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Soil microorganism colonization influenced the growth and secondary metabolite accumulation of *Bletilla striata* (Thunb.) Rchb. F.

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Abstract

Bletilla striata (Thunb.) Rchb. F., a perennial herbaceous plant renowned for its medicinal properties, exhibits growth and secondary metabolite production that are significantly influenced by soil microorganisms. Exploring how soil microorganisms influence the growth and secondary metabolites of *B. striata*, we cultivated sterile seedlings in radiation-sterilized soil inoculated with microbiota from either sandy clay or sandy loam soils. Following a two-year growth period, we employed 16S and ITS Illumina sequencing to analyze the bacterial and fungal communities colonizing the rhizosphere soil, roots, tubers, and leaves of *B. striata*. Concurrently, we assessed the growth indices of the plants and utilized UHPLC-MS/MS to quantify the metabolites in the tubers, with a particular focus on the index component militarine and single bacteria were isolated for verification. Our findings revealed significant variations in the metabolite profiles and growth of *B. striata* across different soil microbial treatments. Specifically, sandy loam microorganisms were found to enhance plant growth, whereas sandy clay microorganisms increased the concentration of secondary metabolites. We identified specific microbes predominantly in loam soil that colonized roots and promoted growth (e.g., *Entrophospora*, *Aspergillus*, *Fusarium*). Similarly, certain microbes in loam soil colonized tubers and enhanced their growth (e.g., *Sphingomonas*, *Hyphomicrobium*). Additionally, microbes predominantly found in sandy soil colonized tubers and stimulated the synthesis of secondary metabolites (e.g., *Myrmecridium*, *Apiotrichum montevideense*). Notably, *Aspergillus versicolor* (B-6), isolated from the rhizosphere soil of *B. striata* after the introduction of sandy loam microorganisms, demonstrated a growth-promoting effect on sterile seedlings upon inoculation. This study elucidates the role of soil microorganisms in colonizing various regions of *B. striata*, thereby modulating its growth and secondary metabolite production. These insights have significant implications for optimizing the yield and quality of *B. striata* in both medicinal and agricultural applications.

Keywords *Bletilla striata*, Soil microbiota, Microbial colonization, Secondary metabolites, Growth and development

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Introduction

In recent years, the study of plant–microbe interactions has gained significant attention. The study of how soil microorganisms affect the growth and secondary metabolite production of medicinal plants has emerged as a key area of interest, given its potential implications for both ecological and pharmaceutical applications. The plant microorganisms, also referred to as the"second genome"of plants, comprises microorganisms inhabiting or adjacent to the plant and plays a vital role in determining the structure of the plant community [1–3]. Rhizosphere microorganisms plays a key role in the interaction between plants and microbes by assisting plant roots in absorbing nutrients from the soil and fostering plant growth [4], interactions between *Astragalus membranaceus* and rhizosphere microorganisms such as *Pseudomonas*, *Bacillus* and *Rhizobia* significantly increased the content of secondary metabolites such as astragaloside, astragaloside IV and Calycosin- 8-C-glucoside in *Astragalus Membranaceus* [5–7]. Arbuscular mycorrhizal fungi (AMF), the most prevalent symbiotic fungi in soil, have been shown to promote plant growth. Specifically, their symbiosis with medicinal plants such as *Panax ginseng* and *Glycyrrhiza uralensis* enhances the accumulation of secondary metabolites, including ginsenosides and glycyrrhetinic acid [8].

The interaction between rhizosphere microorganisms and plants is not confined to the rhizosphere region but also includes colonization within the plant body. For instance, rhizosphere bacteria such as *Streptomyces* can translocate through the xylem to other parts of the plant, providing systemic protection [9]. Research findings indicate that many endophytic microbes may initially originate from the rhizosphere environment and subsequently enter the plant through wounds or natural openings [10]. Upon colonization within plant tissues, rhizosphere fungi can perform a multitude of functions, including the promotion of plant growth and development, the stimulation of secondary metabolite synthesis, and the antagonism against pathogenic microorganisms. Endophytic microorganisms predominantly colonize the roots, stems, and leaves of plants, where they play a pivotal role in enhancing plant stress resistance, fostering plant growth, and facilitating the accumulation of secondary metabolites [11, 12]. For example, the mycelium of *Streptomycetes* (AgN23), was able to colonize the leaf surface, leading to plant resistance against *Alternaria brassicicola* infection in wild-type Arabidopsis plants [13]. Endophytic bacteria capable of producing indole acetic acid significantly enhance the branch length, leaf number, internode elongation, and photosynthetic pigment content in Frankincense Tree [14]. The endophytic bacteria *Bacillus altitudinis* and *Paenibacillus polymyxa* significantly promote plant growth and enhance the content of ginsenosides [15]. Furthermore, *Paenibacillus konkukensis*, *Paenibacillus kribbensis*, and *Paenibacillus taichungensis* mildly induced β-eudesmol and atractylon accumulation in the root of *Atractylodes lance* [16]. *Actinobacteria* and *Proteobacteria*, identified within the root system of Ginkgo biloba, were found to significantly enhance the accumulation of flavonoids [17]. Therefore, endophytic microbes play a significant role in research purposes in enhancing the biomass and secondary metabolites of host plants.

Bletilla striata (Thunb.) Rchb. F., commonly known as the Chinese ground orchid or Baiji, is a species of terrestrial orchid endowed with a wealth of medicinal properties. The remarkable secondary metabolites present in its tubers, such as phenolic compounds, polysaccharides, bibenzyl, dihydrophenanthrene, and diphenanthrene derivatives [18] have been utilized for centuries in traditional Chinese medicine due to their diverse therapeutic properties, including antioxidant and anti-inflammatory effects [19, 20], 1,4-bis [4- (glucosoxy) benzyl] − 2-isobutyl malic acid ester, referred to as militarine, is a novel compound derived from the *B. striata* species, pharmacological experiments have demonstrated its potential to promote cognitive development and delay the aging process [21] additionally to avoiding the development of lung cancer [22]. The soil microorganisms exert significant impacts on the growth and secondary metabolite production of *B. striata* [23]. Although *B. striata* therapeutic benefits are well known, the specific mechanisms by which the soil microorganisms influence the plant's growth and secondary metabolite production remain largely unexplored. The prospects of investigating this information gap are thrilling, as they might facilitate the discovery of novel approaches for cultivating this medicinally significant plant.

Soil microorganisms play an important role in regulating the nutrient absorption, growth and development of orchids and the accumulation of metabolites, but how do soil microorganisms play their role? We speculate that some microorganisms in soil selectively colonize different tissue parts of orchids, thus affecting plant growth and metabolite synthesis, and whether they colonized the plant organs themselves to become endophytic. In the following sections, we will provide more detailed explanations of our experimental procedures, present the findings from our investigation, discuss the implications of these findings, and conclude with a summary of the most important conclusions regarding how soil microorganisms affect *B. striata* growth and the accumulation of secondary metabolites. Our results may provide a basis for optimizing the yield and quality of *B. striata.*

Results

Native microbial community composition in sandy loam and clay soils

16S and ITS Illumina sequencing indicated that the communities of bacteria and fungi in sandy loam soil differed significantly in Bray–Curtis distances, which serve as an indicator of beta diversity, from the corresponding communities in sandy clay soil (Fig. 1A-C). Among the three phyla of fungi that we detected in the two types of soil, Ascomycota was significantly more abundant in sandy loam soil, while Basidiomycota was significantly more abundant in sandy clay soil ($p < 0.05$) (Fig. 1D). Among multiple fungal genera and species with significant differences in abundance between these two soils, we selected 10 whose abundance was significantly higher in sandy clay ($p < 0.05$) (Fig. 1E-F) (e.g., *Cercophora*, *Trichoderma*, *Codinaeopsis*, *Fusicolla*, *Cladorrhinum*, *Exophiala*, *Talaromyces*, *Stachybotrys*, *Metarhizium*, *Saitozyma*, *Mycothermus thermophilus*, *Trichocladium opacum*, *Arthrobotrys superba* and *Exophiala equina*). Conversely, the following fungal genera and species were significantly more abundant in sandy loam soil (e.g., *Sporobolomyces*, *Symmetrospora*, *Erythrobasidium* (*E. hasegawianum*), *Paraconiothyrium*, *Cladosporium*, *Vishniacozyma*, *Myrmecridium*, *Alternaria*, *Ilyonectria*, *Epicoccum*, *Myrothecium*

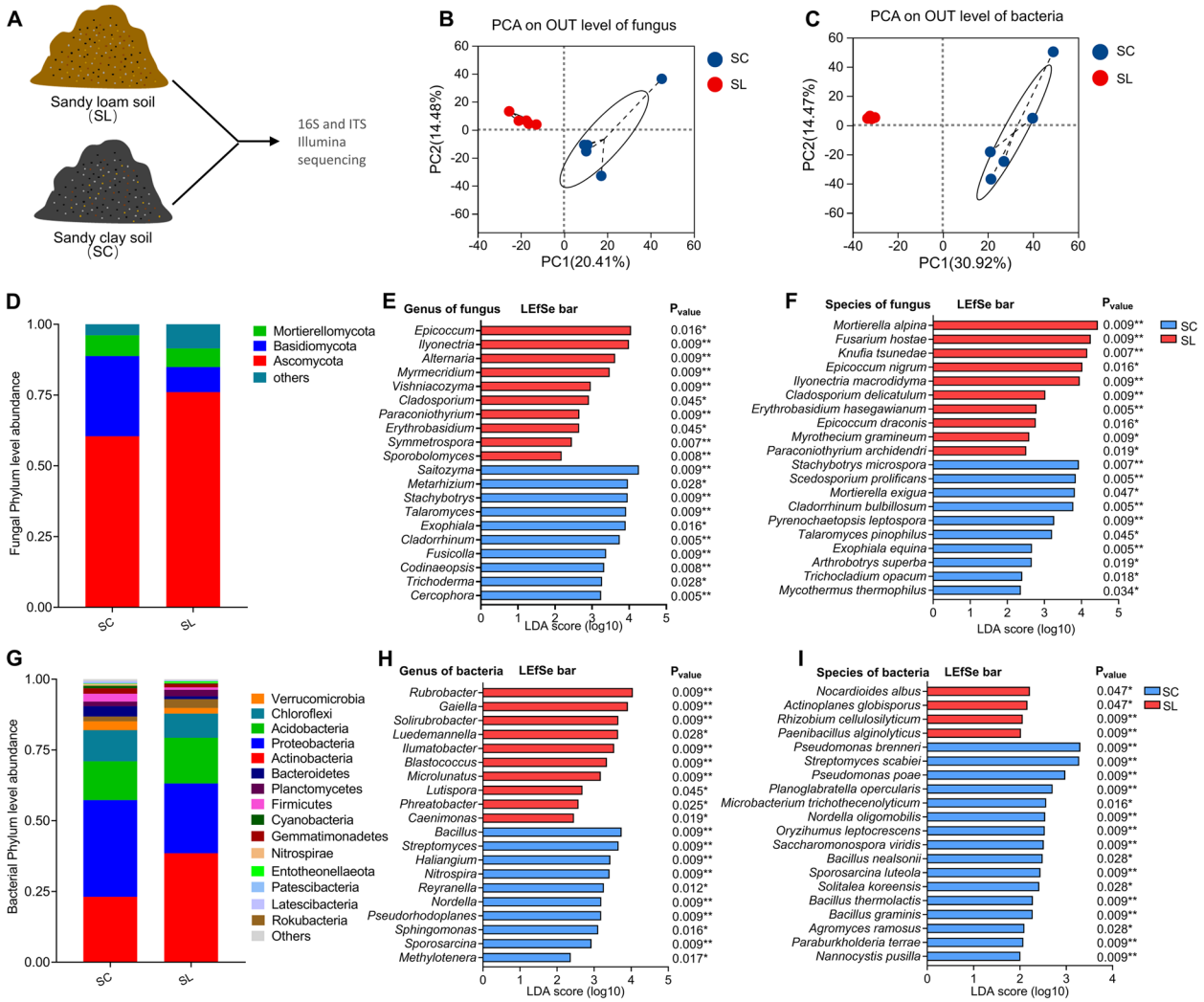

Fig. 1 Different microbial communities in sandy clay soil or sandy loam soil. **A** Analysis of 16S RNA and ITS sequences to characterize microbial communities. **B-C** Analysis of principal components (PCs) of Bray–Curtis distances in (B) fungal and (C) bacterial communities based on operational taxonomic units (OTUs) as an index of beta diversity. **D** Relative abundances of fungal phyla. **E–F** Fungal taxa differing significantly between the soil types based on linear discriminant analysis (LDA) score $\log_{10} > 2.0$ and $P < 0.05$. LEfSe, linear discriminant analysis effect size. **G** Relative abundances of bacterial phyla. **H-I** Bacterial taxa differing significantly between the soil types based on the same criteria as in panels E–F

gramineum, *Knufia tsunedae*, *Fusarium hostae*, and *Mortierella alpina*).

Among the 15 phyla of bacteria that we detected in the two types of soil, Actinobacteria and Rokubacteria were significantly more abundant in the sandy loam soil, while Proteobacteria, Bacteroidetes and Firmicutes were significantly more abundant in sandy clay soil ($p < 0.05$) (Fig. 1G). Among multiple bacterial genera and species with significant differences in abundance between these two soils, select 10 different genera and species for each of the two soils, the abundance of the following bacterial is significantly higher in sandy clay ($p < 0.05$) (Fig. 1H-I) (e.g., *Methylotenera*, *Sporosarcina*, *Sphingomonas*, *Pseudorhodoplanes*, *Nordella*, *Reyranella*, *Nitrospira*, *Haliangium*, *Streptomyces*, *Bacillus*, *Nannocystis pusilla*, *Paraburkholderia terrae*, *Agromyces ramosus*, *Solitalea koreensis* and *Saccharomonospora viridis*). Conversely, the following were significantly more abundant in sandy loam soil (e.g., *Caenimonas*, *Phreatobacter Lutispora*, *Microlunatus*, *Blastococcus*, *Ilumatobacter*, *Luedemannella*, *Solirubrobacter*, *Gaiella*, *Rubrobacter* and *Paenibacillus alginolyticus)*. We comprehensively characterized the distinct microbial communities colonizing sandy clays and sandy loams.

Effects of microbiota transplantation on the structure of microbial communities in *B. striata* rhizosphere soil

We cultivated *B. striata* seedlings for two years in mother soil containing microbiota from sandy clay soil alone, sandy loam soil alone, or both types of soil (Fig. 2A). Consistent with our analyses of the two soil types, we found that the bacterial and fungal communities differed significantly across the three microbiota transplant conditions, based on Shannon and Simpson indices (Fig. 2B) as well as Bray–Curtis distances (Fig. 2C-D, F-G).

Of the nine phyla of fungi that we identified, Ascomycota was significantly more abundant in the soil containing microbiota from sandy loam soil, while Chytidiomycota, Rozellomycota, and Mortierellomycota were significantly more abundant in the soil containing microbiota from sandy clay soil ($p < 0.05$) (Fig. 2E, H). Basidiomycota, in contrast, was significantly more abundant in the soil containing microbiota from both sandy loam and sandy clay soils. Of the eight phyla of bacteria that we identified, none differed significantly in abundance across the three microbiota transplant conditions.

Of the 16 genera and 15 species of fungi whose abundance differed significantly across the three conditions, we found that the following were significantly enriched in soil containing microbiota only from sandy clay soil ($p < 0.05$) (Fig. 2I-J) (e.g., *Botrytis*, *Coniochaeta*, *Myrmecridium*, *Cercophora*, *Tetracladium*, *Cladorrhinum*, *Chytridiomycota sp*, *Serendipita vermifera*, *Rozellomycota sp*, and *Mortierell alpina*). The following were significantly enriched in soil containing microbiota only from sandy loam soil (e.g., *Staphylotrichum*, *Scolecobasidium*, *Plectosphaerella*, *Ramophialophora*, *Mortierella zychae*, *Exophiala equina*, *Staphylotrichum sp*, and *Chaetomium sphaerale*). The following were significantly enriched in soil containing microbiota from the two types of soil (e.g., *Exophiala*, *Powellomyces*, *Exidia*, *Paramyrothecium*, *Serendipita*, *Pyrenochaeta*, *Penicillium sumatraense*, and *Talaromyces cecidicola*).

Of the 14 genera and 15 species of bacteria whose abundance differed significantly across the three conditions, we found that the following were significantly enriched in soil containing microbiota only from sandy clay soil ($p < 0.05$) (Fig. 2K-L) (e.g., *Rubrobacter*, *Demequina*, *Nitrospira*, *Aminobacter*, *Microvirga*, *Thermoactinomyces intermedius*, *Clostridium subterminale*, *Thermobifida fusca YX*, and *Pararhizobium giardinii*). The following were significantly enriched in soil containing microbiota only from sandy loam soil (e.g., *Microbacterium*, *Nordella*, *Reyranella*, *Roseomonas*, *Fictibacillus*, *Streptomyces canus*, *Aneurinibacillus migulanus*, and *Ralstonia solanacearum*). The following were significantly enriched in soil containing microbiota from the two types of soil (e.g., *Roseateles*, *Solibacillus*, *Bradyrhizobium*, *Rhodoplanes*, *Paenibacillus taihuensis*, and *Fictibacillus barbaricus*). We summarized the significant enrichment of fungi and

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Fig. 2 Microbial communities in soil after transplantation of microbiota from different types of soil. **A** Schematic of microbiota transplantation experiments. **B** Alpha diversity in fungal and bacterial communities based on operational taxonomic units (OTUs). Data are mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. SC-MT group, based on two-way ANOVA with Tukey's multiple-comparisons test. **C**-**D** Analysis of principal components (PCs) of Bray–Curtis distances in fungal communities based on operational taxonomic units (OTUs) as an index of beta diversity. The box-and-whiskers histogram on the *right* represents the distribution of the sample on PC 1. $^{***}p < 0.001$ vs. SC-MT and $^{\#\#\#}p < 0.001$ vs. SL-MT, based on one-way ANOVA with Tukey's multiple-comparisons test. **E** Relative abundances of fungal phyla. **F**-**G** Analysis of principal components (PCs) of Bray–Curtis distances in bacterial communities based on operational taxonomic units (OTUs) as an index of beta diversity. The box-and-whiskers histogram on the *right* represents the distribution of the sample on PC 1. $^{**}p < 0.01$ vs. SC-MT and $^{\#\#\#}p < 0.001$ vs. SL-MT, based on one-way ANOVA with Tukey's multiple-comparisons test. **H** Relative abundances of bacterial phyla. **I-J** Fungal taxa differing significantly between the soil types based on linear discriminant analysis (LDA) score $\log_{10} > 2.0$ and $p < 0.05$. LEfSe, linear discriminant analysis effect size. **K-L** Bacterial taxa differing significantly between the soil types based on the same criteria as in panels I-J

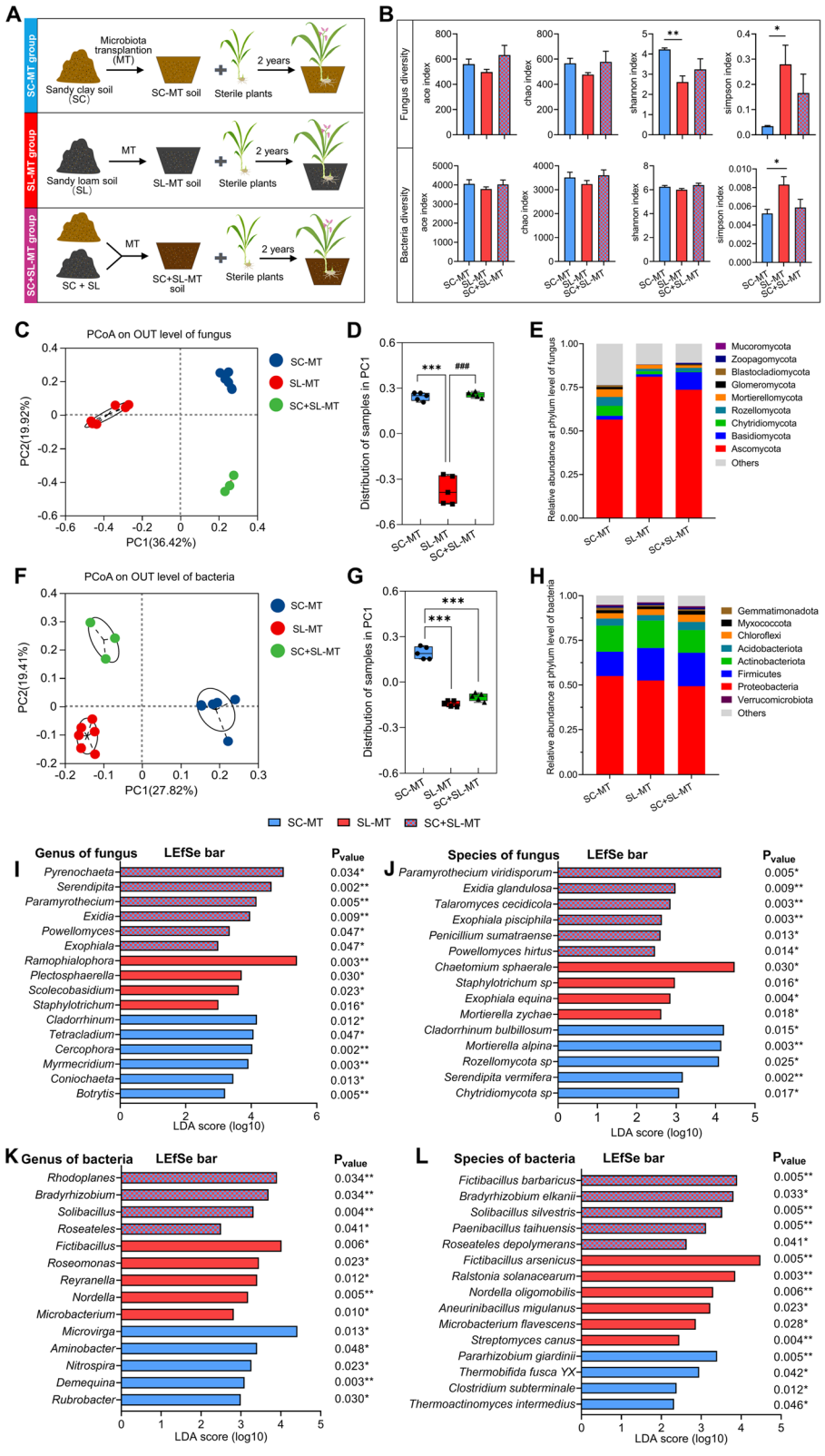

Fig. 2 (See legend on previous page.)

bacteria in *B. striata* rhizosphere soil under each microbiota transplantation condition.

Effect of microbiota transplantation on the composition of endophytic microbial community in *B. striata*

We next examined whether different transplant conditions affected microbial colonization in *B. striata* tissues. Analysis revealed distinct root microbial communities across the three conditions based on diversity indices (Fig. 3A-B). Among the nine fungal phyla identified, Basidiomycota dominated in roots with microbiota from both sandy loam and sandy clay soils. In contrast, *Ascomycota* and *Glomeromycota* were significantly more abundant in roots with microbiota from a single soil type ($p < 0.05$) (Fig. 3C). At the class and genus levels, Mortierellaceae and *Mortierella* were enriched in roots with sandy clay microbiota, Glomeraceae in roots with sandy loam microbiota, and Serendipitaceae, *Zopfiella*, and *Serendipita* in roots with microbiota from both soil types (Fig. 3D).

Among the 14 bacterial phyla identified, Proteobacteria was significantly less abundant, while *Myxococcota* and *Firmicutes* were more abundant, in *B. striata* roots with microbiota from a single soil type compared to roots with microbiota from both soil types ($p < 0.05$) (Fig. 3E). At the genus level, *Nitrospira*, *Ammoniphilus*, *Desulfotomaculum*, *Ruminiclostridium*, *Gaiella*, *Cryptosporangium*, *Amycolatopsis*, *Phaselicystis*, and *Devosia* were enriched in roots with sandy clay microbiota ($p < 0.05$) (Fig. 3F). *Pseudomonas* and *alphaI cluster* were enriched in roots with sandy loam microbiota, while *Prosthecomicrobium*, *Azohydromonas*, *Phyllobacterium*, *Ideonella*, *Comamonas*, and *Caulobacter* were enriched in roots with microbiota from both soil types.

Similar to *B. striata* roots, tuber-colonizing microbial communities exhibited significant compositional variations based on soil microbiota sources. Bacterial communities showed differences in Shannon and Simpson indices (Fig. 4A), while Bray–Curtis distances were comparable between bacterial and fungal communities (Fig. 4B). Among the eight fungal phyla identified, Ascomycota were more abundant in tubers with microbiota from both soil types, whereas Basidiomycota were more abundant in tubers with microbiota from a single soil type ($p < 0.05$) (Fig. 4C). At the class and genus levels, Ceratobasidiaceae were enriched in tubers with sandy clay microbiota, Saccharomycetaceae and *Issatchenkia* in tubers with sandy loam microbiota, and Cladosporiaceae, Hypocreaceae, *Cladosporium*, *Trichoderma*, and *Penicillium* in tubers with microbiota from both soil types (Fig. 4D).

Among the 15 bacterial phyla identified, Proteobacteria were more abundant in *B. striata* tubers with microbiota from both soil types, while Actinobacteriota and Chloroflexi were more abundant in tubers with microbiota from a single soil type (Fig. 4E). At the genus level, *Flavobacterium*, *Sphingobium*, *Hydrogenophaga*, *Microbacterium*, *Sphingomonas*, *Bradyrhizobium*, and *Ralstonia* were enriched in tubers with sandy clay microbiota ($p < 0.05$) (Fig. 4F). *Romboutsia*, *Cloacibacterium*, *Acidovorax*, *Lachnoclostridium*, *Caulobacter*, *Streptococcus*, and *Arthrobacter* were enriched in tubers with sandy loam microbiota, while *Sutterella*, *Pedobacter*, *Bacteroides*, and *Pseudomonas* were enriched in tubers with microbiota from both soil types.

No significant differences in alpha or beta diversity of fungal or bacterial communities were observed in *B. striata* leaves across the three transplant conditions (Fig. 5A-B). However, differences in specific microbial abundances were detected. Among the seven fungal phyla identified, Ascomycota was more abundant in leaves with microbiota from both soil types, while Basidiomycota was more abundant in leaves with microbiota from a single soil type (Fig. 5C). At the class and genus levels, Stachybotryaceae and *Paramyrothecium* were significantly enriched in leaves with sandy clay microbiota ($p < 0.05$) (Fig. 5D).

Among the 15 bacterial phyla identified, Proteobacteria were less abundant in leaves of plants grown in sandy loam soil compared to sandy clay soil, while Firmicutes and Bacteroidota showed the opposite trend (Fig. 5E). The genera *Trichococcus*, *Dorea*, *Limosilactobacillus*, *Capnocytophaga*, *Subdoligranulum*, *Leptotrichia*, and *Actinomyces* were significantly enriched in leaves with microbiota from both soil types ($p < 0.05$) (Fig. 5F). *Sphingorhabdus* was enriched in leaves with sandy clay microbiota. We summarized the significant enrichment

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Fig. 3 Differential colonization of *B. striata* roots by microbiota transplanted from sandy clay soil (SC) or sandy loam soil (SL). **A** Alpha diversity in fungal and bacterial communities based on operational taxonomic units (OTUs). Data are mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. SC-MT group, based on two-way ANOVA with Tukey's multiple-comparisons test. **B** Analysis of principal components (PCs) of Bray–Curtis distances in fungal communities based on OTUs as an index of beta diversity. The box-and-whiskers histogram on the *right* represents the distribution of the sample on PC 1. $^{***}p < 0.001$ vs. SC-MT and $^{\#\#\#}p < 0.001$ vs. SL-MT, based on one-way ANOVA with Tukey's multiple-comparisons test. **C** Relative abundances of fungal phyla. **D** Fungal taxa differing significantly between the soil types based on linear discriminant analysis (LDA) score $\log_{10} > 2.0$ and $p < 0.05$. LEfSe, linear discriminant analysis effect size. **E** Relative abundances of bacterial phyla. **F** Bacterial taxa differing significantly between the soil types based on the same criteria as in panel D

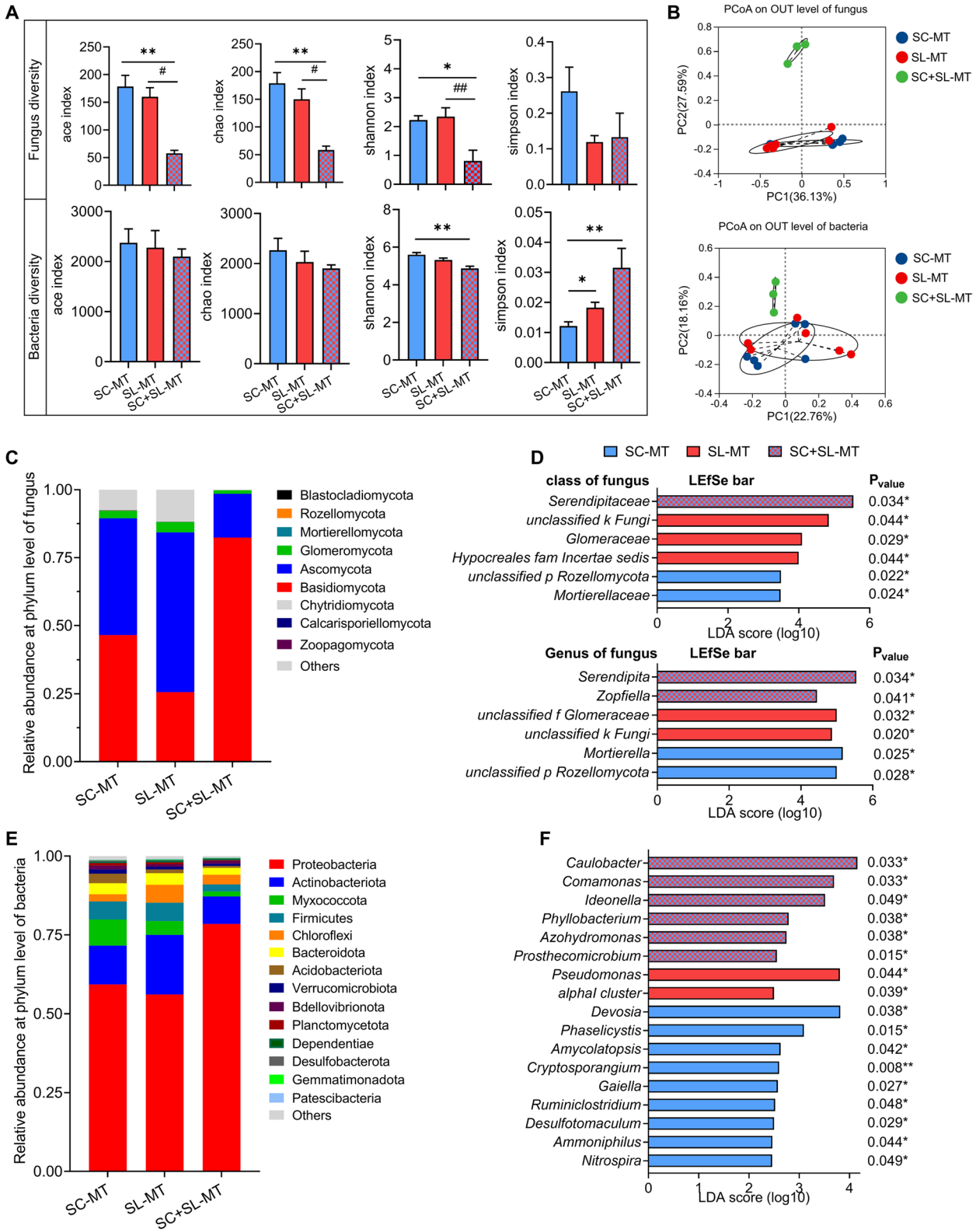

Fig. 3 (See legend on previous page.)

of fungi and bacteria in *B. striata* roots, tubers, and leaves under each microbiota transplantation condition.

Fungal communities in *B. striata* roots, tubers, and leaves exhibited tissue-specific colonization under each microbiota transplantation condition. For instance, in plants with sandy clay microbiota, *Setophoma*, *Myrmetridium*, *Plectosphaerella*, *Fusarium*, and *Chytridiomycota* sp. were specific to roots; *Aspergillus*, *Apiotrichum* (*A. montevideense*), and *Rozellomycota* sp. to tubers; and *Acremonium*, *Sporobolomyces*, *Trichomerium*, *Didymella*, *Vishniacozyma*, *Paramyrothecium* (*P. viridisporum*), *Cladosporium*, *Epicoccum*, *Cercospora*, *Symmetrospora*, *Leptosphaerulina*, *Alternaria*, *Lithophila*, *Articulospora*, *Itersonilia perplexans*, *Symmetrospora coprosmae*, *Trichomerium* sp., *Aspergillus amstelodami*, *Itersonilia pannonica*, *Exophiala* sp., *Buckleyzyma aurantiaca*, and *Symmetrospora symmetrica* were specific to leaves (Fig. 6). Tissue-specific fungi and bacteria under each condition are summarized in Table 1 (Table 1).

The impact of microbial community transplantation on the growth of *B. striata* and its secondary metabolites

We found that the lengths of roots and leaves as well as the fresh and dry weights of roots and tubers varied significantly with microbiota transplant condition ($p < 0.05$). *B. striata* grew significantly better in microbiota transplanted from sandy loam soil (Fig. 7).

The findings of the PCA analysis demonstrated significant disparities in the *B. striata* metabolites following the transplantation of the three distinct soil microorganisms ($p < 0.05$)(Fig. 8A). The Venn diagram analysis uncovered 6010 shared metabolites among the three microbial transplanted *B. striata*. Notably, *B. striata* transplanted with microbes from sandy clay soil exhibited the highest number of unique metabolites (70), while *B. striata* transplanted with microbes from sandy loam soil demonstrated the lowest number of unique metabolites (43) (Fig. 8B). The volcano plot analysis revealed that a total of 424 differentially expressed metabolites were significantly upregulated and 394 differentially expressed metabolites were notably downregulated in *B. striata* transplanted with sandy clay microbes, in comparison to *B. striata* transplanted with sandy loam microbes (Fig. 8C). Hierarchical clustering analysis was conducted on the differential metabolites of the three groups, resulting in the formation of 10 primary clusters. A higher number of metabolites were detected in *B. striata* transplanted with sandy clay microorganisms (Fig. 8D). *B. striata* mainly comprises vitamins, peptides, amino acids, carboxylic acids, nucleotides, nucleosides, cyclic nucleotides, bases, phospholipids, peptide hormones, oligosaccharides, and other metabolites (Fig. 8E). Enrichment analysis of KEGG metabolic pathways revealed that the biosynthesis of various plant secondary metabolites, nucleotide metabolism, phenylalanine metabolism, phenylpropanoid biosynthesis, arginine biosynthesis, and other metabolic pathways were significantly altered in *B. striata* transplanted with sandy clay microorganisms compared with *B. striata* transplanted with sandy loam microorganisms ($p < 0.05$) (Fig. 8F). The results of the visual heatmap analysis revealed that the metabolites were partitioned into two major clusters, each of which was further subdivided into two smaller clusters (Fig. 8G). Among the secondary metabolites present in the tubers of *B. striata*, the concentration of militarine was quantified, and it was found that the levels of militarine in tubers significantly varied with transplant conditions ($p < 0.05$) (Fig. 8H-I).

Correlation analysis allows us to identify specific microorganisms that colonize the roots or tubers and may have a positive effect on the growth and accumulation of secondary metabolites of these tissues (Tables 2– 3**,** Figs. 9 and 10). For example, sandy loams contain several microorganisms that colonize the roots and have a significant positive correlation with root growth: *Entrophospora, Aspergillus, Fusarium, Neocosmospora rubicola,* and *Pseudomonas aeruginosa.* It also contains several microorganisms that colonize the tuber and are significantly positively associated with tuber growth: *Sphingomonas, Bradyrhizobium, Bradyrhizobium elkanii,* and *Hyphomicrobium*. Microorganisms that are significantly positively associated with the accumulation of secondary metabolites include *Cercophora*, *Myrmecridium*, *Reyranella*, *Rhizobacter*, *Chryseobacterium*, *Apiotrichum, Apiotrichum montevideense, Brevundimonas* and *Brevundimonas bullata.*

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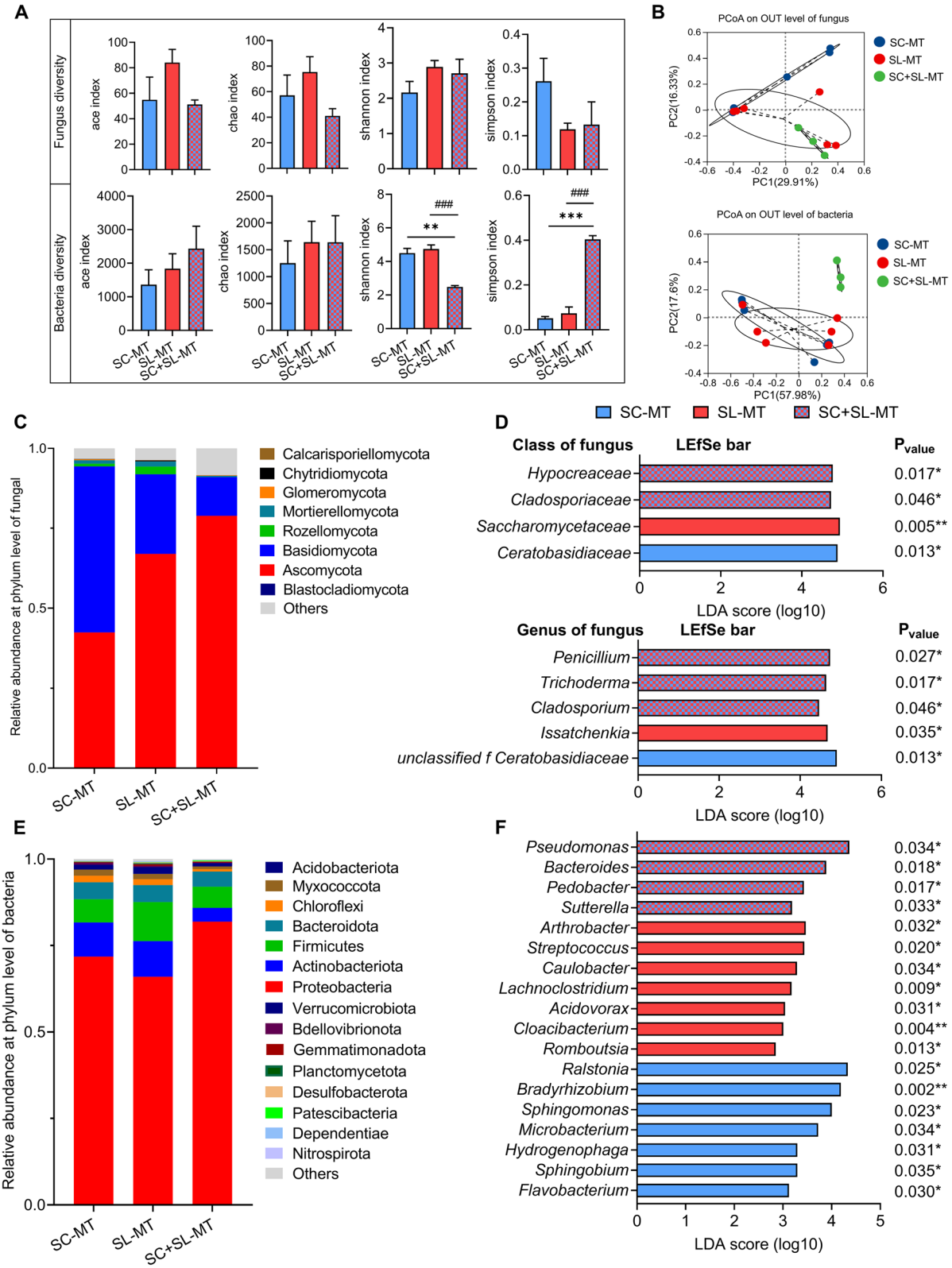
Fig. 4 Differential colonization of *B. striata* tuber by microbiota transplanted from sandy clay soil (SC) or sandy loam soil (SL). **A** Alpha diversity in fungal and bacterial communities based on operational taxonomic units (OTUs). Data are mean ± SEM. *$p < 0.05$, **$p < 0.01$ vs. SC-MT group, based on two-way ANOVA with Tukey's multiple-comparisons test. **B** Analysis of principal components (PCs) of Bray–Curtis distances in fungal communities based on OTUs as an index of beta diversity. The box-and-whiskers histogram on the *right* represents the distribution of the sample on PC 1. ***$p < 0.001$ vs. SC-MT and $^{\#\#\#}p < 0.001$ vs. SL-MT, based on one-way ANOVA with Tukey's multiple-comparisons test. **C** Relative abundances of fungal phyla. **D** Fungal taxa differing significantly between the soil types based on linear discriminant analysis (LDA) score $\log_{10} > 2.0$ and $p < 0.05$. LEfSe, linear discriminant analysis effect size. **E** Relative abundances of bacterial phyla. **F** Bacterial taxa differing significantly between the soil types based on the same criteria as in panel D



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We found that in sandy loams, microbes mainly colonize the roots and have a positive effect on the growth of *B. striata* (e.g., *Entrophospora, Aspergillus, Fusarium, Neocosmospora rubicola, Pseudomonas aeruginosa*) or that colonized tubers and have a positive effect on the growth of *B. striata* (e.g., *Sphingomonas, Bradyrhizobium elkanii, Hyphomicrobium*). We also found that microorganisms mainly in sandy clay, colonize tubers and have positive effects on the accumulation of secondary metabolites of *B. striata* (e.g., *Cercophora*, *Myrmecridium*, *Reyranella*, *Rhizobacter*, *Chryseobacterium*, *Apiotrichum, Apiotrichum montevideense, Brevundimonas* and *Brevundimonas bullata*).

7 Isolation of B- 6 and its effect on *B. striata* growth and development.

After transplanting the sandy loam microorganisms, we isolated 68 strains of fungi from the rhizosphere soil of *B. striata*. According to the results of correlation analysis, there was a significant positive correlation between *Aspergillus* fungi and plant growth. Therefore, the isolated *Aspergillus versicolor* (B- 6) was used to verify the experiment. Phylogenetic tree analysis revealed that B- 6 exhibited the closest genetic relationship with *Aspergillus versicolor* (MH777427.1) (Fig. 11A-B). The height and number of new shoots ($p < 0.05$) in *Aspergillus versicolor* (B- 6) inoculated *B. striata* sterile seedlings were significantly increased compared to the control (Fig. 11C-F). The results of our investigations corroborated the notion that the presence of *Aspergillus versicolor* in sandy loam soil is beneficial to the growth of *B. striata*.

Materials and methods

Materials

Issue cultures were obtained from Guizhou Fusheng Tianhong Ecological Industry Development (Guiyang, China), while sandy clay soil and sandy loam soil were obtained from the *B. striata* Experimental Base of Shibing County (Guizhou, China; 26 57′ 52"N, 108 1′ 33"E). The reference standard for militarine was obtained from Le Meitian Pharmaceutical/De Site Biological (batch DSTDP00250; Chengdu, Sichuan, China).

Methods

Transplantation of soil microorganisms and planting of B. striata

Prior to microbial transplantation, the entire *B. striata* was collected using a"double diagonal"(five-point sampling) technique, including the soil around the *B. striata* roots, which involves taking one sample at the diagonal's midpoint and four additional samples at evenly spaced intervals along the diagonal [24]. The collected samples were transferred to the laboratory at 4° C, and the large pieces of soil around the *B. striata* roots were gently knocked out, only soil particles firmly attached to the *B. striata* tubers and roots (at a distance of approximately 2 mm within the soil layer) are considered [25]. Finally, the five collected samples of soil were evenly mixed to form a single sample.

Sandy clay soil and sandy loam soil were divided into two parts, one of which was irradiated for 3 days with 25 kGy of γ-rays from cobalt- 60, while the other was not. In parallel, mother soil was prepared by mixing nutrient soil, loam, coconut brick, and vermiculite in a volume ratio of 3:1:1:1, then this soil was irradiated for 3 days with cobalt- 60. We prepared 3 sets of experiments, each with 3–6 pots. All pots contained 4,000 mL of irradiated mother soil. (1) To one set of pots we added irradiated sandy loam soil (200 mL) and non-irradiated sandy clay soil (200 mL), corresponding to microbiota transplantation from sandy clay. (2) We added the same volumes of non-irradiated sandy loam soil (200 mL) and irradiated sandy clay soil (200 mL) to another set of pots, which corresponded to microbiota transplantation from sandy loam soil. (3) To the third set of pots we added the same volumes of both types of non-irradiated soil (each 200 mL), corresponding to microbiota transplantation from both types of soil.

Carefully selected *B. striata* seedlings were soaked in a 0.1% mercury disinfectant for 10 min, rinsed with sterilized water, and planted at an equal depth in the center of each pot with approximately 2 cm of soil covering the tubers. Ten sterile seedlings were planted per pot and grown under plastic film coverage in a greenhouse at Guizhou University of Traditional Chinese Medicine (26°23′17.68"N,106°37′43.18"E) to

(See figure on next page.)

Fig. 5 Differential colonization of *B. striata* leaves by microbiota transplanted from sandy clay soil (SC). **A** Alpha diversity in fungal and bacterial communities based on operational taxonomic units (OTUs). Data are mean ± SEM. *$p < 0.05$, **$p < 0.01$ vs. SC-MT group, based on two-way ANOVA with Tukey's multiple-comparisons test. **B** Analysis of principal components (PCs) of Bray–Curtis distances in fungal communities based on OTUs as an index of beta diversity. The box-and-whiskers histogram on the *right* represents the distribution of the sample on PC 1. ***$p < 0.001$ vs. SC-MT and $^{\#\#\#}p < 0.001$ vs. SL-MT, based on one-way ANOVA with Tukey's multiple-comparisons test. **C** Relative abundances of fungal phyla. **D** Fungal taxa differing significantly between the soil types based on linear discriminant analysis (LDA) score $\log_{10} > 2.0$ and $p < 0.05$. LEfSe, linear discriminant analysis effect size. **E** Relative abundances of bacterial phyla. **F** Bacterial taxa differing significantly between the soil types based on the same criteria as in panel D

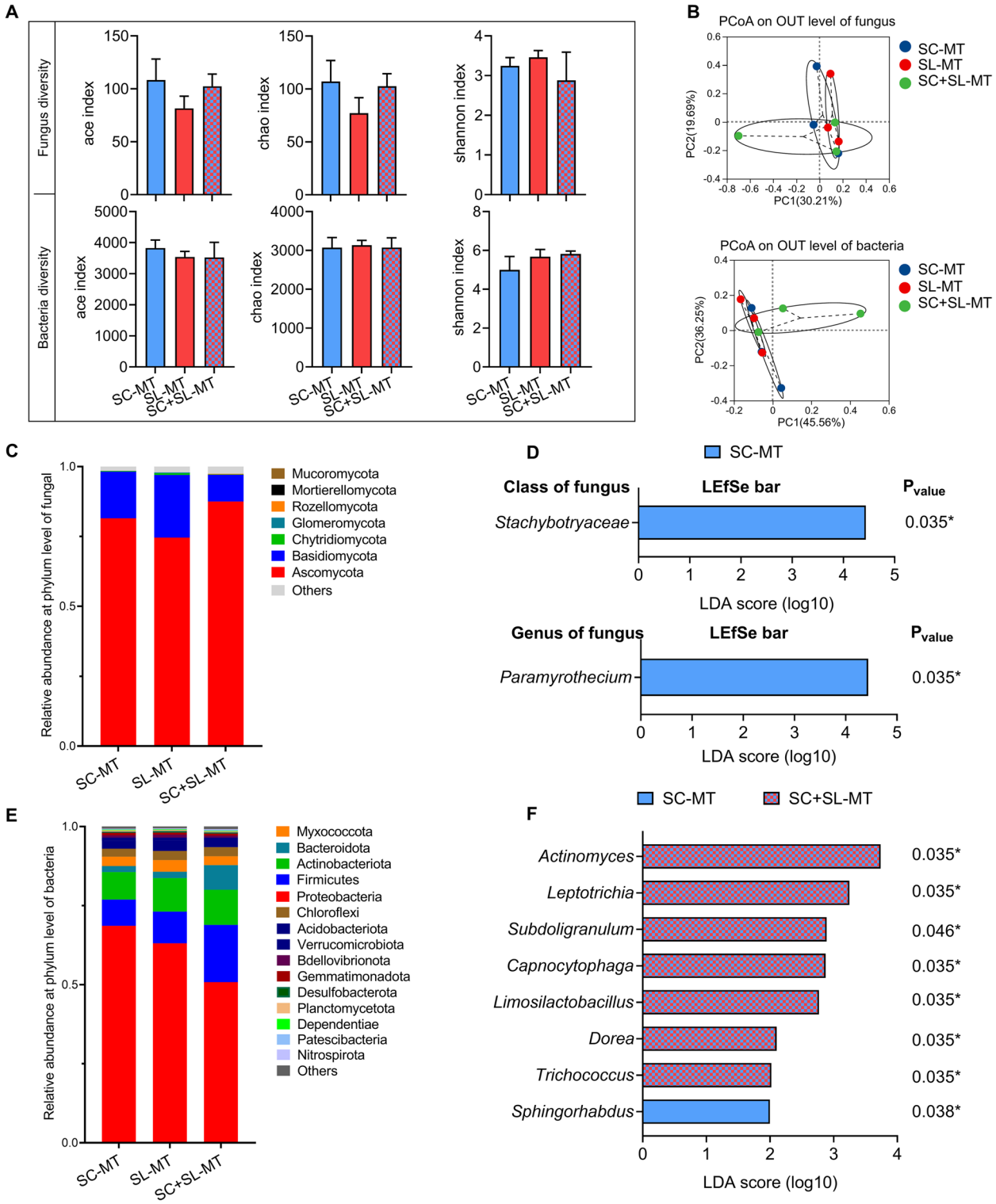

Fig. 5 (See legend on previous page.)

maintain consistency. Maintain the ambient temperature between 23–25 °C and humidity levels between 40–50%. Provide 16 h of light daily from March to November. From December to February of the following year, set the temperature at 4–10 °C, maintain humidity between 50–60%, and expose *B. striata* tubers

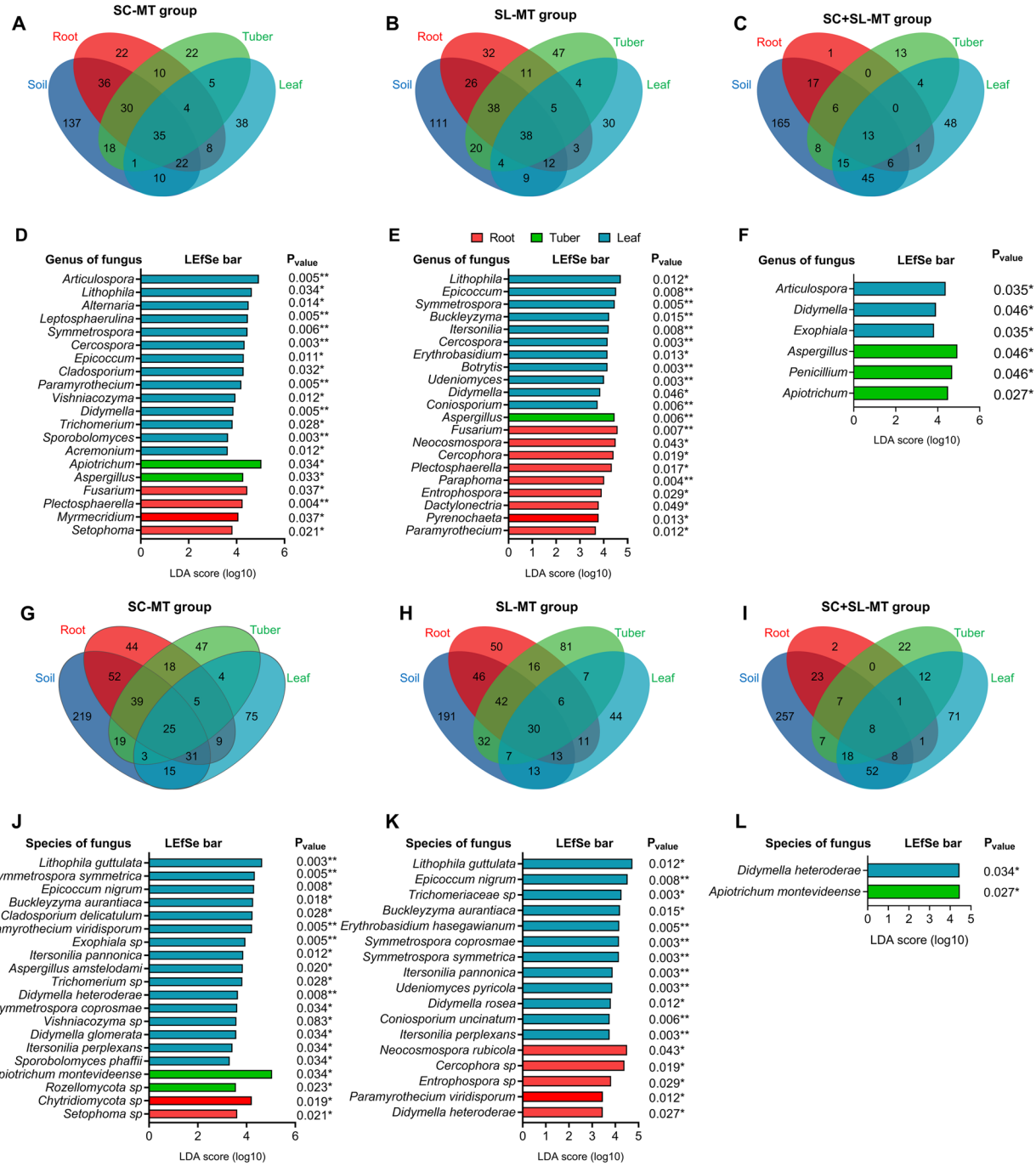

Fig. 6 Differential colonization of *B. striata* tissues by different fungal taxa from different soil types. **A-C** Venn charts of fungal genera in soil, roots, tubers, and leaves. **D-F** Fungal genera whose abundance differed significantly among the three plant tissues within each microbiota transplant condition: (D) SC-MT, (E) SL-MT, (F) SC + SL-MT. The cutoff for significant differences is defined in the legend to Fig. 1E-F. **G-I** Venn charts of fungal species in soil and roots, tubers, and leaves. **J-L** Histograms of fungal species whose abundance differed significantly across *B. striata* roots, tubers and leaves after cultivation in (J) SC-MT, (K) SL-MT or (L) SC + SL-MT soil. The criteria for significant differences were a linear discriminant analysis (LDA) score $\log_{10} > 2.0$ and $p < 0.05$

Table 1 Differential colonization of B. striata tissues by different bacterial taxa according to soil conditions

	Taxonomic level	Root	Tuber	Leaf
Genus level	SC-MT group	Sorangium, Phaselicystis, Reyranella, Lechevalieria, Haliangium, Pseudorhodoplanes, Actinoplanes, Acidibacter, Devosia, Hyphomicrobium	Rubellimicrobium, Microbacterium, Sphingomonas, Pseudomonas	Gaiella, Nocardia, Pseudarthrobacter, Arthrobacter, Ramlibacter, Streptomyces
	SL-MT group	Sorangium, Nannocystis, Caldicoprobacter, Tumebacillus, Phaselicystis, Actinoplanes	Caldicoprobacter, Tumebacillus, Arthrobacter, Chryseobacterium	Sporosarcina, Candidatus_Solibacter, Gaiella, Ureibacillus, Microvirga, Paenibacillus, Lysinibacillus, Rhodoplanes, Fictibacillus, Bacillus
	SC + SL-MT group	Tahibacter, Comamonas, Pseudorhodoplanes, Lechevalieria, Aquabacterium, Rhizobacter, Hyphomicrobium	Sutterella, Parabacteroides, Branchiibius, Pedobacter	Nakamurella, Cutibacterium, Microvirga, Calditerricola, Solirubrobacter, Alkaliphilus, Propionicicella, Prevotella, Laceyella
Species level	SC-MT group	Lactobacillus johnsonii, marine metagenome, metagenome, Bdellovibrio bacteriovorus, Anaeromyxobacter dehalogenans, Rhizocola hellebori, Hyphomicrobium aestuarii, Cryptosporangium japonicum, Pedomicrobium manganicum	Cutibacterium acnes, Microbacterium oxydans	Lactobacillus brevis, Nocardia cyriacigeorgica, Aneurinibacillus danicus, Bacillus thermolactis, Arthrobacter globiformis, Streptomyces scabiei, Methylobacterium adhaesivum
	SL-MT group	Pseudonocardia halophobica, Hyphomicrobium aestuarii, Chthonobacter albigriseus, Anaeromyxobacter dehalogenans, Rhizocola hellebori, Pedomicrobium manganicum, Variovorax paradoxus, Bradyrhizobium elkanii	Clostridium subterminale, Thermobifida fusca YX, Tumebacillus avium, Parabacteroides distasonis, Gordonia sputi, Arthrobacter globiformis	Solibacillus silvestris, Bacillus acidiceler, Ensifer adhaerens, Fictibacillus barbaricus, Ureibacillus thermosphaericus, Fictibacillus arsenicus
	SC + SL-MT group	Roseateles depolymerans, Pararhizobium giardinii, Sphingobium xenophagum, Comamonas aquatica, Lentzea albidocapillata, Variovorax paradoxus	Bacteroides eggerthii, Pseudomonas aeruginosa	Bacillus smithii, Paenibacillus ihbetae, Tepidimicrobium ferriphilum, Symbiobacterium ostreiconchae, Rhodospirillaceae bacterium, Bacillus thermoamylovorans, Lactobacillus brevis, Laceyella sacchari, Brevibacillus formosus, Methylobacterium adhaesivum

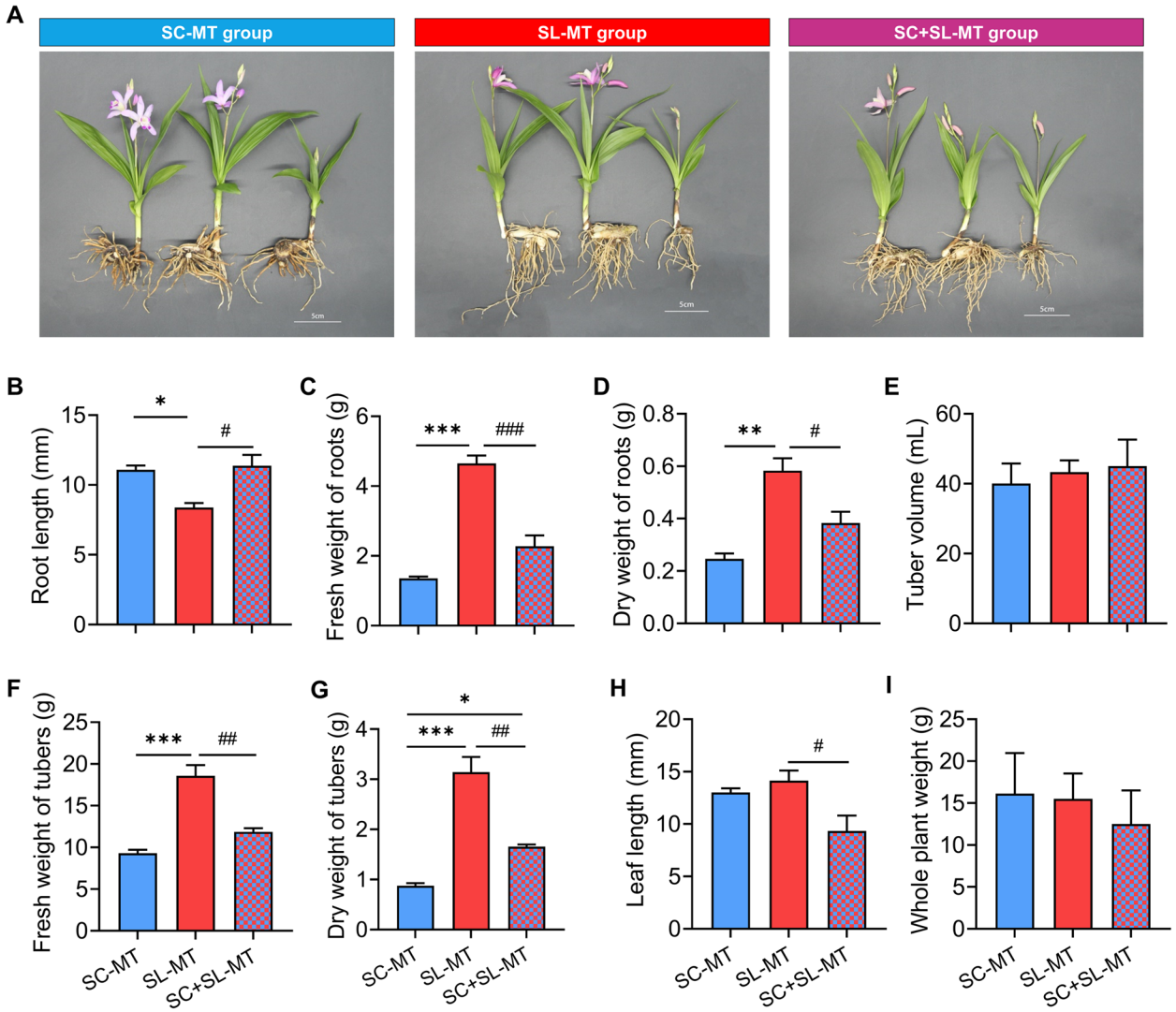

Fig. 7 Influence of microbiota from sandy clay or loam soil on *B. striata* growth. **A** Representative photographs of *B. striata*. **B-I** Assessment of various indicators of plant growth. Data are mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. SC-MT. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. SL-MT. All tests were one-way ANOVA with Tukey's multiple-comparisons test

(See figure on next page.)

Fig. 8 Metabolic profiling of *B. striata* by microbial community transplantation: A comparative analysis. **A** Plots of principal component analysis (PCA) scores for plant samples, with PC1 representing the first principal component and PC2 representing the second principal component. **B** Venn chart of *B. striata* metabolites after microbial transplantation. **C** Volcano plot of SC-MT and SL-MT, in which blue dots indicate significant and down-regulated differential metabolites, red dots indicate significant but up-regulated differential metabolites, and gray dots indicate that they can be detected in the sample but the difference is not significant. **D** Heatmap of hierarchical clustering analysis. The abscissa is used to show the sample name, and the ordinate on the right is used to show the metabolite name. The darker the color, the higher the content of metabolites. The darker the blue, the lower the metabolite content. **E** KEGG metabolite classification. The ordinate is the KEGG compound secondary classification category, and the abscissa is the number of metabolites annotated to this classification. **F** KEGG enrichment map of differential metabolites. The abscissa represents the pathway name and the ordinate represents the enrichment rate. The column color gradient indicates the significance of enrichment. The darker the default color, the more significantly enriched the KEGG term, where Pvalue or FDR < 0.001 is marked as ***, Pvalue or FDR < 0.01 is marked as **, and *P* value or FDR < 0.05 is marked as *. **G** Color-coded correlation heatmaps of the metabolites of the *B. striata* using the strength of Spearman's correlation coefficient (r). Red indicates positive correlation and blue indicates negative correlation. **H** Representative chromatograms of militarine in tubers. **I** Quantitation of levels of militarine in tubers. Data are mean ± SEM. * $p < 0.05$ vs. SC-MT and $^{\#}p < 0.05$ vs. SL-MT, based on one-way ANOVA with Tukey's multiple-comparisons test

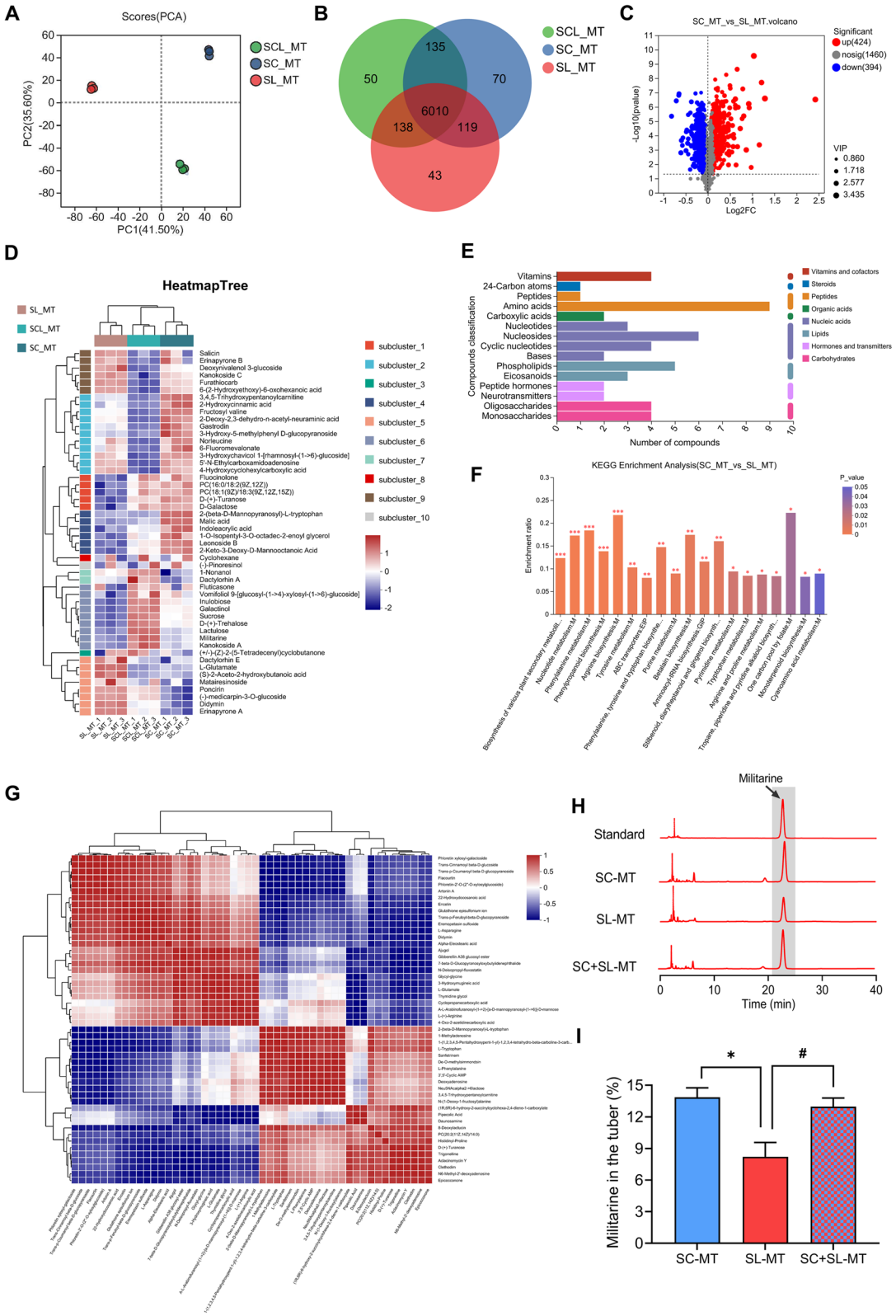

Fig. 8 (See legend on previous page.)

Table 2 Correlations between the abundance of fungal genera or species and the growth or production of secondary metabolites in *B. striata*

Taxon	Growth indicators						Secondary metabolite indicators	
	Root length (mm)		Dry weight of roots (g)		Dry weight of tubers (g)		Militarine in the tuber (%)	
	R-value	*P*-value	*R*-value	*P*-value	*R*-value	*P*-value	*R*-value	*P*-value
g_Cercophora	0.2777	0.5942	0.2639	0.6133	0.0273	0.9591	0.1729	0.7433
g_Plectosphaerella	0.0499	0.9251	0.0785	0.8825	0.4058	0.4247	0.0467	0.9300
g_Paramyrothecium	0.1935	0.7130	0.5412	0.2675	0.2647	0.6121	0.2736	0.5998
g_Pyrenochaeta	0.3465	0.5009	0.3610	0.4821	0.4614	0.3570	0.6164	0.1924
g_Myrmecridium	0.5459	0.2625	0.7186	0.1076	0.3851	0.4510	0.3016	0.5613
g_Apiotrichum	0.6340	0.1765	0.3596	0.4839	0.6986	0.1225	0.9080	0.0123
g_Entrophospora	0.6870	0.1316	0.8806	0.0205	0.4423	0.4423	0.2782	0.5935
g_Mortierella	0.4147	0.4135	0.4320	0.3924	0.5636	0.7355	0.3857	0.4500
g_Aspergillus	0.9392	0.0054	0.7826	0.0658	0.0699	0.8953	0.3226	0.5328
g_Fusarium	0.8900	0.0175	0.6855	0.1328	0.1170	0.8253	0.0982	0.8532
s_Apiotrichum montevideense	0.6404	0.1707	0.3709	0.4692	0.6987	0.1225	0.9508	0.0036
s_Neocosmospora rubicola	0.8267	0.0424	0.5834	0.2241	0.0180	0.9730	0.2699	0.6050
s_Entrophospora sp	0.5165	0.2941	0.6173	0.1917	0.3359	0.5152	0.2637	0.6137
s_Rozellomycota sp	0.3860	0.4496	0.1233	0.8159	0.01846	0.9723	0.1994	0.7049

Data are based on analyses of three *B. striata* samples from each soil type described in Fig. 2A. Root length, dry weight of roots and tubers served as an indicator of growth; militarine, as indicators of secondary metabolite in the tuber of *B. striata*. Correlations were considered significant if $|R| > 0.3$ and $P < 0.05$

Table 3 Correlations between the abundance of bacterial genera or species and the growth or production of secondary metabolites in *B. striata*

Taxon	Growth indicators						Secondary metabolite indicators	
	Root length (mm)		Dry weight of roots (g)		Dry weight of tubers (g)		Militarine in the tuber (%)	
	R-value	*P*-value	*R*-value	*P*-value	*R*-value	*P*-value	*R*-value	*P*-value
g_Reyranella	0.3024	0.5602	0.6660	0.1487	0.1229	0.8165	0.1615	0.7599
g_Rhizobacter	0.2966	0.5681	0.2665	0.6121	0.1389	0.7931	0.0905	0.8647
g_Microbacterium	0.6237	0.1858	0.3497	0.4969	0.3742	0.4649	0.1504	0.7760
g_Sphingomonas	0.4401	0.3824	0.1438	0.7858	0.9077	0.0124	0.9890	0.0002
g_Bradyrhizobium	0.4386	0.3842	0.5645	0.2431	0.9750	0.0009	0.8462	0.0336
g_Ralstonia	0.1932	0.7138	0.1766	0.7379	0.3216	0.5343	0.2732	0.6004
g_Brevundimonas	0.4124	0.4164	0.4849	0.3297	0.4900	0.3238	0.8125	0.0494
g_Aquabacterium	0.7724	0.0718	0.5399	0.2688	0.3622	0.4805	0.3206	0.5355
g_Chryseobacterium	0.5098	0.3015	0.6212	0.1880	0.3839	0.4523	0.3037	0.5585
s_Comamonas aquatica	0.4683	0.3489	0.1776	0.7299	0.5684	0.2392	0.7329	0.0974
g_Hyphomicrobium	0.5568	0.2512	0.4918	0.3217	0.8145	0.0484	0.9916	0.0001
s_Bradyrhizobium elkanii	0.4002	0.4317	0.4069	0.4233	0.9177	0.0099	0.9410	0.0051
s_Pseudomonas aeruginosa	0.6660	0.1487	0.9255	0.0081	0.8040	0.0538	0.7480	0.0873
s_Brevundimonas bullata	0.0710	0.8937	0.4436	0.3782	0.6256	0.1840	0.8822	0.0200
s_Rhizobium phaseoli	0.4429	0.3791	0.3895	0.4453	0.8039	0.0539	0.7622	0.0781

Data are based on analyses of three *B. striata* samples from each soil type described in Fig. 2A. Root length, dry weight of roots and tubers served as an indicator of growth; militarin, as indicators of secondary metabolite in the tuber of *B. striata*. Correlations were considered significant if $|R| > 0.3$ and $P < 0.05$

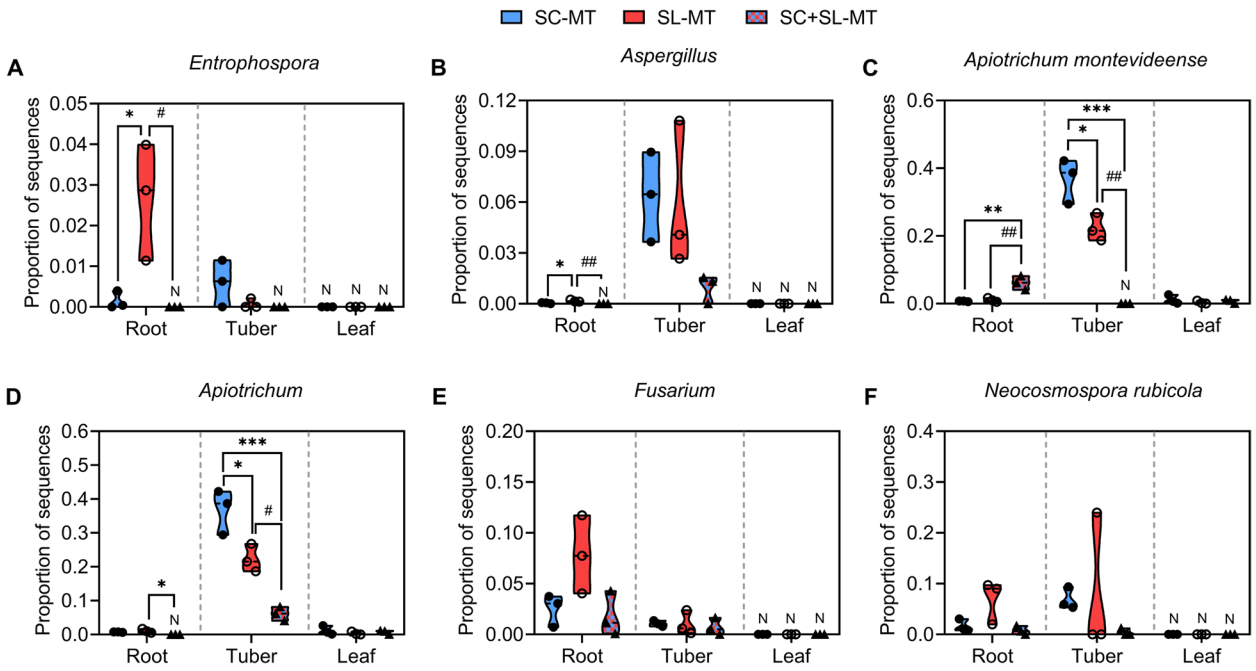

Fig. 9 Relative abundances of fungi in different *B. striata* tissues

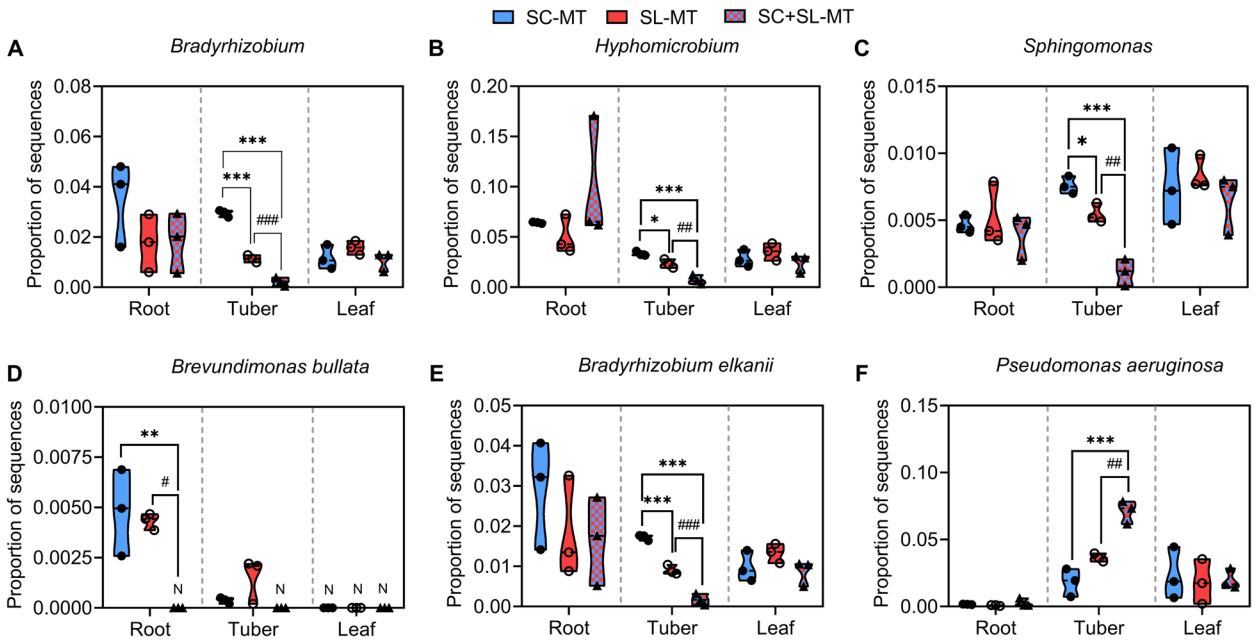

Fig. 10 Relative abundances of bacterial in different *B. striata* tissues

to 12 h of light per day. Administer weekly sterile water irrigation according to specific growth requirements (heating tap water to 121 °C for 20 min and then cooling). Implement manual control measures for pest and disease management.

Biomass of B. striata

In April 2022, we quantified the growth indices of each plant under varying treatment conditions. The total weight of *B. striata* plants, root length, fresh root weight, dry root weight, tuber volume, fresh tuber weight, dry tuber weight, and leaf length were all quantified

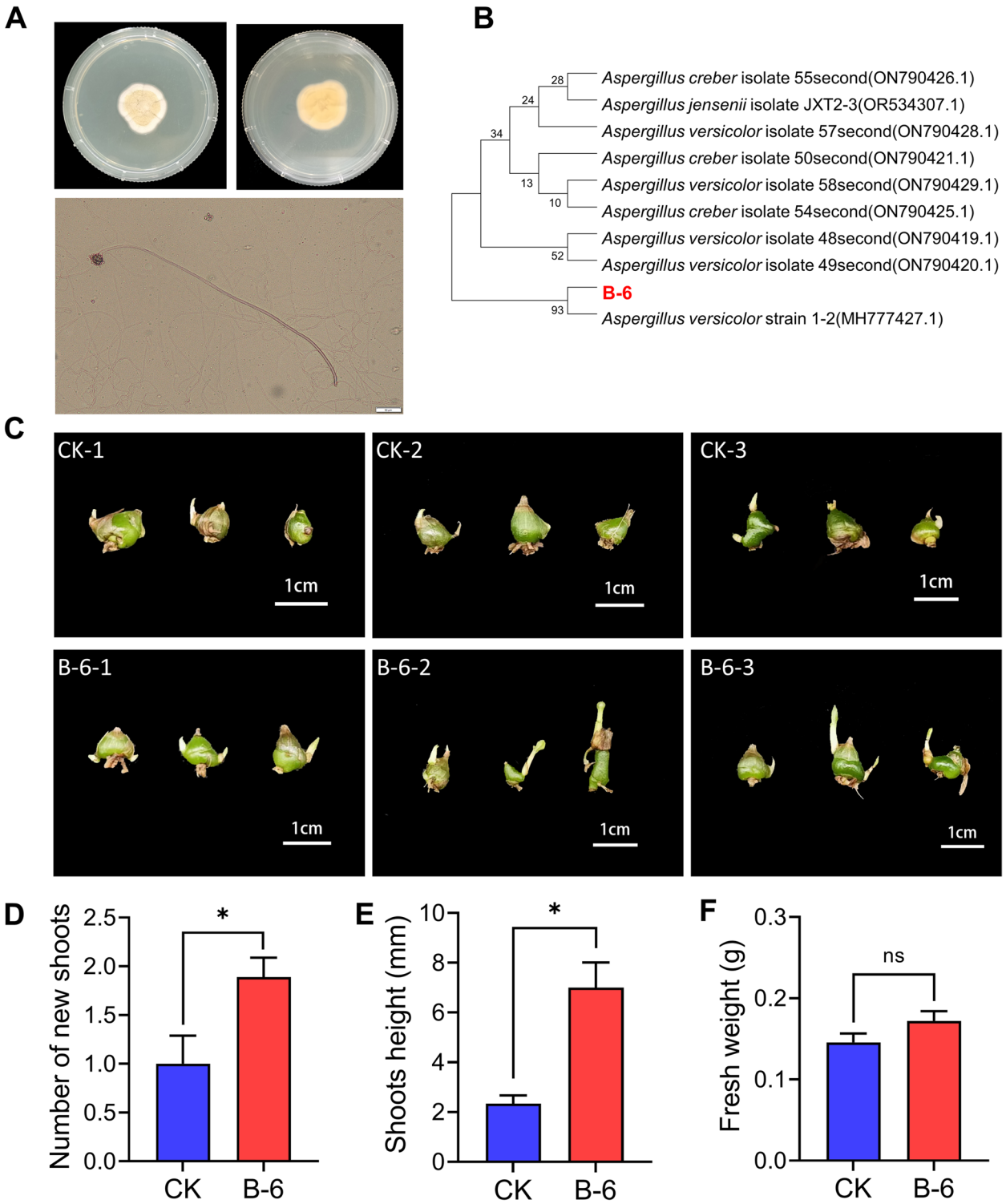

Fig. 11 Isolation of B- 6 and its effect on *B. striata* sterile seedlings growth and development. **A** Growth of B- 6 strains identified from sandy loam soil after 7 days of incubation in nutrient medium of PDA, and micrograph of mycelium of strain B- 6 (fold 20 × 10). The diameter of the Petri dish was 60 mm. **B** The phylogenetic tree of B- 6 was constructed. The fungal DNA sequence fragment of the same genus (bp 500–600) was downloaded from NCBI to construct the phylogenetic tree, and the similarity homology alignment was greater than 90%. **C** *B. striata* sterile seedlings were grown under MS medium conditions and inoculated with B- 6 (20 d). **(D-F)** Assessment of various indicators of plant growth. Data are mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. All tests were one-way ANOVA with Tukey's multiple-comparisons test

as growth indicators. The dry weights of *B. striata* tissues were determined using an electronic scale (Mettler Toledo, Shanghai, China), following oven drying at 60 °C to achieve a constant weight [26].

Sequencing analysis of B. striata microorganisms

Two years post-microbial transplantation, combine soil and *B. striata* in sterile bags, and transfer them to a laboratory environment at 4℃. *B. striata* should undergo laboratory processing to remove any lumpy soil through gentle shaking and tapping [25]. Only soil particles firmly attached to the *B. striata* tubers and roots (at a distance of approximately 2 mm within the soil layer) are considered. We collected rhizosphere soil from each *B.striata* specimen and also obtained *B. striata* roots, tubers, and leaves. The rhizosphere soil, roots, tubers, and leaves from each of the ten *B. striata* samples collected in individual pots were subsequently combined into a single portion. Prior to analysis, the rhizosphere soil sample is preserved at − 80 °C.

For analysis, the leaves were subjected to three consecutive washes using sterile water, followed by a 3-min disinfection process utilizing 75% alcohol. Subsequently, they were washed 2–3 times with sterile water and placed on sterile filter paper to facilitate moisture absorption. Roots and tubers were subjected to a multi-step decontamination process. Initially, they were rinsed 3–4 times in sterile water, followed by a 3-min soak in 75% alcohol. This was followed by another 3–4 rinses with sterile water, and a 7-min soak in 0.1% mercury solution. The specimens were then rinsed 3–4 times more with sterile water, placed on sterile filter paper to absorb moisture, and finally cut into segments approximately 2–3 mm long for roots or pieces with a volume of 2–3 mm3 for tubers using a sterile blade [27]. The segments or pieces were meticulously placed in sterile, self-sealing bags and securely stored at − 80 ℃. The diverse rhizosphere soil, roots, tubers, and leaves of *B. striata*, treated with various conditions, were sampled for 16S and ITS Illumina sequencing analysis.

The E.Z.N.A.® soil DNA kit (Omega Bio-tek, Norcross, GA, U.S.) was employed to extract total microbial genomic DNA from rhizosphere soil and tissue samples of *B. striata*, in accordance with the manufacturer's guidelines. 1% agarose gel electrophoresis was utilized to evaluate the quality of the extracted genomic DNA.

The amplification of the fungal ITS gene was achieved using primer pairs, including the ITS1 forward primer (5'- CTTGGTCATTTAGAGGAAGTAA- 3') and the ITS2 reverse primer (5'- GCTGCGTTCTTCATCGAT GC- 3'). Additionally, the hypervariable region V3-V4 of the bacterial 16S rRNA gene was amplified through the use of primer pairs, such as 799 F (5'-AACMGGATTA GATACCCKG- 3') and 1193R (5'- ACGTCATCCCCA CCTTCC- 3') [28]. The identification and recovery of PCR products were achieved through the application of 2% agarose gel electrophoresis. Subsequently, these products were purified using the PCR Clean-Up Kit (YuHua, Shanghai, China), following the manufacturer's guidelines. The quantities of the purified products were determined employing Qubit 4.0 (Thermo Fisher Scientific, USA).

Eventually, the purified and amplified fragments were coalesced in equimolar proportions and subjected to paired-end sequencing utilizing the Illumina PE300/PE250 platform (Illumina, San Diego, USA). Subsequently, the raw data were uploaded to the NCBI SRA database. The sequences of DNA fragments from sandy loam soil and sandy clay soil are accessible at https://www.ncbi.nlm.nih.gov/sra/PRJNA830871. After microbial transplantation, the DNA sequences of the microorganisms from the *B. striata* rhizosphere soil, roots, tubers, or leaves were uploaded to https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA1012366, https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA1003225.

The analysis of metabolites in B. striata tuber

The tubers from each group of *B. striata* were dried at 50℃, after which the samples from each group were combined into a single sample. These combined samples were then evenly divided into three portions. The processed samples were subsequently sent to Majorbio Bio-Pharm Technology Co. Ltd (Shanghai, China) for UHPLC-MS/MS analysis, utilizing an ACQUITY UPLC HSS T3 column (100 mm × 2.1 mm, 1.8 μm; Waters, Milford, USA) [29]. The mobile phase A consisted of a 95:5% water-acetonitrile mixture (containing 0.1% formic acid), while the mobile phase B consisted of a 47.5:47.5:5% acetonitrile-isopropanol-water solution (also containing 0.1% formic acid). The injection volume was 3 μL, and the column temperature was maintained at 40℃. Mass spectrometry data were collected in both positive and negative modes using a Thermo UHPLC-Q Exactive HF-X mass spectrometer equipped with an electrospray ionization (ESI) source.

The tubers were meticulously prepared as per the instructions, dried at a temperature of 50 °C, and meticulously crushed into a fine powder. A total of three samples were included in each group. According to the standards of *B. striata* of dried tubers specified in the 2020 Pharmacopoeia of the People's Republic of China, the determination of militarine was conducted. An aliquot (0.2 g) of powder was added to 25 mL of 50% (v/v) ethanol in water, the solution was weighed precisely, it was ultrasonicated for 30 min at 120 W and 40 Hz, the solution was weighed again, 50% ethanol solution was added to

adjust for any missing weight, and the final solution was passed through a 0.45 μm filter. An aliquot was analyzed using a 2690 high-performance liquid chromatography system (Waters, Milford, MA, USA), on which the sample was passed through an octadecylsilane bonded silica gel column with a mobile phase of acetonitrile: 0.1% phosphoric acid (22: 78). The two secondary metabolites were detected based on absorption at 223 nm, and the theoretical tray number was at least 2000 [30]. A 1.63 mg/mL solution in 50% (v/v) ethanol in water was made as the militarine reference solution.

Isolation and identification of rhizosphere fungi

Following the transplantation of the sandy loam microorganisms, a portion of 1 g of *B.striata* rhizosphere soil was vigorously shaken in 9 mL of sterile water for a duration of 30 min. Subsequently, 30 μL of soil diluent was carefully aspirated and evenly coated on a potato dextrose agar (PDA) plate [31]. After a continuous cultivation period of 3–7 days at a temperature of 27℃ and a humidity level of 60%, single colonies were selected for further purification and cultivation. In this study, DNA was isolated from individual fungi using a Ezup Column Fungi Genomic DNA Purification Kit (B518259, Sangon Bioengineering (Shanghai) Co., LTD, China), following the kit's instructions. The quality of extracted genomic DNA was assessed via 1% agarose gel electrophoresis, followed by the preparation of templates using the collected DNA. The primers ITS1/ITS4[27]were employed to amplify the fungal ITS gene using a PCR amplifier (C1000 Touch, Bio-Rad, USA). A phylogenetic tree of fungi was constructed based on the Illumina sequencing database and sequence alignment of isolated fungi [32].

Co-culture of B. striata sterile seedlings with potential pro-growth fungi

In this study, we selected fungus, isolated from the *B.striata* rhizosphere soil following the transplantation of sandy loam microorganisms, to co-culture with *B.striata*, in order to investigate the growth-promoting ability of *B. striata*. The fungus was cultured on a potato dextrose agar (PDA) plate for 5 days. Subsequently, the fungus was inoculated into a potato dextrose agar (PDA)[31]broth (150 mL, without agar) and incubated in a constant temperature shaking incubator (27 °C, 180 RPM) for an additional 5 days, with continuous shaking. The mycelium and fermentation broth of the fungus were meticulously separated using sterile gauze, and the fermentation broth of the fungus was subsequently collected. The fermentation broth (2 mL) of the fungus was added to the MS liquid medium (50 mL) [33] followed by the inoculation of three *B. striata* sterile seedlings in each tissue culture flask. The *B. striata* sterile seedlings were then fixed using filter paper [34]. The control group was inoculated with PDA medium (2 mL, without agar) into MS liquid medium (50 mL) under identical treatment conditions as the experimental group. Three tissue culture flasks were inoculated in each group. The cultures were grown in a greenhouse (26°23′17.68"N, 106°37′43.18"E) at Guizhou University of Traditional Chinese Medicine for a duration of 20 days, maintained at a temperature range of 23–25 °C, humidity between 40 and 50%, and provided with 12 h of light per day.

On the twentieth day post-inoculation, we evaluated three physiological parameters: shoot height, the number of new shoots, and fresh weight.

Statistical analyses

Utilizing the Majorbio Cloud platform (https://cloud.majorbio.com), we conducted bioinformatics analyses on plant rhizosphere soil and tissues. Calculate the Mothur v1.30.1 Diversity Index, based on the OTU data, by considering the Ace Index, Chao Richness, Simpson Index, and Shannon Index. Apply principal coordinate analysis (PCoA) and principal component analysis (PCA), utilizing the Bray–Curtis distance, to estimate the degree of similarity between microbial communities in various samples. To identify the significantly numerous bacterial and fungal taxa (genus to species) in various groups (LDA score > 2, $p < 0.05$), Linear Discriminant Analysis (LDA) Effect Size (LEfSe) (http://huttenhower.sph.harvard.edu/LEfSe) was used [35].

The metabolomic data were processed utilizing a freely accessible online cloud platform (cloud.majorbio.com) for Principal Component Analysis (PCA), Venn diagram, identification of significantly differential metabolites, hierarchical clustering heatmap, metabolite correlation analysis, and KEGG pathway annotation and enrichment. Phylogenetic trees were constructed using MEGA 7.0.26 (Molecular Evolutionary Genetics Analysis).

The software program GraphPad Prism 8.0.1 (GraphPad, Chicago, IL, USA) was used for all statistical calculations. For 3–6 separate experiments, data were shown as mean standard error of the mean. Student's two-tailed t tests were used to determine the significance of pairwise comparisons, and one-way ANOVA and Tukey's post hoc tests were used to determine the significance of comparisons involving three or more values. Three levels of significance were used to define significance: significant at $p < 0.05$, very significant at $p < 0.01$, and highly significant at $p < 0.001$.

Discussion

Here we demonstrate that, compared to other plants with economic or medicinal value [36–38], the microbial communities that colonize the roots, tubers and leaves

of *B. striata* can influence its growth and production of secondary metabolites. These findings may help guide efforts to supplement the soil of this medicinally important plant in order to optimize its yield and quality. Our results suggest that *B. striata* grows better in sandy loam soil because its tissues can be colonized by *Fusarium*, *Teleomorph*, *Streptomyces* and *Sphingomonas.* At the same time, we found that levels of the secondary metabolites militarinein tubers were higher in sandy clay soil, which may depend on tissue colonization by *Fusarium* and *Sphingomonas*. The importance of these taxa to *B. striata* growth and quality should be explored further. At the very least, our results suggest that characteristics of soil microbial communities help explain why cultivating *B. striata* in sandy clay soil leads to significantly higher levels of polysaccharides, glycosides, and total phenols than cultivating it in sandy loam soil [23].

Our work identified several microbial taxa that differentially colonized *B. striata* tissues. *Fusarium* wilt TR4 mycolia is mainly colonized in the vascular tissue of the root, and the hyphae in the vascular bundle tissue of the stem cortex in the bulb extend towards the central column [39]. *Fusarium* can colonize plant roots, which is consistent with the findings of our study. *Fusarium oxysporum* can promote the germination of *B. striata* seeds [40], similarly, our study revealed that *Fusarium*, predominantly found in sandy loam soil, plays a positive role in the growth and development of *B. striata*. Future studies should focus on identifying additional Fusarium species associated with the growth and development of *B. striata*. *Streptomyces* strain AgN23 colonizes the leaves of plants with terminated filaments in vesicular structures, penetrating the stomata [13]. *Streptomyces* microorganisms can promote uptake and transport of phosphorus by plants [41], this phenomenon promotes plant growth and development, which is consistent with the findings of our study. Based on these findings, future research can explore additional microbial species that promote the growth in *B. striata*. In our study, correlation analysis suggests that *Sphingomonas* and *Bradyrhizobium* species may have a positive impact on the growth of *B. striata* and the accumulation of its secondary metabolites. Inoculation with *Sphingomonas sp*. and *Micrococcus luteus* can promote the growth of willows (*Salix viminalis* x *caprea*) [42]. *Sphingomonas sp*. SaMR12 first colonizes the lateral root surface and taproot to root hair area of rice, forming biofilm-like structures on the root surface. Then it enters the root hair cell and cortical cell space, ultimately colonizing the vascular tissue of taproot root while also regulating metal absorption and growth of host plants [43]. Our research has revealed that *Sphingomonas* species are capable of colonizing the tubers of *B. striata*, exerting a positive influence on tuber growth. Inoculation with *Bradyrhizobium japonicum* can increase soybean yield and increase levels of nitrogen and phosphorus available in the soil [44], which is consistent with the findings of our study. *Bradyrhizobium elkanii* is a common Rhizobia known to promote nitrogen fixation by plants [45], and ours appears to be the first report that this taxon can promote the synthesis and accumulation of secondary metabolites.

Purified from the rhizosphere soil of sandy loam microorganisms following transplantation, our research identified *Aspergillus versicolor* as a factor capable of enhancing the growth and development of *B.striata*. Related studies have demonstrated that the endophytic fungus *Aspergillus cejii* DMKU-R3G3 enhances plant growth and improves crop yield by producing plant growth-promoting factors, such as phytohormones, antioxidants, and essential nutrients. [46]. *Aspergillus* section Nigri indirectly promotes plant growth by solubilizing phosphate in the soil and producing phytohormones, such as ethylene, which may positively influence plant growth and development [47]. These studies confirm that certain *Aspergillus* species can promote plant growth and development, which is consistent with the findings of our research. Future work should verify our results about the effects of these microbial taxa on growth and secondary metabolites and explore whether they can exert similar effects on other types of plants. Such work should also systematically examine how soil type [48, 49], soil physical and chemical properties [50, 51] and age at planting [52] modulate the beneficial effects of microbes, since these factors are known to affect the composition and diversity of endophytic microbes in plants.

Conclusion

In this study, the microbial communities from sandy clay and sandy loam were inoculated and utilized for the cultivation of Bletilla striata. An analysis was conducted on the specific colonization of rhizosphere microorganisms in different tissues of *B. striata*, which subsequently promotes the growth of *B. striata* and the synthesis of secondary metabolites. Rhizosphere microorganisms were found to colonize the roots, tubers, and leaves of *B. striata* to varying degrees, influencing its growth, development, and the synthesis of secondary metabolites. Microorganisms such as *Entrophospora*, *Aspergillus*, *Fusarium*, *Sphingomonas* and *Hyphomicrobium* are considered to play a significant role in this process.To further validate these findings, *Aspergillus versicolor*, isolated from sandy loam soil after transplantation, was shown to significantly promote the growth and development of *B. striata*. These findings lay the groundwork for the development of specific rhizosphere microbial inoculants

to cultivate *B. striata*, thereby enhancing its yield and quality.

Abbreviations

SC	Sandy clay soil
SL	Sandy loam soil
MT	Microbiota transplantion
SC-MT	Microbiota transplantation from sandy clay soil
SL-MT	Microbiota transplantation from sandy loam soil
SC + SL-MT	Microbiota transplantation from both types of soil
PCA	Principal Component Analysis
PCoA	Principal Coordinate Analysis
LDA	Linear Discriminant Analysis
LEfSe	LDA Effect Size
PDA	Potato dextrose agar medium
MS	Murashige and Skoog medium

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Authors' contributions
Conceptualization, Q.X. and Y.F.; Investigation, C.Y.X.; Methodology, J.Z. and C.H.X.; Project administration, C.H.X. and T.Z.; Resources, Q.Y.; Supervision, J.X.; Validation, W.J.; Visualization, C.Y. and Y.Z.; Writing-original draft, Q.X.; Writing-review & editing, C.H.X.

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Data availability
The datasets generated and analysed during the current study are available in the NCBI Sequence Read Archive (SRA) repository under BioProject accession number PRJNA830871, PRJNA1012366, PRJNA1003225.

Declarations

Ethics approval and consent to participate
This study did not involve animal or human trials.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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