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Influence of environmental and agronomic variables on soil microbiome in citrus orchards: A comparative analysis of organic and conventional farming system

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ABSTRACT

Crop health and productivity depend on the structure and functionality of soil microbiota associated with the root system of plants. The agricultural policy of the European Union promotes organic farming systems to ensure environmental sustainability and food safety. The objective of this study was to investigate the impact of organic farming on soil microbiome in citrus orchards. The soil microbiota of eight conventionally and seven organically managed commercial citrus orchards across eastern Sicily was characterised using Illumina sequencing and BeCrop® primers for PCR amplification. The structure (diversity and relative abundance) and functionality of soil bacterial and fungal communities depended primarily on the sampling site. Other variables influencing the soil microbiome included soil total carbon content, seasonality, rootstock genotype, soil tillage and irrigation system. The latter three exerted differential effects on either bacterial or fungal communities. Conversely, age and visible health status of the tree had negligible influence on both communities. The differences between organically and conventionally managed citrus orchards accounted for a significant proportion of the variability, indicating a relevant effect of the farming system on soil microbiome. Organically managed orchards compared to those managed conventionally exhibited higher microbial diversity and a unique composition of nutrient-cycling microbes. In particular, organic farming promoted beneficial microbial functions, such as nitrogen fixation and phosphorus solubilization. Findings provide insights into the dynamic and complex interactions between environmental variables and soil microbial communities in citrus orchards, confirming the potential of microbial diversity as an indicator of sustainability in agricultural systems.

1. Introduction

Citrus have a prominent role in global fruit production and trade (FAO, 2021; Mukhametzyanov et al., 2024). Spain and Italy are the two major citrus producing countries of the European Union (EU) and among the Italian regions, Sicily (southern Italy) is the first producer, with a total citrus growing area of around 88,000 ha (Spreen et al., 2020; Ciriminna et al., 2024). Organic farming system is expanding in citrus production. However, conventional management still prevails. Since the early 2000s the EU policy has encouraged organic agriculture in order to pursue environmental sustainability and food safety. Presently, the global citrus farmland managed organically is estimated at around 115,000 ha, and Italy and Spain, the two leading organic citrus producers worldwide, account for around 31,000 and 25,000 ha, respectively (Willer et al., 2024). Despite the growing interest in organic citrus farming, no study has yet addressed the effects of EU organic practices

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on soil microbial communities and their ecological functions (Lima et al., 2024). Soil microbial communities are key drivers of agro-ecosystem services such as nutrient cycling, disease suppression, and stress resilience (Turner et al., 2013; Trivedi et al., 2020). However, how these microbial functions respond to organic farming practices in real-world citrus production systems remains poorly understood. In general, the study of soil microbial ecology in citrus orchards focused mostly on the bacterial component (Lima et al., 2024). Moreover, soil health, differently from air and water health, has been only occasionally tackled by EU regulation. Only recently, the European Commission has proposed a directive addressing soil health (Proposal for a directive of the European Parliament and of the Council on soil monitoring and resilience COM(2023) 416), with the ultimate aim of establishing a harmonized soil monitoring system across the member countries. According to the EU soil strategy for 2030, the EU member states will have to define which practices should be implemented and which should be banned to prevent soil degradation.

In recent years, the study of microbial communities associated with plants has been greatly enhanced by the rapid technological progress in metagenomics. As for citrus, metagenomics has been applied to characterize the phylloplane, rhizosphere, and endophytic microbiota (Abdelfattah et al., 2017; Xu et al., 2018; Faddetta et al., 2021; Lombardo et al., 2024).

BeCrop® technology is a cutting-edge tool for measuring microbial processes in soil nutrient cycling. This technology is revolutionizing the study of microbial population dynamics and in recent years has been exploited for the study of plant-associated microbiome in diverse agro-ecosystems (Gobbi et al., 2022; Bansal et al., 2024; Blanco et al., 2024). Using the qualitative and quantitative composition of the microbiome as an input, this technology employs patented algorithms to predict specific microbial activities, which can influence nutrient availability to plants. By profiling soil microbial communities and their functional potentials, BeCrop® can provide valuable insights into the complex network of interactions within the soil microbiome, linking microbial diversity and functionality to soil health and plant productivity.

This study aimed at providing insights into the effect of organic farming system on the structure and functionality of soil microbiome in citrus farmland by complementing high-throughput sequencing with BeCrop® technology. In particular, it evaluated the influence of environmental (geographical area and soil properties) and agronomic factors (rootstock genotype, tree age, tillage frequency and depth, irrigation system, and visible tree health status), season (summer vs. winter), and farming system (organic vs. conventional) on soil microbiome associated with the roots of citrus trees in commercial citrus orchards of Sicily.

2. Material and methods

2.1. Sampling sites

Samples were collected in 15 commercial sweet orange [*Citrus* × *sinensis* (L.) Osbeck] orchards (sampling sites) located in the municipalities of Siracusa, Lentini, Carlentini, Ramacca, and Mineo within the provinces of Catania and Siracusa, in southeastern Sicily (Table 1 and Fig. 1). The extension of a single orchard varied from 10 to around 50 ha.

2.1.1. Features of sampling sites

Samples were collected in eight conventionally and seven organically managed commercial citrus orchards (Table 1). Tree spacing in all orchards was 6 × 4 m. Conventionally managed orchards used herbicides, such as glyphosate (1–2 applications per year at 910 g /ha of a.i. per each application), 2-methyl-4-chlorophenoxyacetic acid (a single application per year at 1.2 L/ha of a.i.), either halauxifen-methyl, florpyr mepthyl and cloquintocet-mexyl (a single application per year at 7.2, 168 and 7.1 g/ha of a.i., respectively) or florasulam and penoxsulam (a single application per year at 7.5 and 15 g/ha of a.i., respectively), oxyfluorfen (a single application per year at 144 g/ha of a.i.) for controlling weeds (herbicides were applied only on the row within a band of around 2 m), paraffinic oils (30–40 L/ha) and synthetic insecticides, such as acetamiprid (1–2 treatments per year at 93 ml/ha of a.i. per each treatment) and/or spirotetramat (a single application per year at 136–200 ml/ha of a.i.) for controlling insect pests, as well as copper fungicides (a single treatment per year at 0.8–1 kg/ha of Cu^{++}) for controlling major diseases caused by fungi and oomycetes (Rovetto et al., 2024). Moreover, conventionally managed orchards were fertilized each year with commercial NPK (20.10.10) complexes at 800–1000 kg/ha. Organically managed orchards, which received subsides from the regional government, were managed according to the EU rules (basically, EU Council Regulation No. 834/2007, European Commission Regulation No. 889/2008 and EU Regulation No, 2018/848): paraffinic oils (30–40 l/ha) were used for controlling insect pests, copper fungicides (a single treatment per year at 0.8–1 kg/ha of Cu^{++}) for controlling diseases caused by fungi and oomycetes, and a commercial product (Biotris, SIRIAC s.r.l., Acate (RG), Italy), an organic mineral complex containing NPK (Ca-Mg-S) 5–8–12 (11−2−13) at 1500–2000 kg/ha as a fertilizer. Moreover, both organically and conventionally managed orchards were fertigated once a year with Fe-EDDHA chelate (0.6–1.2 kg/ha of Fe^{++}). As for other variables, either environmental such as the geographical location (sampling site) and soil physicochemical properties (water content, pH, total carbon, total nitrogen, carbon-to-nitrogen ratio, inorganic carbon, organic carbon and texture class), or agronomic, such as rootstock (sour orange vs.'Carrizo' citrange), tree age (young vs. old), soil tillage frequency (0÷4 times per year) and depth (shallow <15 cm, deep >15 cm), irrigation system (drip, T-shaped sprinklers, microsprinklers), and tree health status as determined by visual observation (vigorous trees with a dense, deep green canopy vs. weak trees with a sparse, i.e., transparent, pale green canopy), detailed information on the sites is summarized in Table 1.

2.2. Collection of soil samples

Sampling activities were carried out in summer (June-July 2021) and replicated in winter (December 2021-January 2022). A total of 150 composite soil samples (75 samples in summer and 75 in winter) were collected from the 15 citrus orchards listed in Table 1 (five samples from each orchard at each sampling time). A square plot of around 2.1 ha was delimited within each orchard and five trees were randomly selected along the two diagonals of the plot at a distance of at least 100 m from each other, excluding the trees along the perimeter of the plot. Soil samples were collected beneath the tree canopy. The top 5 cm layer comprising the litter was removed, then, four soil cores, including fine roots, were sampled in the layer explored by fine roots of citrus trees (depth of approximately 20–30 cm), at four cardinal points, at a distance of 40–100 cm from the trunk. The four soil cores were then pooled together into a single composite soil sample of approximately 1 L. For each 1 L sample, sub-samples of 10 ml were promptly placed in sterile tubes on ice, and transported to the Molecular Plant Pathology Laboratory at the University of Catania for metagenomic processing. These sub-samples were stored at −80°C until molecular analyses were performed. The remaining soil was weighed on-site and immediately brought to the Molecular Plant Pathology Laboratory of the University of Catania, air-dried, reweighed and stored at room temperature until subsequent analyses to determine physicochemical properties.

2.3. Determination of physicochemical properties of soil

The physicochemical properties of the soil samples were analyzed at the Physical Geography Laboratory of the Ruhr-Universität Bochum, Germany. The analyses included the determination of water content (WC), pH, total carbon (TC), total nitrogen (TN), carbon-to-nitrogen ratio (C:N), inorganic carbon (IC), organic carbon (OC), and soil texture class. Before conducting the specific analyses, the soil samples

Table 1
Sampling sites selected in this study, along with the geographic location of the orchard, species and cultivar of the scion, rootstock, age of the tree, farming system, tillage number per year and depth, irrigation system, identification code of soil sample, soil texture and health status of the tree.

Localities	Sampling Site – ID coordinates and altitude[a]	Scion species and cultivar	Rootstock[b] and Tree age[c]	Farming system[d]	Tillage number[e] and depth[f]	Irrigation system	Sample-ID	Soil type[h]	Tree health status[g]
Siracusa (Italy)	CO_01 - Tenuta Giardina; 37°03'43.5'' N; 15°15'30.5'' E 1.4 mt a.s.l.	Sweet orange 'Moro'	Sour orange; old	Org	3 S	Drip	CO_1_P1		V
							CO_1_P2		V
							CO_1_P3	S	V
							CO_1_P4		V
							CO_1_P5		V
	CO_02 - Tenuta Giardina; 37°03'11.2'' N; 15°10'35.1'' E 72.9 mt a.s.l.	Sweet orange 'Valencia'	Sour orange; old	Org	3 S	Drip	CO_2_P1		V
							CO_2_P2		V
							CO_2_P3	SL	V
							CO_2_P4		V
							CO_2_P5		V
	CO_03 -Tenuta Cava Donna; 37°02'29.5'' N; 15°09'29.9'' E 78.3 mt a.s.l.	Sweet orange 'Valencia'	Sour orange old	Conv	NT	Sprinklers (T-shaped)	CO_3_P1		V
							CO_3_P2		V
							CO_3_P3	S	V
							CO_3_P4		V
							CO_3_P5		V
Lentini (Syracusa, Italy)	CO_04 – Tenuta Biviere; 37°18'29.2'' N; 14°57'23.7'' E 20 mt a.s.l.	Sweet orange 'Tarocco'	Citrange 'Carrizo' young	Org	3 S	Sprinklers (T-shaped)	CO_4_P1		V
							CO_4_P2		V
							CO_4_P3	S	W
							CO_4_P4		V
							CO_4_P5		V
	CO_05 – Tenuta Di Cataldo; 37°20'39'' N; 14°58'16'' E 39.5 mt a.s.l.	Sweet orange 'Moro'	Sour orange old	Org	2 D	Sprinkers (T-shaped)	CO_5_P1		W
							CO_5_P2		V
							CO_5_P3	SL	W
							CO_5_P4		W
							CO_5_P5		V
	CO_06 – Tenuta Mario Grimaldi; 37°19'40'' N; 14°49'58'' E 157.3 mt a.s.l.	Sweet orange 'Tarocco'	Sour orange old	Org	4 D	Sprinklers (T-shaped)	CO_6_P1		V
							CO_6_P2		V
							CO_6_P3	SL	W
							CO_6_P4		V
							CO_6_P5		V
	CO_07 – Tenuta Grimaldi; 37°21'05'' N; 14°50'22'' E 56.6 mt a.s.l.	Sweet orange 'Tarocco'	Citrange 'Carrizo' young	Conv	3 D	Sprinklers (T-shaped)	CO_7_P1		V
							CO_7_P2		W
							CO_7_P3	S	V
							CO_7_P4		W
							CO_7_P5		V
	CO_08 – Tenuta Coco; 37°19'53'' N; 14°49'18'' E 99.4 mt a.s.l.	Sweet orange 'Tarocco'	Sour orange old	Conv	4 D	Microsprinklers (inter-row)	CO_8_P1		V
							CO_8_P2		V
							CO_8_P3	SaL	V
							CO_8_P4		V
							CO_8_P5		V
	CO_09 – Tenuta Viglianisi; 37°19'44'' N; 14°49'45'' E 151.5 mt a.s.l.	Sweet orange 'Tarocco'	Sour orange old	Conv	4 D	Microsprinklers (inter-row)	CO_09_P1		V
							CO_09_P2		W
							CO_09_P3	S	V
							CO_09_P4		V
							CO_09_P5		V
Carlentini (Syracuse, Italy)	CO_10 – Tenuta Guastella; 37°18'39'' N; 15°00'40'' E 10.5 mt a.s.l.	Sweet orange 'Tarocco'	Sour orange young	Conv	3 D	Sprinklers (T-shaped)	CO_10_P1		W
							CO_10_P2		V
							CO_10_P3	SL	W
							CO_10_P4		V
							CO_10_P5		V
	CO_11 – Tenuta Calvo; 37°18'47'' N; 15°02'30'' E 7 mt a.s.l.	Sweet orange 'Ovale'	Sour orange old	Org	3 D	Sprinklers (T-shaped)	CO_11_P1		V
							CO_11_P2		W
							CO_11_P3	S	W
							CO_11_P4		V
							CO_11_P5		V
Ramacca (Catania, Italy)	CO_12 – Tenuta Borzi Akiana; 37°29'08'' N; 14°47'09'' E 60.7 mt a.s.l.	Sweet orange 'Tarocco'	Citrange 'Carrizo' young	Org	3 S	Drip	CO_12_P1		V
							CO_12_P2		V
							CO_12_P3	SL	V
							CO_12_P4		V
							CO_12_P5		W
Mineo (Catania, Italy)	CO_13 - Tenuta Sedati;	Sweet orange 'Tarocco'	Sour orange young	Conv	NT	Drip	CO_13_P1		V
							CO_13_P2		V
							CO_13_P3	S	W

(*continued on next page*)

Table 1 (*continued*)

Localities	Sampling Site – ID coordinates and altitude[a]	Scion species and cultivar	Rootstock[b] and Tree age[c]	Farming system[d]	Tillage number[e] and depth[f]	Irrigation system	Sample-ID	Soil type[h]	Tree health status[g]
	37°20'28'' N; 14°41'30'' E 90.5 mt a.s.l.						CO_13_P4 CO_13_P5		V V
	CO_14 - Tenuta Salinella; 37°19'14'' N; 14°41'03'' E 107.5 mt a.s.l.	Sweet orange 'Tarocco'	Citrange 'Carrizo' young	Conv	3 D	Drip	CO_14_P1 CO_14_P2 CO_14_P3 CO_14_P4 CO_14_P5	SL	V V V V V
	CO_15 - Tenuta Serravalle; 37°20'16'' N; 14°41'33'' E 90.9 mt a.s.l,	Sweet orange 'Tarocco'	Sour orange young	Conv	3 D	Microsprinklers (inter-row)	CO_15_P1 CO_15_P2 CO_15_P3 CO_15_P4 CO_15_P5	SL	V V V V V

[a] DATUM WGS84; [b] Sour orange (*Citrus aurantium* L.), 'Carrizo' citrange [*C. sinensis* (L.) Osbeck x *Poncirus trifoliata* (L.) Raf.] [c] young 20÷39 years, old ≥ 40 years; [d] Org = organic, Conv = Conventional; [e] Tillage number (NT= No-tillage,alternatively 2÷ 4 per year); [f] Tillage depth (S=shallow < 15 cm, D=deep ≥ 15 cm); [g] S = silt, SL = silt loam, SaL = sandy loam, U.S.D.A. soil texture classes (IUSS Working Group WRB, 2022);[g] S = silt, SL = silt loam, SaL = sandy loam, U.S.D.A. soil texture classes (Bridges, 1978); [h]V = vigorous, with a deep green and thick canopy, W = weak, with a pale green and transparent canopy.

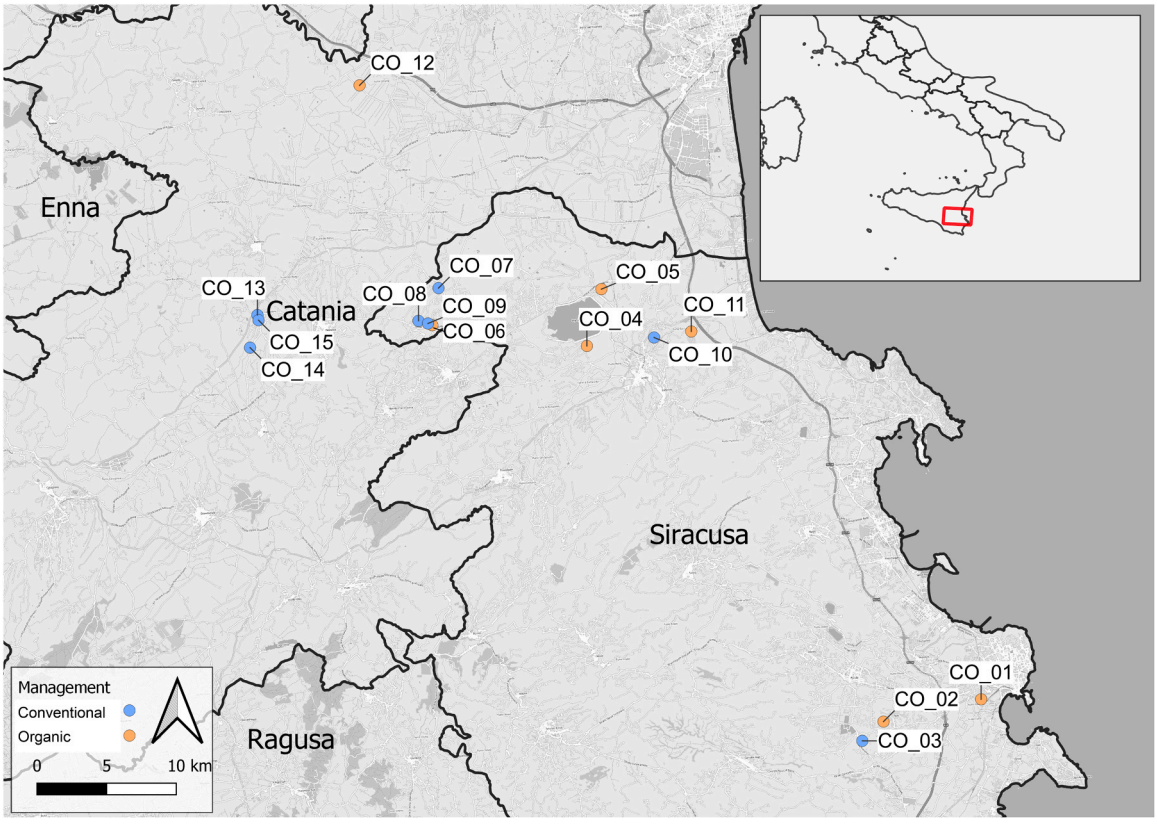

Fig. 1. Geographical location of the 15 citrus orchards located in the municipalities of Siracusa, Lentini, Carlentini, Ramacca, and Mineo in eastern Sicily (Italy).

underwent preliminary preparation, which involved air-drying and sieving through a 2 mm mesh to remove larger residues. These residues were weighed and discarded, while the remaining soil was manually crushed using a porcelain mortar.

The water content of each sample at the time of sampling was determined by calculating the ratio of the dry weight to the wet weight and expressing the result as a percentage of the sample's wet weight.

The pH of the samples was measured using 0.01 M calcium chloride ($CaCl_2$), following the standard DIN ISO 10390:2005–12.

For the determination of TC, TN, and the C:N ratio, the samples were finely ground using a Pulverisette 7 (Fritsch, Idar-Oberstein, Germany), and approximately 750 mg of each sample was analyzed using a Vario Max Cube elemental analyzer (Elementar, Langenselbold, Germany). The measurement was performed twice to ensure accuracy.

Inorganic carbon (IC) was determined by treating 100 mg of soil with 7 ml of phosphoric acid, followed by incineration at 200°C, and quantified using a Solid Sampling Module SSM-5000A of the TOC-L analyzer (Shimadzu, Kyoto, Japan).

Organic carbon (OC) was then calculated as the difference between TC and IC.

For soil texture analysis, approximately 45 g of soil from each sample were subjected to the removal of carbonates and organic matter, following DIN ISO 11277 and the protocol described by Utermann et al. (2000). After treatment, the soil was washed until conductivity fell below 2.0 mS, then dried at 60°C, gently crushed, and sieved through a 0.2 mm mesh. The small quantity of material larger than 0.2 mm was considered negligible and not included in the measurements. The particle size distribution (sand, silt, and clay percentages) was analyzed using laser particle diffraction with an Analysette 22 (Fritsch, Idar-Oberstein, Germany). The results were compared to the World Reference Base for Soil Resources (IUSS 2022) to classify soil texture.

2.4. Soil microbiome analysis

Metagenomic analyses of the microbiome associated with the soil sampled in citrus orchards were conducted by the Biome-Makers laboratory (Valladolid, Spain), to which samples were shipped frozen on ice. The detailed procedures for metagenomic processing are outlined in the following paragraphs.

2.4.1. Environmental DNA (eDNA) extraction and Illumina libraries preparation

For each 10 ml rhizosphere soil sample (in total, 150 samples were analyzed), total eDNA was extracted from three sub-aliquots using the DNeasy PowerLyzer PowerSoil® kit (Qiagen, Hilden, Germany). To analyze the bacterial and fungal communities in the rhizosphere soils of citrus plants, BeCrop custom primers (patent publication number: WO2017096385, Biome Makers) were used for PCR amplifications, specifically targeting the 16S rRNA V4 region and the ITS1 region (Becares and Fernandez, 2017). The amplified DNA fragments were then purified using the KAPA Pure Beads kit (Roche, Basel, Switzerland). The amplification of the 16S and ITS regions was confirmed by electrophoresis on a 2 % agarose gel. Libraries for both 16S and ITS regions were prepared for Illumina sequencing according to a two-step protocol (Gobbi et al., 2019; Liao et al., 2019). The DNA was quantified using a Qubit fluorometer with the Qubit HS Assay (Thermo Fisher Scientific, Waltham, MA, USA). Finally, the DNA libraries were sequenced using an Illumina MiSeq instrument (Illumina, San Diego, CA, USA) with 2 × 300 paired-end reads.

2.4.2. Bioinformatic processing for taxonomical classifications

The paired-end read sequences were processed for primer removal using the Cutadapt software (Martin, 2011). After removing the primers, the sequences were assembled by overlapping at least 100 nucleotides, resulting in longer and more reliable sequences. Then, the assembled sequences underwent a rigorous filtering process based on expected errors, with a maximum threshold value of 1.0, as recommended by Edgar et al. (2015). Following preprocessing to ensure sequence quality, reads with single nucleotide differences were iteratively clustered to form amplicon sequencing variants (ASVs) using the Swarm method (Mahé et al., 2022). De novo chimeras and singletons were subsequently removed. Chimeras were identified and eliminated to avoid false positives, following the methodologies outlined by Edgar and Flyvbjerg, (2015). Finally, the ASVs were classified taxonomically through global alignment with a 97 % identity threshold against reference databases. Specifically, for 16S sequences, the SILVA 138.1 database was used, while the UNITE 8.3 database was employed for ITS sequences (Glöckner et al., 2017; Nilsson et al., 2019).

2.4.3. Computation of microbiome indexes and network properties

Local network properties were determined as described by Ortiz-Álvarez et al. (2021). First, microbial community networks were built separately for 16S and ITS samples, following the methodology of Veech (2013). To create presence-absence meta-networks, rarefied species-level counts were used. Then, pairs of species that appeared together significantly more often or less often than expected were identified. These pairs formed co-occurrence networks (species that often occur together) or co-exclusion networks (species that tend not to appear together). Local network properties were then derived by selecting these species pairs from the meta-network for each individual sample.

In addition, BeCrop® indexes were calculated as per Acedo et al. (2022). BeCrop® is a patented commercial platform for the interpretation of soil microbiomes, which uses amplicon sequencing data (16S rRNA for bacteria and ITS for fungi) as input to infer agronomically relevant functional traits. The taxonomic profiles, obtained through rarefied high-quality amplicon data, are analyzed using a proprietary machine-learning algorithm trained on a curated reference database of annotated soil microbiomes and metagenomes. The BeCrop® database currently includes more than 44.7 million taxonomic references generated through high-throughput NGS studies across diverse cropping systems and soil texture worldwide. These functional indexes evaluate key aspects of soil health by estimating the potential of microbial communities to perform nutrient-related biochemical processes, such as 'calcium transport', 'carbon', 'carbon fixation', 'fermentation', 'inorganic nitrogen consumption', 'manganese transport equilibrium', 'nitrogen', 'organic phosphorus assimilation' and 'potassium consumption'. In this study, BeCrop® indexes were used to evaluate how different management practices, environmental factors, and seasonal variations influence the soil microbiome and, consequently, plant health. This approach has been applied in previous soil microbiome studies, demonstrating the robustness and quality of this technology (Milke et al., 2024). Detailed descriptions of each functional index and the associated microbial groups are provided in Supplementary Table S1.

2.4.4. Statistical analyses

Statistical analyses were performed using phyloseq and vegan packages in R version 4.3.2 (McMurdie and Holmes, 2013; Oksanen et al., 2024). The alpha diversity of microbial communities was determined on rarefied data using the Shannon and Chao1 biodiversity indices. Unsupervised clustering of ITS and 16S soil microbiome was applied using the k-means algorithm. The optimal number of clusters was assessed using silhouette, WSS, and gap statistics. Microbiome indexes, network and physicochemical properties were fitted to a varying set of mixed models. Briefly, these models contained the main effect of environmental factors, including citrus-producing area and soil physicochemical properties, and agronomic factors, including type of rootstock and age of rootstock, tillage practices and depth, irrigation system, and plant vigor. The interactions between management and other factors were included when possible. ITS or 16S clusters were either fixed or random effects, depending on the specific model for microbiome and network indexes. Note that physicochemical properties models did not contain season as a factor since data were available only for summer season. In models that included physicochemical properties as response variables, random effect was always the location. The optimal model for each index was selected based on Akaike's information criterion (AIC) (Supplementary Table S2). Marginal (random and fixed terms) and conditional (fixed terms only) R-squared for each model were determined. Next, ANOVA on fixed terms was applied and subsequent post-hoc comparison across significant factor levels was performed. P-value was corrected for multiple testing using the FDR procedure.

To investigate beta diversity and visualize variations in community structures, Principal Coordinates Analysis (PCoA) based on Bray-Curtis distance was employed. Bray-Curtis dissimilarity was calculated at the ASV level using rarefied feature tables. Permutational Multivariate Analysis of Variance (PERMANOVA) was employed to assess the statistical significance of differences in community composition attributed to experimental factors. Redundancy Analysis (RDA) was performed on summer samples to ascertain which physicochemical properties and

experimental variables were significantly correlated with the observed variations in the microbiome.

The prevalence of conserved prokaryotic and fungal phyla and genera within all soil samples (combined data) from either organically or conventionally managed citrus orchards, collected in either the summer or winter season, was determined at a prevalence threshold of 25 % and a detection limit of 0.01 %. Shared and exclusive taxa numbers at genus level across management practices and seasons were represented in Venn diagrams for both 16S and ITS. Next, differentially abundant (DA) taxa were identified between mainly organic and conventional management practices. Further analyses were performed to examine the effect of tillage depth, frequency and rootstock on microbiome composition, as illustrated in Supplementary Tables S5, S6 and S7. All differential abundance analyses were assessed using negative binomial regression at various taxonomic levels utilizing the edgeR package (Robinson Mark et al., 2010). As for chemical characteristics of soil samples, mean values were compared using the Student's *t*-test.

3. Results

3.1. Physicochemical properties of soils

The physicochemical properties of soil samples are reported in Tables 1 and 2. Most soils belonged to the silt or silt loam texture classes, with the exception of five sandy loam samples from a conventionally managed orchard (CO_08 Tenuta Coco). Overall, physicochemical properties varied across orchards and locations, but did not differ significantly between farming systems, with the only exception of total carbon (TC), which was significantly higher in organically managed soils (4.61 ± 1.67 %) compared to conventionally managed ones (3.85 ± 1.21 %). Other physicochemical properties such as WC, pH, TN, IC, OC and C:N ratio showed overlapping ranges between the two diverse cropping systems. For example, TN content was only slightly higher in organic soils, while pH values remained close to neutral in both groups. However, no difference between the means was statistically significant.

3.2. Variability of core microbiome in soil of organically and conventionally managed citrus orchards

The metagenomic analysis of microbiome of soil samples from organically managed citrus orchards yielded a total 1,632,434 16S- and 997,214 ITS-reads in summer, and 2,754,628 16S- and 2,853,156 ITS-reads in winter, respectively. The metagenomic analysis of soil samples from conventionally managed citrus orchards yielded a total of 1,868,359 16S- and 1,933,049 ITS-reads in summer, and 3,334,785 16S- and 1,158,030 ITS-reads in winter, respectively.

The prevalence of conserved prokaryotic and fungal phyla and genera across all soil samples from both organically or conventionally managed citrus orchards in either summer or winter season was determined at a threshold of 25 % and a detection limit of 0.01 %.

Table 2
Chemical properties of soil samples from conventionally and organically managed citrus orchards. Data are means ± SD of 35 and 40 replicate soil samples for organically and conventionally managed orchards, respectively. Statistically significant differences between management systems were determined using an independent Student's *t*-test (p-value < 0.05 considered significant).

	Organic	Conventional	*t*-test	p-value
pH	7.66 ± 0.04	7.58 ± 0.1	1.75	0.08
TN[a] %	0.18 ± 0.03	0.17 ± 0.06	0.23	0.81
TC[b] %	4.61 ± 1.67	3.85 ± 1.21	2.23	0.02
C:N	25.7 ± 9.99	25.6 ± 12.4	0.04	0.97
IC[c] %	3.15 ± 1.61	2.58 ± 1.50	1.59	0.11
OC[d] %	1.46 ± 0.49	1.39 ± 0.68	0.50	0.61
WC[e] %	0.15 ± 0.04	0.13 ± 0.04	1.47	0.14

[a] Total nitrogen; [b] Total Carbon; [c] Inorganic carbon: [d] Organic carbon; [e] Water content.

3.2.1. Microbiota structure (prokaryotic and fungal phyla)

The comparative analysis of the core microbiota of organically and conventionally managed citrus orchards (Figs. 2 and 3) evidenced that in samples collected in summer, *Proteobacteria*, *Actinobacteriota*, *Crenarchaeota*, and *Acidobacteriota* were the predominant bacterial phyla irrespective of the farming system (Fig. 2, a and b), while in organically managed orchards, differently from conventionally managed orchards (Fig. 2a), the phylum *Planctomycetota* prevailed on *Firmicutes* (Fig. 2b).

In winter, the phylum *Chloroflexi* was detected exclusively in samples from conventionally managed orchards (Figs. 2e and 2f). However, similarly to the samples collected in summer, *Proteobacteria*, *Actinobacteriota*, and *Crenarchaeota* were dominant, irrespective of the farming system.

The analysis of fungal community in soil collected in summer showed that the phylum *Ascomycota* was dominant in both organically and conventionally managed orchards (Figs. 2c and 2d), while *Basidiomycota* and *Mortierellomycota* were comparatively more represented, albeit with minor differences, in soil from conventionally managed orchards (Figs. 2c and 2g).

In soil samples collected in winter, *Ascomycota* was the most prevalent phylum in both conventionally and organically managed orchards (Figs. 2g and 2h). In conventionally managed orchards, the *Basidiomycota* phylum was as prevalent as the *Ascomycota* phylum (Fig. 2g), while in organically managed orchards it was slightly less represented than *Ascomycota* (Fig. 2h). *Mortierellomycota* was the third prevalent fungal phylum in soil from both organically and conventionally managed orchards (Figs. 2g and 2h). The analysis focused on these three main phyla as they were by far the most prevalent ones in soil microbiota. The presence of other phyla was negligible.

3.2.2. Microbiota structure (prokaryotic and fungal genera)

Heatmaps in Fig. 3 depict the prevalence of genera in soil samples from organically (Org) and conventionally (Conv) managed citrus orchards in summer and winter, for prokaryotic (16S region) and fungal (ITS region) communities, respectively.

As for prokaryotic communities, the most represented genera, irrespective of farming system or season, included *Crenarchaeota Nitrososphaera*, *Lutetiella*, *Candidatus Nitrosocosmicus*, *Gaiella*, *Solirubrobacter*, *Rubrobacter*, *Iamia*, *Bacillus*, *Neobacillus*, *Crossiella*, and *Rhodoplanes* (Fig. 3a, b, e and f and Fig. 4a). However, seasonal differences were observed between farming systems. In soil microbiota of conventionally managed orchards, all genera detected in summer were also present in winter (Fig. 3a and e and Fig. 4a). In addition, in winter other genera, such as *Ilumatobacter*, *Sphingomonas*, *Steroidobacter*, and *Bryobacter*, were detected (Fig. 3e and Fig. 4a). A seasonal pattern, was also observed in microbiota of soil samples from organically managed orchards. *Pirellula* and *Vicinamibacter* were exclusively detected in summer (Fig. 3b and Fig. 4a), while *Nitrospira, Bryobacter*, and *Sphingomonas* were exclusively detected in winter (Fig. 3f and Fig. 4a). In summer, *Vicinamibacter* and *Pirellula* were exclusive to samples from organically managed orchards, while no exclusive genera were recorded in microbiota of samples from conventionally managed orchards (Fig. 3a and b and Fig. 4a). In winter, *Ilumatobacter* was exclusively detected in microbiota soil of conventionally managed orchards, while *Nitrospira* was exclusive to samples from organically managed orchards (Fig. 3e and f and Fig. 4a).

As for fungal communities, *Mortierella*, *Penicillium*, *Aspergillus*, *Solicoccozyma*, *Cladosporium*, *Fusarium*, *Lophiotrema*, and *Chrysosporium* were consistently present, irrespective of farming system or season (Fig. 3c, d, g, and h, and Fig. 4a). However, some differences were observed between farming systems and seasons. In soil microbiota of conventionally managed orchards, genera such as *Arthropsis*, *Arachnomyces,* and *Apiotrichum* were detected exclusively in summer, while

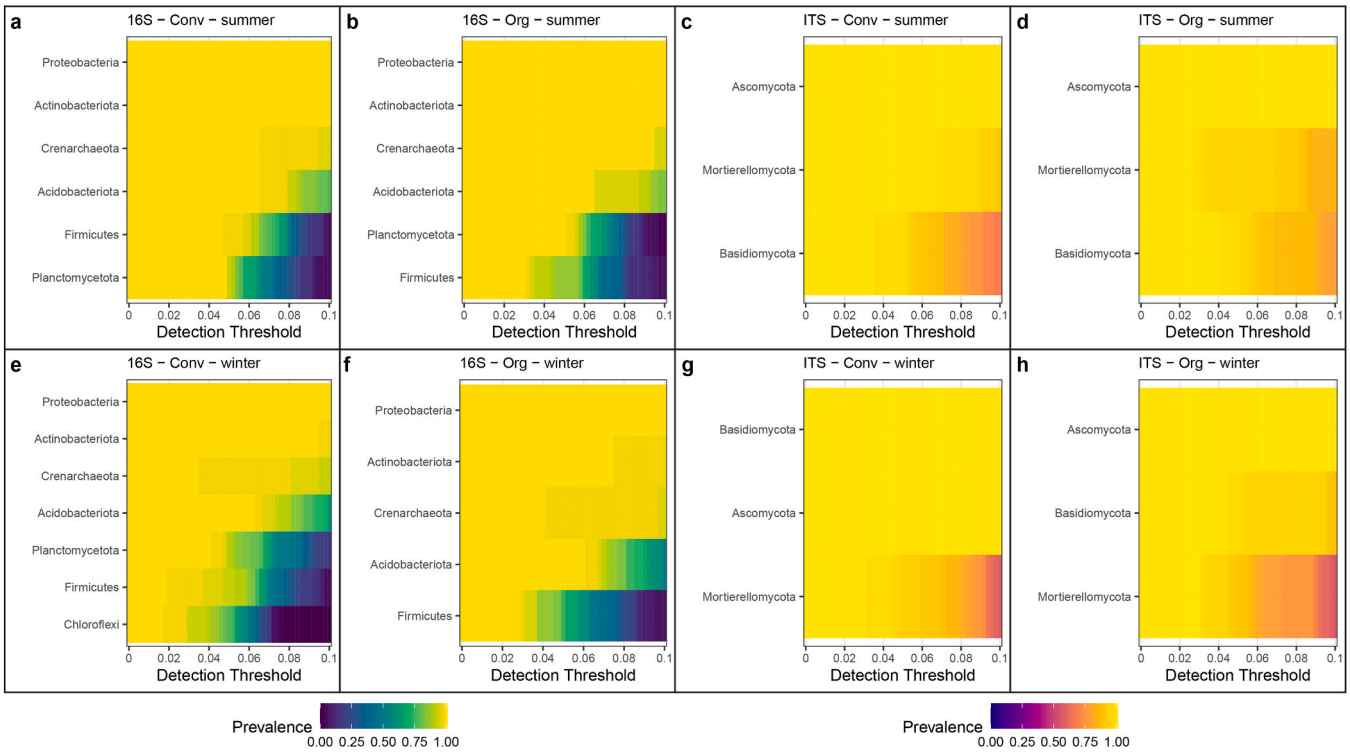

Fig. 2. Heatmaps showing the phylum prevalence proportion across different detection thresholds in organic (Org) and conventional (Conv) management at summer (top) and winter (bottom) for 16S (left) and ITS (right) markers.

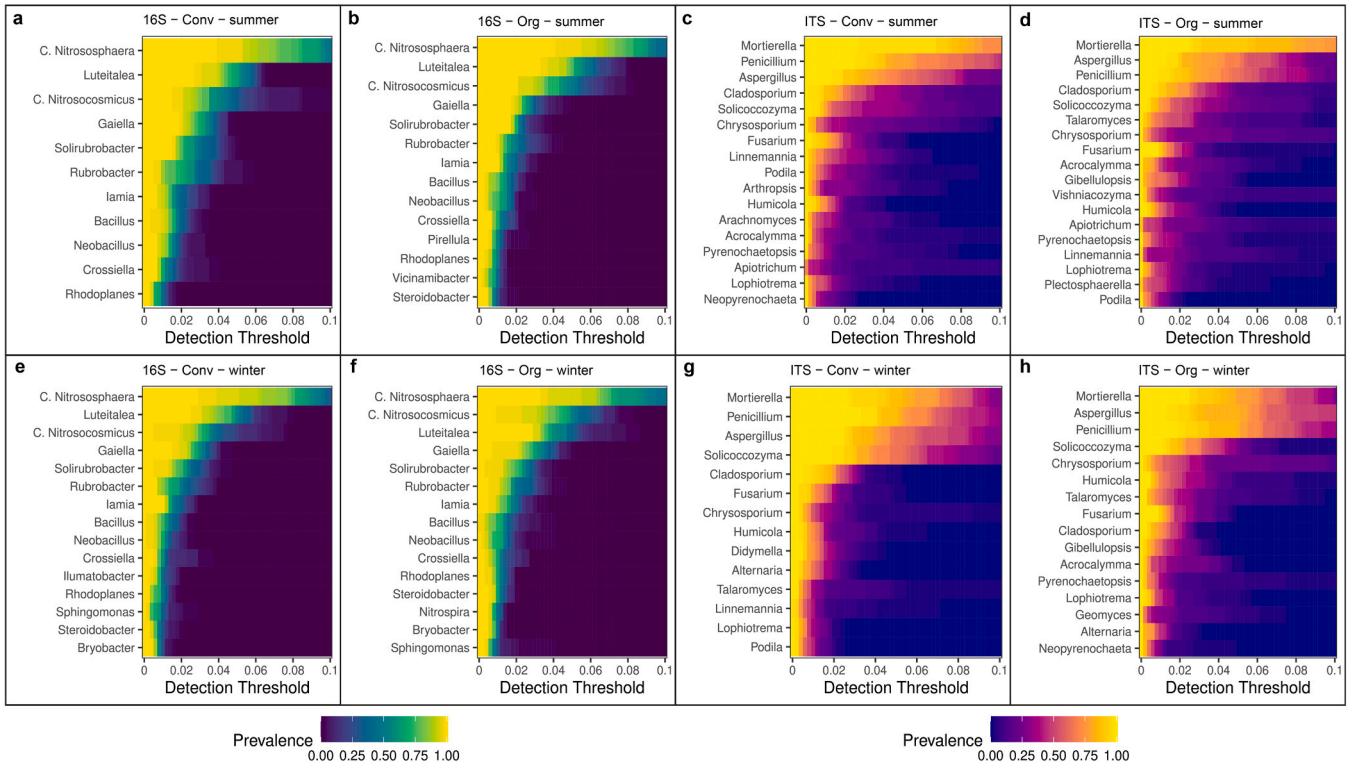

Fig. 3. Heatmaps showing the genus prevalence proportion across different detection thresholds in organic (Org) and conventional (Conv) management at summer (top) and winter (bottom) for 16S (left) and ITS (right) markers (panel a).

genera such as *Didymella*, *Alternaria*, *Talaromyces*, and *Linnemannia* were exclusively detected in winter (Fig. 3c and g and Fig. 4a). Genera, such as *Vishniacozyma*, *Plectosphaerella*, *Apiotrichum* and *Linnemannia,* were detected exclusively in organically managed citrus orchards in summer. *Geomyces*, *Gibellulopsis*, *Alternaria*, and *Talaromyces* were exclusive to the winter season (compare Fig. 3d and h and Fig. 4a). Focusing on fungal

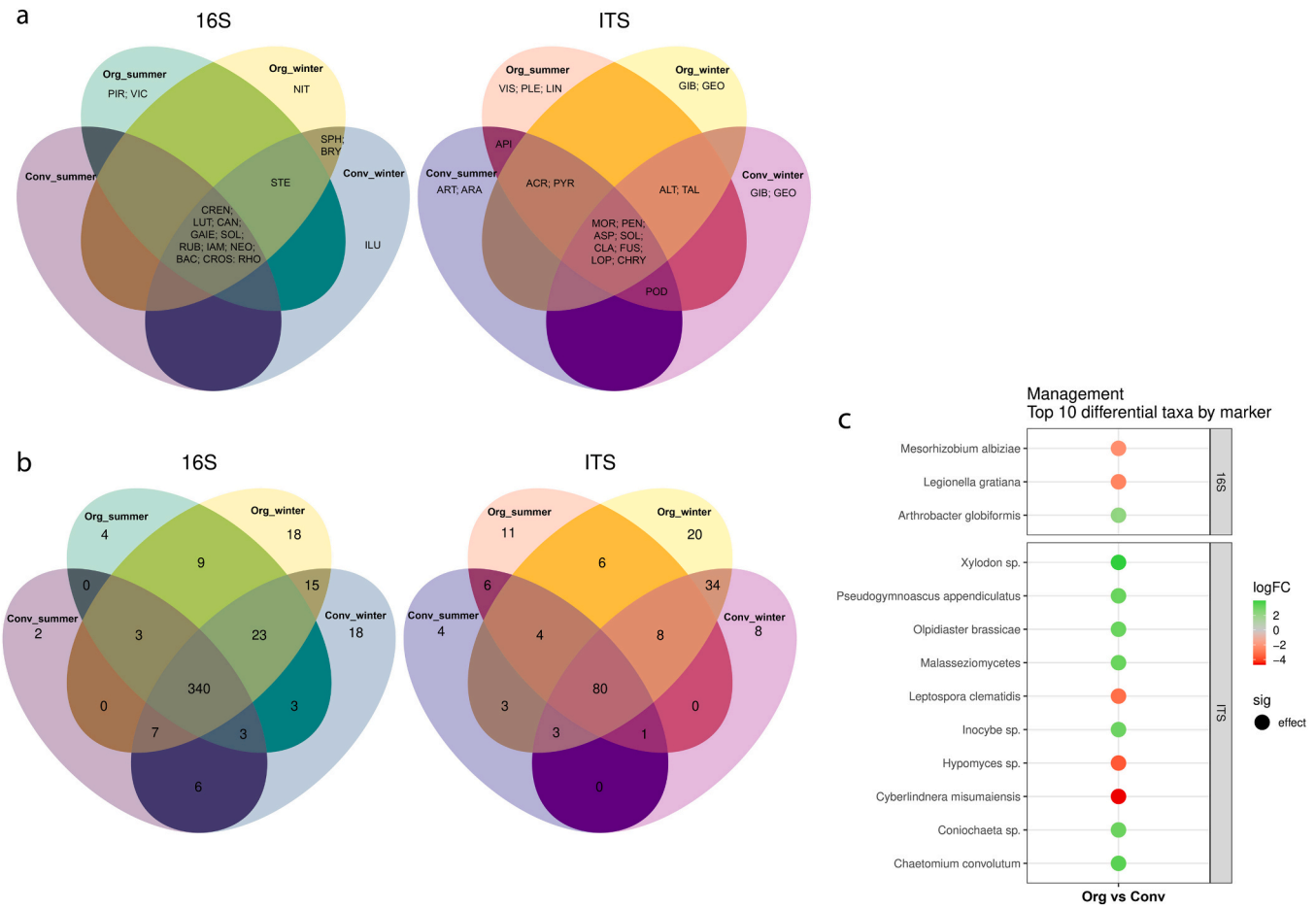

Fig. 4. Comparison among the different members of prokaryotic (left) and fungal (right) taxa at the genus level during summer and winter seasons. Members of prokaryotic taxa (CREN = *Crenarchaeota Nitrososphaera*, LUT = *Lutetiella*, CAN = *Candidatus Nitrosocosmicus*, GAIE = *Gaiella*, SOL = *Solirubrobacter*, RUB = *Rubrobacter*, IAM = *Iamia*, BAC = *Bacillus*, NEO = *Neobacillus*, CROS = *Crossiella*, RHO = *Rhodoplanes*, ILU = *Ilumatobacter*, SPH = *Sphingomonas*, STE = *Steroidobacter*, BRY = *Bryobacter*, PIR = *Pirellula*, VIC = *Vicinamibacter* and NIT = *Nitrospira*). Members of fungal taxa (MOR = *Mortierella*, PEN = *Penicillium*, ASP = *Aspergillus*, SOL = *Solicoccozyma*, CLA = *Cladosporium*, FUS = *Fusarium*, LOP = *Lophiotrema*, CHRY = *Chrysosporium*, ART = *Arthropsis*, ARA = *Arachnomyces*, API = *Apiotrichum*, DID = *Didymella*, ALT = *Alternaria*, TAL = *Talaromyces*, VIS = *Vishniacozyma*, PLE = *Plectosphaerella*, GEO = *Geomyces*, GIB = *Gibellulopsis*, ACR = *Acrocalymma*, and LIN = *Linnemannia*) (panel a). Number of shared and exclusive members of prokaryotic (left) and fungal (right) taxa at the genus level during the summer and winter seasons (panel b). Top 10 differentially abundant taxa (panel c) identified at the species level when comparing organic and conventional managements (adjusted p-value < 0.05) for 16S (top) and ITS (bottom).

communities recorded in summer with respect to agricultural management, *Vishniacozyma*, *Plectosphaerella* and *Linnemannia* were exclusive to samples from orchards under organic management, while *Arthropsis* and *Arachnomyces* were exclusive records in samples from conventional management (compare Fig. 3c and d and Fig. 4a). In winter, *Didymella* and *Linnemannia* were exclusively detected in conventionally managed orchards, whereas *Gibellulopsis* and *Geomyces* were exclusive to samples from organically managed orchards (Fig. 3g and h, and Fig. 4a).

3.2.3. *Variability of microbiota structure in relation to seasonality and farming system*

Venn diagrams (Fig. 4b) depict the frequency of diverse prokaryotic and fungal genera recorded in soil samples based on farming system of orchards (organic versus conventional) and soil sampling season (summer versus winter). As for the seasonal variability of soil prokaryotic communities in relation to the farming system (Fig. 4b), in summer four and two exclusive taxa characterized the soil microbiome of organically and conventionally managed citrus orchards, respectively. No taxa were shared between the soil microbiota of orchards differing in farming system. In winter, 18 bacterial taxa were exclusive to the microbiota of either organically or conventionally managed orchards, while 15 taxa were shared between the soil microbiota of differently managed orchards. When considering the interaction between farming system and seasonality on the assembly of prokaryotic communities, the microbiota of organically managed orchards showed four exclusive taxa in summer and 18 in winter, with nine taxa common to both seasons. In conventionally managed orchards, two exclusive taxa were recorded in summer and 18 in winter, with six taxa common to both seasons. Finally, 340 prokaryotic taxa did not vary with the farming system or season. They were recorded in soil microbiota of both organically and conventionally managed orchards in winter as well as in summer (Fig. 4b).

As for the seasonal variability of fungal communities in relation to the farming system (Fig. 4b), in summer, 11 and four taxa were exclusive to the microbiota of organically and conventionally managed orchards, respectively, while six taxa were shared between the two groups of orchards as separated on the basis of farming system. In winter, 20 and eight taxa were exclusive to the soil microbiota of organically and conventionally managed orchards, respectively, while 34 taxa were shared between the two groups of orchards managed differently. Six out of 31 taxa unique to the soil microbiota of organically managed orchards were detected both in summer and winter, while no taxon out of 12 taxa unique to the soil microbiota of conventionally managed orchards was detected in both seasons. Overall, 80 fungal taxa were not influenced by either agricultural management or seasonality and consequently were

recorded in soil microbiota of both organically and conventionally managed orchards as well as in both summer and winter (Fig. 4b).

3.2.4. Microbiota structure (prokaryotic and fungal species)

The top-ranked differentially abundant species in soil microbiota of surveyed citrus orchards are reported in Fig. 4c and Supplementary Table S3. In the prokaryotic dataset (16S-reads), the top three differentially abundant species were *Mesorhizobium albiziae*, *Legionella gratiana*, and *Arthrobacter globiformis*. These taxa exhibited significant differences in abundance between organically and conventionally managed orchards, with *Arthrobacter globiformis* showing higher abundance in soil microbiota of organically managed orchards (logFC green, p-value < 0.05), while *Mesorhizobium albiziae* and *Legionella gratiana* were the most abundant in soil microbiota of conventionally managed orchards (logFC red, p-value < 0.05). In the fungal dataset (ITS-reads), 10 taxa were the most abundant, including *Xylodon* sp., *Pseudogymnoascus appendiculatus*, *Olpidiaster brassicae*, *Malasseziomycetes*, *Leptospora clematidis*, *Inocybe* sp., *Hypomyces* sp., *Cyberlindnera misumaiensis*, *Coniochaeta* sp., and *Chaetomium convolutum*. Among them, *L. clenmidis*, *Hypomyces* sp. and *C. misumaiensis* were more abundant in conventionally managed soils (logFC red, p-value < 0.05), while the remaining taxa were more abundant in organically managed orchards (logFC green, p-value < 0.05).

3.3. Modelling of microbiome indexes and microbiome network indicators

3.3.1. Farming system and network properties: co-exclusion and co-occurrence

The analyses of data indicated an increase in the proportion of co-exclusion within the prokaryotic and fungal microbial networks of the soil microbiome from organically managed orchards compared to the soil microbiome from conventionally managed orchards (Fig. 5a).

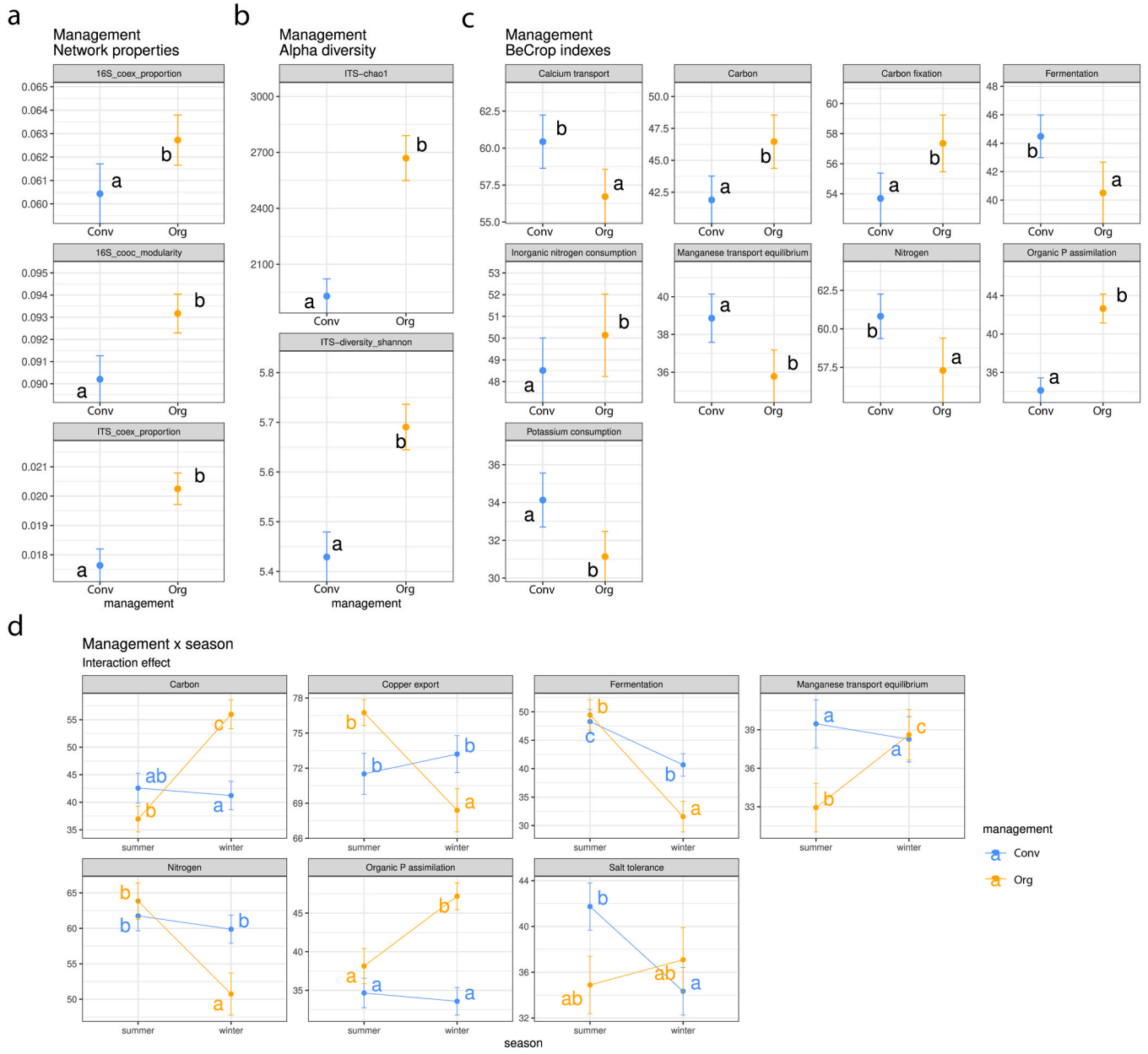

Fig. 5. Network properties (panel a), alpha diversity (panel b) and microbiome BeCrop® indexes (panel c) factorial plots according to management level: organic (Org) and conventional (Conv). Microbiome BeCrop® indexes (panel d) interaction plots according to management level by season. Superscript letters indicate statistically different groups (adj. p-value < 0.05).

The co-occurrence networks were significant only for prokaryotic communities, which showed the highest modularity in soils from organically managed orchards (Fig. 5a).

3.3.2. Influence of management on α-diversity, Chao1 and Shannon indexes

Data processing for alpha diversity indicated that prokaryotic records did not show a significant relationship with the type of farming system. As a result, prokaryotic alpha diversity could not be calculated. Therefore, Fig. 5b presents only the results for fungal alpha diversity. Specifically, the analysis revealed that the highest value of the Shannon diversity index was recorded in soils from organically managed orchards, while the Chao1 index suggested greater species richness in soils from organically managed orchards compared to those from conventionally managed ones.

3.3.3. BeCrop® indexes with focus on management

Results presented in Fig. 5c show the variation of the indexes 'calcium transport', 'carbon', 'carbon fixation', 'fermentation', 'inorganic nitrogen consumption', 'manganese transport equilibrium', 'nitrogen', 'organic P assimilation', and 'potassium consumption' in relation to the farming system of the orchards. Specifically, results indicated that the indexes 'carbon', 'carbon fixation', 'inorganic nitrogen consumption' and 'organic P assimilation' were significantly higher in soils from organically managed orchards compared with those calculated for soils from conventionally managed orchards. All the other indexes showed the highest values in soils from conventionally managed orchards.

3.3.4. BeCrop® indexes with focus on farming system vs. seasonality

The results presented in Fig. 5d show the variation of the following indexes, 'carbon', 'copper export', 'fermentation', 'manganese transport equilibrium', 'nitrogen', 'organic P assimilation', and 'salt tolerance', for orchards managed differently (organically vs. conventionally managed) in relation to seasonality (summer vs. winter). Specifically, in soils from organically managed orchards, significantly higher values of the indexes 'carbon', 'manganese transport equilibrium', and 'organic P assimilation' were observed in winter. Conversely, 'copper export', 'fermentation', and 'nitrogen' showed a higher value in summer. The 'salt tolerance' index did not show any significant seasonal pattern. In soils from conventionally managed orchards, a significant decrease in the indexes for 'carbon', 'fermentation' and 'salt tolerance' was observed. All other indexes showed no significant seasonal variation.

3.3.5. Effects of environmental and management factors on microbial community structure

The analysis revealed significant differences in the structure of microbial communities, which were markedly influenced by both environmental variables, such as geographical location of the orchard (municipality) and soil texture, and agronomic variables, including rootstock genotype (sour orange vs. 'Carrizo' citrange), tree age (young $<$40 years vs. old $\geq$40 years), farming system (conventional vs. organic), tillage frequency (no-tillage and 2, 3, 4 times per year) and depth (shallow $<$15 cm, deep $>$15 cm) and irrigation system (drip, T-shaped sprinklers, microsprinklers), soil sample ID. Specifically, the PERMANOVA analysis (Table 3) indicated that sampling site had the most significant influence on microbial community composition, accounting for 17.55 % of the variance in prokaryotic communities ($p = 0.01$) and 9.45 % in fungal communities ($p = 0.01$). Additionally, the cluster factor had a significant impact, contributing 5.65 % of the variance as regards prokaryotes and 6.33 % as regards fungi (both $p = 0.01$). Soil texture accounted for 4.92 % of the variance for prokaryotes and 2.73 % for fungi ($p = 0.01$). Tillage number contributed 4.24 % of the variance in prokaryotes and 2.91 % in fungi ($p = 0.01$). The influence of the irrigation system accounted for 3.40 % of the variance in prokaryotes and 3.66 % in fungi ($p = 0.01$). Agricultural management practices accounted for 3.19 % of the variance in prokaryotes and 2.24 % in fungi ($p = 0.01$). The rootstock genotype had a moderate impact, accounting for 2.35 % of the variance in prokaryotes and 1.69 % in fungi ($p = 0.01$). Factors such as tillage depth influenced prokaryotic variance by 1.75 % and fungal variance by 1.12 % (both $p = 0.01$). Tree age had a relatively low influence, accounting for 0.90 % of the variance in prokaryotes and 1.77 % in fungi ($p = 0.01$). Finally, the health status of trees as determined by visual observation did not have a significant impact on microbial communities.

Table 3
PERMANOVA analysis of microbiome composition (16S marker for prokaryotes and ITS marker for fungi) as regards management, location, season, cluster and other experimental factors (p-value $<$ 0.05 considered significant).

Marker	16S		ITS	
Variable	p-value	R2	p-value	R2
sampling site	0.01	17.55	0.01	9.45
cluster	0.01	5.65	0.01	6.33
soil texture	0.01	4.92	0.01	2.73
tillage number per year	0.01	4.24	0.01	2.91
sampling season	0.01	3.52	0.01	2.41
irrigation system	0.01	3.40	0.01	3.66
farming system	0.01	3.19	0.01	2.24
rootstock genotype	0.01	2.35	0.01	1.69
tillage depth	0.01	1.75	0.01	1.12
tree age	0.01	0.90	0.01	1.77
tree health status	0.93	0.28	0.66	0.47

The principal coordinate analysis (PCoA) revealed that prokaryotic communities grouped primarily according to the sampling site, while fungal communities were correlated with seasonality (Fig. 6a). In addition, a deeper analysis showed that prokaryotic and fungal communities formed three microbiome groups, clustering primarily in relation to sampling site and, to a lesser extent, according to management practices (Fig. 6b, Supplementary Table S4).

The redundancy analysis (RDA) was conducted to assess the influence of environmental and agronomic variables on microbial community assembly, using models that included (unconstrained) or excluded (constrained) the effect of sampling site (Fig. 7; Table 4).

In prokaryotic communities, the unconstrained model showed that soil tillage frequency had a significant effect ($p = 0.043$), explaining 3.60 % of the variance. Other factors such as rootstock genotype, irrigation system, soil texture, tree age, tillage depth, and management system were not statistically significant ($p > 0.05$), each accounting for between 1.12 % and 3.25 % of the variance. When the sampling site effect was removed, rootstock genotype became significant ($p = 0.029$), explaining 2.26 % of the variance, while all other factors were non-significant (variance explained: 0.98–3.57 %).

In fungal communities, the unconstrained model identified irrigation system ($p = 0.014$; 4.40 %), soil tillage frequency ($p = 0.022$; 4.04 %), farming system ($p = 0.034$; 2.18 %), total nitrogen content ($p = 0.039$; 2.17 %), and rootstock genotype ($p = 0.042$; 2.11 %) as significant predictors. Other variables (e.g., soil texture, tree age, tillage depth) were not significant and explained variance in the range of 1.44–2.11 %. In the constrained model, none of the factors attained statistical significance.

4. Discussion

Findings of this study showed the microbial communities associated with the soil explored by fine roots of citrus trees were influenced by diverse factors. Based on the PERMANOVA analysis both bacterial and fungal communities grouped mostly according to the sampling site, which accounted for the largest proportion of variance. This result is consistent with a previous metagenomics study of tomato root microbiome which demonstrated that the sampling site impacted the bacterial communities more than root/soil interface compartments (Anzalone et al., 2021). The classic microbiological approach in characterizing the soil microbiota of plants distinguishes diverse compartments of the

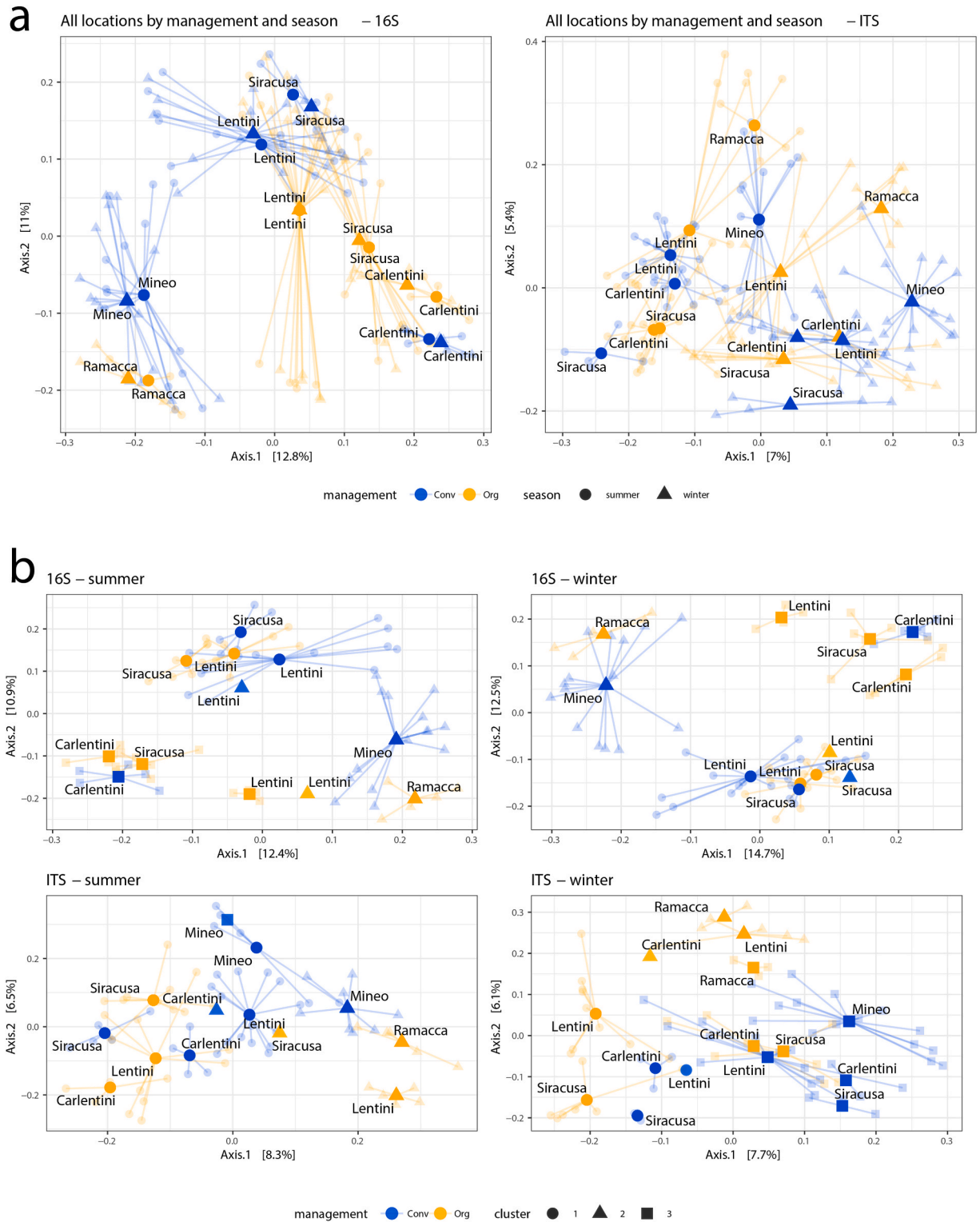

Fig. 6. Principal Coordinate Analysis (PCoA) of the microbial community based on Bray-Curtis distances for 16S and ITS markers annotated management, season and municipality. Centroids are colored by manage-ment, shaped by season and labeled by location (panel a). PCoA split by season of panel a. Centroids are col-ored by management, shaped by cluster and labeled by location (panel b).

interface between root and soil, i.e. rhizosphere, rhizoplane and bulk soil (Gregory, 2006; Barillot et al., 2013; Guo et al., 2024). In this study, the analysis of a large volume of soil from the horizon explored by fine roots was preferred to a fine resolution of microbial communities of root-soil interface compartments, as the focus was on the effects of farming management on soil microbiome of a perennial tree crop with an extended root system. Accordingly, soil sampling conformed to the protocol used by other authors in similar studies, who referred to soil samples containing fine roots as rhizosphere soil in a broader sense (Si et al., 2018; Lazcano et al., 2021). The effect of sampling site on soil microbiota is difficult to decipher as it is the result of complex interactions of several intrinsic and environmental factors. However, in

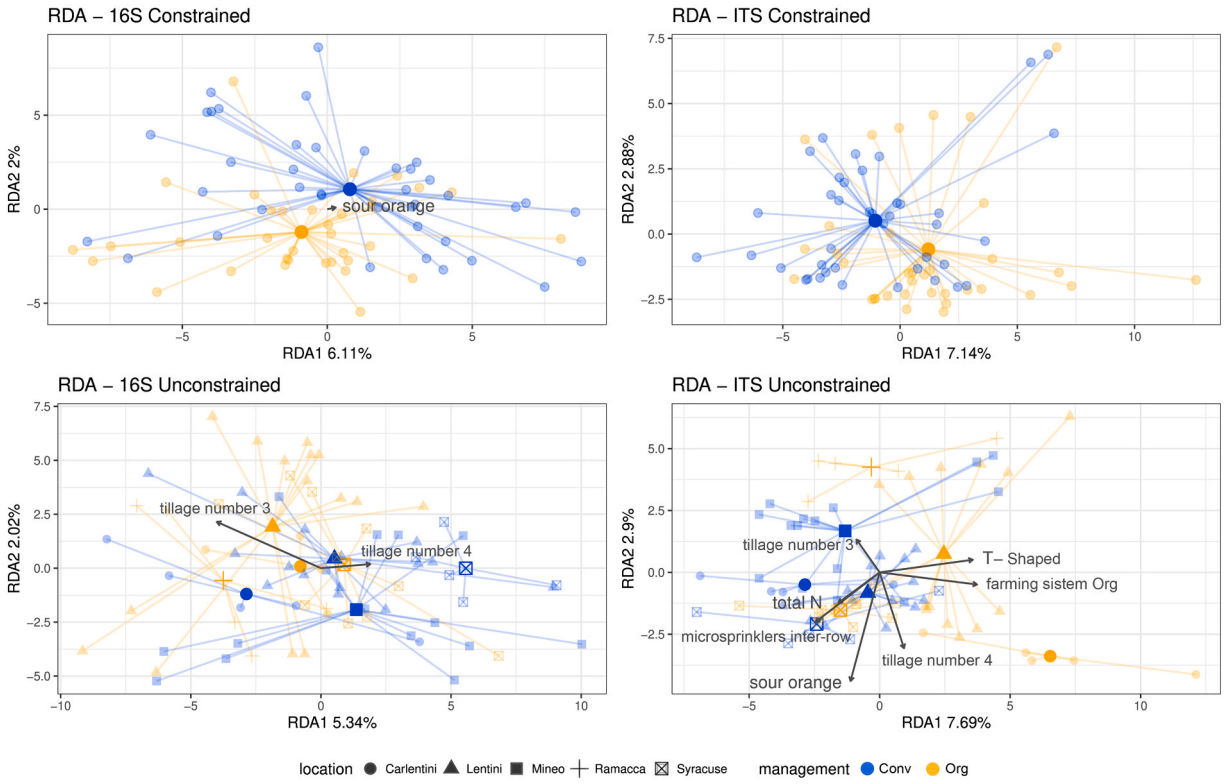

Fig. 7. Redundancy analysis (RDA) for summer samples where municipality effect was removed (top) and included (bottom) with forward model selection using physicochemical properties and experimental factors as linear predictors for 16S and ITS markers (panel c).

Table 4
Variance explained and ANOVA p-value of terms retrieved through forward model selection for redundancy analysis (RDA), either including (unconstrained) or removing (constrained) the sampling site effect for 16S and ITS microbial composition (p-value < 0.05 considered significant).

	Sampling site effect	Included		Removed	
marker	term	p-value	Explained variance (%)	p-value	Explained variance (%)
16S	tillage number	0.043	3.60	0.123	3.38
16S	irrigation system	0.096	3.25	0.077	3.57
16S	soil texture	0.276	2.80	0.324	2.91
16S	rootstock	0.075	1.79	0.029	2.26
16S	tree age	0.252	1.45	0.227	1.58
16S	tillage depth	0.359	1.33	0.485	1.31
16S	farming sistem	0.454	1.26	0.211	1.60
16S	total N	0.730	1.12	0.931	0.98
ITS	irrigation system	0.014	4.40	0.056	4.03
ITS	tillage number	0.022	4.04	0.073	3.89
ITS	soil texture	0.246	2.76	0.393	2.74
ITS	farming system	0.034	2.18	0.196	1.60
ITS	total N	0.039	2.17	0.507	1.23
ITS	rootstock	0.042	2.11	0.289	1.47
ITS	tillage depth	0.105	1.69	0.172	1.70
ITS	tree age	0.223	1.44	0.172	1.69

this study the effects of some of these factors were separated and measured. Along with sampling site, other factors, such as sampling season (summer as opposed to winter), soil texture, frequency and depth of soil tillage, irrigation system (drip, T-shaped sprinklers or microsprinkler), and rootstock genotype (sour orange as opposed to citrange), had a significant impact on both prokaryotes and fungi. In contrast, the influence of tree age and health status was negligible.

4.1. *Impact of organic farming on microbial diversity and functionality*

Interestingly, both prokaryotic and fungal communities of soil microbiome were influenced by the farming system (organic as opposed to conventional). In this respect, results confirmed the efficacy of BeCrop® indexes in fine tuning the analysis of the effects of management system on the structure and functionality of soil microbiome (Acedo et al., 2022). Organically managed orchards compared to those managed conventionally showed a higher microbial diversity as well as a unique composition of nutrient-cycling bacteria, which are crucial for enhancing soil fertility and plant health. Moreover, this study revealed that organic management promotes beneficial microbial functions, such as nitrogen fixation and phosphorus solubilisation. The significant influence of organic management on the enzymatic activity of rhizosphere soil microbiota observed in this study is consistent with the findings of Järvan et al., (2014), who reported noticeable effects of this farming system on the enzymatic activity of soil microbiota. Conversely, no significant difference was observed in most chemical soil properties between organically and conventionally managed citrus orchards, with the exception of TC carbon content which was significantly higher in organically managed orchards. As regards the effects of organic farming system on the physicochemical properties of soil, conflicting reports can be found in the literature. The prevailing opinion is that the transition to organic management improves key soil parameters such as pH, organic carbon, total nitrogen content, and the C:N ratio, with favourable effects on beneficial microbial activity (Canali et al., 2012; Montes-Borrego et al., 2013). However, other factors, such as the inherent soil nature depending mostly on its genesis, may prevail on management practices in conditioning and determining some soil physiochemical properties (Montes-Borrego et al., 2013; Blanco et al., 2024). As a matter of fact,

PERMANOVA analysis revealed that the soil texture exerted a significant effect in shaping both soil bacterial and fungal communities and influencing their functionality in surveyed citrus orchards of eastern Sicily. Silt and silt loam soils retain a higher amount of water and nutrients, supporting diverse microbial populations essential for nutrient cycling processes like carbon sequestration and nitrogen transformations. By contrast, sandy loam soils, which possess a lower water retention capacity, tend to support microbial communities more adapted to fluctuating moisture conditions, resulting in distinct nutrient cycling dynamics (Fierer and Jackson, 2006; Rillig et al., 2017). As for another soil property, soil organic C, it is known that it may be influenced more by other factors such as tree age than by management practices (Pardon et al., 2017; Mercado-Blanco et al., 2018; Zayani et al., 2023). Quite interestingly, in the present study the biochemical functions of rhizosphere soil microbiome as expressed by BeCrop® indices not only showed significant differences between organically and conventionally managed orchards but also a different seasonal pattern in the two farming systems. The indexes for C, Mn transport equilibrium and organic P assimilation of soils from organically managed orchards in summer were higher than in those managed conventionally. By contrast, in winter indexes for Cu export, fermentation and N of soils from organically managed orchards were higher than in conventionally managed orchards. These results are in agreement with other studies indicating an enhancement of C and nutrient dynamics in organically managed systems (Canali et al., 2012; Berthrong et al., 2013; Roussos et al., 2019). Berthrong et al. (2013) highlighted the greater efficiency of respiration per unit of soil organic C in organically managed systems. According to these authors, the mulching of leaves in organic systems contributed to increase the litter biomass in the soil, enhancing microbial enzymatic activity. Moreover, Berthrong et al. (2013) reported that pesticides may have detrimental effects on certain groups of soil microorganisms. To confirm the complex relationships among management practices and biochemical functions of soil microbiome, BeCrop® indices related to Mn, K, and N metabolism were significantly higher in conventionally managed citrus orchards, whereas the index relative to inorganic C consumption was lower than in organically managed orchards. This is consistent with results of previous studies, reporting lower K and N availability in soil of organically managed systems, probably due to the lack of synthetic fertilizer supply (Stalenga, 2007; Canali et al., 2012). Berthrong et al. (2013) observed that microbial communities in soils of organically managed systems were more efficient at mineralizing N than soils of conventionally managed ones. It has been hypothesized that the enhanced metabolism of organic C, P assimilation, and inorganic N consumption in organic systems is linked to a greater microbial diversity (Maeder et al., 2002; Lori et al., 2017). Overall these findings suggest organic management may directly or indirectly influence the biochemical functions of soil microbiome, improving the capacity of soil to retain, metabolize and recycle organic matter, nutrients and even pollutants (Mauro et al., 2015; Rillig et al., 2017).

4.2. Composition and ecological structure of soil microbiome

As for the effects of farming system on the diversity of soil microbiota in citrus orchards results of this study indicate that there were no great differences between core microbiota of organically and conventionally managed orchards, while there were noticeable differences in the seasonal pattern of microbial communities between the two farming systems. *Proteobacteria*, *Actinobacteriota*, and *Acidobacteriota*, were the predominant bacterial phyla across both systems, consistently with previous global mapping studies of soil microbial communities of the citrus rhizosphere (Xu et al., 2018). However, in winter *Chloroflexi* was detected exclusively in conventionally managed orchards, suggesting a possible effect of management system on bacterial community composition. *Proteobacteria*, *Actinobacteriota*, and *Acidobacteriota* remained dominant in both management systems across seasons, highlighting their fundamental role in citrus rhizosphere soils. Additionally, *Crenarchaeota* and *Firmicutes* were present in both management systems across all seasons. The fungal communities were predominantly composed of the phylum *Ascomycota*, regardless of management practices or season. During winter, *Basidiomycota* was the second most prevalent phylum, particularly in conventionally managed orchards, while *Mortierellomycota* prevailed in summer, once again particularly in conventionally managed orchards. All three phyla were detected in both summer and winter. These findings are consistent with the results of Wu et al. (2021), who identified *Ascomycota* and *Mortierellomycota* as the most represented fungal phyla in soil microbiota. Similarly, Xi et al. (2023) reported *Ascomycota* as the predominant phylum in soil mycobiota, with higher relative abundance of *Basidiomycota* compared to *Mortierellomycota*. It is noteworthy that in winter, the bacterial genus *Nitrospira* was exclusively present in the core microbiome of soil from organically managed orchards. This genus plays a pivotal role in the nitrification process, converting ammonia (NH^{3+}) to nitrate (NO3-), a N form available to plants (Rice et al., 2016; Li et al., 2019). The exclusive detection of *Nitrospira* in soils of organically managed orchards during winter, together with the significantly higher BeCrop® index for inorganic nitrogen consumption, indicate a potential increase in microbial activity related to nitrogen cycling under organic management.

Another relevant difference of the soil microbiota between organic and conventional farming systems is the richness in fungal taxa observed in organically managed orchards, particularly in winter. The number of fungal taxa detected in rhizosphere soil of organically managed orchards was 54, as opposed to 25 detected in soil of conventionally managed orchards. In particular, *Chaetomium convolutum*, a well-known biocontrol agent (BCA), exhibited differential abundance in organically managed orchards. This BCA has been demonstrated to be effective against a wide range of plant diseases, including root rot of citrus (Hung et al., 2015). Similarly, *Sphingobacterium*, which has been demonstrated to be a potential BCA of citrus diseases (Ezrari et al., 2021) and *Arthrobacter globiformis*, which has been reported to confer salt tolerance (Stassinos et al., 2022), were significantly more abundant in soil of organically managed orchards. Based on these findings, it can be speculated that organic management may improve the resilience of citrus trees to biotic and abiotic stresses. As for the effects of environmental factors on the structure of rhizosphere soil microbiota in citrus orchards, PCoA analysis showed that the prokaryotic component was predominantly influenced by geographical location and soil texture, while the fungal component was influenced by the irrigation systems and exhibited significant seasonal variation. These findings are consistent with those of Mercado-Blanco et al. (2018), which reported that fungal communities were sensitive to seasonal changes and irrigation systems. Also bacterial communities fluctuate in response to seasons, which exert their influence more on α-diversity than β-diversity of these communities (Lauber et al., 2013). Results of this study indicated that both prokaryotic and fungal communities were significantly impacted by both the frequency and depth of tillage, confirming previous research indicating that microbial communities exhibit greater stability under reduced soil management regimes (Bevivino et al., 2014).

A general aspect highlighted by the analysis of the 16S (prokaryotes) and ITS (fungi) microbial networks is the significantly higher proportion of co-exclusion associated with organic management compared to those observed in conventionally managed orchards. Co-exclusion refers to the tendency of two species not to coexist in the same environment or ecological niche, often due to direct competition or antagonism. An increased level of co-exclusion might indicate that, in organically managed contexts, microorganisms may actively inhibit the presence or expansion of competitors, fostering a more competitive yet dynamically balanced microbial ecosystem. A study of other authors, focusing on the impact of organic versus conventional management on the diversity and community structure of bacteria and fungi in tea plantations, demonstrated that under organic management soil micro-ecological networks are significantly more complex and stable (Huang et al., 2023). This

would indicate that organic management fosters cooperative relationships among microbial species, enhancing soil resilience, while conventional management results in less complex microbial networks that are more susceptible to environmental stress (Huang et al., 2023). Overall, these findings suggest that organic management promotes a more resilient soil ecosystem. In the present study, the Shannon index for soil microbiome fungi was found to be significantly higher in organically managed orchards compared to those managed conventionally, which is in agreement with previous studies that demonstrated an increase in soil microbial diversity under organic management (Schmidt et al., 2019; Suyal et al., 2021; Huang et al., 2023). Similarly, in the present study the Chao1 index indicated significantly higher richness in organically managed citrus orchards. Scotton et al., (2020) found that transitioning from conventionally to organically managed citrus orchards in Brazil increased soil fungal diversity, as a consequence of a progressive reduction in the use of chemical substances. Similarly, Panelli et al. (2017) observed that organically managed and no-tillage crop soil hosted richer fungal consortia compared to those managed conventionally, with a predominance of *Ascomycota*.

As suggested by previous studies, higher microbial diversity can limit the invasion capacity of phytopathogens by saturating available ecological niches and increasing competition for resources (Van Elsas et al., 2012). Additionally, organic management appears to significantly influence the formation of more discrete and specialized prokaryotic networks, characterized by a significant increase in co-occurrence modularity. This implies that groups of microorganisms tend to form well-defined subcommunities, within specialized ecological niches (Wang et al., 2021). This type of organization reflects a complex and stratified microbial ecosystem, where intra-group interactions are more prevalent than those between different groups. For example, the modularization of microbial networks was observed in arid agricultural ecosystems, where the combined effect of irrigation and fertilization practices increased the modularity of bacterial networks and was positively correlated with soil fertility and crop productivity (Ye et al., 2021). The greater co-occurrence of microbial community members in organic agriculture would indicate a higher ecological balance and increased complexity, potentially making microbiomes of organically managed systems more resilient to environmental stress (Banerjee et al., 2019; Parasuraman et al., 2019; Mannaa and Seo, 2021).

The analysis of the structure and functionality of soil microbiome complements other methods such as Life Cycle Assessment (LCA) and Carbon Footprint (CF) in evaluating the sustainability of organic farming systems in citriculture (Ribal et al., 2017, 2019). Moreover, it paves the way toward the development of strategies based on engineered synthetic microbial consortia (McCarty and Ledesma-Amaro, 2019; Duncker et al., 2021; Mahmud et al., 2021; Contreras-Salgado et al., 2024; Gómez-Lama Cabanás and Mercado-Blanco, 2025;), which might be a breakthrough to manage plant diseases, enhance crop productivity and improve ecological sustainability in agriculture.

5. Conclusions

This is the first study dealing with the effects of environmental variables and farming systems on the structure and functionality of both prokaryotic and fungal communities residing in the soil explored by fine roots of citrus trees in the Mediterranean region. Sampling site accounted for the highest proportion of variance observed in these microbial communities underscoring both the complexity of interactions among driving forces that shape them and the importance of spatial factor in evaluating the role of cropping system and single management practices. No great differences were observed between core microbiota of organically and conventionally managed citrus orchards, indicating a certain resilience of microbial soil communities associated to a permanent crop. However, the finely tuned analysis of data using BeCrop® indexes revealed differences in seasonal patterns of microbial communities between conventionally and organically managed orchards. Moreover, organic farming system fostered richer and more diverse soil microbial communities and enhanced their functionality in terms of nutrient cycling. In particular, it favoured nitrogen fixation and organic phosphorus solubilisation. This finding adds arguments in favour of organic agriculture and has relevance for fertilization of citrus orchards. The analysis of major factors shaping the structure and conditioning the ecology of soil microbiome provides helpful information to improve the health, resilience and efficiency of citriculture in the Mediterranean region.

CRediT authorship contribution statement

Sebastiano Conti Taguali: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rhea Pöter:** Writing –review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Francesco Aloi:** Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Clara Fernández-Trujillo:** Writing – review & editing, Visualization, Software, Methodology, Data curation. **Alberto Acedo:** Software, Methodology, Formal analysis, Data curation. **Federico La Spada:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Maria Giulia Li Destri Nicosia:** Writing – review & editing, Supervision, Funding acquisition. **Antonella Pane:** Writing – review & editing, Supervision, Resources, Investigation. Leonardo Schena: Writing – review & editing, Supervision, Funding acquisition. **Santa Olga Cacciola:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

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Declaration of competing interest

Alberto Acedo is a cofounder and Clara Fernández-Trujillo are current employees of Biome Makers. All the other authors declare no conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.micres.2025.128260.

Data availability

The raw bacterial (16S) and fungal (ITS) amplicon sequencing data have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1242073

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