobacteria, Firmicutes, and Basidiomycota, all three citrus hosts shared a core microbiome, consistently present across fruit samples. Bacterial diversity and composition were negatively influenced by copper treatments, whereas biological products (chitosan, sweet orange essential oils and their mixtures) had minimal or no negative impacts. Fungal communities, including potential pathogens, appeared less sensitive to treatments. Network analysis confirmed that copper altered microbial interactions, increasing mutual exclusion relationships between bacterial taxa compared to untreated or biologically treated samples, which were dominated by positive interactions. A parallel survey of cultivable microbiota from the same samples identified potential biocontrol agents (BCAs) against *Colletotrichum gloeosporioides* and *Alternaria alternata*. Notably, cross-referencing cultivable BCAs with core Amplicon Sequence Variants (ASVs) showed that 81.7 % of bacterial core members represent potential biocontrol agents. This study highlights the importance of management practices for sustaining beneficial microbiomes. Furthermore, it establishes a valuable resource of core-associated BCAs, offering promising avenues for the biological control of fungal pathogens.

1. Introduction

Citrus holds a critical role in Mediterranean agriculture, but its production is currently facing several challenges, especially those associated with plant pathogens (Barbieri et al., 2023). Current management strategies still rely on unsustainable practices (i.e., chemicals and copper-based antimicrobials), highlighting the need for eco-friendly alternatives (Lamichhane et al., 2018). In this context, citrus microbiome has emerged as a promising resource for improving and developing sustainable alternatives that enhance citrus health in several ways, including improved disease resistance, fruit quality, and potentially even stress tolerance (Trivedi et al., 2020; Zhang et al., 2021b). Substantial progresses have been achieved to elucidate the composition and functions of citrus microbiome, particularly in the root environments, where microbial communities play a central role in plant health and nutrition (Lombardo et al., 2024b; Xu et al., 2018). Multiple studies have investigated the microbiome of above-ground plant compartments, including flowers, flush and leaves (Abdelfattah et al., 2017), due to their crucial contributions to plants vegetative and reproductive cycles (Wu et al., 2020; Xi et al., 2023; Xu et al., 2023). In contrast, the citrus fruit microbiome, despite its potential impact on fruit health has received comparatively limited attention. This is largely due to the fruit's less stable microbial communities, which are shaped by harsh external conditions, such as temperature fluctuations, UV exposure,

* Corresponding authors.
E-mail addresses: monia.lombardo@phd.unict.it (M.F. Lombardo), gabriella.cirvilleri@unict.it (G. Cirvilleri).

https://doi.org/10.1016/j.micres.2025.128346
Received 30 June 2025; Received in revised form 10 September 2025; Accepted 19 September 2025
Available online 23 September 2025

desiccation and agricultural practices (Zhang et al., 2021a). Despite these conditions, the fruit microbiome has been proved to be resilient and functionally specialized, and exerts significant impact on fruit health and quality, disease resistance and postharvest storage (Droby et al., 2022; Jing et al., 2023; Solanki et al., 2024). Importantly, pre-harvest factors, including pathogens, phytosanitary treatments, climate, agricultural practices, or soil properties, profoundly influence the fruit microbiome functionalities by shaping and selecting its members (Wicaksono et al., 2023). A deeper understanding of these selection dynamics is crucial to identify agricultural practices that promote beneficial microbial communities inhabiting the fruit (Solanki et al., 2024; Wassermann et al., 2019). Fruit diseases and their management are the most significant preharvest challenges, such as Alternaria brown spot (ABS) and anthracnose, caused by *A. alternata* and *Colletotrichum* species, respectively in citrus fruit (Vitale et al., 2021). These diseases are exacerbated by climate change conditions, particularly increased rainfall during warm seasons, which heighten disease pressure and drive extensive use of fungicide sprays, while in organic citrus systems, only copper-based products are approved for disease control. Yet, the intensive use of synthetic (i.e., fludioxonil and pyraclostrobin-based) and inorganic (i.e., copper-based) fungicides have raised concerns due to their environmental toxicity, risks to human health, development of fungicide resistance, and adverse impacts on soil health, non-target organisms, and finally microbial biodiversity (Avenot and Michailides, 2015; Çayır et al., 2016; Chitolina et al., 2021; Gama et al., 2020;; Lamichhane et al., 2018; Lee et al., 2019). Due to these concerns, and in line with increasing regulatory restrictions, there is growing need to reduce fungicides application, particularly copper in organic production (Katsoulas et al., 2020). This has led to intensified research into alternative products, including natural derivatives and lower-risk compounds, that could reduce or replace copper use (Lamichhane et al., 2018). Among the most promising alternatives to conventional fungicides are biological products such as plant extracts, basic substances, and biopolymer formulations (Lamichhane et al., 2018). Multi-year field trials have demonstrated that treatments like chitosan (biopolymer derived from chitin) and sweet orange essential oil (a plant extract) or their mixture can effectively reduce symptoms of ABS and anthracnose across diverse citrus hosts and environments, offering economic and ecological advantages (Lombardo et al., 2023; 2024a). However, despite their disease-suppressive potential, the broader ecological effects and collateral microbiome perturbation of these treatments, particularly on the native fruit-associated microbiota, remain insufficiently understood. To date, copper-based fungicides has been proved to negatively shape the structure and dynamics of plant-associated microbiome (Gobbi et al., 2020). In citrus, a recent study on the phyllosphere supported that copper treatments can disrupt beneficial microbial associations (Carvalho et al., 2020). Nevertheless, current understanding of how copper-based and biological treatments affect the microbiome of the fruit surface is limited. To address this knowledge gap, we investigated the composition of the citrus carposphere (fruit surface) microbiome and whether biological or copper treatments might affect the microbial communities and potential biocontrol agents (BCAs). To comprehensively analyse microbiome composition and identify potential BCAs, we employed a combined approach using both cultivation-dependent and cultivation-independent (Amplicon Sequencing) techniques, which have been used in postharvest citrus microbiome studies (Blacutt et al., 2020; Jing et al., 2023; Penyalver et al., 2022). Understanding the core members of the microbiome with biocontrol potential provides a strong foundation for developing fruit-associated probiotics that can adapt to their host and persist over time (Zhang et al., 2021a). In citrus, several core taxa have already been reported with disease suppression activity. For example, *Bacillus* strains isolated from citrus tissues have shown field efficacy against post bloom fruit drop over consecutive seasons (Klein et al., 2016). Beyond individual strains, synthetic microbial consortia composed of selected core taxa, including *Bacillus*, *Pantoea*, and *Curtobacterium* genera, have shown promising results. Their chitinase-related activities have been effective in suppressing *Penicillium digitatum* and *Geotrichum candidum* (Zhang et al., 2021b). Other citrus-associated core taxa also exhibit antagonistic interactions with major pathogens. For instance, *Pantoea* isolates have been found to inhibit *Liberibacter crescens*, the culturable surrogate of *Candidatus* Liberibacter asiaticus, the causal agent of citrus Huanglongbing (Blacutt et al., 2020), while *Curtobacterium* species have been implicated in modulating *Xylella fastidiosa* infections (Azevedo et al., 2016). *Pseudomonas* species are well-recognized for their versatile antagonistic mechanisms and their potential as effective BCA (Poveda et al., 2021; Trivedi et al., 2011). The extensive study of *Pseudomonas* species and their established role as a core member of the citrus microbiome (Penyalver et al., 2022; Xu et al., 2018; Zhang et al., 2021b) further supports their potential as an effective BCA against citrus pathogens (Poveda et al., 2021; Trivedi et al., 2011;). While *Methylobacterium* genera, widely reported as citrus core members, are classified as methylotrophic bacteria. These microorganisms are capable of using plant-derived methanol (CH_3OH) or methane (CH_4) as sole source of carbon and energy, inducing plant immune defence and protection against UV radiation (Sohrabi et al., 2023; Vorholt, 2012), underscoring their specialized activity as epiphytic microbes.

Thus, this study aimed to: (i) elucidate the microbial composition of the citrus carposphere of three citrus hosts (one lemon and two oranges), (ii) evaluate the impact of phytosanitary treatments (biological and copper) on microbial communities, and (iii) identify core microbial taxa with potential for biocontrol activity for future application. Furthermore, we hypothesized that copper-based treatments have stronger suppressive effect on the bacterial community than on fungal community, and that the citrus fruit microbiome harbours core microbe members with biocontrol potential against *A. alternata* and *C. gloeosporioides.* Insights from this study could guide the development of disease management strategies that preserve microbial diversity while maintaining a sustainable and effective disease control.

2. Materials and methods

2.1. Field treatments and sample collection

As an integral part of our previous research, fruit samples were collected from three citrus host: lemon [*C. limon* (L.) Osbeck] 'Femminello Siracusano 2KR' and sweet orange [*C. sinensis* (L.) Osbeck] 'Tarocco Tapi' and orange 'Tarocco Scirè'. The experimental field trial was arranged in a completely randomized block design, and field treatment procedures align with the protocols previously described (Lombardo et al., 2024). Plants were treated for two consecutive growing seasons to control natural infection of anthracnose, caused by *Colletotrichum* spp., or ABS, caused by *A. alternata.*, with the following commercial products: (1) a standard copper-based fungicide Kop-Twin [Chimiberg-DIACHEM® (13.3 % tribasic copper sulphate and 8.9 % copper hydroxide)], (2) Chitosano Biorend® (Bioplanet; 1.9 % chitosan hydrochloride), (3) a plant extract-based product, PREV-AM® PLUS [Ascenza; 5.8 % Sweet orange essential oil (SOEO)]; and (4) a mixture, Chitosan + SOEO (Mixture). The trial also comprised untreated plants as the negative control.

At harvesting time, from each citrus host and treatment, mature fruits, free from injuries and uniformly shaped were selected. Fifteen fruits were collected for each treatment (untreated control, copper, chitosan, SOEO, mixture), for a total of 75 fruits from each citrus host. Each fruit was aseptically transferred into separate polyethylene sterile bags and submerged with 200 mL sterile buffered peptone water (BPW, pH = 7.0 ± 0.2) supplemented with 0.02 % (v/v) Tween 80 (Gomba et al., 2017). Microbial epiphytes of fruit surfaces were dislodged by shaking for 1 h at 150 rpm, at 20 °C and then sonicated at room

temperature in a sonication water bath, for 8 min at 200 W and 50 Hz. From obtained washing solution, aliquots were used for both isolation of carposphere cultivable microbial population and for amplicon sequencing.

2.2. Cultivable microbial population

To isolate and quantify the cultivable microbial population from fruit surface, serial ten-fold dilutions of the washing solutions were plated on Nutrient Agar (NA, Oxoid, Basingstoke Hempshire, UK) supplemented with 100 mg/L of cycloheximide (AppliChem GmbH, Darmstadt, Germany), and on Potato Dextrose Agar (PDA, Oxoid) supplemented with 100 mg/L of chloramphenicol (AppliChem GmbH). After incubation at 25 °C in the dark for two-four days, the number of microorganisms was counted. For each fruit sample, four replicates were analysed. Data were calculated as CFU (colony forming units per cm^2 and expressed as Log_{10} CFU/cm^2. All data were checked for normal distribution by Shapiro-Wilk normality test, considering a significance level of 0.05. To evaluate data homogeneity, Levene's test at p-value$<$0.05 was employed. Significant differences among the counts of cultivable microbial population between differently treated fruits were determined with one-way analysis of variance (ANOVA) and Tukey HSD post hoc test at p-value$<$ 0.05.

2.3. Amplicon sequencing of 16S rRNA and ITS rDNA

The washing solution was also processed to obtain pellet for DNA extraction and subsequent amplicon sequencing. Specifically, four tubes for each fruit (four subsamples) were centrifuged at 12,000 rpm for 30 min at 4° C. The supernatant was discarded, the pellet was resuspended in 1 mL of sterile distilled water, and centrifuged at 10,000 × g for 10 min at 4 °C. The supernatants were discarded, and the subsamples were pooled together, obtaining one sample/fruit and three samples per treatment, which were stored at −20 °C until use. Each sample was subjected to DNA extraction using a MoBio Powersoil DNA extraction kit (MoBio Laboratories Inc. Carlsbad, CA, USA) according to the manufacturer's instructions. The DNA quality and quantity were investigated and by running the extracted DNA on a 1.5 % agarose gel. 16S and ITS amplicon library preparation and sequencing were performed by IGA Technology Services (Udine, Italy). Primers were selected according to Earth Microbiome Project (2025) protocol (earthmicrobiomeproject.org). For bacterial community profiling, the 16S rDNA V4 region was amplified with primers 515 F (5'- GTGYCAGCMGCCGCGGTAA-3') and 806 R (5'-GACTACNVGGGTWTCTAAT-3'). Peptide nucleic acid (PNA)-clamping was applied during the first 16S rRNA amplification step to block amplification of host chloroplast and mitochondrial 16S rRNA gene sequences. For fungal community profiling, primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') were used to amplify part of the ITS region of fungal rRNA operon. After quality control, quantification and normalization of the DNA libraries, 250-bp paired-end reads were generated from the NovaSeq6000 sequencing platform (Illumina, San Diego, CA). All sequences were deposited in the NCBI Short Read Archive BioProject PRJNA1014910.

2.4. Bioinformatics and data processing

Bioinformatics analyses were performed by importing the raw sequences into R software (v.4.2.1). The FASTQ files were processed using the *DADA2* pipeline (v.1.24) (Callahan et al., 2016). Sequence reads were filtered, de-replicated and de-noised using *DADA2* recommended parameters. Paired-end sequences were merged, and chimeras were removed. To obtain the taxonomic information, ASVs tables were compared against SILVA database for 16S and UNITE database for ITS, using the RDP Naive Bayesian Classifier algorithm throughout *assignTaxonomy* function (Wang et al., 2007). To generate a clean dataset and feature tables, the ASVs merged sequences, defined as "Unknown" at the kingdom and phylum rank, and "Chloroplast", "Mitochondria" and "Eukaryota" were removed from the 16S ASVs table; similarly, "Unknown" at the kingdom and phylum rank, "Bacteria" or "Plant" from the ITS ASVs table were removed. The relative abundance tables for taxa were generated based on the read count for each taxon across samples by using the total-sum scaling (TSS) method (Weiss et al., 2017).

2.5. Microbiome data analysis

Data analyses were conducted by importing the produced data into Rstudio and analysed though *phyloseq* (v.1.40.0) tool (McMurdie and Holmes, 2013). Diversity metrics were calculated for each sample with not-rarefied data (McMurdie and Holmes, 2014). Cumulative Sum Scaling (CSS) (Paulson et al., 2013) transformed bacterial and fungal data for community composition analyses. The effect of the variables "citrus host", "treatment", and their interaction on α diversity (Shannon index) was performed using two-way ANOVA, and differences were analysed with one-way ANOVA and Tukey's HSD post hoc test. Tests with p-value < 0.05 were considered significant. The Bray Curtis dissimilarity was utilized to evaluate β diversity and then visualized on Principal Coordinates Analysis (PCoA). The β dispersion was performed using the *betadisper* function on *vegan* (v. 2.2.6) package (Oksanen et al., 2014). A Permutational Multivariate Analysis of Variance (PERMANOVA) was performed using the *Adonis* function (Anderson, 2001) on *vegan* package, with a permutation number of 999 available and both "citrus host" and "treatment" as the test factors.

Based on the abundance profiles, phyla and families with significantly differential abundances across treatments (copper, chitosan, SOEO and mixture versus the corresponding untreated control) within each citrus host were determined using *DESeq2* (v. 1.36.0) (Love et al., 2014). *P*-values for multiple testing were corrected using the BH method. Tests with adjusted p-value$<$ 0.05 were considered significant.

Core members were calculated using the occurrence of association across samples as the criterion (Lombardo et al., 2024b), using *Core* function in *microbiome* (v. 1.18.0) package (Lahti and Shetty, 2017). The ASVs that were present in more than 75 % of the samples were considered as core ASVs for each citrus host. These core ASVs were then cross-referenced to identify the common core ASVs.

Cytoscape version 3.10.2 and the add-on "CoNet" were used to perform network analysis of significant ($q \geq 0.05$) (Shannon et al., 2003) co-occurrence and mutual exclusion patterns of the microbiomes as described by Wasserman et al. (2019).

2.6. Screening of antagonistic bacterial isolates against Colletotrichum gloeosporioides and Alternaria alternata

A census of the cultivable microbiota was performed to collect bacterial strains naturally occurring on the fruit surface to select potentially BCAs. Distinct, non-overlapping bacterial colonies exhibiting morphological diversity (colour, size, shape) were picked and streaked on NA to obtain pure cultures. Pure cultures were tested for Gram staining by using the 3 % KOH test (Schaad et al., 2001) and streaked on King's medium B agar (KB) (King et al., 1954) to check for fluorescent isolates. Colonies were stored at −20 °C.

In vitro antagonism assays against *C. gloeorporioides* (*Cg*) and *A. alternata* (*Aa*) strains, belonging to the laboratory collection, were performed using the dual-culture test. On each plate bacterial colonies were spot inoculated on PDA plates approximately 25 mm from the centre of the plate. After 48 h at 25 ± 1 °C, a mycelium plug (5 mm diameter) was placed in the centre of each plate and incubated at 25 ± 1 °C for 7 d. A double Petri dish assay was used to test the antifungal activity of volatile organic compounds (VOCs) produced by bacterial strains against *Cg* and *Aa* (Parafati et al., 2015). The fungal growth (cm) was measured five days after inoculation. Petri dishes with only *Cg* or *Aa* served as control. The experiments were performed twice.

The mycelial growth reduction (M.G.R.) was calculated according to

Panebianco et al., (2022). The antagonistic activities for each strain were measured and scored according to an arbitrary scale ranging from 0 to 4, categorized as follows: class 0 = no antagonism; class 1 = 1–5 mm (<25 %); class 2 = 6–10 mm (26–50 %); class 3 = 11–15 mm (51–75 %); class 4 ≥ 15 mm (>75 %). A chi-square test of independence was performed to assess the relationship between two categorical variables, "treatment" and "effectiveness class (0-to-4)". The analysis was conducted after creating a contingency table to summarize the distribution of observations across treatment groups and effectiveness classes. Standardized residuals, Cramér's V measure of association, and post hoc pairwise comparisons were calculated using the *DescTools* package.

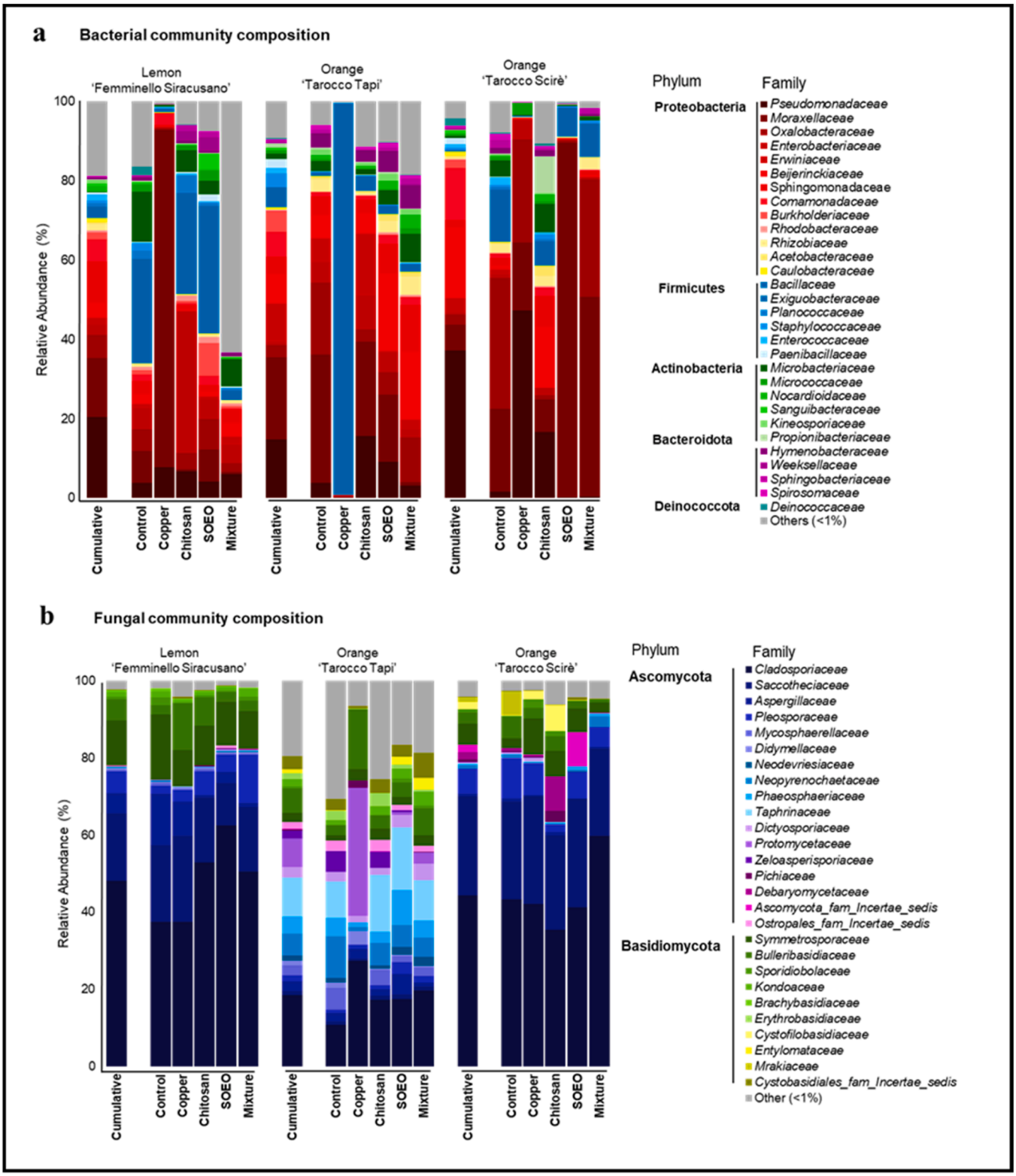

Fig. 1. Taxonomic composition and distribution of bacterial (a) and fungal (b) communities in citrus surface grouped by treatments (control, copper, chitosan, SOEO and mixture) associated to three citrus hosts (lemon 'Femminello Siracusano', orange 'Tarocco Tapi' and orange 'Tarocco Scirè') at Phylum and Family-level.

2.7. Molecular identification of bacterial isolates

A total of 120 bacterial strains (40 from each citrus host, equally from each treatment), selected among those showing the most effective antagonistic activity, were subjected to 16S rRNA gene sequencing. Bacterial DNA was obtained by thermal lysis of cells and the 16S rRNA gene-encoding region was PCR-amplified using the universal primer pair forward 27 F (5'-AGAGTTTGATCMTGGCTCAG-3') and reverse 1492 R (5'-TACGGYTACCTTGTTACGACTT-3') (Lane, 1991), followed by sequencing. After low quality trimming, high quality nucleotide sequences were blasted using the NCBI GenBank database and aligned by Clustal W using MEGA11 software (v.11.03.13) (Tamura et al., 2021). The aligned sequences were tested for their phylogenyand a Maximum Likelihood tree was built including 16S rRNA gene sequences of the related type strains (Saitou and Nei, 1987). The 120 16S rRNA gene sequences were then used to intercept those bacteria that showed a $\geq$ 98 % similarity to the ASV common core members (Jing et al., 2023). Each ASV reference sequence assigned to the core-citrus microbiome comprised the "query" sequences and then intercepted into the network analysis. In this study, nucleotide sequences of 117 antagonistic bacterial strains were deposited in the GenBank database under accession numbers OR394435 - OR394551.

3. Results

3.1. Sequencing outputs of citrus carphospere microbiome community

After quality filtering and removal of non-target sequences, a total of 14,223,727 for 16S and 7,055,852 for ITS high-quality reads was retained, with an average of 948,248 and 470,390 reads per samples (Table S1 and S2). ASVs assigned to nontarget sequences [Prokaryota dataset: eukaryota (n = 1; 0.01 %), chloroplast (n = 71; 1.68 %), mitochondria (n = 344; 5.66 %), unknown bacterial kingdom (n = 27; 0.44 %), unknown phylum (n = 147; 2.42 %); Eukaryota dataset: unknown fungal phylum (n = 1629; 35.09 %)] were removed. The curated ASVs contained a total of 5511 unique ASVs for bacterial microorganisms (37 phyla, 77 classes, 174 orders, 296 families, 729 genera, 354 species), with an average of 717 ASVs per sample. The fungal community contained a total of 3013 different ASVs (8 phyla, 30 classes, 91 orders, 255 families, 574 genera, 856 species), with an average of 407 ASVs per sample (Table S3).

3.2. Taxonomic composition of the citrus carposphere microbiome

The taxonomic composition of the citrus carposphere was largely consistent across the citrus hosts, with varying relative abundances (Fig. 1a, b). Specifically, the dominant bacterial phyla (>1 % of relative abundance) were Proteobacteria (lemon 'Femminello Siracusano', 67.5 %; orange 'Tarocco Tapi', 63.6 % and orange 'Tarocco Scirè' 80.8 %), Firmicutes (20.5 %, 22.0 % and 9.5 %), Actinobacteriota (8.6 %, 7.4 % and 6.7 %) and Bacteroidota (2.7 %, 5.2 % and 2.1 %) (Fig. 1a). Within Proteobacteria, the predominant families were Pseudomonadaceae, Moxarellaceae, Oxalobacteraceae, Enterobacteraceae, Erwiniaceae, Beijerinckiaceae, and Sphingomonadaceae whereas Bacillaceae was the most represented family within the Firmicutes (Fig. 1a). The fungal community was mainly composed of Ascomycota (78.8 %, 74.8 % and 85.8 %), and Basidiomycota (20.9 %, 19.1 % and 14.0 %) (Fig. 1b). Within the largest taxonomic group of the Ascomycota, Cladosporiaceae, Saccotheciaceae, Pleosporaceae and Aspergillaceae were the most represented families.

3.3. Changes in bacterial and fungal taxonomic composition

The relative abundance of ASVs was analysed, revealing taxa that were either enriched or depleted by copper or alternative treatments for each citrus cultivar when compared to the untreated control. *DESeq2* analyses revealed a significant impact of copper treatment on the citrus microbiome taxonomic composition, with a more pronounced effect on the bacterial ASVs distribution compared to fungal ASVs (Supplementary Figure 1). Specifically, through the study of the associated higher taxonomic level (phylum and family), copper-treated fruits displayed a Proteobacteria-dominated community in both lemon 'Femminello Siracusano' (98 %) and orange 'Tarocco Scirè' (96 %), and a Firmicutes-dominated community in orange 'Tarocco Tapi' (99 %) (Fig. 1a), resulting in the depletion of 10, 16 and 7 bacterial phyla when compared to the untreated control (Supplementary Data S1). At family taxonomic level, the reduction was reflected in the depletion of 85, 107 and 65 bacterial families in copper-treated samples (Supplementary Data S2). In contrast, alternative treatments induced minimal (in oranges) or no depletion (in lemon) with some enriching occurring of both bacterial phyla and families compared to the untreated controls (Supplementary Data S1 and S2). For example, the highest enrichment of family taxa was recorded by chitosan in 'Tarocco Scirè' (a total of 102 enriched families), followed by SOEO in 'Tarocco Tapi' (57) and Mixture in 'Tarocco Tapi' (26) and 'Tarocco Scirè' (28).

The fungal taxonomic composition remained largely unaffected by the treatments, with fewer depletions observed (Supplementary Data S1). However, the highest number of depleted taxa was recorded in copper-treated samples, with 18 families in lemon and 45 families in orange 'Tarocco Tapi' (Supplementary data S2).

3.4. Impact of treatments on carposphere microbial diversity

The induced modification by field treatments and citrus host on bacterial and fungal microbiome was assessed. Alpha and beta diversity metrics revealed distinct trends between the two kingdoms (Table 1). Based on alpha diversity indices (Supplementary Table S4, S5), Shannon diversity within the bacterial community was primarily driven by field treatment (R^2 = 0.47) rather than host (R^2 = 0.05). Notably, copper

Table 1
Effects of single factors (citrus hosts and treatments) and their interactions on alpha (Shannon index) and beta (Bray-Curtis distance) diversities on bacterial and fungal communities. Statistical analyses on alpha diversity were performed using two-way ANOVA. Statistical analyses for beta diversity were performed using Permutational Multivariate Analysis of Variance (PERMANOVA)*.

Bacterial community	Source of variation	Df	Sum of Sqs	R^2	F value	*P*-value
Alpha diversity	Citrus host	2	3.338	0.05	6.58	< 0.01
	Treatment	4	30.09	0.47	29.64	< 0.001
	Citrus host: Treatment	8	22.84	0.35	11.25	< 0.001
	Residuals	30	7.61	0.11		
Beta diversity	**Source of variation**	**Df**	**Sum of Sqs**	**R^2**	**F value**	***P*-value**
	Citrus host	2	1.70	0.22	8.77	< 0.001
	Treatment	4	1.25	0.16	3.21	< 0.001
	Citrus host: Treatment	8	1.83	0.23	2.35	< 0.001
	Residual	30	7.71	0.38		
Fungal community	**Source of variation**	**Df**	**Sum of Sqs**	**R^2**	**F value**	***P*-value**
Alpha diversity	Citrus host	2	18.21	0.78	292.71	< 0.001
	Treatment	4	1.15	0.05	9.21	< 0.001
	Citrus host: Treatment	8	2.93	0.13	11.756	< 0.001
	Residuals	30	0.93	0.04		
Beta diversity	**Source of variation**	**Df**	**Sum of Sqs**	**R^2**	**F value**	***P*-value**
	Citrus host	2	3.32	0.43	19.89	< 0.001
	Treatment	4	0.71	0.09	2.13	< 0.001
	Citrus host: Treatment	8	1.27	0.16	1.90	< 0.001
	Residual	30	2.51	0.32		

*Df = degrees of freedom; Sum of Sqs=Sum of Squares; R^2=coefficient of determination; F test of fixed effects, *p*-value associated to F.

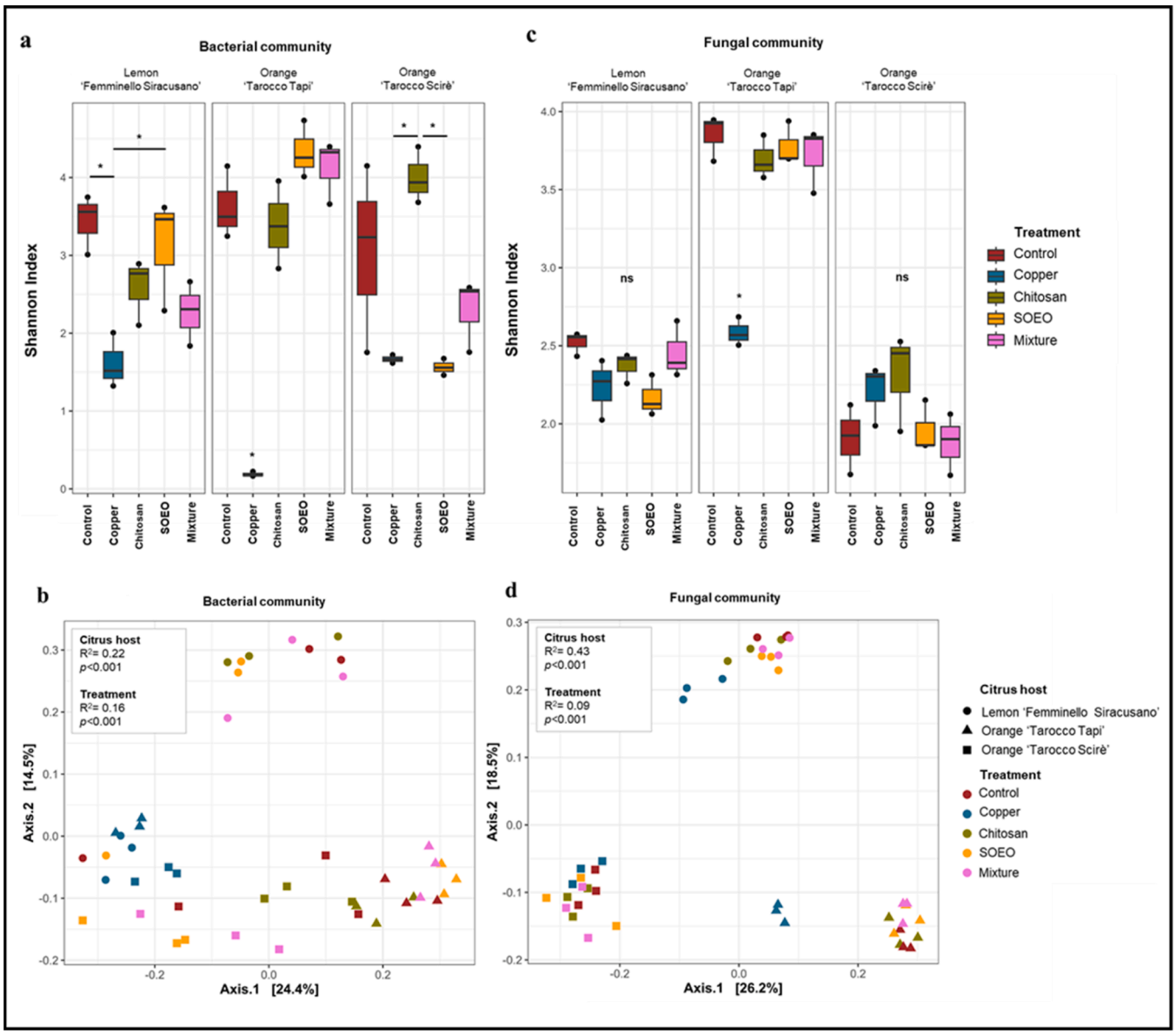

Fig. 2. Shannon index of bacterial (a) and fungal (c) communities in citrus fruit surface grouped by treatments (control, copper, chitosan, SOEO and mixture) associated to three citrus hosts (lemon 'Femminello Siracusano', orange 'Tarocco Tapi' and orange 'Tarocco Scirè'); Shannon indices were statistically compared between treatments within the same citrus host. Asterisks indicate significant differences (p-value<0.05) computed with one-way ANOVA, ns= not significant. Centre value represents the median of index; Principal Coordinates Analysis (PCoA) based on the Bray-Curtis distance of bacterial (b) and fungal (d) community in citrus fruit surface for each citrus host. Points are colored by treatment. P-values and R^2 values were obtained using a permutational multivariate analysis of variance (PERMANOVA).

treatment consistently reduced bacterial diversity across all hosts compared to untreated controls (Fig. 2a). This reduction in Shannon index was statistically significant for lemon 'Femminello Siracusano' and orange 'Tarocco Tapi' (Fig. 2a). Conversely, no significant differences in Shannon diversity were observed following treatment with biological products compared to the control. Beta diversity analysis of the bacterial communities revealed host-driven effect (R^2 = 0.22, $p < 0.001$). Lemon samples clustered separately from the two 'Tarocco' varieties, indicating a more distinct bacterial composition (Fig. 2b). Interestingly, treatment effects were also observed on beta diversity (R^2 = 0.16, $p < 0.001$). Copper-treated samples clustered together regardless to citrus host, suggesting a significant impact of copper on bacterial community structure (Fig. 2b).

In contrast to bacteria, fungal Shannon diversity was predominantly influenced by the citrus host (R^2 = 0.78) with minimal influence of treatments (R^2 = 0.05). No significant differences in alpha diversity were observed within each host following treatment with biological products (Fig. 2c). However, a significant decrease in Shannon diversity was observed in copper-treated 'Tarocco Tapi' oranges. Beta diversity analysis of the fungal communities revealed host-specific clustering (R^2 = 0.43; Fig. 2d). Notably, copper-treated samples from 'Tarocco Tapi' orange and, to a lesser extent, 'Femminello Siracusano' lemon deviated from their respective host clusters. Supporting the amplicon sequence data, the corresponding culturable bacterial population exhibited greater sensitivity to treatments (Supplementary Figure 2a), while. fungal populations only displayed a slight decrease following copper treatment in 'Tarocco Tapi' oranges (Supplementary Figure 2b).

3.5. *Alteration of co-occurrence network interactions*

Significant co-occurrence and mutual exclusion patterns within bacterial and fungal networks were analysed to investigate how

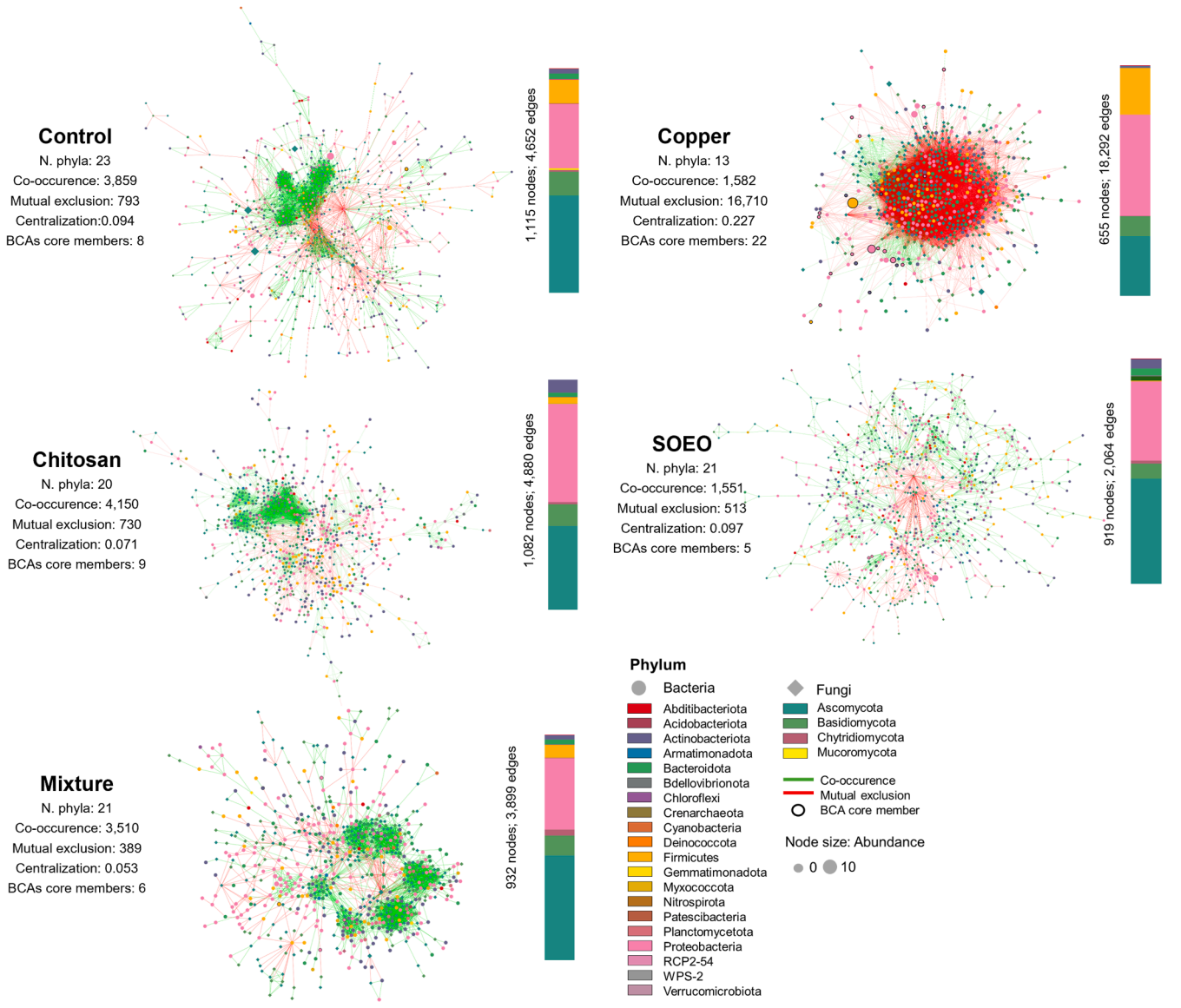

Fig. 3. Significant co-occurrence and mutual exclusion relationships among carposphere-associated microbiota of citrus fruit in different treated samples: untreated control (control), copper, chitosan, sweet orange essential oil (SOEO), and mixture. Only significant interactions are shown ($r \geq 0.05$). The nodes of each network are coloured according to phylum affiliation and sized according to the abundance of the taxon. The edges connecting the nodes are represented by green lines to indicate co-occurrence interactions (positive) and by red for mutualistic exclusions (negative). Nodes highlighted with a black border represent isolated BCA that matched with common core members. Relative abundance of nodes present in the network is reported at phylum level in the bar chart for each treatment.

different treatments influence network connectivity. The untreated control network displayed the highest node count (1115), representing the most diverse microbial community, encompassing a total of 23 distinct phyla (Fig. 3). Compared to the control, alternative treatments resulted in moderate reductions in both node count and represented phyla. In contrast, copper treatment caused a substantial decrease in network diversity, reflected by a significantly lower number of nodes (655) and fewer present phyla (13). The distribution of edges also differed between treatments. In the control and organically treated networks, over 75 % of the edges were positive, indicating a prevalence of co-occurrence interactions between nodes. In contrast, while copper treatment significantly increases the overall number of edges (18,292), connection was dominated (91 %) by mutual exclusion between nodes. The copper network exhibited a higher network centralization (0.227) compared to the other treatments, suggesting a smaller number of nodes played a central role in connecting the network under the copper treatment.

3.6. Characterization and distribution of Colletotrichum and Alternaria genera

As target pathogens of our investigation, a species-level investigation of *Colletotrichum* and *Alternaria* genera was performed using 16S data, resulting in the discrimination of distinct species (Supplementary Data S3). The analysis revealed the presence of six taxa affiliated with the *Colletotrichum* genus, for a total of four species: *C. gloeosporioides, C. acutatum* (both represented by two distinct ASVs), *C. novae-zelandiae* and *C. karstii*. Notably, among the identified species, *C. gloeosporioides* exhibited the highest abundance, being present in all examined orchards and with an occurrence of 100 % within each sample of 'Tarocco' hosts. Statistical analysis using non-parametric analysis of variance indicated no significant alterations ($p = 0.99$, p-value$= 0.97$, p-value$= 0.84$, Kruskal-Wallis) in the relative abundance of this genus as a consequence of treatments across all three orchards (Figure S3a). In relation to *Alternaria*, we identified 25 taxa associated with this genus

(Supplementary Data S3), corresponding to 13 distinct species: *A. alternata* and *A. metachromatica* (each represented by three distinct ASVs), *A. infectoria* (represented by five distinct ASVs), *A. rosae*, *A. nepalensis*, *A. chlamydosporigena* (each represented by two distinct ASVs), *A. tumida A. sorghi*, *A. multiformis*, *A. lolii*, *A. japonica*, *A. hordeicola,* and *A. brassicicola*. Among them, *A. alternata* exhibited the highest abundance across all three orchards. Additionally, one ASV related to *A. alternata* (ASV5) and one related to *A. infectoria* (ASV42) were detected in all samples from each citrus hosts. Statistical analysis revealed no significant changes between treatments for *Alternaria* genus (p-value=0.68, p-value=0.23, p-value= 0.21, Kruskal-Wallis) (Figure S3b)

3.7. Shared core members

Core members were calculated for each citrus host (Supplementary Data 4, 5, 6) and then filtered to identify the common members. Following the filtering, one hundred and ten bacterial and forty-five fungal ASVs were identified to belong to the core shared microbiome (Supplementary Data S7, Fig. 4a, b). The 110 common bacterial core taxa (Fig. 1c) were primarily identified as different members of the highly abundant Proteobacteria (58 different taxa, 14 families, 23 genera), Firmicutes (15 different taxa, 6 families, 8 genera), Actinobacteriota (23 different taxa, 7 families, 15 genera), and Bacteroidota (13 different taxa, 4 families, 5 genera). The phylum Deinococcota was represented by a single taxon. Within Proteobacteria, the Alphaproteobacteria class is mainly composed of Sphingomonadaceae (9 taxa), Beijerinckiaceae (6 taxa) and Rhizobiaceae (4 taxa) families, while Gammaproteobacteria class mainly included Pseudomonadaceae (6 taxa), Oxalobacteraceae (6 taxa), Moraxellaceae (6 taxa), and Enterobacteriaceae (5 taxa). Within Firmicutes, the major bacterial class Bacilli harboured Bacillaceae (3 taxa), Enterococcaceae (2 taxa), Exiguobacteraceae (2 taxa), Planococcaceae (5 taxa) and Staphylococcaceae (2 taxa). The Actinobacteria class was predominantly represented by the families Microbacteriaceae (11 taxa) and Micrococcaceae (4 taxa). The 45 common fungal core taxa (Fig. 4d) were primarily members of the highly abundant Ascomycota (27 taxa, 11 families, 13 genera) and Basidiomycota (17 taxa, 10 families, 11 genera). The phylum Chytridiomycota was represented by a single taxon. Ascomycota, the most prominent phylum, was primarily represented by the Dothideomycetes class (22 taxa), which included 13 genera, with two taxa assigned to the *Alternaria* genus.

3.8. Identification of bacterial strains associated with core members as potential biological control agents

In this study, we isolated and analysed a total of 447 bacterial strains from the fruit surface (carposphere) of differently treated citrus hosts: 101 from lemon 'Femminello Siracusano', 217 from orange 'Tarocco Tapi', and 129 from orange 'Tarocco Scirè' (Table S9). These strains exhibited diverse morphologies and were predominantly Gram-negative in lemons ('Femminello Siracusano', 61.4 %) and 'Tarocco Tapi' oranges (56.7 %), while 'Tarocco Scirè' oranges displayed a higher prevalence of Gram-positive bacteria (57.4 %). Additionally, a total of 42 bacteria (9.4 %) exhibited fluorescence (Table S9). The isolated bacteria were screened for their ability to inhibit the growth of *Cg* and *Aa*. Based on their inhibition scores (classes 0–4), the majority of antagonistic bacteria fell within the intermediate-low effectiveness classes (classes 0–2), while some strains displayed strong inhibition (class 3–4), with the 19.9 % and 4.8 % belonging to class 3 and 4 against *Aa*, while the 17.5 % and 2 % against *Cg*. Our screening identified the most effective bacteria primarily isolated from the lemon fruit surface (8.9 % of isolates belonged to class 4 against *Aa*), demonstrating strong inhibitory activity. This percentage was lower for 'Tarocco Tapi' orange (6.9 %), while 'Tarocco Scirè' orange lacked class 4 isolates entirely. Among the 447 strains, 37.8 % demonstrated VOCs production, leading to varying degrees of reduction in pathogen growth. The more efficient antagonistic strains were confirmed from lemon 'Femminello Siracusano' (56 % of VOCs-producing strains), with a higher number of VOCs producer bacteria and higher efficacy class observed when tested against *Aa*. While these trends suggesting a potential influence of citrus host on the antagonist effectiveness, the chi-square test revealed no significant treatment influence on the distribution of antagonist effectiveness classes within each citrus host (Supplementary Figure S4).

In accordance with the findings from the antagonism assays, a further selection of the best antagonistic bacteria was performed to establish a collection of potential BCAs. A total of 120 bacterial isolates were selected and identified (Table S7). Fourteen different families were encountered (Fig. 4a): Pseudomonadaceae (n = 32, 26.7 %), Erwiniaceae (n = 28, 23.3 %), Burkholderiaceae (n = 21, 17.5 %), Bacillales (n = 15, 12.5 %), Microbacteriaceae (n = 6, 5 %), Staphylococcaceae (n = 5, 4.2 %), Oxalobacteraceae (n = 3, 2.5 %), Enterobacteriaceae (n = 3, 2.5 %), Xanthomonadaceae (n = 1, 0.8 %), Micrococcaceae (n = 1, 0.8 %), Paracoccaceae (n = 1, 0.8 %), Sphingomonadaceae (n = 1, 0.8 %), Alcaligenaceae (n = 1, 0.8 %) and Sanguibacteraceae (n = 1, 0.8 %) (Fig. 5a). In Fig. 5b a dendrogram showing the phylogenetic relationships of bacterial strains isolated from the citrus fruit surface and the type strains of the putative bacterial species identified by the BLASTN analysis is displayed.

A high overlap was found between antagonistic isolates and the core microbiome, with 98 out of 120 (81.7 %) antagonistic bacterial isolates matching core ASVs (Table S7, Fig. 4c). Network analysis confirmed the presence of BCAs core members across all treatments, although varying in number and abundance (Fig. 3). Interestingly, although copper treatment significantly altered microbial communities in terms of composition and diversity (Figs. 1, 2 and Supplementary Data 5, 6), the network analysis identified 22 core-associated ASVs with BCAs. These BCAs predominantly exhibited positive interactions with other bacteria and negative interactions with fungi. For example, ASV 1316, associated with the genus *Klebsiella*, formed 22 connections, including the only four negative interactions with fungal taxa, potentially including phytopathogens like *Ramularia*. Conversely, ASVs 370 (*Bacillus*), 3776 (*Massilia*), 4225, and 4230 (*Pseudomonas*) - some of the most abundant taxa in the network - exclusively formed positive interactions with other bacteria and among BCAs.

4. Discussion

Understanding the composition and dynamics of the fruit microbiome is crucial for developing sustainable agricultural practices that promote fruit health (Droby and Wisniewski, 2018). By identifying and fostering beneficial microbial communities it is possible to enhance fruit quality, resistance to diseases and yield (Droby et al., 2022). Our study contributes to this goal by revealing a complex citrus microbiome dominated by Ascomycota and Basidiomycota fungal phyla, and Proteobacteria as the most abundant bacterial phylum, followed by Firmicutes, Actinobacteriota, and Bacteroidota. This overall composition aligns with the established knowledge of citrus fruit microbiomes (Gomba et al., 2017; Jing et al., 2023; Kumar et al., 2021). While beta diversity analyses highlighted distinct microbial assemblages among citrus hosts, suggesting the influence of host genotype on microbiome assembly (Xu et al., 2023; Lombardo et al., 2024b), a core group of taxa was shared across all investigated cultivars. Our identification of a core citrus fruit microbiome expands upon previous research primarily focused on other plant compartments, such as the rhizosphere and phyllosphere (Blaustein et al., 2017; Ginnan et al., 2020; Munir et al., 2020; Xu et al., 2018). The identified core taxa comprised genera previously reported as core members of the citrus fruit surface microbiome, including *Acinetobacter, Bacillus*, *Chryseobacterium*, *Curtobacterium, Hymenobacter*, *Massilia*, *Methylobacterium*, *Sphingomonas*, *Alternaria* and *Penicillium* (Jing et al., 2023), supporting the concept of a shared citrus holobiont.

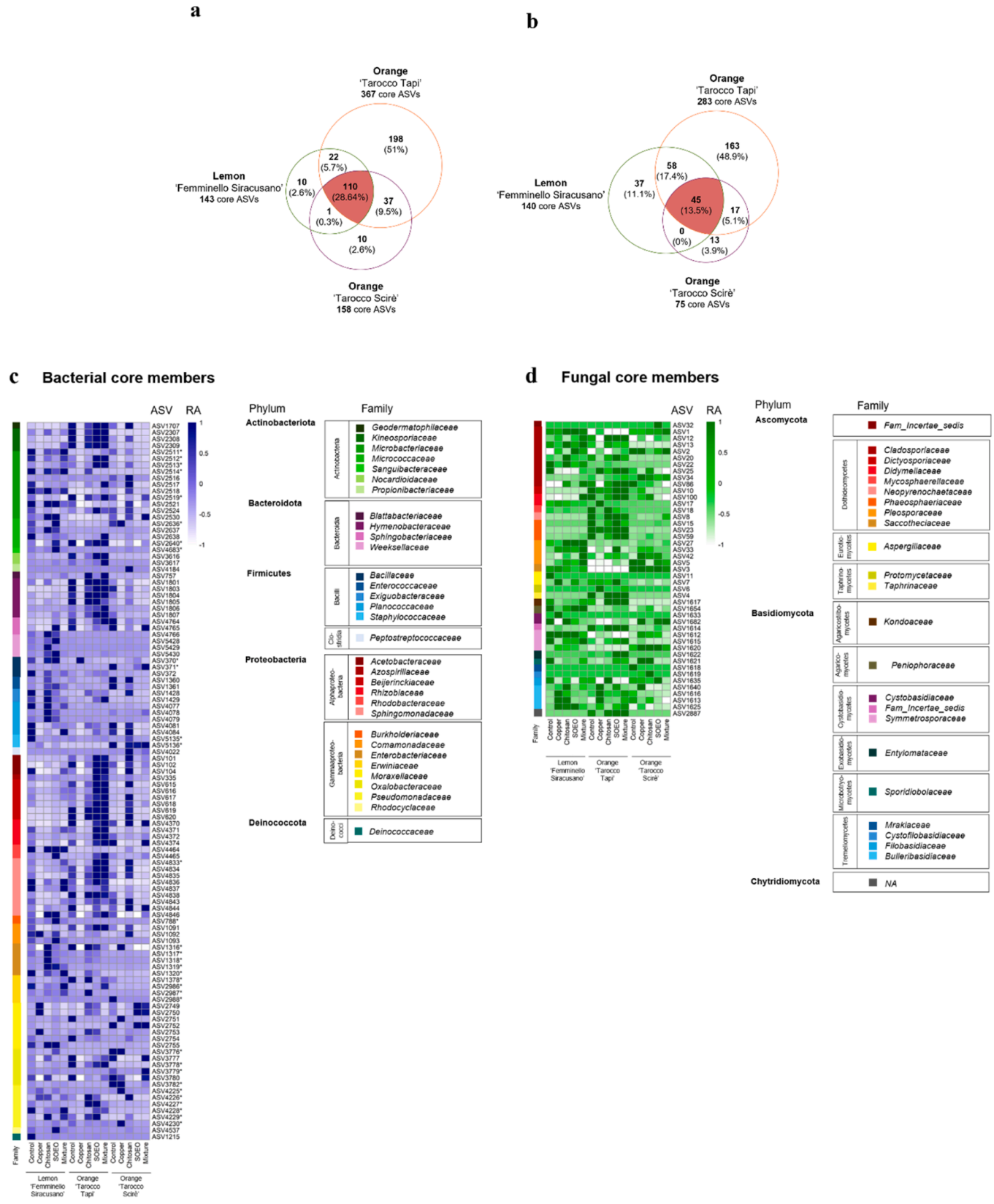

Fig. 4. Venn diagram demonstrating unique (not present in any other type of samples) and shared bacterial (a) and fungal (b) core ASVs of citrus fruit surface across three citrus hosts. Relative abundance of 110 core bacterial (c) and 45 fungal (d) ASVs in the carposphere of citrus fruits across citrus host and treatment. Bacterial ASVs marked with * correspond to isolated Biological Control Agents (BCAs). The color gradient from dark to white indicates the relative abundance of each taxon, with blue representing higher abundance and white lower abundance. The scale is based on the relative abundance (RA) of ASVs, normalized by row using the Log10 (x + 1) transformation of observed reads. The color bars on the right side of the heatmap indicate the family to which each ASV belongs.

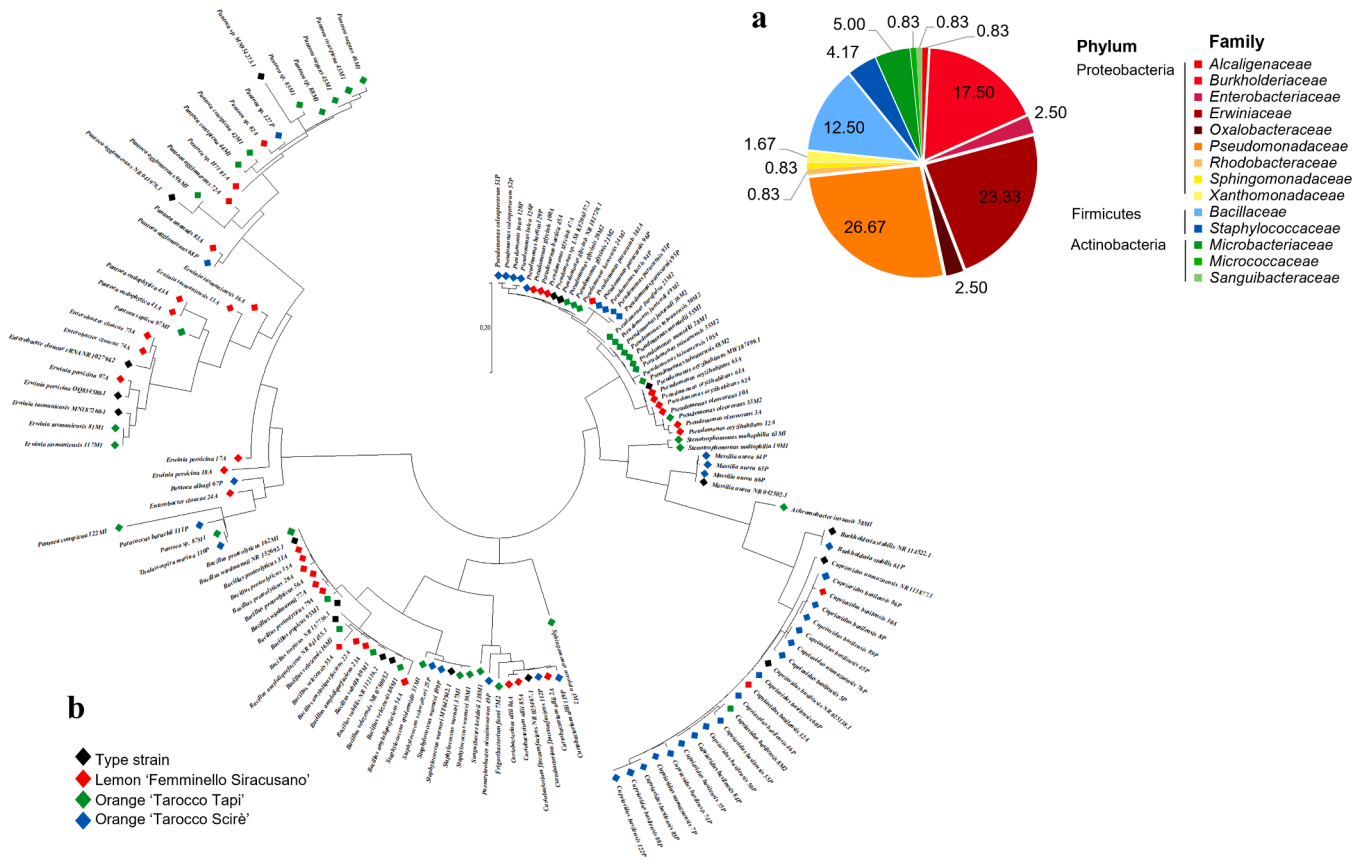

Fig. 5. (a) Family distribution (%) of bacterial antagonistic strains isolated from citrus carposphere of three citrus hosts pooled together. (b) Phylogenetic tree based on 16S rRNA gene sequences of 120 bacterial strains isolated from the carposphere of citrus fruit from different citrus host corresponding. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (2021).

Cultivating a thriving and balanced fruit microbiome through appropriate agricultural practices is essential for optimizing plant-microbe interactions and maximizing the benefits for both the plant and the environment (Delitte et al., 2021). Thus, having established a baseline understanding of the citrus carposphere microbiome and its core members, we next investigated the impact of two-years field-applied phytosanitary treatments (biological and copper-based products) on microbial community. Considering field-based microbiome dynamics, previous studies have highlighted the influence of the whole farming systems on plant microbiomes, including organic and conventional approaches (Abdelfattah et al., 2016; Kecskeméti et al., 2016; Wassermann et al., 2019), but few focused on the effect of treatment applications (Cernava et al., 2019). For instance, organically managed apples showed higher microbial diversity than conventionally grown ones (Wassermann et al., 2019), while only minor shifts were observed in grape berries (Kecskeméti et al., 2016). More recently, Wu et al. (2023) demonstrated that neutralized phosphorous acid suppressed powdery mildew on cucumber while preserving the leaf microbiome, indicating the potential of environmentally friendly treatments to combine disease control with microbiome stability. Our results confirm that the carposphere microbiome of citrus is influenced by the application of phytosanitary treatments (Gomba et al., 2017; Kumar et al., 2021), exhibiting differential responses to biological or copper-based products. Specifically, in copper treated fruit, we observed a significant decline in bacterial diversity, with the community becoming dominated by a limited number of families, suggesting that copper may selectively eradicate a substantial portion of bacterial taxa (Carvalho et al., 2020). However, the effect was not uniform across taxa: while an overall reduction was evident in bacterial diversity, as evidenced by both alpha and beta diversity indices, cultivar-specific pattern emerged, with the carposphere of 'Tarocco Tapi' exhibiting an exclusive presence of *Bacillaceae* family, and lemon 'Femminello Siracusano' and 'Tarocco Scirè' displaying an exclusive dominance of the *Proteobacteria* phylum. Among taxa most severely depleted by copper were citrus-associated core beneficial members, such as *Curtobacterium*, *Erwinia*, *Klebsiella*, *Pantoea*, *Massilia*, *Methylobacterium* or *Sporobolomyces* (Fig. 4c, d). These genera were consistently reported as beneficial and part of citrus core microbiota in previous studies (Ginnan et al., 2020; Jing et al., 2023; Munir et al., 2020; Penyalver et al., 2022). The sensitivity of those taxa to copper would underscore the collateral perturbation posed by the ecotoxicological profile of copper to beneficial microorganisms (Carvalho et al., 2020; Lamichhane et al., 2018; Gobbi et al., 2020).

These findings suggest that, despite the overall reduction in bacterial diversity, specific taxa may exhibit differential resilience or susceptibility to copper exposure. Given the inherent variability in microbial communities within the carposphere, we speculate that other factors (e.g., fruit exposure, the chemical composition of individual carpospheres, and climatic conditions during harvest) not assessed in this study, could further influence the structure and stability of the indigenous microbial community (Wicaksono et al., 2023). These negative impacts, previously observed in the phyllosphere of citrus and grape (Carvalho et al., 2020; Gobbi et al., 2020), are now confirmed for the fruit microbiome. In contrast to copper, biological treatments did not significantly alter the microbial community structure, as indicated by comparable diversity indices to the control (Fig. 1a, b), and manly enriched the core beneficial members.

The observed differences in microbial community response likely stem from the broad-spectrum antimicrobial activity of copper, in

contrast to the more targeted properties of chitosan and SOEOs, particularly active against fungi (Jing et al., 2014; Rajestary et al., 2021;). Chitosan activity depends strongly on formulation and physicochemical properties (molecular weight, degree of deacetylation, pH, concentration) and on the method of application (e.g., coatings, controlled-release), which in turn influence whether the treatment primarily suppresses fungi, alters resource availability, or elicits host defences (López-Moya et al., 2019; Poznański et al., 2023; Xing et al., 2015;). Consequently, empirical outcomes are heterogeneous: some studies report little change in overall bacterial community structure after chitosan treatment (Lopez-Nuñez et al., 2025), with Gram-positive bacteria more sensitive due to cell wall composition, whereas others report selective enrichment of putatively beneficial taxa (e.g., *Lactobacillus*, *Weissella*, *Bacillus*, *Pseudomonas*) in coated fruit (Rai et al., 2024). By contrast, fungi are generally reported as highly susceptible to chitosan than bacteria, although sensitivity varies with taxon and developmental stage (Xing et al., 2015), and plant-pathogenic fungi, such as *Alternaria* and *Fusarium*, are always reported as highly sensitive to chitosan in microbiome studies (Lopez-Nuñez et al., 2025), although exceptions exist among taxa adapted to particular membrane/cell-wall chemistries. Similarly, the effects of essential oils (EOs) are highly composition- and formulation-dependent. Although specific data on SOEO in planta are limited, EOs in general tend to inhibit fungi more strongly than bacteria (Nazzaro et al., 2017), with Gram-positive bacteria typically more susceptible than Gram-negative taxa (Akarca and Sevik, 2021). Encapsulated EO formulations have been shown to stimulate some bacterial groups (e.g., actinomycetes) while suppressing fungal populations in soil (Chmiel et al., 2022). Taken together, chitosan and SOEO can indirectly shape microbial communities by selectively removing susceptible taxa, favouring tolerant or opportunistic groups, and by modifying host surface chemistry and micro-niche structure; these dynamics should therefore be considered when proposing biological alternatives to copper.

Nevertheless, the fungal community remained largely unaffected by either biological or copper treatments, including pathogenic fungal genera, such as *Colletotrichum* and *Alternaria*. This lack of distinction could be attributed to non-mutually exclusive factors, such as the sampling of asymptomatic, marketable fruits which likely harboured a relatively low pathogen load and therefore limited detectable treatment effects and methodological constraints related to low microbial biomass on the carposphere, which can reduce sensitivity for detecting shifts in less abundant taxa.

Nonetheless, these findings emphasize the presence of pathogenic fungal genera as integral components of the citrus carposphere microbiome.

These considerations echo previous research in which drenching treatments produced affected bacterial diversity but not he fungal community (Gomba et al., 2017; Kumar et al., 2021). Accordingly, differences in the abundance of the major pathogenic genus *Penicillium* were not detected among differently treated fruits by Kumar et al. (2021). Moreover, the pronounced decline on the bacterial community underscores the greater vulnerability of bacteria to copper treatments compared to fungi, also supported by recent findings in the field of microbiome studies (Jiang et al., 2023; Xu et al., 2022). The differential vulnerability could be attributed to fundamental differences in cell structure and physiology (Cervantes and Gutierrez-Corona, 1994). Bacterial cell membranes, primarily composed of phospholipids, are more susceptible to disruption by copper ions compared to the rigid fungal cell walls, which provide a greater degree of protection. Additionally, the different mechanisms to tolerate heavy metals developed by fungi may contribute to their resilience to copper stress, compared to bacteria (Rajapaksha et al., 2004). Results from network analysis confirmed the potential detrimental effect of copper treatments on microbial community dynamics, revealing significant alteration and remodelling of microbial interactions and topological indices (Jiang et al., 2023), while fewer differences were recorded in biological-treated samples. Compared to the control, copper-treated samples exhibited increased network complexity (number of degree), but marked in negative interactions, suggesting potential shifts in microbial associations under copper stress. Notably, although the network structure and community were altered, BCAs associated with the core ASVs were part of the network even in copper-treated fruits. The selected BCAs were not influenced by applied treatments, demonstrating their stable association with citrus fruit microbial community. Since in our study the network correlations roughly predicted the benefits of BCAs against fungal pathogens, such as *Alternaria* and *Colletotrichum* ASVs in the network, investigation aimed at clarifying the impact of field treatments and selected BCAs on citrus pathogens during preharvest conditions are needed. This would facilitate the development of strategies aimed at reducing the pathogen load in the field, with significant implications for postharvest storage (Wassermann et al., 2022). While in vitro assays provide an essential first screening step, we acknowledge the limitations in predicting BCAs performance under field conditions. Further steps, including field trials and assays targeting additional functional traits, are required to validate their potential and clarify their mechanisms of action for future applications.

In conclusion, this work has shed new light on the composition of the citrus fruit microbiome and how phytosanitary products influence the microbial community and biological control agents. We confirmed the toxicological risk of copper on microbial communities and highlighted the need for alternative biological products that could preserve beneficial microbial communities. A sustainable alternative strategy should demonstrate efficacy in reducing disease symptoms without causing detrimental effect to plant beneficials (Kong et al., 2018). In line with these considerations, the biological products evaluated in our study, which exhibited effective disease control against ABS and anthracnose of citrus fruit (Lombardo et al., 2023; 2024a), did not significantly perturb the beneficial microbial composition. Thus, we suggest them as potential candidates for reducing copper usage in agriculture and promoting beneficial microbiomes. Moreover, we confirmed that integrating cultural-dependent and independent techniques is a potentially effective approach to lay the foundation for the selection and application of highly specialized bioinoculants for disease management.

5. Conclusion

This study explored the structure of the citrus carposphere microbiome and its response to field treatments, including conventional copper compounds and alternative approaches. By combining cultivation-dependent and high-throughput sequencing methods, we demonstrated the unsustainability of copper as an inducer of microbiome disturbance. In contrast, alternative treatments with biological products had a moderate or negligible impact, preserving the microbial balance. Importantly, we identified core microbial community members across treatments. Among these, several bacterial strains showed potential as BCAs, indicating their possible role in suppressing pathogenic fungi. These findings support the use of alternative treatments as viable and more sustainable options to copper in citrus disease management. They also highlight the potential of leveraging stable core microbiota for the development of microbiome-informed biocontrol strategies, promoting both plant health and environmental sustainability.

CRediT authorship contribution statement

Monia Federica Lombardo: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Ahmed Abdelfattah:** Writing – review & editing, Validation. **Vittoria Catara:** Writing – review & editing, Methodology. **Nian Wang:** Writing – review & editing, Validation, Investigation, Conceptualization. **Gabriella Cirvilleri:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by Organic PLUS project, that received funding from European Union's Horizon 2020 Research and Innovation Programme [774340 – Organic PLUS "Pathways to phase-out contentious inputs from organic agriculture in Europe" (2018–2022)]; University of Catania, Italy [PIA.CE.RI. 2020–2022 Linea 2, Research Project MEDIT-ECO)]; and Fondi di Ateneo 2020–2022 – Linea Open Access.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.micres.2025.128346.

Data availability

Data will be made available on request.

References

Abdelfattah, A., Cacciola, S.O., Mosca, S., Zappia, R., Schena, L., 2017. Analysis of the fungal diversity in citrus leaves with greasy spot disease symptoms. Microb. Ecol. 73, 739–749. https://doi.org/10.1007/s00248-016-0874-x.

Abdelfattah, A., Wisniewski, M., Droby, S., Schena, L., 2016. Spatial and compositional variation in the fungal communities of organic and conventionally grown apple fruit at the consumer point-of-purchase. Hortic. Res. 3, 16047. https://doi.org/10.1038/hortres.2016.47.

Akarca, G., Sevik, R., 2021. Biological activities of *Citrus limon* L. and *Citrus sinensis* L. peel essential oils. J. Essent. Oil Bear. Plants 24 (6), 1415–1427. https://doi.org/10.1080/0972060X.2021.2022000.

Anderson, M.J., 2001. Permutation tests for univariate or multivariate analysis of variance and regression. Can. J. Fish. Aquat. Sci. 58, 626–639. https://doi.org/10.1139/f01-004.

Avenot, H.F., Michailides, T.J., 2015. Detection of isolates of *Alternaria alternata* with multiple-resistance to fludioxonil, cyprodinil, boscalid and pyraclostrobin in California pistachio orchards. Crop Prot. 78, 214–221. https://doi.org/10.1016/j.cropro.2015.09.012.

Azevedo, J.L., Araújo, W.L., Lacava, P.T., 2016. The diversity of citrus endophytic bacteria and their interactions with *Xylella fastidiosa* and host plants. Genet. Mol. Biol. 39 (4), 476–491. https://doi.org/10.1590/1678-4685-GMB-2016-0056.

Barbieri, H.B., Fernandes, L.S., Pontes, J.G.D.M., Pereira, A.K., Fill, T.P., 2023. An overview of the most threating diseases that affect worldwide citriculture: main features, diagnose, and current control strategies. Front. Nat. Prod. 2, 1045364. https://doi.org/10.3389/fntpr.2023.1045364.

Blacutt, A., Ginnan, N., Dang, T., Bodaghi, S., Vidalakis, G., Ruegger, P., Peacock, B., Viravathana, P., Vieira, F.C., Drozd, C., Jablonska, B., Borneman, J., McCollum, G., Cordoza, J., Meloch, J., Berry, V., Salazar, L.L., Maloney, K.N., Rolshausen, P.E., Roper, M.C., 2020. An in vitro pipeline for screening and selection of Citrus-Associated microbiota with potential Anti-"*Candidatus Liberibacter asiaticus*" properties. Appl. Environ. Microbiol. 86, e02883-19. https://doi.org/10.1128/AEM.02883-19.

Blaustein, R.A., Lorca, G.L., Meyer, J.L., Gonzalez, C.F., Teplitski, M., 2017. Defining the core citrus Leaf- and Root-Associated microbiota: factors associated with community structure and implications for managing huanglongbing (Citrus Greening) disease. Appl. Environ. Microbiol. 83, e00210-17. https://doi.org/10.1128/AEM.00210-17.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from illumina amplicon data. Nat. Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869.

Carvalho, C.R., Dias, A.C., Homma, S.K., Cardoso, E.J., 2020. Phyllosphere bacterial assembly in citrus crop under conventional and ecological management. PeerJ 8, e9152. https://doi.org/10.7717/peerj.9152.

Çayır, A., Coşkun, M., Coşkun, M., 2016. Genotoxicity of commercial fungicide cabrio plus on human cell. Cytotechnology 68, 704–1697. https://doi.org/10.1007/s10616-015-9919-0.

Cernava, T., Chen, X., Krug, L., Li, H., Yang, M., Berg, G., 2019. The tea leaf microbiome shows specific responses to chemical pesticides and biocontrol applications. Sci. Total Environ. 667, 33–40. https://doi.org/10.1016/j.scitotenv.2019.02.319.

Cervantes, C., Gutierrez-Corona, F., 1994. Copper resistance mechanisms in bacteria and fungi. FEMS Microbiol. Rev. 14 (2), 121–137. https://doi.org/10.1111/j.1574-6976.1994.tb00083.x.

Chitolina, G.M., Silva-Junior, G.J., Feichtenberger, E., Pereira, R.G., Amorim, L., 2021. Distribution of alternaria alternata isolates with resistance to quinone outside inhibitor (QoI) fungicides in Brazilian orchards of tangerines and their hybrids. Crop Prot. 141, 105493. https://doi.org/10.1016/j.cropro.2020.105493.

Chmiel, M., Drzymała, G., Bocianowski, J., Komnenić, A., Baran, A., Synowiec, A., 2022. Maltodextrin-coated peppermint and caraway essential oils effects on soil microbiota. Plants 11 (23), 3343. https://doi.org/10.3390/plants11233343.

Delitte, M., Caulier, S., Bragard, C., Desoignies, N., 2021. Plant microbiota beyond farming practices: a review. Front. Sustain. Food Syst. 5, 624203. https://doi.org/10.3389/fsufs.2021.624203.

Droby, S., Wisniewski, M., 2018. The fruit microbiome: A new frontier for postharvest biocontrol and postharvest biology. Postharvest Biology and Technology 140, 107–112. https://doi.org/10.1016/j.postharvbio.2018.03.004.

Droby, S., Zhimo, V.Y., Wisniewski, M., Freilich, S., 2022. The pathobiome concept applied to postharvest pathology and its implication on biocontrol strategies. Postharvest Biol. Technol. 189, 111911. https://doi.org/10.1016/j.postharvbio.2022.111911.

Earth Microbiome Project, 2025. Protocols and Standards: Earthmicrobiome https://earthmicrobiome.org/protocols-and-standards/. (Accessed November 2023).

Gama, A.B., Baggio, J.S., Rebello, C.S., Lourenco, S.D.A., Gasparoto, M.C.D.G., da Silva Junior, G.J., Peres, N.A., Amorim, L., 2020. Sensitivity of colletotrichum acutatum isolates from citrus to carbendazim, difenoconazole, tebuconazole, and trifloxystrobin. Plant Dis. 104, 1621–1628. https://doi.org/10.1094/PDIS-10-19-2195-RE.

Ginnan, N.A., Dang, T., Bodaghi, S., Ruegger, P.M., McCollum, G., England, G., Vidalakis, G., Borneman, J., Rolshausen, P.E., Roper, M.C., 2020. Disease-Induced microbial shifts in citrus indicate Microbiome-Derived responses to huanglongbing across the disease severity spectrum. Phytobiom. J. 4, 375–387. https://doi.org/10.1094/PBIOMES-04-20-0027-R.

Gobbi, A., Kyrkou, I., Filippi, E., Ellegaard-Jensen, L., Hansen, L.H., 2020. Seasonal epiphytic microbial dynamics on grapevine leaves under biocontrol and copper fungicide treatments. Sci. Rep. 10, 681. https://doi.org/10.1038/s41598-019-56741-z.

Gomba, A., Chidamba, L., Korsten, L., 2017. Effect of postharvest practices including degreening on citrus carpoplane microbial biomes. J. Appl. Microbiol. 122, 1057–1070. https://doi.org/10.1111/jam.13396.

Jiang, R., Wang, M., Chen, W., 2023. Heavy metal pollution triggers a shift from bacteria-based to fungi-based soil micro-food web: evidence from an abandoned mining-smelting area. J. Hazard. Mater. 459, 132164. https://doi.org/10.1016/j.jhazmat.2023.132164.

Jing, J., Wang, W., Deng, L., Yi, L., Zeng, K., 2023. A core epiphytic bacterial consortia synergistically protect citrus from postharvest disease. Food Chem. 407, 135103. https://doi.org/10.1016/j.foodchem.2022.135103.

Jing, L., Lei, Z., Li, L., Xie, R., Xi, W., Guan, Y., Sumner, L.W., Zhou, Z., 2014. Antifungal activity of citrus essential oils. J. Agric. Food Chem. 62 (14), 3011–3033. https://doi.org/10.1021/jf5006148.

Katsoulas, N., Løes, A.-K., Andrivon, D., Cirvilleri, G., De Cara, M., Kir, A., Knebl, L., Malińska, K., Oudshoorn, F.W., Willer, H., Schmutz, U., 2020. Current use of copper, mineral oils and sulphur for plant protection in organic horticultural crops across 10 European countries. Organic Agriculture 10 (S1), 159–171. https://doi.org/10.1007/s13165-020-00330-2.

Kecskeméti, E., Berkelmann-Löhnertz, B., Reineke, A., 2016. Are epiphytic microbial communities in the carposphere of ripening grape clusters (*vitis vinifera* L.) different between conventional, organic, and biodynamic grapes? PLoS One 11, e0160852. https://doi.org/10.1371/journal.pone.0160852.

King, E.O., Ward, M.K., Raney, D.E., 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44, 301–307. PMID: 13184240.

Klein, M.N., Da Silva, A.C., Kupper, K.C., 2016. *bacillus subtilis* based-formulation for the control of postbloom fruit drop of citrus. World J. Microbiol. Biotechnol. 32 (12), 205. https://doi.org/10.1007/s11274-016-2157-6.

Kong, Z., Hart, M., Liu, H., 2018. Paving the way from the lab to the field: using synthetic microbial consortia to produce High-Quality crops. Front. Plant Sci. 9, 1467. https://doi.org/10.3389/fpls.2018.01467.

Kumar, A., Zhimo, V.Y., Biasi, A., Feygenberg, O., Salim, S., Wisniewski, M., Droby, S., 2021. Impact of packhouse treatments on the peel microbiome of mandarin fruit (cv. Orr). Postharvest Biol. Technol. 176, 111519. https://doi.org/10.1016/j.postharvbio.2021.111519.

Lahti, L., Shetty, S., 2017. Microbiome R package. Bioconductor. https://doi.org/10.18129/B9.bioc.microbiome.

Lamichhane, J.R., Osdaghi, E., Behlau, F., Köhl, J., Jones, J.B., Aubertot, J.-N., 2018. Thirteen decades of antimicrobial copper compounds applied in agriculture. A review. Agron. Sustain. Dev. 38, 28. https://doi.org/10.1007/s13593-018-0503-9.

Lane, D.J., 1991. "16S/23S rRNA sequencing. In: Stackebrandt, E., Goodfellow, M. (Eds.), Nucleic Acid Techniques in Bacterial Systematics. John Wiley and Sons, Chichester, pp. 115–175.

Lee, G.-H., Hwang, K.-A., Choi, K.-C., 2019. Effects of fludioxonil on the cell growth and apoptosis in T and B lymphocytes. Biomolecules 9, 500. https://doi.org/10.3390/biom9090500Li.

Lombardo, M.F., Panebianco, S., Azzaro, A., Catara, V., Cirvilleri, G., 2023. Assessing copper-alternative products for the control of pre- and postharvest citrus anthracnose. Plants 12, 904. https://doi.org/10.3390/plants12040904.

Lombardo, M.F., Panebianco, S., Azzaro, A., Timpanaro, G., Polizzi, G., Cirvilleri, G., 2024a. Copper-alternative products to control anthracnose and Alternaria Brown spot on fruit of Tarocco sweet oranges and lemon in Italy. Crop Prot. 176, 106520. https://doi.org/10.1016/j.cropro.2023.106520.

Lombardo, M.F., Zhang, Y., Xu, J., Trivedi, P., Zhang, P., Riera, N., Li, L., Wang, Y., Liu, X., Fan, G., Tang, J., Coletta-Filho, H.D., Cubero, J., Deng, X., Ancona, V., Lu, Z., Zhong, B., Roper, M.C., Capote, N., Catara, V., Pietersen, G., Al-Sadi, A.M., Xu, X., Wang, J., Yang, H., Jin, T., Cirvilleri, G., Wang, N., 2024b. Global citrus root microbiota unravels assembly cues and core members. Front. Microbiol. 15, 1405751. https://doi.org/10.3389/fmicb.2024.1405751.

López-Moya, F., Suarez-Fernandez, M., Lopez-Llorca, L., 2019. Molecular mechanisms of chitosan interactions with fungi and plants. Int. J. Mol. Sci. 20. https://doi.org/10.3390/ijms20020332.

Lopez-Nuñez, R., Prieto-Rubio, J., Bautista, I., Lidón-Cerezuela, A., Valverde-Urrea, M., Lopez-Moya, F., Lopez-Llorca, L., 2025. Chitosan reduces naturally occurring plant pathogenic fungi and increases nematophagous fungus *purpureocillium* in soil under field conditions. Front. Agron. https://doi.org/10.3389/fagro.2024.1502402.

Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550. https://doi.org/10.1186/s13059-014-0550-8.

McMurdie, P.J., Holmes, S., 2013. Phyloseq: an r package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8, e61217. https://doi.org/10.1371/journal.pone.0061217.

McMurdie, P.J., Holmes, S., 2014. Waste not, want not: why rarefying microbiome data is inadmissible. PLoS Comput. Biol. 10, e1003531. https://doi.org/10.1371/journal.pcbi.1003531.

Munir, S., Li, Y., He, Pengfei, Huang, M., He, Pengbo, He, Pengjie, Cui, W., Wu, Y., He, Y., 2020. Core endophyte communities of different citrus varieties from citrus growing regions in China. Sci. Rep. 10, 3648. https://doi.org/10.1038/s41598-020-60350-6.

Nazzaro, F., Fratianni, F., Coppola, R., Feo, V.D., 2017. Essential oils and antifungal activity. Pharmaceuticals 10, 86. https://doi.org/10.3390/ph10040086.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H. and Wagner, H., 2014. Vegan: Community Ecology Package. R Package Version 2.2-0. http://CRAN.Rproject.org/package=vegan.

Panebianco, S., Lombardo, M.F., Anzalone, A., Musumarra, A., Pellegriti, M.G., Catara, V., Cirvilleri, G., 2022. Epiphytic and endophytic microorganisms associated to different cultivar of tomato fruits in greenhouse environment and characterization of beneficial bacterial strains for the control of post-harvest tomato pathogens. Int. J. Food Microbiol. 279. https://doi.org/10.1016/j.ijfoodmicro.2022.109861.

Parafati, L., Vitale, A., Restuccia, C., Cirvilleri, G., 2015. Biocontrol ability and action mechanism of food-isolated yeast strains against botrytis cinerea causing post-harvest bunch rot of table grape. Food Microbiol. 47, 85–92. https://doi.org/10.1016/j.fm.2014.11.013.

Paulson, J., Stine, O., Bravo, H., et al., 2013. Differential abundance analysis for microbial marker-gene surveys. Nat Methods 10, 1200–1202. https://doi.org/10.1038/nmeth.2658.

Penyalver, R., Roesch, L.F.W., Piquer-Salcedo, J.E., Forner-Giner, M.A., Alguacil, M.D. M., 2022. From the bacterial citrus microbiome to the selection of potentially host-beneficial microbes. New Biotechnol. 70, 116–128. https://doi.org/10.1016/j.nbt.2022.06.002.

Poveda, J., Roeschlin, R.A., Marano, M.R., Favaro, M.A., 2021. Microorganisms as biocontrol agents against bacterial citrus diseases. Biol. Control 158, 104602. https://doi.org/10.1016/j.biocontrol.2021.104602.

Poznański, P., Hameed, A., Orczyk, W., 2023. Chitosan and chitosan nanoparticles: parameters enhancing antifungal activity. Molecules 28. https://doi.org/10.3390/molecules28072996.

Rai, S., Singh, S., Pathak, N., Sharma, S., 2024. Assessing the potential of chitosan based coatings in modulation of bacteriome on tomato carposphere. Postharvest. Biol. Technol. 209, 112684. https://doi.org/10.1016/j.postharvbio.2023.112684.

Rajapaksha, R.M.C.P., Tobor-Kapłon, M.A., Bååth, E., 2004. Metal toxicity affects fungal and bacterial activities in soil differently. Appl. Environ. Microbiol. 70, 2966–2973. https://doi.org/10.1128/AEM.70.5.2966-2973.2004.

Rajestary, R., Landi, L., Romanazzi, G., 2021. Chitosan and postharvest decay of fresh fruit: Meta-analysis of disease control and antimicrobial and eliciting activities. Compr. Rev. Food Sci. Food Saf. 20 (1), 563–582. https://doi.org/10.1111/1541-4337.12672.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. https://doi.org/10.1093/oxfordjournals.molbev.a040454.

Schaad, J.B., Jones, W., Chun, 2001. Laboratory guide for identification of plant pathogenic bacteria (third ed.). American Phytopathological Society (APS Press), St. Paul, Minnesota, United States. https://doi.org/10.1046/j.1365-3059.2001.00635.x.

Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 13, 2498–2504. https://doi.org/10.1101/gr.1239303.

Sohrabi, R., Paasch, B.C., Liber, J.A., He, S.Y., 2023. Phyllosphere microbiome. Annu. Rev. Plant Biol. 74 (1), 539–568. https://doi.org/10.1146/annurev-arplant-102820-032704.

Solanki, M.K., Wang, Z., Kaushik, A., Singh, V.K., Roychowdhury, R., Kumar, M., Kumar, D., Singh, J., Singh, S.K., Dixit, B., Kumar, A., 2024. From orchard to table: significance of fruit microbiota in postharvest diseases management of citrus fruits. Food Control 165, 110698. https://doi.org/10.1016/j.foodcont.2024.110698.

Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: molecular evolutionary genetics analysis version 11. Mol. Biol. Evol. 38, 3022–3027. https://doi.org/10.1093/molbev/msab120.

Trivedi, P., Leach, J.E., Tringe, S.G., Sa, T., Singh, B.K., 2020. Plant–microbiome interactions: from community assembly to plant health. Nat. Rev. Microbiol. 18, 607–621. https://doi.org/10.1038/s41579-020-0412-1.

Trivedi, P., Spann, T., Wang, N., 2011. Isolation and characterization of beneficial bacteria associated with citrus roots in florida. Microb. Ecol. 62 (2), 324–336. https://doi.org/10.1007/s00248-011-9822-y.

Vitale, A., Aiello, D., Azzaro, A., Guarnaccia, V., Polizzi, G., 2021. An Eleven-Year survey on field disease susceptibility of citrus accessions to colletotrichum and alternaria species. Agriculture 11 (6), 536. https://doi.org/10.3390/agriculture11060536.

Vorholt, J.A., 2012. Microbial life in the phyllosphere. Nat. Rev. Microbiol. 10, 828–840. https://doi.org/10.1038/nrmicro2910.

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261–5267. https://doi.org/10.1128/AEM.00062-07.

Wassermann, B., Abdelfattah, A., Cernava, T., Wicaksono, W., Berg, G., 2022. Microbiome-based biotechnology for reducing food loss post harvest. Curr. Opin. Biotechnol. 78, 102808. https://doi.org/10.1016/j.copbio.2022.102808.

Wassermann, B., Kusstatscher, P., Berg, G., 2019. Microbiome response to hot water treatment and potential synergy with biological control on stored apples. Front. Microbiol. 10, 2502. https://doi.org/10.3389/fmicb.2019.02502.

Weiss, S., Xu, Z.Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., Lozupone, C., Zaneveld, J.R., Vázquez-Baeza, Y., Birmingham, A., Hyde, E.R., Knight, R., 2017. Normalization and microbial differential abundance strategies depend upon data characteristics. Microbiome 5, 27. https://doi.org/10.1186/s40168-017-0237-y.

Wicaksono, W.A., Buko, A., Kusstatscher, P., Cernava, T., Sinkkonen, A., Laitinen, O.H., Virtanen, S.M., Hyöty, H., Berg, G., 2023. Impact of cultivation and origin on the fruit microbiome of apples and blueberries and implications for the exposome. Microb. Ecol. 86 (2), 973–984. https://doi.org/10.1007/s00248-022-02157-8.

Wu, P.-H., Chang, H.-X., Shen, Y.-M., 2023. Effects of synthetic and environmentally friendly fungicides on powdery mildew management and the phyllosphere microbiome of cucumber. PLoS One 18 (3), e0282809. https://doi.org/10.1371/journal.pone.0282809.

Wu, Y., Qu, M., Pu, X., Lin, J., Shu, B., 2020. Distinct microbial communities among different tissues of citrus tree *citrus reticulata* cv. *chachiensis*. Sci. Rep. 10, 6068. https://doi.org/10.1038/s41598-020-62991-z.

Xi, M., Deyett, E., Stajich, J.E., El-Kereamy, A., Roper, M.C., Rolshausen, P.E., 2023. Microbiome diversity, composition and assembly in a California citrus orchard. Front. Microbiol. 14, 1100590. https://doi.org/10.3389/fmicb.2023.1100590.

Xing, K., Zhu, X., Peng, X., Qin, S., 2015. Chitosan antimicrobial and eliciting properties for pest control in agriculture: a review. Agron. Sustain. Dev. 35 (2), 569–588. https://doi.org/10.1007/s13593-014-0252-3.

Xu, J., Wang, Y., Zhang, Y., Riera, N., Li, J., Clark, K., Tao, J., Chen, H., Wen, J., Ma, W., Liu, H., Wang, N., 2023. Host genetic traits underlying the composition/assembly of the citrus microbiome. PBIOMES 09-22-0059-R Phytobiomes J.. https://doi.org/10.1094/PBIOMES-09-22-0059-R.

Xu, J., Zhang, Y., Zhang, P., Trivedi, P., Riera, N., Wang, Y., Liu, X., Fan, G., Tang, J., Coletta-Filho, H.D., Cubero, J., Deng, X., Ancona, V., Lu, Z., Zhong, B., Roper, M.C., Capote, N., Catara, V., Pietersen, G., Vernière, C., Al-Sadi, A.M., Li, L., Yang, F., Xu, X., Wang, J., Yang, H., Jin, T., Wang, N., 2018. The structure and function of the global citrus rhizosphere microbiome. Nat. Commun. 9, 4894. https://doi.org/10.1038/s41467-018-07343-2.

Xu, M., Su, M., Zhang, A., Niu, Y., Xu, Y, L., 2022. Combined phenanthrene and copper pollution imposed a selective pressure on the rice root-associated microbiome. Front. Microbiol. 13, 888086. https://doi.org/10.3389/fmicb.2022.888086.

Zhang, H., Serwah Boateng, N.A., Ngolong Ngea, G.L., Shi, Y., Lin, H., Yang, Q., Wang, K., Zhang, X., Zhao, L., Droby, S., 2021a. Unravelling the fruit microbiome: the key for developing effective biological control strategies for postharvest diseases. Comp. Rev. Food Sci. Food Saf. 20, 4906–4930. https://doi.org/10.1111/1541-4337.12783.

Zhang, Y., Trivedi, P., Xu, J., Roper, M.C., Wang, N., 2021b. The citrus microbiome: from structure and function to microbiome engineering and beyond. Phytobiom.s J. 5, 249–262. https://doi.org/10.1094/PBIOMES-11-20-0084-RVW.