Characterizing the Alterations in the Phyllosphere Microbiome in Relation to Blister Blight Disease in Tea Plants

Shuyuan Liu
Northwestern Agricultural and Forestry University
Nini Guo
Northwestern Agricultural and Forestry University
Jiayi Jin
Northwestern Agricultural and Forestry University
Qiqi Zhang
Northwestern Agricultural and Forestry University
Youben Yu (yyben@163.com)
Northwestern Agricultural and Forestry University

Research Article

Keywords: Tea plant, Phyllosphere microbiome, Tea blister blight, Microbiome diversity, Microbial community

Posted Date: January 17th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-3862672/v1

Additional Declarations: No competing interests reported.

Abstract

Background

Tea blister blight is a highly significant leaf disease of tea plants (*Camellia sinensis* (L.) O. Kuntze) that adversely affects global tea production. While the influence of commensal microbes on disease development has been observed, the overall impact of the phyllosphere microbiome and its response to pathogen invasion in tea leaves has largely not been explored. For this investigation, we utilized a blend of 16S ribosomal RNA (16S rRNA) and internal transcribed spacer (ITS) amplicon information to analyze the changes in the phyllosphere microbiome concerning different degrees of blister blight disease.

Results

The results showed that the fungal community on healthy leaves had greater alpha diversity than that on diseased leaves. However, there were no significant differences in the bacterial Sobs, Chao 1, or Shannon indices between healthy and diseased tea leaves. Principal coordinate analysis (PCoA) was employed to distinguish the microbial communities of tea plants with blister blight disease from those of healthy plants. Distinct operational taxonomic units (OTUs) were identified at different disease developmental stages using effect size analysis via linear discriminant analysis (LefSe). Moreover, redundancy analysis conducted at both the phylum and genus levels provided additional evidence of disparities in the bacterial and fungal compositions between healthy and diseased tea leaves. These findings suggested the occurrence of potential interactions between beneficial and pathogenic microorganisms within the phyllosphere region. To examine the main connecting nodes in the microbial interaction network, a co-occurrence analysis was performed. Certain nonpathogenic microorganisms, such as *Pseudomonas*, *Aureionas*, and *Bulleromyces*, could serve as key taxa within the network and hold promise as effective biological control agents against tea blister blight. Furthermore, the alterations in key biochemical constituents in tea leaves were examined, and the presence of abundant ECGs and select alkane components was shown to potentially contribute positively to the ability of tea plants to resist fungal infection.

Conclusions

We provide the dynamic characterization of in the phyllosphere microbiome of tea leaves responses to the development of tea blister blight disease. These results will help deepen the understanding of the relationship between the phyllosphere microbiome and tea plant health.

Background

In the natural environment, plants engage in continuous interactions with a wide variety of microorganisms, including protists, fungi, bacteria, viruses, and nematodes [1]. These microorganisms

inhabit various plant structures or tissues, such as the rhizosphere (soil-root interface), phyllosphere (air–plant interface), and endophytes (plant tissue interior) [2]. Numerous lines of evidence have consistently indicated that fungi and bacteria hold a dominant position within the microbial community and significantly influence the overall performance and survival of plants. The crucial role of microbial interactions in the rhizosphere is to enhance plant health and productivity by facilitating plant nutrient absorption, promoting nitrogen fixation, and stimulating hormone production [3–5]. Some microorganisms can release bioactive substances or change the balance of nutrients, thus improving the ability of plants to withstand abiotic and biotic stresses [6–8]. However, microbial competition for resources and space with plants, as well as the potential induction of diseases, can have detrimental effects on plant yield and quality [9]. Therefore, it is crucial to elucidate the variety and organization of microorganisms to achieve superior and abundant agricultural output.

Initial investigations have primarily concentrated on examining the structural and functional attributes of rhizosphere microorganisms, which can promote plant growth [10, 11]. Notably, the parts of plants that are visible above the ground, especially the leaves, serve as an extremely plentiful environment for microorganisms on a global scale. Bacteria dominate the phyllosphere, with cell densities ranging from 10^6 to 10^7 cells per square centimeter of leaf area [12]. Filamentous fungi, yeast strains, protists, and bacteriophages also colonize this environment [12]. Phyllosphere microbiomes play a critical role in leaf surface environments, and their surroundings can affect plant fitness in natural communities and agricultural quality and productivity. Several studies have demonstrated that epiphytic bacteria can produce biosurfactants, which facilitate the movement of nutrients through a hydrophobic cuticle and into favorable growth conditions [13, 14]. Certain species of *Methylobacterium* have been found to utilize methanol as their primary carbon source during the colonization of various plant species, with some of these strains exhibiting the ability to enhance plant fitness and improve survival prospects [15, 16]. Some specific ecological groups of fungi have shown the capacity to generate a wide variety of unique bioactive compounds that efficiently alleviate environmental pressures such as ultraviolet rays, reactive oxygen species (ROS), and drought [17]. Certain species within the *Pseudomonas*, *Bacillus*, and *Trichoderma* genera have been extracted from the abundant leaf microbiota and effectively employed as biological control agents for safeguarding plants [18–20]. However, investigations of phyllosphere microbial structure and function have been relatively limited.

While microorganisms affect plants, these plants in turn also influence the microbial community composition and quantity on their leaves, resulting in an adaptive coevolutionary relationship between phyllosphere microbes and plants [21]. Phytochemicals, the secondary metabolites synthesized by plants, are recognized as significant regulators of microbial activity [22]. Microorganisms primarily rely on plant metabolites as their main sources of carbon and nitrogen [23]. However, the bioactive metabolites exhibit considerable variation among plant species, potentially influencing growth and colonization dynamics. The beneficial role of flavonoids in facilitating the establishment of endosymbiotic relationships between legume roots and nitrogen-fixing bacteria has been firmly established [24]. Citric and fumaric acids can be released by tomato roots and are reportedly used to attract plant growth-promoting rhizobacteria [25].

The microbial community in soils and cucumber growth have been shown to be influenced by *p*-coumaric acid [26]. Moreover, some plants, such as camomile, thyme and eucalyptus plants, can even exude unique antimicrobial metabolites to resist pathogen infections [27].

Tea, obtained from the young foliage of the tea plant (*Camellia sinensis* (L.) O. Kuntze), has become widely popular worldwide as a drink because of its wide range of beneficial compounds, such as flavonoids, alkaloids, and theanine [28]. Given its preference for warm and humid climates, tea plants are vulnerable to various fungal pathogens [29]. Tea blister blight disease, caused by *Exobasidium vexans*, poses a significant threat to tea production. This disease primarily affects young leaves, leading to yield losses of up to 25–30%, particularly in susceptible cultivars [30]. Spraying fungicides is still the primary method of controlling this disease in tea fields and might involve significant costs and fungicide residue problems [30]. Investigating the response of the phyllosphere microbiome at the community level following pathogen invasion could enhance the understanding of the interactions between microbes and plants, as well as the relationships between pathogens and other microbes, and could provide guidance for developing and applying biocontrol microorganisms for tea plants.

The present study examined the temporal variations in phyllosphere bacterial and fungal communities during the progression of tea blister blight disease. A comparative analysis was conducted to assess microbial diversity, community composition, and intramicrobial interactions between healthy and infected leaves. Furthermore, we examined the biochemical compounds found in tea leaves affected by tea blister blight disease. These investigations will enhance the overall understanding of the relationships among microbes, hosts, and pathogens, providing valuable insights for the development and application of biological control microorganisms in tea cultivation.

Methods

Tea Sample Collection

Tea plants (*Camellia sinensis* cv. Fuding dabai) were grown in the gardens of Northwest A&F University Tea Experimental Station (located in Xixiang, Shaanxi, China, at coordinates 32°57'43" N, 107°40'12" E). In September 2022, fresh third leaves with only one typical tea blister-blight symptom were collected and classified into the following three groups based on established criteria: yellow, transparent patches that formed in the early stage (S1); circular blisters that appeared and were coated by white powdery basidiospores in the middle stage (S2); and necrotic spots that formed in the late stage (S3) [31]. Healthy leaves at the same leaf position were used as control samples. For further analysis, the samples were kept at a temperature of -80°C.

Determination of Biochemical Compounds

To ascertain the overall polyphenol content (TP), the Folin–Ciocalteu technique was used [32]. As per the China National Standard (GB/T 8314 – 2013), the ninhydrin reaction at 570 nm was utilized to determine the amino acid concentration. The soluble protein concentration was assessed by the Coomassie brilliant

blue G250 method. The amount of water soluble carbohydrates was determined as described by Morris [33]. High-performance liquid chromatography with UV detection (HPLC-UV, Agilent 1100VL, Agilent Technologies, Inc., Santa Clara, CA, USA) was used to identify and measure caffeine, gallic acid, and catechins. The SPME-GC–MS technique was utilized to analyze the volatile compounds by employing a solid-phase microextraction device coupled with gas chromatography (Agilent 7697A)/mass spectrometry (Agilent 7890A). Our previous work provided a description of the parameters and conditions employed in the analysis of HPLC-UV and SPME-GC–MS [34, 35].

Genomic DNA Extraction and Sequencing

Following thawing at a temperature of 25°C, 10 g of tea leaves were transferred to polypropylene test tubes containing 40 mL of sterile water and orbit-shaked at 150 rpm for 1 h at room temperature. The suspensions were separated using 0.22 μm vacuum filtration units (EMD Millipore, Billerica, MA), and the filtered sediment was used as a sample for the extraction of microbial DNA via HiPure Soil DNA Kits (Magen, Guangzhou, China). The V5-V7 region of the bacterial 16S rRNA gene and internal transcribed spacer (ITS2) region of the fungus were amplified via PCR using the primers listed in Table S1. All amplifications were performed in triplicate in 50 μL reactions containing 1.0 μL of DNA template (50 ng), 1.5 μL of dNTPs (2.5 mmol/L), 1.5 μL of each primer (10 μmol/L), 10 μL of 5× Q5 reaction buffer, 10 μL of 5× Q5 High GC Enhancer, 0.3 μL of Q5 High-Fidelity DNA Polymerase, and 24.2 μL of ddH_2O. The thermal cycler conditions consisted of initial denaturation at 95°C for 5 min; 30 cycles of 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min; and a final extension at 72°C for 7 min. The genes were purified using an AxyPrep DNA Gel Extraction Kit from Axygen Biosciences, Union City, US, and quantified using an ABI StepOnePlus Real-Time PCR System (Life Technologies). The sequence libraries were pooled into equimolar quantities and paired-end sequenced (PE250) on the Illumina platform (San Diego, US).

Bioinformatic analyses

The study involved filtering the raw data using FASTP v0.18.0[36] and then merging with FLASH[37]. Using the UPARSE v9.2.64 pipeline[38], the filtered tags were grouped into operational taxonomic units (OTUs) with a similarity of ≥ 97%. Chimeric sequences were eliminated using the UCHIME algorithm v4.1[39]. The classification of the representative sequences was performed using the RDP classifier v2.2 [40] based on the SILVA database v132 [41] and UNITE database v8.0 [42] with the aid of a Bayesian naive model. The sequence data were stored in the NCBI Sequence Read Archive (SRA) database under accession number PRJNA1050022.

Statistical analyses

Rarefaction curves were drawn to assess sequencing depth. QIIME v1.9.1 was used to compute the alpha diversity indices. A principal coordinate analysis (PCoA) plot was generated using the R ‘vegan’ package v2.5.3 based on the weighted UniFrac distance. The sequence data for bacteria and fungi were normalized using the centered log-ratio transformation method [43]. The UpSet plot was visualized graphically with the R ‘UpsetR’ package v1.3.3. Visualization of the abundance statistics for each taxon was conducted using the R ‘circlize’ package v0.4.7. Heatmap visualizations were also performed using

the R ‘pheatmap’ package v1.0.12. A co-occurrence network was constructed using the R ‘igraph’ package v1.1.2 based on the Spearman correlation coefficient. SPSS statistical software (SPSS, Chicago, USA) was used to examine significant differences, for which the p value was less than 0.05.

Results

Diversity of the microbial community

Tea blister blight disease is a severe condition that affects tea plantations (Fig. 1A). A pale yellow translucent spot emerged on the leaf subsequent to infection by *E. vexans* (Fig. 1B, S1 stage), subsequently leading to the formation of circular blisters resulting from the indentation and protrusion of both the upper and lower leaf surfaces. As spores were produced, the convex surface on the underside of the leaf assumed a white and velvet-like appearance (Fig. 1B, S2 stage). Following sporulation, the blisters transitioned to a brown hue, and necrotic spots formed (Fig. 1B, S3 stage). High-throughput sequencing was employed to assess alterations in the microbial community within tea leaves affected by blister blight disease. A total of 1,550,953 reads from 16S and 1,566,187 reads from ITS were acquired following the elimination of low-quality reads from 12 samples. From these reads, 14,190 operational taxonomic units (OTUs) were generated, encompassing 3,792 bacteria and 10,398 fungi (Supplementary Table S1). All the samples exhibited a plateau-like pattern in the rarefaction curves (Supplementary Fig. S1), suggesting that the sequencing depth was sufficient for further analysis.

The diversity and microbial community composition of tea leaves were determined during blister blight disease. In our study, the α diversity of the fungal communities tended to decrease in response to disease stress (Table 1). These findings indicate that the richness and diversity of the fungal community are correlated with the stage of blister blight disease. Tea leaves at the late stage (S3) of blister blight disease exhibited the lowest fungal Sobs, Chao 1, and Shannon indices, as shown in Table 1. However, there was no significant difference in the bacterial Sobs, Chao1 estimator, or Shannon index between healthy tea leaves and blister blight disease-infected tea leafs (Table 1). The PCoA showed that the different tea samples tended to cluster well together (Fig. 2). The diversity and composition of microorganisms on the epidermis of tea leaves were significantly affected by the development of blister blight disease, as indicated by the β diversity of bacteria and fungi.

Table 1
Alpha diversity of bacterial and fungal communities on the surface of tea leaves at different blister blight disease stages

Samples	Bacteria			Fungi		
	Sobs	Chao 1	Shannon	Sobs	Chao 1	Shannon
H	269.00 ± 11.36 [a]	296.34 ± 11.97 [a]	4.57 ± 0.46 [a]	756.00 ± 29.60 [a]	829.84 ± 36.32 [a]	4.85 ± 0.31 [a]
S1	243.00 ± 38.69 [a]	293.12 ± 28.85 [a]	4.51 ± 0.48 [a]	712.33 ± 13.58 [ab]	812.52 ± 24.39 [ab]	4.25 ± 0.49 [ab]
S2	268.67 ± 11.59 [a]	307.35 ± 15.27 [a]	4.79 ± 0.03 [a]	684.67 ± 13.05 [bc]	761.59 ± 6.78 [bc]	3.58 ± 0.14 [bc]
S3	282.00 ± 60.23 [a]	308.86 ± 57.81 [a]	4.54 ± 0.42 [a]	642.00 ± 51.18 [c]	721.74 ± 33.74 [c]	3.25 ± 0.38 [c]

Note: H, healthy leaves; S1, tea leaves in the early disease stage; S2, tea leaves in the middle disease stage; S3, tea leaves in the late disease stage. The values are expressed as the means ± standard errors. Lowercase letters in the same row indicate significant differences at $p < 0.05$

Dynamics of the microbial community throughout the development of blister blight disease on tea leaves

An UpSet plot was created to visualize unique OTUs present at different developmental stages of blister blight disease (Fig. 3). Out of the 589 distinct OTUs, 197 OTUs (making up 33.62% of all OTUs) were found in every sample, suggesting that the stages of blister blight disease had a relatively low level of bacterial diversity. Sixty-three OTUs were found only in H, and 32, 53, and 53 OTUs were unique to S1, S2, and S3, respectively. Of the fungal sequences, 455 OTUs, accounting for 30.76% of the total OTUs, were shared by all the samples. According to the present results, 165, 94, 170, and 68 OTUs were uniquely present at the H, S1, S2, and S3 stages, respectively.

Fungal and bacterial community succession during the onset of tea blister blight disease was further investigated (Fig. 4 and Supplementary Data Sheet 1). The predominant phylum in the tea leaf samples was *Proteobacteria*. Its abundance in the H period reached 93.62% and decreased due to disease infection. In contrast, the second-highest abundance of *Actinobacteria* increased from 4.43–13.26% as blister blight development staged (Fig. 4A). The abundance of *Bacteroidetes* was also affected by tea blister blight disease, which was 0.37%, 1.72%, 1.94%, and 7.73% at the H, S1, S2, and S3 stages, respectively (Fig. 4A). The fungi included mainly *Ascomycotafa* (representing 79.31%-86.23% of the total fungi detected) and *Basidiomycota* (representing 12.84%-20.23% of the total fungi detected) (Fig. 4B).

A heatmap was created for in-depth analysis using the 35 genera with the greatest abundance. Figure 5 shows a substantial difference between the H stage and the S1-3 stage, indicating a significant difference in the composition of bacteria and fungi between the samples infected and uninfected by tea

blister blight disease. The abundance of bacteria such as *Aureimonas*, *Bacteroides*, *Microbacterium*, *Roseomonas*, *Variovorax*, and *Vibrio* decreased in the diseased tea samples, while the abundance of *Actinomycetospora*, *Geodermatophilus*, and *Pantoea* increased (Fig. 5A). Among the fungi, the abundances of *Erythrobasidium, Septoria*, *Sporobolomyces*, *Strelitziana*, and *Taphrina* were significantly lower in diseased tea samples than in healthy leaves, and the abundances of *Cladosporium*, *Colletotrichum*, *Exobasidium, Didymella*, and *Paraphoma* were greater (Fig. 5B).

Analysis of the co-occurrence network among microbial communities

To investigate the connections between microorganisms, a co-occurrence network was constructed using Spearman correlation analysis (with correlation > 0.65 for positive correlations and < − 0.65 for negative correlations, $p < 0.05$). The networks that were created contained a total of 73 nodes and 137 edges (Fig. 6 and Supplementary Data Sheet 2). The larger the node is, the more important the genus in the microbial community is. Based on the network connectivity statistics, bacterial genera such as *Aureionas*, *Bulleromyces*, *Geodermatophilus*, *Pseudomonas*, and *Neisseria* were among the hubs. For fungi, the genera *Alternaria*, *Bulleribasidium, Colletotrichum, Coniothyrium, Sporobolomyces, Sphaerulina, Setophoma, Symmetrospora, and Ophiognomonia* served as the main connecting nodes. The examination of microbial communities revealed both positive and negative correlations. Visualization of the network revealed that a majority of the edges were positively correlated (105) and that a smaller number of edges were negatively correlated (32) (Fig. 6A). Positive correlations were predominantly observed within fungi (43) or between fungi and bacteria (44), while negative correlations were primarily observed between fungi and bacteria (15) (Fig. 6B). *Bulleribasidium* was positively correlated with *Bulleromyces*, *Strelitziana*, *Quadrisphaera*, *Actinomycetospora*, *Erythrobasidium*, and *Bullera*. *Alternaria* was positively correlated with *Photobacterium*, *Neisseria*, *Dissoconium*, *Sporobolomyces*, *Sphaerulina*, and *Symmetrospora* but negatively correlated with *Colletotrichum*. *Spaerullina* was negatively correlated with *Septoria*, *Setophoma*, *Phaeosphaeria*, *Leptospora*, and *Ramularia*.

Response of the main chemical constituents of tea leaves to blister blight disease

The contents of tea polyphenols, catechins, alkaloids, and volatile components in tea leaves at different blister blight disease development stages were analyzed (Tables S3 and S4). Figure 7A shows a PLS-DA score plot that exhibited distinct segregation among the H, S1, S2, and S3 samples, suggesting notable variations in the main chemical constituents in tea leaves at different blister blight disease development stages. The effective predictive ability of the PLS-DA model without overfitting was demonstrated by R2 and Q2 permutation tests (Fig. 7B). A total of 15 significantly different metabolites were identified (variable importance projection > 1 and $p < 0.05$) and visualized via heatmap analysis (Fig. 8). The content of catechins markedly decreased during infection, with a slight increase in the S2 stage followed by a decrease in the S3 stage. EGCG displayed the same pattern as total catechins. ECG decreased during

infection, while EC and EGC were observed during S2-S3 and S3, respectively. Alkane components were identified as characteristic volatile components in different disease stages. The levels of tetradecane, heptadecane, 2,4-dimethylheptane, and 1-chlorohexadecane substantially decreased as the disease progressed. In contrast, the level of pentadecane started increasing with the intensity of the infection and reached a maximum in the S2 stage. The levels of hexadecane and 2,6,10-thrimethythridecane were significantly greater in the S2 stage than in the S3 stage. The concentrations of icosane and 2,6,10-thrimethytridecane tended to decrease first and then increase again. Additionally, the levels of palmitic acid were significantly greater in the S1 and S3 stages.

A partial Mantel test was applied to compare distance-corrected dissimilarities of microbial communities with distance-corrected dissimilarities of the main chemical components (Fig. 9). ECG, 1-chlorohexadecane, heptadecane, icosane, EGCG, 2-4-dimethylheptane, and catechins were positively correlated with the OTUs of the fungal community, with Mantel's r = 0.45, 0.40, 0.31, 0.29, 0.29, 0.23 and 0.22, respectively, while hexadecane was negatively correlated with the OTUs of the fungal community, based on Mantel's r = − 0.24. Of the bacterial sequences, 2-4-dimethylheptane and 1-chlorohexadecane showed strong positive correlations with the OTUs, with Mantel's r = 0.39 and 0.24, respectively.

Discussion

The phyllosphere encompasses a diverse array of microorganisms that interact with host plants and have an impact on their overall health and function. While numerous studies have concentrated on investigating plant–pathogen interactions and disease mechanisms, the regulatory mechanisms governing the defense of the entire phyllosphere community against plant diseases have been largely overlooked. Therefore, our study primarily centers on examining the alterations in the diversity and composition of microorganisms present in tea leaves throughout the various developmental stages of tea blister blight disease. This investigation holds immense importance in facilitating a comprehensive assessment of the role played by microorganisms in maintaining a healthy ecosystem and aiding in the identification of specific microorganisms with antagonistic properties against pathogens.

To examine the variety of microbial communities, the study employed α diversity analysis, specifically the Sobs, Chao1, and Shannon indices. The results showed that there was a notable reduction in the richness and diversity of the fungal community (Table 1), suggesting a strong negative correlation between fungal α diversity and the progression of tea blister blight disease. Conversely, the bacterial α diversity remained largely unaffected during pathogen infection. This difference suggested that the dependence of bacterial and fungal communities on host plants differs, as highlighted by Li et al. [44]. Specifically, fungal communities appear to be more susceptible when the host plant is attacked by plant–pathogenic microorganisms. PCoA was employed to visually represent β diversity and demonstrated clear clustering patterns between control samples and tea leaves at different disease stages. Additionally, the microbiota composition was found to be significantly dissimilar among the different disease stages (Fig. 2). This dissimilarity was further supported by the presence of distinct proportions of operational taxonomic units (OTUs) in leaves at various disease stages (Fig. 3). Previous studies have reported alterations in the

diversity and community structure of plant-associated microbes when subjected to pathogenic microorganisms [45, 46]. The alterations observed in the phyllosphere microbiota of healthy and diseased tea leaves imply the occurrence of interactions between beneficial and pathogenic organisms within the phyllosphere regions, potentially influencing the activation or suppression of plant immune responses.

It is widely believed that the majority of these phyllosphere bacteria are nonpathogenic and confer benefits to plant growth and resistance against pathogens [47]. Examination at the phylum level indicates that *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* are the primary constituents of bacterial communities found in different plants, such as lettuce, spinach, wheat, apple, and naturally existing plants [48–50]. These findings are consistent with previous findings, suggesting that *Proteobacteria* is the main phylum discovered on tea leaves, comprising a percentage ranging from 77.85–93.62% (Fig. 4). Among the *Proteobacteria*, *Sphingomonas*, a member of the *α-Proteobacteria* class, was the most prevalent community member. Previous studies have demonstrated the plant-protective effects of *Sphingomonas*, including the suppression of disease symptoms and a reduction in pathogen growth [19, 48]. The observed disease suppression did not result in a significant alteration in the abundance of *Sphingomonads* (Fig. 5). *Sphingomonads* can utilize a wide range of substrates, facilitated by the presence of various transport proteins, including TonB-dependent receptors [51]. This capability serves as an alternative adaptive mechanism for competing with pathogenic microorganisms in resource-limited environments, exhibiting a remarkable affinity. Additionally, *Methylobacterium* represents a prevalent and beneficial bacterial population within the phyllosphere, offering advantages to plants. In the S1 stage, the *Methylobacterium* count decreased to 56.5% of that found in healthy leaves (Fig. 5). However, in the S2 and S3 stages, the expression of these genes rebounded to a level comparable to that of healthy leaves (Fig. 5). *Methylobacterium* species can utilize plant-derived methanol as their primary carbon source and enhance plant immune defense and protection against UV radiation, although the specific mechanisms involved in these defense processes have not been identified [52]. The genus *Klebsiella* is widely distributed in various environments, including humans, animals, and plants [53]. Under normal circumstances, *Klebsiella* bacteria are typically nonpathogenic to plants [54]. For example, *Klebsiella pneumoniae*, a nitrogen-fixing plant endophyte, is known to promote plant growth, but it is also a common opportunistic pathogen that causes pneumonia in humans [55]. The prevalence of *Klebsiella* was found to be highest during the S1 stage of tea leaf growth (Fig. 5), potentially increasing the risk of microbial transmission to humans. Several *Aureimonas* strains have been obtained from plant leaves [56, 57]. Notably, in healthy tea leaves, *Aureimonas*, which encompasses several species that participate in the cycling of carbon and nitrogen [58], was more prevalent (Fig. 5).

Ascomycota is the largest phylum of the fungal kingdom [59] and aligns with the findings of our study. The genera *Cladosporium*, *Didymella*, and *Colletotrichum* within the *Ascomycota* phylum were more prevalent in diseased S1-3-stage tea leaves than in healthy leaves, suggesting their role as etiological agents. *Cladosporium* species are commonly observed in various plant species, such as wheat, *Heterosmilax japonica*, and *Atriplex canescens*, where they serve as significant and abundant plant pathogens [60–62]. Two *Didymella* species (*D. theae* and *D. theifolia*) have been identified and characterized as the causative agents responsible for the occurrence of tea leaf brown–black spot

disease [63]. *Colletotrichum*, a genus comprising more than 189 species, is widely recognized as a prominent plant pathogen on a global scale [64]. Among these species, *Colletotrichum camelliae* is a dominant fungal pathogen affecting tea plants, leading to the development of anthracnose disease in tea leaves, which ultimately exerts a significant impact on tea yield and quality [65, 66]. In contrast, the presence of disease stress resulted in a notable decrease in the populations of *Septoroa*, *Strelitziana*, and *Taphrina*, potentially attributable to the loss of their competitive advantage in the acquisition of space and nutrients in the face of pathogenic competition. *Basidiomycota* exhibited a relatively lower abundance. The genus *Exobasidium* within *Basidiomycota* encompasses more than 170 species worldwide [67]. *E. vexans*, which belongs to a certain group within *Exobasidium*, primarily affects the delicate foliage and stems of tea plants [31]. During the S1-3 stage, the relative abundance of *Exobasidium* exhibited 2.3-fold, 15.8-fold, and 15.4-fold greater than that of healthy leaves. The noted increase aligns with the changes occurring in tea leaves at various phases of tea blister blight disease. Our findings were consistent with the changes observed in endophytic fungal communities, indicating an increase in the proportion of *Exobasidium* [68]. Moreover, the advancement of tea blister blight ailment additionally resulted in a slight increase in the proportionate prevalence of *Bulleromyces* and *Hannaella*, accompanied by a minor decrease in the proportionate prevalence of *Sporobolomyces* and *Erythrobasidium*.

The diversity and composition of the microbiota in healthy and diseased tea leaves suggest the occurrence of potential interactions between beneficial and pathogenic microorganisms in the phyllosphere region. Within host plants, nonpathogenic microorganisms rapidly proliferate and compete for limited resources [69, 70]. Various antimicrobial compounds, including phenazines, pyoluteoria, pyrrolnitrin, cyclic lipopeptide surfactants, and bacteriocins, can be synthesized by microorganisms to antagonize and inhibit the colonization and propagation of pathogens [55, 71, 72]. Furthermore, interactions between plants and microorganisms also trigger induced systemic resistance (ISR), which primes the entire plant to react to both abiotic and biotic stressors [18, 20]. Cooccurrence analysis further revealed the intricate nature of microbial interactions, encompassing both positive and negative interactions (Fig. 6). Highly connected OTUs were identified as the main connecting nodes and major players in microbial community formation [22]. These OTUs, which included *Pseudomonas*, *Aureionas*, and *Bulleromyces*, were found to be nonpathogenic microorganisms and have the potential to be utilized as beneficial biological control agents against tea blister blight disease.

The phyllosphere microbiome was found to be closely associated with the biosynthesis of plant metabolic substances. The presence of carbon-based nutrients on leaves is widely believed to be a significant determinant of the colonization of phyllosphere microbes [17]. In turn, plants can actively shape their associated microbial communities through the synthesis of bioactive substances. Among these substances, phenolic compounds serve as the primary chemical defense materials in plants, accumulating notably at infection sites and triggering the production of reactive oxygen species [73]. Catechins, the primary phenolic compounds found in tea plants, have a significant impact on the defense mechanism of tea plants against pathogen assaults [74, 75]. Gray blight-infected leaves exhibited elevated levels of GC and EC, which may enhance the resistance of tea plants to pathogen-induced harm

[75]. After being inoculated with *C. fructicola*, the levels of EGCG and C in the ZC108 cultivar (resistant) increased more than those in the LJ43 cultivar (susceptible), while there was no notable alteration in the levels of EC and EGC in ZC108 [76]. This study revealed significant accumulations of the nonester catechins EC and EGC after infection with tea blister blight disease (Fig. 8). It is reasonable to hypothesize that catechin monomers fulfill distinct functions in safeguarding tea plants against various pathogens. Researchers have consistently been fascinated by the role of volatile compounds in inter- and intraplant communication, as shown by their proven capacity to trigger herbivore resistance through jasmonic acid and enhance cold and drought tolerance in tea plants [77, 78]. The availability of volatile compounds in plant roots as a means of fighting infection has been highlighted in several studies [79, 80]. The antimicrobial activity of various components of alkanes, such as tetradecane, pentadecane, hexadecane, heptadecane, heneicosane, and docosane, has been demonstrated [81, 82]. The increased concentration of hexadecane, icosane, and 2,6,10-trimethythridecane (Fig. 8) in tea leaves affected by blister blight disease stress may positively regulate the immune response against fungal infection. Furthermore, positive and negative correlations between operational taxonomic units (OTUs) and the main biochemical components were observed (Fig. 9), potentially leading to the selective enrichment of phyllosphere microorganisms.

Conclusion

The objective of our research was to examine the changes in phyllosphere microorganisms throughout the various phases of tea blister blight infection. As the disease progressed, the fungal population exhibited a decrease in both richness and diversity, while no notable differences were detected in the bacterial Sobs, Chao 1, or Shannon indices between healthy and diseased tea leaves. The composition of the bacterial and fungal communities in the phyllosphere of tea plants affected by blister blight disease exhibited notable dissimilarities compared to that of the phyllosphere of unaffected plants. The co-occurrence analysis conducted in this study delved deeper into the primary connecting microorganisms and revealed the occurrence of intricate interactions among them. The biosynthesis of plant metabolic substances was influenced by the occurrence of tea blister blight disease. Certain components, such as catechins and alkanes, exhibited strong correlations with alterations in the microbial community, suggesting their potential for mitigating fungal infection in tea plants. Enhancing the understanding of the fluctuating dynamics of phyllosphere microbial communities during various stages of disease progression will facilitate the cultivation of biocontrol microorganisms, curtail reliance on chemical interventions, and yield superior tea products.

Declarations

Acknowledgements

We thank the Guangzhou Genedenovo Biotechnology Co., Ltd. for sequencing the sample. We thank the Instrument-sharing Platform of the College of Horticulture of Northwestern Agricultural and Forestry University for instrument sharing.

Author Contributions

SL: Conceptualization, supervision, writing – original draft, funding acquisition. NG: Data curation, Visualization, Project administration. JJ: Data curation, Visualization. QZ: Investigation, Data curation. CG: supervision, writing – review & editing. Y.Y: supervision, writing – review and editing, funding acquisition.

Funding

This research was supported by the National Natural Science Foundation of China (32202549), the China Agriculture Research System of MOF and MARA (CARS-19), the National Key Research and Development Program (2022YFD1602003), and the County Scientific and Technological Innovation Experimental Demonstration Station (2023XYSF-06).

Availability of data and materials

The data presented in this study is deposited in the NCBI database under BioProject accession number PRJNA1050022.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Figures

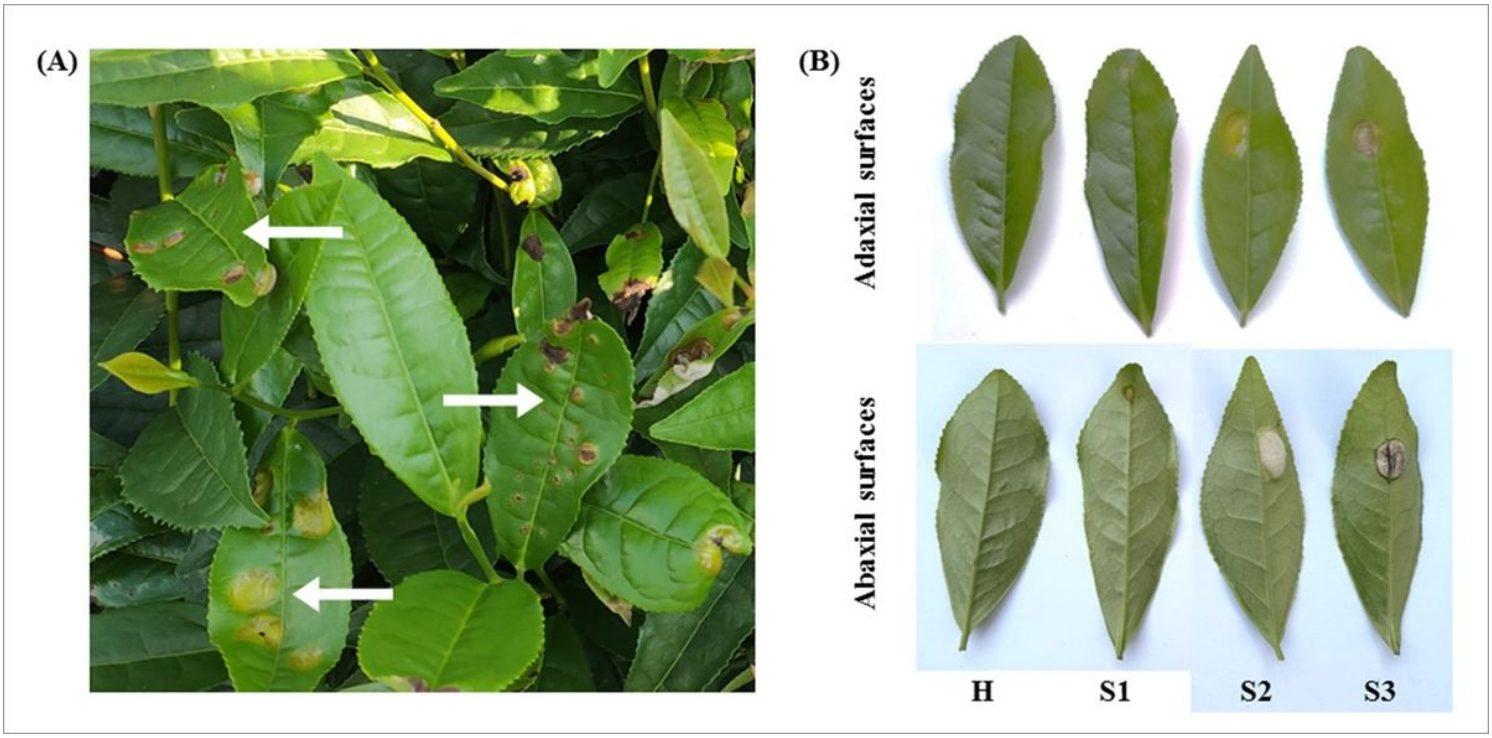

Figure 1

Phenotypes and disease symptoms of leaves infested with tea blister blight. (A) Appearance of tea blister blight disease in tea plantations; (B) Disease phenotypes of tea leaves at different tea blister blight disease stages; H, healthy leaves; S1, tea leaves in the disease early stage; S2, tea leaves in the disease middle stage; S3, tea leaves in the disease late stage

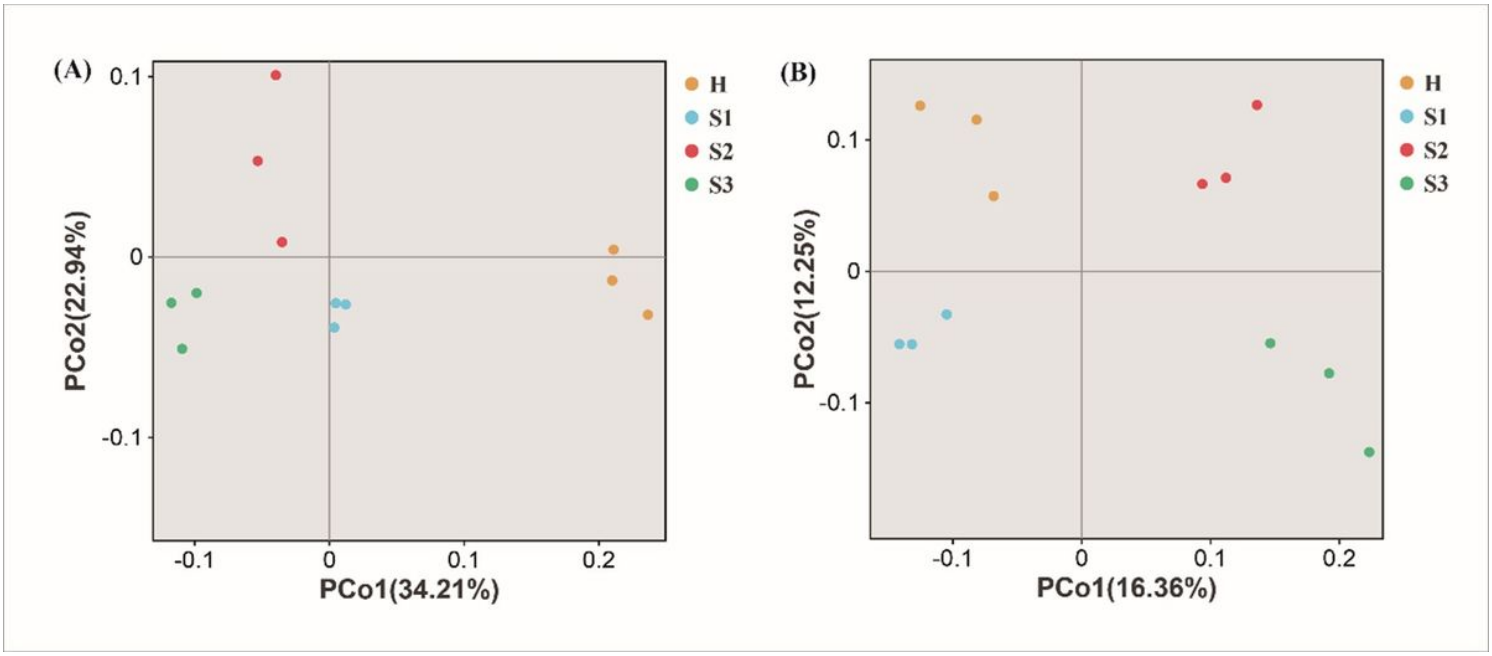

Figure 2

PCoA plots of the microbial community based on unweighted UniFrac distances. (A) Bacterial community; (B) Fungal community; H, healthy leaves; S1, tea leaves in the early disease stage; S2, tea leaves in the middle disease stage; S3, tea leaves in the late disease stage

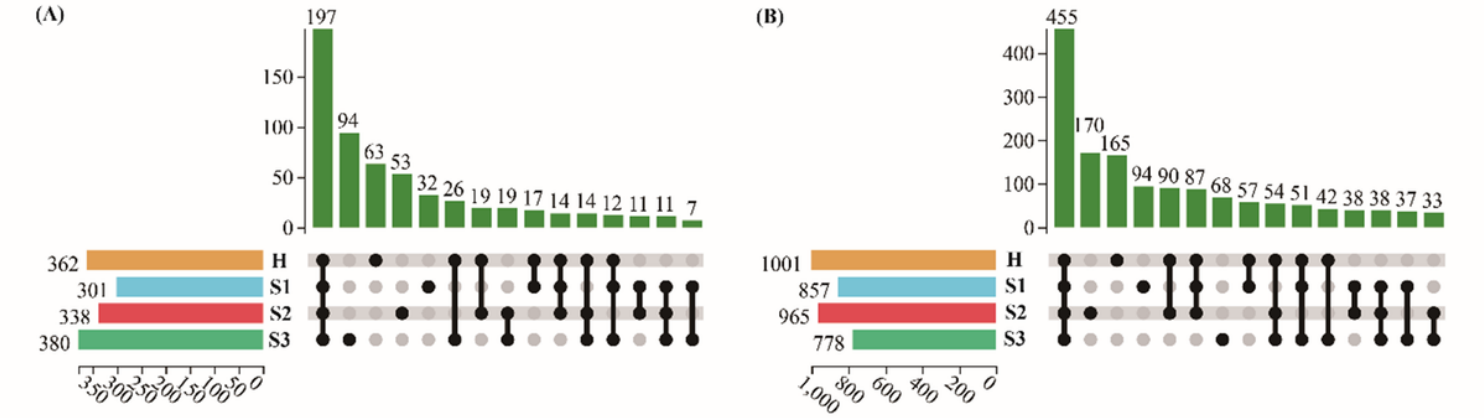

Figure 3

UpSet plot of OTUs of the microbial community at different disease development stages. (A) Bacterial community; (B) Fungal community; H, healthy leaves; S1, tea leaves in the disease early stage; S2, tea leaves in the disease middle stage; S3, tea leaves in the disease late stage; Dark circles indicate samples containing accessions, and connecting bars indicate multiple overlapping samples

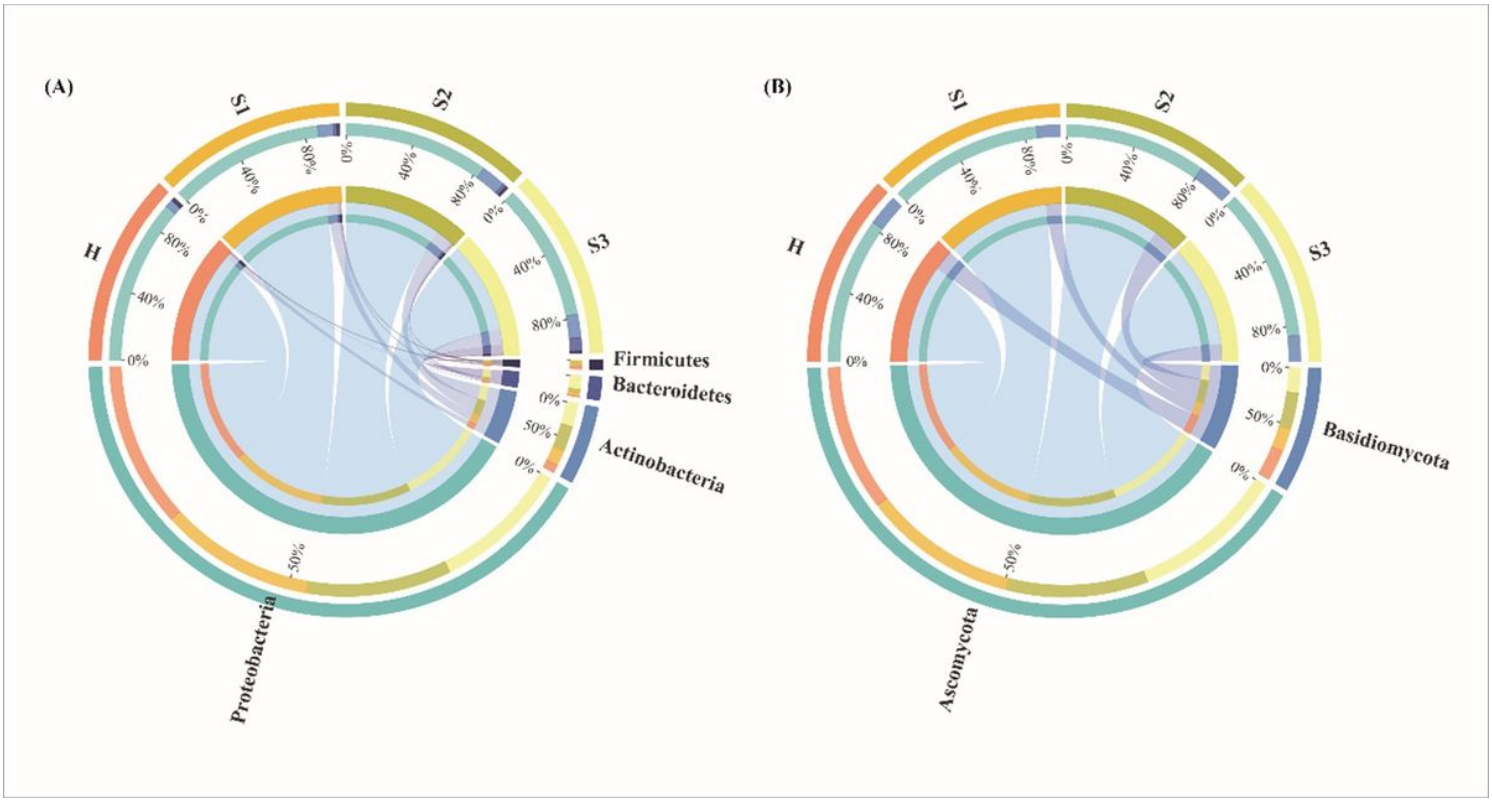

Figure 4

Differences in the microbial community at different disease development stages at the phylum level. (A) Bacterial community; (B) Fungal community; H, healthy leaves; S1, tea leaves in the disease early stage; S2, tea leaves in the disease middle stage; S3, tea leaves in the disease late stage

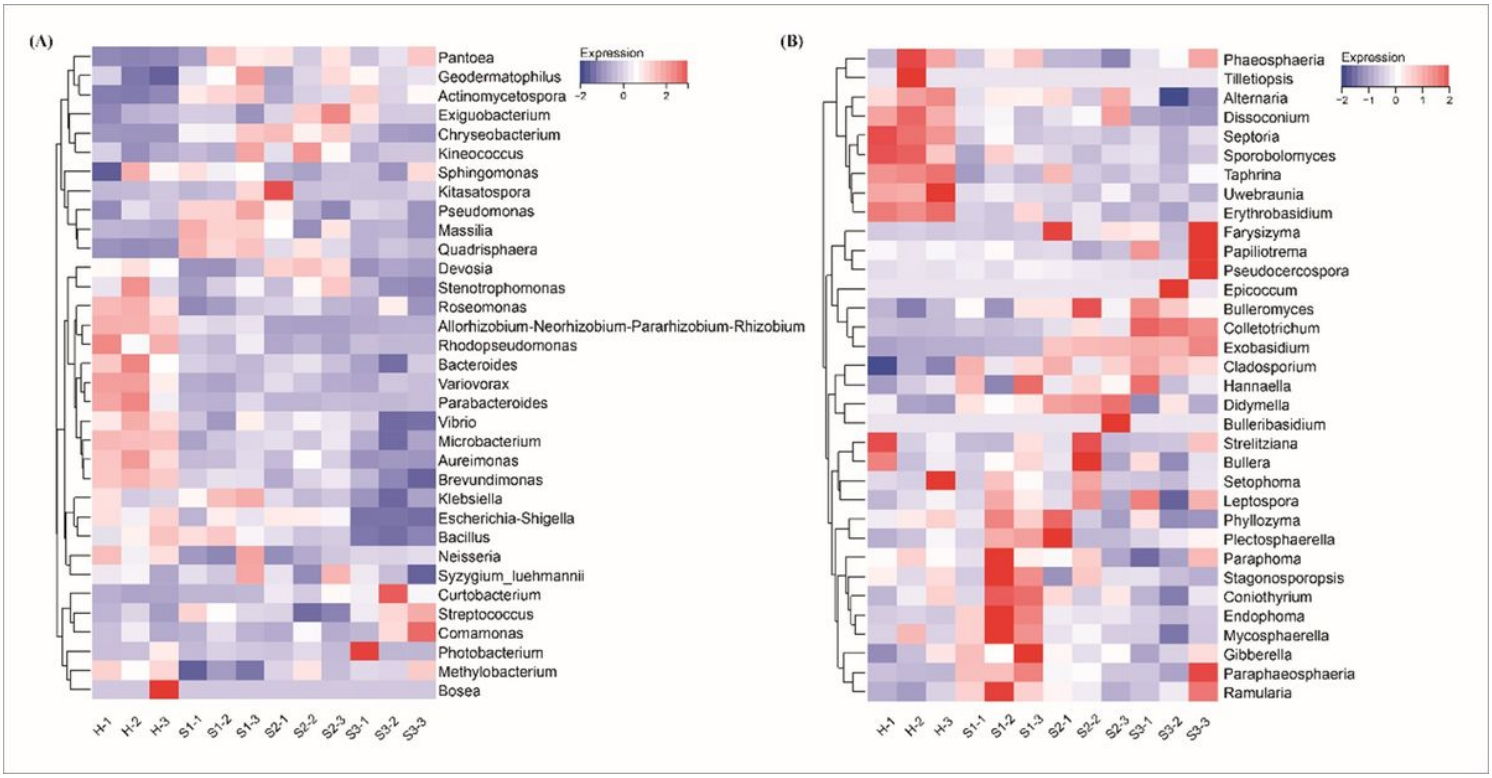

Figure 5

Heatmap of the 35 most abundant genera in the bacterial and fungal communities at different disease development stages. (A) Bacterial community; (B) Fungal community. H, healthy leaves; S1, tea leaves in the early disease stage; S2, tea leaves in the middle disease stage; S3, tea leaves in the late disease stage. The samples are clustered according to the similarities among their constituents and arranged in a horizontal order. Red represents the more abundant genera in the corresponding group, and blue represents the less abundant genera

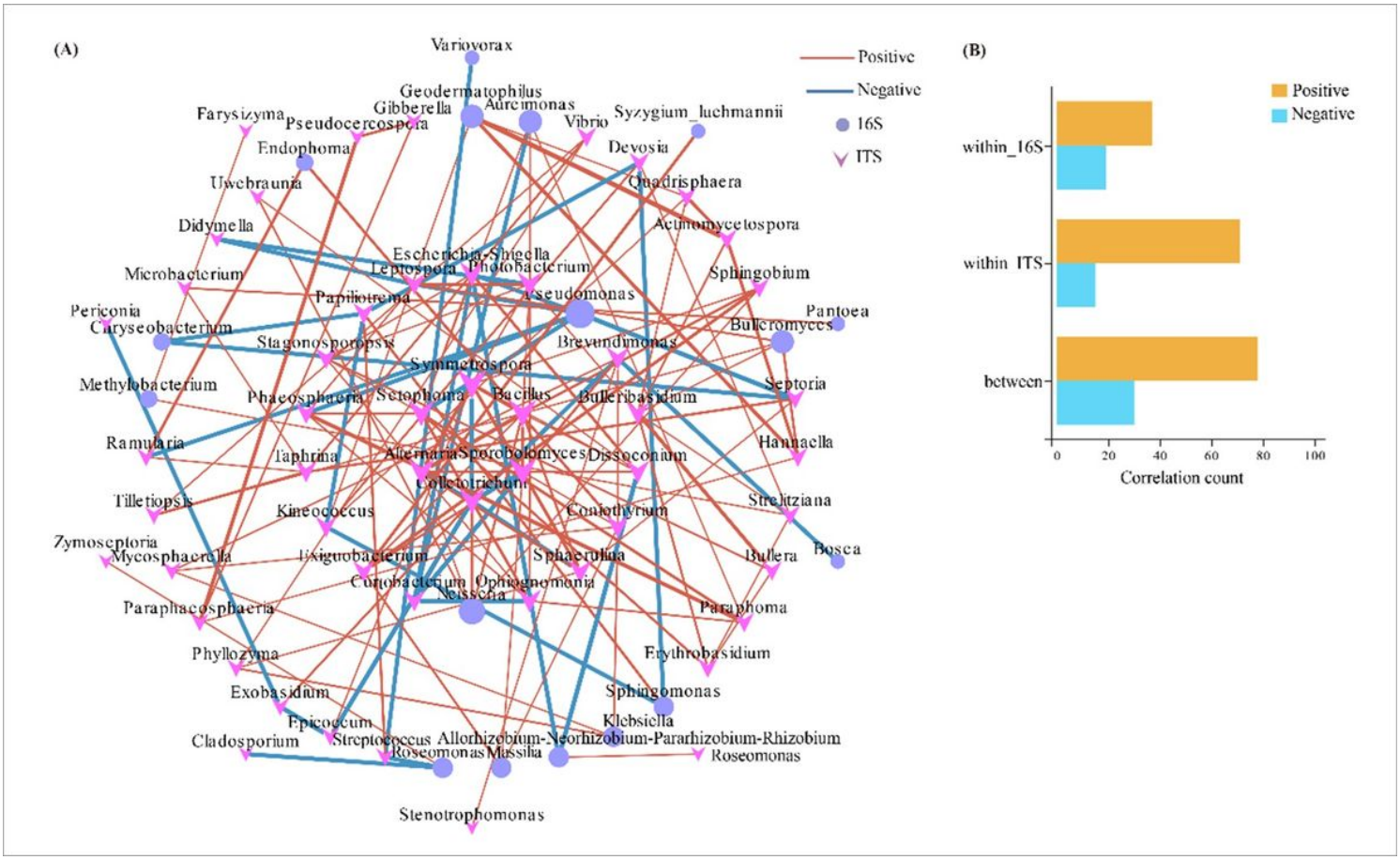

Figure 6

Co-occurrence network among the bacterial and fungal communities. All correlated OTUs were visualized in a network, where OTUs were set as nodes and the correlations were set as edges. OTUs that were identified as indicator OTUs in an indicator analysis and that also appeared in the co-occurrence were shown as larger nodes

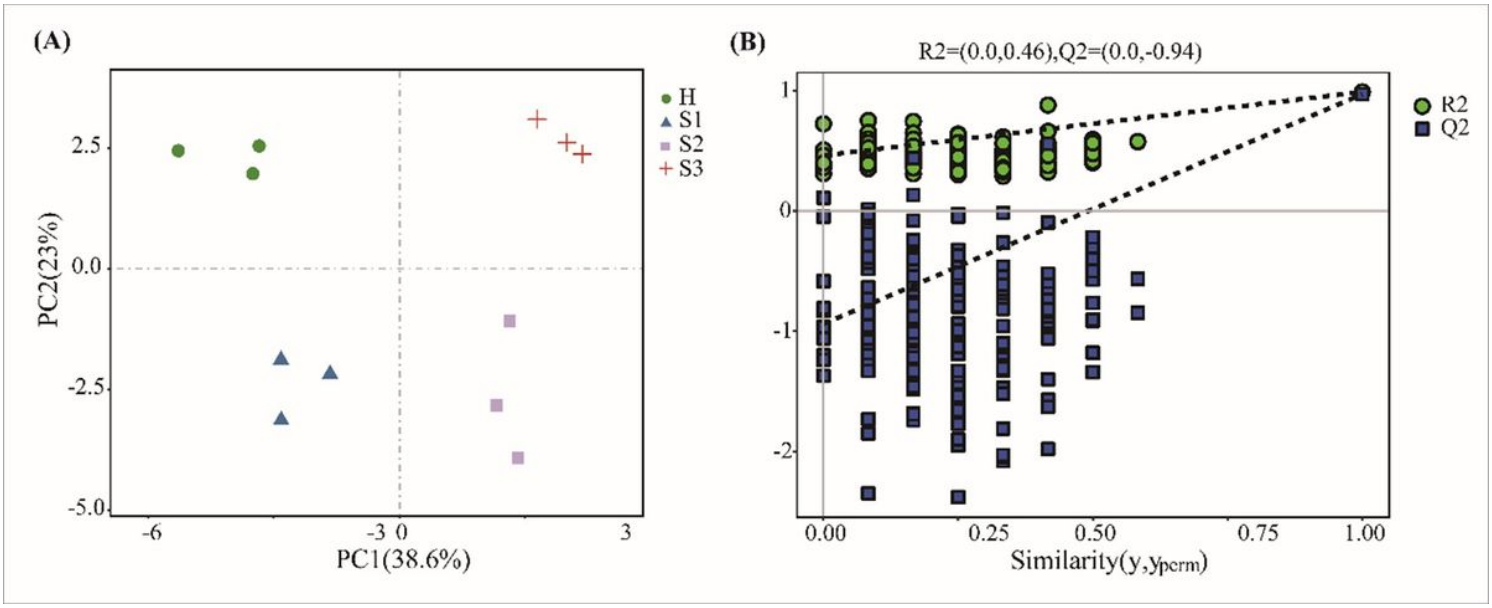

Figure 7

Multivariate statistical analysis for the differences in the metabolites of tea leaves at different disease development stages. (A) PLS-DA score plot; (B) permutation tests for the PLS-DA score plot. H, healthy leaves; S1, tea leaves in the early disease stage; S2, tea leaves in the middle disease stage; S3, tea leaves in the late disease stage

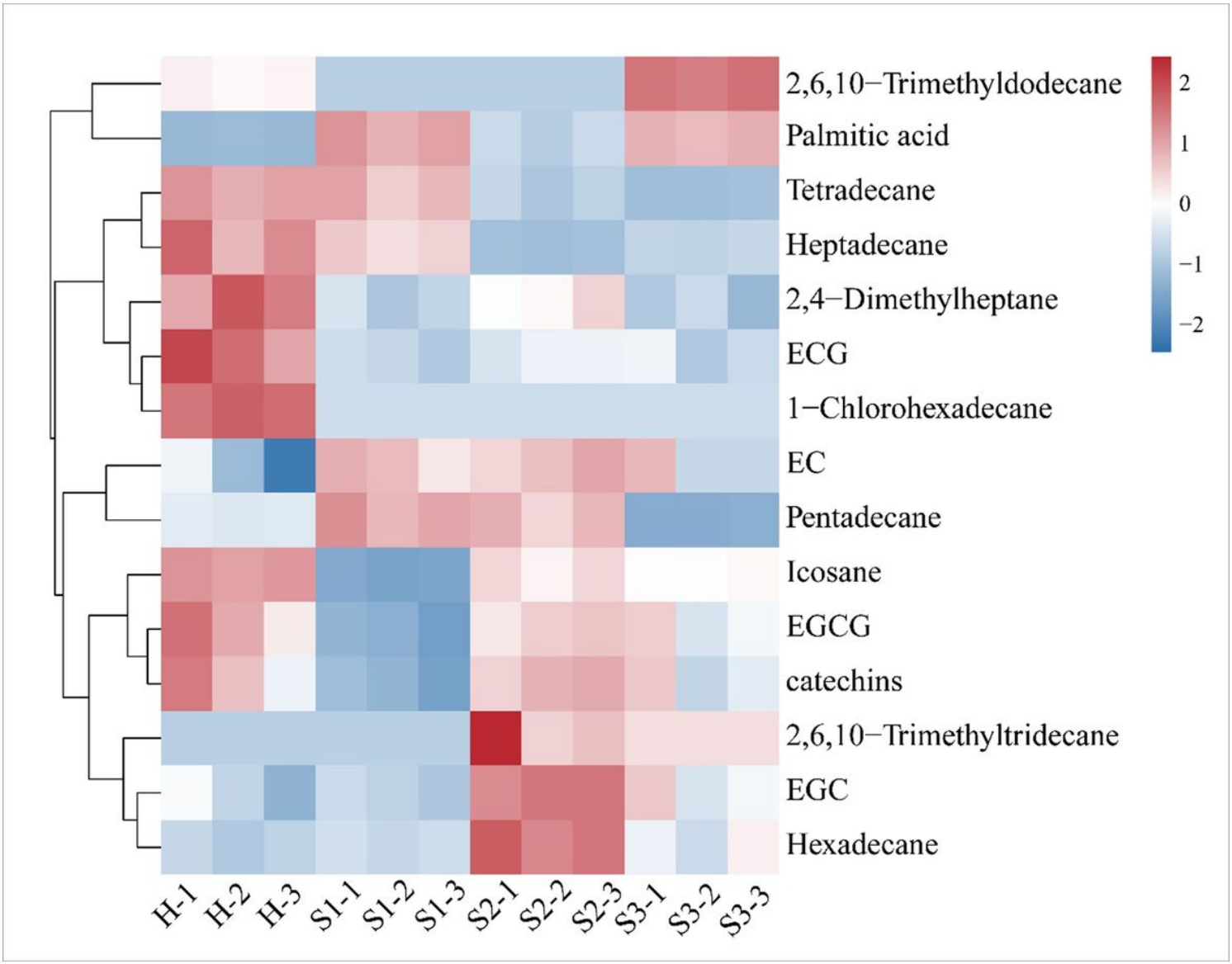

Figure 8

Heatmap analysis of differentially abundant metabolites. H, healthy leaves; S1, tea leaves in the early disease stage; S2, tea leaves in the middle disease stage; S3, tea leaves in the late disease stage. The samples are clustered according to the similarities among their constituents and arranged in a horizontal order. Red represents the more abundant genera in the corresponding group, and blue represents the less abundant genera

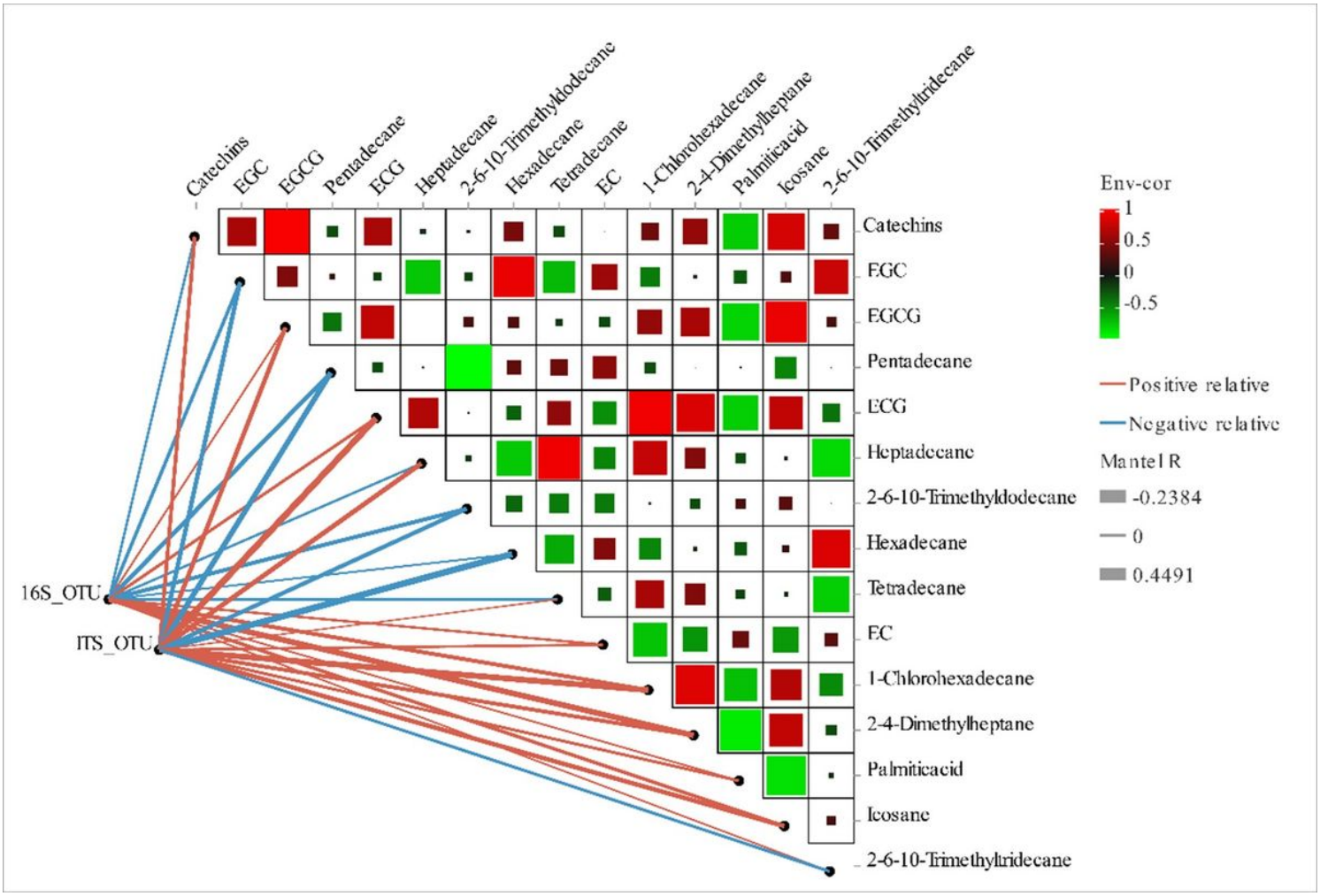

Figure 9

Correlation of microbial communities with main biochemical components determined by the partial Mantel test. Pairwise comparisons of metabolites are shown with a color gradient denoting Spearman's correlation coefficients. OTU composition in relation to metabolites determined by partial Mantel tests. Edge width corresponds to Mantel's R statistic for the corresponding distance correlations

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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