

Article

Peach Buds' Microbiome Profiling Reveals Cultivar-Specific Signatures Associated with TCSB Susceptibility

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Abstract

The plant microbiome plays a pivotal role in host development and resilience against biotic and abiotic stresses. In perennial crops like peach, microbial communities inhabiting dormant buds—critical yet vulnerable organs—may influence disease outcomes and plant fitness. This study characterized the bacterial and fungal communities associated with the buds of three peach cultivars differing in susceptibility to Twig Canker and Shoot Blight (TCSB). Amplicon-based profiling revealed distinct microbiome signatures across cultivars, shaped by host genotype. The highly tolerant ‘Catherina’ harbored a structured and relatively diverse community enriched in beneficial bacterial genera such as *Pseudomonas*, *Sphingomonas*, and *Curtobacterium*, alongside protective yeasts including *Aureobasidium* and *Cladosporium*. In contrast, the susceptible cultivar ‘Pavaro®-Pav 1605’ hosted a less balanced microbiome, marked by enrichment of opportunistic pathogens such as *Alternaria* and *Diaporthe*, as well as the bacterial lineage 1174-901-12. The intermediate cultivar ‘Lami®.COM’ displayed a transitional profile enriched in *Sphingomonas*, *Pelomonas*, and *Vishniacozyma*. Differential abundance analyses confirmed cultivar-specific enrichment patterns, underscoring the influence of genotype in shaping microbiota composition and potential disease outcomes. These findings support the integration of microbiome-based approaches into sustainable disease management via beneficial microbial promotion, early detection of harmful consortia, and microbiome-informed breeding to foster resilient, low-input peach cultivation systems.

Keywords: peach (*Prunus persica* (L.) Batsch); twig canker and shoot blight (TCSB); endophytic microbiota; host–microbiome interactions; sustainable biocontrol



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1. Introduction

Re-emerging plant diseases represent an escalating threat to global agriculture and food security, driven by multiple interconnected factors including climate change, global trade, monoculture cultivation practices, and the introduction of pathogens into novel environments [1,2]. This threat is especially evident in stone fruit agriculture, where crops such as peach (*Prunus persica*) are increasingly vulnerable to both endemic and newly emerging fungal pathogens, with climate change, intensive cultivation, and global trade amplifying vulnerability [3–5]. The unpredictable nature of these diseases is often attributed to the emergence of new pathogen strains, increased genetic recombination,

and shifts in host range, processes that are further intensified by environmental changes and anthropogenic activity [6,7]. Managing these diseases in peach orchards remains particularly challenging due to the broad host range of many pathogens (e.g., *Monilinia* spp., *Diaporthe amygdali*, *Plum Pox virus*, *Taphrina deformans*), the lack of curative treatments for some of them, and the difficulty of predicting disease dynamics under shifting ecological conditions [4,5]. Consequently, robust surveillance systems, rapid diagnostic tools, and integrated multidisciplinary research approaches are critical to mitigate future outbreaks and ensure sustainable peach production systems.

In this context, understanding the ecological drivers that shape pathogen emergence and re-emergence, particularly in peach-growing regions, is critical for forecasting threats and informing control strategies. Ecological conditions, such as rising temperatures and altered precipitation patterns, can modify the distribution, lifecycle, and virulence of fungal pathogens affecting peach trees. Indeed, climate change, particularly rising temperatures and shifting humidity patterns, can extend the geographic range and seasonal activity of fungal diseases, enhance pathogen fitness, and promote the evolution of traits such as thermotolerance and increased virulence [8,9].

Environmental disturbances, including storms and flooding events, further facilitate the dispersal of fungal spores and the colonization of new habitats [10]. These changing ecological conditions also favor local adaptation and ecological speciation, including host shifts, which allow fungi to exploit novel plant hosts and niches, often giving rise to more aggressive or resilient pathogen populations. Moreover, environmental stressors can compromise plant immune responses, increasing host susceptibility and contributing to disease outbreaks. For example, recent flooding events in key fruit-producing areas of the Po Valley have led to significant tree mortality and yield reduction—especially in vulnerable species such as stone fruits and kiwifruit [11]. Beyond the direct physiological stress on host plants, flooding induces hypoxic or anoxic soil conditions that disrupt biogeochemical and microbial processes, altering the composition and functionality of the plant-associated microbiota. These shifts may further compromise plant resilience and facilitate the establishment of soilborne and opportunistic pathogens, ultimately increasing the risk of disease outbreaks [9,10,12,13]. A deeper understanding of these ecological dynamics is critical for informing disease surveillance efforts and developing integrated, adaptive strategies to manage fungal threats in agroecosystems [10].

In addition to ecological drivers, plant genotype plays a fundamental role in shaping the dynamics and outcomes of plant disease. The genetic makeup of plants determines their resistance or susceptibility to specific pathogens, influencing both the severity of infection and the composition of pathogen communities [14]. Variation among host genotypes can result in differential pathogen multiplication, with some genotypes facilitating greater pathogen proliferation and disease spread, while others act as barriers that suppress infection. For instance, populations dominated by tolerant genotypes may inadvertently support higher pathogen loads, potentially increasing the risk of regional outbreaks [13]. In contrast, the presence of resistant genotypes can mitigate disease impact by limiting pathogen success. The interaction between genotype and environment ($G \times E$) further complicates disease outcomes, as the resistance conferred by a given genotype may vary under different environmental conditions, leading to local adaptation of both hosts and pathogens [15,16]. Importantly, genetic diversity within crop populations can act as a buffer against widespread epidemics, whereas uniform monocultures are more susceptible to pathogen invasion and the rapid evolution of virulence. Overall, plant genotype not only affects individual plant health but also shapes broader ecological and evolutionary dynamics in plant–pathogen interactions.

Buds are dynamic structures whose morphology, chemical profile, and physiological activity change markedly across phenological stages. These transitions create shifting ecological niches that are selectively colonized by microbial taxa, ultimately shaping the phyllosphere microbiome. The structural and developmental dynamics of buds provide heterogeneous surface properties, nutrient availability, and temporal windows that favor microbial succession. Morphological traits such as bud size, shape, and internal anatomy (e.g., fertile whorl development, vascular connections) generate microhabitats that can modulate microbial colonization and community diversity. Likewise, changes in chemical characteristics—including pH, soluble solids, and acidity—during bud development act as selective forces, favoring microorganisms adapted to specific physicochemical conditions [17,18]. In peach, the dormant phase represents a crucial physiological bottleneck, during which buds rely on stored reserves and exhibit reduced metabolic activity. This period of low physiological turnover may influence the establishment and persistence of endophytic and epiphytic microorganisms, including both beneficial taxa and potential pathogens. Since bud health is directly linked to flowering success and subsequent fruit set, the composition of the bud-associated microbiome is of particular importance for understanding plant resilience.

Understanding how host genotype and ecological context interact is critical when examining specific pathosystems. As a case in point, re-emerging Twig Canker and Shoot Blight (TCSB) in peach orchards is now understood as a multifaceted disease caused by a broad spectrum of fungal agents, such as *Diaporthe* spp., *Cytospora* spp., *Phoma glomerata*, *Phomopsis amygdali*, *Botryosphaeria dothidea*, and *Calosphaeria pulchella* [19–22]. Notably, the composition and aggressiveness of these pathogens vary geographically and in response to local environmental conditions [19,23]. Climate parameters, particularly temperature and moisture, are key drivers of pathogen activity; for instance, seasonal changes directly affect the proliferation and infectivity of *P. glomerata* and *D. eres*, underscoring potential climate-change impacts [19,24]. Additionally, assessments of commercial peach and nectarine cultivars reveal uniformly high levels of susceptibility, reflecting a scarcity of effective resistance traits in current breeding material [5,25]. The concurrent presence of multiple fungi—with entry points via wounds, buds, or leaf scars—further complicates disease management and elevates the risk of outbreaks [26]. These findings highlight the urgent need for integrated monitoring and comprehensive control measures in peach production. Despite the global diversity of TCBS pathogens, surveys in Italian peach orchards have consistently implicated *Diaporthe amygdali* as the putative principal causal agent of trunk canker and shoot blight in the main productive regions (e.g., Emilia-Romagna).

This study explores the possible involvement of resident microbial communities in enhancing peach cultivar tolerance against Twig Canker and Shoot Blight (TCSB). To this end, a pool of peach cultivars exhibiting contrasting responses to TCSB was selected based on long-term field monitoring of symptomatic orchards and information gathered from growers. The cultivars included ‘Pavoro[®]-Pav 1605’ (identified as highly susceptible), ‘Lami[®].COM’ (intermediate tolerance), and ‘Catherina’ (more tolerant). Dormant floral and wood buds from each cultivar were sampled for high-throughput microbiome profiling to elucidate potential associations between cultivar-specific microbial communities and differential susceptibility to TCSB.

2. Results

2.1. Sequencing Output and ASVs Recovery

Illumina sequencing generated 1.44M raw bacterial paired-end reads. After quality filtering, chimera removal, and filtering of the unassigned ASVs, a total of 158 taxa were re-

tained across all samples for downstream analysis. Similarly, fungal ITS sequencing yielded 1.27M raw fungal paired-end reads, accounting for 368 taxa after filtering operations.

2.2. Bacterial Community Composition

A first screening at the phylum level indicated that the bud-associated bacterial communities in all cultivars were dominated by Proteobacteria (86.6%), Actinobacteriota (4.4%), and Bacteroidota (3.2%) (Figure S1). Notably, ‘Lami[®].COM’ exhibited an intermediate profile between ‘Catherina’ and ‘Pavaro[®]-Pav 1605’, with additional phyla like Firmicutes (4.3%) and Chloroflexi (1.5%), which were absent or present in low amounts in the other two cultivars.

Genus-level analysis revealed that *Sphingomonas*, *Delftia*, *Massilia*, and *Methylobacterium* were among the most abundant across all cultivars (Figure 1). The bud-associated bacterial community in ‘Catherina’ was dominated by a handful of genera with high and relatively consistent abundances across replicates. *Pseudomonas* was the most prevalent, representing between 25.6% and 31.8% of the total community in each sample. *Sphingomonas* followed closely, accounting for 15.0% to 24.7% of total ASVs. Members of *Curtobacterium* comprised 6.4% to 12.8% of the microbiome, while *Methylobacterium*–*Methylorubrum* ranged from 9.0% to 11.0%. Other notable genera included *Massilia* (8.4–10.0%), *Hymenobacter* (5.4–7.3%), *Luteibacter* (3.1–4.6%), and *Pantoea* (≈3.0%).

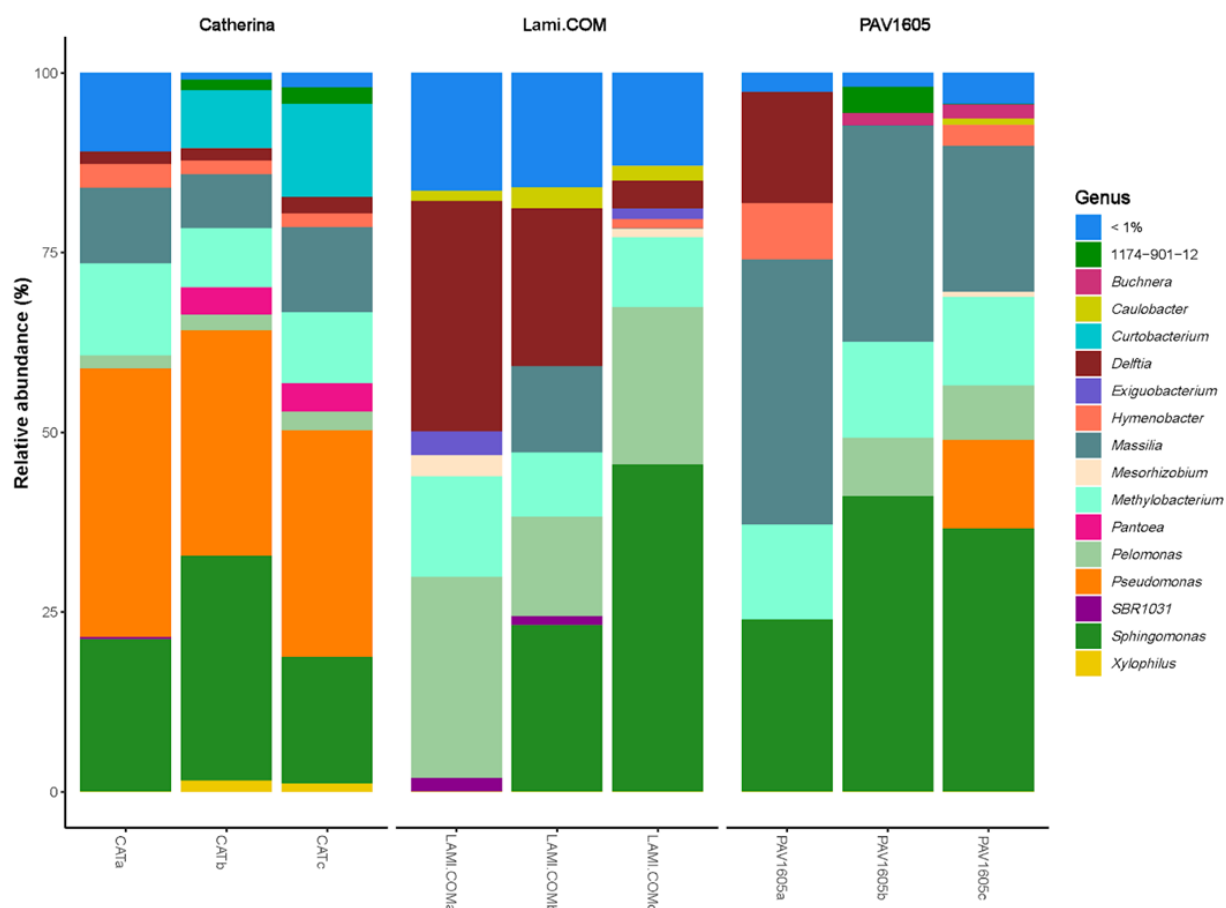


Figure 1. Comparison of bacterial community composition at the genus level across three peach cultivars.

Overall, ‘Lami[®].COM’ buds harbored a microbiome characterized by a few highly dominant taxa, particularly members of the Sphingomonadaceae and Comamonadaceae families, alongside a moderate presence of methylotrophic and soil-associated genera. The buds’ microbiome of ‘Lami[®].COM’, representing intermediate tolerance to TCBS, displayed

a distinct assemblage dominated by *Sphingomonas*, *Delftia*, and *Pelomonas*. *Sphingomonas* was the most abundant genus in two replicates (41.3% and 21.5%), averaging approximately 31% across the three replicates. The genus *Delftia* accounted for 20.4% to 31.4% of the community, while *Pelomonas* contributed between 19.9% and 27.4%. Other notable taxa included *Methylobacterium–Methylobacterium*, which comprised roughly 8.2% to 13.7% of reads, and *Massilia*, present at 7.6% to 11.2%. Less abundant genera such as *Fronthabactans* (4.9–7.1%), *Exiguobacterium* (3.3%), *Mesorhizobium* (2.8%), *Caulobacter* (2.6%), and an unclassified SBR1031 lineage (1.9%) completed the profile (Figure 1).

In the most susceptible cultivar ‘Pavaro[®]-Pav 1605’, the bud-associated bacterial community was predominantly composed of *Sphingomonas* and *Massilia*, both consistently abundant across the replicates. The genus *Sphingomonas* accounted for between 22.1% and 39.4% of the community, while *Massilia* ranged from 20.1% to 33.4%. Members of the *Methylobacterium–Methylobacterium* genus also featured prominently, accounting for approximately 11.9% to 14.2%, and *Delftia* contributed around 14.0% of the community just in one sample. Notably, *Pseudomonas* appeared at a lower level compared to ‘Catherina’ microbiome composition, with a maximum of 11.9%. Other taxa, including *Pelomonas* (6.8–7.7%), *Hymenobacter* (2.7–7.1%), and *Buchnera* (6.9%), were detected at moderate abundances. A lineage designated as 1174-901-12 was also present at low levels (2.2–3.5%) (Figure 1).

2.3. Fungal Community Composition

As expected in woody plant tissues, the fungal communities among the three different cultivars were overwhelmingly dominated by Ascomycota (52%). Basidiomycota represented a secondary component (48%), primarily consisting of wood-inhabiting saprobes and yeast-like taxa involved in nutrient cycling and potential antagonism of pathogenic fungi (Figure S2).

Following the phylum-level overview, fungal communities at the genus level were investigated to identify key taxa that may influence cultivar tolerance. In the tolerant cultivar ‘Catherina’, the bud mycobiome was overwhelmingly dominated by *Aureobasidium*, which comprised roughly 42–48% of the total across replicates. The next most abundant genus was *Cladosporium*, accounting for approximately 18–20%, followed by *Filobasidium* at 13–17%. Genera with moderate representation included *Alternaria* (9–11%), while *Vishniacozyma* and *Papiliotrema* contributed less than 5% of the community (Figure 2).

In ‘Lami[®].COM’ plants, the fungal community of dormant buds was similarly dominated by a few key genera, though with a distinct distribution compared to ‘Catherina’. *Aureobasidium* remained the most prevalent taxon, comprising approximately 42.6–43.6%. *Vishniacozyma* followed, accounting for roughly 24.4–25.6%, while *Cladosporium* represented about 19.2–21.2% of the community. *Alternaria* contributed around 9.8–10.6%, and *Filobasidium* was detected at low levels (≈ 1.1 –1.5%). A minor presence of *Stemphylium* ($<0.3\%$) completed the profile (Figure 2).

The bud mycobiome of ‘Pavaro[®]-Pav 1605’ was likewise dominated by *Aureobasidium*, which comprised 41.8–45.7% of reads across replicates. Unlike more tolerant cultivars, ‘Pavaro[®]-Pav 1605’ exhibited a markedly higher relative abundance of *Alternaria*, accounting for 18.3–20.0% of the community. The genus *Cladosporium* also featured prominently at 18.2–18.6%. *Filobasidium* represented a substantial fraction (≈ 11.0 –11.6%), while *Vishniacozyma* was present at lower levels (≈ 3.0 –4.8%). Minor constituents included *Dioszegia* (≈ 1.4 –1.6%) and *Neocucurbitaria* (≈ 0.7 –0.9%). Notably, the pathogen genus *Diaporthe* was detected at low but consistent levels (0.33–0.67%) only in this cultivar. Other minor taxa, such as *Taphrina* and *Genolevuria*, were each found at $<0.5\%$ (Figure 2).

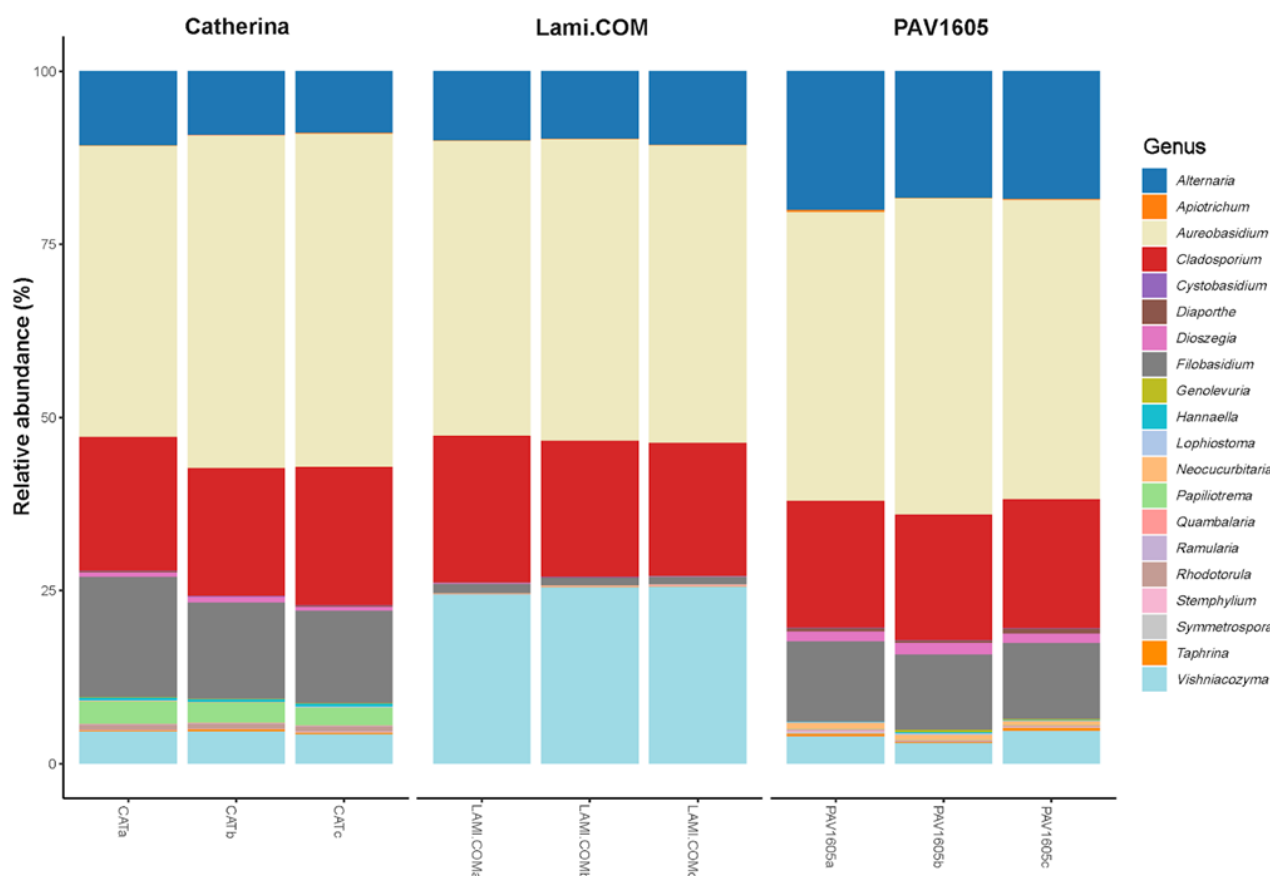


Figure 2. Taxonomic profiles of fungal genera associated with distinct peach cultivars based on relative abundance.

2.4. Communities' Diversities and Dissimilarities

The within-sample alpha diversity was assessed using three complementary metrics: observed ASV richness, Chao1 richness estimator, and Shannon diversity index (Figure 3). For bacterial communities (Figure 3A), all three indices consistently showed that 'Catherina' exhibited the highest diversity, followed by 'Lami[®].COM', with 'Pavaro[®]-Pav 1605' displaying the lowest values.

Fungal community diversity, assessed using the same indices (Figure 3B), also ranked 'Catherina' as the most diverse cultivar. In contrast, 'Lami[®].COM' showed the lowest values across all indices. Although 'Pavaro[®]-Pav 1605' exhibited a Shannon index similar to 'Catherina' (both around 1.8), its distribution was notably more variable, as reflected by a wider interquartile range. In contrast, 'Lami[®].COM's Shannon index centered near 1.6.

Bray–Curtis–based Principal Coordinates Analysis (PCoA) revealed distinct separation of microbial communities according to peach cultivar (Figure 4). For bacterial communities (Figure 4A), the first two axes explained a considerable portion of the variance—26.3% on axis 1 and 24.5% on axis 2—with clear clustering patterns for 'Catherina', 'Lami[®].COM', and 'Pavaro[®]-Pav 1605'. Similarly, fungal communities (Figure 4B) exhibited marked cultivar-specific segregation, with axis 1 accounting for 84% and axis 2 for 11.3% of the variation. In both cases, PERMANOVA analyses confirmed that cultivar identity significantly contributed to the dissimilarity (Tables S2 and S3).

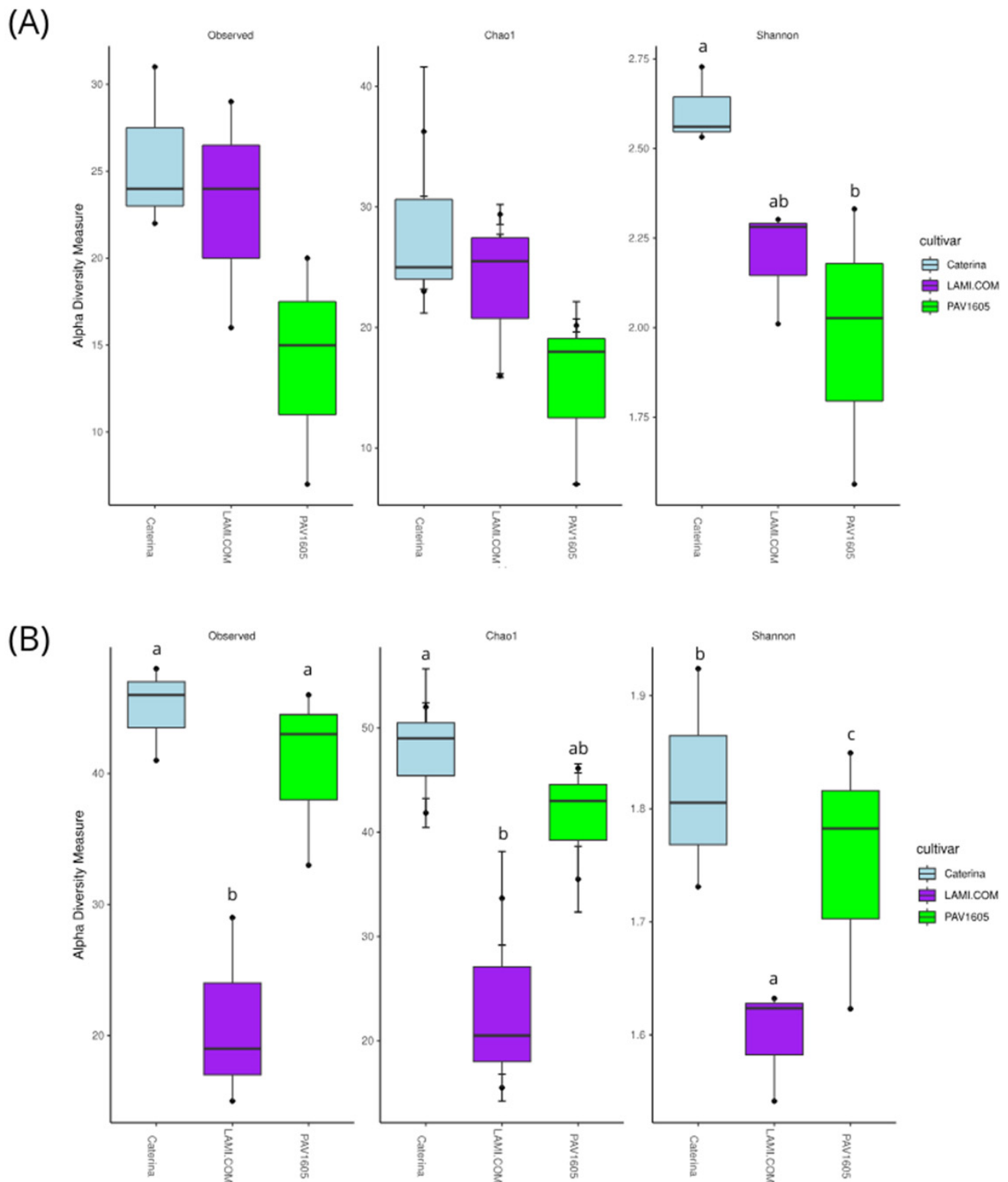


Figure 3. Alpha diversity metrics (Observed species, Chao1, and Shannon indexes) of bacterial (A) and fungal (B) communities across three peach cultivars ('Catherina' = light blue, 'Lami®.COM' = purple, and 'Pavoro®-Pav 1605' = green). Different letters above boxplots indicate significant differences between cultivars based on Tukey's Honest Significant Difference (HSD) test ($p < 0.05$).

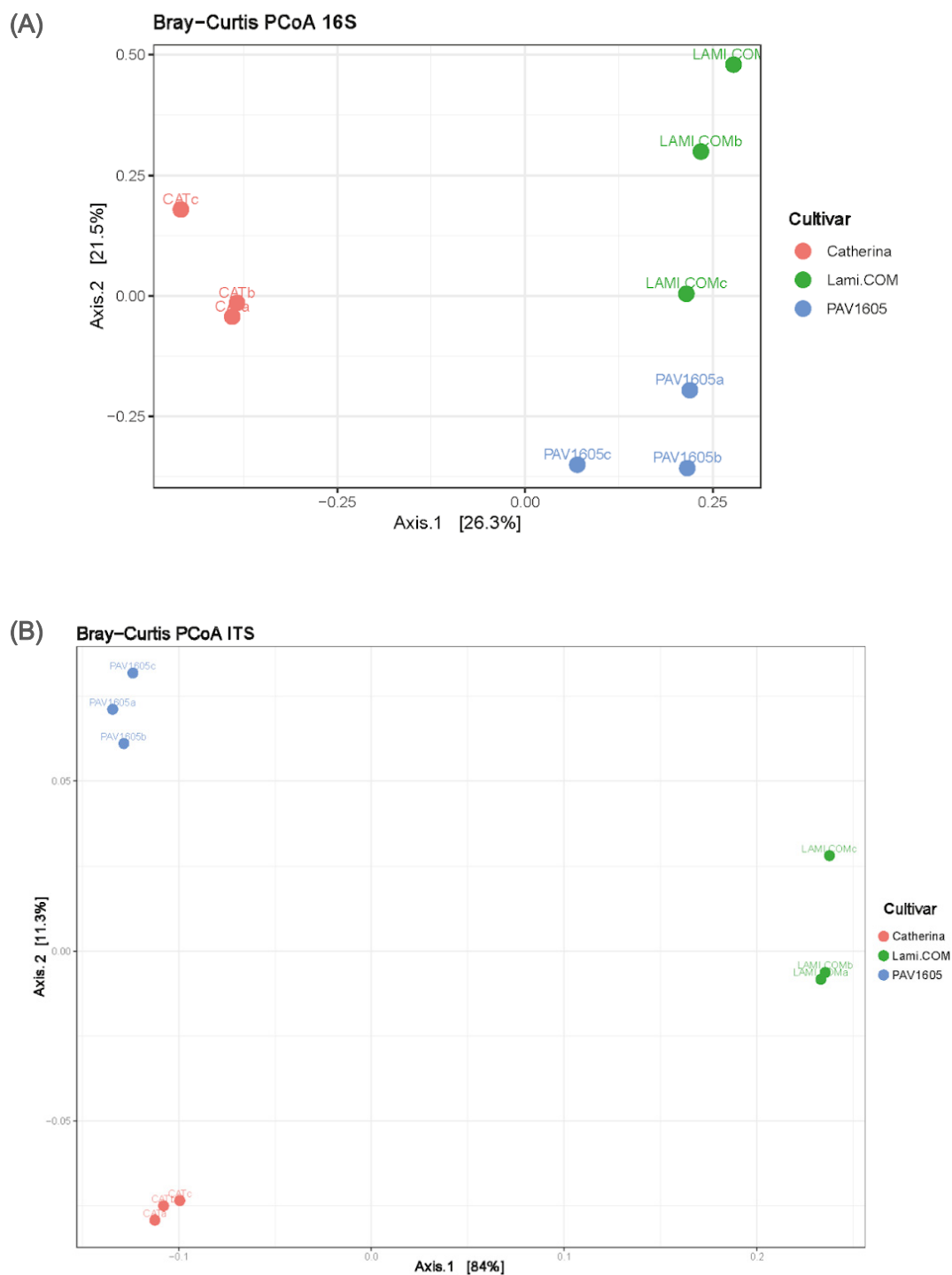


Figure 4. Principal Coordinate Analysis (PCoA) based on Bray–Curtis dissimilarity showing differences in (A) bacterial and (B) fungal community structure across three peach cultivars: ‘Catherina’ (red), ‘Lami[®].COM’ (green), and ‘Pavoro[®]-Pav 1605’ (blue).

2.5. Differential Abundance of Key Microbial Taxa

The differential abundance analysis highlighted distinct cultivar-specific microbial assemblages in the peach bud microbiome (Figure 5), with Z-scores revealing distinct community profiles across the three peach cultivars. ‘Lami[®].COM’ exhibited a strong enrichment of several bacterial genera, including *Aquabacterium*, *Caulobacter*, *Delftia*, *Fronthabians*, *Mesorhizobium*, *Pelomonas*, *Vishniacozyma*, and *Exiguobacterium*, all of which showed Z-scores exceeding +1. These same genera were comparatively depleted ($Z < 0$) in both

‘Catherina’ and ‘Pavaro[®]-Pav 1605’, indicating a cultivar-specific shift in bacterial composition favoring ‘Lami[®].COM’. In contrast, ‘Catherina’ displayed a higher relative abundance of several fungal and bacterial taxa. Notably, *Aureobasidium*, *Curtobacterium*, *Luteibacter*, *Pantoea*, *Papiliotrema*, *Pseudomonas*, and *Rhodotorula* showed Z-scores near or above +1.15 in ‘Catherina’, while being reduced in the other cultivars. Among these, *Aureobasidium*, *Papiliotrema*, and *Rhodotorula* are yeast-forming fungi commonly associated with fruit surfaces and stress-adapted phyllosphere communities. Their enrichment suggests that ‘Catherina’ may selectively favor yeast-like fungi, which could influence surface colonization dynamics, microbial competition, or interactions with the host plant’s physiology. This yeast-dominated fungal signature sets ‘Catherina’ apart from both ‘Lami[®].COM’ and ‘Pavaro[®]-Pav 1605’. The microbiome of ‘Pavaro[®]-Pav 1605’ was marked by moderate enrichment of both bacterial and fungal taxa. Genera such as *Alternaria*, *Diaporthe*, *Dioszegia*, *Massilia*, *Methylobacterium*–*Methylorubrum*, *Neocucurbitaria*, and *Sphingomonas* all showed Z-scores above +1 in ‘Pavaro[®]-Pav 1605’ but were consistently less abundant in ‘Catherina’ and ‘Lami[®].COM’. Although the amplitude of these enrichments was smaller compared to the other cultivars, they indicate a characteristic and balanced microbial assemblage associated with ‘Pavaro[®]-Pav 1605’. Several genera, including *SBR1031*, *Xylophilus*, and *1174-901-12*, exhibited variable but modest Z-score deviations across cultivars. These lineages may represent relatively stable taxa across the phyllosphere community, although their ecological roles remain unclear. Overall, the observed differences highlight cultivar identity as a key factor shaping microbial assemblages in the peach phyllosphere, with bacterial enrichment distinguishing ‘Lami[®].COM’ and fungal dominance contributing to the unique profile of ‘Catherina’.

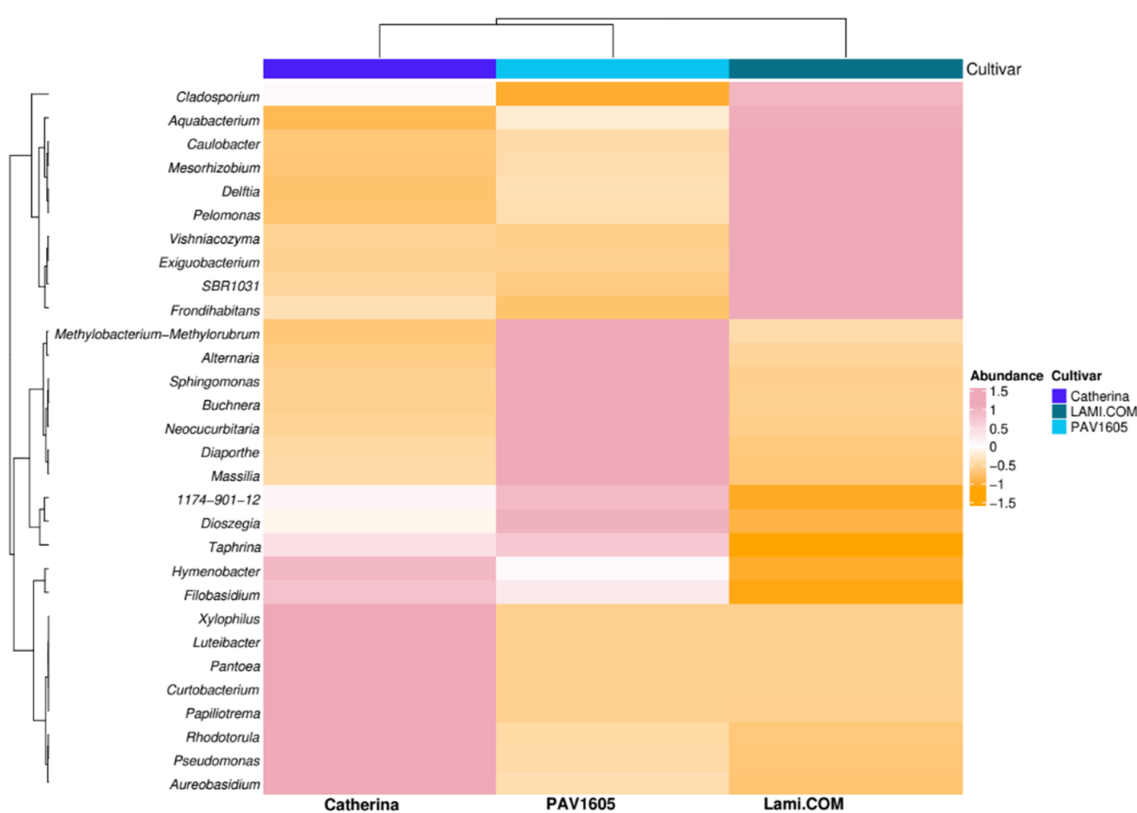


Figure 5. Heatmap showing the Z-scored relative abundance of the top 30 microbial genera across three peach cultivars. The rows represent the genera (annotated by kingdom), the columns represent the cultivars, and the colour scale represents the Z-score (orange = low; pink = high). The dendrograms depict the hierarchical clustering of both the genera and the cultivars.

3. Discussion

The plant microbiome plays a crucial role in regulating host health, development, and resilience to biotic and abiotic stresses. Microbial communities associated with plant tissues can contribute to nutrient acquisition, modulate hormone signaling, enhance immune responses, and suppress pathogens through competitive or antagonistic interactions. In perennial crops such as peach, which are subject to recurring infections and long-term environmental exposures, the composition and stability of microbial assemblages, particularly in vulnerable tissues like buds, can significantly influence disease outcomes and plant vigor. Understanding how host genotype shapes these communities is essential for developing targeted strategies that harness the microbiome to improve crop performance.

This study reveals cultivar-specific microbiome signatures in dormant peach buds, suggesting a strong interplay between host genotype and microbial assemblage in shaping potential responses to TCSB. The evident distinctions in bacterial and fungal communities across the three cultivars examined—‘Catherina’ (tolerant), ‘Lami[®].COM’ (intermediate), and ‘Pavoro[®]-Pav 1605’ (susceptible)—emphasise the significance of microbiome composition in plant health and disease resilience. At the phylum level, all cultivars shared a core bacterial community dominated by Proteobacteria, consistent with previous findings in plant phyllospheres and endospheres [27–29]. However, genus-level analysis revealed meaningful distinctions. The highly tolerant cultivar ‘Catherina’ was found to harbour a relatively diverse and stable microbiome enriched in *Pseudomonas*, *Sphingomonas*, *Curtobacterium*, and yeast-associated fungi such as *Aureobasidium*, *Papiliotrema*, and *Rhodotorula*. These genera are distinguished by their capacity to exhibit beneficial traits for plants, encompassing antagonism against pathogens, the production of antimicrobial compounds, the formation of biofilms, and the modulation of plant immune responses [30–33]. In particular, *Pseudomonas* spp. have been well-documented as biocontrol agents [30,34], while yeasts such as *Aureobasidium* have been shown to outcompete fungal pathogens through nutrient competition and surface colonization [35–37]. The coexistence of these microbial groups may contribute synergistically to the suppression of twig canker and shoot blight (TCSB) pathogens and to enhanced plant fitness during dormancy. In contrast, the susceptible cultivar ‘Pavoro[®]-Pav 1605’ exhibited a less diverse microbiome, dominated by *Massilia*, *Sphingomonas*, and *Methylobacterium*–*Methylorubrum*, with notable enrichment of fungal genera such as *Alternaria* and *Diaporthe*. The detection of *Diaporthe* only in ‘PAV1605’ suggests either a latent infection or a permissive microenvironment favourable to its colonization. Furthermore, the relatively high abundance of *Alternaria*, a known opportunistic pathogen, may be indicative of a dysbiotic fungal community that is susceptible to pathogenic outbreaks. The lower bacterial diversity observed in ‘PAV1605’ may compromise its microbiome stability and resistance to pathogen invasion, consistent with the concept of a protective microbial shield in tolerant genotypes. The bacterial community in ‘Pavoro[®]-Pav 1605’ showed higher variability across replicates compared to the fungal community, which may reflect genuine biological differences rather than technical artifacts. Subtle environmental and physiological factors can influence microbial assemblages even under standardized sampling conditions. In particular, the consistently low abundance of *Sphingomonas* observed across replicates suggests a cultivar-specific pattern, in line with previous reports showing that *Sphingomonas* abundance varies strongly among host genotypes and tissues in other plants [3]. The intermediate profile of ‘Lami[®].COM’ lends further support to the hypothesis of a genotype-microbiome link. Despite its reduced diversity compared to ‘Catherina’, its microbiome was uniquely enriched in genera such as *Pelomonas* and *Delftia*, all of which are associated with environmental resilience and nutrient cycling [36–38]. It is noteworthy that Lami[®].COM also exhibited a significant presence of *Vishniacozyma*, a yeast genus frequently associated with cold tolerance and

stress adaptation [39–41]. *Alternaria* was detected as part of the fungal community inhabiting dormant buds of ‘Lami[®].COM’. This genus includes both latent pathogens and endophytes, depending on host and environmental context. Since our study was based on metabarcoding, we cannot determine the functional lifestyle of *Alternaria* in this specific case. However, as buds were asymptomatic at the time of sampling, *Alternaria* likely persisted in a latent or endophytic state rather than causing disease. These findings suggest that, in the absence of direct antagonistic effects, the microbial community in ‘Lami[®].COM’ may nevertheless modulate plant responses by supporting general physiological resilience.

Alpha and beta diversity metrics further confirmed the cultivar-specific structuring of microbial communities. ‘Catherina’ consistently exhibited the highest bacterial and fungal diversity, while ‘Lami[®].COM’ displayed the lowest fungal richness. Principal coordinate analysis and PERMANOVA tests reinforced the non-random distribution of microbial profiles, demonstrating a strong effect of cultivar identity on both bacterial and fungal community structures. The results of differential abundance analyses provided further support for these associations, highlighting taxa that were uniquely enriched in each cultivar. The yeast-dominated profile of ‘Catherina’ may offer an unexplored mechanism of disease suppression, perhaps through niche exclusion or modulation of bud surface conditions. Concurrently, the enrichment of soil- and rhizosphere-associated bacteria in ‘Lami[®].COM’ indicates a more transitional microbial assemblage, potentially inherited from the root zone or surrounding environment.

The results of this study emphasise the pivotal role of the bud-associated microbiome in modulating cultivar-specific tolerance to TCSB. The structure, diversity, and functional potential of microbial communities appear to mirror the host’s intrinsic defense capacity and actively influence disease dynamics. In future research, it will be essential to functionally validate the roles of key microbial taxa, such as *Pseudomonas*, *Aureobasidium*, and *Curtobacterium*, through co-inoculation assays, gnotobiotic plant systems, and metatranscriptomic approaches. The application of these methodologies will facilitate the elucidation of the direct antagonistic effects exerted by specific microbes on pathogens, as well as their capacity to modulate host physiology and enhance resistance. It is important to note that these findings establish the foundation for the development of microbiome-informed biocontrol strategies that are aimed at enriching beneficial taxa in susceptible cultivars. The utilisation of protective microbes, such as *Pseudomonas*, *Papillotrema*, or *Aureobasidium*, has the potential to facilitate the suppression of pathogens through mechanisms including competitive exclusion and metabolic interference. Concurrently, the identification of potentially deleterious microbial consortia—such as the co-occurrence of *Diaporthe* with the unidentified lineage 1174-901-12 in the susceptible cultivar—underscores the efficacy of microbiome-based diagnostics for expeditious disease detection and targeted intervention.

The findings of this study could provide a foundation for designing analogous experiments in other areas where symptoms of TCSB have been reported, or in areas where such symptoms have yet to be observed. Furthermore, the identification of various microbial consortia, both directly and indirectly implicated in resistance and/or susceptibility to the disease, could be extended to other peach cultivars.

In the long term, insights into genotype–microbiome interactions could drive the adoption of microbiome-assisted breeding programs, selecting for cultivars that naturally support protective microbial assemblages or that respond favorably to beneficial inoculants. Integration of microbiome profiling into peach cultivation and crop protection strategies offers a promising pathway towards more resilient, low-input production systems that are aligned with the principles of sustainable agriculture.

4. Materials and Methods

4.1. Peach Buds Collection

Dormant wood and floral buds were collected in February 2025 from three peach (*Prunus persica* L. Batsch) cultivars ('Catherina', 'Lami[®].COM', and 'Pavaro[®]–Pav 1605') grown in a commercial orchard located in Cotignola (Ravenna Province, Northern Italy, 44°20'57.0" N, 12°12'10.1" E). All trees were managed under standard agronomic practices, without chemical treatments during the dormant period. Sampling was performed on one-year-old shoots. To minimize position-related variability, buds were excised randomly from different portions of the branches. For each cultivar, buds were collected from five independent groups of trees, with ten buds per tree (total $n = 50$ per cultivar). The collected buds were subsequently pooled to generate three independent biological replicates per cultivar, each consisting of a subset of buds randomly drawn from the total material. Both wood and floral buds were included in the sampling.

Immediately after excision, buds were placed into sterile bags on ice and then kept at 4 °C. Surface sterilization was performed by sequential immersion in 70% ethanol for 1 min, 1% sodium hypochlorite for 2 min, and a final rinse in sterile distilled water for 5 min. Sterilized buds were blotted dry on sterile paper towels, flash-frozen and ground in liquid nitrogen, and stored at –80 °C until DNA extraction.

4.2. DNA Extraction and Sequencing

For each cultivar, the three biological replicates were processed independently throughout all downstream analyses. Genomic DNA extraction was performed following a modified protocol from Cheng et al. [42]. Briefly, 1 ml of extraction buffer (2% CTAB, 1.5M NaCl, 100 mM Tris-HCl (pH 8), 20 mM EDTA (pH 8), 2% PVP, 2% fresh beta-mercaptoethanol) pre-heated at 65 °C, was added to 100 mg of frozen tissues, vortexed for 5 min, and let incubate at 65 °C. After 30 min, 1 ml of chloroform: isoamyl alcohol (24:1) solution was added to the tubes and shaken vigorously to form a complete emulsion. The tubes were then centrifuged at 10,000 rpm for 10 min at RT. The aqueous phase was transferred to new fresh tubes and two volumes of cold (–20 °C) EtOH (95%) were added, following an incubation period of 1 h at –20 °C. The precipitated DNA was recovered after a centrifugation step at 10,000 rpm for 10 min, and 600 µL of 1 M NaCl solution was added and incubated at 65 °C for 10 min. After incubation, 300 µL of phenol:chloroform:isoamyl alcohol (25:24:1) was added to each tube, vortexed, and centrifuged at 10,000 rpm for 10 min to let the phases separate. The fresh aqueous phase was transferred to new tubes, and 500 µL of chloroform was added to clear the phenol residual. Total DNA was precipitated from the aqueous phase by adding 1 mL of cold (–20 °C) EtOH (95%), followed by incubation at –20 °C for 1 h. After a centrifugation step at 10,000 rpm for 20 min, the DNA pellet was washed twice with cold (–20 °C) EtOH (70%) and let dry, to remove residual ethanol, under the hood at RT for 1 h. Genomic DNA was resuspended in 70 µL of TE buffer and incubated at 4 °C overnight to facilitate its complete elution. DNA concentration was assessed with a Qubit[™] 4 fluorometer (Invitrogen, Waltham, MA, USA) using the Qubit[™] dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA).

4.3. PCR Amplification and Library Preparation

A preliminary PCR was first conducted to evaluate the amplification efficiency of extracted DNA. For this assay, 1 ng of DNA was amplified under standard conditions with primers described in Table S1, using PCR BIO Taq Mix Red (PCR Biosystems Ltd., London, UK). Thermocycling consisted of an initial denaturation at 95 °C for 3 min, followed by 30 cycles of 95 °C for 30 s, 57 °C for 45 s, and 72 °C for 1 min, with a final extension at 72 °C for 10 min. Amplification products were visualized on a 1.5% agarose gel. After this check,

gDNA aliquots were sent to Biomarker Technologies (BMK) GmbH (Münster, Germany) for library preparation and sequencing with an Illumina PE250 platform.

4.4. Data Summary and Bioinformatic Analysis

Raw sequencing reads were first subjected to quality control using the QIIME 2 platform [43], where primer sequences and low-quality bases (Phred score < 20) were trimmed. Paired reads were then merged, and chimeric sequences were removed with the DADA2 plugin to generate high-resolution Amplicon Sequence Variants (ASVs). Taxonomic assignment of bacterial and fungal ASVs was performed against the SILVA 138 database [44] and the NCBI Fungi RefSeq ITS project (PRJNA177353), respectively, using the classifier algorithm *consensus-blast*. Downstream analyses of microbial communities, including relative taxonomic abundance, alpha diversity (Observed, Chao1, and Shannon indices), and beta diversity, were conducted in R (v4.2.3) using the *phyloseq* (v1.52) and *vegan* (v2.7-1) packages, as described by Turco et al. [45] and Cardacino et al. [46]. Differences in alpha diversity among peach cultivars were assessed using one-way analysis of variance (ANOVA), followed by Tukey's Honest Significant Difference (HSD) post-hoc test for pairwise comparisons. Principal Coordinates Analysis (PCoA) was used to visualize sample clustering by cultivar, and permutational multivariate analysis of variance (PERMANOVA) tested for significant differences in community composition. To explore variation in microbial composition across peach cultivars, tables of abundances for bacterial and fungal genera were combined into a single dataset. The genus-level relative abundances were grouped by cultivar, and the 30 most abundant genera across all samples were selected for visualization. Z-scores were calculated for each genus by centering and scaling the abundance values (mean = 0, standard deviation = 1) across cultivars. This enabled the identification of relative enrichment or depletion patterns. The resulting standardized matrix was visualized as a heatmap using the *ComplexHeatmap* (v2.24.1) package in R, and hierarchical clustering with Euclidean distance and complete linkage was applied to both the genera and the cultivars.

5. Conclusions

This study provides the first comprehensive characterisation of bacterial and fungal communities associated with dormant wood and floral buds of multiple peach (*Prunus persica*) cultivars with different levels of tolerance to twig canker and shoot blight (TCSB). The results obtained demonstrate that cultivar genotype exerts a significant influence on the composition and relative abundance of the bud microbiome, thus revealing patterns that may be relevant for disease susceptibility. While varietal differences in disease resistance are influenced by environmental conditions, agronomic practices, and host physiology, the present study's sampling strategy was devised to minimise these external factors in order to focus on genotype-associated microbial patterns. It is noteworthy that the abundance of certain bacterial taxa, such as *Sphingomonas*, exhibited variation among the different cultivars. In addition, the presence of fungi, including *Alternaria*, was observed as latent endophytes within asymptomatic buds. This finding suggests the potential for these fungi to persist without manifesting symptoms of TCSB, thereby underscoring the intricate interactions that occur between the host genotype and the microbial communities. The contrasting enrichment of beneficial taxa such as *Pseudomonas* and *Aureobasidium* versus potentially harmful genera like *Alternaria* and *Diaporthe* highlights the dual role of the microbiome as both a protective shield and a reservoir of latent pathogens.

This work marks a preliminary step in comprehending the function of the bud microbiome in peach health and TCSB susceptibility. The study establishes a foundation for future research that will integrate climatic, agronomic, and physiological data. This

research will explore the functional significance of microbial taxa in promoting resistance or susceptibility to pathogens. These insights have the potential to inform cultivar selection and microbiome-based strategies for sustainable peach production.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/stresses5030060/s1>. Figure S1: Relative abundance of bacterial phyla in three peach cultivars. Stacked barplots represent the taxonomic composition of bacterial communities at the phylum level for each replicate; Figure S2: Phylum-level relative abundance of fungal taxa in association with three peach cultivars. Stacked barplots represent the taxonomic composition of bacterial communities at the phylum level for each replicate; Table S1: List of primers used for amplicon sequencing of bacterial 16S rRNA and fungal ITS regions. The table includes primer names, target regions, and nucleotide sequences; Table S2: PERMANOVA (adonis) results testing the effect of peach cultivar on bacterial community composition based on Bray–Curtis dissimilarity; Table S3: PPERMANOVA (adonis) analysis of beta diversity (Bray–Curtis dissimilarity) assessing the significance of differences in fungal community composition among peach cultivars.

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