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Cover crop-based under-row dry mulching enhances phyllosphere and rhizosphere microbial biodiversity in a non-irrigated vineyard

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Abstract

Background In viticulture, temporary cover crops and organic mulching are sustainable practices that enhance biodiversity, improve soil fertility, and strengthen grapevine health/resilience, particularly in Mediterranean regions. However, their impact on microbial communities associated with grapevines in non-irrigated vineyards remains largely unexplored. Inter-row soil management included a cereal-based cover crop (CC), a mixed cereals, legumes and brassicas cover crop (MC), and a control with alternating soil tillage and spontaneous grass (GT). In spring, cover crops were terminated to form a dry mulch under the vine rows.

Results At veraison, under-row dry mulching significantly maintained higher soil water availability and reduced soil temperature by approximately 2.5 °C compared to the GT treatment. CC, in particular, enhanced grapevine physiological performances. These different soil conditions positively shaped the rhizosphere microbiome by maintaining higher microbial richness and promoting nutrient-cycling microorganisms (e.g., *Bradyrhizobium* sp., *Nitrospira japonica*) in both CC and MC. In contrast, the GT treatment selectively favored drought-tolerant plant growth-promoting rhizobacteria (PGPR) taxa such as *Bacillus zanthoxyli*, *Gaiella occulta*, *Roseiflexus* sp., *Pseudarthrobacter* sp., and *Paenibacillus* sp. In the phyllosphere, the abundance of *Erysiphe necator*, the powdery mildew agent, was lower in CC and MC, which also showed a higher presence of *Aureobasidium pullulans*, a species reported in the literature as a potential biocontrol agent.

Conclusions Our results suggest that under-row dry mulching, by modifying soil conditions, can have a positive effect on grapevine microbial richness and biodiversity during the dry summer period, serving as an indicator of improved vineyard agroecosystem health and sustainability.

Keywords Cover crops, Dry mulching, Microbiome, Rhizosphere, Phyllosphere, Vineyard

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Introduction

Cover cropping is a sustainable soil vineyard management strategy offering multiple agronomic and environmental benefits, such as erosion control, weed suppression, enhanced nutrient cycling, and improved soil fertility [1]. In perennial crops like the grapevine, cover crops help to maintain long-term soil health. Under specific conditions, they can also reduce costs by decreasing the need for fuel, fertilizers, herbicides, and plant protection products [2, 3]. In regions with high winter and spring rainfall, cover crops can help mitigate waterlogging, nitrogen leaching, soil erosion, while simultaneously controlling vine vigor to enhance grape quality [4, 5]. Conversely, in Mediterranean regions, where water scarcity and irregular summer rainfall limit crop production, inter-row vegetation can increase the competition for water and nutrients, potentially leading grapevines to face severe drought stress [6]. To address these challenges, temporary winter cover crops, terminated in spring before grapevine flowering as green manure or mulch, can be an effective strategy to enhance soil fertility, suppress weeds, and improve vine health, and increase grape quality [7–9]. Moreover, these practices represent a practical application of circular economy principles [10].

Dry mulching with cover crop residues (applied between and under vine rows), reduces water runoff, improves water infiltration capacity, enhances water retention, increasing moisture availability for plant roots and reduce evaporation [11–14]. Mulching also regulates soil temperature, reduces evaporation, and suppresses weed growth, reducing competition for nutrients and water [15]. Recently, Cunial et al. [16] found that under-row mulching with a mix of winter cover crops enhanced the agronomic and physiological performance of grapevines, compared to alternate tillage and spontaneous grass.

Cover crops may also affect soil microbial diversity and function, key indicators of soil health that strengthen resilience in perennial agriculture [17]. Microorganisms play essential roles in nutrient cycling, soil structure maintenance, humus formation, and pathogen suppression [18]. As shown by Ingels et al. [18], cover crop-induced increases in microbial biomass and activity improve soil physical and biological processes, thereby contributing to more stable soil functioning under changing environmental conditions [19]. Moreover, soil microbiota interacts directly and indirectly with plants, promoting their growth, health, and productivity [20, 21]. Together with their microbiota, plants form a holobiont [22] that enhances resilience to abiotic and biotic stresses, underscoring its role in agricultural sustainability and ecosystem resilience [23].

Several studies have investigated the impact of soil management on soil microbial communities in vineyards [3, 9, 20, 24–28]. For example, Likar et al. [26] examined fungal and bacterial communities in soils from organic and conventional vineyards, both managed with permanent cover crops, and found that microbial responses were influenced by biogeographic factors, vineyard management practices, and broader environmental conditions. However, relatively few studies have focused on grapevine-associated microorganisms in the rhizosphere and phyllosphere, particularly in relation to cover cropping and dry mulching.

The phyllosphere, which includes all above-ground plant organs is primarily colonized by microorganisms from indigenous plant populations and external sources such as soil, air, or neighboring plants [29]. Meanwhile, the rhizosphere, the soil zone directly influenced by roots and their exudates, hosts microbes predominantly derived from the surrounding soil environment [30]. Root exudates serve as a major nutrient source for microbes, driving intense microbial interactions in the rhizosphere and shaping population density and activity [31]. While some studies suggest that phyllosphere communities share similarities with soil microbial communities under natural conditions [32, 33], other research indicates distinct microbial populations between these two niches [34, 35]. Given the limited knowledge in this area, particularly in grapevines, further studies are needed to better understand these interactions.

Moreover, microbial communities could be influenced by the timing of cover crop termination and species selection. Proper timing is essential to minimize nutrient and water competition between vines and cover crops [6, 8, 9] and may alter microbial communities. Cover crop diversity affects soil microbial activity and enhances biodiversity [36]. For instance, legumes improve nitrogen availability through biological fixation, while non-legumes contribute more biomass, enriching soil organic matter and converting soluble nutrients into stable micro- and macro-elements [37, 38]. Mixed cover crop systems integrate these benefits by increasing microbial biomass carbon (MBC), promoting higher microbial biodiversity [37], influencing water uptake, root density traits, and soil aggregate stability [39].

As plant-microbiome dynamics are influenced by phenological stages and seasonal weather conditions [40, 41], our study primarily focuses on the effects of soil management treatments on the microbial community at two specific sampling times: before cover crop termination (pre-flowering) and mid-summer (veraison), which represent key vineyard conditions that have not yet been investigated from a microbiological perspective, particularly regarding dry mulching practices.

This study aims to (i) evaluate the effects of different soil management practices on the abundance, composition, and predicted functions of microbial communities

in the grapevine rhizosphere and phyllosphere before cover crop termination (around pre-flowering) and at veraison when the dry mulch is present under the rows; and (ii) explore potential associations between microbial community dynamics, vine performance, and soil water status.

Materials and methods

Plant material and experimental design

The study was carried out in 2023 using a non-irrigated cv. Barbera vineyard (*Vitis vinifera* L.) grafted onto the 420A (*Vitis berlandieri* × *Vitis riparia*), a rootstock known for its low vigor, moderate drought tolerance, and good adaptation to calcareous and humid soils. This vineyard, established in 2001, is located at "Tenuta Pernice" estate in Castelnovo Val Tidone, Italy (44°97' N, 9°42' E), within the Colli Piacentini wine district.

The vineyard rows were North–South oriented, with a 2.4 m × 1 m vine spacing (between row and in the row distance, respectively) resulting in a density of 4166 vines/ha. The vines were trained to a unilateral Guyot training system featuring a vertically shoot-positioned (VSP) canopy with main wire at 80 cm above ground and three upper catch wires, for a total canopy height of about 2.2–2.3 m. Standard organic viticulture practices, aligned with the regional protocols, were applied throughout the trial. Phytosanitary management followed the Integrated and Organic Production Bulletin of the Regional Phytosanitary Consortium of Piacenza. Copper-based products were applied preventively against downy mildew, sulfur was used against powdery mildew, and sexual confusion, implemented using natural biodegradable polymer dispensers releasing controlled amounts of synthetic pheromones, was adopted for the control of the grapevine moth (*Lobesia botrana*).

Seasonal weather data, including daily minimum (T_{min}), mean (T_{mean}), and maximum (T_{max}) air temperature (°C), as well as rainfall (mm), were recorded by a weather station located nearby the vineyard.

The experimental design was a randomized block with three blocks, each including three treatments applied over three consecutive years from 2021 to 2023. Each block consisted of 15 vines per treatment, 45 vines per treatment in total, arranged in a vine row and the two adjacent interrows managed to the assigned practice. The treatments involved two inter-row winter cover crops with cereal-based species (CC), a balanced mix of cereal, legume, and brassica species (MC), and a control consisting of alternating rows with inter-row spontaneous grass and inter-row tillage (GT).

CC, using the commercial product Humusfert (Padana Sementi Elette s.r.l., Padova, Italy), consisted of 56% *Hordeum vulgare* (barley), 27% *Avena strigosa* (bristle oat), 10% *Trifolium incarnatum* (crimson clover), 4% forage rape (Ringo), and 3% *Phacelia tanacetifolia* (Angelia variety). MC, using the commercial product Stratus (Padana Sementi Elette s.r.l., Padova, Italy), included 24% *Avena strigosa* (Iapa 61 variety), 24% *Triticosecale* (Dublet variety), 20% *Vicia sativa* (Marianna variety), 7% *Trifolium incarnatum* (crimson clover), 7% *Vicia benghalensis* (hairy vetch), 6% *Raphanus sativus* (Octopus variety), 4% *Phacelia tanacetifolia* (Boratus variety), 4% *Armoracia rusticana* (horseradish), and 4% *Brassica carinata* (Ethiopian mustard). CC is characterized by a predominance of cereals, with a C/N ratio of 26–28. The MC mixture is considered balanced because it contains a functional equilibrium of cereals, legumes, and brassicas, resulting in a C/N ratio of 20–22. Both mixtures are commercially available and commonly used in northern Italy, including the Piacenza area.

The two inter-row winter cover crops were sown annually at a rate of 80 kg/ha in fall (October 6, 2023; Day of the Year, DOY 275), and spontaneous grass was present under the row until termination in spring (June 1, 2023; DOY 152). Termination included mulching under the row strip using a Spit Green mulcher (Falc srl, Faenza, Italy) when the cover crop reached full flowering. In GT, spontaneous grass, mainly composed of mixed native species dominated by Poaceae, grew in the inter-rows and under-the-vine strip was mowed when the average grass height exceeded approximately 35 cm, with the cut biomass left in place.

Under-row mulching was applied to the soil strip beneath the vine row, including both the area directly under the vines and the inter-vine space, for a total surface of approximately 0.5 m^2 between two adjacent vines. Mulching was quantified by manually collecting and weighing the biomass within this area and expressed as kg/m^2. A subsample of approximately 500 g was used to determine fresh weight (FW) and dry weight (DW). For DW determination, samples were oven-dried at 105 °C for 48 h until a constant weight was achieved.

Microbiome analyses

Sample collection

Soil and leaf samples for microbial analysis were collected on two representative dates: before cover crop termination on DOY 129 (pre-flowering stage, BBCH 65) and mid-summer on DOY 199 (veraison stage, BBCH 81), following the BBCH scale described by Lorenz et al. [42]. Veraison occurred on DOY 197.

Within each treatment, three healthy and uniform vines were randomly selected per block for sample collection. Samples collected from each vine were pooled to form a composite sample, representing one biological replicate per block. This resulted in three biological replicates per treatment at each sampling date, for a total of 18 soil samples.

Soil sampling involved extracting soil cores at a depth of 25–30 cm, approximately 20 cm from the vine trunk. Two soil subsamples were collected per vine. Samples were immediately stored in sterile bags on dry ice during collection and later kept at − 20 °C. In the laboratory, all roots recovered from the soil cores were collected to ensure sufficient and representative material for DNA extraction. The roots were first shaken to remove loosely attached soil, and the remaining soil adhering to the root surface was carefully scraped off with sterile forceps to obtain the rhizosphere fraction, following the procedure described by Ambrosini and Passaglia [43]

Leaf sampling was conducted by collecting five healthy leaves per vine, with no visible symptoms of powdery mildew, taken from the 4th and 7th nodes, resulting in 15 leaves per biological replicate. Leaf samples were stored following the same procedure as for soil samples. The phyllosphere fraction was collected following the method described by Perazzoli et al. [33]. Briefly, for each biological replicate, leaves were washed in 300 ml of an isotonic solution containing 0.01% Tween 80 for 15 min using a BagMixer 400S (Interscience, Saint Nom la Bretêche, France). The solution was then centrifuged at 4000 rpm for 20 min to collect the pellet, which was subsequently used for DNA extraction.

DNA extraction, high-throughput sequencing, sequence data processing and PICRUSTs analyses

Total genomic DNA from both the rhizosphere and phyllosphere was extracted from 0.25 g of homogenized samples using the FastDNA SPIN Kit for Soil (MP Biomedicals, Irvine, CA, USA), following the manufacturer's instructions. DNA quantification was performed using the Quant-iT™ HS dsDNA assay kit (Invitrogen, Paisley, UK) and measured with a Qubit 2.0 fluorometer (Invitrogen, Paisley, UK). A negative control for DNA extraction was performed using the kit without any sample.

For bacterial community analysis, the V3-V4 hypervariable region of the 16S rRNA gene was amplified using universal primers 343F (5′-TACGGRAGGCAGCAG-3′) and 802R (5′-TACNVGGGTWTCTAATCC-3′) [44]. The reaction mix, thermal cycling conditions, and nested PCR were carried out as described by Vaccari et al. [45]. In brief, PCR reactions (25 µL) included 12.5 µL Phusion Flash Master Mix, 1.25 µL of each primer (10 µM), 1 ng DNA template, and 8µL of nuclease-free water. The program was 95 °C for 5 min; 20 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 30 s; and a final extension at 72 °C for 10 min. To allow multiplexing, a nested PCR was carried out using first-step products as templates and 10 cycles.

A negative control for PCR amplification was included using nuclease-free water instead of template DNA. As a positive control, the genome of *Priestia megaterium* strain from our collection was used as template. For fungal community analysis, the Internal Transcribed Spacer (ITS) regions (ITS1 and ITS2) of the ribosomal RNA (rRNA) gene were amplified using the universal primers ITS-1 (5'-TCCGTAGGTGAACCTGCGC-3') and ITS-2 (5'-GCTGCGTTCTTCATCGATGC-3′) [46]. The reaction mix and nested PCR conditions were the same as those used for bacteria, with a slight modification of the thermal cycling program, which consisted of 94 °C for 4 min, followed by 28 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min, and a final extension at 72 °C for 7 min.

Amplicons from the second amplification step were pooled, multiplexed, and purified using the solid-phase reversible immobilization (SPRI) method (Agencount AMPure XP kit; REF A63880, Beckman Coulter, Italy). Sequencing was performed by Novogene UK (Cambridge, UK) using a TruSeq DNA sample preparation kit for amplicon library preparation and the Novaseq 6000 Illumina instrument for obtaining 250 pb paired-end reads. Reads were quality-checked, denoised, and filtered using the DADA2 plug-in in QIIME2 (https://docs.qiime2.org/2024.10/plugins/available/dada2/), demultiplexed paired-end reads were processed with qiime dada2 denoise-paired using the default trimming and truncation parameters (trim-left-f = 0, trim-left-r = 0; trunc-len-f = 0, trunc-len-r = 0). DADA2's default quality filtering was used, which removes reads containing ambiguous bases and reads exceeding the default expected-error threshold, and chimeric sequences were removed using the consensus method; the remaining non-chimeric amplicon sequence variants (ASVs) were retained for downstream analyses. Taxonomic assignment was performed on the V3–V4 region of the bacterial 16S rRNA gene using a custom reference database derived from SILVA release 138.2, and on the ITS1–ITS2 region of the fungal ribosomal DNA using a custom reference database derived from UNITE release 9.0. To ensure consistency with the amplified regions, full-length 16S sequences from SILVA were trimmed to the primer-defined V3-V4 region, and full-length ITS sequences from UNITE were trimmed to the primer defined ITS1–ITS2 region using Cutadapt (https://docs.qiime2.org/2024.10/plugins/available/cutadapt/).

This approach ensured that both classifiers were trained on sequences corresponding precisely to the targeted amplicon regions. Further details of the methodology are provided by De Bernardi et al. [47].

The Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2; Version 2.5.1) [48] was utilized to predict functional categories based on the taxonomic composition of the samples. PICRUSt2 exploits 16S sequencing data to estimate the abundance of enzymes and metabolic pathways within

each sample. To predict the functional potential of the bacterial communities, the Kyoto Encyclopedia of Genes and Genomes (KEGG) [49] and MetaCyc [50] database was used. The output from PICRUSt2 predictions were further analyzed using the Statistical Analyzer of Metagenomic Profiles (STAMP) software (Version 2.3.1) [51] using ANOVA followed by Tukey–Kramer post hoc tests and Benjamini–Hochberg False Discovery Rate (FDR) multiple-test correction to avoid type 1 and 2 errors. Pairwise comparisons confronted to the control (GT) were additionally performed using Welch's *t*-test. All pathways were presented in the MetaCyc format [50].

Single-leaf function and soil water status

In 2023, leaf gas exchange, pre-dawn water potential (Ψ_{PD}), leaf gas exchange and midday leaf water potential (Ψ_{MD}) were measured on DOY 199. Ψ_{PD} was measured at 04:00 am, using a Scholander pressure chamber (Model 3500, Soilmoisture Equip. Corp., Santa Barbara, CA). Leaf gas exchanges were measured between 12:00 pm and 1:30 pm under saturating light (PAR > 1200) [52], and ambient temperature and relative humidity with a portable gas exchange system (LCi-SD, ADC Bioscientific Ltd, Hoddesdon, UK) equipped with a broad-leaf cuvette chamber featuring a 6.25 cm^2 window and an airflow set at 200 mL min^{-1}. Two mature mid-shoot leaves per vine were measured yielding a total of 18 leaves per treatment.

Following gas exchange measurements, the same leaves were used to measure Ψ_{MD} using the same instrument for Ψ_{PD}.

Soil water potential (SWP) and soil mean temperature (soil T_{mean}) data were collected throughout the season using nine TEROS-21 sensors (METER Group, Washington, USA), three per each treatment. The sensors were placed at a depth of 30 cm along the row axis in early spring 2021, positioned approximately halfway between two adjacent vines. Data were periodically recorded using a handheld ProCheck multifunctional meter (Decagon Devices, Pulman, WA). Instantaneous Water Use Efficiency (WUEe) was calculated as the ratio of net CO_2 assimilation (A) to transpiration (E), while intrinsic Water Use Efficiency (WUEi) was calculated as the ratio of A to stomatal conductance (gs).

Statistical analyses

Microbial statistical analyses and graph plotting were primarily conducted using MicrobiomeAnalyst software [53]. Rhizosphere and phyllosphere datasets, separated by phenological stage, were rarefied independently to the minimum library size of each dataset. Alpha diversity was calculated using the Chao1 and Shannon indices in the Phyloseq package, while beta diversity was estimated through principal coordinates analysis (PCoA) based on Bray–Curtis dissimilarity. Differentially abundant taxa across treatments were identified using the Linear Discriminant Analysis Effect Size (LEfSE) algorithm [54]. The MicrobiomeAnalyst implementation of LEfSE was employed, with the threshold for the logarithmic Linear Discriminant Analysis (LDA) score set at 3.5 and the Original p-value cutoff at 0.1, as recommended by Segata et al. [54]. Bacterial and fungal amplicon sequence variants (ASVs) shared among treatments at the two phenological stages were obtained through Venn diagram analysis using the VennDiagram package (v.1.7.3) in R (v. 4.1.1). The representative ASVs in each sample were identified using BLAST against the NCBI 16S rRNA database for species-level classification. Matches were considered valid when sequence identity was ≥ 98.7% and query coverage ≥ 95%, corresponding to species-level identification.

Additional microbial community analyses, graph plotting, soil water potential and physiological data analyses and microbiome-grapevine-water status correlation, were performed in R v. 4.1.1. One-way ANOVA with Student–Newman–Keuls (SNK) post-hoc tests were used specifically for soil water potential, and vine physiological data. Bray–Curtis microbiome dissimilarities were correlated with soil water status and physiological variables using Mantel tests (9999 permutations; FDR-adjusted p-values). The R analysis depended on the following packages: reshape2 (v. 1.4.4), microeco (1.15), vegan (2.7–1), ggplot2 (v. 3.5.1), dplyr (v. 1.1.4), and ALDEx2 (v. 1.36.0) [55].

Results

Seasonal weather trend and mulching biomass

In 2023, a total of 80 mm of rainfall was recorded between the first sampling and cover crop termination (DOY 129–152). During the same period, the average T_{mean} was 15 °C, and the average T_{max} was 20 °C. This was followed by a prolonged dry period that persisted until 30 days after the second sampling, during which only 32 mm of rainfall was recorded (DOY 152–228), with no rain in the 18 days preceding the veraison sampling. During these 18 days, the average T_{mean} reached 28 °C and the average T_{max} was 34 °C, with several days exceeding 35 °C starting from DOY 191 (Fig. 1).

Under-the-row green mulching residues were more abundant in MC (3.435 kg/m^2 DW) compared to CC (1.628 kg/m^2 DW), while the DW/FW ratio exhibited the opposite trend.

Seasonal soil water potential, soil temperatures and vine physiology at veraison

The seasonal soil water potential and soil temperature are shown in Fig. 2. All treatments exhibited similar SWP during the early part of the season. From DOY 87 to DOY 129, SWP was higher (less negative) in the GT treatment compared to the others, until abundant rainfall

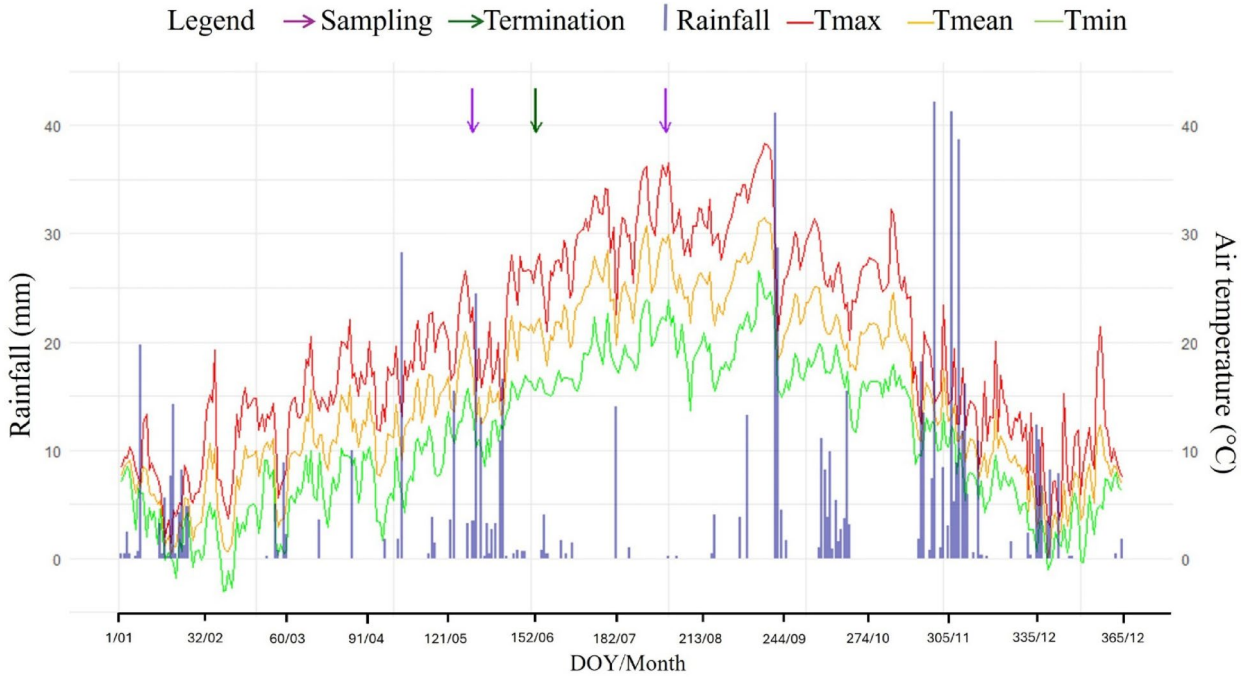

Fig. 1 Seasonal pattern of air temperatures and rainfall in 2023 at the experimental site. The graph shows the maximum (T_{max}), mean (T_{mean}) and minimum (T_{min}) air temperature and rainfall from 1 Jan to 31 Dec 2023. Purple arrows indicate soil and leaves sampling dates (DOY129 and DOY199), and green arrow indicates the cover crop termination (DOY152). *DOY* Day Of the Year

increased SWP uniformly across all treatments by DOY 144. After cover crop termination (DOY 152), all treatments showed a marked decrease in SWP until DOY 166. Throughout the remaining growing season (DOY 166–255), CC treatments maintained significantly higher SWP values, stabilizing around – 0.25 MPa, compared to GT and MC. The latter (MC) recovered to the same SWP level as CC by harvest (DOY 255). In contrast, GT consistently maintained lower SWP values, remaining stable around – 0.35 MPa until harvest (Fig. 2a).

The soil temperature trend (Fig. 2b) showed similar values between treatments until the first sampling. Afterward, GT maintained cooler soil temperature at 30 cm depth compared to both cover crop mulching treatments, with the largest differences observed at veraison, when the temperature difference between GT and MC/CC was approximately 2.5 °C.

At veraison, A, WUEe, WUEi and Ψ_{PD} differed significantly between treatments (Table 1). Vines in the CC treatment exhibited higher A, WUEe and WUEi values compared to those in MC and GT, while the GT treatment showed lower Ψ_{PD} than both under-vine dry mulching treatments (CC and MC).

Temporal and spatial comparison of bacterial and fungal community structure in the phyllosphere and rhizosphere

After paired-end alignments, quality filtering and deletion of chimeric, singletons, mitochondrial and chloroplast sequences, a total of 5,488,880 fungal internal transcribed spacer (ITS) sequences and 2,087,944 bacterial 16S rRNA sequences were retained.

In both the phyllosphere and rhizosphere, the Chao1 diversity estimator revealed significant differences between fungal and bacterial communities (p-value < 0.001), with bacteria exhibiting higher richness in both compartments. From pre-flowering to veraison, a significant shift in bacterial richness was observed, with a reduction in the rhizosphere and an increase in the phyllosphere (p-value < 0.001).

Fungal diversity, as measured by the Chao1 index, ranged from 93.1 to 135.4 in the phyllosphere and from 105.3 to 184.23 in the rhizosphere. Bacterial diversity in the phyllosphere ranged from 357.1 to 465.8 at pre-flowering and increased to a range of 530.7 to 652 at veraison. Conversely, in the rhizosphere, bacterial diversity ranged from 1158.2 to 1272.4 at pre-flowering but decreased to a range of 578.4–700.8 at veraison. [Additional files 1, Table S1].

PCoA analysis of total fungal (R-squared 0.58 and p-value < 0.001) and bacterial (R-squared 0.60 and p-value < 0.001) communities revealed a significant distinct clustering based on both the grapevine compartment (phyllosphere vs rhizosphere) with higher variability in the phyllosphere and the phenological stage (pre-flowering vs veraison). Notably, community

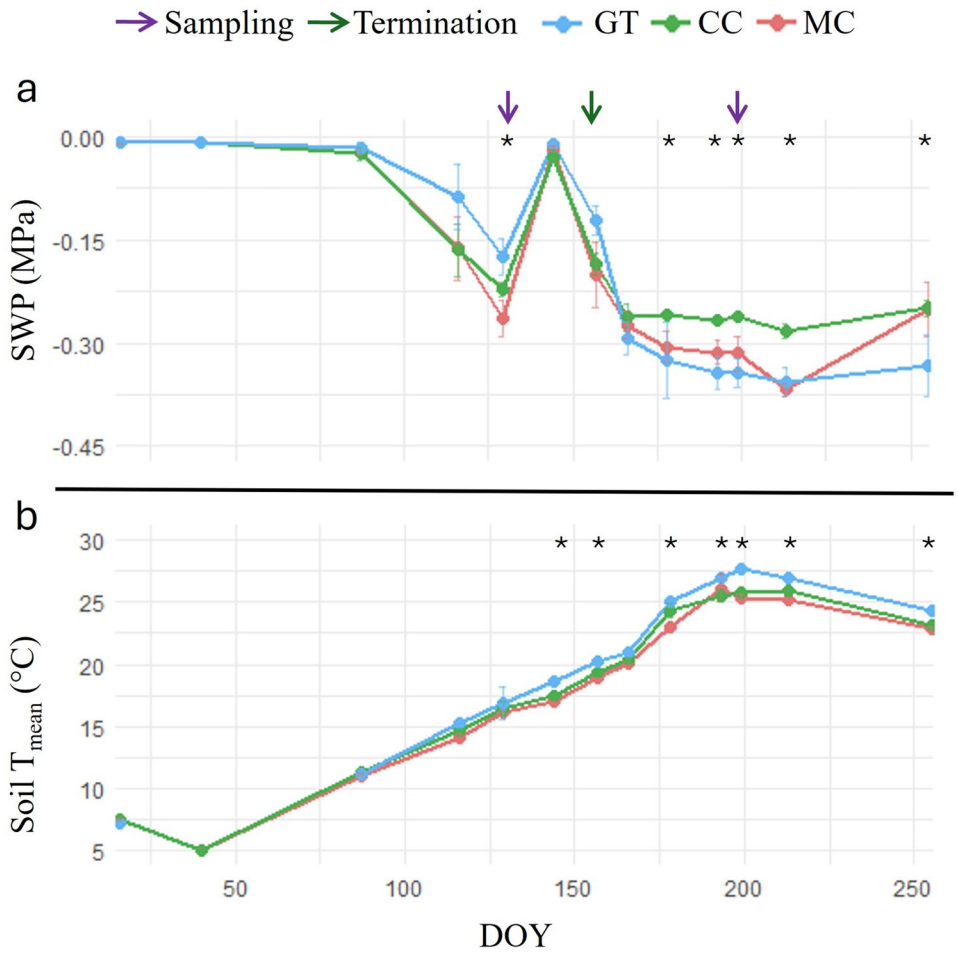

Fig. 2 Seasonal soil water potential (**a**) and soil mean temperature (**b**) at 30 cm depth. The graph shows the soil water potential (SWP) (**a**) and soil mean temperature (soil T_{mean}) (**b**) at 30 cm depth for under-rows subjected to a cereal-based cover crop mulching (CC), to a mixed cover crop mulching (MC) and in a controlled grassed under-row (GT). Purple arrows indicate soil and leaves sampling dates (DOY129 and DOY199) and green arrow indicates the cover crop termination (DOY152). Vertical bars represent standard errors (n = 3).* represent statistically significant differences with $p < 0.05$. *DOY* Day Of the Year

Table 1 Grapevine leaf gas exchange and water status across the treatments at veraison

Treatment	E	g_s	A	WUEe	WUEi	Ψ_{MD}	Ψ_{PD}
CC	3.13	0.079	8.10a	2.5a	102.6a	− 1.31	− 0.58a
MC	2.60	0.055	5.09b	1.9b	89.8b	− 1.30	− 0.63a
GT	2.31	0.048	4.41b	1.9b	94.0b	− 1.34	− 0.73b
Sig	ns	ns	**	**	**	ns	**

Leaf gas exchange parameters taken at veraison (DOY199): transpiration rate (E, mmol m^{-2} s^{-1}), stomatal conductance (gs, mol m^{-2} s^{-1}), assimilation (A, µmol m^{-2} s^{-1}), (WUE_e) Instantaneous water use efficiency (WUE_e), *WUEi* intrinsic water use efficiency (WUE_i), midday leaf water potential (Ψ_{MD}, MPa) and pre-dawn water potential (Ψ_{PD}, MPa). *CC* Cereal-based cover crop mulching, *MC* Mixed cover crop mulching and *GT* control grassed under-row. Within column, in case of significant F test, mean separation was performed by SNK test. *$p < 0.05$, ** $p < 0.01$, *ns* not significant

variability was higher in the phyllosphere than in the rhizosphere.

Shared microbial assemblages and taxonomic composition under the row across treatments

Before cover crop termination (pre-flowering)

At pre-flowering, in both the phyllosphere and rhizosphere, the bacterial and fungal community composition showed no significant differences in terms of relative abundance across the treatment.

Phyllosphere 42.8% of bacterial ASVs (Fig. 3c) and 41.7% of fungal ASVs (Fig. 3d) were shared. Unique ASVs accounted for 8% to 12%, with pairwise overlaps remaining low at 5% to 9%. The most predominant bacterial families were *Hymenobacteraceae*, *Rhizobiaceae*, *Sphin-*

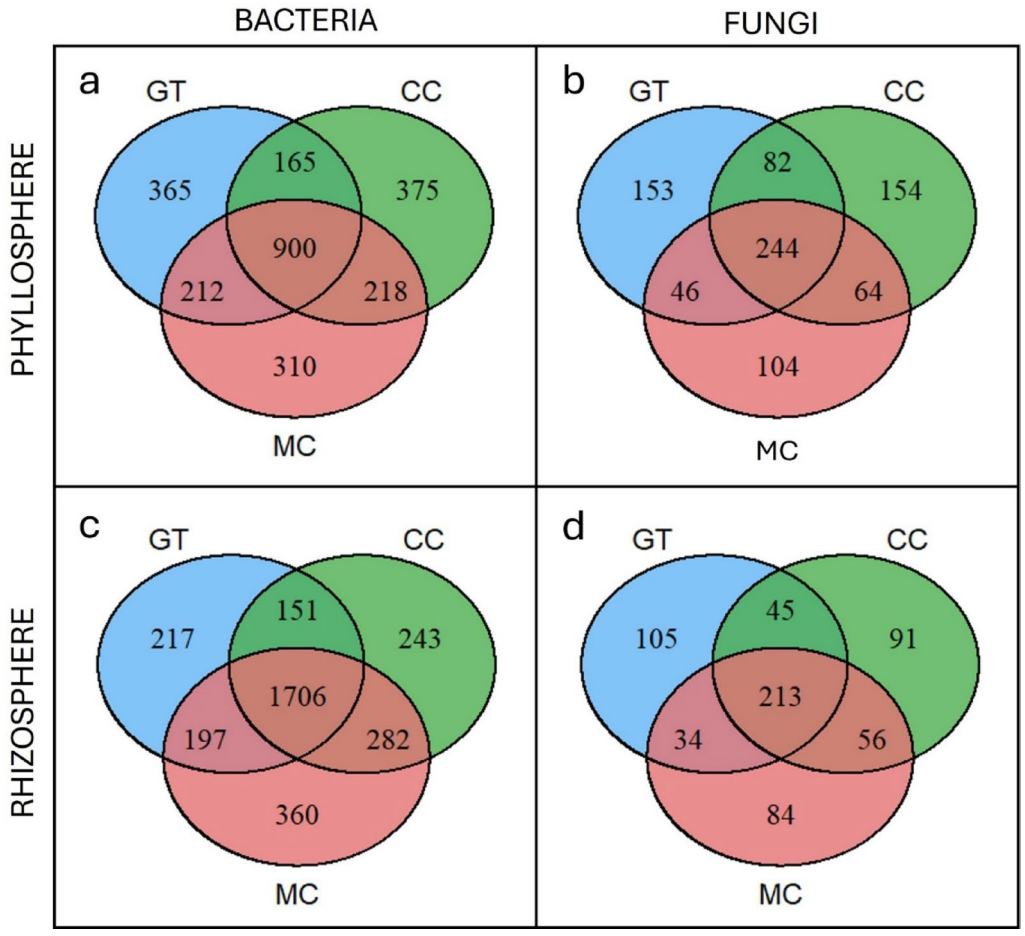

Fig. 3 Shared and unique bacterial and fungal ASVs across treatments at pre-flowering in phyllosphere and rhizosphere. Venn diagram shows the common and exclusive bacterial and fungal ASVs of the phyllosphere (**a** and **b**, respectively) and rhizosphere (**c** and **d**, respectively). *CC* Cereal-based cover crop mulching, *MC* Mixed cover crop mulching and *GT* control grassed under-row

gomonadaceae, and *Bacillaceae*, while the most predominant fungal species were *Blumeria graminis*, *Stemphylium vesicarium*, and *Aureobasidium pullulans* [Additional file 2, Figure S1 and S5].

Rhizosphere In the rhizosphere, 46.3% of bacterial ASVs (Fig. 3a) and 41.5% of fungal ASVs (Fig. 3b) were shared across treatments. Unique ASVs contributed 8% to 14% of the total ASVs detected across the treatments, with pairwise overlapping similarly ranging from 5 to 9%. The dominant bacterial families included *Anaerolineaceae*, *Chthoniobacteraceae*, *Gemmatimonadaceae*, *Vicinamibacteraceae*, and *Xanthobacteraceae*, while the predominant fungal species were *Onygenales* sp. and *Solicoccozyma aerea* [Additional file 2, Figure S2 and S6].

Dry mulching and spontaneous grass (veraison stage)

Phyllosphere At veraison, phyllosphere microbial communities showed a combination of shared and treatment-specific taxa. Overall, 42.8% of bacterial ASVs (Fig. 4b) and 33.5% of fungal ASVs (Fig. 4d) were shared across treatments. Unique fractions ranged from 8.7% to 16.9%, while pairwise overlaps varied between 3.7 and 12%.

Firmicutes were more abundant in GT (69.1%) than in MC (49.5%) and CC (46.6%), whereas *Proteobacteria* were proportionally higher in MC (33.8%) and CC (36.7%) compared to GT (16%). At the family level, MC showed higher relative abundances of *Rhizobiaceae* (6%) and *Sphingomonadaceae* (8%) compared to CC (1% and 3%) and GT (1.3% and 1.9%). Conversely, GT was enriched in *Bacillaceae* (60%) relative to CC (30.3%) and MC (38.8%). *Enterobacteriacea*e was more abundant in CC (20.9%) than in MC (3.3%) and GT (< 1%) [Additional file 2, Figure S3].

Regarding fungal taxa, *Aureobasidium pullulans* was predominant in MC (58%) and CC (48.7%) but significantly lower in GT (27.6%) ($p < 0.05$). In contrast, *Erysiphe necator* and *Pseudopithomyces rosae* were more abundant in GT (12% and 13.4%) than in MC (< 0.2% and 4.7%) and CC (< 0.2% and 4.7%) ($p < 0.01$) [Additional file 2, Figure S7].

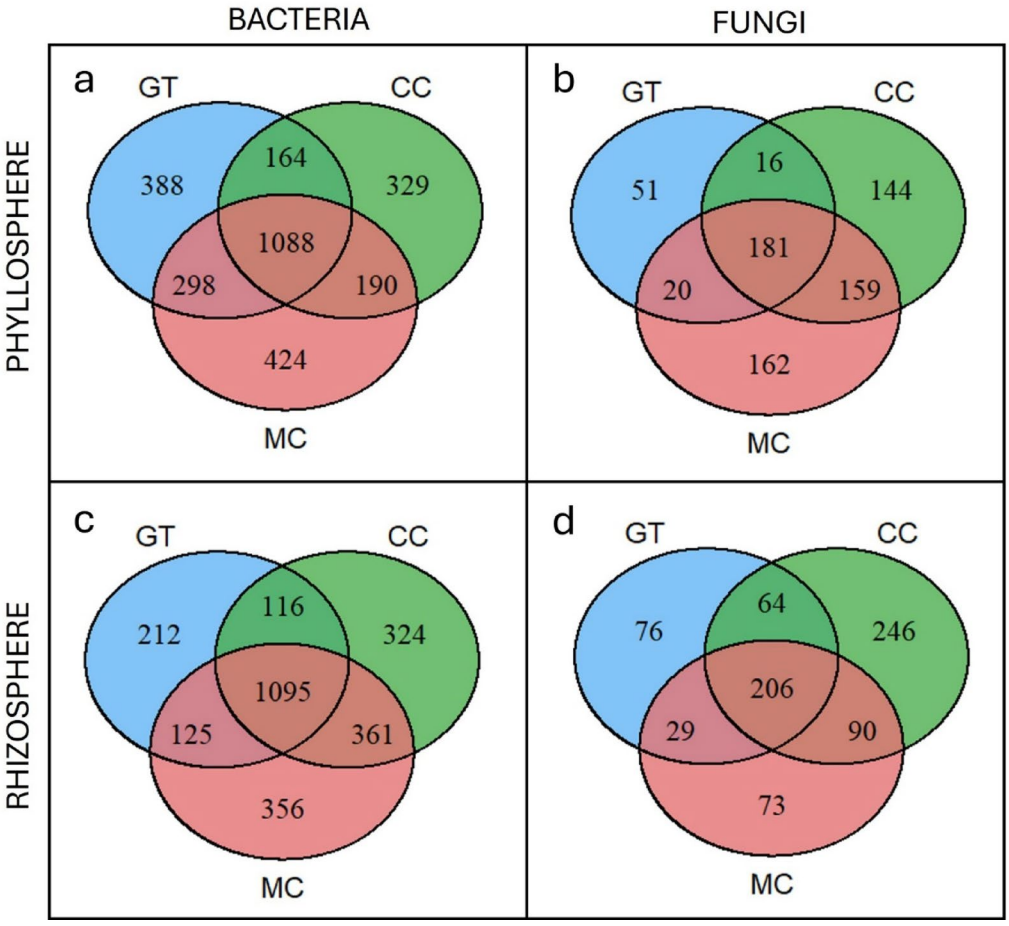

Fig. 4 Shared and unique bacterial and fungal ASVs across treatments at veraison in phyllosphere and rhizosphere. Venn diagram shows the common and exclusive bacterial and fungal ASVs of the phyllosphere (**a** and **b**, respectively) and rhizosphere (**c** and **d**, respectively). *CC* Cereal-based cover crop mulching, *MC* Mixed cover crop mulching and *GT* control grassed under-row

Rhizosphere In the rhizosphere, 40.2% of bacterial ASVs (Fig. 4a) and 34.8% of fungal ASVs (Fig. 4c) were shared across treatments. Unique contributions ranged from 8.4 to 14.3%, with pairwise overlaps between 2.9 and 14.4%.

Actinobacteriota (47.6%) and *Chloroflexi* (31.8%) were more abundant in GT compared to MC (29.9% and 15.9%) and CC (30.8% and 15.1%). Conversely, *Proteobacteria* and *Acidobacteria* were more represented in MC (14.5% and 18.7%) and CC (16.3% and 14.8%) than in GT (6.8% and 3.4%). At the family level, *Roseiflexaceae* (9.8%) and *Solirubrobacteraceae* (4.6%) were enriched in GT relative to MC (2.4% and 2.3%) and CC (3.3% and 2.6%). In contrast, *Chthoniobacteraceae*, *Gemmatimonadaceae*, and *Vicinamibacteraceae* were more abundant in MC and CC (3.3–5.5%), while remaining below 1% in GT [Additional file 2, Figure S4].

For fungal taxa, *Solicoccozyma aerea* was significantly more abundant in MC (15.2%) and CC (10.6%) compared to GT (2.9%), whereas *Onygenales* sp. was markedly enriched in GT (40%) relative to CC (6.5%) and MC (6.2%) ($p < 0.01$) [Additional file 2, Figure S8].

Treatments impact on phyllosphere and rhizosphere microbial richness and diversity at veraison

Phyllosphere

In the phyllosphere, for bacterial communities, alpha diversity did not differ among treatments, whereas beta diversity revealed distinct clustering patterns ($p < 0.05$). Axis 1 accounted for 48.3% of the variation and Axis 2 for 22.5%. GT was negatively correlated with both Axis 1 and Axis 2, while MC and CC showed positive correlations with Axis 2 and Axis 1, respectively.

For fungal communities, beta diversity also showed a clear separation among treatments ($p < 0.05$). Axis 1 explained 85.9% of the variation and Axis 2 explained 7%. GT was positively correlated with Axis 1, whereas MC and CC were negatively correlated.

Rhizosphere

In the rhizosphere, both alpha and beta diversity of bacterial and fungal communities differed significantly across treatments (Fig. 5). Alpha diversity was assessed using richness (Chao1 index) and diversity (Shannon index).

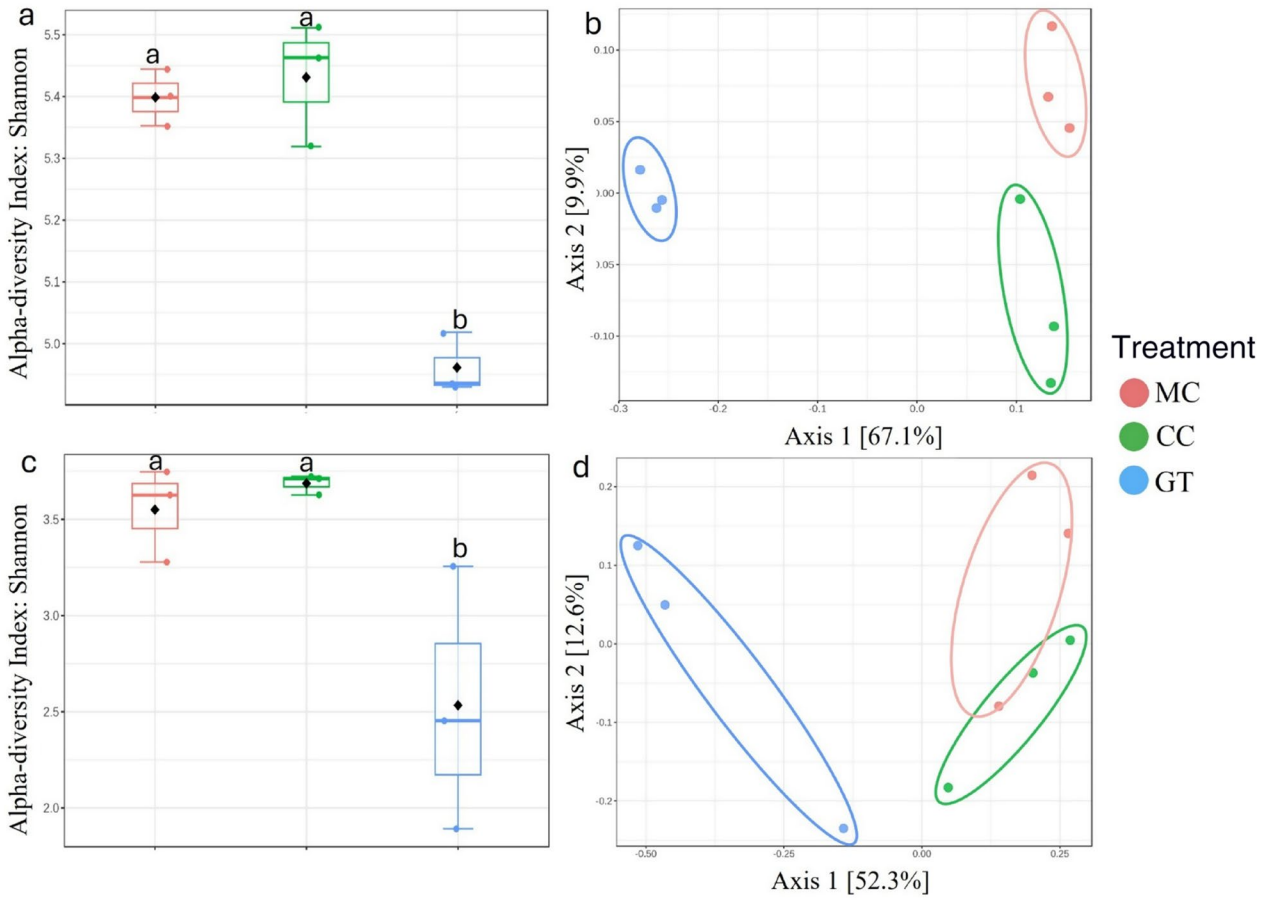

Fig. 5 Bacterial and fungal alpha and beta diversity in the rhizosphere at veraison (ASV level). Alpha diversity and beta diversity of bacterial (**a** and **b**, respectively) and fungal (**c** and **d**, respectively) communities at veraison across treatments. Alpha diversity with *p*-value < 0.001 (bacteria) and < 0.05 (fungal); beta diversity with *p*-value < 0.01 (bacteria) and < 0.05 (fungal). *CC* Cereal-based cover crop mulching, *MC* Mixed cover crop mulching and *GT* control grassed under-row

Bacterial richness and diversity (Fig. 5a) were significantly lower in GT (577 and 4.9, respectively) compared to MC (669 and 5.4) and CC (681 and 5.4) ($p < 0.001$). Similarly, fungal richness and diversity (Fig. 5c) were reduced in GT (105 and 2.54, respectively) relative to MC (180 and 3.55) and CC (184 and 3.68) ($p < 0.05$).

Bacterial beta diversity (Fig. 5b) differed significantly among treatments ($p < 0.01$), with Axis 1 explaining 67.1% and Axis 2 explaining 9.9% of the variation ($R^2 = 0.75$). GT was negatively correlated with Axis 1, whereas MC and CC were positively correlated with Axis 1; additionally, MC showed a positive correlation with Axis 2, while CC correlated negatively with Axis 2.

Fungal beta diversity (Fig. 5d) also differed among treatments ($p < 0.05$), with Axis 1 and Axis 2 explaining 52.3% and 12.6% of the variation, respectively. GT showed a negative correlation with Axis 1, while MC and CC were positively correlated. LEfSe was performed to find dissimilarities in taxa among treatments. In [Additional files 1, Table S2, S3, S4 and S5] are reported the *p*-value and the LDA score of all the bacterial and fungal dissimilarities at the features level across the treatments at veraison in the phyllosphere and rhizosphere. LEfSe analysis identified specific biomarkers for each treatment. In the phyllosphere, 7 bacterial genera and 9 fungal species (Fig. 6a), and in the rhizosphere, 19 bacterial genera (Fig. 6b) and 26 fungal species.

Mantel tests detected no significant associations between Bray–Curtis dissimilarities and vine physiological or soil water variables.

Effect of under-the-row dry mulching and spontaneous grass on bacterial metabolic pathways

At pre-flowering, no significant differences were detected between treatments in either compartment. At veraison, no significant differences were observed in the phyllosphere, whereas the rhizosphere showed significant differences, particularly between the control (GT) and the treatments MC and CC (Fig. 7).

The first two principal components (PC1: 89.5%, PC2: 7.3%) explained most of the variation in predicted functional profiles, with GT samples clustering separately on

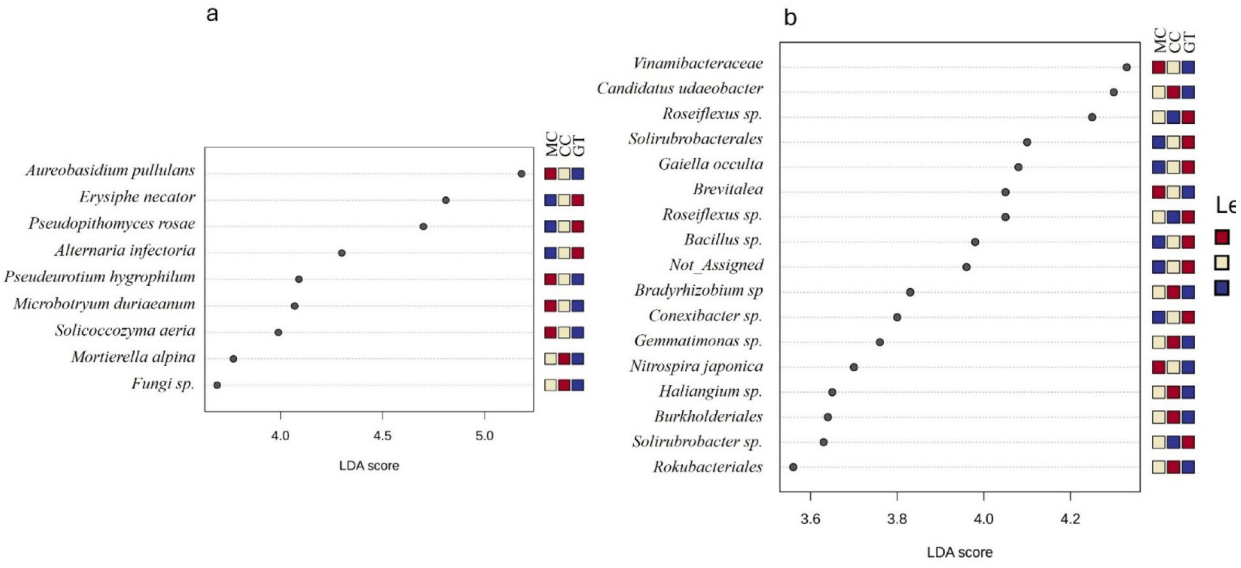

Fig. 6 LEfSe analysis of phyllosphere fungal and rhizosphere bacterial communities at veraison across treatments. Graphical summary of LEfSe analysis. Significant taxa are ranked in decreasing order by LDA score x-axis. The mini heatmap to the right of the plot indicates whether the taxa are higher (red) or lower (blue) predicted relative abundance in each group. Fungi at specie level in the phyllosphere (**a**) and Rhizobacteria at genus/species level (**b**) at veraison. *CC* Cereal-based cover crop mulching, *MC* Mixed cover crop mulching and *GT* control grassed under-row

the negative PC1 side. Pairwise comparisons with the control (GT) revealed 213 active features differentiating MC from GT [Additional file 3, Figure a] and 94 differentiating CC from GT [Additional file 3, Figure b]. Detail information on MetaCyc pathways, relative abundances, and associated *p*-values is provided in [Additional file 1, Table S6].

Some of the pathways identified in the predicted bacterial metagenome are associated with key plant growth-promoting rhizobacteria (PGPR) traits.

A notable observation was a non-significant trend (p-value = 0.1) toward higher relative abundance of genes associated with L-tryptophan and chorismate biosynthesis in the GT treatment compared to MC and CC. Significant differences ($p < 0.05$) were also observed for pathways involved in oxidative stress responses, such as arginine, ornithine, and proline metabolism, which exhibited higher relative abundance in GT.

Conversely, pathways related to nitrogen cycling, including nitrifier denitrification, urate biosynthesis, and inosine 5'-phosphate (IMP) biosynthesis, showed higher relative abundance in MC and CC than in GT.

Discussion

Three consecutive years of distinct soil management practices, including winter cover crops terminated and applied as under-the-row mulch, as well as the traditional inter-row alternation of tillage and spontaneous grass [56], were likely enabled for a more robust assessment of treatment effectiveness on grapevine microbial communities. Similar to Labarga et al. [57], who reported significant shifts in bacterial community composition between mulch and bare soil, likely due to the gradual decomposition of organic matter leading to lasting effects on soil microbial structure.

The summer season of 2023 marked a prolonged drought period, which likely stressed the grapevine under non-irrigation management.

Mulching under the vine row is known to conserve soil moisture [15, 58], as confirmed by the CC treatment, which maintained higher soil water potential during summer compared to MC and GT. A lower dry weight-to-fresh weight ratio typically indicates less lignified, more labile biomass (as expected for this mix, which included a high proportion of legumes and brassica species), generally associated with faster decomposition due to lower C/N ratio and enhanced microbial activity [15, 59]. Although decomposition was not directly measured, this faster turnover likely reduced the MC treatment's ability to maintain a persistent mulch layer, thereby diminishing its capacity to buffer soil moisture over time. However, the composition and thickness of both mulches likely shielded the soil from direct solar radiation, maintaining soil temperatures approximately 2.5 °C lower than in the GT treatment. A similar result was reported by Wang et al. [60], who observed a 1.7 °C reduction in maximum and minimum soil temperatures at a 10 cm depth near the grapevine roots in the mulched treatment, as compared to the tilled control. Since soil temperature is a key factor influencing both plant growth and microbial activity [61], maintaining a cooler environment during

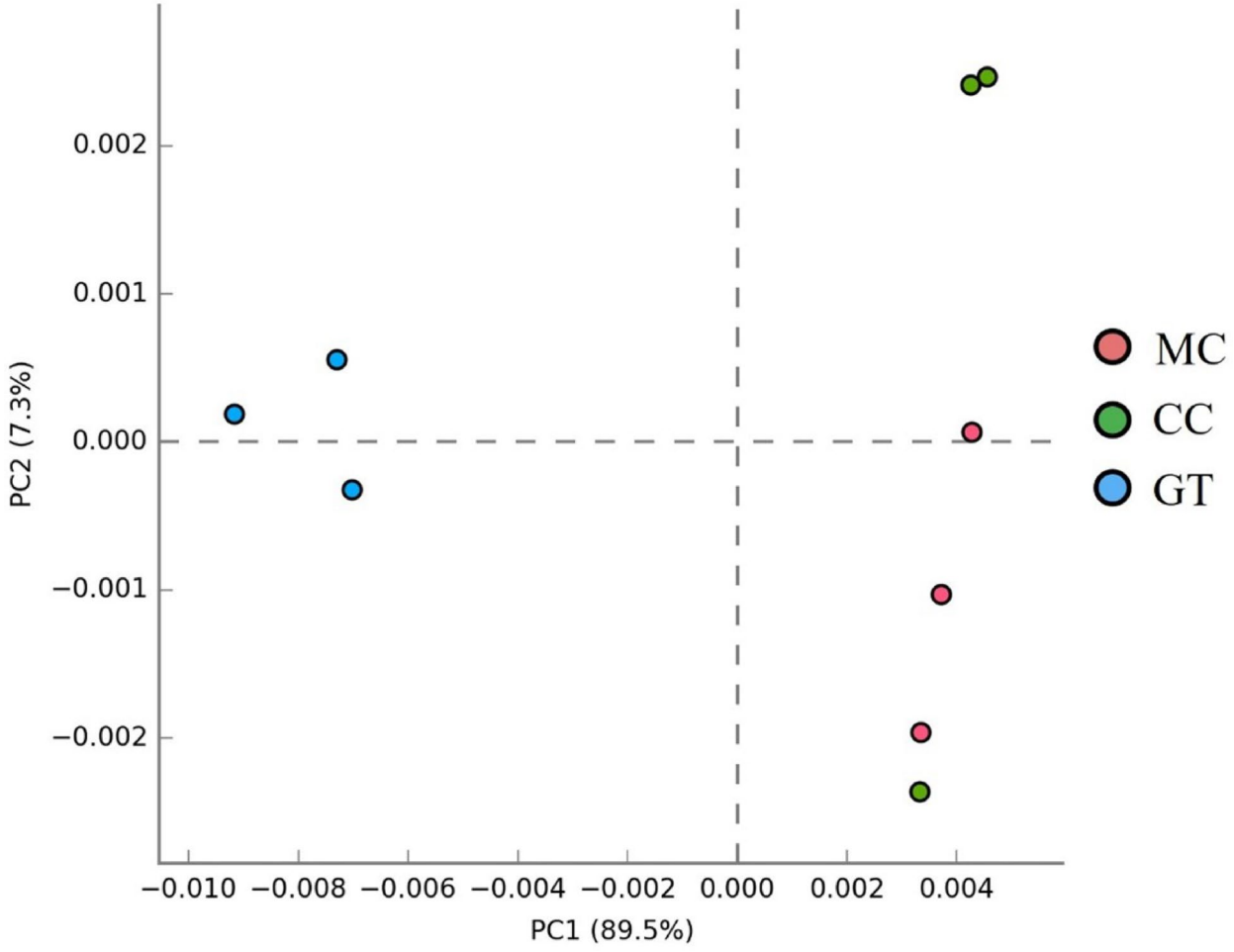

Fig. 7 Predicted functional metabolic profile of rhizosphere microbial communities across the treatments at veraison. PCA plot of PICRUSt-predicted functional metabolic pathways. Statistical differences in functional metabolic pathways were assessed using ANOVA (p-value < 0.05) with Tukey–Kramer post-hoc test. *CC* Cereal-based cover crop mulching, *MC* Mixed cover crop mulching and *GT* control grassed under-row

very hot periods could positively affect biological processes in the soil [62].

In all treatments, grapevines experienced drought stress, as indicated by both Ψ_{MD} and Ψ_{PD} values. Grapevines in the CC treatment appeared to use water more efficiently, whereas those in the MC and GT treatments showed lower WUEe and WUEi exhibiting an isohydric-like response by closing their stomata to limit gas exchange. Nevertheless, the GT treatment showed lower Ψ_{PD} values compared to CC and MC. Since Ψ_{PD} is commonly used to assess vine water status [63] and reflects the water equilibrium between the plant and the soil before the onset of transpiration, this suggests reduced water availability and greater drought stress in the control treatment. This may be attributed to potential water competition between the grapevine and the living grass present in both the inter-row and under row strips.

It is important to consider that the average mismatch of approximately − 0.35 MPa between SWP and Ψ_{PD} at 30 cm depth may be attributed to the point-based nature of the sensor measurement, which is not fully representative of the spatial distribution of the root system and, consequently, of the actual root water uptake zone.

The above-ground microbiome is strongly affected by environmental factors such as air temperature fluctuations, humidity, radiation, and wind [35]. Moreover, the limited lifespan of leaves and their nature as discrete structures further contribute to the high variability of phyllosphere microbial communities. This helps explain the marked differences in microbial diversity observed between the phyllosphere and the rhizosphere, as also reported in previous studies [34, 35, 40, 64].

Previous studies support our findings about various factors influencing microbial communities. For example, Novara et al. [9] demonstrated that temporary cover crops enhance the soil microbial carbon pool, slightly increase microbial diversity, and reduce the need for chemical nitrogen fertilizers.

At pre-flowering, before cover crop termination, grapevine microbial diversity and composition were

not significantly affected by the different treatments, likely due to the similar under-row management, which allowed the growth of spontaneous vegetation. This early-season stability is consistent with previous findings suggesting that microbial communities tend to remain relatively unchanged in the absence of environmental stressors, such as drought [34]. In contrast, other studies have shown that long-term and more differentiated soil management practices can significantly influence microbial communities. For example, Likar et al. [26] found that ecological vineyard management, based on permanent cover crops, absence of herbicides, and reduced soil disturbance, strongly shaped both bacterial and fungal communities in grapevine rhizosphere soils. Similarly, Burns et al. [28] reported that cover cropping had a greater influence on soil bacterial composition than no-tillage alone, underlining the importance of plant-derived inputs in shaping soil microbial communities.

From pre-flowering to veraison, grapevine rhizosphere bacterial richness generally declined across all treatments. This trend may be associated with the drought stress experienced by the grapevines during the summer dry period, as indicated by similar Ψ_{MD} values across treatments, even though Naylor and Coleman-Derr [65] observed stable alpha diversity under drought conditions. However, MC and CC treatments maintained higher rhizosphere microbial richness compared to GT, likely due to more favorable soil conditions at the approximately 25–30 cm depth of rhizosphere sampling. Supporting this, Labarga et al. [57] showed that organic mulching influences bacterial communities in vineyards, indicating potential enhancement of microbial richness. Yet, mulching effects can vary with soil depth and mulch type; for instance, Zhou et al. [66] reported a reduced bacterial diversity near the surface under straw mulch. Additionally, soil disturbances such as tillage have been found to increase bacterial diversity but reduce fungal diversity, highlighting the complexity of soil management impacts on microbial communities [67].

Moreover, microbial beta diversity reflected the likely similar soil conditions under dry mulching in MC and CC, such as soil temperature. In contrast, the distinct rhizosphere community in GT may be attributed to the absence of mulch and potential differences in soil properties. The different floristic compositions of MC and CC, as dry mulching, had a limited influence on the grapevine microbiome. Whereas, the lower water availability observed in GT, as indicated by $\Psi_{PD,}$ likely promoted the selection of drought-tolerant taxa, such as those belonging to the Actinobacteria phylum. Barnard et al. [68], Santos-Medellín et al. [69], and Naylor et al. [70] reported an increase in Actinobacteria under environmental stressors like drought. Actinobacteria are considered oligotrophic K-strategists [71], characterized by slow growth, low nutrient requirements, and the production of secondary metabolites that help them cope with stress [72]. Nevertheless, we acknowledge that Actinobacteria are a highly diverse phylum, and some studies have also described members as copiotrophic taxa rapidly responding to labile organic matter inputs [71, 73, 74]. This highlights the ecological heterogeneity of Actinobacteria, which may adopt different life strategies depending on the environmental context. In contrast, MC and CC treatments showed a higher abundance of Proteobacteria, which are typically more prevalent in nutrient-enriched soils. Although nutrient availability was not directly assessed, it is plausible that three consecutive years of winter cover cropping combined with dry mulching increased the accumulation of organic matter in the soil. Since organic matter is a key indicator of soil fertility, its accumulation may have improved soil structure, enhanced chemical properties, and created a more favorable environment for microbial communities to thrive [75].

Under-row cover crop mulching appeared to significantly influence fungal communities in the phyllosphere. This effect may be attributed to the higher microbial diversity in the rhizosphere, which can influence aboveground communities indirectly through plant-mediated processes, together with the physical barrier created by the mulch, which likely reduced the splash dispersal of fungal propagules [32, 76].

Our results identified specific microbial genera contributing to the observed differences between cover crop mulching treatments (MC and CC) and the control (GT) at veraison, based on LEfSe analysis. In the rhizosphere, a significant enrichment of nutrient-cycling microorganisms was observed, including fungi (*Sirastrachys* sp., *Mortierella* sp., *Circinella* sp.) and bacteria (*Nitrospira japonica*, *Bradyrhizobium* sp., *Gemmatimonas* sp.), in MC and CC compared to GT.

Among fungi, *Mortierella* is a known phosphate-solubilizing organism playing a crucial role in phosphorus cycling in the rhizosphere [77]. Additionally, *Sirastachys* and *Circinella* are saprotrophic fungi likely involved in organic matter decomposition. Regarding bacteria, *Nitrospira japonica*, a nitrate-oxidizing bacterium (NOB), has recently been identified as capable of complete ammonia oxidation [78]. This genus is often linked to increased nitrogen availability from mineral fertilization [79]. Since both CC and MC contain a percentage of legumes, their presence likely stimulates nitrifiers, potentially enhancing nitrogen cycling in the soil [9]. Moreover, *Bradyrhizobium* is a nitrogen-fixing bacterium recognized also for its plant growth-promoting properties due to its production of indole-3-acetic acid (IAA), cytokinins, and gibberellins [80]. Furthermore, *Gemmatimonas* has been described as a polyphosphate-accumulating strain, contributing to phosphorus storage and cycling [81].

These enhancements in nutrient-cycling microorganisms likely stem from the increase in organic matter derived from cover crop residues, which serve as a source of organic carbon. This hypothesis is supported by PICRUSt2 analyses, which revealed an increase in microbial pathways associated with nitrogen cycling, including nitrifier denitrification, urate biosynthesis, and inosine 5'-phosphate (IMP) production. These changes suggest an enhanced microbial capacity for nitrogen cycling in the rhizosphere. A review on cover crops and the soil microbiome in tree crops highlights that organic residues like mulch enhance denitrification, thereby reducing nitrate (NO_3^-) to nitrogen gas (N_2) and limiting nitrate leaching [82]. The enrichment of urate biosynthesis pathways may reflect improved nitrogen storage and transport within the plant, while the increase in IMP production indicates greater potential for purine biosynthesis, essential for energy metabolism and nucleic acid synthesis [43]. Together, these findings suggest a shift in microbial functional potential that may promote more efficient nitrogen use by plants.

The GT treatment appeared to have recruited specific drought-adapted or potentially beneficial microorganisms, likely reflecting the influence of shifts in soil conditions and vine water status on grapevine-associated microbiomes. For example, Carbone et al. [83] reported that severe water stress can substantially alter rhizosphere fungal communities, including an enrichment of arbuscular mycorrhizal fungi such as *Funneliformis*. In our study, the microorganisms potentially recruited under GT included plant growth-promoting rhizobacteria (PGPR) such as *Bacillus zanthoxyli*, *Blastococcus* sp., *Gaiella* sp., *Roseiflexus* sp., *Pseudarthrobacter* sp., and *Paenibacillus* sp., which may contribute to grapevine adaptation under water-limited conditions. Multiple species of *Bacillus* and *Paenibacillus* are known to promote plant growth and health through mechanisms such as phytohormone production, nutrient solubilization, and pathogen suppression [84]. Among Actinobacteria, an ecologically versatile phylum well adapted to arid conditions [85], *Blastococcus* have been identified as keystone taxa in soils subjected to various environmental stressors [86]. Similarly, *Gaiella*, originally described by Albuquerque et al. [87], was later reported as the dominant Actinobacteria genus in *Vitis vinifera* cv. Pinot Noir vineyard under integrated pest management (IPM) practices [88], highlighting its potential relevance in vineyard microbiomes. *Pseudoarthrobacter* has also been found in comparable environments and under conventional tillage systems [9], suggesting a role in soil functioning across diverse management strategies. Within the phylum Chloroflexota, *Roseiflexus*, a thermophilic, filamentous, photosynthetic bacterium lacking chloroplasts, has been suggested as a potentially beneficial microorganism in soil ecosystems [89]. Together, these microbial taxa may contribute to grapevine adaptation to environmental change and improved stress resilience [90].

In support of this, predictive functional profiling hinted at a potential enrichment in microbial pathways involved in indole-3-acetic acid (IAA) production and proline in the GT treatment. Proline acts as an osmoprotectant and plays a role in microbial biofilm formation, potentially strengthening plant–microbe interactions under stress conditions.

Another interesting finding is the potential role of cover crops in modulating grapevine diseases. At veraison, an increased abundance of *Aureobasidium pullulans* in CC and MC treatments correlated with a reduced presence of *Erysiphe necator*, the grapevine powdery mildew agent, in the phyllosphere. Known for its resilience to environmental stresses, *A. pullulans* is a well-documented biocontrol agent that competes for nutrients and space, produces antifungal compounds, and activates plant defences [91, 92]. The lower abundance of *A. pullulans* in GT, along with a higher prevalence of *E. necator*, further supports this relationship. Additionally, cover crops may reduce foliar pathogen dispersal by acting as a physical barrier, as their height before termination in late May limited splash from soil to canopy [73], unlike the control, where one side of the canopy was directly exposed to a tilled inter-row.

Conclusion

To the best of our knowledge, this is the first study to investigate both bacterial and fungal communities associated with *Vitis vinifera* in the phyllosphere and rhizosphere of a rainfed vineyard where winter inter-row cover crops are terminated and retained as under-row dry mulch during summer. The study integrates different plant developmental stages, soil moisture, temperature dynamics and vine performance.

Our findings indicate that using winter cover crops as dry under-row mulch significantly increased soil water availability and reduced soil temperature by 2.5 °C compared to the control during the summer dry period. These changes supported better vine water status and enhanced photosynthetic performance, particularly in the cereal-based mulch, which is agronomically relevant as it helps mitigate drought stress during critical phenological phases. Microbial analyses showed that under these improved soil conditions, both MC and CC treatments maintained higher bacterial and fungal richness and supported a greater abundance of nutrient-cycling taxa, including *Mortierella*, *Circinella*, *Nitrospira*, *Bradyrhizobium*, and *Gemmatimonas*. These shifts indicate enhanced functional capacity for nitrogen transformation, phosphorus solubilization, and organic matter turnover, consistent with PICRUSt2 predictions showing

enrichment in nitrogen-cycling pathways. In contrast, the unmulched control selectively recruited drought-tolerant and stress-adapted taxa such as Actinobacteria (*Blastococcus*, *Gaiella*, *Pseudarthrobacter*) and PGPR (*Bacillus*, *Paenibacillus*), likely reflecting an adaptive response to harsher soil conditions. Moreover, under-row dry mulching treatment showed an increased abundance of *Aureobasidium pullulans* and a reduced presence of *Erysiphe necator* in the phyllosphere, suggesting that mulching may help reduce foliar pathogen pressure through both microbiological and physical.

Future research should further elucidate the mechanisms by which cover crops and organic mulching influence grapevine fungal and bacterial populations and assess their direct impact on vineyard agroecosystem functioning, long-term productivity, and sustainability.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40793-026-00854-2.

Additional file1 (XLSX 203 kb)

Additional file2 (DOCX 1,023 kb)

Additional file3 (PDF 328 kb)

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Author contributions
GC: Methodology, Visualisation, Investigation, Data curation, Writing—original draft, Writing—review & editing. FV: Bioinformatic analysis, FD: Investigation, SP: Conceptualisation, Writing—review & editing, MG: Conceptualisation, TF: Supervision, Writing—review & editing EP: Supervision, writing-review & editing

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Data availability
Sequence data were submitted to the National Centre for Biotechnology Information Sequence Read Archive (BioProject ID PRJNA1258297).

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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