

Soil microbiome dynamics of *Cannabis sativa* L. under biotic stress induced by *Tetranychus urticae*

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Abstract

The microbiome associated with *Cannabis sativa* L. plays a crucial role in plant growth and health, although the mechanisms by which it is modulated in response to different types of stress during cultivation remains under investigation. In this study, the bacterial microbiome of both rhizospheric and bulk soil associated with a therapeutic *C. sativa* variety was characterized across three stages of the cultivation cycle (early vegetative, late vegetative, and late flowering), comparing healthy plants and those under stress induced by *Tetranychus urticae*. In addition to microbial profiling, plant physiological parameters were assessed, along with the analysis of cannabinoid and terpene profiles in floral tissues. Analyses of alpha diversity, community structure, discriminant taxa (LEfSe), and functional predictions (PICRUSt2) were performed using 16S rRNA gene sequencing data. The results revealed a decrease in plant growth-promoting genera such as *Pseudomonas* and members of the family *Oxalobacteraceae* in stressed plants, and a relative increase in members of the *Gemmatimonadaceae* family and *SBR1031* genus (*Chloroflexota*), particularly during the late flowering stage. Functional predictions further indicated that in control conditions the rhizosphere community exhibited higher metabolic activity, enriched in pathways related to replication, transcription and protein synthesis, whereas under stress, functions shifted toward resource recycling and metabolic flexibility. These findings suggest that biotic stress triggers a functional and structural reorganization of the microbiota, favoring more resilient yet less beneficial communities for plant development. This study provides novel evidence of the interaction between insect, plant, and microbiota, with both agronomic and biotechnological implications.

Introduction

The cultivation of *Cannabis sativa* L. for therapeutic purposes has gained increasing relevance in recent years. However, it remains a relatively underexplored species from both physiological and ecological perspectives, making it important to study its cultivation under various environmental conditions and its interactions with beneficial microorganisms, pathogens and herbivores. There is a pressing need to optimize management practices aimed at maximizing production quality and stability, and to elucidate the mechanisms by which the plant and its microbiome respond to stress conditions. Some studies have advanced the characterization of the microbiome associated with *Cannabis sativa* L., highlighting its relationship with factors such as soil type, plant genotype, and developmental stage. Winston et al. (2014) analyzed the microbiota of five *Cannabis* spp. cultivars in rhizosphere, endorhiza, and bulk soil, finding that microbial composition in soil and rhizosphere correlated with edaphic properties, while the endorhizal community exhibited cultivar-specific structures. Complementarily, Comeau et al. (2020) investigated the dynamics of the microbiome across different growth stages and among cultivars of distinct chemotypes. They identified *Pseudomonadota* and *Actinomycetota* as the dominant phyla and reported significant shifts in microbial composition across developmental stages, with *Actinomycetota* increasing during flowering. No direct correlation was found between microbiome composition and cannabinoid profiles. Notably, cultivar-related differences were more pronounced during early growth stages.

One promising approach for enhancing the growth and overall health of cannabis plants involves the use of plant-growth-promoting bacteria (PGPBs) (Aunkam 2024). Interactions between *Cannabis* and PGPB have been reported in several studies, especially involving *Bacillus* spp. and *Pseudomonas* spp. (Aunkam 2024; Comeau 2021). *Pseudomonas* spp., including *P. fluorescens*, *P. protegens*, and *P. putida*, have been consistently identified as dominant members of the *Cannabis* microbiome across diverse cultivars and environmental conditions (Balthazar 2022). Rhizosphere microbiomes have co-evolved with their hosts to optimize nutrient acquisition and immune responses (Bakker et al. 2018). It was described that plants actively modulate their rhizospheric microbiome through root exudates, particularly under biotic stress. These compounds not only serve as nutrients but also act as chemical signals that promote interactions with beneficial microbes. Stress conditions can alter exudate composition, enabling plants to attract specific microbes that aid in stress mitigation. (Wankhade et al. 2025). Biotic stresses affecting plant–microbiome interactions have been predominantly studied in the context of pathogenic fungi, while insect-related stress remains less underexplored. Under insect-induced biotic stress, some studies in other plant species have shown relevant plant–microbe interactions. For example, in *Zea mays*, infestation by *Spodoptera littoralis* triggers the release of root exudates enriched in benzoxazinoids (BX), which promote the colonization of microorganisms capable of inducing systemic resistance (ISR) (Hu et al., 2018). This gap is particularly evident in *Cannabis sativa*, where the influence of environmental stressors on microbiome dynamics is still poorly understood.

Tetranychus urticae is one of the main agricultural pests of Cannabis, causing significant crop losses. This phytophagous mite feeds on mesophyll cells, piercing them and leading to chlorosis, chlorophyll degradation, reduced photosynthetic rate, stomatal closure, and tissue dehydration (Kostanda et al. 2022; Rosa-Díaz 2023). Damage caused by this pest was associated with altered production and profiles of secondary metabolites, including increased concentrations of cannabinoids and terpenes in inflorescences (Kostanda et al. 2022). Nevertheless, it is not yet established whether the effect of pest infestation on cannabinoid and terpene profiles is genotype-dependent, potentially varying among different cultivars. Although plant–pathogen–microbiome interactions were explored in other crops of agricultural interest, this approach remains limited in *Cannabis*. Therefore, the hypothesis of this study is that biotic stress induced by *Tetranychus urticae* triggers both structural reorganization of the *Cannabis sativa* L. rhizosphere microbiome, promoting microbial communities that are more stress-resilient but less beneficial for plant development. This shift is reflected in a reduction of plant growth-promoting bacteria (PGPB) in stressed plants, an increase in environmentally adapted taxa, and physiological changes in the plant, including reduced photosynthetic efficiency and alterations in floral terpene profiles. In this context, we worked with a local therapeutic variety of *Cannabis sativa* L. (Cepa Argentina Terapéutica 1 – Proyecto Cannabis y Salud – Jardín del Unicornio – CIM – CONICET – UNLP), comparing healthy plants and those subjected to biotic stress induced by *T. urticae*. We characterized the dynamics of the microbiome across three key stages of the life cycle—early vegetative, late vegetative, and late flowering—considering two microenvironments: the rhizosphere and bulk soil. Likewise, we assessed plant physiological parameters, terpene and cannabinoid floral profiles in order to analyze the impact of *T. urticae* on this cultivar.

Materials and methods

Cultivation of Cannabis plants

The study was conducted using the cultivar CAT 1, chemotype II, with a 2:1 CBD:THC ratio (Vaccarini 2025). This is a photoperiod-dependent cultivar, with a predominant terpene profile composed of myrcene, β -caryophyllene, and α -pinene. The experiment included a control group (healthy plants) and a biotic stress treatment (plants inoculated with *Tetranychus urticae*) over a 10-week period—comprising four weeks of vegetative growth and six weeks of flowering. Plants were cultivated indoors under controlled humidity and temperature conditions (25 °C and 50% RH), using separate grow tents for each treatment (1 meter deep and wide, 2 meters high). Lighting was provided by 300-watt LED panels (Bellavita, SMD 5000K + 660nm + 730nm), with a PPFD of 400 $\mu\text{mol}/\text{m}^2/\text{s}$ and an 18/6 h light/dark photoperiod during the vegetative stage, and 1000 $\mu\text{mol}/\text{m}^2/\text{s}$ with a 12/12 h photoperiod during flowering. Each tent was equipped with ventilation and air intake/extraction systems. Plants were grown in 7-liter pots containing a mixture of 80% commercial substrate (Growmix Multipro, Terrafertil) and 20% locally produced compost. The commercial substrate consists of Sphagnum peat moss, fine bark compost, perlite, and a pH adjuster. It has a moisture content of 55–65%, pH between 5.00 and 5.80, and electrical conductivity (EC) ranging from 0.20 to 0.60 mS/cm. The organic matter content is 80–85%, with ash content of 15–20%, and a carbon-to-nitrogen (C/N) ratio of 30–35. Soluble nutrients in saturated extract include nitrate (250–450 ppm), phosphate (30–100 ppm), and potassium (200–300 ppm). From a physical standpoint, it has a dry bulk density of 140–180 kg/m³, particle density of 1600 kg/m³, total porosity of 85–90%, water-holding capacity of 55–65%, and air porosity of 25–30%. Physical analyses were performed according to European standard UNE-EN 13041:2007. No additional fertilization or synthetic agrochemical treatments were applied during the cultivation period.

Plants were irrigated three times per week using water with an electrical conductivity of 500 $\mu\text{S}/\text{cm}$ and a pH of 6.0, at a volume equivalent to 10% of the pot volume (700 mL). A total of 18 cuttings were produced for the experiment, with 9 plants allocated to each treatment. Cuttings were obtained by cloning from two CAT 1 mother plants maintained under continuous vegetative growth in the same environmental conditions used in the trial. All tools used for cutting were sterilized with 70% ethanol. Fifteen-centimeter-long branches were cut at a 45° angle and placed in water for 24 hours. The basal portion of each stem was then gently scraped and treated with rooting hormone (King Clone, SKOG®), after which the cuttings were placed in an aeroponic cloning machine (La Máquina de hacer esquejes®) for 12 days until root development reached 10–12 cm. At that point, they were transferred to their final pots to initiate the vegetative stage. For the stress treatment, three *T. urticae* individuals (two females and one male) were inoculated on the first leaves of each plant using a fine brush at the start of the vegetative stage. The sex of the mites was determined under an optical microscope by observing abdominal morphology. Control plants were regularly inspected to ensure the absence of mites.

Secondary metabolite and physiological parameter analysis

Throughout the experiment, various physiological parameters were assessed at four key time points during cultivation: weeks 2, 4, 6, and 8. Week 2 corresponded to the early vegetative stage, week 4 to late vegetative, and week 8 to late flowering. Measurements of chlorophyll a fluorescence (Fv/Fm, ETR, and NPQ) were performed using modulated chlorophyll fluorescence (MINI-PAM II). Additional measurements included relative water content in leaves (RWC%), greenness index (SPAD-502 Minolta), and stomatal conductance (porometer Decagon SC-1). Chlorophyll fluorescence analyses were conducted on the youngest fully expanded leaves, previously dark-adapted for 30 minutes. The same type of leaves was also used for porometer and SPAD measurements, and for fluorescence measurements it was further ensured that the selected leaves were not shaded. RWC% was calculated by extracting three leaf discs per plant using 1 mL Eppendorf tubes. The discs were soaked in distilled water for 24 hours to obtain turgid weight, then dried at 30 °C until reaching constant weight. Relative water content was calculated based on the ratio between turgid and dry weight. At the end of the cycle, inflorescences were harvested and subjected to controlled drying at 18 °C and 50% relative humidity for 10 days, after which the total dry weight of flowers (g) was determined. The cannabinoid profile was analyzed by high-performance liquid chromatography (HPLC-UV DAD), following the protocol described by De Backer et al. (2009) with minor modifications (Sedan et al. 2021). Terpene content (expressed in ppm/g of dry flower) was determined using gas chromatography with flame ionization detection (GC-FID), according to the methodology described by Giese et al. (2015), with slight modifications (Bugvila 2022).

Soil microbiota sampling

To analyze the soil microbiome, samples were collected at three stages of the plant life cycle: early vegetative (week 2), late vegetative (week 4), and late flowering (week 8). At each time point, three plants per treatment were selected as biological replicates, which resulted in a progressive reduction in the total number of plants throughout the experiment. Samples were collected in sealed nylon bags and stored under refrigeration (4 °C) until processing. Plants were carefully removed from their pots, and samples were taken from two soil zones: bulk soil (the soil not attached to the roots) and the rhizosphere. To obtain the rhizospheric fraction, roots were shaken inside the sampling bags to detach the soil tightly adhered to the root surface, following the methodology described by Comeau et al. (2021). Total DNA was extracted from each individual sample using the Spin Soil DNA extraction kit (Macherey-Nagel) for subsequent metabarcoding analysis. Sequencing was performed by Novogen Inc. using the Illumina Novaseq platform, targeting the V4 region of the 16S rRNA gene with primers 515F/806R.

Bioinformatic and Statistical Analysis

Bioinformatic processing was performed using QIIME 2 (v. 2024-5). A total of 7,285,036 raw sequences were obtained and subjected to quality control using the q2-dada2 plugin, applying a quality-based trimming step to retain positions with Q scores > 30. Sequences were then dereplicated, merged, and chimera artifacts were removed. Taxonomic assignment of amplicon sequence variants (ASVs) was

performed using the pre-trained Naïve Bayes classifier available in QIIME 2, based on the SILVA 138 database (99% identity), specifically trimmed to the V4 region amplified with primers 515F/806R. Sequences classified as mitochondria, chloroplasts, or eukaryotes were removed. Additionally, a minimum frequency threshold of 0.01% was applied to eliminate sequencing errors inherent to the MiSeq platform. Rarefaction curves, alpha diversity metrics (Shannon index, observed richness) were performed in RStudio (version 2025.05.0+496) using the phyloseq, vegan, and ggplot2 packages. In addition, relative abundance bar plots were generated, and LEfSe analysis (Segata et al. 2011) was used to identify differentially represented taxa between conditions. Ternary plots were also constructed to explore distribution patterns of dominant genera across different developmental stages of the plant life cycle. Functional prediction analysis was performed with PICRUSt2 using the ASV table as input and the KEGG database for metabolic function annotation (Douglas et al., 2020). This approach generated a matrix of relative abundances for each KO, which was subsequently analyzed with the STAMP (Statistical Analysis of Metagenomic Profiles) software. Functional differences across treatments (control vs. stress), microenvironments (rhizosphere vs. bulk soil), and plant life cycle stages were evaluated using the non-parametric Kruskal–Wallis test, followed by the Games–Howell post-hoc comparison and multiple testing correction with the Benjamini–Hochberg false discovery rate (FDR). Additionally, effect size (η^2) was calculated to estimate the magnitude of the differences between groups.

Results

Physiological response to *Tetranychus urticae* infestation

Infestation by *Tetranychus urticae* progressed throughout the cultivation cycle, reaching its highest population density during the flowering stage. At this point, generalized chlorosis was observed on the leaves, along with the presence of webs covering both leaves and inflorescences (Fig. 2 C and D). Chlorophyll *a* fluorescence analyses revealed physiological alterations associated with the progression of biotic stress, showing increasing differences between treatments toward the final stage of cultivation, which coincided with the peak development of the pest. The Fv/Fm parameter showed a gradual decrease over time in both groups, with more pronounced differences during late flowering: 0.835 ± 0.03 (control) and 0.827 ± 0.01 (stress) in week 2; 0.763 ± 0.02 (control) and 0.724 ± 0.01 (stress) in week 4; 0.743 ± 0.01 (control) and 0.715 ± 0.01 (stress) in week 6; and 0.693 ± 0.01 (control) versus 0.631 ± 0.04 (stress) in week 8 (Fig. 3). Similarly, the effective quantum yield of photosystem II (Φ_{PSII}) showed a progressive decline: 0.692 ± 0.008 (control) and 0.701 ± 0.008 (stress) in week 2; 0.717 ± 0.01 (control) and 0.697 ± 0.02 (stress) in week 4; 0.639 ± 0.03 (control) and 0.586 ± 0.006 (stress) in week 6; and 0.559 ± 0.03 (control) versus 0.322 ± 0.053 (stress) in week 8. The electron transport rate (ETR) showed variable behavior between treatments: 41.4 ± 4.03 (control) and 42.0 ± 4.3 (stress) in week 2; 51.9 ± 9.3 (control) and 73.6 ± 3.69 (stress) in week 4; 111.0 ± 13.72 (control) and 91.8 ± 11.16 (stress) in week 6; and finally, 145.0 ± 12.29 (control) versus 80.8 ± 15.19 (stress) in week 8. Lastly, the non-photochemical quenching component (qNP) showed an increase in stressed plants toward the end of the experiment:

1.53 ± 0.24 (control) and 1.34 ± 0.14 (stress) in week 2; 1.14 ± 0.08 (control) and 1.23 ± 0.009 (stress) in week 4; 0.96 ± 0.03 (control) and 1.59 ± 0.10 (stress) in week 6; and 0.42 ± 0.27 (control) versus 2.44 ± 0.10 (stress) in week 8 (Fig. 3). These results showed a progressive reduction in photochemical efficiency and electron transport in stressed plants across the cultivation cycle, accompanied by an increasing diversion of excess energy into non-photochemical pathways, indicative of damage to photosystem II.

Leaf relative water content (RWC%) progressively decreased throughout the cultivation cycle, with more pronounced differences toward the end. In week 2 (early vegetative), the control treatment recorded a value of 74.3% ± 2.53, while biotically stressed plants reached 69% ± 2.31. In week 4 (late vegetative), values were 71.3% ± 3.51 for the control and 63.7% ± 2.78 for the stress treatment. In week 6, RWC was 70.9% ± 3.81 in control and 59.2% ± 2.31 in stressed plants. Finally, in week 8 (late flowering), RWC was 68.7% ± 1.53 in the control and 29.3% ± 3.79 under biotic stress, with statistically significant differences observed only at this final stage (p = 0.0009). Both the greenness index (SPAD) and stomatal conductance (SC) varied over time, with significant differences between treatments only during late flowering. At this stage, the greenness index was 58.5 ± 2.9 in the control and 16.9 ± 3.0 in stressed plants (p = 0.00006). Stomatal conductance was also significantly higher in the control group (167.2 ± 39.1 mmol·m⁻²·s⁻¹) compared to the biotically stressed group (75.9 ± 5.3 mmol·m⁻²·s⁻¹) (p = 0.0537) in the latest stage (Fig. 4). Biotic stress led to a decline in leaf water status, accompanied by reduced stomatal conductance and lower greenness index values, with the greatest impact observed during the flowering stage.

At the productive level, the average dry weight of inflorescences was 26.33 ± 0.70 grams in control plants, whereas it dropped to 16.13 ± 1.06 grams under biotic stress, representing a 38.7% reduction in floral yield. Regarding cannabinoid content, total CBD was 74.02 ± 1.3 mg/g in the control group and 74.95 ± 1.2 mg/g in stressed plants. For total THC, the values were 41.4 ± 0.8 mg/g and 47.7 ± 0.7 mg/g, respectively. The sum of total cannabinoids reached 132.09 ± 0.38 mg/g in the control and 133.65 ± 0.29 mg/g under stress. No significant differences were detected in cannabinoid content or profile between treatments. In contrast, total terpene content (ppm) showed significant differences between treatments (p = 0.009), being higher in biotically stressed plants (892.2 ± 65.2 ppm) compared to control plants (633.0 ± 53.8 ppm), representing a 40.9% increase. In addition to the increase in total terpene content, statistically significant differences were observed in the concentrations of individual terpenes. In particular, β-caryophyllene increased from 174.9 µg/g in control to 277.0 µg/g under biotic stress (p = 0.0017), α-humulene rose from 59.5 µg/g to 100.5 µg/g (p = 0.0022), and guaiaol increased from 38.6 µg/g in control to 56.7 µg/g under stress conditions (p = 0.0091).

Table 1. Quantification of secondary metabolites in *Cannabis sativa* L. under control and biotic stress conditions. Cannabinoid content is expressed as (mg/g) of flower dry weight, and inflorescence dry weight is expressed in grams. * indicates significant differences between treatments (p < 0.05).

Treatment	Control	Biotic Stress
Total CBD (mg/g)	74,02±1,3	74,95±1,2
Total THC (mg/g)	41,4 ±0,8	47,7±0,7
Total Cannabinoids (mg/g)	132,09 ±0,38	133,65 ±0,29
Inflorescences Dry Weight (g)	26,3±0,4	16,3±1,1*

Microbiome Diversity Analysis

The inoculation effect on bacterial community structure during the bioaugmentation process was studied through 16S rRNA gene metabarcoding at different times of incubation. Time points were selected considering the observed PAH elimination kinetics (days 15 and 58 for all treatments and day 0 only for the control). Rarefaction curves of the analyzed samples showed that the sequences retrieved were enough to cover most of the sample diversity. In the alpha diversity analysis (Shannon index), no statistically significant differences were found between groups; however, relevant patterns were observed in the mean values and data dispersion. (Supplementary material, Figure 1 and 2). Under control conditions, bulk soil showed a mean Shannon index of 6.63 with tightly clustered values (range: 6.58–6.67), while the rhizosphere had a similar mean (6.59) but displayed greater variability, including both the lowest (2.23) and highest (6.89) values in the dataset. Under biotic stress, both bulk soil (mean = 6.67) and rhizosphere (mean = 6.41) showed more homogeneous values and narrower ranges (bulk: 6.41–6.91; rhizosphere: 5.58–6.70) (Fig. 7). Although no statistically significant differences were detected between growth stages ($p > 0.05$), a decreasing trend in microbial diversity was observed from early vegetative (mean = 6.91) to late flowering (mean = 6.58). Data dispersion was highest during the flowering stage, particularly in rhizosphere samples under control conditions, where outliers were observed both on the low (min = 2.23) and high ends (max = 6.89). These results reflect a relative stability in alpha diversity throughout plant development, with greater heterogeneity during the final stage.

Community Composition at Phylum and Class Levels

At a global scale, the analysis of bacterial communities in soil and rhizosphere samples of *Cannabis sativa* throughout its life cycle and under different experimental conditions (control vs. biotic stress) revealed that both communities were dominated by the phyla *Pseudomonadota* (44.9%), *Bacteroidota* (10.2%), *Actinomycetota* (8.05%), *Acidobacteriota* (7.44%), and *Chloroflexota* (5.96%). The rhizosphere showed a slightly higher proportion of *Pseudomonadota* (47.0% to 42.8% in bulk soil), while bulk soil exhibited a somewhat greater abundance of *Acidobacteriota* (7.1% to 6.7% in the rhizosphere). Over the course of the plant's life cycle, the relative abundance of *Actinomycetota* increased progressively, from 5.61% in the early vegetative stage to 8.75% in the late vegetative stage, reaching 9.77% during late flowering. When comparing treatments, a reduction in the relative abundance of *Pseudomonadota* was observed under biotic stress (41.2%) compared to the control (48.6%). Conversely, phyla such as

Chloroflexota (7.45% under stress vs. 4.47% in control), *Gemmatimonadota* (6.04% under stress vs. 5.05% in control), and *Planctomycetota* (3.14% under stress vs. 2.44% in control) showed increased proportions under stress. Additionally, *Deinococcota* was exclusively detected in stressed samples (1.69%), and was absent from control treatments.

Genus-Level Shifts

Genus-level relative abundances were analyzed across different experimental conditions (Fig. 5). In the Control – Rhizosphere group, during early vegetative stage, the most abundant genera were *Massilia* (14.73%), *Rhodanobacter* (8.42%), *Burkholderia-Caballeronia-Paraburkholderia* (4.36%), *Devosia* (3.67%), and *Micropepsaceae-uncultured* (3.60%). In late vegetative stage, dominant genera included *Stenotrophomonas* (9.15%), *Enhydrobacter* (7.98%), *Brevundimonas* (6.52%), *Pseudomonas* (3.66%), and *Vicinamibacterales-uncultured* (3.26%). During late flowering, *Vicinamibacterales-uncultured* (3.81%), *Sandaracinaceae-uncultured* (3.76%), *Devosia* (2.90%), *Rhodanobacter* (2.82%), and *Gemmatimonadaceae-uncultured* (2.75%) were most abundant. Under Stress – Rhizosphere conditions, early vegetative stage was dominated by *Rhodanobacter* (5.51%), *Vicinamibacterales-uncultured* (4.32%), *SBR1031* (4.21%), *Massilia* (2.39%), and *Gemmatimonadaceae-uncultured* (2.36%). In late vegetative stage, most abundant genera were *Vicinamibacterales-uncultured* (5.45%), *Chryseolinea* (3.63%), *Gemmatimonadaceae-uncultured* (3.24%), *Devosia* (3.15%), and *Sandaracinaceae-uncultured* (2.51%). During late flowering, *SBR1031* (7.22%), *Sandaracinaceae* (3.51%), *Gemmatimonadaceae-uncultured* (3.19%), *Devosia* (2.85%), and *Chryseolinea* (2.69%) were dominant.

In the Control – Bulk Soil group, early vegetative stage was dominated by *Rhodanobacter* (7.48%), *S0134_terrestrial_group* (2.54%), *Vicinamibacterales-uncultured* (2.43%), and *Devosia* (2.40%). In late vegetative stage, *Vicinamibacterales-uncultured* (5.20%), *Devosia* (3.08%), *Sandaracinaceae-uncultured* (2.77%), *Blrii41* (2.69%), and *Chryseolinea* (2.35%) were the most abundant. In late flowering, dominant genera included *Vicinamibacterales-uncultured* (4.96%), *Sandaracinaceae-uncultured* (4.72%), *Chryseolinea* (3.36%), *Devosia* (3.17%), and *Microscillaceae-uncultured* (3.16%). In the Stress – Bulk Soil group, early vegetative stage included *Rhodanobacter* (5.57%), *Chryseolinea* (2.87%), *Devosia* (2.71%), *Blrii41* (2.61%), and *Vicinamibacterales-uncultured* (2.57%). In late vegetative stage, *Massilia* (4.71%), *Vicinamibacterales-uncultured* (4.45%), *Sandaracinaceae-uncultured* (4.10%), *Blrii41* (2.63%), and *Devosia* (2.49%) were most represented. This pattern was repeated in late flowering, with the same dominant genera.

Comparative ternary plots were generated for the Control and Biotic Stress groups, including only genera with relative abundance >1% in at least one life stage (Supplementary material, Fig. 6). The Control group showed clearer phenological stage differentiation, with characteristic genera associated with specific phases. In early vegetative stage, *Massilia* (~85% Early Veg), *Pandora* (70% Early Veg), *Rhodanobacter* (75% Early Veg), *Rhodoferrax* (65% Early Veg), and *Stenotrophomonas* (60% Early Veg) stood out. In the late vegetative stage, *Devosia* (60% Late Veg), *Massilia* (50% Late Veg), *Delftia* (80% Late Veg), and *Pandora* (65% Late Veg) were most prominent. In late flowering, *Enhydrobacter* (67% Late F) and

Chryseolinea (60% Late F) and Planctinomicetota - CL500-3 (60% Late F) were strongly associated, being absent or scarce in earlier stages. In contrast, the Stress group showed a more homogeneous distribution, without clear stage-specific segregation. Across the cycle, only a few genera showed marked preference for late flowering: *Devosia* (evenly distributed, slightly biased toward ~45% Late Veg, ~40% Late F), *IS-44* (~90–95% Late F), and *Enhydrobacter* (almost 100% Late F). Additionally, several plant growth-promoting bacteria (PGPB) genera observed in the Control group were absent under stress, while unique taxa such as *Truepera*, *Candidatus-Nitrosopumilus*, *SBR1031*, and *Lacunisphaera* appeared exclusively in the Stress group.

Differential LEfSe Analysis: Environmental Factors and Growth Stages

LEfSe analysis at the genus level (Fig. 4, Supplementary material Table 1 and 2.) identified differentially abundant taxa between Control and Stress conditions, as well as between microenvironments (rhizosphere and bulk soil). In the rhizosphere under control conditions, a significant enrichment of *Pseudomonas* (LDA = 3.93; $p = 0.0228$) and *Delftia* (LDA = 3.52; $p = 0.0085$) was detected. In contrast, under biotic stress, the rhizosphere showed higher relative abundance of *Gemmatimonadaceae* (LDA = 3.90; $p = 0.0096$). In bulk soil from the control group, enriched genera included *S0134_terrestrial_group* (LDA = 3.87; $p = 0.0167$), *Blrii41* (LDA = 3.85; $p = 0.0376$), *Rhodothermaceae* (LDA = 3.60; $p = 0.0074$), *MND1* (LDA = 3.51; $p = 0.0234$), and *TRA3-20* (LDA = 3.41; $p = 0.0023$). In contrast, in bulk soil under biotic stress, *Lacunisphaera* was the only differentially abundant genus identified (LDA = 3.80; $p = 0.0438$). Regarding the plant growth cycle, LEfSe analysis revealed significant differences in bacterial community composition among phenological stages, irrespective of treatment. In the early vegetative stage, enriched taxa included *Rhodanobacter* (LDA = 4.56; $p = 0.0001$) and an unclassified group (*Unknown*), LDA = 3.77; $p = 0.0082$). During the late vegetative stage, a significant increase in *Vicinamibacterales* (LDA = 4.08; $p = 0.0021$) and *Vicinamibacteraceae* (LDA = 3.61; $p = 0.0163$) was detected. In late flowering, differentially enriched genera included *Sandaracinaceae* (LDA = 4.24; $p = 6.5 \times 10^{-5}$), *Iamia* (LDA = 3.96; $p = 3.3 \times 10^{-6}$), *Microscillaceae* (LDA = 3.93; $p = 0.0087$), *Gemmatimonadaceae* (LDA = 3.84; $p = 0.025$), *Hirschia* (LDA = 3.78; $p = 1.5 \times 10^{-5}$), and *Steroidobacter* (LDA = 3.66; $p = 1.7 \times 10^{-5}$).

Predicted Pathways of the Cannabis Bacterial Microbiome

Functional prediction analysis performed with PICRUSt2 revealed differences across life cycle stages and microenvironments and provided the assignment of KOs to specific taxa within each experimental group (e.g., rhizosphere under stress at late flowering), enabling the linkage of functional profiles with dominant taxa and the subsequent discussion of their ecological roles (data not shown).

In the control group, major functions were primarily associated with growth and gene expression. High values were observed in genes related to protein synthesis (RPL24e [K02906], RPS30e [K02994]; 50S/30S ribosomal proteins), transcription (rpoB K03043; RNA polymerase β -subunit), and tRNA maturation/modification (TAN1/THUMPD1 [K14835], tRNA acetyltransferase; TRM5 [K07446]/PUS10 [K06179], tRNA modification), as well as genes involved in cell signaling and replication (Ser/Thr protein

kinase [K08884]). Additional functions included the initiation of membrane glycosylation (ALG7, dolichyl-phosphate GlcNAc phosphotransferase; glyco-conjugate biosynthesis) and the biosynthesis of lipopolysaccharides (LPS) (wbdC/mtfC [K03272], α -1,3-mannosyltransferase of the O-antigen) (Supplementary material figure 6). Under biotic stress, functions related to translation, transcription, tRNA modification, glycosylation, and LPS synthesis were markedly reduced, suggesting an energy-saving strategy and repression of microbial growth. At the same time, functions related to defense, metabolic reconfiguration, and resource recycling were enriched. Among these, *exoH* [K16555], involved in succinoglycan biosynthesis, promotes exopolysaccharide production and biofilm formation, enhancing protection and adherence in the rhizosphere. Complementarily, *ypdF* [K01265] (aminopeptidase) contributes to peptide degradation, while *pps/ppsA* [K01007] (pyruvate water dikinase) participates in phosphoenolpyruvate recycling, conferring metabolic flexibility. Finally, *bgtA* [K02030], encoding an ATP-binding protein of amino acid transport systems (arginine, lysine, histidine, and glutamine), is associated with efficient amino acid uptake and utilization (Supplementary material figure 6).

Discussion

The analyses conducted in this study revealed spatiotemporal variations in the bacterial microbiome associated with *Cannabis sativa* L. under both control and biotic stress conditions. In terms of alpha diversity, no statistically significant differences in the Shannon index were observed between treatments, microenvironments, or developmental stages ($p > 0.05$), suggesting a relative stability in bacterial community richness and evenness throughout the cultivation cycle. Our results align with those of Comeau et al. (2020), who also found no significant differences in alpha diversity across the phenological development of the plant. However, other authors reported a decline in diversity from bulk soil to the rhizosphere, reflecting a progressive filtering of the microbiome toward root tissues (Winston et al., 2023; Comeau et al. 2020). Despite the apparent stability in alpha diversity, biotic stress induced a notable reconfiguration in the taxonomic composition and abundance of the microbial community, with particularly strong effects in the rhizosphere. At a global level, the microbiome was dominated by the phyla *Pseudomonadota*, *Bacteroidota*, *Actinomycetota*, *Acidobacteriota*, and *Chloroflexota*, partially consistent with previous studies that describe a predominant bacterial core composed of *Pseudomonadota*, *Actinomycetota*, and *Acidobacteriota* in *Cannabis* (Winston et al. 2014; Comeau et al. 2020). The rhizosphere exhibited a higher proportion of *Pseudomonadota*, while bulk soil showed a greater relative abundance of *Acidobacteriota*, consistent with the patterns reported by Winston et al. (2014). Across the cultivation cycle, a progressive increase in *Actinomycetota* was observed, particularly during late flowering and under stress conditions—a pattern also reported by Comeau et al. (2020).

Although *Pseudomonadota* was the dominant phylum in both treatments, biotic stress induced a structural reconfiguration characterized by its relative decrease, accompanied by an increase in *Chloroflexota*, *Gemmatimonadota*, and *Planctomycetota*. This shift suggests that certain microbial groups may be adapted to, or selected under, adverse environmental conditions. The presence of *Chloroflexota* under stress is particularly noteworthy, as it includes microorganisms associated with

extreme environments and known for their abilities in inorganic carbon fixation, halogen utilization, denitrification processes, and active participation in biogeochemical cycles (Freches 2024). In our functional analysis, we found that Chloroflexi is linked to central carbon metabolism, amino acid biosynthesis and degradation, redox regulation, exopolysaccharide biosynthesis and biofilm formation. This is supported by the presence of several key KOs related to carbon metabolism: alcohol dehydrogenase (*adh* [K00001]), myo-inositol dehydrogenase (*io/G* [K00010]), malate dehydrogenases (*mdh* [K00019/K00024/K00029]), glyceraldehyde-3-phosphate dehydrogenase (*gapdh* [K00134]), and phosphofructokinase (*pfk* [K21071]); biofilm formation and polysaccharides: UDP-glucose 6-dehydrogenase (*ugd* [K00012]), *gtrB* [K20534], and *pslG* [K21000]; and secondary metabolism and stress response: glucose-6-phosphate dehydrogenase (*zwf* [K00036]), hydroxymethylglutaryl-CoA reductase (*mvaA* [K00054]), and *nnrR* (CRP/FNR regulator [K21564]). These features highlight their metabolic versatility and suggest an enhanced capacity to exploit root-derived substrates, maintain redox balance, and promote biofilm formation. The phylum *Gemmatimonadota* is commonly found in various soil types, ranging from agricultural and livestock soils to contaminated environments (Fawaz, 2013). A large proportion of reported members from this phylum have been detected in arid soils, suggesting adaptation to low-moisture environments (DeBruyn et al., 2011). In our dataset, members of this phylum carried functional markers consistent with osmotic adaptation, including proline dehydrogenase (*prodh* [K00318]), betaine-aldehyde dehydrogenase (*betB* [K00130]), and sorbitol dehydrogenase (*sord/gutB* [K00008]). Additionally, genes related to exopolysaccharide biosynthesis were present, such as UDP-glucose 6-dehydrogenase (*ugd* [K00012]), which supports both biofilm formation and water retention in the extracellular matrix.

In addition to these taxonomic shifts, functional predictions obtained with PICRUSt2 provided further insights into how the microbiome responds to biotic stress. Under control conditions, the rhizosphere community displayed a higher metabolic activity, enriched in pathways related to replication, transcription, translation, and cell wall biosynthesis, reflecting microbial microorganisms actively supporting plant growth. In contrast, stress conditions were associated with a reduction of these growth-related functions and an enrichment in pathways linked to defense, resource recycling, and metabolic flexibility, including exopolysaccharide biosynthesis and amino acid transport. These results suggest that biotic stress not only drives the microbiome structure but also drives a functional reorganization toward strategies that enhance microbial resilience and adaptation, even at the expense of processes beneficial for plant development.

Rhizosphere vs. Bulk Soil

When comparing the rhizosphere and bulk soil at the functional level, the rhizosphere was enriched in pathways related to protein synthesis, transcription, tRNA maturation, and the biosynthesis of membrane components and polysaccharides. This profile suggests a metabolically more active microbial community oriented toward growth. In contrast, bulk soil exhibited a lower representation of biosynthetic functions, reflecting a less dynamic microbial metabolism. At the genus level, within the Control group and specifically in the rhizosphere, a high abundance of plant growth-promoting bacteria (PGPB) was

observed, including *Massilia*, *Devosia*, *Burkholderia-Caballeronia-Paraburkholderia*, *Stenotrophomonas*, *Brevundimonas*, and *Pseudomonas*. LEfSe differential analysis identified *Pseudomonas* and *Delftia* as discriminant genera, along with the families *Oxalobacteraceae*, *Pseudomonadaceae*, *Rhizobiaceae*, *Xanthobacteraceae*, *Nitrosomonadaceae*, and *Rhodothermaceae*, all primarily represented in the rhizosphere under control conditions.

Many of these taxa are well-documented for their beneficial roles in plant development. For example, *Massilia* has root-colonizing abilities and growth-promoting effects (Gerrewey et al. 2021) while the *Burkholderia-Caballeronia-Paraburkholderia* complex is associated with nitrogen recycling, mineral solubilization, and phytohormone production (Wallnery et al., 2022). *Devosia* also includes PGPB-active members (Chhetri et al. 2022). Among the most prominent genera, *Pseudomonas* has been shown to improve yield and quality in cannabis cultivation, as well as to confer protection against biotic and abiotic stresses (Balthazar et al. 2022; Comeau et al. 2021). The functional predictions revealed that *Massilia*, *Devosia*, *Pseudomonas*, and the *Burkholderia–Caballeronia–Paraburkholderia* complex harbor multiple markers linked to plant-beneficial pathways. Among these, indole-3-glycerol phosphate synthase (*trpC* [K01609]) and indolepyruvate ferredoxin oxidoreductase [K04090] indicate the potential for tryptophan-dependent IAA biosynthesis. In parallel, alkaline phosphatases (*phoA/phoB* [K01077]; *phoD* [K01113]) highlight pathways associated with phosphate solubilization. Additional functions linked to nitrogen cycling were also consistently detected across these taxa, including nitrite reductases (*nirB/nirD* [K00362/K00363]), multiple subunits of nitrate/nitrite oxidoreductases (*narGHIJ*, *nxrAB* [K00370–K00374]), and assimilatory nitrate reductases (*nasA/C* [K00372]). Collectively, these features reinforce the role of these bacteria in nutrient turnover and growth promotion of *Cannabis sativa*.

Under biotic stress, LEfSe analysis identified only *Gemmatimonadaceae* as a discriminant taxon in the rhizosphere. A marked absence or reduction of the aforementioned PGPB genera was observed, with the exception of *Massilia* and *Devosia*, which were present in low abundance. Instead, taxa that were not detected under control conditions dominated, such as *SBR1031* (*Chloroflexota*), *Gemmatimonadaceae* (*Gemmatimonadota*), and *Vicinamibacterales* (*Acidobacteriota*), suggesting a functional shift in the microbial community under stress. The data reflect a stress-driven reconfiguration of the rhizosphere microbiome, evidenced by a reduced presence of beneficial bacterial genera compared to control conditions, and a proliferation of *Gemmatimonadaceae* and *SBR1031* (*Chloroflexota*) under stress. *Gemmatimonadaceae* has been associated with soils characterized by low moisture and low carbon content (Bao, 2025) while *SBR1031* has been linked to phenolic compound degradation and anaerobic fermentation (Cai et al., 2025). In our dataset, both groups carried functions linked to osmotic adaptation, such as proline dehydrogenase (*prodh* [K00318]) and betaine-related pathways including betaine-aldehyde dehydrogenase (*betB* [K00130]), together with UDP-glucose 6-dehydrogenase (*ugd* [K00012]) involved in exopolysaccharide biosynthesis and biofilm formation. *SBR1031* encoded enzymes like quercetin 2,3-dioxygenase (*pir* [K00454]) and NADP-dependent alcohol dehydrogenase (*yqhD* [K00001]), suggesting specialization in phenolic compound degradation and root-exudate metabolism, whereas *Gemmatimonadaceae* was characterized by metal resistance systems (ArsR regulators [K03892], *czcD/zitB* efflux pumps [K03455]) and fermentative metabolism (*ldhA* [K00016]), conferring resilience in

nutrient- or oxygen-limited niches. Notably, *Gemmatimonadaceae* also carried the predicted marker indole-3-glycerol phosphate synthase (*trpC* [K01609]), indicating a potential capacity for tryptophan-dependent IAA biosynthesis. In addition, both groups harbored predicted markers of phosphorus mobilization, including alkaline phosphatases (*phoD* [K01113], *phoA* [K01077]) and the Pho regulon regulator (*phoB* [K07657]), suggesting a shared ability to contribute to organic phosphorus mineralization under limiting conditions. Collectively, these features indicate that biotic stress selectively enriches bacterial lineages capable of combining resource utilization, biofilm-mediated persistence, nutrient mobilization, and resistance to environmental stressors, thereby restructuring the rhizosphere microbiome. Biotic stress reduced the number of differentially enriched taxa, particularly in the rhizosphere, indicating a loss of functional complexity under stress, with *Gemmatimonadaceae* and SBR1031 emerging as a key taxa.

In contrast to the rhizosphere, the bacterial community in bulk soil exhibited greater compositional stability between treatments. While the rhizosphere showed higher sensitivity to biotic stress, bulk soil maintained a relatively consistent set of taxa such as *Devosia*, *Vicinamibacterales*, *Chryseolinea*, and *Blrii41*. Under control conditions, LEfSe analysis identified *S0134_terrestrial_group*, *Blrii41*, *MND1*, and *TRA3-20* as discriminant taxa—groups previously reported in agricultural soils subjected to biological or chemical treatments (Wu et al. 2024). Enriched families also included *Nitrosomonadaceae*, *Nitrososphaeraceae*, and *Vicinamibacterales*, the latter known for its ability to inhabit both the rhizosphere and bulk soil (Wei et al. 2023). In stressed bulk soil, *Lacunisphaera* stood out as a differentially abundant taxon. The functional analysis of *Lacunisphaera* revealed the presence of genes associated with metal resistance and homeostasis, including zinc D-Ala-D-Ala carboxypeptidase (*vanY* [K08641]), zinc protease (*pqqL* [K08642]), as well as $Zn^{2+}/Mn^{2+}/Fe^{2+}$ transporters (*troA/mntA/znuA* [K02009], *troB/mntB/znuC* [K02010/K02011]). These are complemented by the regulators and enzymes of the arsenic system, including the transcriptional repressor (*arsR* [K03892]) and arsenate reductase (*arsC* [K00537]), responsible for the detection and detoxification of As(V), together with Fe^{2+} transporters (*feoA* [K04759], *feoB* [K04758]) operating under reducing conditions.

These findings suggest that the rhizosphere responds in a more dynamic and specific manner to changes in plant physiological state and environmental conditions, harboring taxa more directly associated with plant–microbe interactions. In contrast, bulk soil represents a more conservative environment, dominated by generalist edaphic taxa that persist regardless of plant status.

Changes Across the Life Cycle

Throughout the life cycle of *Cannabis sativa*, changes in the structure of the soil microbial community were observed. At a general level, LEfSe analysis highlighted *Rhodanobacteraceae* as a biomarker during the early vegetative stage. This family, present under both control and stress conditions, maintained high relative abundances and has been associated with nitrogen reduction processes in soil (Huang, 2021). This was also shown through PICRUSt2 predictions, where enrichment of key denitrification genes was observed, including nitrate reductase subunits (*narGHIJ* [K00370–K00374]), nitrite reductases (*nirS* [K15864], *nirK* [K00368]), nitric oxide reductase (*norB* [K04561]), and nitrous oxide reductase (*nosZ*

[K00376]). In the early vegetative stage under control conditions, the rhizosphere showed high abundances of PGPB taxa, including *Massilia*, *Burkholderia-Caballeronia-Paraburkholderia*, and *Devosia*. In contrast, the rhizosphere microbiome of stressed plants in this stage displayed a distinct structure, with the presence of alternative functional groups such as *Gemmatimonadaceae*, members of the phylum *Chloroflexota* (e.g., *SBR1031*), and *Vicinamibacterales*. Although the impact of *Tetranychus urticae* in plants was still low at this stage compared to later phases, these early differences between treatments suggest an initial reconfiguration of the microbial community, in which some groups that may be occupying the ecological niche of the *Pseudomonadota* PGPB taxa dominant under the control conditions.

During the late vegetative stage, LEfSe analysis identified *Vicinamibacteraceae*, *Comamonadaceae*, and *Flavobacteriaceae* as differentially abundant taxa. These groups are linked to organic matter recycling, polymer degradation, and participation in soil trophic networks (Willems 2014; Meng et al. 2025). PICRUSt2 analysis predicted that *Vicinamibacteraceae* encoded enzymes for the degradation of structural polymers and aromatics, such as cellulases/endoglucanases (*celA* [K01179]), xylanases (*xynA* [K01181]), lignin peroxidases (*lip* [K00430]) and protocatechuate dioxygenase (*pcaG/H* [K00448/K00449]), suggesting a role as primary degraders. *Comamonadaceae* were predicted to be associated with intermediate metabolite processing and sulfur mobilization, supported by sulfite reductase (*cysI/J* [K00380/K00381]) and alkanesulfonate monooxygenase (*ssuD* [K04091]). *Flavobacteriaceae* combined both strategies, showing versatile functions in polymer breakdown (β -glucosidase [K05349], chitinase [K01183]) and trophic interactions via cross-feeding of aromatic intermediates. Under control conditions, the dominant genera included *Stenotrophomonas*, *Enhydrobacter*, *Brevundimonas*, and *Pseudomonas*, several of which—particularly *Pseudomonas*—are well known for their PGPB characteristics during active plant growth. Although beneficial microorganisms remained present, a transition in dominant composition was evident, as families such as *Burkholderiaceae* and *Rhodanobacteraceae*, which had been predominant in earlier stages, were no longer detected. Under biotic stress, the high abundance of *Vicinamibacteraceae* observed previously persisted, while *Gemmatimonadaceae* further increased its relative abundance. *Chryseolinea* and *Devosia* were also notable in this stage, though genera like *Rhodanobacter*, *Massilia*, and *SBR1031* showed decreased abundance.

In late flowering, the main biomarkers identified (LDA > 3) included the families *Sandaracinaceae*, *Iamiaceae*, and *Gemmatimonadaceae*. The first two are rarely reported in plant-associated contexts and are linked to polysaccharide degradation (Mohr et al., 2012). In *Cannabis sativa* L. the flowering stage is characterized by high phosphorus demand, with this nutrient being transported to floral tissues, impacting leaf biomass and cannabinoid production (Shiponi 2021). In this context, the presence of *Gemmatimonadaceae* may contribute to phosphorus availability in soil during this stage. Wen et al. (2025) reported that their abundance correlates positively with available phosphorus in agricultural soils. Consistently, PICRUSt2 predictions indicated the presence of key phosphatases such as acid phosphatase (*phoN* [K09474]), alkaline phosphatase (*phoD* [K01113]), and exopolyphosphatase (*ppx*-

gppA [K01525]), in the *Gemmatimonadaceae* family, supporting the role of this taxon in phosphate bioavailability.

Under control conditions, while PGPB genera from the phylum *Pseudomonadota* predominated in vegetative stage, later flowering was marked by an increase in phyla such as *Acidobacteriota* (*Vicinamibacterales*), *Myxococcota* (*Sandaracinaceae*), and *Bacteroidota* (*Chryseolinea*), suggesting a microbial succession pattern, with genera related to plant growth dominating vegetative phases, and a transition toward more generalist taxa during flowering. In biotically stressed plants, *SBR1031* (*Chloroflexota*) reached its highest relative abundance in the late flowering stage, becoming the dominant taxon in the rhizosphere. Additionally, groups detected in earlier stages, such as *Gemmatimonadaceae*, remained highly abundant throughout the cycle under this treatment. Overall, the stressed rhizosphere showed a succession of taxa associated with soil nutrient degradation, while PGPB genera like *Devosia* and *Chryseolinea* remained in low abundance. The dominance of *SBR1031* in late flowering is particularly noteworthy, as there are no prior reports linking this genus specifically to rhizospheric associations in plants. Its high abundance may be related to the peak of physiological stress recorded in the plants during this stage, suggesting a microbial shift in soil composition induced by biotic stress.

Throughout the developmental cycle of *Cannabis sativa*, bulk soil exhibited a relatively stable microbial composition, with persistent presence of typically edaphic genera such as *Vicinamibacterales*, *Chryseolinea*, and *Devosia*, under both control and biotic stress conditions. Although subtle differences between treatments were observed, the overall community structure remained relatively constant. *Vicinamibacterales* is known for its ability to degrade complex organic compounds (Wei, 2024). Our functional predictions revealed multiple enzymes, including endoglucanases [K01179], xylanases [K01181], chitinases [K01183], and aromatic compound degradation enzymes such as protocatechuate dioxygenase (*pcaG/H* [K00448/K00449]) and catechol 1,2-dioxygenase (*catA* [K03381]), supporting their role as primary degraders of complex organic matter. Under control conditions, genera such as *Rhodanobacter* were more abundant during early stages, while *Vicinamibacterales* dominated during late vegetative and flowering phases. In bulk soil under biotic stress, the microbial composition was similar to that of the control, although with increased representation of *Massilia*, a genus with known plant growth-promoting functions, and *Blrii41*, a group associated with organic soils and decomposition processes (Zhang et al. 2025).

Physiological and Metabolic Responses to Biotic Stress

In our experiment, *Cannabis sativa* L. cultivar CAT 1 exhibited physiological responses typically associated with *T. urticae* damage, including stomatal closure, loss of turgor, and decreased photosynthetic parameters. Differences between treatments were most pronounced during the late flowering stage, coinciding with the visible symptoms such as leaf chlorosis and webbing around leaves and inflorescences. Chlorophyll *a* fluorescence analysis, which estimates the amount of absorbed light energy directed toward photochemistry, enabled us to assess the functional status of photosystem II (PSII) under biotic stress conditions (Moustakas 2022). Parameters such as maximum quantum

efficiency (Fv/Fm), effective quantum yield (Φ PSII or Y(II)), and electron transport rate (ETR) all showed reductions under stress. Conversely, non-photochemical quenching (NPQ), associated with energy dissipation as heat, increased compared to the control, confirming a loss of efficiency in light energy utilization that would otherwise be allocated to photochemistry. The Fv/Fm parameter, which reflects the percentage of functional photosystem II reaction centers, is particularly informative: values around 0.8 are typically considered normal, whereas declines indicate damage to the photosynthetic apparatus (Tambussi & Graciano, 2010). A gradual decrease in control plants is expected as the growth cycle progresses, especially during late flowering, while stressed plants exhibit an even stronger reduction due to stress-induced damage. Decreased Φ PSII and Fv/Fm values, together with elevated NPQ, are commonly observed under environmental stress and have been documented in leaves affected by agricultural pests (Pérez-Bueno, 2019). Similarly, low Fv/Fm and greenness index values were reported in *Cannabis* under biotic stress caused by *Sclerotinia sclerotiorum* (Rodríguez-Yzquierdo et al., 2023).

Leaf relative water content (RWC%) was also affected by biotic stress, showing a progressive decline throughout the plant's life cycle. During the late flowering stage, RWC% dropped to 29.3% in infested plants, compared to 68.7% in control plants. The greenness index, an indirect measure of chlorophyll concentration and thus of the plant's nutritional status, also decreased significantly under stress. While control plants maintained values within the optimal range reported for *Cannabis* (40–60 SPAD units; Tang et al. 2017), infested plants exhibited an average of 16.9 ± 3.0 , reflecting a substantial decline in chlorophyll levels. These results can be explained by the feeding behavior of *T. urticae*, a phytophagous mite that punctures leaf underside cells using its stylet, leading to cell content loss, mesophyll tissue damage, and tissue dehydration—all of which negatively impact photosynthesis (Kostanda 2022). Stomatal conductance was also significantly reduced under biotic stress during late flowering, in line with studies indicating that herbivory can trigger stomatal closure as a defense mechanism (Lin et al. 2022). Stomatal closure, in addition to regulating water loss, hinders mite stylet access through natural openings, forcing insertion between epidermal cells (Díaz et al. 2023). These findings point to progressive dehydration in cultivar CAT 1 as a consequence of mite infestation, compromising the plant's water balance. As a response, plants exhibited compensatory mechanisms such as reduced stomatal conductance, a common reaction to spider mite attack. However, this comes at the cost of limiting CO₂ uptake, which negatively affects photosynthesis and vegetative growth (Taiz and Zeiger 2003). The observed 38.7% reduction in floral yield under stress conditions may result from damage to photosystems, reduced chlorophyll levels, and stomatal closure. Chloroplast degradation caused by rising mite populations ultimately impacts both the quantity and quality of crop production (Mutthuraju et al. 2024).

Regarding secondary metabolites, a 40.9% increase in total terpene content was observed under stress, particularly in β -caryophyllene, α -humulene, and guaiaol. This trend aligns with prior findings: plants respond to herbivory by increasing the synthesis and emission of volatile compounds such as terpenes, which serve defensive or signaling functions (Kutty & Mishra 2023). The response observed in CAT 1 mirrors that reported by Kostanda and Khatib (2022), who also documented the accumulation of β -caryophyllene and α -humulene in plants infested by *T. urticae*, suggesting a conserved metabolic

response. However, unlike their findings, our study detected no significant differences in total cannabinoid (CNB) concentrations, which may be due to genotypic differences among the cultivars analyzed. This indicates that the metabolic response to biotic stressors is not only influenced by the nature of the stress but also by the specific genetic profile of each *Cannabis sativa* L. variety.

Conclusion

Overall, the results analyzed confirm our hypothesis that the presence of *Tetranychus urticae* profoundly alters plant physiology, floral yield and metabolites of therapeutic interest, as well as the soil microbiome associated with the rhizosphere. We propose that stress induced by *T. urticae* in the CAT 1 cultivar promotes a functional and taxonomic reconfiguration of the microbiome, in which beneficial groups are replaced by taxa more tolerant to adverse conditions. Under stress conditions, a lower presence of PGPB groups was observed, along with a greater abundance of taxa related to nutrient degradation and solubilization processes in altered soils. Among the discriminant taxa identified under these conditions, the family Gemmatimonadaceae stood out, together with the increased relative abundance of *SBR1031* (*Chloroflexota*), particularly during the late flowering stage. These findings represent key aspects of the rhizosphere microbiota restructuring. Although *Gemmatimonadaceae* has been widely reported as a taxon associated with abiotic stress conditions—such as high salinity, drought, or low nutrient availability—the results of this study reveal its prominent presence under biotic stress induced by *Tetranychus urticae*. This observation suggests that its resilience may not be limited to physicochemical factors alone, but could also extend to ecosystem imbalances generated by biotic interactions such as herbivory. In this context, we propose *Gemmatimonadaceae* as a potential microbial biomarker of stress in agroecosystems, given its ability to persist and increase in relative abundance under both abiotic and biotic adverse conditions.

Infestation by *T. urticae* not only causes visible damage to foliar tissues in *Cannabis*, but also induces significant alterations in the soil microbial community, potentially affecting associations with plant growth-promoting bacteria. This restructuring, which does not necessarily imply a loss of diversity, could have profound ecological implications in the functionality of the soil ecosystem and the plant's ability to sustain beneficial relationships under environmental pressure. Our results show that the effects of aerial biotic stress can extend to belowground compartments, altering key processes such as nutrient acquisition and physiological homeostasis. In this context, identifying genotypes capable of sustaining critical physiological functions under biotic pressure—together with a resilient microbiome—represents a promising strategy for characterizing therapeutic cannabis cultivars adapted to sustainable, high-quality production systems. Understanding the impact of pests beyond the aerial parts of the plant allows for the integration of more holistic agronomic approaches and the design of management strategies tailored to medicinal crops. This study provides novel evidence of the interaction between insect, plant, and microbiota, with both agronomic and biotechnological implications. The findings reinforce the importance of integrating eco-physiological and microbiological perspectives to understand plant responses to stress conditions.

Declarations

Author contribution

All authors contributed to the study conception, design, material preparation, data collection, and analysis. The first draft of the manuscript was written by Lucas Pinto, Patricio de Urraza, Bibiana Coppotelli and Darío Andrinolo. All authors commented on previous versions of the manuscript, read, and approved the final manuscript.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on request.

Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

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Figures

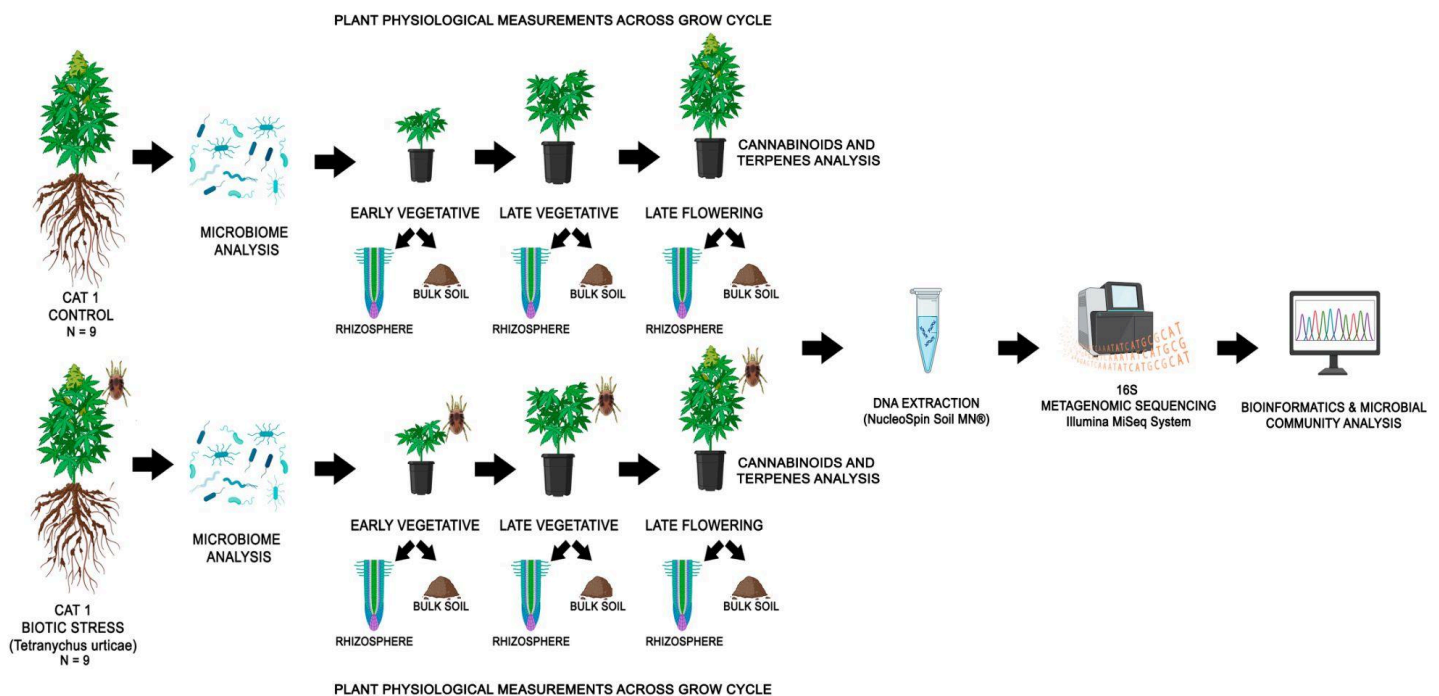


Figure 1

Graphical scheme of the experimental design. Overview of the experimental workflow applied to CAT 1 under control and biotic stress conditions. The scheme illustrates the main experimental stages: microbiome analysis in rhizosphere and bulk soil across the grow cycle, plant physiological measurements, cannabinoid and terpene profiling, DNA extraction, 16S rRNA gene sequencing using Illumina MiSeq, and microbial community bioinformatics analysis.



Figure 2

Images of CAT 1 at the late flowering stage. Panels a and c correspond to the control group, while b and d show plants under biotic stress. Note the webbing produced by the mite and the aggregation of individuals at the apex of the inflorescence, which is absent in the control group.

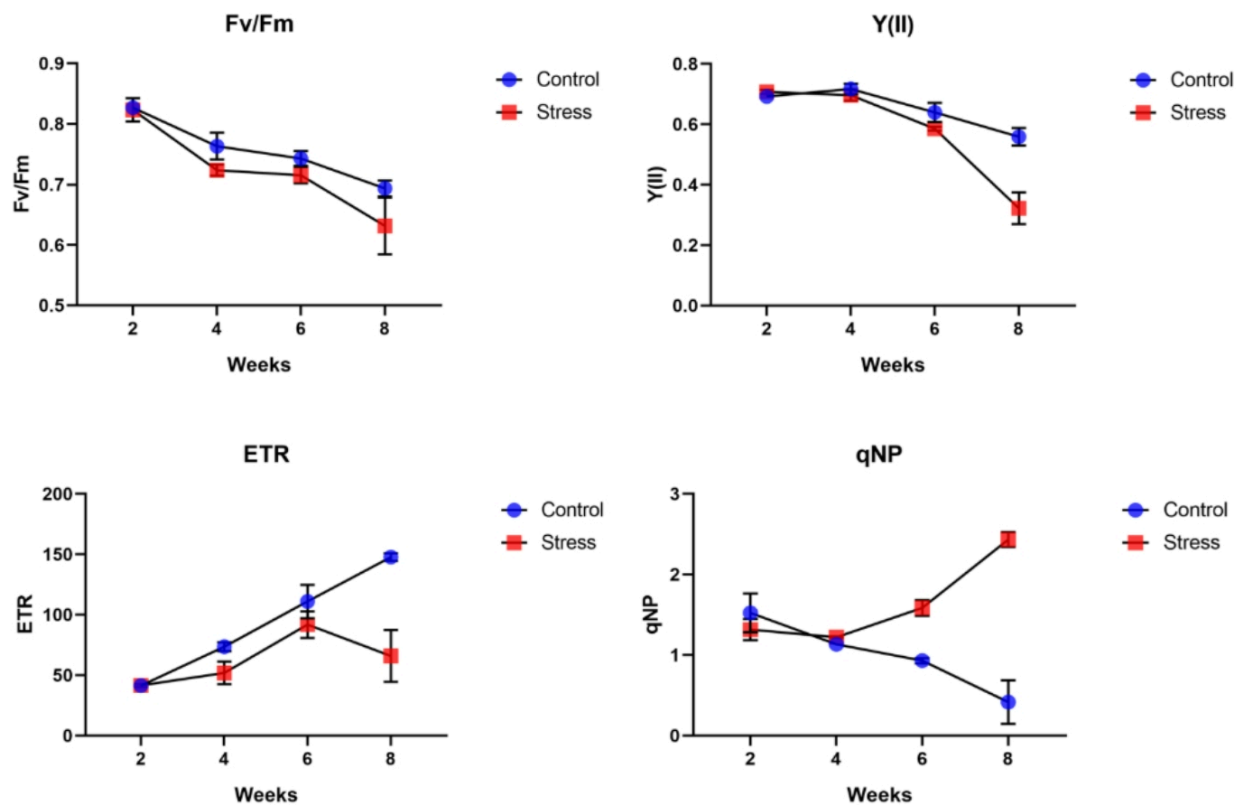


Figure 3

Variation of chlorophyll *a* fluorescence parameters (Fv/Fm, Φ PSII, ETR, and qNP) in *Cannabis sativa* L. plants under control and biotic stress treatments throughout the life cycle.

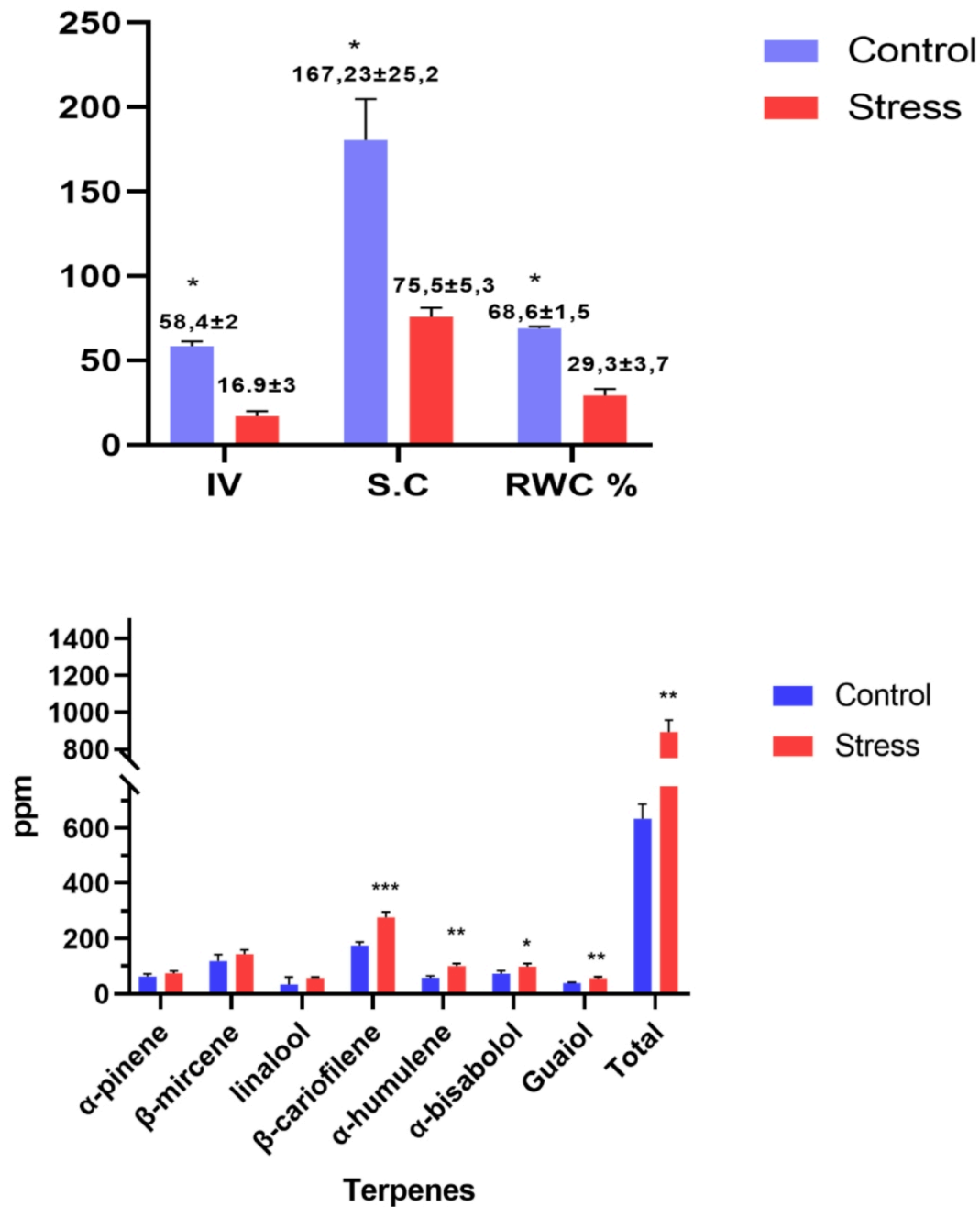


Figure 4

Physiological parameters in *Cannabis sativa* under control and biotic stress conditions. Top: Bar graph showing greenness index, stomatal conductance, and relative water content (RWC %). Bottom: Bar graph showing terpene content (ppm) in floral tissues. Asterisks indicate significant differences: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ (FDR-corrected). Data are presented as mean $\pm$ standard deviation ($n = 3$).

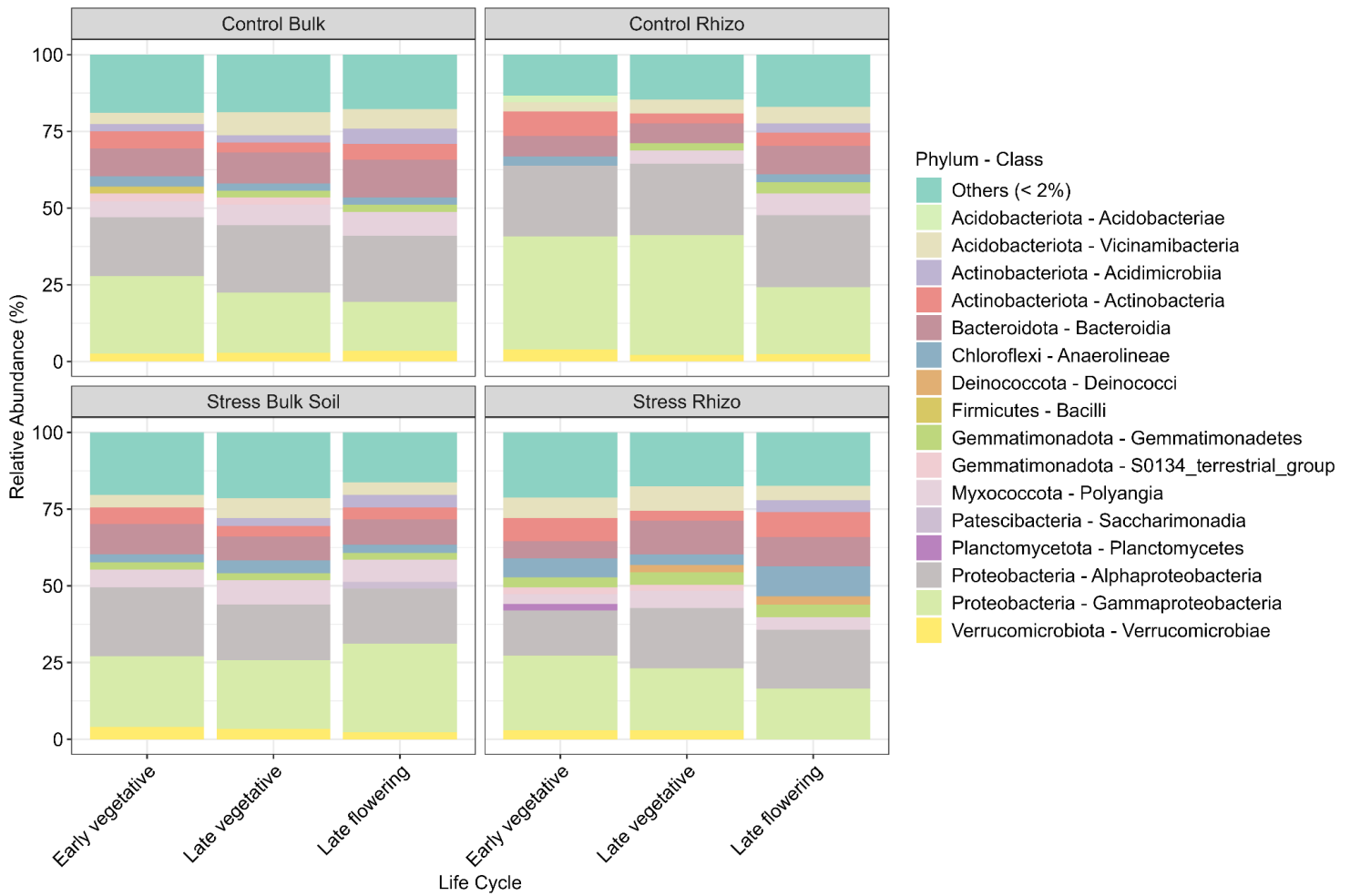


Figure 5

Bar plots showing the relative abundance of the bacterial community in soil and rhizosphere of *Cannabis sativa* at the phylum–class level, according to microenvironment (bulk vs. rhizosphere), experimental condition (control vs. biotic stress), and life cycle stage (early vegetative, late vegetative, and late flowering). Bars represent the average relative abundance of each taxon across experimental combinations. Taxa with relative abundance < 2% were grouped as “Others.”

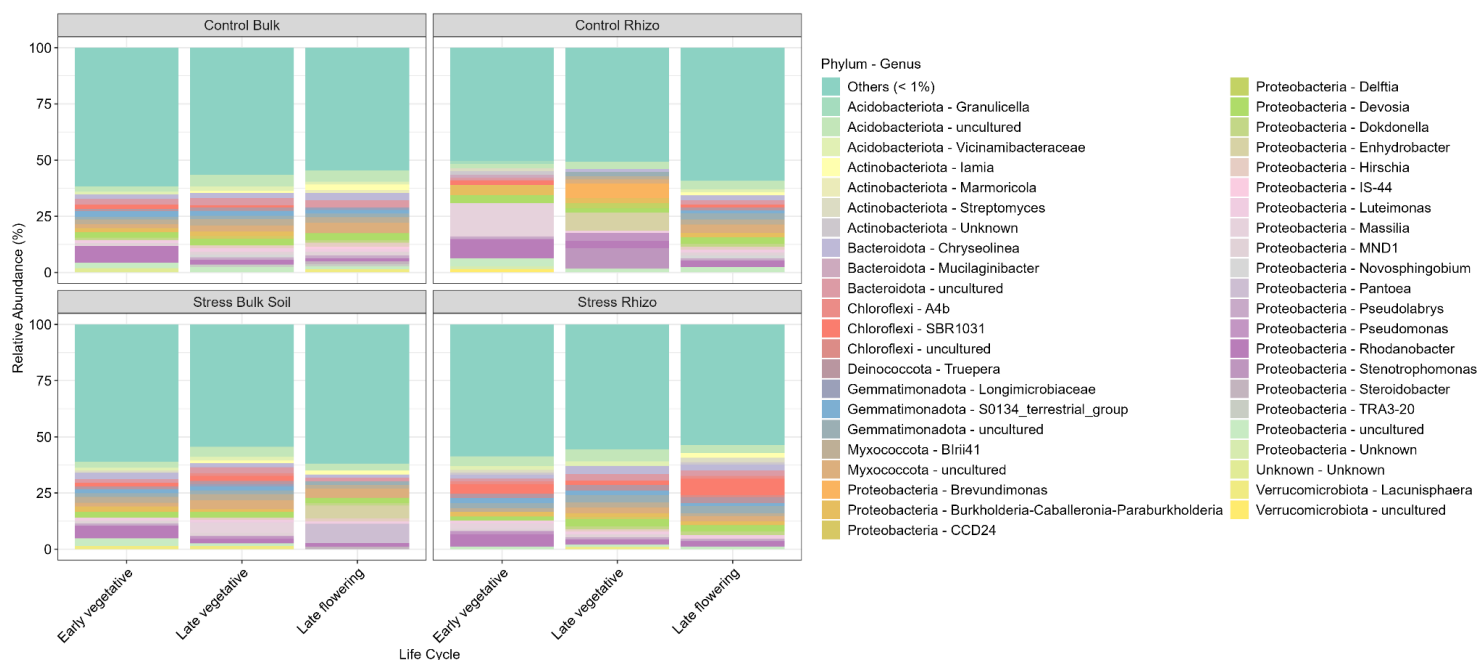


Figure 6

Barplots of the relative abundance of bacterial genera in bulk soil and rhizosphere of *Cannabis sativa* under control and biotic stress conditions, across three plant life cycle stages (early vegetative, late vegetative, and late flowering). The plot displays the average proportions of genera with relative abundance $\geq 1\%$, color-coded by phylum and labeled by genus. Genera with relative abundance $< 1\%$ were grouped as "Others." These genera together accounted for more than 50% of the total community composition. The values shown represent the average of biological triplicates for each treatment.

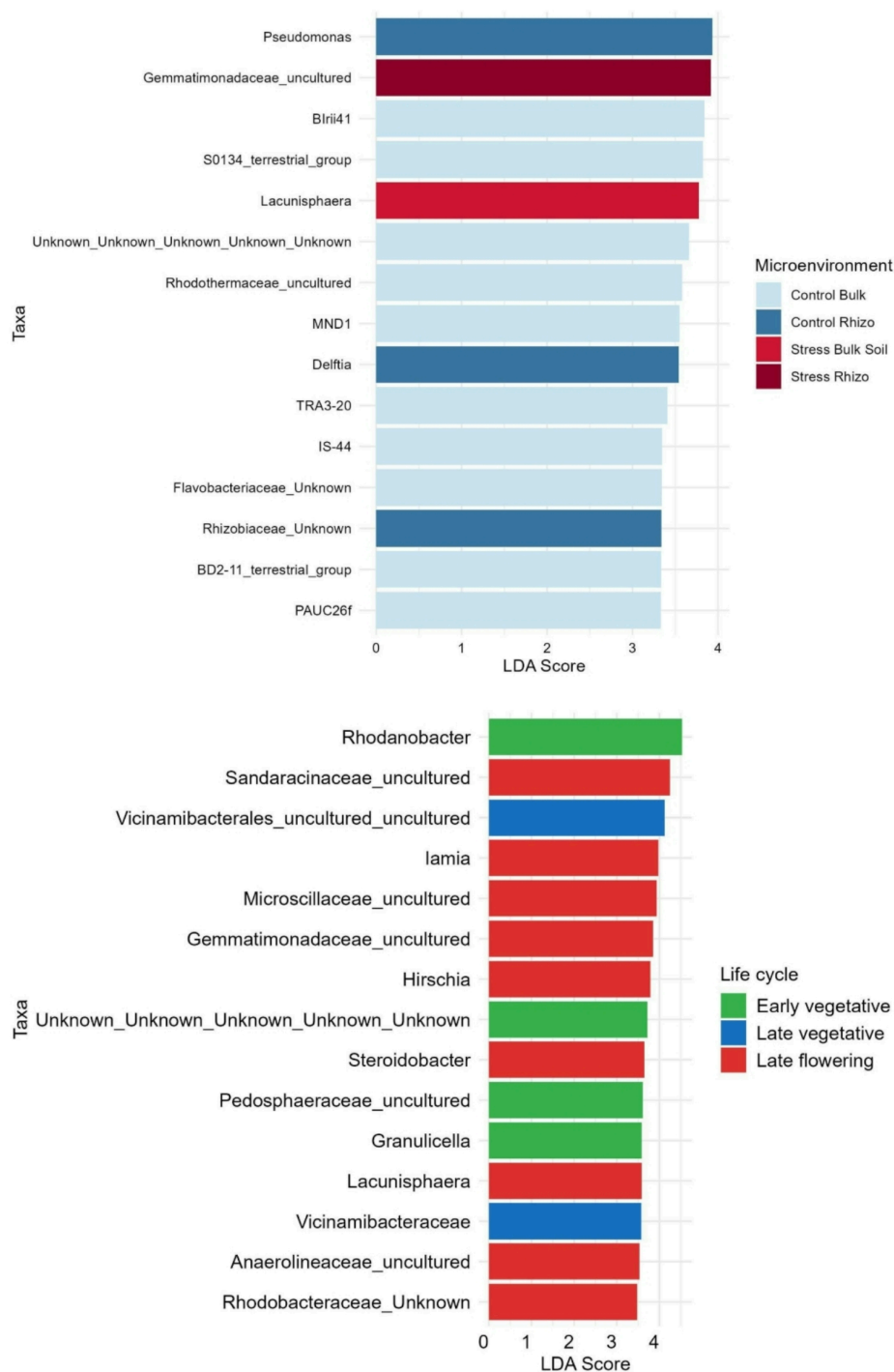


Figure 7

Fig. 8.a Top: LEfSe analysis at the genus level integrating both control and biotic stress treatments, as well as microenvironment (rhizosphere and bulk soil). Bottom: LEfSe analysis at the genus level grouped by plant life cycle stages (early vegetative, late vegetative, and late flowering).

Supplementary Files

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