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The blueberry phyllosphere microbiota: tissue-specific core communities and their stability across cultivars and years

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Summary
Blueberries are critical for food production due to their widespread consumption and nutritional value. Beyond agriculture, wild Vaccinium species play essential ecological roles, including supporting pollinators and enhancing soil health. This dual importance underscores their relevance to both food security and ecosystem sustainability. The fruit-associated microbiome, both internal and surface-dwelling, includes a wide range of microorganisms. These microbial communities play a dual role: they influence fruit quality (e.g., taste, texture, shelf life) and are also involved in the degradation processes that occur during fruit senescence or postharvest storage.". Despite their importance, the specific factors shaping the microbiomes of blueberry fruits, as well as their relationship with other above-ground parts of the plant and their stability over different years, remain poorly understood. We conducted a field experiment to characterize the taxonomic composition of fungal and bacterial communities colonizing the leaves and the surface and pulp of fruits on a collection of 10 different cultivars of blueberry over two years. Independently from the sampling time, pulp of the fruit, surface and leaves harbor specific and distinct microbiomes. A major factor determining the microbiome of blueberry fruits and leaves was plant cultivar, followed by tissue. We further identified the core microbiome for each plant tissue and demonstrated that core taxa account for the dominant fraction of the microbiota of each plant. As trade and production of blueberries is expanding, our results provide a foundation for advancing the development of targeted microbiome management strategies, with potential applications in enhancing plant health and productivity.

Keywords Metabarcoding, Fungi, Bacteria, Plant microbiome, Core microbiome, Biodiversity, Climate change, Network analysis

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Introduction

Plants are colonized by complex microbial communities that play an essential role in various aspects of their physiology and development through processes such as pathogenesis and nutrient cycling, ultimately contributing to plant health [36, 77, 85, 121]. Each plant tissue has different morphological and physiological characteristics that create a set of diverse micro-habitats [129, 130], offering a multitude of distinct ecological niches that host specific microbial communities [25, 70]. In the phyllosphere, i.e. the aboveground parts of plants, microbes play a role in carbon and nitrogen cycles, growth promotion, stress tolerance, and biological control of pathogens [116].

The sources and environmental reservoirs of the phyllosphere microbiomes are various, including soil, seeds, the surrounding environment, and even the air [116]. For instance, it has been proposed that soil is the primary source of microbial colonization in maize, barley, wheat, and grapevine [83, 143]. Airborne communities play a crucial role in the early colonization of *Arabidopsis thaliana* [78]. While microorganisms can be introduced through various pathways, their composition and diversity are primarily shaped by plant genotype and environmental conditions [30]. Among land plants, *Vaccinium spp.* (thereafter "blueberries") are long-lived woody perennial members of the Ericaceae family [72]. Blueberries are widely used in food (beverages, jams), cosmetics, and pharmaceuticals [50, 57, 67, 146]. Their long shelf life makes them ideal for export and transportation, with global production and consumption recently surpassing one million tons [62]. Given their adaptability to harsh conditions across various latitudes, blueberries also might also serve as an ideal model for studying how climatic and environmental factors shape microbiome diversity in wild and cultivated berry fruits [88]. Despite their economical importance, little is known about the composition and functions of the microbiome of blueberry plants [61]. The majority of existing studies have focused on rhizosphere and endosphere [64, 96] and on their influence on plant adaptation against abiotic stresses such as dryness and extreme temperatures [2, 3, 44, 131]. Only a few studies have considered the composition of the aboveground microbiome of the blueberry plant showing that bacterial communities tend to be more abundant and prevalent than fungal ones in floral tissues such as nectar, although opposite patterns with yeast dominance have also been reported. Furthermore, microbial community composition appears to be strongly shaped by site-specific factors including pollinator activity, local climate, and agricultural practices. Recent work has also isolated epiphytic bacteria from blueberry flowers and fruits with antagonistic activity against postharvest fungal pathogens, supporting the importance of tissue-specific microbiomes in biocontrol strategies (e.g. *B. velezensis* and *A. spathodeae*) [22, 95, 106, 119]. Microbial community structure in blueberries is influenced by climate, impacting both diversity and abundance [87]. Other key determinants include soil properties [24], cultivation and management practices [133], and plant genotype, particularly in shaping fungal communities [102, 106]. Moreover, blueberry fruits are exposed to several pathogens in their life cycle, such as mummy berry (*Monilinia vaccinii-corymbosi*) [9], *Phytophthora*, *Colletotrichum spp*. and *Botrytis cinerea*, which are responsible for several diseases [14, 84].

While the role of microbial communities in pathogen biocontrol, host physiology, resilience and adaptation is well established, there is still a significant knowledge gap regarding how environmental factors, particularly annual climatic variations, shape the microbial communities associated with blueberry plants. Fluctuations in temperature, rainfall patterns, humidity, and seasonal duration across different years can alter the structure and diversity of plant-associated microbiomes. These shifts may have critical implications for biocontrol efficacy, as the abundance, composition, and activity of beneficial microorganisms capable of suppressing pathogens can vary over time. Understanding how climatic factors influence these communities is essential to develop stable, reliable microbiome-based strategies for disease management and to promote sustainable crop production. For example, warmer temperatures or prolonged drought conditions may favor drought-tolerant microbial taxa, while cooler and wetter conditions may promote the proliferation of specific microbial groups. Furthermore, genotype variations among cultivars and differences in plant tissues also influence the composition and dynamics of microbial communities inhabiting aboveground plant parts [32]. Finally, the relative importance of core microbial species shared across plant cultivars and tissues, as well as that of taxa specific to certain tissues or individual plants, remains poorly understood.

To address these knowledge gaps, we explored the above-ground microbiome (fungi and bacteria) of cultivated *Vaccinium spp.* using the metabarcoding technique in order to: *i*) Investigate whether different plant tissues exhibit specific compositional patterns and assess the extent to which they share microbial taxa, with the aim of identifying microorganisms characteristic of each microniche; *ii*) assess the major driving factors that influence the community composition, including host cultivar; iii) define the core microbiome specific for each tissue i*v)* explore the interactions within and between fungal and bacterial communities to identify potential candidates for biocontrol.

Comprehensive studies integrating these complex interactions are crucial for advancing our understanding

of plant health, enhancing stress resilience, and developing sustainable crop management strategies. As one of the most widely consumed fruits, blueberries exemplify the link between human, animal, and environmental health at the basis of the "One Health" concept. Fruit microbiota might be transferred to humans through consumption, and have an impact on gut microbiome and health status, particularly in early life [20]. Blueberry consumption supports gut microbiome diversity, suggesting potential prebiotic effects [68], promotes gut health, and may help prevent chronic diseases [49]. Understanding and managing microbial communities in food production and fermentation are key to ensuring safety and sustainability [138]. Further research on blueberry microbiota, especially in the processes that lead to the formation of fruit and leaf microbiomes, is essential to maximize health benefits and optimize cultivation practices.

Materials and methods

Plant materials and sampling

Ten blueberry (*Vaccinium corymbosum*) cultivars were selected from the germplasm collection of the Edmund Mach Foundation (FEM), located in Trentino-Alto Adige, Italy (46.0744° N, 11.2334° E). The site lies within a predominantly mountainous region, characterized by extensive forested areas and agricultural systems adapted to steep terrain through terracing. The local alpine climate features warm summers, cold winters, and increasingly frequent extreme weather events associated with climate change [7, 28]. For each cultivar, samples from three plants were collected from three compartments: leaves (30 samples), fruit surface (30 samples), and fruit pulp (30 samples), resulting in a total of 90 samples per year. Sampling was repeated over two consecutive years, yielding a total of 180 samples (Table S1); no phytosanitary treatments were applied during the first year, whereas in the second year, a copper-based treatment (tribasic copper sulfate, Hattrick 30WG) was applied at a concentration of 150 g/hL. Berries were harvested at stage 7 of the BBCH scale that subdivides the developmental cycle of the plants into ten clearly recognizable and distinguishable longer-lasting developmental phases [82, 134], visually homogeneous by size and showing a complete blue surface color; the white or red rings around the pedicel scar were considered to be genotype-specific. Fruits and leaves were collected using sterile tweezers and shears, placed in sterile plastic bags, then stored on ice and later stored at −20°C, until downstream analysis.

DNA extraction and sequencing

Sample preparation

Epiphytic (fruit and leaf surface) microbiome

The epiphytic extraction was made using 150 ml of sterile washing solution (0.9% NaCl, 0.01% Tween 80) was added, and then incubated on an orbital shaker at 400 rpm for 1 h [13]. At the end of the incubation, the water was filtered using sterile filtration apparatus (2GPU02RE Stericup Quick Release Millipore Express PLUS 0.22 µm PES, 250 ml Merck Millipore) to recover as much biological material as possible. The filters were used for DNA extraction using DNeasy PowerWater kit QIAGEN according to the manufacturer's protocol. DNA extractions were performed in batches ranging from 4 to 24 samples. For each extraction batch, a negative control (no biological material) was included and processed using the same DNA extraction and PCR amplification protocols as for the experimental samples. No DNA amplification was detected in any of the negative controls, confirming the absence of contamination during the extraction.

Endophytic (pulp) microbiome

The endophytic extraction required a different procedure [16]. Fifteen fruits were randomly selected for each replicate and washed in 90% ethanol once and then rinsed three times in sterile Milli-Q water to minimize contamination from the surface of the fruit. The disinfected surface of fruit was aseptically peeled with a disinfected scalpel. The pulp tissue of the fruit portion was aseptically removed and cut into smaller pieces using a sterile scalpel blade, and used for DNA extraction using the DNeasy PowerSoil Pro Kit by QIAGEN, according to the manufacturer's protocol. DNA was quantified using the NanoDrop™ 8000 Spectrophotometer (Thermo Fisher Scientific Inc. Waltham, Massachusetts, United States) according to the protocol provided by the manufacturer. DNA extractions were performed in batches ranging from 4 to 24 samples. For each extraction batch, a negative control (no biological material) was included and processed using the same DNA extraction and PCR amplification protocols as for the experimental samples. No DNA amplification was detected in any of the negative controls, confirming the absence of contamination during the extraction.

Amplicon sequencing

For fungi, the ITS1 region was amplified using ITS1F (5'-CTT GGT CAT TTA GAG GAA GTAA-3') and ITS2 (5'-GCT GCG TTC TTC ATC GAT GC-3') primers [47, 132] with overhang Illumina adapters. The PCR were carried out with a total volume of 25 µL, containing 1 µL of each primers (10 µM of each one), 0.25 µL of FastStart High Fidelity PCR System (Roche), 18,75 µL of nuclease-free water (Sigma–Aldrich), 2.5 [10X] Buffer, 0.5 µL dNTP and 1 µL of DNA (5–10 ng/µL). PCR were performed by using the GeneAmp PCR System 9700 (Thermo Fisher Scientific); the conditions were: initial denaturation at 95°C for 3 min, 35 cycles of denaturation

at 95°C for 20 s, annealing at 50°C for 45 s, extension at 72°C for 90 s, followed by a final extension at 72°C for 10 min.

For bacteria, we amplified the sub-region V3-V4 of the 16S gene using the 341 F (5'- CCT ACG GGN GGC WGC AG –3') and 805R (5'- GAC TAC NVG GGT WTC TAA TCC –3') primers [66] with overhang Illumina adapters. The PCR were carried out with a total volume of 25 µL, containing 5 µL of each primer (1 µM), 12,5 µL MIX KAPA HiFi HotStart ReadyMix (Roche) and 2,5 µL of DNA (5–10 ng/µL).

PCR were performed by using the GeneAmp PCR System 9700 (Thermo Fisher Scientific); the conditions were: initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s, followed by a final extension at 72°C for 5 min.

The obtained amplicons were purified using the CleanNGS (CleanNA), following the manufacturer's instructions. Afterward, a second PCR was used to apply dual indices and Illumina sequencing adapters Nextera XT Index Primer (Illumina), by 7 cycles PCR (16 S Sequencing Library Preparation, Illumina). The amplicon libraries were purified using CleanNGS (CleanNA), and the quality control was performed on a Tapestation 4150 platform (Agilent Technologies, Santa Clara, CA, USA). Finally, all barcoded libraries were pooled in an equimolar way and sequenced on an Illumina® MiSeq (PE300) platform in pair ends (2 × 300 bp) (MiSeq Control Software 2.5.0.5 and Real-Time Analysis software 1.18.54.0). Library construction and NGS were performed at the sequencing platform of the Edmund Mach Foundation (San Michele all'Adige, Italy). Negative controls were also sequenced for quantification of possible contaminants.

Bioinformatic analysis

The raw reads, already demultiplexed, were analyzed using the MICCA (MICrobial Community Analysis) v1.7.2 bioinformatics pipeline [4]. Sequence pairs were merged to obtain consensus sequences. Primers were trimmed and sequences that did not contain these primers were discarded. Quality filtering was performed using an Expected Error Rate of 0.75 for ITS and 16 S, with the minimum read length set to 200 for ITS and 400 for 16 S. Reads were clustered into Amplicon Sequence Variants (ASVs) using the UNOISE protocol [38]. Finally, taxonomy was assigned using the "UNITE 9.0" for the fungal component and "RDP classify 2.14" for the bacterial component. To ensure the absence of contaminants after sequencing, the R package "Decontam" version 1.26.0 [33, 34] was using negative controls as reference. The analysis yielded a negative result, confirming the absence of contamination in the dataset.

Statistical analysis

Alpha diversity and beta diversity

Due to the lower number of reads for endophytic communities, alpha and beta diversity analysis were carried out using different rarefactions depending on the analyzed tissues. Samples were rarefied to 3,000 reads for bacteria and 10,000 for fungi for the whole tissues (pulp, leaf and fruit surfaces) comparisons, while in comparisons including only epiphytic communities (leaf and fruit surfaces) the rarefaction was 10,000 reads for bacteria and 30,000 for fungi. Three alpha diversity indices were computed, namely Chao1, Shannon Diversity, and Simpson Dominance [41, 65, 135] using the "estimate_richness" function from the R package "Phyloseq version 1.46.0" [80]; Chao1 indices were used to estimate richness; measurement of ASVs expected in samples given all the microbial species that were identified in the samples. The Shannon diversity index was a measure of species diversity in a community and taking into account the number of species present and their relative abundance, high values of this index indicated a higher biodiversity. The Dominance-Simpson index was another widely used diversity index in ecology; this ranges from 0 to 1, values close to zero indicate that all species were equally present, while a value close to 1 indicates that few species dominated the sample. The Wilcoxon-Mann-Whitney test was employed for the statistical analysis of alpha diversity in R Studio [109]; the adjustment method used for *p*-value was Holm-Bonferroni. For beta diversity analysis, the Bray-Curtis dissimilarity index was used to assess the difference between the plant tissues, which were visualized with a PCoA (Principal Coordinates Analysis). Statistical significance was assessed using PERMANOVA (Permutational multivariate analysis of variance) with pairwiseAdonis v0.4 R package [79]. The parameters considered in this analysis were difference in year, difference in tissues (for epiphytes), and difference in plant cultivar compared to the reference set of pairwise distances between samples from the same year, plant cultivar and tissues. Enrichment of genera in the different tissues was assessed using the negative binomial distribution analysis implemented in R package DESeq2 [76]. Inter-Kingdom associations: The SPRING (Semi-Parametric Rank-based approach for INference in Graphical model) [98, 141] method implemented in the R package NetCoMi (Network Construction and Comparison for Microbiome) v 1.1.0 [98] was used to infer the network of associations between taxa. bacterial and fungal relative abundances have been merged into a single ASVs table and then aggregated at the Genus level. Only ASVs present in at least 30 samples have been considered. The SPRING method uses the modified centered log-ratio transform (mclr) to deal with spurious correlations due to compositionality. Subnetworks of strongly

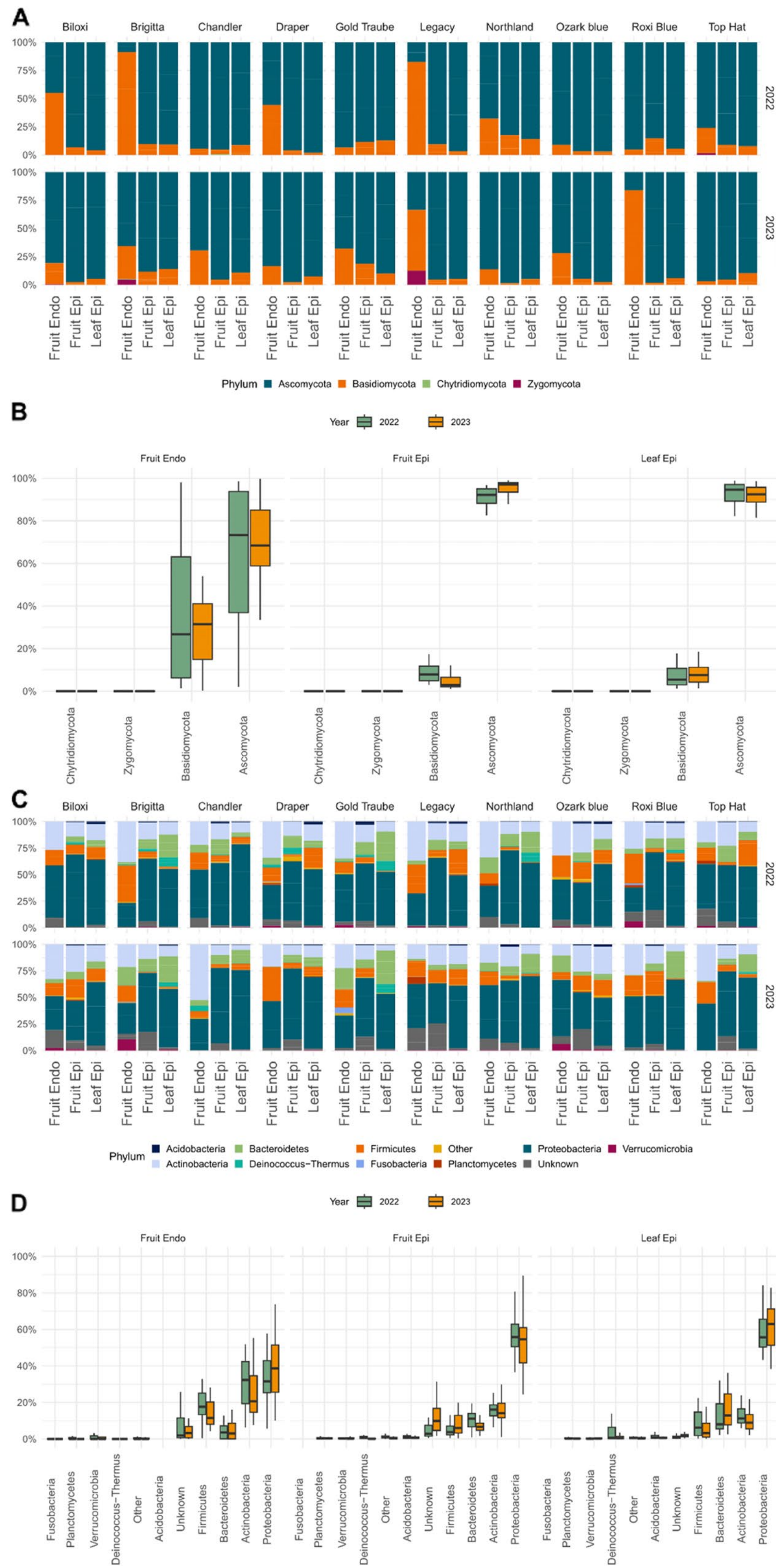

Fig. 1 (See legend on next page.)

(See figure on previous page.)

Fig. 1 Phylum-Level Composition of fungal and bacterial Communities in Blueberry Cultivars, showing only the ten most abundant taxa for each part of cultivars, a total of 4 different taxa for fungi and 11 different taxa for bacteria. **A** Stacked bar charts showing the relative abundance of fungal phyla across different blueberry cultivars and tissue types for 2022 and 2023; **B** Box plots illustrating the relative abundance of fungal phyla in different tissues for the same years, highlighting variability between tissue types and years; **C** Stacked bar charts depicting the relative abundance of bacterial phyla across blueberry cultivars and tissue types for 2022 and 2023; **D** Box plots showing the relative abundance of bacterial phyla in different tissues for the same years, indicating differences in bacterial community composition between tissue types and years

interacting taxa have been identified via greedy optimization of modularity using the cluster_fast_greedy method of the R package igraph v2.0.3.

Results

Raw data analysis

After quality filtering, we obtained a total of 11,023,303 quality-filtered ITS and 12,148,449 16 S sequencing reads. For ITS reads, the dataset included 4,056,471 fungal reads from leaf epiphytes, 4,402,685 fungal reads from fruit epiphytes, and 2,564,147 fungal reads from fruit endophytes. For 16 S reads, recognizing the challenges associated with amplifying plant DNA using primers targeting the V3-V4 region, particularly in fruit pulp, all sequences classified as "Chloroplast" and "Mitochondria" were removed. After filtering we obtained 2,911,912 bacterial reads for leaf epiphytes (originally 3,871,098; reads removed 959,186); 2,160,618 bacterial reads for fruit epiphytes (originally 4,084,827; reads removed 1,924,209); 318,518 bacterial reads for fruit endophytes (originally 4,188,247; reads removed 3,869,729). The filtered reads were classified into 10,750 Amplicon Sequence Variants (ASVs) for 16 S and 3,008 ASVs for ITS.

Taxonomic profiles reveal compositional differences between epiphytic and endophytic niches

In all samples, the most prevalent fungal phyla were *Ascomycota* and *Basidiomycota* (Fig. 1-A & B and Tables S2, S3, S4) while *Chytridiomycota* and *Zygomycota* were found only in a few samples and at low relative abundance. At the genus level (Fig. S1 A & B and Tables S5, S6, S7), *Davidiella* was the most abundant, followed by *Phoma*, *Alternaria*, *Malassezia*, *Aureobasidium*, *Botrytis*, *Cryptococcus*, *Leptosphaerulina*, *Rhodotorula*, *Taphrina*, and *Mortierella*. The compositional analysis of bacteria at the phylum level (Fig. 1-C & D and Tables S8, S9, S10) shows a wide variety of taxa present. The phylum *Proteobacteria* was dominant across all samples, followed by *Actinobacteria*, *Bacteroidetes*, and *Firmicutes*. We could identify compositional patterns that distinguish the endophytic and epiphytic communities, with certain taxa exclusively present in specific tissues. For instance, *Acidobacteria* were found on the leaf and fruit surfaces tissues but not in the pulp fruit, while *Fusobacteria*, which were present in small amounts in the pulp tissues, were absent in the leaf and fruit surface. At the genus level (Fig. S1 A & 1-B and Tables S11, S12, S13), we found that the most abundant taxa were *Massilia*, *Hymenobacter*, *Sphingomonas* and *Methylobacterium* for the fruit and leaf surface, while *Micrococcus*, *Staphylococcus*, *Enhydrobacter and Corynebacterium* was the most abundant in fruit pulp.

To corroborate the observed differences in the taxonomic profiles of the different tissues, we analyzed the abundance data for both bacteria and fungi (Fig. S2 A&B) with DESeq2 [35]. At phylum level we found a significant difference in fungal communities of fruit pulp and both fruit and leaf surface (Tables S14, S15, S16), while no statistical differences were found when comparing fruit and leaf surfaces. At genus level (Tables S17, S18, S19) we observed a significant difference in the comparison of fruit pulp with surface of leaf and fruit that involves several taxa such as: *Leptosphaerulina* and *Rhodotorula* presents only on the surface of fruit and leaves or *Mortierella* and *Malassezia* that are present only in the fruit pulp. Moreover we observed a significant difference also between fruit and leaf surface. Amongst the taxa that showed a significant difference, *Davidiella* and *Botrytis* had higher abundance on the fruit surface and *Leptosphaerulina* was more abundant on the leaf surface.

For the bacterial communities, at phylum level (Tables S20, S21, S22) we found that *Acidobacteria*, *Fusobacteria* and *Actinobacteria* were significantly less abundant in fruit pulp than on fruit and leaf surfaces, while the comparison between the surface of fruit and leaf showed a significant difference only for *Deinococcus-Thermus* and *Bacteroidetes*. At the genus level (Tables S23, S24, S25) we found significant differences between fruit pulp and both surface tissues. Significant differences were found in the relative abundance of *Sphingomonas*, *Deinococcus*, *Hymenobacter*, *Methylobacterium* and *Lactobacillus* that were present only on fruit and leaf surface while taxa present only in the fruit pulp were *Corynebacterium*, *Paracoccus*, *Enhydrobacter*, *Pseudomonas* and *Propionibacterium.* The comparison of fruit and leaf surface showed fewer significant differences, some examples are: *Methylobacterium*, present mostly on leaf surface or *Exiguobacterium* predominant on the fruit surface.

Alpha diversity reveals contrasting patterns for bacteria and fungi across plant niches

To characterize the richness and diversity of the microbial communities in the different tissues, after rarefaction we computed the Chao1 estimator of species richness, the Shannon entropy index of diversity and the Simpson

index of dominance. Fruit pulp had a significant lower richness than fruit and leaf surfaces both for bacteria and fungi (Table S26). Comparing fruit and leaf surfaces (Fig. 2-A), we found for fungi significantly higher values for leaves than for fruits (Chao1, p-value = 0.008; Shannon, p-value = 0.008; Simpson, p-value = 0.005). Analyzing alpha diversity in relation to the year of sampling we found that Shannon and Simpson had significantly higher values in the first year of sampling in leaf communities, while for fruit epiphytes we observed a significant difference only in the Shannon index (Fig. S3). For bacteria (Fig. 2-B), we found no significant difference

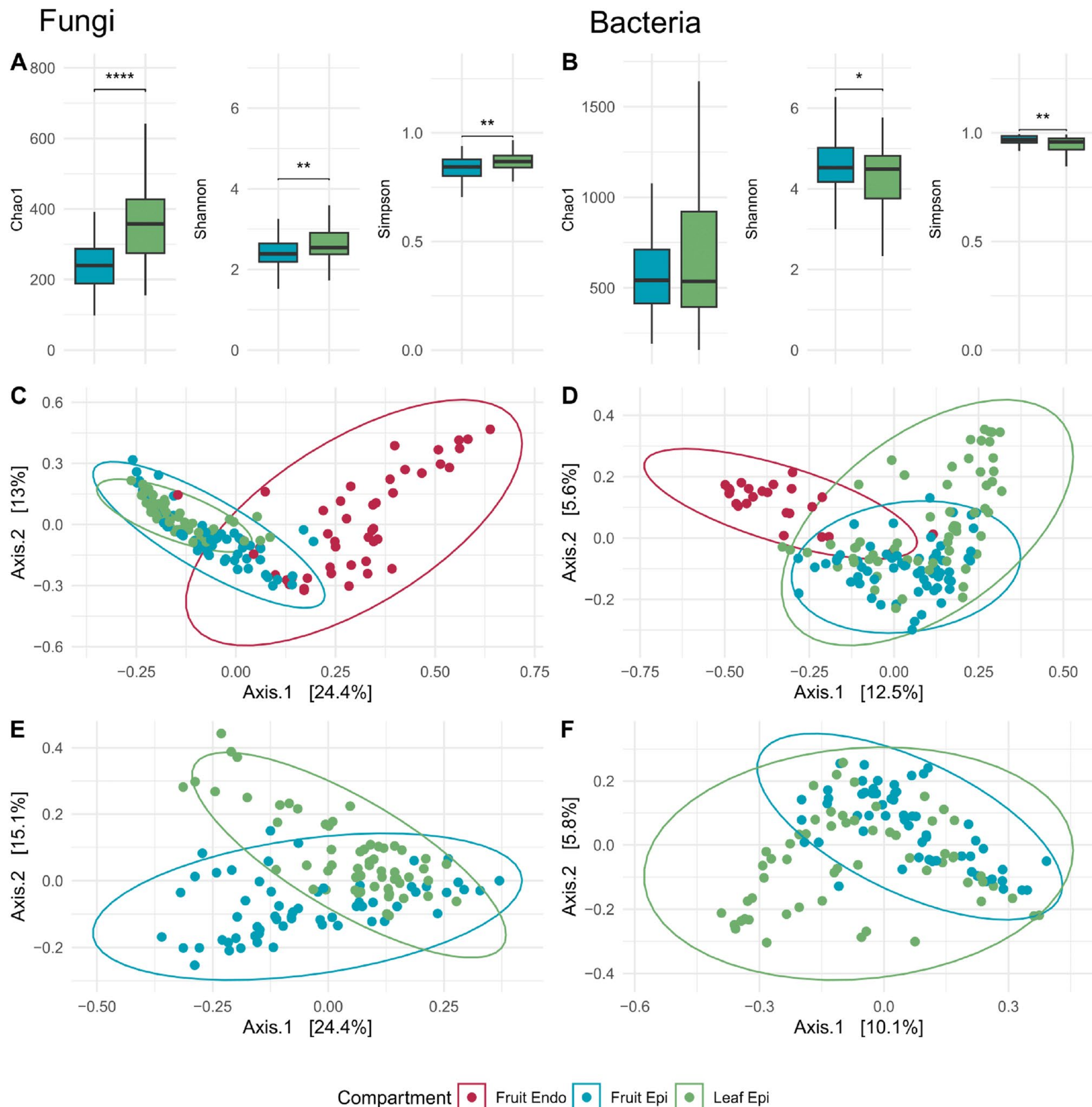

Fig. 2 Alpha and beta diversity show significant differences across tissues (p-value: "****"=0.0001; "***"=0.001; "**"=0.01; "*"=0.05). **A** Alpha Diversity Indices of fungi communities in fruit epiphyte (Blue) and leaf epiphyte tissues (Green); **B** Alpha diversity indices of bacteria communities in fruit epiphyte (Blue) and leaf epiphyte tissues (Green); **C** PCoA plot showing fungal communities, highlighting a separation between fruit endophytes and both fruit and leaf epiphytes. However, fruit and leaf epiphytes showing an overlap; **D** PCoA plot depicting bacterial communities, with clear separation between fruit endophytes and the epiphytic tissues; **E** PCoA plot focusing on fungal communities in epiphytic tissues, indicating some overlap but distinct clustering between fruit epiphytes and leaf epiphytes; **F** PCoA plot of bacterial communities in epiphytic tissues, showing more pronounced overlap between fruit epiphytes and leaf epiphytes

between the species richness measured by the Chao1 index (p-value = 0.54) between leaf and fruit epiphytic communities, while both the Shannon entropy and the Simpson dominance index were significantly higher in fruits than in leaves (Shannon, p-value = 0.046; Simpson, p-value = 0.0064). The analysis of alpha diversity comparing the different years of sampling showed an effect on richness of leaf epiphytes (Fig. S4). Indeed, while the Shannon and Simpson indexes did not vary significantly, we found that the Chao1 index for leaf epiphytes was significantly higher in the second year of sampling than in the first year. No significant differences were found for fruit epiphytes.

Beta diversity shows that plant tissues and genotype have a significant impact on microbial communities

For fungi (Fig. 2-C), we found significant differences in the overall beta-diversity analysis (PERMANOVA, p-value = 0.001, R^2 = 0.21027). Similarly, a Pairwise-PERMANOVA (Table S27) highlighted that the greatest differences occurred between endophytic and epiphytic communities, but we detected a difference also among epiphytic communities due to plant variety and year of sampling (Year: p-value = 0.005, R^2 = 0.0171; Plant Variety: p-value = 0.001, R^2 = 0.13045). Focusing on the analysis on epiphytes, we found statistically significant differences based on niche, year and plant variety (p-value = 0.001). Taken together these variables account for 40.68% of the variability. In particular, we found that the most important factor was plant variety (p-value = 0.001, R^2 = 0.26739), followed by niche (p-value = 0.001, R^2 = 0.0962) and year (p-value = 0.001, R^2 = 0.0437). For endophytic communities, we found significant differences considering year (p-value = 0.015, R^2 = 0.0518) and plant variety (p-value = 0.001, R^2 = 0.311).

For bacteria (Fig. 2-D) we observed a division among the three niches, which was particularly evident when comparing fruit endophytic communities with epiphytic fruits and leaf communities. A PERMANOVA statistical test on niches, year and variety of plant showed that these factors had a statistically significant effect (Niches: p-value = 0.001, R^2=0.10414; Year: p-value = 0.001, R^2=0.02001; Plant variety: p-value = 0.001, R^2=0.10549). Most significant differences were observed between endophytic and epiphytic communities; however, there were also significant differences between the epiphytic communities of the fruit and leaf surface (Fig. 2-E&F). Focussing on epiphytic communities, we found that plant variety, niche and year accounted for 21.7% of the variability (p-value = 0.001). Similarly to fungi, plant variety was the most relevant factor (p-value = 0.001, R^2 = 0.151), followed by niche (p-value = 0.001, R^2 = 0.03881) and year of sampling (p-value = 0.001, R^2 = 0.0264). Taken together, these data show that, although with some correction due to plant tissues and year, the plant variety was the dominating factor to determine the variability of the plant epiphytic bacterial communities.

To further characterize the relative importance of the different factors (tissues, plant cultivar and year) that impact the structure of the microbial communities, we compared the distribution of the pairwise Bray-Curtis dissimilarities between samples in which only one factor varies. The boxplot (Fig. 3) represents the dissimilarity between microbial communities in relation to factors change. The analysis of fungal epiphytes (Fig. 3-A) shows significant dissimilarity when comparing the reference set that shares the same plant variety, year, and tissues. For the bacterial epiphytes (Fig. 3-B), communities appear to be heterogeneous with values close to 1. Also here we found that plant variety, year and tissues have a significant influence on the heterogeneity of communities. For the fungal endophytic communities (Fig. 3-C) we found that plant variety and year show more heterogeneous communities, while for bacterial endophytic communities (Fig. 3-D) we found that only year has an influence on the heterogeneity of the communities. Additionally, when comparing the communities, the fungal communities were found to be less heterogeneous than the bacterial ones, as confirmed by the Wilcoxon-Mann-Whitney test (Table S27), which revealed significant differences (p-value < 0.005).

Prevalent taxa accounts for a large fraction of the microbiome reads in all tissues

To correlate the relative abundance of different genera with their prevalence and quantify the relative importance of the sample-specific and shared taxa, for each analyzed tissue we calculated the fraction of the total number of reads belonging to genera with a specific prevalence (Fig. 4). The results showed that in all tissues, both for bacteria and fungi, the highest fraction of the communities is attributable to genera present in over 90% of the samples; demonstrating that, despite differences in taxonomic composition amongst samples, the dominating part of the microbiome is composed by widely distributed taxa. This phenomenon is particularly noteworthy in the case of fungi on fruit and leaf surfaces, as the most prevalent genera constitute nearly 100% of the microbiota, while for bacteria the corresponding fraction was lower. Compared to the epiphytic communities, the endophytic microbiome was less homogeneous among the various samples, in particular for fungi.

Interesting differences between bacteria and fungi appeared when we repeated the analysis at the ASV level (Fig. S5). While for fungi the fraction of each sample attributable to prevalent ASVs was still well above 75% for both leaf and fruit surfaces, this fraction decreased drastically for bacteria. This finding was correlated to

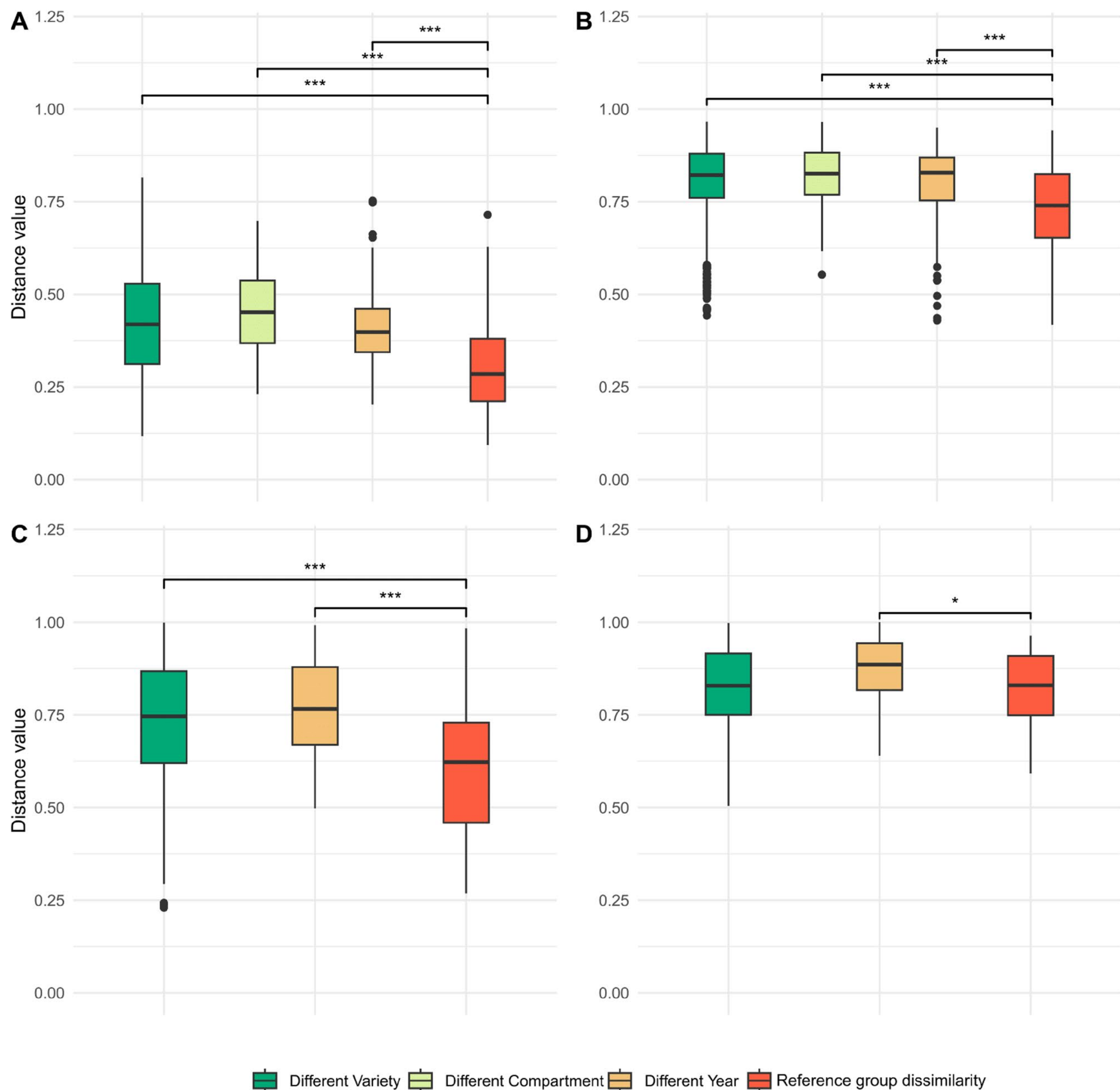

Fig. 3 Bray-Curtis Dissimilarity Analysis for fungi and bacteria. The figure presents the variation in dissimilarity values across different parameters: different variety, different tissues, different year, and no differences (*p*-value: "****"=0.0001; "***"=0.001; "**"=0.01; "*"=0.05). **A** The dissimilarity for epiphytic communities of fungi; **B** The dissimilarity for epiphytic communities of bacteria; **C** The dissimilarity for endophytic communities of fungi; **D** The dissimilarity for endophytic communities of bacteria

the greater genetic variability of bacterial genera compared to fungal genera. Indeed, we found a generally lower level of genetic complexity within fungal genera (Fig. S6) than within bacterial genera (Fig. S7). The most extreme example was the bacterial genus *Hymenobacter* that included more than 700 distinct ASV, while the most diverse fungal genus *Mortierella* included 20 distinct ASVs. Moreover, the within-genus variability was correlated with prevalence. Counting the number of samples in which a given genus was present and correlating this number to the number of distinct ASV that were classified in the same genus, we found that genera present in the largest number of samples also included the highest number of different ASVs (Figs. S8, S9), showing that while at higher taxonomic level there is a high degree of homogeneity across the different plants and tissues, this is accompanied by a large genomic variability at finer levels of taxonomic classification, with a large number of plant and tissues-specific strains for most prevalent taxa. The level of genetic diversity was particularly high for the

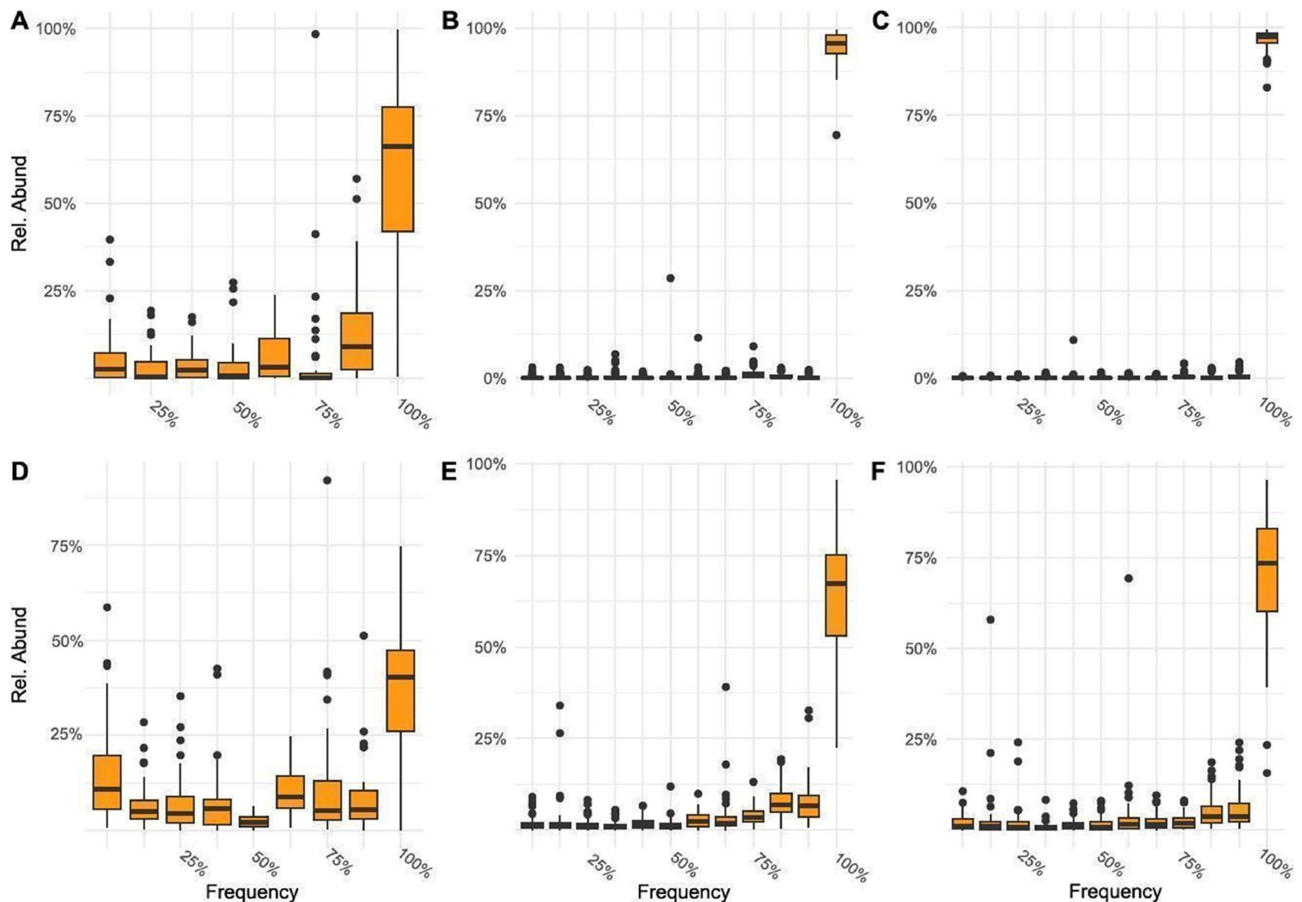

Fig. 4 Box plots of the relative abundance of Genus in blueberry endophytic and epiphytic niches, summarized by their frequency of occurrence across samples in order to analyze the prevalent taxa; each panel represents a specific tissue: **A** Endophytic fungi communities in fruits; **B** Epiphytic fungi communities on leaves; **C** Epiphytic fungi communities on fruits; **D** Endophytic bacteria communities in fruits; **E** Epiphytic bacteria communities on leaves **F** Epiphytic bacteria communities on fruits

most prevalent bacterial genera. Additionally, it is interesting to note the marked increase of bacterial taxa specific to a small number of samples when the analysis is conducted at the ASV level. Taken together, these results suggest that dispersal limitations have an impact on the colonization of plants by specific strains, while, at higher taxonomic level, each environment nonetheless selects a limited number of taxa. In the next section we will explore how this set of core taxa are conserved across tissues and years, in an attempt to define a core microbiome of the phyllosphere of this species.

Microbial communities share a core of common microbial taxa across plant cultivars and tissues

Given the dominant role of the most prevalent taxa in the microbiome of each tissues as shown in Fig. 4 and the importance of plant variety in determining the structure of the microbiome, we explored if it was possible to define a tissues-specific core microbiome that included the taxa that were present in the specific tissues all plant genotypes. Moreover, leveraging on the availability of data over two consecutive years on the same plants, we asked if this core microbiome was conserved over time and, finally, if, given their close proximity, the endophytes and epiphyte tissues of fruit and leaf share the same set of core taxa, or if the different physical properties were sufficient to trigger a different composition. We thus constructed a presence/absence matrix at the genus level, where a genus was considered present if its abundance was greater than 0, using unrarefied data to increase the sensibility of the analysis. Using these data, we defined a taxa as "core" if it was present in a given year and tissues in at least 9/10 plant genotypes in one of the triplicates (Fig. 5). As expected given the lower number of sequenced reads, we found both for bacteria and fungi a smaller core for the endophytic tissues (11 and 12 taxa for the first and second year for fungi, 19 and 14 genera taxa for the first and second year for bacteria, respectively) that for the fruit and leaf surface (56, 60 for fruit and 77, 85 for leaf core genera in the first and second year for fungi and 104, 104 for fruits and 89, 127 for leaf core genera for the first and second year for bacteria,

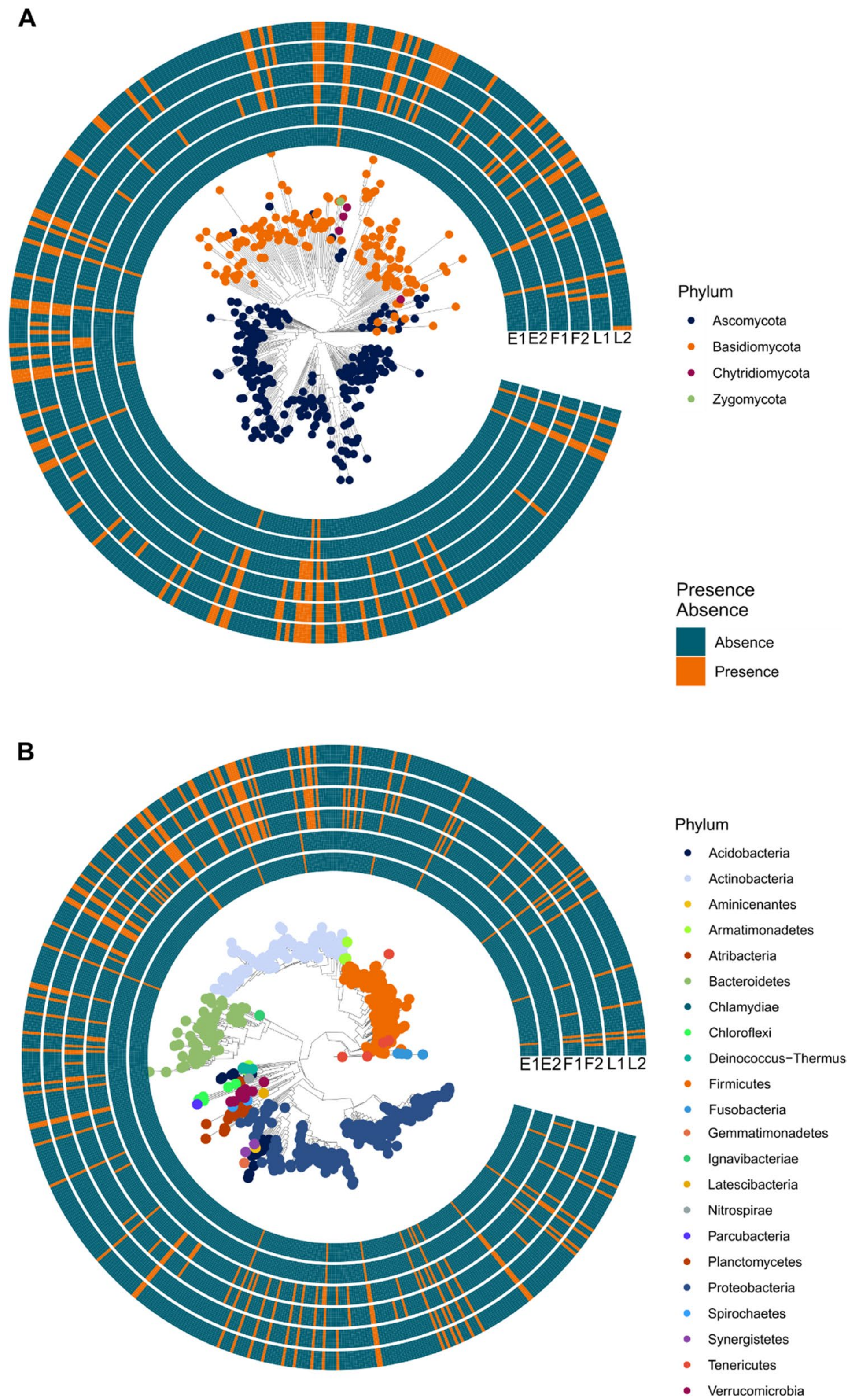

Fig. 5 (See legend on next page.)

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Fig. 5 Tissues microbial core composition show tissue-specific taxa and shared taxa. **A** Tree of fungal microbial core at the genus level in witch are considered only the genera that appear in 9 out of 10 plants; the labels are at the Phylum level, surrounded by six matrices: E1 first year fruit endophytes, E2 second year fruit endophytes, F1 first year fruit epiphytes, F2 second year fruit epiphytes, L1 first year leaf epiphytes, L2 second year leaf epiphytes that indicate the presence/absence of the genus in cultivars analyzed; **B** Tree of bacteria microbial core at the genus level in witch are considered only the genera that appear in 9 out of 10 plants; the labels are at the Phylum level, surrounded by six matrices: E1 first year fruit endophytes, E2 second year fruit endophytes, F1 first year fruit epiphytes, F2 second year fruit epiphytes, L1 first year leaf epiphytes, L2 second year leaf epiphytes, that indicate the presence/absence of the genus in each one plant in cultivars variety analyzed

respectively). Comparing the tissues-specific core microbiome across the two years, we found that each tissue exhibited a core similarity greater than 50% between the two years (Table S28). Moreover, comparing the set of core taxa across tissues, we found that for both fungi (Fig. S10) and bacteria (Fig. S11) most of the core taxa were shared across the epiphytic tissues, while the core taxa of the endophytic fruit tissues were a subset of the taxa present on the surface of fruits and leaves.

In the core analysis (Tables S29, S30), we detected the presence of ubiquitous genera across different tissues, while others were tissue-specific. Fungal genera such as *Davidiella*, *Leptosphaerulina*, and *Botrytis* were present in all tissues, though with varying abundance percentages. Some genera, such as *Taphrina* and *Pleospora*, were found exclusively in the surface tissues of fruit and leaf, while *Dioszegia* and *Myrothecium* were specific to the leaf tissue, and *Udeniomyces* to the fruit. We also identified a core microbiome for bacteria, highlighting widely distributed genera in all the tissues such as *Methylobacterium*, *Micrococcus* and *Massilia*, with notable variations in abundance, similar to what was observed for fungal genera. Other bacterial genera showed tissue specificity: *Gaiella* and *Rathayibacter* were found only in surface tissues, *Exiguobacterium* and *Aridibacter* were exclusive to the fruit, and *Micromonospora* was exclusive to the leaf.

Co-occurrence network identifies negative correlations between fungal and bacterial taxa

In order to characterize interactions between bacterial and fungal taxa, we built co-occurrence networks using the SPRING method (see Methods) separately for fruit and leaves epiphytes. To reduce the number of nodes and improve interpretability, taxa were merged at the genus level and only genera present in at least 30 samples were analyzed. In total, the networks included 148 genera for leaf samples (76 bacteria and 72 fungi) and 137 genera for fruit samples (89 bacteria and 48 fungi). The five most connected bacterial genera were *Pseudonocardia*, *Streptomyces*, *Nocardioides*, *Deinococcus and Corynebacterium* in leaves samples and *Nocardioides*, *Saccharibacteria_genera_incertae_sedis*, *Mycobacterium*, *Blastococcus*, and *Micrococcus* in fruits. For fungi, the most connected genera were *Vuilleminia*, *Dioszegia*, *Erythrobasidium*, *Coniothyrium* and *Exophiala* in leaves, and *Hyphodermella*, *Cryptococcus* and *Exophiala*.

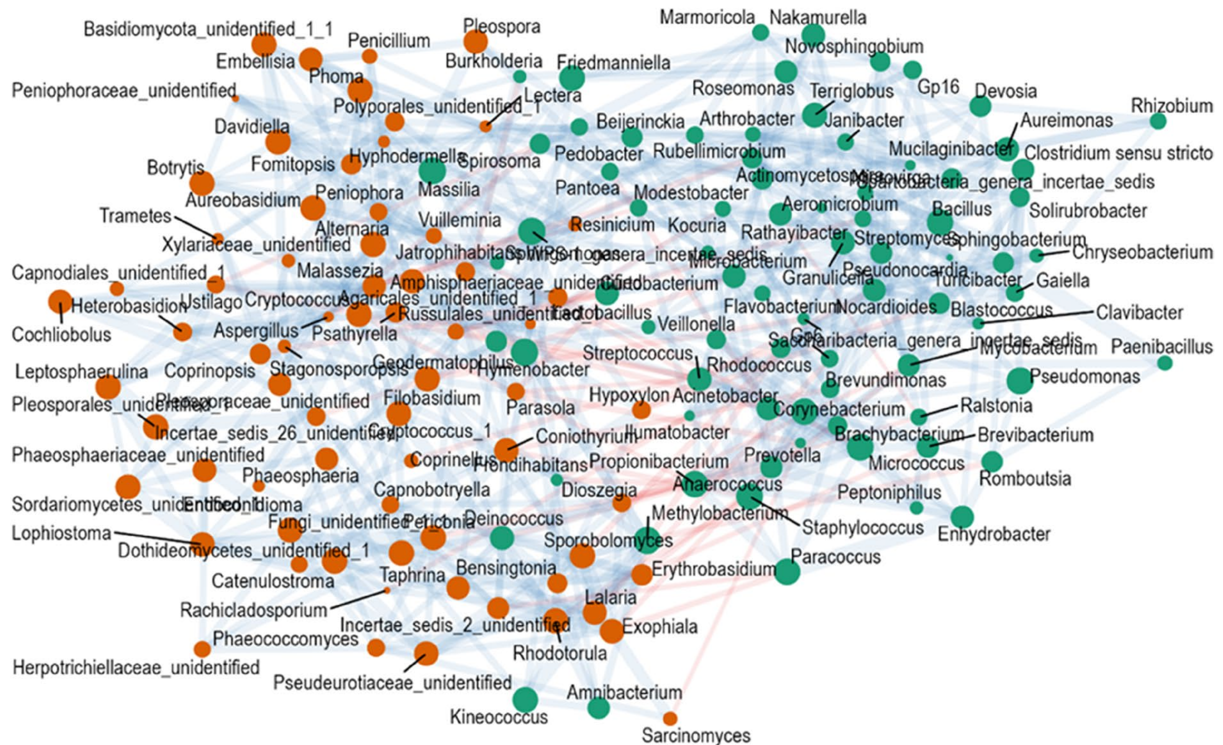
As shown in Fig. 6, both for leaves and fruit samples the bacterial and fungal communities formed two distinct subnetworks. Most of the positive connections were within the same Kingdom (bacteria vs. bacteria and fungi vs. fungi), and negative connections were mainly across Kingdoms (bacteria vs. fungi and fungi vs. bacteria) as shown in Fig. S12 where we plot the number of positive and negative interactions within and across Kingdoms stratified by Phylum. From this figure it is evident that while the vast majority of interactions within each Kingdom is positive, a large fraction of inter-Kingdoms interaction is negative both in leaves and fruits, and for all bacterial and fungal Phyla. However, we also found positive interactions across the two Kingdoms. In particular, bacteria of the genera *Deinococcus*, *Jatrophihabitans*, *Methylobacterium*, *Massilia*, *Hymenobacter* interact positively with fungi, in particular with the genera *Rhodotorula*, *Vuilleminia*, *Resinicium*, *Taphrina*, *Bensingtonia*, and *Pleospora*. The groups of strongly interacting taxa were identified using a community detection algorithm (Fig. S13).

Discussion

We conducted a field experiment over two consecutive years in ten different accessions of blueberries to *i)* investigate the microbiome of fruits and leaves; *ii)* assess the relative importance of year, cultivar or tissue in shaping the structure of the microbiota colonizing the phyllosphere of a perennial fruit plant; *iii)* define a core microbiome of leaves and fruits, and measure their overlap to understand if, despite their proximity, these two environments recruit different microbial communities; *iv*) measure the stability of leaf and fruit microbiomes over consecutive years, in particular of their core and *v)* characterize the network of correlations between bacterial and fungal taxa, in an attempt to understand their mutual interactions.

The blueberry microbiome

Using metabarcoding, we performed a comprehensive comparison of the structure and composition of both bacterial and fungal components of the plant-associated microbiota, across above-ground tissues, over two consecutive years. Considering the fungal component, the fruit pulp was dominated by the *Basidiomycetes*, in contrast to what has been observed in other studies on *V. myrtillus*, which indicates *Ascomycota* as the most



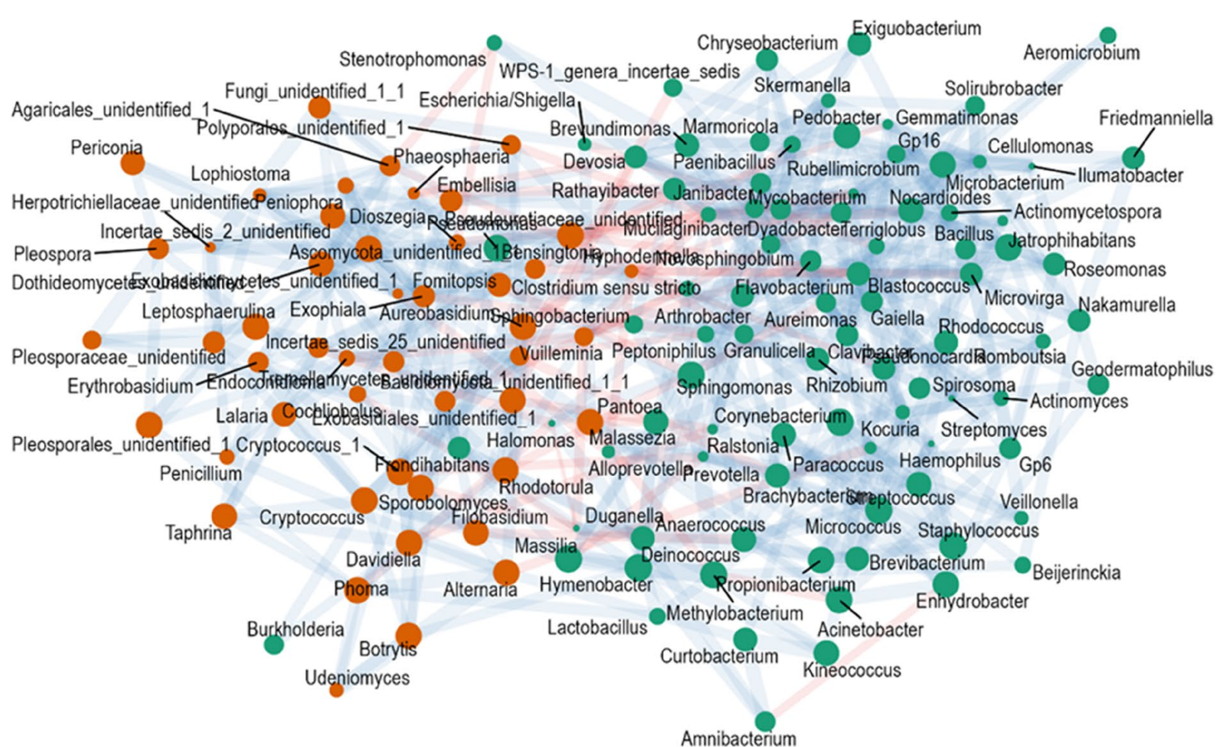


Fig. 6 Co-occurrence network of the bacterial (green) and fungal (orange) taxa at the Genus level. Size of nodes is proportional to prevalence, color of links indicates positive (blue) or negative (red) associations, width of links is proportional to strength of association. Two distinct subnetworks formed mostly by fungal and bacterial taxa are clearly visible, as well as the pattern of mostly positive intra-subnetwork and negative inter-subnetwork interactions

abundant phylum [88], while leaf and fruit surfaces were dominated by Ascomycetes as was observed for *Vitis vinifera* and *Vaccinium spp.* [13, 73]. At finer taxonomic level (i.e. genera), *Davidiella* and *Botrytis* were found to be shared between all the tissues, as already observed in grapevine [18, 108, 115]. Although generally considered pathogenic, some taxa from the *Davidiella* genus (e.g. *D. tassiana*) have been isolated from apparently healthy vines [93]. The absence of a direct link between the presence of these pathogens and disease suggests that their proliferation might be regulated by a healthy microbiota. This balance, however, is disrupted in cases of dysbiosis, leading to a loss of microbial equilibrium and potentially increasing the risk of disease development [8]. For this reason and to adjust to mutated conditions, plants are thought to actively reshape their microbiota in conditions of stress [127]. We also found that *Mortierella* and *Malassezia* were consistently present in the fruit pulp, while *Rhodotorula* and *Leptosphaerulina* were present mostly on fruit and leaf surfaces; similar results were observed in plants such as grapevine, tomato, apples, strawberries and white clover [125, 144]. *Malassezia* is commonly known as a fungus associated with animal environments, particularly the skin of mammals. However, it has been shown that this genus has a broad ecological distribution, having been detected in various habitats including both marine and terrestrial environments [6, 17, 117], although the debate of a possible human origin of this organism in marine environment is still ongoing [101]. Moreover, Malassezia has been identified through metagenomics analysis from endophytic tissues of Serjania erecta [45] suggesting the ability to also colonize plant environments; this is supported by its phylogenetic position, closely related to plant pathogens, suggesting the hypothesis of an evolutionary origin linked to plant hosts, followed by a subsequent adaptation to animal environments [136]. Despite the fact that we cannot completely rule out the possibility that the presence of Malassezia is linked to cultural practices, we believe that its consistent detection across the vast majority of samples makes this explanation unlikely.

For bacteria, we found taxa from the phylum *Proteobacteria* in all the tissues. This was expected as this phylum is the largest and most diverse bacterial lineage, which also includes plant pathogens and parasites [91]. In line with another study on *V. corymbosum*, all tissues were colonized by taxa from the phyla *Actinobacteria* and *Firmicutes* [1] although at lower relative abundance. Differently, we found that taxa from the phylum *Acidobacteria* were present both in fruit and leaf surface, but not in the pulp, while *Fusobacteria* were present only in the fruit pulp. At the genus level, *Massilia*, which is commonly isolated from cultivated plants [105], was found across all the three tissues while *Hymenobacter*, *Sphingomonas* and *Methylobacterium* were found only in fruit and leaf surfaces. Moreover, *Corynebacterium*, *Enhydrobacter*, *Paracoccus and Pseudomonas*, that have been found able to promote plant growth through enhanced nutrition, were found only in the fruit pulp in line with other studies on apple, rice and soybean [23, 71, 75]. Also found exclusively in the fruit pulp were members of the genus *Propionibacterium.* Despite including some well known human pathogens, members of this genus were found also as agricultural plant endophytes [19, 120].

In terms of richness and diversity, the fruit pulp hosted a simpler microbiota, including a significantly lower richness compared to the surface of fruit and leaf. This result is consistent with a previous study on blueberries [40, 119]. Smaller, but significant differences, could be found also in diversity and richness between leaf and fruit surfaces and across different plant cultivars. In particular, in fungal epiphytic communities we found higher richness and diversity in leaves that might indicate a more favorable habitat able to sustain a more diverse community [51]. For bacteria, although observed differences in richness were not statistically significant, diversity was higher in the fruit surfaces. This contrasting behavior between bacteria and fungi has been observed in topsoil habitats [10, 29] and constitutes a characteristic feature of the interactions between fungal and bacterial communities. In fact, fungal–bacterial competition driven by nutrient availability has been identified as one of the major forces shaping microbial communities globally [94, 128].

Within-species plant cultivar diversity shapes the microbiome of blueberries

The importance of host-associated and environmental factors shaping microbial richness and diversity in plants are still not fully understood [12, 32, 54]. Previous studies have highlighted that the composition of microbial communities in the phyllosphere is shaped by variables such as biogeography, climate, agricultural practices, and plant cultivars [60, 73, 126]. While plant genetics plays an important role, further investigation into this aspect is necessary, as it has been observed that it does not serve as a universal driver in determining microbial composition [90]. In this work, by characterizing samples collected from plants that share the same location and management, we focus on the relative importance of cultivars, tissue and year in modulating the structure and composition of the phyllosphere microbiota [71]. We found that the tissue type was the main determinant for the microbial composition. The analysis of β-diversity showed that the interior of the fruit was colonized by a microbiome distinct from that of the surface of fruits and leaves, that were much more similar to each other. Focusing only on epiphytic communities, we found that the three studied factors, namely plant variety, tissue and

years, could explain only a relatively small fraction of the measured diversity (40.68% in the case of Fungi and 21.7% for Bacteria); suggesting that other factors, including possibly stochastic variability, are important in determining the structure of the phyllosphere microbiota. Amongst the considered factors, the major driver was host variety followed by tissues, while year of sampling was the least important factor; suggesting that variability due yearly climatic fluctuations have a limited impact on perennial plants. Notably, despite the absence of phytosanitary treatments in the first year and the application of a tribasic copper sulfate treatment (Hattrick 30WG, 150 g/hL) in the second year, no significant shifts in microbial diversity were observed between years, further supporting the limited impact of this treatment under the studied conditions. In the literature there are contrasting reports concerning the relative importance of environmental and genetic factors in shaping plant-associated microbial communities. In some cases, such as different *Vitis vinifera* cultivars, host genotype was identified as the primary driver [52, 99], while other studies on blueberry, maize and rice report other drivers such as agricultural practice, growing stage, plant health status or seasonal variation [11, 73, 119, 121]. Due to its complexity, it has been proposed that both host species and environment are co-determinants of microbial communities in the phyllosphere [71].

The microbiome of fruit and leaves is dominated by a core of ubiquitous taxa that are conserved in different years

In an attempt to distinguish taxa that are specific components of the phyllosphere microbiome from those that are transient colonizers, we defined a tissue specific core microbiome, i.e. the set of species that are consistently found in a given tissue. Several studies have demonstrated the presence of a core microbiome in the roots and rhizosphere [129, 130, 137], and in the phyllosphere, across different species, genotypes, growing stage, season and geographical locations [5, 73, 116]. However the quantitative importance of core taxa has not been fully characterized before, as well as its year to year variability in a perennial plant. We demonstrated the dominance of the core microbial taxa in the above ground microbiome showing that the largest fraction of the microbiota is composed by a relatively small number of taxa that are widely shared across plant cultivars and time. In some cases these taxa are specific to a given tissue, while in others they are shared across tissues. These results might be influenced by the homogeneity of environmental conditions and cultural practices considered here, as all plants were located in the same experimental field. An extension to consider different locations and environmental conditions will be the subject of future studies.

For fungi, *Davidiella* was detected across all tissue types. This genus represents the teleomorph of *Cladosporium*, which includes numerous plant-pathogenic species. Consequently, *Davidiella* species are generally considered plant pathogens, although their pathogenicity may vary depending on the specific species and host [48]. *Alternaria*, *Leptosphaerulina* and *Botrytis*, that were found to be part of the core fungal microbiota of all the tissues, are known as fungal pathogens or parasites [14, 74, 122, 142]. *Aureobasidium*, also part of the core microbiota shared across the tissues, has been shown to have antagonistic properties against pathogens [103, 112]. *Cryptococcus*, present in the core fungal microbiota, is a genus involved also in biocontrol for blue mold in apples and postharvest decay [53, 55, 86]. *Sporobolomyces* is known for its role as a natural antagonist for *B. cinerea* [21, 59]. Beyond the genera shared across all tissues, we identified tissue-specific core taxa: specifically, *Taphrina* and *Pleospora*, known as a plant pathogen [26, 124], were found to be exclusive to the fruit and leaf surface. *Dioszegia* and *Myrothecium*, well known as plant pathogens and found on leaves in other studies [46, 100] were present only on leaf surfaces. *Myrothecium* is a globally distributed genus capable of colonizing diverse hosts that exhibits both saprophytic behavior, thriving in soil and decomposing plant matter, and pathogenic activity, affecting a wide range of species [139]. Some *Myrothecium* strains infect economically important crops like coffee, rice, and mulberry, causing leaf blight and leaf spot. Notably, *M. verrucaria* produces diverse enzymes with insecticidal and nematophagous properties and shows strong virulence against weeds, highlighting its potential as a bioherbicide [31], while *Udeniomyces* is exclusive in fruit surface, consistent with observations in grape studies [34]. This contrasts with previous findings where it was isolated from leaf tissues [89].

As for fungi, the bacterial core microbiome showed several genera shared across all three tissues. The genus *Massilia*, shared between all the tissues, has been detected also in other plants such as tomatoes and grapevines [111, 114]. *Methylobacterium*, also part of the core microbiome, is involved in regulation of phytohormone levels, in growth-promoting processes and exhibits biocontrol activities against pathogens [81, 145]. *Micrococcus*, belonging to the core microbiome, was proposed as a biocontrol agent against the post-harvest pathogen such as *B. cinerea*, *P. expansum* and *A. uvarum* [97, 104]. The core microbiomes of fruit and leaf surfaces are characterized by *Gaiella* and *Rathayibacter;* the latter is a plant pathogen [118]. Moreover, we identified specific genera present only on the fruit surface such as *Exiguobacterium*, of which some strains have been proposed as biocontrol agents against *Peronophythora* [58]. While *Micromonospora* was detected in our study only

on leaves, other studies demonstrated that this genus is able to colonize different tissues of plants from the roots to the leaves [15, 107].

Variations in core microbiome composition across different tissues may be driven by their distinct chemical and physical properties. For instance, leaves have a larger surface area, trichomes, stomata, and defensive compounds, along with lower nutrient availability [92]. In contrast, fruit surfaces are smoother, nutrient-rich, and contain high sugar levels [39]. The functions associated with the core microbiome could significantly contribute to health, productivity and resistance to plant pathogens [54, 43, 69]. Despite the overall healthy condition of the plants analyzed, we observed not only well-known plant pathogens such as *Botrytis* or *Leptosphaerulina* but also microorganisms that may enhance plant health by coping with biotic and abiotic stress such as *Methylobacterium* or *Micrococcus* [63, 110]. Defining the annual core allows us to evaluate its stability over time and analyze its response to climate change [113]. A deeper understanding of the core exhibited by different tissues will provide valuable insight into consistently present taxa and transient ones, highlighting potential key players which may be exploited for developing effective approaches to enhance productivity under biotic and abiotic stress conditions [121, 123, 140].

Network analysis show intra-kingdom interactions

Network co-occurrence analysis revealed key taxa that likely mediate interactions between fungi and bacteria. Our findings identified two distinct subnetworks, one bacterial and one fungal, each dominated by positive intra-kingdom associations. In contrast, inter-kingdom interactions were predominantly negative, consistently with previous reports that demonstrated that negative interactions between bacteria and fungi in the roots of *A. thaliana* are essential for plant health [10, 37, 56, 94, 128]. However, we identified a few genera with positive interactions across both kingdoms: *Friedmanniella*, *Massilia*, *Deinococcus*, and *Kineococcus* in leaves, and *Hymenobacter*, *Lactobacillus*, *Methylobacterium*, and *Pseudomonas* in fruits. These microorganisms likely play key roles in mediating bacterial-fungal interactions. In the future, this approach may serve as a useful tool for identifying potential biocontrol agents against pathogens and promoting plant growth [27].

This study provides a detailed analysis of the microbial communities associated with *Vaccinium spp.*, highlighting the complexity of interactions between plant tissues and their microbiota. By characterising important aspects like tissue-specific compositional patterns, the core microbiota, and year-to year variability, our study is the first step for future investigations aimed at better understanding the interactions among microorganisms colonizing Blueberry plant tissues and how they can help in improving cultural practices to reduce reliance on chemical inputs and help address challenges related to climate change, including drought, pest outbreaks, and extreme weather events [42]. A major limitation of this study was the sampling of plants that were grown in the same location, a fact that could be responsible for the relative homogeneity of the core microbiome across plants and tissues. However, this lack of geographical representativeness of our study design allows us to concentrate on other factors, namely plant variety, tissue, and year of sampling, that might be obscured by considering plants from different geographical locations. Geographical factors and farming systems will be the object of future studies.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06871-6.

Supplementary Material 1.

Supplementary Material 2.

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Authors' contributions

C.C, L.G. and C.D. designed the study.M.G., B.F., M.A., M.C. collected samples.M.G., E.S., S.L. and M.P. produced metabarcoding sequences.M.G., C.C., M.D-B., C.D. analyzed data.M.G. prepared figures and tables.M.G, L.G, M.D-B., C.C. and C.D. wrote the main manuscript text.All authors reviewed the manuscript.

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Data availability

Metagenomes raw data are available under the ENA accession PRJEB82098. The R code used for analysis is available on Zenodo under doi : https://doi.org/10.5281/zenodo.14499578.

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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