Cuscuta chinensis as a mobile conduit for plant microbiota assembly and functional transfer

Yingying Zhou
Guizhou University
Jiasi Zhong
Guizhou University
Zhima Zhaxi
Guizhou University
Shuangshuang Hou
Guizhou University
Bingnan Ma
Guizhou University
Qingsong Ran
Guizhou University
Xia Liu
Guizhou University
Yanfeng Han
Guizhou University
Chunbo Dong
cbdong@gzu.edu.cn
Guizhou University

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Abstract

To investigate how *Cuscuta chinensis* parasitism affects host microbiomes, we performed 16S rRNA gene and ITS high-throughput sequencing of *C. chinensis* parasitizing *Schefflera heptaphylla*, *Pueraria montana*, and *Humulus scandens*, along with parasitized and unparasitized host stems. Parasitism significantly reshaped host microbial community structure. We identified core differential taxa driving these changes, including *Allorhizobium*, *Massilia*, *Xanthomonas*, *Cladosporium*, among others, which formed significant modular correlation networks with plant pathogen-related functions, thus driving functional shifts in host microbiomes. *C. chinensis* and parasitized stems were enriched in plant pathogen, nitrogen fixation, and wood saprotroph functions, whereas unparasitized stems preferentially enriched saprotrophic functions. Source-tracking analysis revealed active bidirectional microbial exchange between *C. chinensis* and parasitized stems. Although sampled from conspecific individuals, *C. chinensis* microbiomes showed significant compositional and functional divergence across host species, indicating host specificity. These findings provide new insights into parasitic plant–host–microbe tripartite interactions.

Introduction

The genus *Cuscuta* is the only holoparasitic lineage within the family Convolvulaceae, comprising approximately 200 described species. These plants are nearly achlorophyllous and lack true roots and leaves (Nickrent 2020). Taking *Cuscuta chinensis* as a representative species, its vegetative body consists of filiform, twining stems 1–3 mm in diameter, which can infect aerial parts of herbaceous plants and also parasitize woody shrubs and even trees (Hartenstein et al. 2023). In China, *C. chinensis* is a well-documented traditional Chinese medicine, first recorded in *Shennong Bencao Jing*, with therapeutic effects including tonifying the liver and kidney, consolidating essence, reducing urination, improving eyesight, and preventing miscarriage (Huang et al. 2023). However, due to its obligate holoparasitic lifestyle, *Cuscuta* can infest multiple host species across diverse plant families, causing substantial yield losses and economic damage in agricultural production (Jhu and Sinha 2022).

Like other parasitic plants, *Cuscuta* develops hemispherical haustoria, which are specialized organs for host attachment, tissue penetration, vascular connection, and interorganismal material exchange. Haustorium formation is regulated by host-derived haustorium-inducing factors. Upon penetrating host stems or roots, haustoria establish direct connections with the host vascular system, enabling bidirectional transport of water, nutrients, proteins, nucleotides, pathogens, and even retrotransposons (Yoshida et al. 2016). In recent years, the mechanisms underlying haustorium-mediated interactions have been explored at multiple levels. At the genetic level, signal exchange and horizontal gene transfer occur between *Cuscuta campestris* and its hosts (Yang et al. 2019). At the molecular level, microRNAs from *Cuscuta* can target host mRNAs, while host RNAs can be translated into proteins within *Cuscuta* tissues (Park et al. 2021). Regarding cellular structure, the cell wall plays critical roles in the four key stages of *Cuscuta* parasitism: adhesion, host recognition, penetration, and vascular connection (Takagawa and Yokoyama 2025). For physical factors, light quality, intensity, and photoperiod regulate

the parasitic process (Bawin and Krause 2024), and tactile stimuli under far-red light can induce haustorium development (Tada et al. 1996). In terms of chemical signals, Runyon et al. used a controlled experimental system to demonstrate that parasitic plants respond to host volatiles and identified several monoterpenes as active components in host attraction (Runyon et al. 2006).

Microorganisms also play indispensable roles in the interaction between *Cuscuta* and its hosts. Specific microbial taxa can exert distinctive effects on both *Cuscuta* and host plants. For instance, combined application of silicate-solubilizing bacteria and potassium silicate promotes *C. campestris* germination while alleviating oxidative stress in host plants caused by parasitism (Aliverdi et al. 2025). *Alternaria alternata* strains Cbp6 and Cbp7 significantly inhibit *Cuscuta japonica* growth without obvious adverse effects on host plants, showing promising potential for biological control (Liu et al. 2024). Furthermore, *C. campestris* has been employed as a vector plant for microbial transmission to investigate pathogen–host interactions (Li et al. 2021).

Although progress has been made in studies focusing on individual microbial strains, research from the perspective of the entire microbial community remains limited. Plant endophytes, as key components of the plant microbiome, exert multifunctional roles in nutrient acquisition, nitrogen fixation, phytohormone synthesis, and stress tolerance (Papik et al. 2020), and can substantially influence the quality and yield of medicinal plants (Jia et al. 2016). Currently, several critical research gaps exist in this field. First, although studies have reported shared fungal endophytes between parasitic plants and their hosts, the composition and characteristics of the shared microbiota among *Cuscuta* individuals parasitizing different host plants remain unclear (Suryanarayanan et al. 2000). Second, patterns of community diversity change are controversial. For example, in the *Cuscuta salina–Ambrosia psilostachya* system, endophytic fungal and mycoviral diversity was higher than that in the host community; however, whether this pattern is universal or whether host diversity may decrease under certain conditions remains unresolved (Feldman et al. 2012). Third, the impacts of *Cuscuta* parasitism on microbial communities vary across host backgrounds, yet existing studies have mostly focused on soil and rhizosphere habitats, with systematic comparisons of plant endophytic communities still lacking (Brunel et al. 2020). Additionally, one study found that *Cuscuta epithymum* and its host *Ziziphus lotus* shared highly similar endophytic communities, but whether this phenomenon is widespread across other host–parasite systems requires further verification (Radouane et al. 2024).

Against this background, this study collected stem samples of *C. chinensis* parasitizing three distinct host plants—*Schefflera heptaphylla*, *Pueraria montana*, and *Humulus scandens*—along with parasitized and unparasitized stems of the three host species. Using high-throughput sequencing technology, we systematically analyzed the composition and diversity dynamics of bacterial and fungal communities, explored the impacts of *C. chinensis* parasitism on different hosts, and aimed to reveal microbial communication between *C. chinensis* and its hosts.

Materials and Methods

Sample collection and processing

In September 2025, stem samples of *C. chinensis* parasitizing *S. heptaphylla*, *P. montana*, and *H. scandens*, as well as corresponding parasitized and unparasitized host stems, were collected in Guiyang, Guizhou Province, China. All samples were collected under aseptic conditions, snap-frozen in liquid nitrogen, and stored at − 80°C until processing. A total of nine experimental groups were established: *C. chinensis* parasitizing *S. heptaphylla* (EZCT), *S. heptaphylla* stems parasitized by *C. chinensis* (EZCJ), unparasitized *S. heptaphylla* stems (EZCW), and analogous groups for *P. montana* (PLGT, PLGJ, PLGW) and *H. scandens* (LCHT, LCHJ, LCHW), with four biological replicates per group. For analytical purposes, samples were divided by host system into the *S. heptaphylla* system (EZC: EZCT, EZCJ, EZCW), the *P. montana* system (PLG: PLGT, PLGJ, PLGW), and the *H. scandens* system (LCH: LCHT, LCHJ, LCHW). Furthermore, all *C. chinensis* samples were pooled into group T (EZCT, PLGT, LCHT), parasitized stems into group J (EZCJ, PLGJ, LCHJ), and unparasitized stems into group W (EZCW, PLGW, LCHW).

DNA extraction, PCR amplification, and high-throughput sequencing

Bacterial and fungal genomic DNAs were extracted from *C. chinensis* stems, parasitized host stems, and unparasitized host stems using bacterial and fungal genomic DNA extraction kits (Beijing Biomarker Biotechnology Co., Ltd.), respectively. The bacterial 16S rRNA gene was amplified using primers 27F (5'-CADACTCCTACGGGAGGC-3') and 1492R (5'-ATCCTGTTTGMTMCCCVCRC-3'). The fungal ITS region was amplified using primers ITS1 (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TGCGTTCTTCATCGATGC-3'). The PCR reaction was performed in a 25 μL volume containing 12.5 μL of 2× Taq PCR Master Mix, 1 μL of each forward and reverse primer, 2 μL of DNA template, and sterile ddH_2O to reach the final volume. For bacterial amplification, the thermal cycling conditions were: pre-denaturation at 95°C for 5 min; 35 cycles of denaturation at 92°C for 30 s, annealing at 50°C for 30 s, and extension at 68°C for 2 min; followed by a final extension at 78°C for 8 min. For fungal amplification, the conditions were: pre-denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min; followed by a final extension at 72°C for 10 min. After validation via 1% agarose gel electrophoresis, qualified PCR products were submitted to Beijing Biomarker Biotechnology Co., Ltd. for paired-end sequencing on the Illumina NovaSeq 6000 platform.

Sequencing data processing

Raw sequencing data were processed using QIIME2 2020.6. High-quality sequences were denoised using the DADA2 algorithm to generate amplicon sequence variants (ASVs), with a feature filtering threshold of minimum sequence abundance ≥ 2. Taxonomic annotation of bacterial 16S rRNA gene sequences was performed against the SILVA database (https://www.arb-silva.de/, version 138) using a combined BLAST and Bayesian classifier approach. For fungal ITS sequences, taxonomic annotation

was conducted against the UNITE database (https://unite.ut.ee/, version 10.0) using the same combined method.

Data analysis

Alpha diversity analysis. Chao1 index and Simpson index, were calculated for each sample using Mothur v1.30.1 (http://www.mothur.org/wiki/Schloss_SOPAlpha_diversity).

Beta diversity analysis. Principal coordinate analysis (PCoA) was performed based on Bray–Curtis distances, and PERMANOVA (Adonis) test was used to assess the significance of differences in community composition among groups.

Differential taxa screening. Linear discriminant analysis effect size (LEfSe) was applied to identify differentially abundant microbial taxa between parasitism-associated groups (T + J) and healthy group W within each host system. Based on these results, differential taxa among different sample types within each host system were further identified. The thresholds were set as linear discriminant analysis (LDA) score > 3.0 and $p < 0.05$.

Genus–function association analysis. Based on the relative abundances of core differential genera and the abundances of key functional guilds across samples, Spearman correlation coefficients were calculated using the psych package in R ($|r| > 0.4$, $p < 0.05$). The pheatmap package was used to visualize correlation patterns between core genera and key functions in heatmaps. Meanwhile, interaction networks were constructed from significant genus–function pairs using the igraph package and visualized with Gephi software (v0.10) to analyze topological properties, including node number, edge number, average degree, and modularity coefficient.

Functional annotation. Bacterial community functions were annotated using the FAPROTAX database (http://www.ehbio.com/ImageGP/index.php/Home/Index/FAPROTAX.html) to predict potential roles in biogeochemical cycling (Cydzik-Kwiatkowska and Zielińska 2016). Fungal community functions were annotated using the FUNGuild database (http://www.stbates.org/funguild/), with confidence levels restricted to “probable” and “highly probable” (Nguyen et al. 2016).

Microbial source-tracking analysis. Microbial source tracking was conducted using SourceTracker software based on a Bayesian algorithm. Unparasitized host stems and parasitized host stems were set as potential source pools to estimate the contribution of each source to the endophytic microbiota of *C. chinensis*. Subsequently, unparasitized host stems and *C. chinensis* were set as potential source pools to quantify source contributions to the endophytic microbiota of parasitized host stems. The analysis was run with 10,000 iterations, and the average contribution rate of each source was computed.

Results

Effects of parasitism on host microbial diversity and community restructuring

Wilcoxon rank-sum tests were used to compare alpha diversity between parasitized stems (J) and unparasitized stems (W) within each host system (Fig. 1a,b). In the *S. heptaphylla* system, parasitism significantly reduced both bacterial and fungal Simpson indices ($p < 0.05$), whereas the Chao1 index showed no significant change. In the *P. montana* system, parasitism only significantly increased the fungal Chao1 index, with no significant differences in bacterial or fungal Simpson indices or bacterial Chao1 index. In the *H. scandens* system, parasitism exerted no significant effects on bacterial or fungal Chao1 and Simpson indices. Overall, *C. chinensis* parasitism had limited impacts on host alpha diversity.

Further PCoA based on Bray–Curtis distances and PERMANOVA (Adonis) tests revealed clear separation of microbial communities among *C. chinensis* (T), parasitized stems (J), and unparasitized stems (W) across all three host systems (Fig. 1c–e). Specifically, bacterial and fungal communities differed significantly among the three groups in the *S. heptaphylla* system; similar significant separations were observed in the *P. montana* system. In the *H. scandens* system, bacterial communities were distinctly separated, and although fungal communities differed significantly overall, sample points of LCHT and LCHJ were relatively close. Notably, the UPGMA cluster analysis showed consistent separation patterns: in all three systems, *C. chinensis* and parasitized stems first clustered together and then grouped with unparasitized stems (Fig. 1f,g). These results indicate that parasitism restructured host microbial community structure and rendered the overall communities of *C. chinensis* and parasitized stems more similar. PCoA plots further supported this conclusion: along PCoA1, *C. chinensis* (T) and parasitized stems (J) clustered in the same direction, while unparasitized stems (W) occupied the opposite direction. Along PCoA2, *C. chinensis* (T) and parasitized stems (J) were generally distributed in distinct directions, except for fungal communities in the *H. scandens* system where T and J were closely clustered.

Core differential microbiota driving community restructuring

To identify microbial taxa responsible for shifts in host microbial community structure, LEfSe analysis was performed to screen parasitism-associated differential microbes. *C. chinensis* (T) and parasitized stems (J) in each system were combined into a “parasitism-associated group” (T+J) and compared with unparasitized stems (W) ($LDA > 3.0$, $p < 0.05$) to identify genera consistently enriched in T+J across all three host systems. Results showed (Fig. 2; Online Resource 1, Table S1) that among bacteria, *Allorhizobium*, *Massilia*, *Xanthomonas*, and *Polaromonas* were significantly enriched in T+J across all three systems; among fungi, *Cladosporium*, *Fusarium*, *Dioszegia*, and *Leptospora* were also significantly enriched in T+J, whereas only *Microdochium* was significantly enriched in W. These cross-system universally enriched genera constituted the core differential microbiota of the parasitic interaction, suggesting their specialized functions in parasitism adaptation.

To further characterize the distribution patterns of these core differential taxa in *C. chinensis* and parasitized stems, three-group comparisons (T vs J vs W) were conducted via LEfSe to identify characteristic biomarkers for each group (Fig. 2; Online Resource 1, Table S2). Genera specifically enriched in *C. chinensis* (T) included *Massilia* and *Fusarium*; those uniquely enriched in parasitized stems

(J) included *Xanthomonas*, *Polaromonas*, *Cercospora*, and *Dioszegia*; the genus specifically enriched in unparasitized stems (W) was *Microdochium*; *Allorhizobium*, *Cladosporium*, and *Leptospora* were significantly enriched in both T and J across all three systems.

In addition, each host system harbored unique biomarkers (Fig. 2; Online Resource 1, Table S2). For example, in the *S. heptaphylla* system, *C. chinensis* -specific biomarkers included *Pseudomonas* and *Colletotrichum*; parasitized stem-specific biomarkers included *Sphingomonas*; and unparasitized stem-specific biomarkers included *Halomonas*. In the *P. montana* system, *C. chinensis* -specific biomarkers included *Stenotrophomonas*, while *Candidatus_Phytoplasma* was exclusively enriched in parasitized *P. montana* stems. In the *H. scandens* system, unparasitized stem-specific biomarkers included *Methylobacterium-Methylorubrum* and *Pseudoascochyta*.

Collectively, parasitism induced a conserved suite of cross-system core differential microbiota, while host identity conferred system-specific biomarkers, reflecting host-mediated selective shaping of microbial

C. chinensis parasitism remodels host microbial functions

To explore functional impacts of parasitism, bacterial and fungal community functions were predicted using FAPROTAX and FUNGuild databases, and functional differences were compared among *C. chinensis* (T), parasitized stems (J), and unparasitized stems (W) (Fig. 3a,b; Online Resource 1, Table S3). Parasitized groups (T and J) were significantly enriched in functions related to plant pathogens, nitrogen fixation, and wood saprotrophy, whereas unparasitized stems (W) were preferentially enriched in undefined saprotrophic functions. This functional reorganization pattern was highly consistent across the three host systems. Specifically, bacterial functions including plant pathogen and nitrogen fixation were significantly higher in T and J than in W, while fermentation and aromatic compound degradation were more abundant in W. For fungal functions, plant pathogen and wood saprotroph were significantly enriched in T and J, whereas undefined saprotroph was more prevalent in W.

Spearman correlation analysis revealed significant and well-structured associations between core differential microbiota and key functions (Fig. 3c; Online Resource 1, Table S4). For nitrogen fixation, *Allorhizobium*, *Massilia*, *Leptospora*, *Polaromonas*, *Dioszegia*, and *Cladosporium* showed significant positive correlations, all of which were enriched in parasitized groups (T, J, or T+J). In contrast, *Microdochium*, a genus specific to healthy stems, exhibited a highly significant negative correlation with nitrogen fixation. For pathogenic functions, bacterial plant pathogens were significantly positively correlated with *Allorhizobium*, *Massilia*, and *Leptospora*; fungal plant pathogens were positively correlated with *Cladosporium*, *Fusarium*, and *Cercospora*; and animal pathogens were strongly positively correlated with *Fusarium*, *Cladosporium*, *Allorhizobium*, and *Massilia*. Wood saprotroph function also showed strong synergy with parasitism-enriched genera, with *Cladosporium*, *Fusarium*, *Leptospora*, *Allorhizobium*, and *Massilia* all displaying significant positive correlations. Functions enriched in unparasitized stems showed the opposite association pattern: undefined saprotroph, aromatic compound degradation, and fermentation were positively correlated only with *Microdochium*, while

nitrogen fixation, plant pathogen, and animal pathogen functions were negatively correlated exclusively with *Microdochium*.

The genus–function interaction network constructed from these significant correlations visually demonstrated modular relationships between core differential microbiota and key functions (Fig. 3d). In this network, *Allorhizobium* and *Massilia* emerged as highly connected hub nodes, forming dense linkages with nitrogen fixation, plant pathogen, animal pathogen, and wood saprotroph functions, indicating their central roles in the functional network of parasitized groups. *Cladosporium* and *Fusarium* formed a strongly connected module with wood saprotroph, animal pathogen, and fungal plant pathogen functions, with high node degrees, suggesting tight functional synergy between these two fungi in parasitized tissues. Additionally, *Polaromonas* and *Xanthomonas* were mainly linked to plant saprotroph and plant pathogen functions, while *Dioszegia* was negatively correlated with endophyte function and positively correlated with plant saprotroph function.

Source convergence and functional differentiation of the *C. chinensis* microbiome across hosts

To assess host effects on the *C. chinensis* microbiome, alpha diversity was first compared among *C. chinensis* samples from different hosts (EZCT, PLGT, LCHT). Kruskal–Wallis tests showed no significant differences in bacterial Chao1 and Simpson indices among the three groups, whereas the fungal Simpson index differed significantly ($p = 0.0231$), with no significant change in the Chao1 index (Fig. 4a). This indicates no universal significant differences in alpha diversity of *C. chinensis* communities across host species.

SourceTracker analysis was then performed to identify the origins of *C. chinensis* microbiota. With *C. chinensis* as the sink and parasitized/unparasitized stems as potential sources, results showed that the *C. chinensis* microbiome mainly originated from parasitized stems (Fig. 4b; Online Resource 1, Table S5): for bacteria, the mean contribution of EZCJ to EZCT was 78.3%, PLGJ to PLGT was 50.5%, and LCHJ to LCHT was 65.1%; for fungi, the corresponding values were 86.4%, 80.9%, and 61.7%, respectively. Contributions from unparasitized stems were all below 15%. Source tracking with parasitized stems as the sink also confirmed that *C. chinensis* was a major microbial source for parasitized stems (Fig. 4c; Online Resource 1, Table S6): for bacteria, the mean contribution of EZCT to EZCJ was 74.1%, PLGT to PLGJ was 73.5%, and LCHT to LCHJ was 82.3%; for fungi, the corresponding values were 72.9%, 76.9%, and 89.1%, respectively. Source-tracking analysis revealed high similarity between microbial communities of *C. chinensis* and parasitized stems, which served as the primary mutual microbial sources. These findings demonstrate active bidirectional microbial exchange between *C. chinensis* and parasitized stems, with haustoria likely acting as conduits for microbial migration.

Despite being conspecific, *C. chinensis* individuals from different hosts exhibited significant divergence in community composition (Fig. 4e). PCoA analysis showed complete separation of both bacterial (PCoA1 = 47.06%, PCoA2 = 29.44%, $R^2 = 0.762$, $p = 0.001$) and fungal (PCoA1 = 34.69%, PCoA2 = 24.93%, $R^2 = 0.564$, $p = 0.001$) communities among the three *C. chinensis* groups, indicating that host identity is a decisive factor shaping the community composition of the *C. chinensis* microbiome.

Functional prediction further revealed functional differentiation of the *C. chinensis* microbiome across hosts (Fig. 4d; Online Resource 1, Table S3). For bacterial functions, *C. chinensis* parasitizing *S. heptaphylla* (EZCT) was significantly enriched in arsenite oxidation detoxification and dissimilatory arsenite oxidation; *C. chinensis* parasitizing *P. montana* (PLGT) was enriched in animal parasites or symbionts, human-associated, and nitrogen metabolism-related functions; *C. chinensis* parasitizing *H. scandens* (LCHT) was enriched in fermentation and human gut-associated functions. For fungal functions, the three *C. chinensis* groups displayed relatively similar functional profiles, but PLGT showed relative enrichment in fungal parasite and epiphyte functions.

Discussion

This study found that *C. chinensis* parasitism exerted limited effects on host microbial alpha diversity but caused significant separation in beta diversity. This pattern is consistent with the observation of unchanged alpha diversity in the *C. epithymum–Z. lotus* system reported by Radouane et al., but contrasts with their finding of non-significant beta diversity differences (Radouane et al. 2024). In other holoparasitic plant systems, Xi et al. (in *Orobanche cumana*–sunflower) and Fitzpatrick et al. (in *Orobanche hederae–Hedera* spp.) also reported that parasitism did not alter host alpha diversity but significantly modified beta diversity (Xi et al. 2022; Fitzpatrick and Schneider 2020). The significant impact on beta diversity can be explained by the fact that microbial community assembly is generally regulated by both environmental filtering and dispersal (Langenheder et al. 2012). In this study, the parasitic state likely acted as a strong environmental filter, dominating community assembly and leading to pronounced shifts in beta diversity. The pattern of limited alpha diversity effects but significant beta diversity changes has been attributed to selective rearrangement triggered by pathogen invasion in the tobacco bacterial wilt rhizosphere microbiome, where opportunistic taxa proliferate while dominant microbes maintain relative abundance balance (Xiang et al. 2026). This explanation suggests that *C. chinensis* parasitism alters microbial communities mainly through species turnover rather than net gains or losses in overall diversity.

Host identity also strongly influences the *C. chinensis* microbial community. Microbiomes of *C. chinensis* from different hosts were clearly separated in PCoA plots; although their core functional profiles were relatively similar, each group was enriched in distinct specific functions. We propose that *C. chinensis* employs host-specific parasitic strategies, i.e., host specificity. Schneider et al. revealed cryptic host specificity in the holoparasitic plant family Orobanchaceae, providing an evolutionary framework for host-dependent specificity in the *C. chinensis* microbiome (Schneider et al. 2016).

After characterizing bidirectional shifts in *C. chinensis* and host microbial community structure, we identified ten core differential microbial taxa that likely drive community changes. All genera enriched in T + J, T, and J (e.g., *Allorhizobium*, *Fusarium*) were significantly positively correlated with plant pathogen function, and functional prediction showed that plant pathogen function was significantly higher in T and J than in W. In contrast, *Microdochium*, enriched in W, was significantly positively correlated with undefined saprotroph function. These results indicate that *C. chinensis* parasitism shifts host microbial

community functions toward pathogenicity. The well-known pathogen *Xanthomonas*, enriched in J, belongs to Xanthomonadaceae and is also enriched in hosts of the holoparasitic plant *Orobanche cumana* (Xi et al., 2022). This functional shift aligns with the previous view that parasitic plants are potential vectors of plant pathogens (Savov et al. 2024).

A seemingly paradoxical observation is that *C. chinensis* parasitism enriched not only prominent pathogenic microbes such as *Xanthomonas*, *Fusarium*, *Cladosporium*, and *Cercospora*, but also classic plant growth-promoting bacteria (PGPB) including *Massilia* and *Allorhizobium* (An et al. 2020; Ma et al. 2013; Bensch et al. 2012; Groenewald et al. 2013; Holochová et al. 2020; Yang et al. 2025). Why do these pathogens and beneficial bacteria coexist, and why do their functional profiles overlap (e.g., *Allorhizobium* and *Massilia* are positively correlated with animal and plant pathogen functions, while *Cladosporium* is positively correlated with nitrogen fixation)? We propose that haustorium penetration creates wounds in host tissues, providing entry routes for pathogens; in response, hosts sensing pathogenic stress actively recruit beneficial microbes for antagonistic defense. For instance, plants infected with downy mildew actively recruit a bacterial consortium comprising *Microbacterium*, *Stenotrophomonas*, and *Xanthomonas* from the rhizosphere to synergistically enhance disease resistance and promote growth in themselves and their progeny (Berendsen et al. 2018). Similarly, cabbage infected with *Fusarium* recruits beneficial *Pseudomonas* strains that suppress pathogen growth and enhance host resistance (Ping et al. 2024). This mechanism well explains the strong positive correlations between nitrogen fixation and multiple key genera. Nitrogen is widely involved in plant stress responses, modulating disease resistance and susceptibility by regulating plant growth and physiology, pathogen virulence, and rhizosphere conditions (Huber and Watson 1974; Fagard et al. 2014). Thus, beneficial microbes may enhance plant nitrogen status via nitrogen fixation to improve pathogen resistance. Meanwhile, as an obligate holoparasite, *Cuscuta* completely depends on hosts for nitrogen (Yuncker 1932); it may acquire not only inorganic nitrogen but also critical nitrogen-fixing microorganisms from host tissues.

In addition to nitrogen fixation, parasitized groups (T and T + J) were significantly enriched in wood saprotroph function associated with cell wall degradation, with the strongest correlations observed for *Cladosporium* and *Fusarium*. This may be because *C. chinensis* requires haustoria to penetrate host xylem and phloem during invasion. Studies have shown that *C. campestris* secretes pectin methylesterase, polyphenol oxidase, and polygalacturonase during haustorial invasion—enzymes highly similar to those involved in plant pathogen virulence (Nun and Maye 1999; Furuhashi et al. 2011). Thus, *C. chinensis* may cooperate with these pathogenic fungi in wood degradation to facilitate successful haustorial invasion.

The mechanisms by which *C. chinensis* parasitism alters host microbial community structure and function likely include the following. First, direct microbial transmission via haustoria. Our source-tracking analysis confirmed that 50%–86% of the *C. chinensis* microbiome originated from parasitized stems, and 72%–89% of the microbiome in parasitized stems was derived from *C. chinensis*. Yoshida et al. noted that haustoria are core organs for material exchange between parasitic plants and hosts,

allowing migration of diverse biomolecules including pathogens (Yoshida et al. 2016). Furuhashi et al. also verified open xylem and phloem connections between *Cuscuta* and hosts, enabling bidirectional transport of mRNAs and proteins (Furuhashi et al. 2011). Second, parasitism modifies host physiological status and microenvironment, driving shifts in microbial community structure and function. *C. campestris* parasitism significantly alters host stem physiology, including nutrient redistribution and secondary metabolite accumulation (Landi et al. 2022). These changes reshape tissue microenvironments and exert selective pressure on microbiota. Furthermore, *Cuscuta reflexa* secretes cell wall-degrading enzymes (e.g., xyloglucan endotransglucosylase/hydrolases, XTHs) during invasion, causing local microinjuries and cell wall modifications that create new niches for colonization and migration of specific microbes such as pathogens (Olsen and Krause 2017; Vaughn 2003). Third, hosts may actively regulate microbial communities through defense responses. For example, tomato recognizes a *Cuscuta* cell wall protein via the CuRe1 receptor to initiate defense, which may modify root exudation and thereby modulate microbiome assembly and stress tolerance (Hegenauer et al. 2020). Similarly, *Fusarium*-infected cabbage recruits beneficial *Pseudomonas* to suppress pathogens and enhance resistance (Ping et al. 2024).

In summary, this study provides microbial evidence for interorganismal communication between *C. chinensis* and its hosts, proposes mechanistic frameworks for parasitism-induced microbial community remodeling, and offers a new perspective for understanding material and information flow among parasitic plants, hosts, and microorganisms. Future research should focus on integrated multi-omics analyses, synthetic microbial community construction, and further exploration of molecular and chemical mechanisms underlying microbiome impacts on *C. chinensis*, with the goal of developing green biopesticides to control *C. chinensis* growth.

Statements & Declarations

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

Conceptualization: Yingying Zhou, Chunbo Dong; Literature investigation: Yingying Zhou, Jiasi Zhong, Qingsong Ran, Zhima Zhaxi; Formal analysis: Yingying Zhou; Writing – original draft preparation:

Yingying Zhou; Visualization: Yingying Zhou; Writing – review & editing: Yingying Zhou, Chunbo Dong, Jiasi Zhong, Xia Liu, Shuangshuang Hou, Bingnan Ma, Qingsong Ran, Yanfeng Han; Funding acquisition: Chunbo Dong; Project administration: Chunbo Dong; Supervision: Chunbo Dong, Jiasi Zhong.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

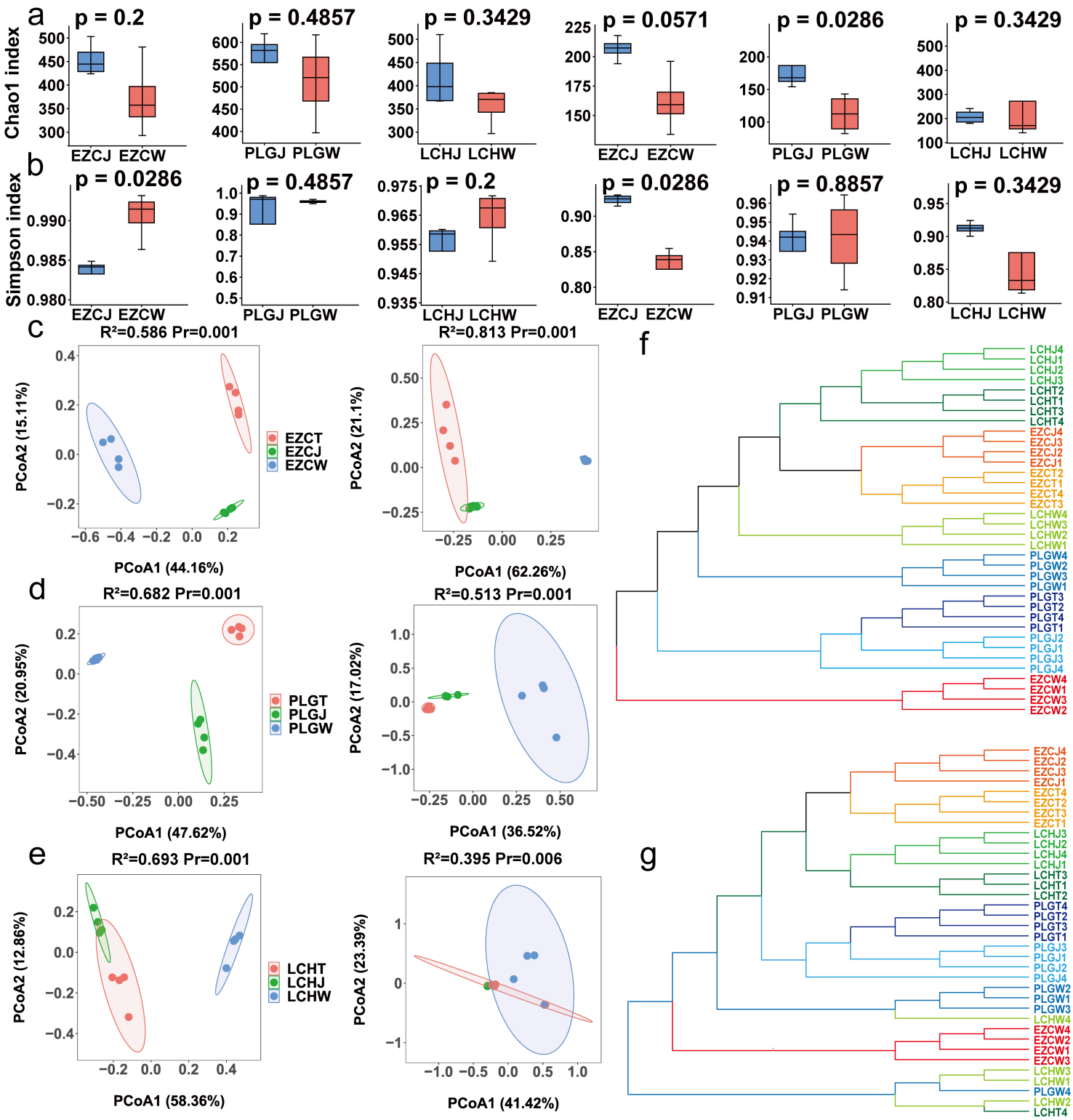

Figure 1

Comparison of microbial diversity across three host plant systems. (**a**) Chao1 index of microbiota in three host systems (three on the left: bacteria; three on the right: fungi). (**b**) Simpson index of microbiota in three host systems (left: bacteria; right: fungi). (**c–e**) PCoA analysis of microbial communities in *S. heptaphylla* (c), *P. montana* (d), and *H. scandens* (e) systems (left: bacteria, right: fungi). (f–g) UPGMA

cluster analysis based on Bray-Curtis distance for bacterial (f) and fungal (g) communities across the three host systems

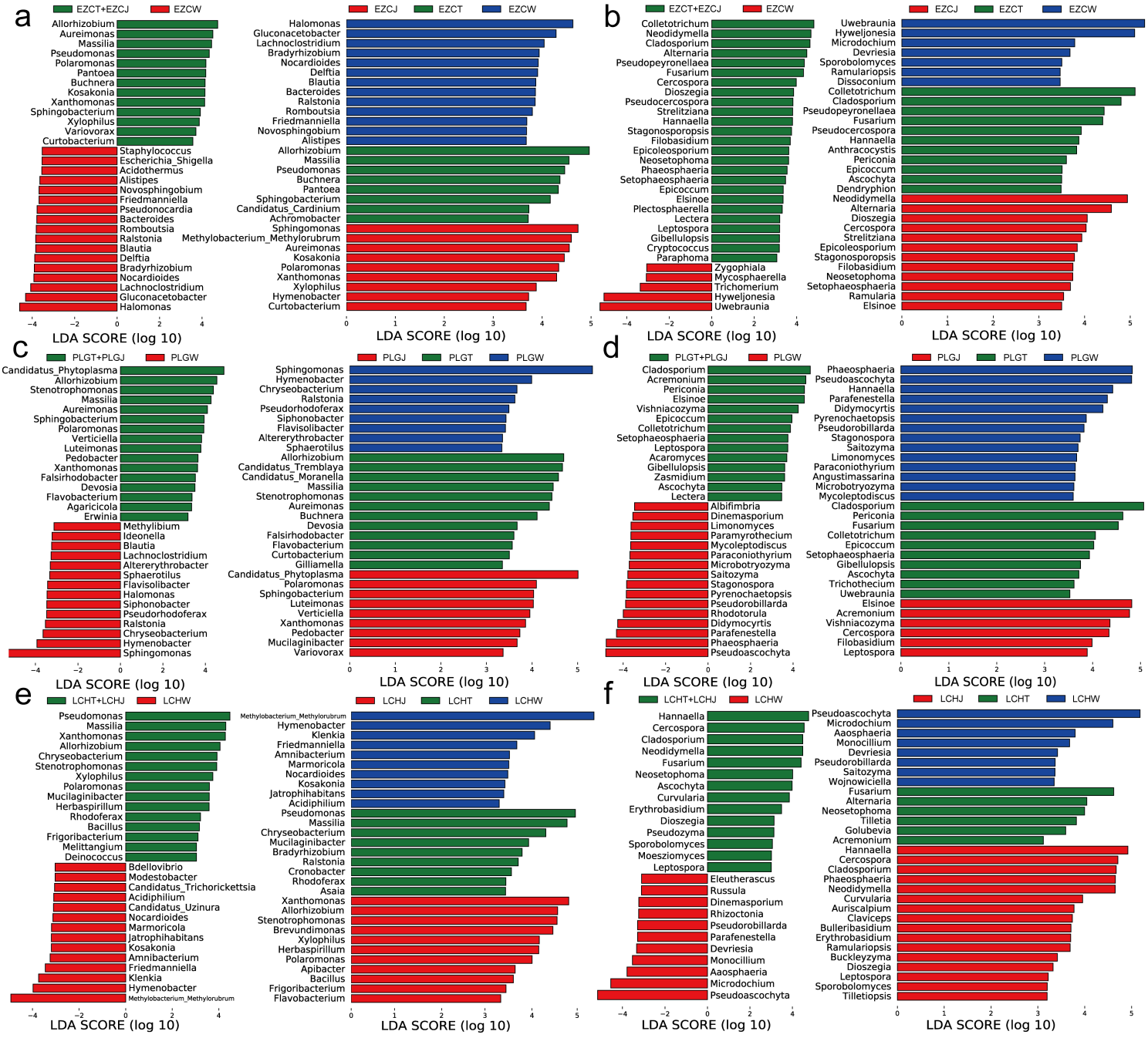

Figure 2

Differential microbial taxa among microbial communities of the three host systems. (**a,b**) LEfSe analysis of bacterial (a) and fungal (b) biomarkers in the *S. heptaphylla* system (LDA > 3.0, $p < 0.05$). (**c,d**) LEfSe analysis of bacterial (c) and fungal (d) biomarkers in the *P. montana* system (LDA > 3.0, $p < 0.05$). (**e,f**) LEfSe analysis of bacterial (e) and fungal (f) biomarkers in the *H. scandens* system (LDA > 3.0, $p < 0.05$)

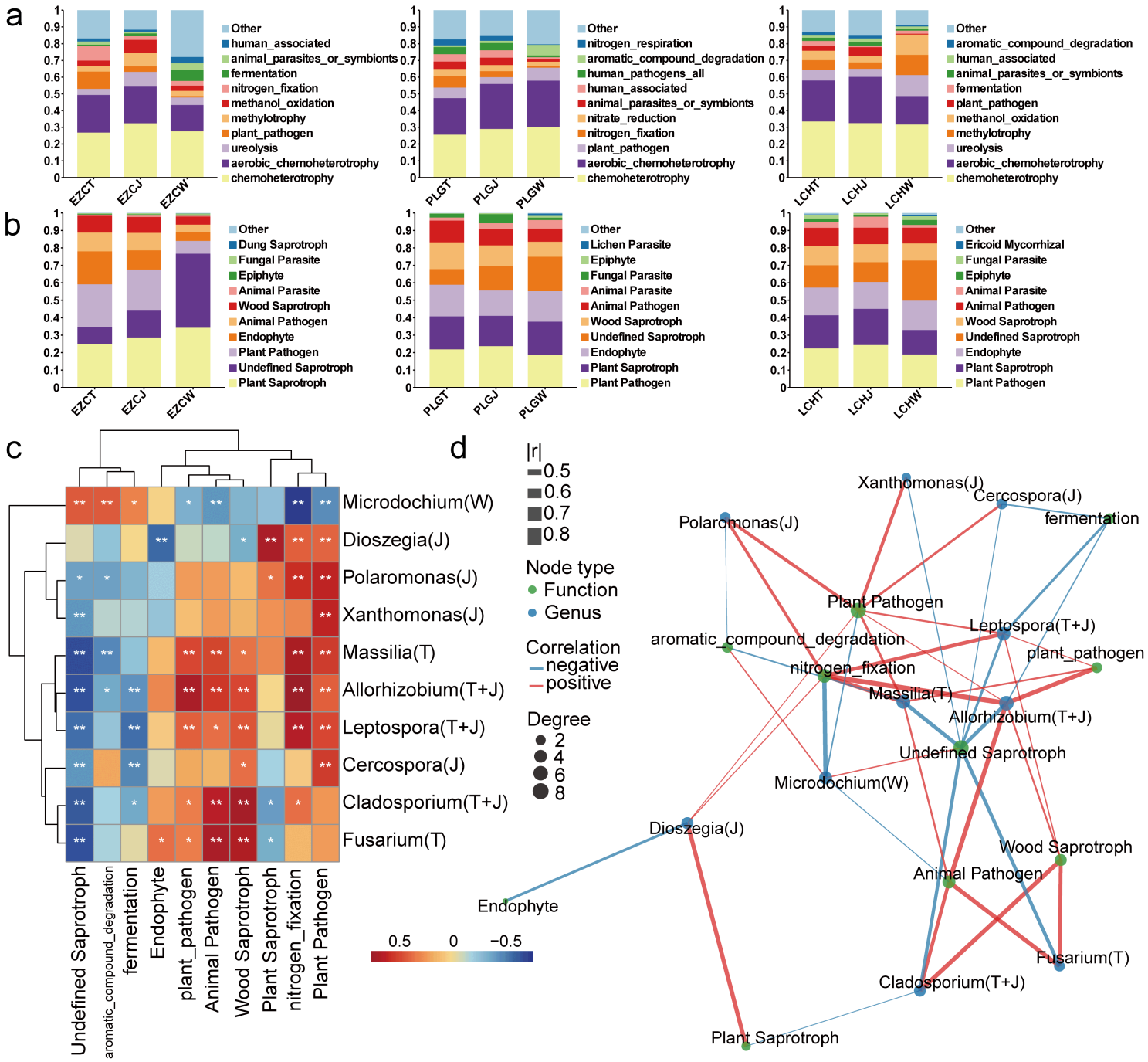

Figure 3

Microbial functional prediction and correlation analysis between core microbiota and core functions across three host systems. (**a**) Functional composition of bacterial communities across the three host systems. (**b**) Functional composition of fungal communities across the three host systems. (**c**) Correlation heatmap of core microbiota and core functions. (**d**) Correlation network of core microbiota and core functions

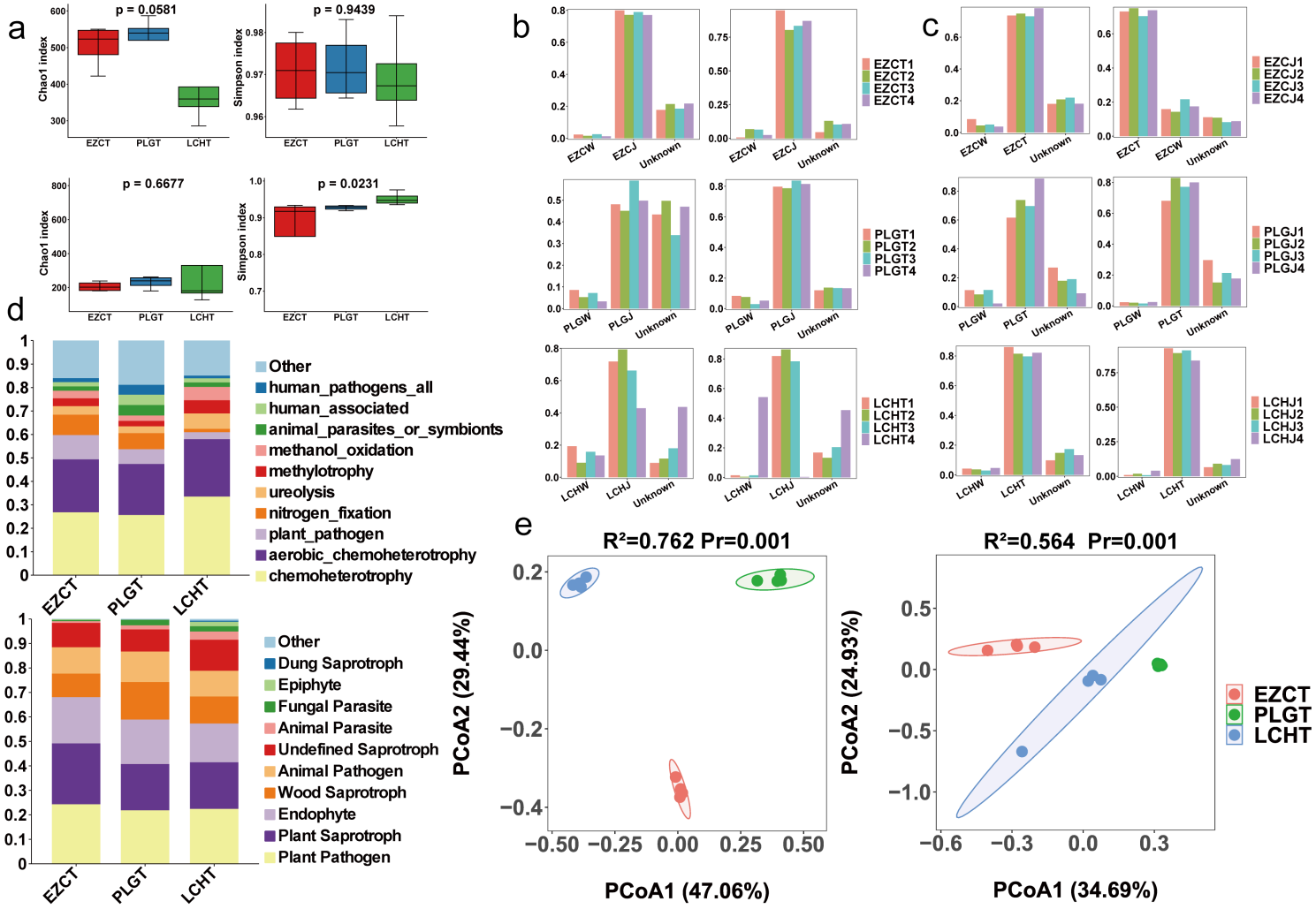

Figure 4

Alpha diversity, source contribution, community structure, and functional differentiation of the *C. chinensis* microbiome parasitizing different host plants. (**a**) Alpha diversity of *C. chinensis*microbiome from different hosts, showing bacterial Chao1 (top left), fungal Chao1 (bottom left), bacterial Simpson (top right), and fungal Simpson (bottom right) indices. (**b**) Source tracking analysis with *C. chinensis* as the sink using bacterial (left) and fungal (right) communities across *S. heptaphylla*, *P. montana*, and *H. scandens*systems. (**c**) Source tracking analysis with parasitized stems as the sink. (**d**) Functional composition of *C. chinensis* -associated bacterial (top) and fungal (bottom) communities. (**e**) PCoA of *C. chinensis* bacterial (left) and fungal (right) communities from different host systems

Supplementary Files

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