

1 **Analysis of the Structure of Tea Plant Phyllosphere Endophytic Microorganisms**

2 **Under the Influence of ZnO NPs**

3 **Screening and Random Forest Analysis of Differential Endophytic** 4 **Microorganisms**

5 To clarify which microbial groups are responsible for the differences in the phyllosphere endophytic
6 microorganisms of tea plants caused by ZnO NPs, we used LefSe analysis to screen for differentially
7 abundant microorganisms. There were 3 important biomarkers affecting the bacterial community
8 structure differences between CKED and T1ED, with CKED dominated by *Oxalobacteraceae*, and
9 T1ED dominated by *Sphingomonadaceae* (Fig.28A). There were 5 important biomarkers affecting
10 the bacterial community structure differences between CKED and T2ED, with CKED dominated
11 by *Oxalobacteraceae*, and T2ED dominated by *Agrobacterium radiobacter*, *Microbacterium*,
12 *Microbacteriaceae*, and *Sphingomonas* (Fig.28B). There were 37 important biomarkers affecting
13 the fungal community structure differences between CKED and T1ED, with CKED dominated by
14 *Lophiostoma*, *Lophiostomataceae*, *Pleosporales*, *Uwebraunia musae*, *Ascomycota*, *Aureobasidium*
15 *pullulans*, *Sarocladium strictum*, *Acremonium alternatum*, *Taphrinaceae sp*, and *Monographella*
16 *cucumerina*, while T1ED was dominated by *Sordariomycetes*, *Hypocreales*, *fungus sp P1S11*, and
17 *Pyrenochaeta* (Fig.28C). There were 39 important biomarkers affecting the fungal community
18 structure differences between CKED and T2ED, with CKED dominated by *Lophiostomataceae*
19 *Lophiostoma* *Ascomycota* *Dothideomycetes* *Uwebraunia musae* *Uwebraunia* *Sarocladium strictum*
20 *Aureobasidium pullulans* *Hypocreales* *family Incertae sedis* *Acremonium alternatum*
21 *Monographella cucumerina* *Sarocladium* *Taphrinaceae sp* and *Microcyclospora*, while T2ED was
22 dominated by *Pyrenochaeta inflorescentiae* *Pyrenochaeta* *Pleosporales family Incertae sedis* *fungus*

23 *sp PIS11 unclassified Fungi unclassified Fungi unclassified Fungi unclassified Fungi unclassified*

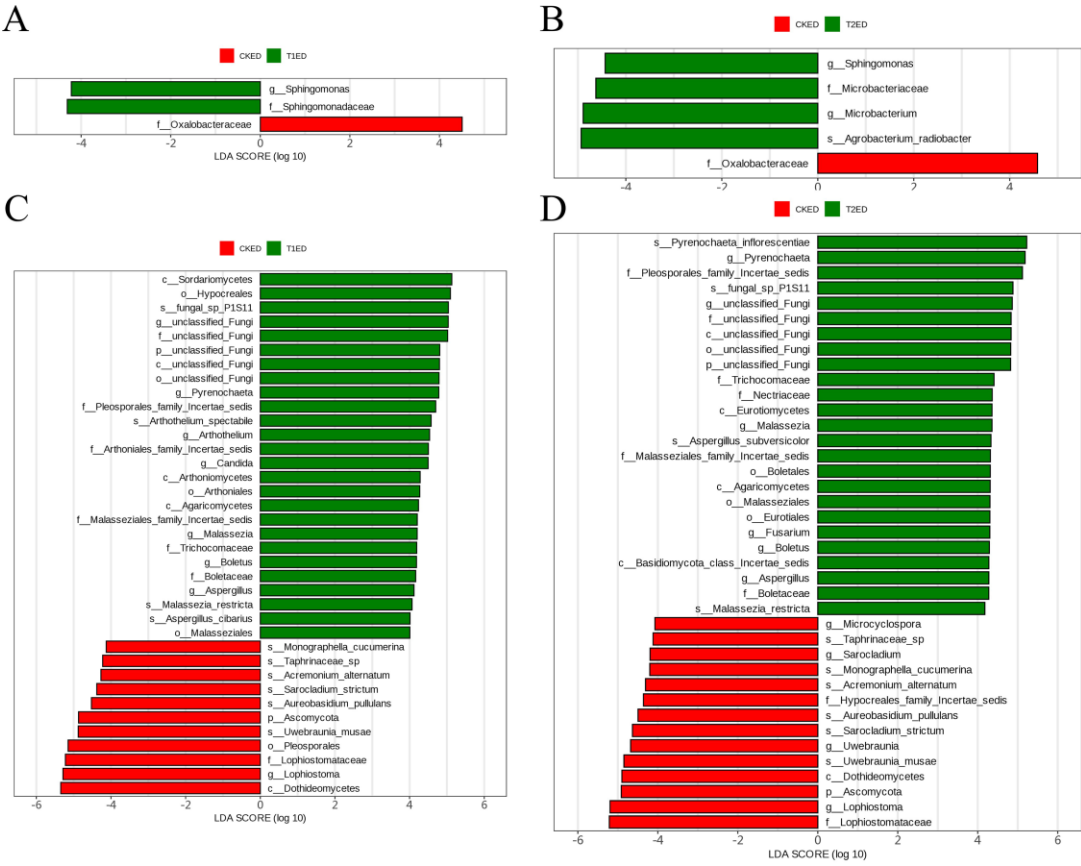
24 *Fungi Trichocomaceae Nectriaceae Eurotiomycetes Malassezia Aspergillus subversicolor*

25 *Malasseziales family Incertae sedis Boletales Agaricomycetes Eurotiales Fusarium Boletus*

26 *Basidiomycota class Incertae sedis Aspergillus Boletaceae and Malassezia restricta* (Fig.28D). The

27 results indicate that ZnO NPs have a significant impact on both phyllosphere endophytic bacteria

28 and fungi in tea plants.



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30 **Fig.28 Significant species with differential bacterial abundance between CKED and T1ED**

31 **under the influence of ZnO NPs (A); significant species with differential bacterial abundance**

32 **between CKED and T2ED (B); significant species with differential fungal abundance between**

33 **CKED and T1ED (C); significant species with differential fungal abundance between CKED**

34 **and T2ED (D). The LDA value distribution bar chart in Fig.28 shows species with an LDA**

Score greater than the set value (default is 4), i.e., biomarkers with statistical differences between groups, and the length of the bar chart represents the impact size of the differential species.

To identify key species of phyllosphere endophytic microorganisms in tea plants under the influence of ZnO NPs, similar to epiphytic microorganisms, we conducted a random forest analysis (Fig.29). *Sphingomonas* and *Colletotrichum* could explain the largest changes in the endophytic bacterial and fungal communities, respectively, at 50mg L⁻¹ ZnO NPs; *Microbacterium* and *Nadsonia* could explain the largest changes in the bacterial and endophytic fungal communities, respectively, at 100mg L⁻¹ ZnO NPs. The AUC was 1 for both, indicating good accuracy and discrimination ability.

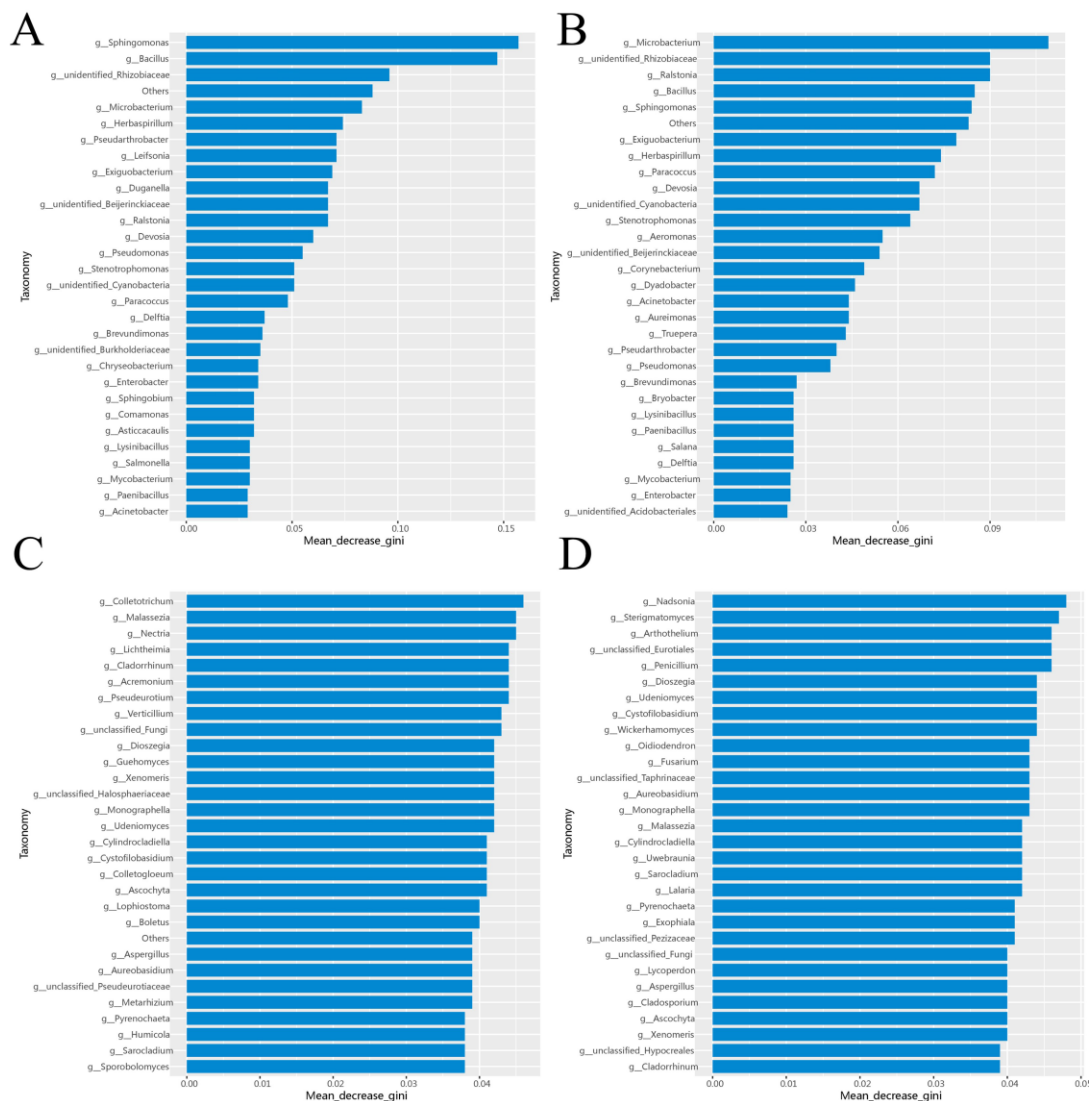


Fig.29 Key species of phyllosphere endophytic microorganisms (at the genus level) under the influence of ZnO NPs. Key endophytic bacteria in tea plants under the influence of T1 (A); key endophytic fungi under the influence of T2 (B); key endophytic bacteria under the influence of T1 (C); key endophytic bacteria under the influence of T2 (D).

Functional Clustering Analysis of Phyllosphere Endophytic Bacteria and Fungi

To analyze the impact of ZnO NPs on the functions of phyllosphere endophytic bacteria in tea plants, similar to epiphytic microorganisms, we used Tax4Fun2 for functional annotation and clustering.

As shown in Fig.30A, the bacterial functions in CKED were mainly enriched in Fatty acid biosynthesis, Biotin metabolism, and Glyoxylate and dicarboxylate metabolism. The bacterial functions in T1ED were primarily clustered in Carbon fixation pathways in prokaryotes, Biofilm formation - *Vibrio cholerae*, and Oxidative phosphorylation. The bacterial functions in T2ED were primarily clustered in Metabolic pathways, Biosynthesis of amino acids, Ribosome, Amino sugar and nucleotide sugar metabolism, Biosynthesis of antibiotics, Purine metabolism, Pyrimidine metabolism, Biosynthesis of secondary metabolites, ABC transporters, Glycolysis/Gluconeogenesis, and Carbon metabolism.

To analyze the impact of ZnO NPs on the functions of phyllosphere endophytic fungi in tea plants, similar to epiphytic microorganisms, we used FunGuild for functional annotation and clustering. As shown in Fig.30B, the fungal functions in CKED were mainly clustered in Plant Pathogen-Undefined Saprotroph, Ectomycorrhizal, Ericoid Mycorrhizal, Undefined Saprotroph, Endophyte-Insect Pathogen, Fungal Parasite-Undefined Saprotroph, Leaf Saprotroph, Animal Pathogen-Endophyte-Epiphyte-Plant Pathogen-Undefined Saprotroph, Fungal Parasite-Litter Saprotroph, Animal Pathogen-Endophyte-Fungal Parasite-Plant Pathogen-Wood Saprotroph, Animal Pathogen-Endophyte-Fungal Parasite-Lichen Parasite-Plant Pathogen-Wood Saprotroph, Animal Pathogen-Endophyte-Epiphyte-Undefined Saprotroph, Plant Pathogen. The fungal functions in T1ED were mainly enriched in Endophyte-Litter Saprotroph-Undefined Saprotroph, Fungal Parasite, Animal Pathogen-Endophyte-Endosymbiont-Epiphyte-Soil Saprotroph-Undefined Saprotroph, Endomycorrhizal-Plant Pathogen-Undefined Saprotroph, Lichenized, Dung Saprotroph-Ectomycorrhizal-Litter Saprotroph-Undefined Saprotroph, Plant Pathogen-Wood Saprotroph,

Endophyte-Plant Pathogen. The fungal functions in T2ED were mainly enriched in Ectomycorrhizal-Fungal Parasite-Plant Saprotroph-Wood Saprotroph, Endophyte-Plant Pathogen-Wood Saprotroph, Dung Saprotroph-Undefined Saprotroph-Wood Saprotroph, Animal Pathogen, Animal Pathogen-Endophyte-Lichen Parasite-Plant Pathogen-Soil Saprotroph-Wood Saprotroph.

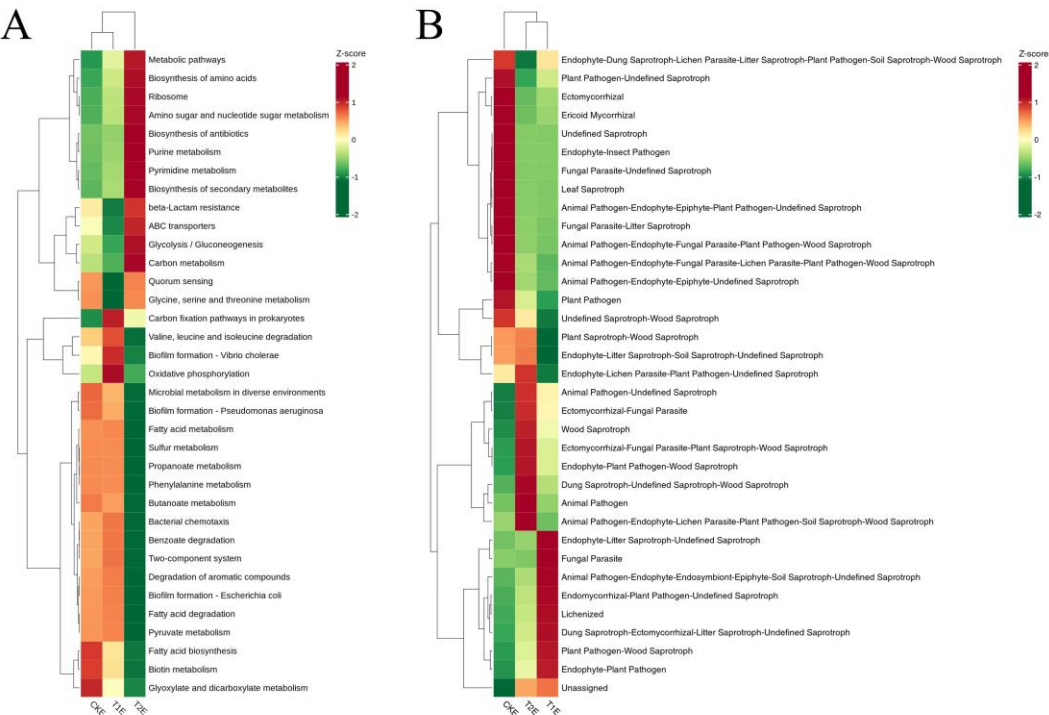


Fig.30 Heatmaps of functional annotation clustering of phyllosphere endophytic bacteria based on Tax4Fun2 (A) and phyllosphere endophytic fungi based on FunGuild (B) across different groups. Horizontally, the heatmaps represent functional annotation information; vertically, they represent sample information. The squares indicate relative abundance, with redder colors indicating higher relative abundance and bluer colors indicating lower relative abundance.