Environmental Microbiome

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Rain-shelter cultivation promotes grapevine health by altering phyllosphere microecology in rainy areas

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Abstract

Grapes are a globally significant fruit crop, but their cultivation is often challenged by leaf diseases, which limit industrial productivity. Rain-shelter cultivation has emerged as a sustainable agricultural strategy to mitigate these challenges. This study examines the effects of rain-shelter cultivation, compared to open-air cultivation, on the microclimate within the grape canopy and the microbial ecology of the grape phyllosphere. The research focused on two cultivation methods: rain-shelter and open-air cultivation. Key environmental factors such as temperature, relative humidity, and light intensity within the grape canopy were measured during the growing season. The study also explored how these conditions influence the biodiversity, stability, and functional roles of phyllosphere microbiota, particularly focusing on the community assembly processes of bacteria and oomycetes, and the efficacy of culturable microorganisms in combating grape leaf diseases. The results showed that rain-shelter cultivation signifcantly reduced leaf humidity, increased canopy temperature, and decreased light intensity, regardless of weather conditions. This approach led to a significant decrease in the incidence of grape downy mildew without affecting the overall Shannon diversity index of phyllosphere microbes. At the Class level, there was a reduction in Cystobasidiomycetes, Bacteroidia, Brocadiae, and Phycisphaerae, while Oligoflexia levels are significantly increased under rain-shelter conditions. Genus-level analysis revealed significant reductions in plant pathogens such as *Erysiphe*, *Alternaria*, and *Cercospora*. The study found that rain-shelter cultivation shifts fungal community assembly from stochastic to deterministic processes, while bacterial networks showed increased stability. Additionally, the beneficial microorganism *Pseudomonas aeruginosa* exhibited a preventive effect against grape leaf diseases, enhancing grape berry quality by increasing puncture resistance and leaf internode length. These findings provide understanding of the complex relationship between grape canopy microclimate, disease management, and microbial dynamics suggesting rain-shelter cultivation as a viable strategy for sustainable grape production, it offers insights into the research and development of future biological control agents.

Keywords Grape, Rain shelter cultivation, Phyllosphere microecology, Microbial network

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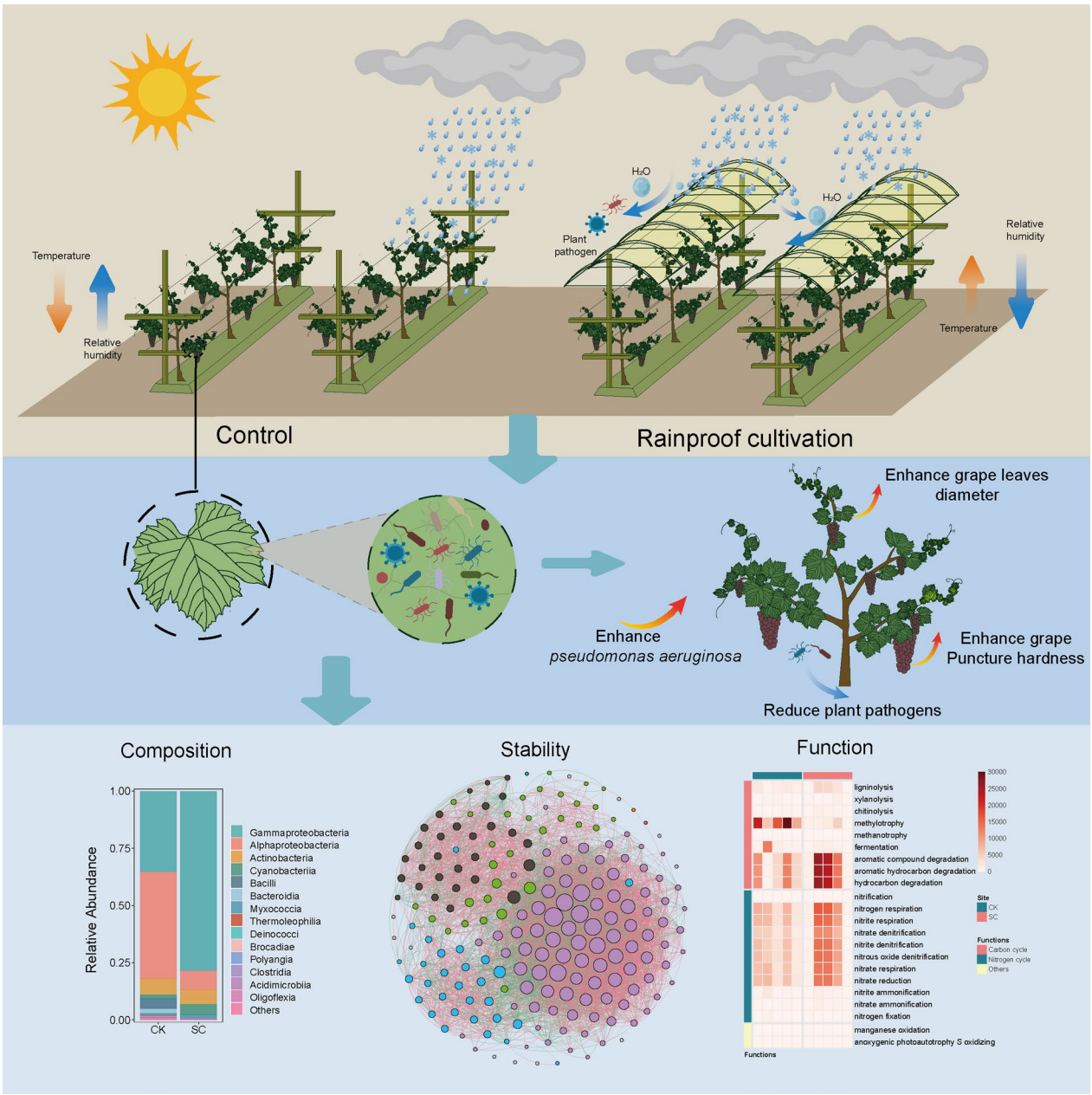
Graphical abstract



Introduction

Grapes, including wine grapes and table grapes, are among the most important fruit species cultivated worldwide, with significant economic and cultural value. In 2022, the global grape planting area reached 7.39 million hectares, yielding at total production of 87.62 million tons of grapes, China is the top major grape-producing country contributing alone a 12.67 million tons to the total production [22]. Despite its extensive production, the viticulture industry faces major challenges due to the increase of diseases caused by changing climatic conditions. Grape downy mildew, caused by *Plasmopara viticola* (Berk. & Curt.) [30], is a critical disease of grapevines, affecting all green tissues and causing crop yield losses of 40–90% in the field [30, 65]. In severe cases, infected leaves die, and the infection can also spread to

the fruits, leading to significant yield losses [24]. Chemical methods currently serve as the primary approach for preventing and controlling grape downy mildew. However, the widespread application of chemical pesticides has led to the development of varying levels of resistance in many production regions [19, 25]. Additionally, excessive pesticide application contributes to environmental pollution, contaminates drinking water, and leaves residues in crops that pose significant health risks [61, 66]. In response to these adverse effects, the European Union has implemented restriction on the use of chemical fungicides, emphasizing the development of disease-resistant grape varieties and the promotion of biological control strategies [35]. As a result, there is an urgent need to develop sustainable and effective disease management practices.

Rain-shelter cultivation, distinct from greenhouse cultivation, functions as a protective barrier, shielding plants from rainfall [14]. By preventing rainfall from reaching the crops, this method establishes a stable growth environment leading to significant improvement in both crop yield and quality. For example, it is increasingly adopted in regions where the ripening season overlaps with the rainy season, proving beneficial for crops such as grapes, pears, strawberries, and *Panax notoginseng* [9, 81, 82]. In China, especially in Yunnan Province, rain-shelter cultivation has been widely adopted to mitigate rain-related diseases such as grape downy mildew. Some research indicates that rain-shelter cultivation can prevent damage from excessive rainfall and hail, reduces disease occurrence, and provide significant protective benefits [64]. Rain-shelter cultivation contributed to a decrease in the apparent infection rate, average temperature, relative humidity, and leaf wetness duration of 'Kyoho' grapes, leading to a reduction in the severity of downy mildew, compared to open-field cultivation, rain-shelter cultivation delayed the onset of the disease, thus reducing the disease incidence in plants [5, 78]. Rain-shelter treatment protected the plants from rainfall and reduced the relative humidity of the canopy. It also demonstrated significant inhibitory effects on white rot, gray mold, and brown spot [14]. Additionally, wines produced from grapes cultivated under rain-shelter conditions exhibit increased monoterpene concentration [80]. The implementation of rain-shelter cultivation in Yunnan has significantly advanced the viticulture industry. Over the past decade, the grape planting area has expanded to 46.69 thousand hectares, positioning Yunnan as a major table grape-producing region in China. To sustain the healthy development of the grape industry and address challenges such as disease management, this study aims to utilize rain-shelter cultivation to reduce the incidence of natural diseases in grapes, such as downy mildew. Furthermore, it seeks to investigate the mechanisms through which rain-shelter environment influence phyllosphere microbial communities to enhance grapevine health.

Understanding the microbial communities on grape leaves is essential to this exploration. The phyllosphere of plants hosts a diverse array of microorganisms, including fungi, bacteria, cyanobacteria, viruses, and actinomycetes. These microorganisms interact through mutual promotion, exclusion, and competition for nutrients [27, 74]. These microbial communities are associated with specific plant functional traits and can influence plant physiological functions, enhancing mineral absorption, plant biomass, and chlorophyll content to promote plant growth [3, 33]. A study demonstrated that these microbial communities significantly influence plant health and productivity by promoting growth and enhancing pathogen resistance [8]. In a recent study, rain-shelters influenced the composition and community structure of plant phyllospheric microorganisms in 'Cabernet Sauvignon' grapes which resulted in decreased OTUs of fungi and bacteria in the soil, along with reduced diversity of leaf and branch fungi [32].

However, studies on the impact of rain-sheltered cultivation primarily focus on soil microorganisms, with limited investigations on leaf microorganisms, particularly leaf oomycetes. Additionally, it is not yet clear which functional bacteria within the microbial community are influenced by rain-sheltered environments and whether these changes promote the health of 'Red Globe' grapes. This study aims to address these knowledge gaps by using Illumina high-throughput sequencing technology in conjunction with bioinformatics analyses. The objectives are to: (a) determine the effects of rain-sheltered habitats on the structure, composition, and assembly of phyllospheric fungi, bacteria, and oomycetes on 'Red Globe' grape leaves; (b) identify the functional roles of key microorganisms in rain-sheltered environments; and (c) investigate the regulatory mechanisms of phyllospheric microbial communities on grape health in these environments. The findings from this research will provide a theoretical foundation for future rain-sheltered cultivation practices, contributing to sustainable and environmentally friendly production of 'Red Globe' grapes.

Materials and methods

Plant material and study site

The grapevine variety used in this study was 'Red Globe', during the grapevine growth period, which was 10 years old and located at the Grape Practice Base of Yunnan Agricultural University, Yunnan Province, China, the average annual temperature is 21 °C, the average precipitation is 1031.7 mm, and the altitude is 1873 m (coordinates: 102°10′ E, 25°23′ N), The experimental plot

was selected in a pesticide-free area, and the soil type was classified as sandy loam. The experimental grape planting shed had the following dimensions: arch shed height × side height × width × length = 3.0 × 1.5 × 4.5 × 1 2.0 m. The cultivation method employed was shed cultivation, with each shed containing two rows of vines, and plant spacing of 2.0 m × 1.0 m. A randomized block design was employed to subject grapes to either rain-sheltered cultivation (SC) or open-air cultivation (CK), with each treatment comprising over 30 grape vines. During the experiment, watering was carried out according to the normal management mode of the base. During the study period, the daily average temperature, daily average relative humidity, and daily average light intensity of the grape canopy curtain were measured on sunny and rainy days.

Sample collection

After 180 days of rain shelter cultivation, grape leaves were collected from both the treatment and control groups. A standardized sampling protocol was followed where, three grape vines with similar growth patterns were selected. Young leaves were harvested from each vine, ensuring consistency in their orientation and height above ground. Leaves showing damage or irregular growth were excluded from the sampling. Each sample was labeled, and approximately 2 g of grape leaves were taken and subsequently stored in 50 mL sterile centrifuge tubes. The collected leaves were immediately frozen in dry ice and transported to the laboratory for future analysis. These leaves were used to isolate beneficial microorganisms within phyllosphere.

Sample pretreatment

The field-collected samples collected were processed and weighed on a clean bench. For every gram of sample, 10 mL of potassium phosphate buffer (0.1 M, pH = 8.0) was added, followed by ultrasonication for 15 min at 40 kHz. The mixture was vortexed at 200 rpm/min for 10 s at room temperature, rinsed back and forth 15 times, and the resulting eluate was collected and transferred to a low-temperature centrifuge. This procedure was repeated twice. The eluates from each group were combined and centrifuged at 13,000 rpm for 10 min. The supernatant was discarded, and the precipitate was collected. A portion of the sample was used for microbial analysis, while the remaining portion was used for the isolation of cultivable microorganisms.

Determination of antibacterial activity of culturable microorganisms

Cultivable microorganisms from the leaf surface were primarily isolated using potato dextrose agar (PDA) medium. Two grams of the previously centrifuged precipitate were serially diluted to concentrations of t 1×10^{-1}/mL, 1×10^{-2}/mL, 1×10^{-3}/mL, 1×10^{-4}/mL, and 1×10^{-5}/mL. The diluted samples were then spread on agar plates using the spread plate method and incubated for three days. After incubation, individual colonies were isolated, purified, and stored for subsequent molecular biological identification. *Pseudomonas aeruginosa*, isolated from grape leaves phyllosphere, was stored in a strain storage box. Prior to the subsequent experiment, the strain was cultured using PDA medium to ensure its viability.

Following the activation of pathogenic bacteria, the antagonistic potential of the isolated biocontrol bacteria was evaluated using the plate confrontation method. A 5 mm diameter tool was used to extract a bacterial colony from the culture dish containing pathogens, which was then placed in the center of PDA medium. Simultaneously, the leaf disk floating method was employed to determine the biocontrol bacteria's ability to manage grape downy mildew. A 10 μL bacterial suspension was pipetted onto four points—top, bottom, left, and right—each positioned 2.5 cm from the bacterial colony. The culture medium inoculated only with the pathogen served as the control. Each treatment was replicated three times. The inoculated plates were incubated at 25 °C in a constant temperature chamber. After 5 to 7 days, the radius of the pathogen colonies in both groups was measured.

The method for determining the isolated *Pseudomonas aeruginosa* was as follows: the DNA of *Pseudomonas aeruginosa* was extracted using the kit provided by Kunming Shuoyang Technology Co., Ltd., and the bacterial 16SrDNA universal primers 27F/1492R were used for amplification. The primers were 27F:(5′-AGAGTTTGATCCTGGCTCAG-3′),1492R:(5′-TACGGCTACCTTGTTACGACTT-3′) [67]. PCR reaction system: Nuclease-free H_2O 19 μL, 2 × PCR Taq Master Mix 25 μL, Forward primer (10 μM/L) 2.0 μL, reverse primer (10 μM/L) 2.0 μL, DNA template 2.0 μL, total volume 50 μL, PCR amplification process: 95 ℃ pre-denaturation, 4 min 30 s; 94 ℃ denaturation 40 s, annealing at 55 ℃ for 45 s, extension at 72 ℃ for 1 min, for a total of 30 cycles; reaction at 72 ℃ for 10 min, and storage at 4 ℃. The PCR product was detected by 1% agarose gel electrophoresis, and then sequenced by Kunming Shuoyang Technology Co., Ltd.

Determination of grape berry quality assessment

Subsequently, the beneficial microorganisms were transformed into a bacterial suspension of 1×10^{6}/mL. This suspension was applied as a foliar spray in the vineyards to evaluate its efficacy in controlling downy mildew and its effect on grapevine growth parameters. Simultaneously, a portion of the collected leaves was used to isolate

beneficial microorganisms on the leaf phyllosphere. After isolation, these beneficial microorganisms were prepared into a 1×10^6/mL bacterial suspension and sprayed in the field vineyards to evaluate its control over downy mildew and its effect on grape growth indicators.

The field was divided into four randomized plots, and three sets of bacterial suspension individually sprayed, while sterile water was used as a control. Fruit samples were collected from the four plots during the fruit maturity period. Five 'Red globe' grapevines were selected from each plot, and 20 berry were randomly obtained from each 'Red globe' grapevine. The horizontal and vertical diameters of the 'Red globe' berry were measured using a vernier caliper (Shenzhen Sicheng Resources Technology Co., Ltd. Mitutoyo CDN-PC). The puncture hardness of the fruit was measured using a handheld fruit hardness meter (manufacturer: Zhejiang Top Instrument Co., Ltd.; model: GY-3). The sugar content and acidity were measured using a sugar-acid all-in-one machine (Model: PAL-BX|ACID2). The analyzer was first calibrated with clean water, then dried with paper, and 0.5 mL of juice was extracted from the fruits for subsequent measurement of sugar content and acidity, each measurement was repeated 3 times.

RNA extraction, cDNA synthesis and PCR amplification

The precipitate obtained during the pretreatment process was subjected to RNA extraction, cDNA synthesis, and PCR amplification. Total RNA was extracted from the microbial community present in grape leaves using the Soil RNA Extraction Kit (Major bio, Shanghai, China) according to the manufacturer's protocol. The integrity of the RNA extracts was verified on 1% agarose gels, and their concentration and purity were assessed using a Nano Drop 2000 UV–Vis spectrophotometer (Thermo Scientific, Wilmington, USA). Complementary DNA (cDNA) was synthesized from the total RNA using the HiScript II Reverse Transcriptase Kit (Novozyme, China) and subsequently quantified using a Qubit fluorometer (Life Technologies) [34].

To amplify the fungal ITS1 gene, the primer pair ITS1F_ITS2R was used [72], le bacterial amplification employed the primers 338F_806R [69]. Additionally, the oomycete amplification was performed using the ITS1-3F_ITS1-4R primers [12]. All PCR assays were conducted using an ABI Gene Amp® 9700 PCR Thermal Cycler (ABI, California, USA). The total PCR reaction was 20 uL and contained: 4 μL of 5×reverse-prime Fast Pfu buffer, 2 μL of dNTPs (2.5 mM), 0.8 μL of forward primer (5 μM), 0.8 μL of reverse primer (5 μM), 0.4 μL of reverse-prime Fast Pfu DNA polymerase, 1 μL of template DNA 10 ng/μL, and added ddH_2O to reach a total volume of 20 μL, the PCR control was ddH_2O. PCR amplification was carried out with denaturation at 95 °C for 3 min, followed by denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 45 s, a single extension at 72 °C for 10 min, and termination at 10 °C. PCR products were then extracted from 2% agarose gels, purified using the Axy Prep DNA gel extraction kit (Axygen Biosciences, Union City, CA, USA), following the manufacturer's instructions, and quantified using Quantus (Promega, USA). The purified amplicons were pooled for sequencing on the Illumina MiSeq PE300 platform, and the sequencing work was contracted to Shanghai Meiji Biotechnology Co., Ltd. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (PRJNA1112534, PRJNA1112550, PRJNA1112321, PRJNA1192574. https://www.ncbi.nlm.nih.gov).

Statistical analysis

Sequencing data were processed by separating and quality filtering the raw gene sequencing reads using Fastp (version 0.20), followed by merging with FLASH (version 1.2.7). Clustering of operational taxonomic units (OTUs) was conducted using UPARSE (version 7.1) (http://drive5.com/uparse/) with a similarity threshold set at 97% [16, 59], during which chimeric sequences were identified and removed. The classification of each OTU representative sequence was performed using the Ribosomal Database Project (RDP) classifier (version 2.2) (http://rdp.cme.msu.edu/), referencing the Unite (version 8.0) and Silva (version 138) databases with a confidence threshold of 0.7.

Statistical analyses, including the incidence and disease index, differences between groups, and collinearity network complexity, were conducted using GraphPad (version 8.0) (https://www.graphpad.com/) through ANOVA. The isolated strain sequences underwent clustering using MEGA (version 11.0), and evolutionary analysis was performed using the Maximum Likelihood method. Intergroup differences in the alpha diversity index were evaluated via Mothur (version 1.30.2) in conjunction with the student's t-test.

Nonmetric multidimensional scaling (NMDS) analysis was performed using the Bray–Curtis distance algorithm and ANOSIM, employing 999 permutations to assess group differences. At the phylum level of the phyllosphere microbial community, the Wilcoxon rank sum test was used to examine intergroup differences, with a two-tailed confidence interval and correction for multiple testing using the false discovery rate (FDR) method along with Bootstrap confidence interval calculations. At the genus level, multi-level species difference discriminant analysis was conducted using LEfSe, with the Linear Discriminant Analysis (LDA) value indicating species effect size on group differences,

applying a threshold of 3 and a stringent All-against-all multi-group comparison method. Microbial functional predictions were conducted with Funguild (version 1.0) [38], PICRUSt2 (version 2.2.0) [13] and FAPROTAX (version 1.2.1) [31]. All the above analyses were performed on the Shanghai Major Biotechnology Co., Ltd. platform (https://www.majorbio.com/web/www/index).

Collinearity networks were calculated using R (version 4.3.3) along with the Tidyverse, SpiecEasi, and igraph packages. Supplemented by visualization and further analysis using the Hiplot platform (https://hiplot.com.cn/home/index.html), Gephi (version 7.0) (https://gephi.org/), Lianchuan Bio Cloud Platform (https://www.omicstudio.cn/tool?order=complex), and Adobe Illustrator (version 25.2.1) (https://www.adobe.com/products/illustrator/campaign/pricing.html).

Results

Effects of rain-shelter cultivation on grape canopy temperature, humidity, and light intensity

The results indicated that there were 26 sunny and cloudy days and a total of 45 rainy days during the test period (Supplementary Table 1). The average temperature, relative humidity, and light intensity trends in both treatment groups were found to be similar. On sunny days, the average total temperature of the grape leaf canopy under the SC treatment was slightly higher than that of the CK group, but not statistically significant. Similarly, the average relative humidity and light intensity were slightly lower than those of the CK group, but the differences were not statistically significant (Fig. 1A–C). On rainy days, the temperature and light intensity trends were similar to those on sunny days, but the SC group significantly reduced the average relative humidity of the grape leaf canopy (Fig. 1B–D). Subsequently,

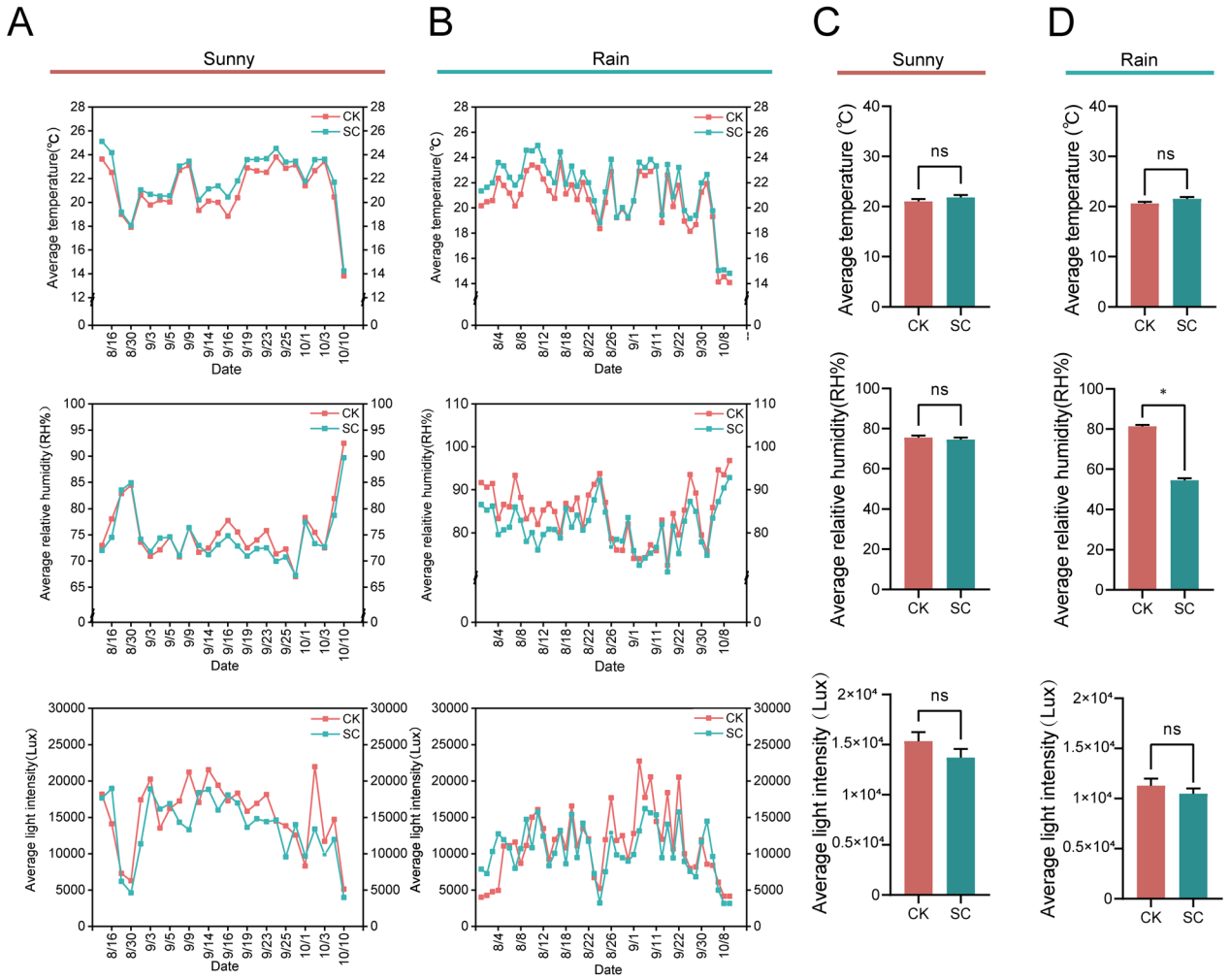


Fig. 1 Changes and differences in daily average temperature, relative humidity, and light intensity on sunny and rainy days under different conditions. CK: control group; SC: rain shelter cultivation group. **A**/**C**: sunny day; **B**/**D**: rainy day, "ns" means no significant difference, $*p < 0.05$

comparisons were made between the daily maximum and minimum temperatures, maximum and minimum relative humidity, and maximum and minimum light intensity during the test period. Statistical analysis of light intensity was conducted, indicating that sunny days and rainy days had a greater impact on relative humidity during the test period. Rainy days lead to higher relative humidity in the field. (Supplementary Fig. 1A-B). Further analysis demonstrated that the SC treatment significantly increased the maximum leaf temperature (Sunny and rainy days increased by 2.82 ℃ and 2.87 ℃) and significantly reduced the maximum relative humidity (Sunny and rainy days decreased by 1.1% and 0.69%) ($p < 0.05$) but had no significant effect on the other indicators (Supplementary Fig. 1C-D).

Incidence of grape downy mildew

The results indicated that grape downy mildew incidence under rain-shelter cultivation was significantly reduced, with an average incidence below 30%, compared to 80% under open-air cultivation ($p < 0.05$). The disease index for downy mildew was recorded below 10, which was significantly lower than that of the control group ($p < 0.05$) (Fig. 2A, B). Microbial sequencing analysis revealed a significant reduction in the relative abundance of *Plasmopara viticola* following the SC treatment ($p < 0.05$) (Fig. 2C).

Phyllosphere microbial diversity and composition

High-throughput sequencing analysis yielded 482,094, 446,206, and 333,316 valid sequences following amplification with ITS, 16S rRNA, and specific oomycete primers, respectively. The table below (Supplementary Table 2) displays the valid sequences for each sample. Using cluster analysis, all reads were grouped into operational taxonomic units (OTUs) based on 97% similarity. A total of 398 fungal, 1,619 bacterial, and 14 oomycete OTUs were identified. The dilution curve of the Shannon index for grape leaf microbiota demonstrated that, at a 97% similarity classification level, the diversity curves for fungi, bacteria, and oomycetes gradually approached a stable maturation point. This indicate that the sequencing data were sufficient to capture the majority of microbial species present on the grape leaf microbiota (Supplementary Fig. 2A–C).

Effects of rain-shelter cultivation on the alpha diversity of grape leaf microbial communities

The diversity indices presented in Table 1 indicated that higher values correspond to greater richness and diversity within the phyllosphere microbial community. Conversely, a larger Simpson indicated lower microbial diversity in grape samples. The fungal coverage index of both sample groups exceeded 0.99, signifying a high coverage rate and reliable sequencing results. In the CK group, the microbial community exhibited the greatest diversity among bacteria, followed by fungi and oomycetes. After the rain-shelter cultivation treatment, the order of richness remained consistent. The results demonstrated that rain-shelter cultivation treatment reduced the richness (ACE) and diversity (Shannon) of fungi and bacteria in the grape phyllosphere, though these changes were not statistically significance ($p > 0.05$) (Fig. 2D).

Effects of rain-shelter cultivation on the beta diversity of grape phyllospheric fungal communities

Beta diversity of grape phyllosphere microbial communities at the OTU level was analyzed using NMDS to explore potential shifts in microbial communities induced by rain-shelter cultivation (Fig. 2E, F). Similar to the results in the alpha diversity assessment, differences were observed between replicates in the rain-shelter cultivation and control treatments, likely due to the presence of numerous low-abundance taxa in the grape phyllosphere. Statistical analysis revealed a significant and clear separation was identified between the rain-shelter cultivation treatment and the control group. The central points of the samples were located in distinct quadrants, indicating that rain-shelter cultivation treatment had a significant impact on the beta diversity of fungal communities ($p < 0.05$; $r = 0.67$; $stress = 0.10$), a substantial effect on the beta diversity of bacterial communities ($p < 0.05$; $r = 0.5$; $stress = 0.07$), but no significant impact on the beta diversity of oomycete OTUs ($p > 0.05$; $r = 0.01$; $stress = 0.00$) (Fig. 2G).

Effects of rain-shelter cultivation on class-level composition with grape phyllosphere microorganisms

At the fungal class level, Dothideomycetes and Leotiomycetes were the predominant bacteria in both treatments, with average relative abundances of 60.1% and 10.91%, respectively, followed by Tremellomycetes (9.16%) and Agaricomycetes (4.87%). Following the rain-shelter cultivation treatment, the relative abundance of Dothideomycetes and Tremellomycetes increased by 0.98% and 3%, respectively, compared to the control sample, while Leotiomycetes and Agaricomycetes decreased by 10.77% and 1.87%, respectively (Fig. 3A). Upon statistical analysis, it was determined that among all species with a relative abundance higher than 0.01%, Cystobasidiomycetes experienced a significant reduction under the rain-shelter cultivation treatment ($p < 0.05$) (Supplementary Fig. 3A).

The community composition of bacteria at the class level was analyzed similarly to fungi. It was found that Gammaproteobacteria and Alphaproteobacteria were the major bacteria in both treatments, with average

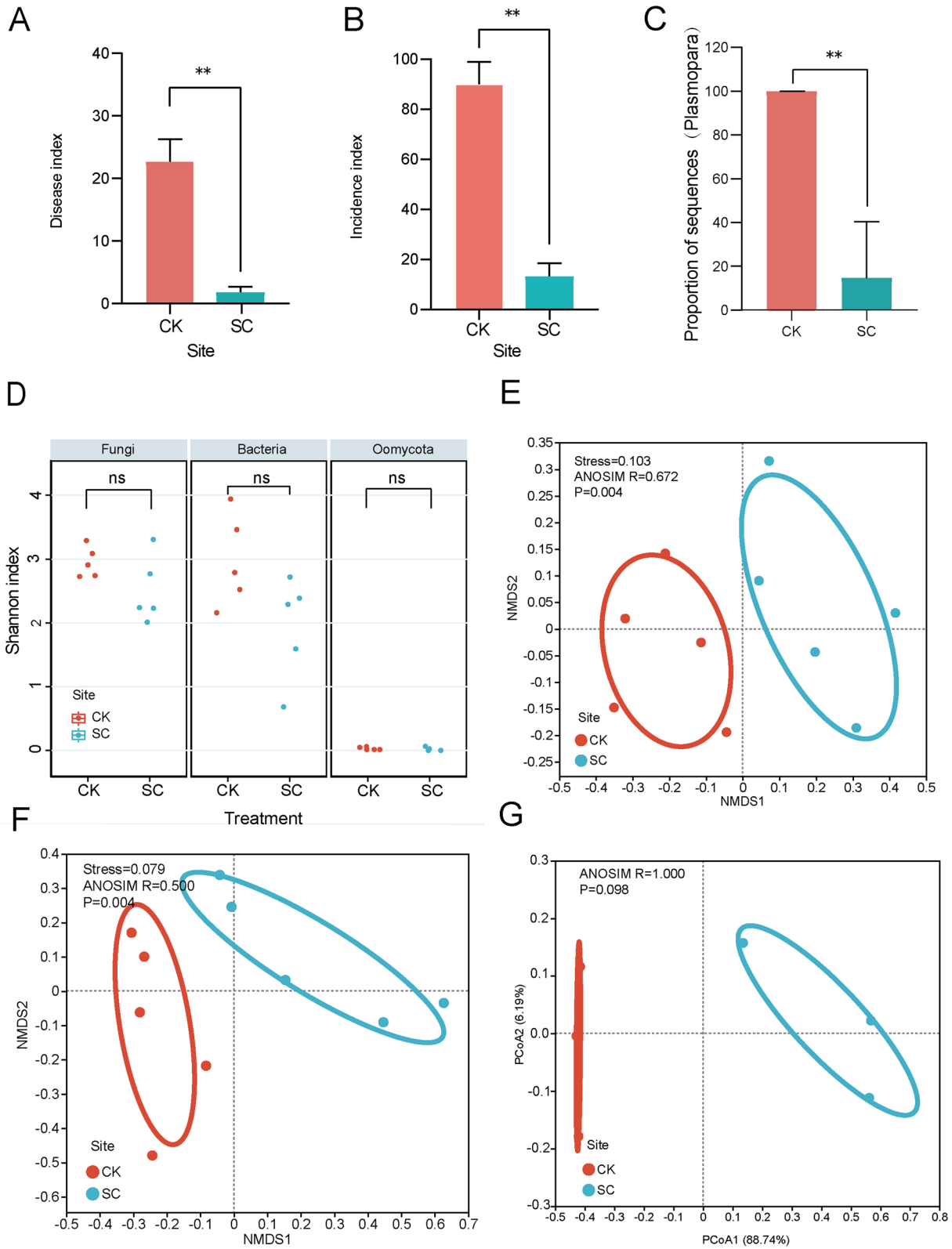


Fig. 2 Field disease occurrence and leaf microbial diversity results in rain shelter cultivation and control group. **A** Field disease index of grape downy mildew, **B** Field incidence of grape downy mildew, **C** Relative abundance of grape monosporus, **D** Shannon index of grape leaf microorganisms under different treatments, **E** Fungal NMDS analysis results, **F** Bacterial NMDS analysis results, **G** Oomycete PCoA analysis results, CK: control group; SC: rain shelter cultivation group. Note: "ns" means no significant; $^{**}p < 0.01$

Table 1 Diversity index table of two groups of grape samples

Estimators	Microbial taxa	BY	CK	*p* value	*Q*-value
Sobs	Fungal	56.00 ± 51.66a	92.80 ± 48.54a	0.28	0.28
	Bacteria	274.00 ± 74.64a	531.60 ± 200.80b	0.03	0.16
	Oomycetes	5.40 ± 1.52a	6.40 ± 2.07a	0.41	0.82
Simpson	Fungal	0.16 ± 0.06a	0.09 ± 0.02a	0.06	0.16
	Bacteria	0.37 ± 0.28a	0.15 ± 0.08a	0.14	0.20
	Oomycetes	0.90 ± 0.22a	0.99 ± 0.01a	0.35	0.82
Ace	Fungal	58.35 ± 53.50a	122.48 ± 47.92a	0.09	0.16
	Bacteria	405.37 ± 225.91a	616.47 ± 268.70a	0.22	0.26
	Oomycetes	9.75 ± 10.30a	12.23 ± 10.23a	0.72	0.85
Chao	Fungal	58.22 ± 55.62a	112.88 ± 54.41a	0.16	0.19
	Bacteria	356.36 ± 136.03a	615.49 ± 265.59a	0.09	0.18
	Oomycetes	6.90 ± 2.50a	8.60 ± 6.39a	0.60	0.85

The results shown in the table are expressed as "mean ± standard". According to the ANOVA test, different letters indicate significant differences ($p < 0.05$)

relative abundances of 56.90% and 27.49%, respectively, followed by Actinobacteria (6.71%), Cyanobacteria (3.19%), and Bacilli (2.36%). Following the rain-shelter cultivation treatment, Gammaproteobacteria and Cyanobacteria were increased (Fig. 3B). The increase in Gammaproteobacteria following rain-shelter cultivation was highly significant ($p < 0.01$), while Alphaproteobacteria, Actinobacteria, and Bacilli showed a decrease, with Alphaproteobacteria exhibiting a significant declining trend ($p < 0.01$) (Supplementary Fig. 3B). Further statistical analysis of bacterial taxa with relative abundances exceeding 0.01% indicated significant reductions in Bacteroidia, Brocadiae, and Phycisphaerae under the rain-shelter cultivation treatment, while Oligoflexia exhibited a significant increase. As expected, all samples were classified under the class Oomycetes following amplification with oomycete-specific primers (Supplementary Fig. 4).

Effects of rain-shelter cultivation on genus composition of grape phyllosphere microorganisms

At the genus level, *Cladosporium* and *Erysiphe* were identified as the predominant fungal genera in both treatments, with *Alternaria* and *Epicoccum* following in abundance. Post-rain shelter cultivation, *Erysiphe*, *Alternaria,* and *Epicoccum* decreased by 10.62%, 7.07%, and 4.41%, respectively, while *Cladosporium* exhibited a marked increase of 20.51%. Similarly, *Methylobacterium-Methylorubrum* and *Pseudomonas* emerged as the dominant bacterial genera in both treatments, with *Ralstonia* and *Rhodococcus* in subsequent positions. In the rain shelter cultivation treatment, the relative abundance of *Pseudomonas*, *Ralstonia*, and *Rhodococcus* were increased by 7.36%, 4.69%, and 2.47%, respectively, while *Methylobacterium-Methylorubrum* decreased significantly by 34.47% (Supplementary Fig. 5).

The LDA analysis identified variations in fungal and bacterial species between the rain-shelter cultivation and open-air cultivation. The magnitude of the LDA score reflected the extent to which species abundance contributed to the observed differences. Among fungal species, *Cladosporium* was significantly enriched in the rain-shelter cultivation treatment.In contrast, *Erysiphe, Cercospora*, *unclassified_f_Microascaceae, Neocosmospora*, *Epicoccum*, *Symmetrospora*, *Pseudombrophila*, *Chaetomium*, *Didymella*, and *Gibberella* were significantly enriched in the control. For bacterial species, *Delftia* and *Blastomonas* showed significant enrichment, while *Methylobacterium-Methylorubrum*, *Sphingomonas*, *Hymenobacter*, *Kineococcus*, *CL500-29_marine_group*, *norank_f__norank_o__norank_c__KD4-96*, and *Azospirillum* were significantly enriched in the control group (Fig. 3C).

The findings indicated the presence of *Erysiphe*, *Alternaria*, *Cercospora*, and *Colletotrichum* in the SC group, with a moderate decrease in their abundance. The reductions in *Erysiphe*, *Alternaria*, and *Cercospora* were statistically significant ($p < 0.05$) (Fig. 3D).

Assembly of microbial communities under rain-shelter cultivation

The findings revealed that regardless of the weather conditions, the temperature had a negative correlation with the main grape diseases (*Erysiphe*, *Botrytis*, *Colletotrichum*). Specifically, on sunny days, the relative humidity showed a significant positive correlation with the anthracnose pathogen, *Colletotrichum*, and a significant negative correlation with *Cercospora* (Fig. 4A). Temperature demonstrated a negative correlation with the *Erysiphe*, relative humidity and light intensity displayed a positive correlation with it, reaching significance for

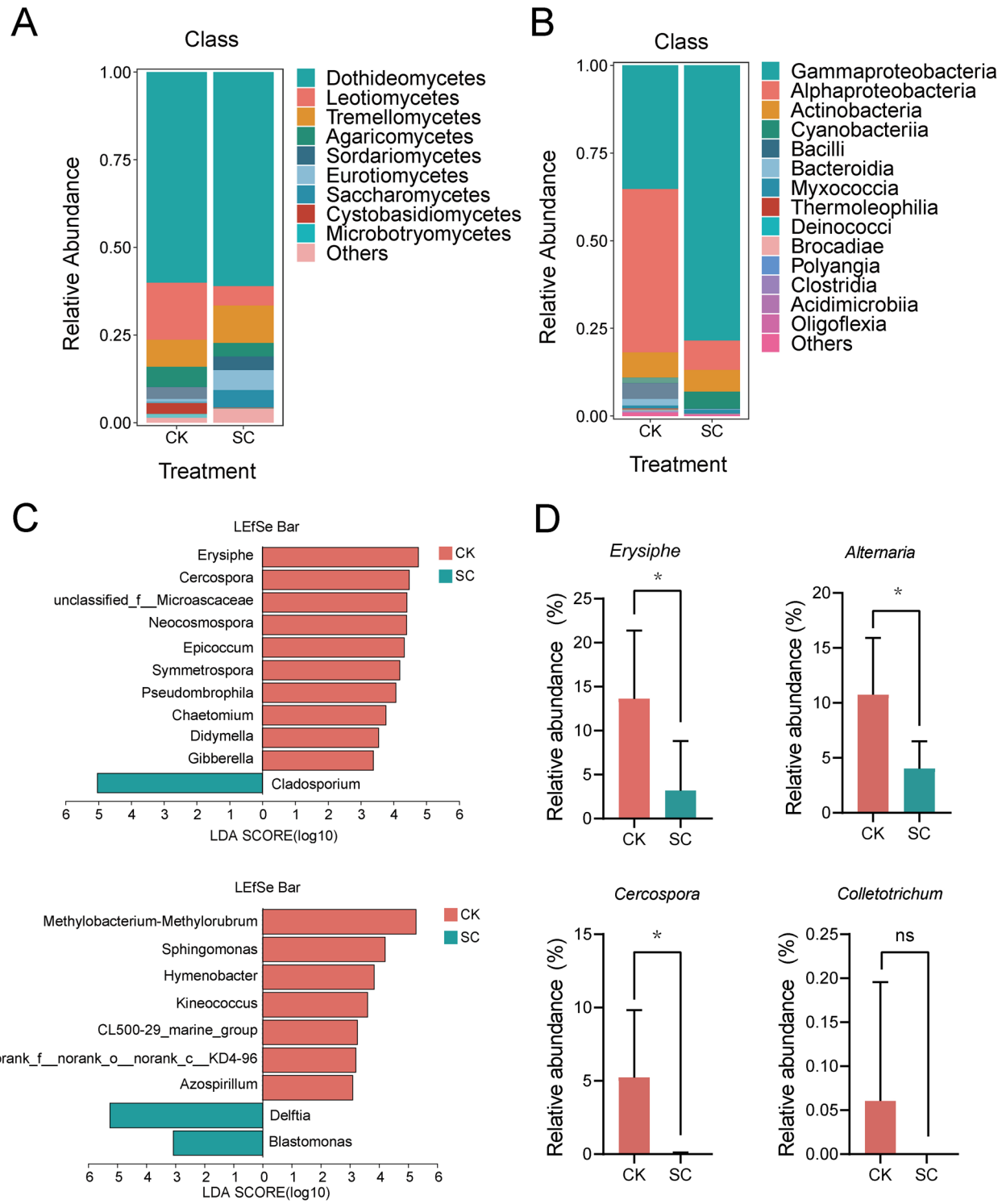


Fig. 3 Microbial composition of grape phyllosphere on rain-shelter cultivation and control groups. **A** Results of species composition at the fungal class level; **B** Species composition at the bacterial class level, **C** LEfse analysis results of grape phyllosphere fungi and bacteria, **D** Analysis of relative abundance differences of main pathogens in the grape phyllosphere. Note: "ns" means no significant; *$p < 0.05$

light intensity on rainy days ($p < 0.05$) (Fig. 4B). The fungal db-RDA results demonstrated a total of 31.71% of the variation, with the most influential factors being rainy day relative humidity (RR), and temperature (TR), while light intensity (LR) had the least impact. In comparison, the bacterial db-RDA results explained 43.73% of the variation, with temperature (T) and light intensity (L) on sunny days being the most influential factors, and the least impact observed for rainy day light intensity (LR) (Fig. 4C, D).

In the SC treatment, the NST value of the fungus fell below 50% (Fig. 4E–G), indicating the dominance of the deterministic process. The bacterial NST values in both the SC and CK groups were also below 50%, suggesting deterministic process dominance, but the bacterial NST values in the SC group were significantly lower than those in the CK group ($p < 0.01$). The trend of the oomycete community in the SC and CK groups aligned with that of the bacterial community. The NST value of the SC group was significantly lower than that of the CK group and remained below 50% ($p < 0.01$). These values indicate that the fungal community transitioned from stochastic to determinism processes after rain-shelter cultivation, and rain-shelter cultivation altered the aggregation mechanism of the fungal community ($p < 0.001$). Both the bacterial and oomycete communities in the rain-shelter cultivation and control groups were dominated by deterministic processes, and rain-shelter cultivation significantly impacted the degree of aggregation in the bacterial and oomycete communities ($p < 0.01$).

Phyllosphere microbial network properties

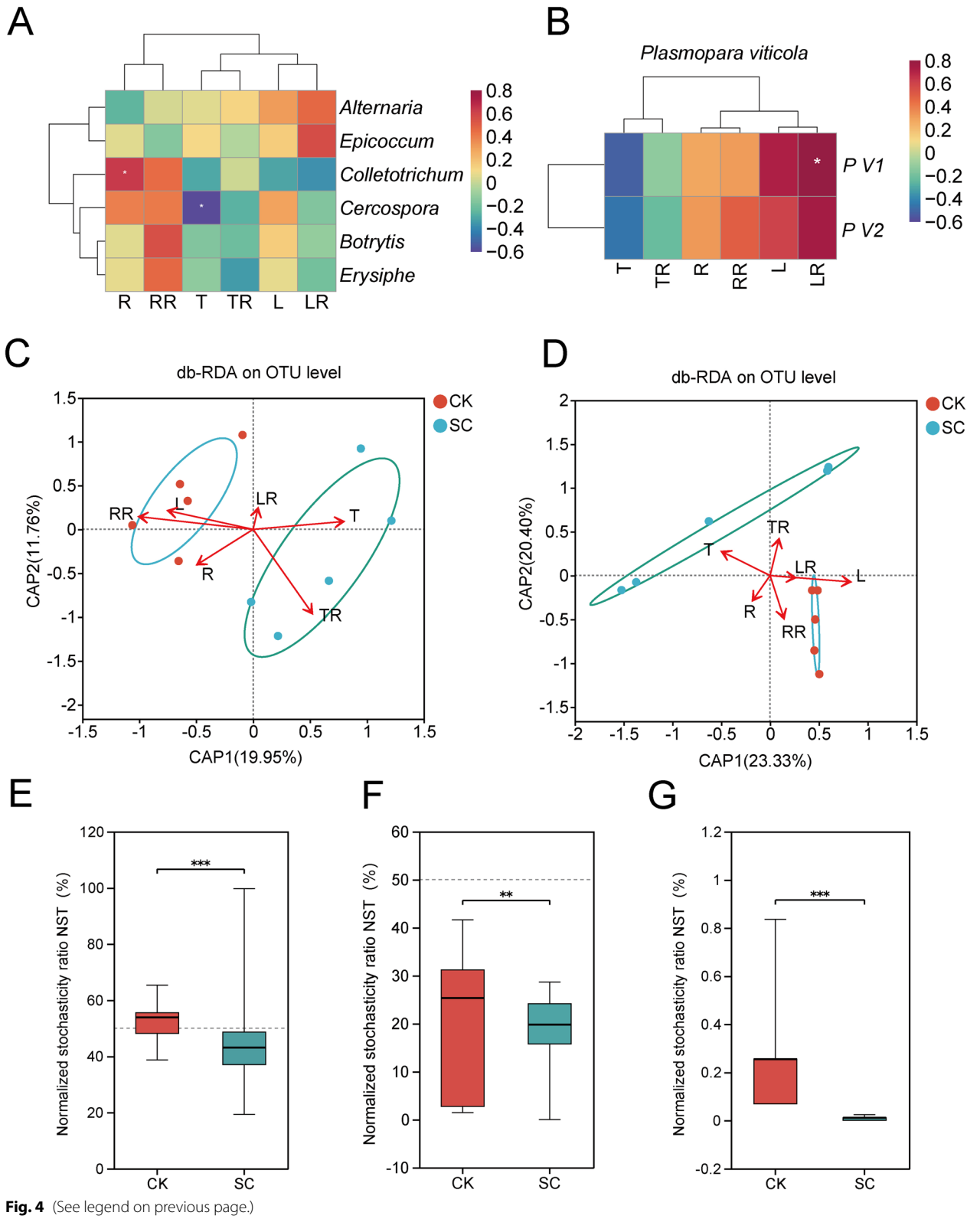
A microbial collinear network based on SparCC correlation was constructed to demonstrate the impact of rain-shelter cultivation on the structure of the grape phyllosphere microbial community (Fig. 5A) [70]. The results revealed significant differences in the symbiotic patterns of plant phyllosphere fungi and bacteria under different treatments. The results of the collinear network topology index showed that the network complexity of grape phyllosphere fungi increased significantly after rain-shelter cultivation. In contrast, the network complexity of bacteria decreased significantly after rain-shelter cultivation. Network topology index analysis indicates that after SC, the number of fungal nodes, edges, average clustering coefficient, and modularity all decreased (Fig. 5B, Supplementary Table 3); however, the number of modules remained unchanged, and the lower network modularity may have an adverse effect on stable co-occurrence patterns. At the same time, SC significantly reduced the complexity of the bacterial network ($p < 0.05$) (Fig. 5D). After SC, the number of bacterial nodes, edges, and modularity all decreased, while the average clustering coefficient and modularity increased (Fig. 5B, Supplementary Table 3). To further assess the stability of the microbial network, we removed the influence of network nodes on natural connectivity. For the fungal network, natural connectivity significantly decreased after SC, indicating lower stability. In the bacterial network, natural connectivity decreased significantly after CK, while the decrease in the SC group was less pronounced, indicating that the bacterial network's stability was higher following SC (Fig. 5C).

Predicted functional properties

The FAPROTAX functions of the bacteria in the two groups of treatments were predicted, and the results of their involvement in the carbon and nitrogen cycles were analyzed. The findings indicated that bacterial functions were mainly concentrated in aromatic compound degradation, aromatic hydrocarbon degradation, hydrocarbon degradation, and nitrogen metabolism-related pathways (Fig. 6A). Methylotrophy and methanotrophy were significantly reduced after SC treatment (Fig. 6B). All bacterial OTUs were functionally predicted with PICRUSt2, and the relative abundance of the bacterial community showed a significant positive correlation with the KEGG Orthology (KOs), with a more apparent trend in the CK group (Fig. 6C). Top 10 KOs with significant differences were further screened for Mantel's analysis, and most of them showed negative correlations with the SC group. In the CK group, apart from Acyl-ACP dehydrogenase, D-methionine transport system substrate-binding protein, and Transmembrane sensor, the correlations included Acyl-ACP dehydrogenase, GntR and MocR family aminotr, Cytochrome C, and Outer-membrane receptor for ferric coprogen and ferric-rhodotorulic acid, which were negatively correlated. The remaining

(See figure on next page.)

Fig. 4 Correlation between environmental factors and phyllosphere microorganisms under rain-shelter cultivation and open-air cultivation, and the impact of rain-shelter cultivation on community microbial assembly. **A**/**B** Correlation between fungal pathogens, oomycete pathogens, and environmental factors, **C** Fungal db-RDA analysis results; **D** Bacterial db-RDA analysis results; **E**–**G** Fungi, Normalized stochastic ratio (NST) results for bacteria and oomycetes. Values above and below 50% of the NST threshold represent stochastic processes and deterministic processes respectively. Note: sunny light intensity (L), sunny temperature (T), sunny relative humidity (R), rainy light intensity (LR), rainy temperature (TR), rainy relative humidity (RR), "ns" means no significant; *$p < 0.05$; **$p < 0.01$, ***$p < 0.001$



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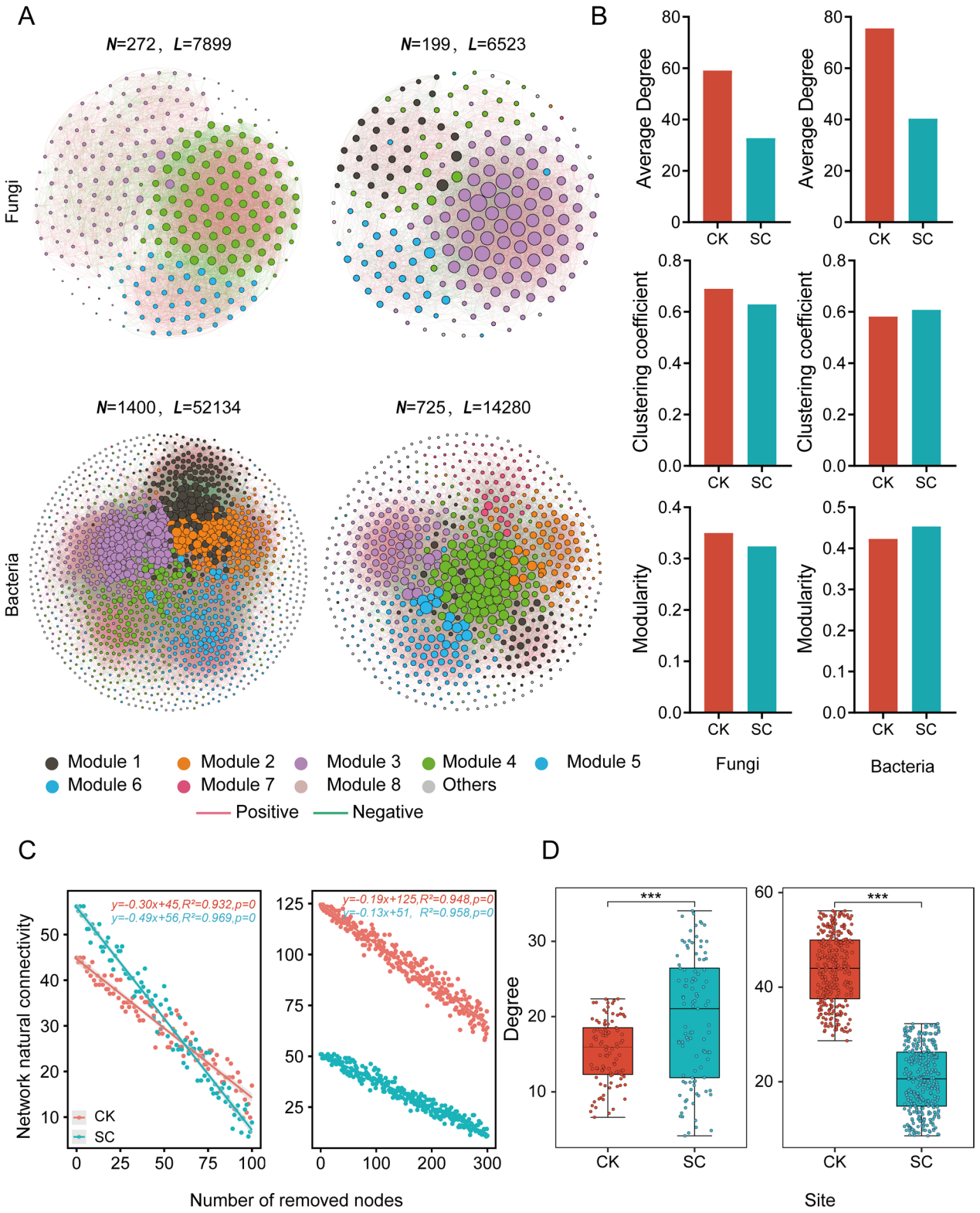

Fig. 5 Co-occurrence patterns and network properties. **A** A single node represents a single OTU, its size is positively related to the node degree, and the color represents the module. Edges indicate significant SparCC correlations with $r > 0.6$ and $p < 0.05$. The red line represents positive correlation, and the green line represents negative correlation; **B** Fungal and bacterial network attribute index, with fungi on the left and bacteria on the right; **C** Network stability test, different colors represent different treatments, and the equation represents changes in natural connectivity. **D** Community complexity analysis, ***, $p < 0.001$

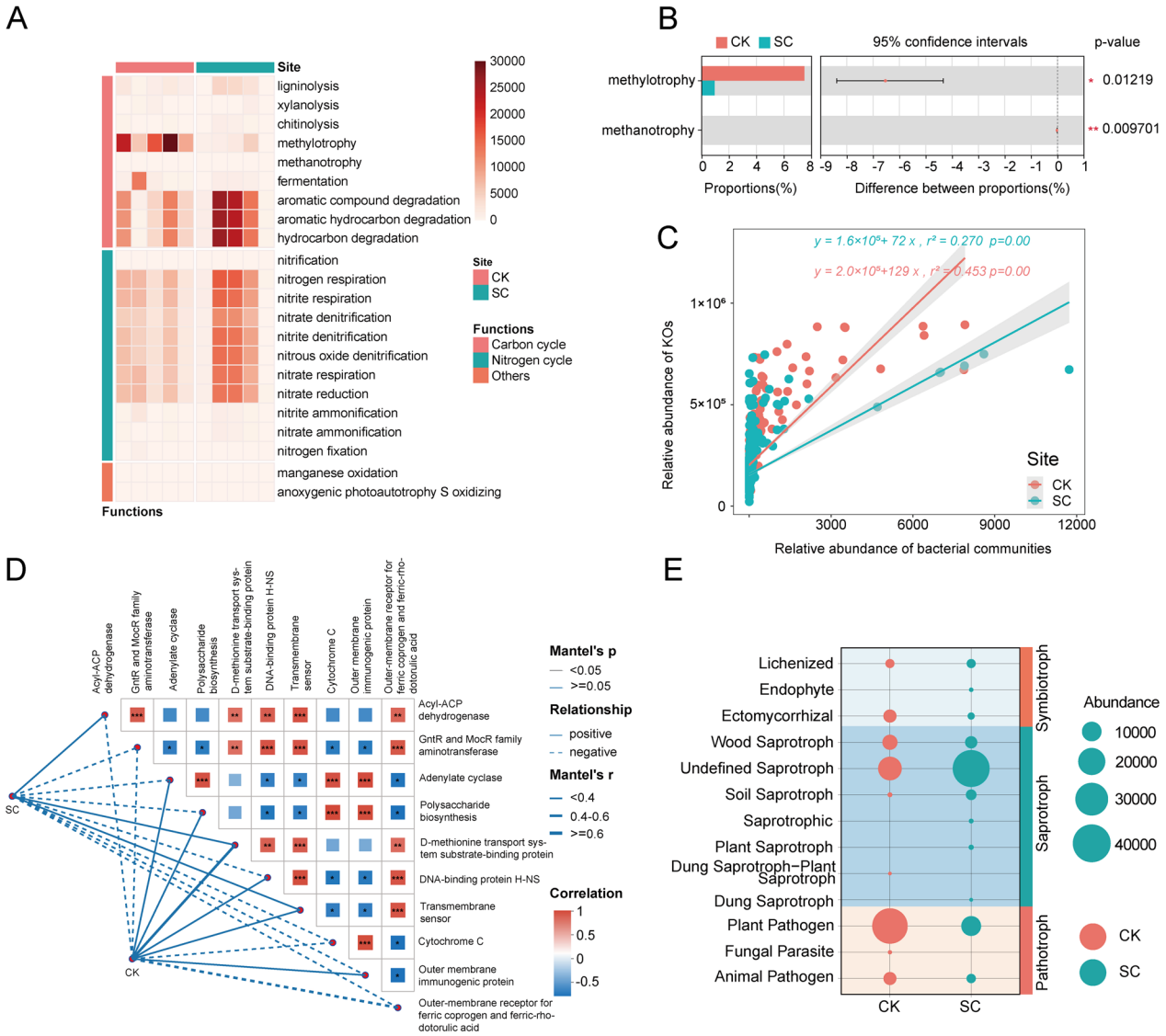

Fig. 6 Putative functions of microbial communities. **A** Relative abundance of bacterial functional groups related to nutrient cycling predicted by FAPROTAX. Relative abundance is scaled by log10 migration. The color blocks on the left represent different functional groups, and the color blocks on the top represent rain-shelter cultivation and control treatments; **B** Bacterial functional groups with significant differences; **C** Between bacterial communities and KOs Correlation, **D** Mantel correlation diagram shows KOs with significant differences; **E** Funguild is used to analyze the functional characteristics of the fungal community; the size of the node represents the prediction strength, and the different color blocks on the right represent different nutritional types

KOs showed a positive correlation with the CK group treatment (Fig. 6D). Funguild was employed to predict the function of fungi, and symbiotroph; saprotroph; and pathotroph fungi were selected for analysis. In the SC treatment, among symbiotroph fungi, lichenized fungi did not change, the endophytic fungi increased, and the ectomycorrhizal fungi decreased. Among saprotrophic fungi, undefined saprotrophs predominated, while wood saprotrophs and dung saprotrophs—along with plant saprotrophs—exhibited a decrease. Conversely, soil saprotrophs, saprotrophic fungi, plant saprotrophs, and dung saprotrophs showed an increase. Regarding pathotrophic fungi, plant pathogens were prevalent, whereas both fungal parasites and animal pathogens decreased in the SC group (Fig. 6E).

Resistance of culturable microorganisms on the phyllosphere to grape pathogens

Random forest analysis identified important microorganisms, including 26 fungal genera and 34 bacterial genera

(Fig. 7A, B). Combined with the culturable microorganisms isolated from the leaf phyllosphere, the microbial strain was ultimately selected. Upon sequencing and identification, it was determined that its taxonomic status was *Pseudomonas aeruginosa* (Fig. 7C). In a laboratory confrontation assay against grape downy mildew, grape anthracnose, and grape gray mold, *Pseudomonas* bacteria demonstrated a strong inhibitory effect on these pathogens. The diameter of pathogenic colonies treated with *Pseudomonas aeruginosa* was significantly smaller compared to those in the control group ($p < 0.001$) (Fig. 7C).

Effects of culturable microorganisms on the phyllosphere of grape fruit quality

The *Pseudomonas* bacteria isolated from the phyllosphere were formulated into a bacterial suspension and applied to 'Red Globe' grape plants during the fruit setting period. The results indicated that the *Pseudomonas* strain significantly increased the puncture resistance and leaf node interval length of the grape fruits, as well as their acidity ($p < 0.01$). However, it reduced the berry's sugar content and had no significant effect on other measured indicators (Fig. 7D).

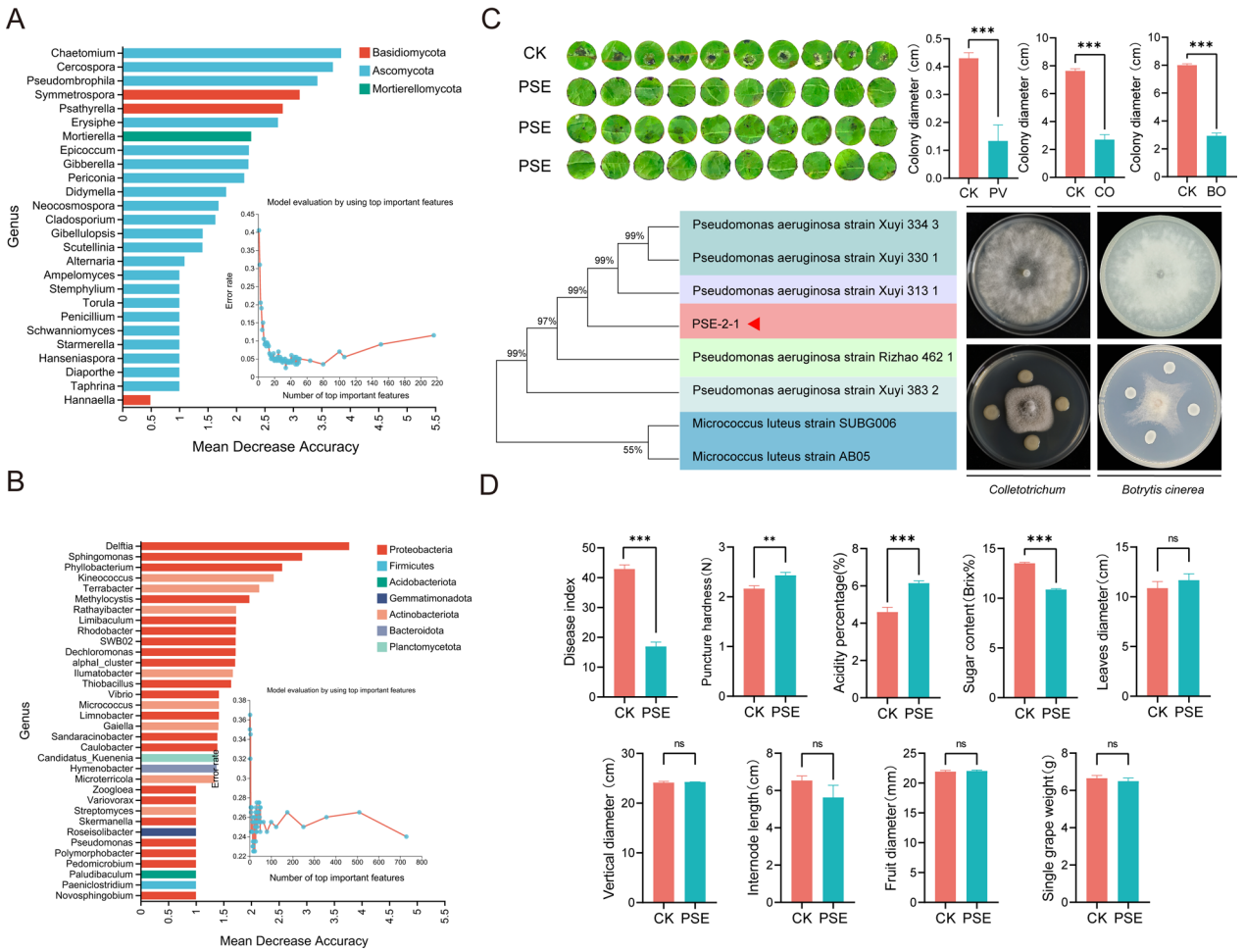

Fig. 7 Main functions of key microorganisms on the grape phyllosphere. **A** Random forest analysis of key fungal species on the grape phyllosphere at the OTU level; **B** Random forest analysis of key bacterial OTU species; **C** The inhibitory effect and Colony diameter of key species *Pseudomonas aeruginosa* on grape leaf disc inoculation of downy mildew, culture medium inoculation of anthracnose and gray mold, and the evolutionary tree indicates the species classification status of PSE, "PV" means "*Plasmopara viticola*", "CO" stands for "*Colletotrichum*", and "BO" is "*Botrytis cinerea*" "PSE" is *Pseudomonas aeruginosa treatment*; **D** Respectively indicate the disease index of grape downy mildew after the PSE bacterial suspension was sprayed into the field, and the changes in grape fruit-related quality indicators. Note: "ns" means no significant, **, $p < 0.01$, ***, $p < 0.001$

Discussion

Previous studies utilizing high-throughput sequencing to explore phyllosphere microbial communities under rain-shelter cultivation have predominantly focused on fungi and bacteria, leaving a gap in understanding the role of oomycetes in these environments. Our study uniquely addresses this gap by incorporating oomycete-specific primers in addition to the commonly used fungal and bacterial primers. This comprehensive approach allows for a more detailed analysis of the community structure and interactions among fungi, bacteria, and oomycetes. By elucidating these complex microbial dynamics, our findings provide new insights into how rain-shelter cultivation can be optimized to enhance grapevine health, particularly by managing downy mildew and other pathogens more effectively in high-rainfall areas.

Effects of rain shelter cultivation on plants and plant diseases

In recent years, rain-shelter cultivation has gained significant recognition and has rapidly developed within China's grape production industry. Studies have demonstrated. Studies have demonstrated that this method increased total photosynthetic accumulation and fruit yield, particularly in cherries grown in southwest China, while reducing the incidence of fruit cracking. This led to enhanced vegetative growth and improved fruit quality in cherry trees. [64]. Similarly, when bayberries were cultivated under rain-shelter conditions, there was a significant reduction in the presence of *Acetobacter* and *Gluconobacter*, which in turn decreased sugar consumption and disease incidence, thereby improving fruit quality quality [77]. This present study also observed a significant reduction in the incidence of downy mildew, a primary grape disease, under rain-shelter cultivation. Other research has indicated that compared with open-field cultivation, rain-shelter cultivation resulted in higher temperature and humidity, greater diurnal changes, reduced light intensity, and lower average soil moisture content, all of which contributed to improved fruit quality. Specifically, the average temperature under rain-shelter conditions is 2–3 °C higher than in open-air cultivation, and relative humidity was reduced by up to 5.79% [32]. This study also found higher temperatures and lower light intensity on both sunny and rainy days under rain-shelter cultivation.

Microbial composition and function

In this study, we performed a taxonomic analysis of microbial communities based on the transcriptional activity of microbes in the grape phyllosphere, which has provided a clearer understanding of the functional status of these communities. Notably, *Erysiphe necator* and *Plasmopora viticola* are specialized pathogenic fungi; conventional DNA-based high-throughput sequencing techniques may not adequately reflect their survival levels on host plant leaves. Analyzing microbial transcriptional activity helps identify metabolically active microbes under specific conditions, revealing their true role in ecological processes [45].

However, it is essential to recognize that this approach may introduce certain biases. Future studies should combine microbial abundance, transcriptomics, and metabolomics to better understand microbial community dynamics and ecological functions, offering a stronger foundation for managing grapevine diseases.

Durán et al. [15] observed that the fungal classes Dothideomycetes, Leotiomycetes, and Tremellomycetes, as well as the bacterial class Bacilli, exhibit negative correlations with other fungal and bacterial species in *Arabidopsis* roots, suggesting that these taxa may play a crucial role in regulating fungal-bacterial interactions in *Arabidopsis* roots. In the present study, Dothideomycetes demonstrated a relative abundance exceeding 60%, with values of 60.11% under rain-shelter cultivation and 61.09% under open-air conditions. Dothideomycetes comprises adiverse group of saprophytic known for decomposing plant tissues and includes some pathogenic species. This suggests that rain-shelter conditions may promote the accumulation and colonization of Dothideomycetes, potentially enhancing their tissue decomposition capabilities [11]. Additionally, Leotiomycetes were the second dominant microbial group on the grape phyllosphere, with many members being plant pathogens. The relative abundance of Leotiomycetes decreased from 16.29% to 5.02% under rain shelter cultivation, indicating that this cultivation may reduce the prevalence of Leotiomycetes on the grape phyllosphere.

Tremellomycetes, the third most abundant bacterial genus identified in this study, includes species known for their edible and medicinal properties. These fungi produce extracellular polysaccharides with antioxidant properties and the ability to inhibit starch-digesting enzymes, thereby affecting starch decomposition and delaying glucose release. This characteristic plays a significant role in the development of hypoglycemic drugs [50, 83]. In this study, the abundance of *Tremellomycetes* increased from 7.66 to 10.66% following rain-shelter cultivation, suggesting that this cultivation method may enhance the growth of grape phyllosphere microbiota with antioxidant capabilities.

Cystobasidiomycetes another fungal class, has been reported to contribute significantly to the resistance of elm trees against biotic and abiotic stresses. Inoculation of elm seedlings with Cystobasidiomycetes induced a

transient defense effect mainly mediated by Salicylic acid (SA), resulting in a reduction in the load of pathogenic bacteria, thus having a certain positive effect on plants [43]. However, in this study, the abundance of Cystobasidiomycetes was significantly reduced after rain-shelter cultivation treatment, indicating that rain-shelter cultivation method also impacts the relative abundance of beneficial microbial taxaon plant leaves.

Proteobacteria, Actinomycetes, Cyanobacteria, and Bacilli are the dominant bacterial groups? in the grape leaf phyllosphere. Proteobacteria are known for their strong adaptability, with some species carrying genes that interact beneficially with plants [6]. For example, rhizobia, which belong to the *α*-Proteobacteria, form a symbiotic relationship with legumes, acting as nitrogen-fixing endosymbionts [40]. Additionally, certain *Pseudomonas* species within the Proteobacteria phylum have denitrification abilities, allowing them to remove nitrate-nitrogen from plants without accumulating nitrites, and they can also eliminate nitrogen and chromium [76]. In this study, the average relative abundance of Proteobacteria on the grape leaf phyllosphere exceeded 80%, with an overall increase observed after rain-shelter cultivation.

Cyanobacteria can perform oxygenic photosynthesis and nitrogen fixation, producing various bioactive compounds that contribute to phosphate solubilization, soil fertility improvement, and increased crop productivity [36]. They also exhibit resistance to fungal and oomycete pathogens. For instance, *Microcystis aeruginosa* has been shown to have antifungal effects against mycotoxin-producing fungi [42], and some cyanobacterial compounds have demonstrated antifungal effects against *Aspergillus flavus* [55]. In this study, the increased presence of cyanobacteria after rain shelter cultivation suggests these microorganisms may be recruited to enhance nitrogen fixation in grapevines, thereby promoting their growth and development. Bacilli are known to harbor numerous beneficial microorganisms that promote plant growth, development, and soil fertility. Among them, *Bacillus* species, which are extensively studied, are particularly recognized for their production of antibiotics, enzymes, and iron carriers, all of which are significant for sustainable agricultural development [2]. Singh et al. [57] demonstrated that certain *Bacillus* genes effectively reduce the incidence of wilt in *Cicer arietinum* and significantly enhance plant growth and dry matter. Additionally, Pandey et al. [52] found that plant growth-promoting bacteria, such as natural cold-resistant *Bacillus* species, can improve nutrient utilization efficiency in host plants, leading to increased crop yields. However, in this study, the abundance of Bacilli decreased after rain shelter cultivation, indicating that *Bacilli* may be better suited for open-air environments. This suggests that rain-shelter cultivation may reduce the relative abundance of *Bacillus*-like microorganisms that are beneficial to plants.

Bacteroidia and Brocadiae are classes that include anaerobic bacteria [20]. Phycisphaerae, a group of bacteria involved in nitrate removal from plants, was significantly reduced under rain-shelter cultivation conditions [73]. In contrast, Oligoflexia, an aerobic bacterial class known for its unique adaptability, experienced a significant increase following rain shelter cultivation. This increase may be attributed to higher oxygen concentrations in the rain shelter compared to open air during the day, which could account for the observed reduction in anaerobic bacteria [39]. Additionally, oomycetes are recognized as potential biological threatsto high-value economic crops, including grapes, where they act as pathogens [63].

The fungal microorganisms with the highest relative abundance in both the rain-shelter and controlwere *Cladosporium*, *Erysiphe, Alternaria,* and *Epicoccum*, all of which belong to the phylum Ascomycota. *Cladosporium* is the predominant bacterial genus affecting grape leaf microorganisms. While many species within this genus are molds that can have adverse effects on the health of humans, animals, and plants [62], some *Cladosporium* species produce metabolites, such as isocladosporin and 5′-hydroxyasperentin, which are effective in controlling anthracnose and black mold [68]. Singh et al. [56] also found that *Cladosporium* dominates the fungal microbiota of the grape phyllosphere, consistent with the findings of this study. These results suggest that *Cladosporium* exhibits a significant increase after rain-shelter cultivation, indicating that the self-regulation and balance of the phyllosphere may contribute to maintaining the healthy growth of grapes. The pathogenic fungus *Erysiphe*, which causes grape powdery mildew, was significantly reduced after rain-shelter cultivation. This finding aligns with Du et al. [14], who reported that various rain-shelter cultivation methods inhibit grape powdery mildew. Differences in rain shelter conditions can lead to variations in the grape phyllosphere microenvironment and the incidence of powdery mildew incidence conditions, ultimetely affecting the relative abundance of phyllosphere microorganisms. Currently, research on grape phyllosphere microorganisms under rain-shelter cultivation is limited. *Alternaria* is a plant pathogen capable of infecting a wide range of crops, including potatoes, tomatoes, and carrots [17]. It has a strong adaptability to temperature and can cause grape rot throughout most of the growing season [37]. In this study, *Alternaria* was detected in both the rain shelter and control treatments, with relative abundances of 10.88% and 3.81%, respectively, suggesting that rain shelter cultivation may help mitigate diseases caused by *Alternaria*. *Epicoccum* has the

potential to enhance the biomass of sugarcane roots and control pathogens affecting sugarcane, indicating significant application potential [18]. Furthermore, *Epicoccum* exhibits effective antibacterial activity against various pathogens, including *Phytoplasma*, *Pythium*, *Monilinia* [29, 41, 48]. *Colletotrichum*, the pathogen responsible for grape anthracnose, can lead to substantial reductions in grape yield, increased volatile acidity, gluconic acid, and malic acid content, ultimately deteriorating the taste of grapes and related products. *Cercospora*, which causes leaf diseases in beets (*Beta vulgaris* L.), can inflict severe damage under severe conditions [46, 58, 75]. This study observed a reduction in both *Colletotrichum* and *Cercospora*- key plant pathogens associated with grape diseases under rain-shelter cultivation, suggesting this cultivation can decrease the relative abundance of plant pathogens and therefore promoting plant health.

The bacterial genera with the highest relative abundance were *Methylobacterium-methylorubrum*, *Pseudomonas*, *Ralstonia*, and *Rhodococcus*. Among these, only *Rhodococcus* belongs to the phylum *Actinobacteria*, while the others are classified within the Proteobacteria phylum. *Methylobacterium-Methylorubrum* is commonly found in the rhizosphere soil of wheat [1], though its specific functions and mechanisms of influence on plants remain unclear. Many plant-associated *Pseudomonas* species are known to promote plant growth by inhibiting pathogenic microorganisms, synthesizing growth hormones, or enhancing plant disease resistance [53]. Some *Pseudomonas* species also can produce gluconic acid (GA), solubilize insoluble phosphates, and stimulate the growth of pea plants [51]. Additionally, *Pseudomonas* can increase the availability of phosphorus in the soil by solubilizing it and increase its effectiveness for plants [60]. In this study, the relative abundance of *Pseudomonas* increased from 7.05 to 14.41%, indicating that the rain-shelter cultivation elevated the proportion of the beneficial bacteria, which may contribute to enhanced plant resistance. *Ralstonia* is a eutrophic autotrophic organism that can absorb carbon dioxide through a high-energy demand material cycle and has the *Cbb* gene encoding the enzyme of this cycle [4]. *Rhodococcus* is a non-spore-forming aerobic bacterium that metabolizes a wide variety of exogenous compounds and can biodegrade aromatic and nitrile compounds, as well as certain degradation effect on pesticides [44]. The results indicate that rain-shelter cultivation may create conditions more favorable for the survival of *Ralstonia*, which can absorb carbon dioxide, and *Rhodococcus*, which can degrade harmful compounds.

In rain-shelter cultivation treatments, *Delftia* and *Blastomonas* have been identified as free-living bacteria with significant functions. *Delftia* is recognized for its ability to enhance the performance of rhizobia-inoculated strains during co-inoculation of alfalfa and clover. Furthermore, when used in conjunction with *bradyrhizobia*, *Delftia* can regulate the chemical composition of plant tissues, facilitate nutrient absorption, and support the overall health of soybeans. Moreover, *Delftia* is considered a promising candidate for the treatment of water and industrial wastewater contaminated with high levels of Cr (VI) [7, 47]. *Blastomonas* has been documented to stimulate tiller production in rice plants, indicating its potential role in promoting plant growth [54].

Microbial networks and assembly

Interactions among microorganisms can significantly influence microbial communities and their functions. In this study, the complexity of the grape leaf fungal network was found to increase following rain-shelter cultivation. The number of edges and modules within the network's topological index also increased, indicating that rain-shelter cultivation promotes the formation of cooperative connections among fungal species on grape leaves. However, compared to the CK group, the modularity of the fungal symbiotic network after SC treatment was lower, that the diversity of leaf fungi was partially inhibited, potentially affecting the stability of the microbial network. In the bacterial symbiotic network of grape leaves, there was an observed increase in network stability, characterized by network complexity, fewer network nodes, and fewer edges. Previous research has suggested that different types of network stability have varying relationships with microbial complexity [71], and the evaluation of network stability remains unclear under multiple environmental factors [28]. The decline in microbial diversity inevitably may lead to a loss of potential ecosystem functions [23]. Similarly, this present study observed a decrease in the relative abundance of certain major plant pathogens, which may have feedback effects on the microbial network.

The assembly process of microbial communities is crucial microbial function [49], and is influenced by ecological diversity in natural ecosystems [26]. In this study, rain-shelter cultivation altered the temperature and humidity of the grape leaf canopy layer, consequently changing the microecological environment for microorganisms. Post-rain-shelter cultivation, the fungal community assembly shifted from a stochastic to a deterministic process, significantly altering the aggregation mechanism, with bacteria and oomycetes showing deterministic dominance. However, their NST values were significantly lower than those in the control group. Previous research has indicated that the microbial community succession is initially driven by stochastic processes [10], and strong disturbances can temporarily govern microbial

community assembly through stochastic processes [21]. During the niche differentiation stage, niche differentiation and microbial interactions control community assembly, with stochastic processes playing a crucial role [79]. In this study, open-air cultivation provided a a more favorable colonization environment for grape downy mildew pathogens, characterized by higher humidity and prolonged water retention. Conversely, rain-shelter cultivation disrupted these favorable conditions, which is a key principle in disease control.

Variations in environmental conditions exist between rain shelter cultivation and control treatments, leading to spatial heterogeneity in stress and impacts on microorganisms, thereby resulting in differences in microbial composition. Further investigation is needed to explore the specific effects of these factors on microorganisms in depth.

Conclusions

This study has demonstrated that rain-shelter cultivation significantly reduced relative air humidity on rainy days, increased leaf canopy temperature, and substantially decreased the incidence of grape downy mildew. Additionally, it influenced the beta diversity of phyllosphere microbial community during the growth of 'Red Globe' grapes. The results indicate that the bacterial community in the phyllosphere is more complex than the fungal community. Rain-shelter cultivation reduced the colonization and distribution of key pathogens including *Plasmopara viticola*, *Colletotrichum*, *Alternaria*, *Erysiphe*, and *Cercospora* within the vineyard. Moreover, rain-shelter cultivation enhanced the complexity of phyllosphere fungi and increased the stability of phyllosphere bacteria. Following rain-shelter cultivation, the assembly process of the fungal community shifts from stochastic to deterministic, while the assembly of bacterial and oomycete communities remained predominantly governed by stochastic processes. The beneficial microorganism *Pseudomonas aeruginosa* present on the phyllosphere was also found to exert inhibitory effects on the primary diseases of grapes. Furthermore, rain-shelter cultivation was shown to increase the puncture resistance of grape berries and the length of leaf internodes. These findings highlight the impact of rain-shelter cultivation on microclimate, disease incidence, and the composition and assembly of phyllosphere microbial communities. This study provides a theoretical foundation for maintaining the diversity and balance of grape phyllosphere microorganisms. In regions with heavy rainfall, rain-shelter cultivation offers a viable management strategy that can be promoted as an alternative to the use of chemical agents. The information derived from predicting microbial functions is inherently limited, underscoring the importance of integrating transcriptomics and metagenomics for more precise analysis aimed at identifying the functions of affected microorganisms.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40793-025-00708-3.

Additional file1.

Acknowledgements
Not applicable.

Author contributions
H: Conceptualization, Sampling, Investigation, Formal analysis, Visualization, Writing—original draft. R: Writing—review & editing, F: Sampling, Investigation, Formal analysis. D: Sampling, Investigation. Y: Sampling, Investigation, Z: Writing—review & editing, L: Formal analysis, W: Formal analysis. D: Conceptualization, Formal analysis. Z: Conceptualization. Z: Conceptualization, Resources. Y: Formal analysis. Z: Conceptualization, Resources. D: Conceptualization, Resources, Visualization, Writing—review & editing. All authors reviewed the manuscript.

Funding
This work was supported by the National Natural Science Foundation of China (32360712), Yunnan Province Agricultural Joint Special Project (202101BD070001-125), and the Yunnan Provincial Academician Exploration Funding Project (2018HA009).

Availability of data and materials
The datasets generated in the current study are available in NCBI repository. (PRJNA1112534, PRJNA1112550, PRJNA1112321, PRJNA1192574, https://www.ncbi.nlm.nih.gov.)

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors agree to publish.

Competing interests
The authors declare no competing interests.

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Received: 1 November 2024 Accepted: 15 April 2025
Published online: 22 May 2025

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