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Qingqing Xu

Guizhou University of Traditional Chinese Medicine

Yan Fu

Guizhou University of Traditional Chinese Medicine

Jinqiang Zhang

Guizhou University of Traditional Chinese Medicine

Chunyun Xu

Guizhou University of Traditional Chinese Medicine

Changgui Yang

Guizhou University of Traditional Chinese Medicine

Qingsong Yuan

Guizhou University of Traditional Chinese Medicine

Jiao Xu

Guizhou University of Traditional Chinese Medicine

Weike Jiang

Guizhou University of Traditional Chinese Medicine

Yongping Zhang

Guizhou University of Traditional Chinese Medicine

Tao Zhou

Guizhou University of Traditional Chinese Medicine

Chenghong Xiao

xiaochenghong1986@126.com

Guizhou University of Traditional Chinese Medicine

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The colonization of soil microorganism affected the growth and the accumulation of secondary metabolites of *Bletilla striata* (Thunb.) Rchb. F.

Qingqing Xu ^{a†}, Yan Fu ^{a†}, Jinqiang Zhang ^a, Chunyun Xu ^a, Changgui Yang ^a, Qingsong Yuan ^a, Jiao Xu ^a, Weike Jiang ^b, Yongping Zhang ^b, Tao Zhou ^{a*}, Chenghong Xiao ^{a*}

^a Resource Institute for Chinese and Ethnic Materia Medica, Guizhou University of Traditional Chinese Medicine, Guiyang, China

^b College of Pharmacy, Guizhou University of Traditional Chinese Medicine, Guiyang 550025, China.

[†] Qingqing Xu and Yan Fu contributed equally to this work.

* **Correspondence:** Corresponding Author: Tao Zhou*, E-mail: taozhou88@163.com; Chenghong Xiao*, E-mail: xiaochenghong1986@126.com.

Abstract

Bletilla striata (Thunb.) Rchb. F., a perennial herbaceous plant known for its medicinal properties. The growth and secondary metabolite production of *B. striata* can be influenced by soil microorganisms. To investigate the soil microbes that enhance *B. striata* growth and quality, we planted *B. striata* sterile seedlings in radiation-sterilized soil amended with microbiota from sandy clay or sandy loam soils. After two years of growth, we conducted 16S and ITS Illumina sequencing to identify bacteria and fungi colonizing *B. striata* rhizosphere soil, roots, tubers, and leaves. We determined the growth indices of the *B. striata*, simultaneously, we used UHPLC-MS/MS to determine metabolites in *B. striata* tubers, finally, we quantified the index component militarine in *B. striata*, we found significant differences in *B. striata* metabolites among different soil microbial treatments, with sandy loam microorganisms promoting the growth of *B. striata*, while sandy clay microorganisms increased the content 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 montevidense*). The *Aspergillus versicolor* (B-6) isolated from the *B. striata* rhizosphere soil after transplantation of sandy loam microorganisms, after inoculation with B-6 with *B. striata* sterile seedlings, it was found that B-6 could promote *B. striata* sterile seedlings growth. Our analysis uncovers that the soil microorganisms can colonize various regions of *B. striata*, thereby influencing its growth and secondary metabolite production. These findings hold implications for enhancing the yield and quality of *B. striata* in both medicinal and agricultural contexts.

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

1 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]. The endophytic microorganisms primarily inhabit

plant organs, have the potential to directly or indirectly impact plant growth and development[4, 5], they can enhance the stress tolerance of host plants, as well as increase their yield and accumulation of secondary metabolites. For example, the mycelium of *Streptomyces* (AgN23), was able to colonize the leaf surface, leading to plant resistance against *Alternaria brassicicola* infection in wild-type *Arabidopsis* plants[6]. Additionally, *Bacillus pumilus* strain JPVS11 enhanced the growth attributes of rice[7]. Furthermore, *Paenibacillus konkukensis*, *Paenibacillus kribbensis*, and *Paenibacillus taichungensis* mildly induced β -eudesmol and atractylon accumulation in the root of *Atractylodes lance*[8]. Therefore, endophytic microbes play a significant role in research purposes in enhancing the biomass and secondary metabolites of host plants.

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[9], plant root exudates can alter the diversity and function of microbial populations by providing a nutritious environment for rhizosphere bacteria[10, 11]. Precisely, it has been documented that plants secrete specific metabolites to attract microorganisms. The microorganisms are capable of perceiving and responding to these metabolites, so the microbes assemble around the plant. The microorganisms that gather around the plant feed back to the plant by providing nutrients, providing a suitable environment, and influencing metabolite accumulation [12]. In order to colonize the plant body, the microorganisms associated with the root surface traverses into the plant's interior, transforming into an endophytic microorganisms capable of stimulating plant growth and secondary metabolite accumulation[7, 13-15].

Bletilla striata (Thunb.) Rehb. 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[16]have been utilized for centuries in traditional Chinese medicine due to their diverse therapeutic properties, including antioxidant and anti-inflammatory effects[17, 18], 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[19]additionally to avoiding the development of lung cancer[20]. The soil microorganisms exert significant impacts on the growth and secondary metabolite production of *B. striata* [21]. 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*.

2 Results

2.1 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 gramineum*, *Knufia tsuneda*, *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., *Methylothermus*, *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*, *Microthecium*, *Blastococcus*, *Ilumatobacter*, *Luedemannella*, *Solirubrobacter*, *Gaiella*, *Rubrobacter* and *Paenibacillus alginolyticus*). We comprehensively characterized the distinct microbial communities colonizing sandy clays and sandy loams.

2.2 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 Chytridiomycota, 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 *Mortierella 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 bacteria in *B. striata* rhizosphere soil under each microbiota transplantation condition.

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

Next, we analyzed whether the different transplant conditions resulted in variations in the colonization of *B. striata* tissues by microbial communities. Indeed, the microbial communities within the roots differed across the three conditions in terms of various diversity indices (**Fig. 3A-B**). Among the nine phyla of fungi that we identified, in the root of *B. striata* containing microbiota from both sandy loam and sandy clay soils, Basidiomycota has the highest abundance, while Ascomycota and Glomeromycota were significantly more abundant, in roots of *B. striata* in soil containing microbiota from either soil type alone than in roots of *B. striata* in soil containing microbiota from both soil types ($p < 0.05$) (**Fig. 3C**). Of the six classes and six genera of fungi whose abundance in roots differed significantly across the three transplant conditions, Mortierellaceae and *Mortierella* were significantly enriched in roots of plants in soil containing microbiota from sandy clay soil; Glomeraceae, in roots of plants in soil containing microbiota from sandy loam soil; and Serendipitaceae, *Zopfiella* and *Serendipita*, in roots of plants in soil containing microbiota from both types of soil (**Fig. 3D**).

Among the 14 bacterial phyla that we identified, Proteobacteria was significantly less abundant, while Myxococcota and Firmicutes were significantly more abundant, in the roots of *B. striata* in soil containing microbiota from either soil type alone than in roots of *B. striata* in soil containing microbiota from both soil types ($p < 0.05$) (**Fig. 3E**). Of the 17 genera of bacteria whose abundance in roots differed significantly across the three transplant conditions, the following were significantly enriched in soil containing microbiota from sandy clay soil ($p < 0.05$) (**Fig. 3F**): *Nitrospira*, *Ammoniphilus*, *Desulfotomaculum*, *Ruminiclostridium*, *Gaiella*, *Cryptosporangium*, *Amycolatopsis*, *Phaselicystis*, and *Devosia*. *Pseudomonas* and *alphaI* cluster were significantly enriched in roots of plants in soil containing microbiota from sandy loam soil, while the following were significantly enriched in roots of plants in soil containing microbiota from the two types of soil: *Prosthecomicrobium*, *Azohydromonas*, *Phyllobacterium*, *Ideonella*, *Comamonas*, and *Caulobacter*.

As with *B. striata* roots, we found that microbial communities colonizing tubers differed significantly in composition depending on the soil source of the microbiota. These differences were visible across bacterial communities as different Shannon and Simpson indices (**Fig. 4A**), yet Bray-Curtis distances of the bacterial communities were similar to those of the fungal communities (**Fig. 4B**). Among the eight fungal phyla that we identified, Ascomycota were significantly more abundant in tubers in *B. striata* in soil containing microbiota from both soil types than in tubers of *B. striata* in soil containing microbiota from only one soil type, while the opposite was true of Basidiomycota ($p < 0.05$) (**Fig. 4C**). Of the four fungal classes and five fungal genera whose abundance in tubers differed significantly across the three transplant conditions, *Ceratobasidiaceae* were significantly enriched in tubers of plants in soil containing microbiota from sandy clay

soil, *Saccharomycetaceae* and *Issatchenkia* were enriched in tubers of plants in soil containing microbiota from sandy loam, and *Cladosporiaceae*, *Hypocreaceae*, *Cladosporium*, *Trichoderma*, and *Penicillium* were enriched in tubers of plants in soil containing microbiota from both soil types (**Fig. 4D**).

Of the 15 bacterial phyla that we identified, Proteobacteria were significantly more abundant in tubers of *B. striata* in soil containing microbiota from both soil types, while the opposite was true of Actinobacteriota and Chloroflexi (**Fig. 4E**). Of the 19 bacterial genera whose abundance in tubers differed significantly across the three transplant conditions, the following were significantly enriched in tubers of *B. striata* in soil containing microbiota from sandy clay soil ($p < 0.05$) (**Fig. 4F**): *Flavobacterium*, *Sphingobium*, *Hydrogenophaga*, *Microbacterium*, *Sphingomonas*, *Bradyrhizobium*, and *Ralstonia*. The following were enriched in tubers of plants in soil containing microbiota from sandy loam soil: *Romboutsia*, *Cloacibacterium*, *Acidovorax*, *Lachnoclostridium*, *Caulobacter*, *Streptococcus*, and *Arthrobacter*. The following were enriched in tubers of plants in soil containing microbiota from both soil types: *Sutterella*, *Pedobacter*, *Bacteroides*, and *Pseudomonas*.

Although we did not detect significant differences in alpha or beta diversity of fungal or bacterial communities in leaves of *B. striata* grown under the three transplant conditions (**Fig. 5A-B**), we did detect significant differences in the abundance of certain fungi and bacteria. Among the seven identified fungal phyla, Ascomycota was significantly more abundant in the leaves of *B. striata* in soil containing microbiota from both soil types than in the leaves of *B. striata* in soil containing microbiota from either soil type alone; the opposite was true of Basidiomycota (**Fig. 5C**). The abundances of fungal class Stachybotryaceae and fungal genus *Paramyrothecium* were significantly enriched in leaves of plants in soil containing microbiota from sandy clay soil ($p < 0.05$) (**Fig. 5D**).

Among the 15 bacterial phyla that we identified, Proteobacteria was significantly less abundant in leaves of plants in sandy loam soil than in leaves of plants in sandy clay soil, while the opposite was true of Firmicutes and Bacteroidota (**Fig. 5E**). The following bacterial genera were significantly enriched in leaves of plants in soil containing microbiota from both types of soil: *Trichococcus*, *Dorea*, *Limosilactobacillus*, *Capnocytophaga*, *Subdoligranulum*, *Leptotrichia*, and *Actinomyces* ($p < 0.05$) (**Fig. 5F**). *Sphingorhabdus* was enriched in leaves of plants in soil containing microbiota from sandy clay soil. We summarized the significant enrichment of fungi and bacteria in *B. striata* roots, tubers and leaves under each microbiota transplantation condition.

When we compared the fungal communities across the three plant tissues (roots, tubers, leaves) for *B. striata* grown under a given condition of microbiota transplantation, we detected several fungi that seemed to colonize only one of the three tissues. For example, in plants in soil containing microbiota from sandy clay soil, *Setophoma*, *Myrmecridium*, *Plectosphaerella*, *Fusarium*, and *Chytridiomycota* sp colonized specifically the root; *Aspergillus*, *Apiotrichum* (*A. montevidense*), and *Rozellomycota* sp colonized specifically the tuber; and the following colonized specifically the leaves: *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* (**Fig. 6**). We summarized the fungi and bacteria that specifically colonized each tissue under each microbiota transplant condition. (**Table 1**).

2.4 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, Fig. 9-10). 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 montevidense*, *Brevundimonas* and *Brevundimonas bullata*.

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 montevidense*, *Brevundimonas* and *Brevundimonas bullata*).

2.5 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*.

3 Figures

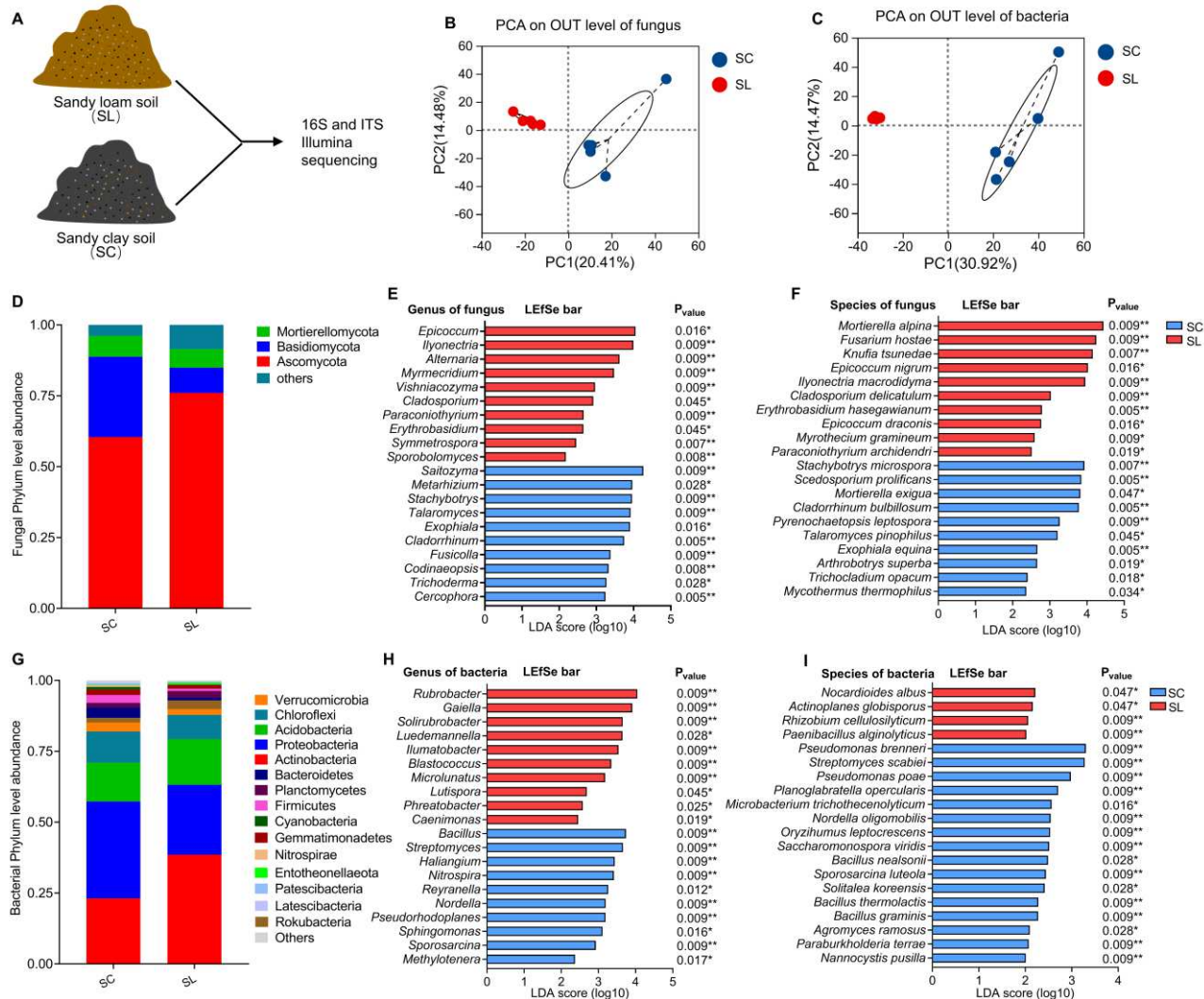


Figure 1. Different microbial communities in sandy clay soil or sandy loam soil. Sandy clay soil (SC) or sandy loam soil (SL) of the same type used in microbiota transplant experiments was analyzed using microbial metagenomics. (A) Analysis of 16S RNA and ITS sequences to characterize microbial communities. (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.

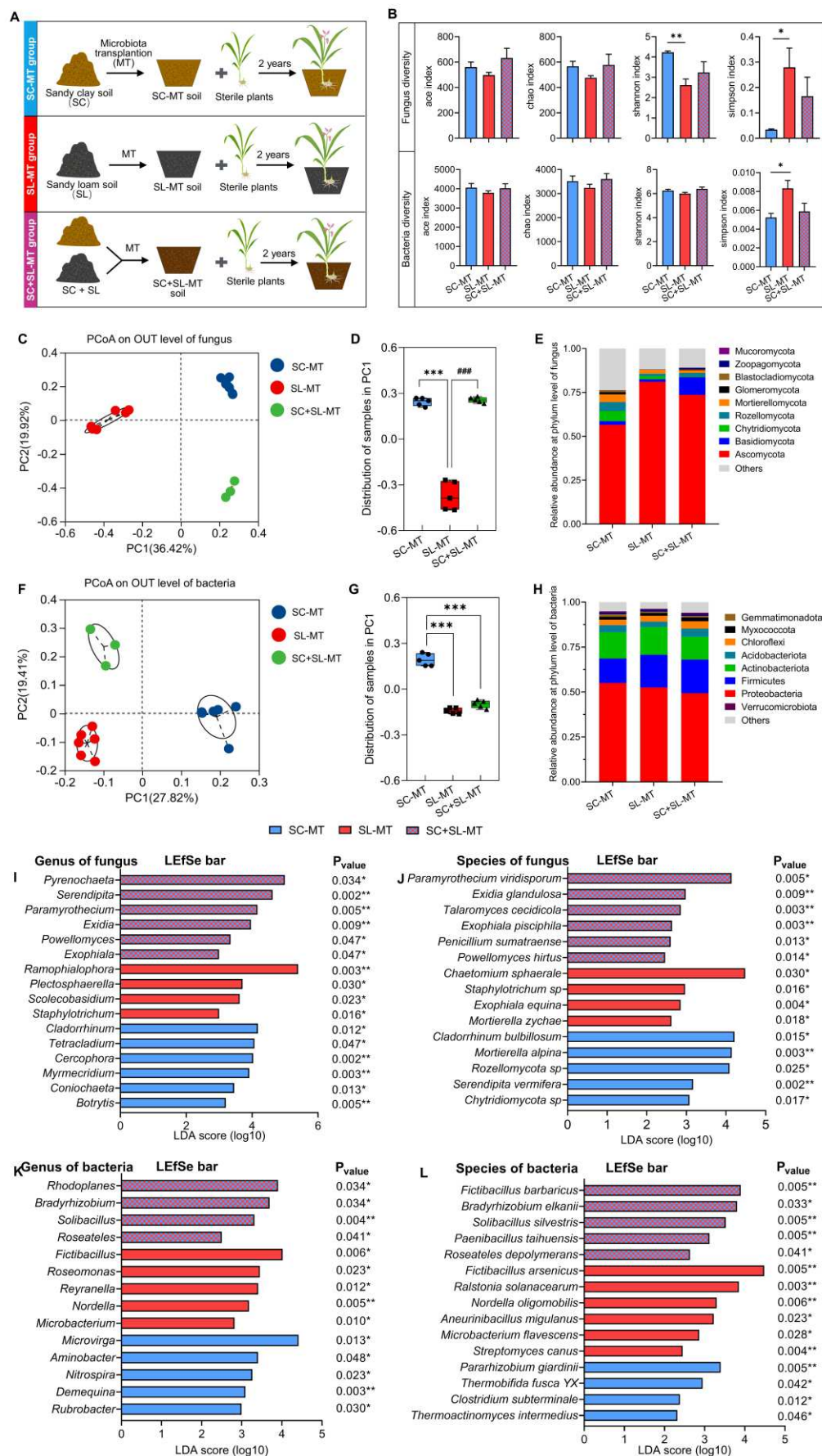


Figure 2. Microbial communities in soil after transplantation of microbiota from different types of soil. Mother soil was radiation-sterilized, then inoculated with sandy clay soil alone (SC-MT), sandy loam soil alone (SL-MT), or the two types together (SC+SL-MT).

(A) Schematic of microbiota transplantation experiments. **(B)** Alpha diversity in fungal and bacterial communities based on operational taxonomic units (OTUs). Data are mean $\pm$ 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. ** $p < 0.001$ 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.

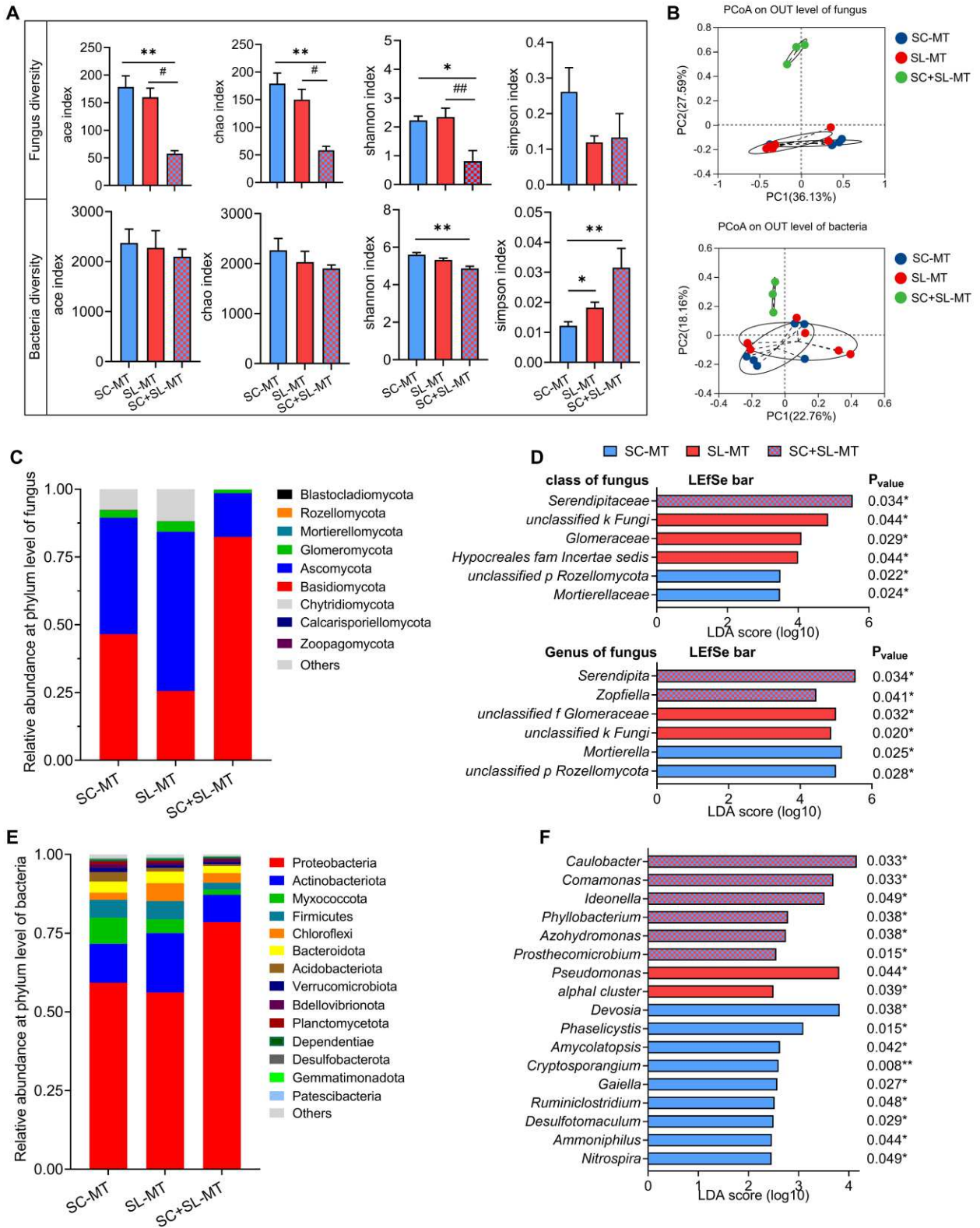


Figure 3. Differential colonization of *B. striata* roots by microbiota transplanted from sandy clay soil (SC) or sandy loam soil (SL). Roots of *B. striata* were cultivated under the three microbiota transplant conditions in Figure 2A and analyzed using microbial metagenomics.

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

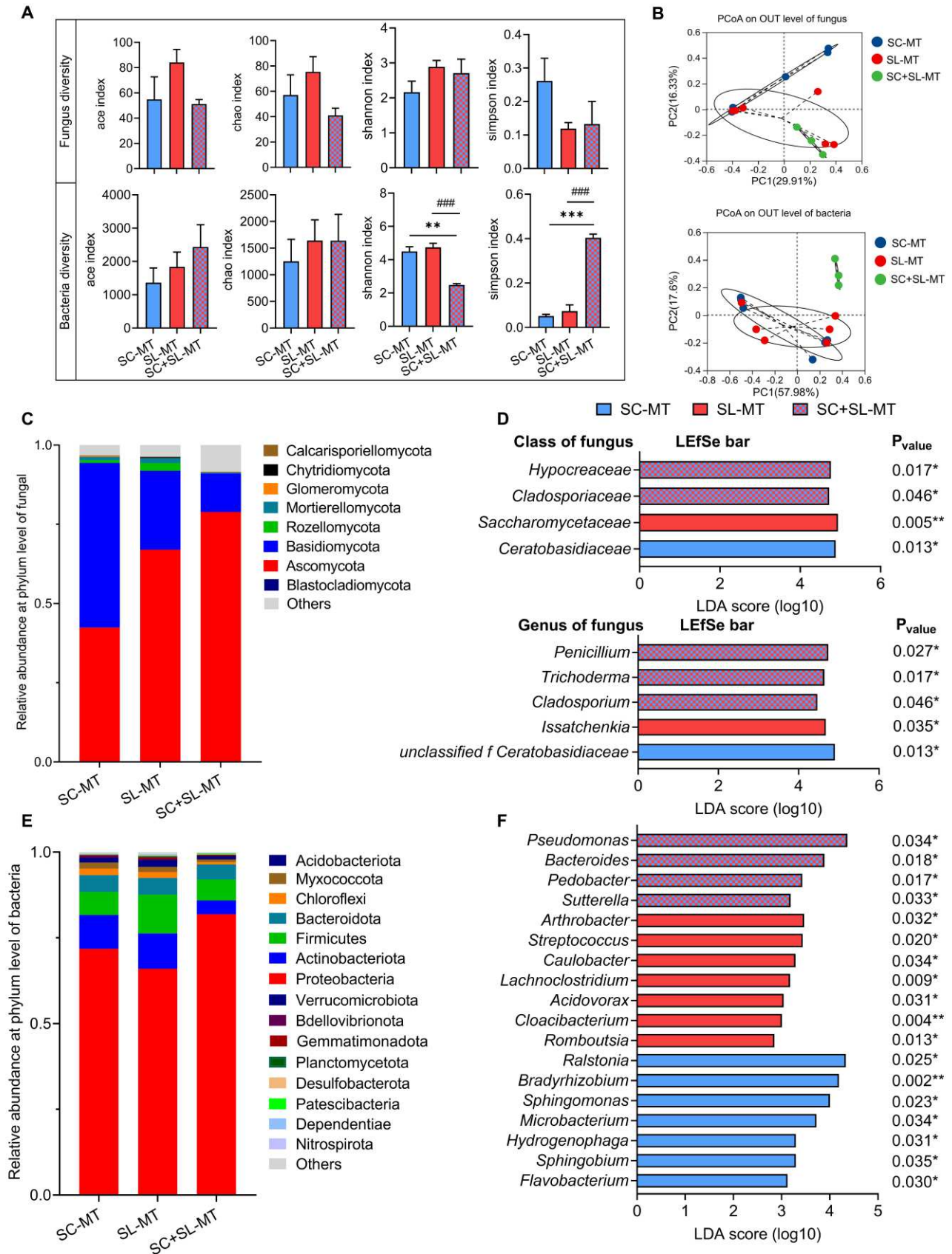


Figure 4. Differential colonization of *B. striata* tuber by microbiota transplanted from sandy clay soil (SC) or sandy loam soil (SL). Tubers of *B. striata* were cultivated under the three microbiota transplant conditions in Figure 2A and analyzed using microbial metagenomics.

(A) Alpha diversity in fungal and bacterial communities based on operational taxonomic units (OTUs). Data are mean $\pm$ 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.

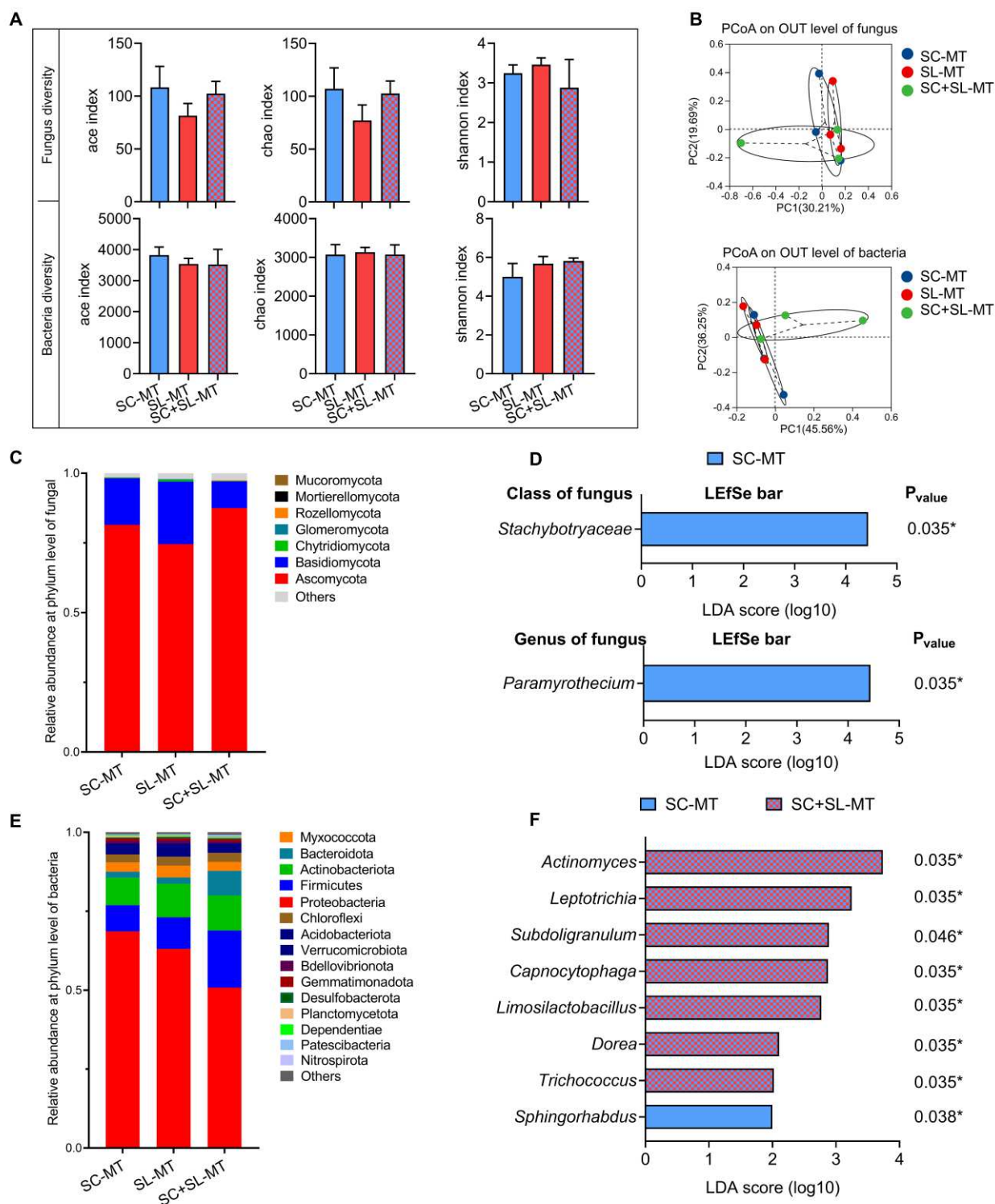


Figure 5. Differential colonization of *B. striata* leaves by microbiota transplanted from sandy clay soil (SC) or sandy loam soil (SL). Leaves of *B. striata* were cultivated under the three microbiota transplant conditions in Figure 2A and analyzed using microbial metagenomics.

(A) Alpha diversity in fungal and bacterial communities based on operational taxonomic units (OTUs). Data are mean $\pm$ 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.

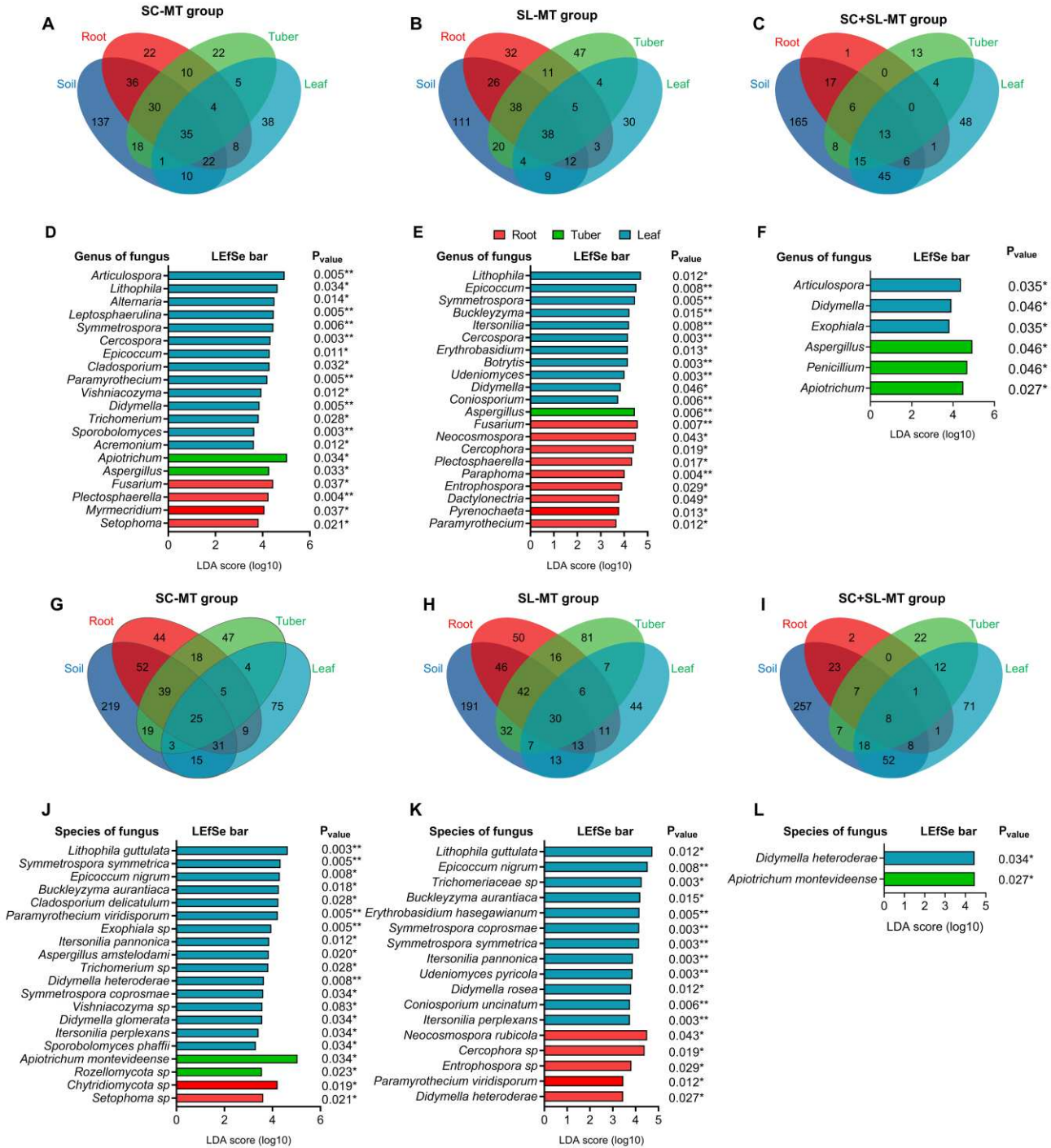


Figure 6. Differential colonization of *B. striata* tissues by different fungal taxa from different soil types. Roots, tubers, and leaves of *B. striata* were cultivated under the three microbiota transplant conditions in Figure 2A and analyzed using microbial metagenomics. Results for the original soil without *B. striata* cultivation ("soil") are shown for comparison. (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 Figure 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 $p < 0.05$.

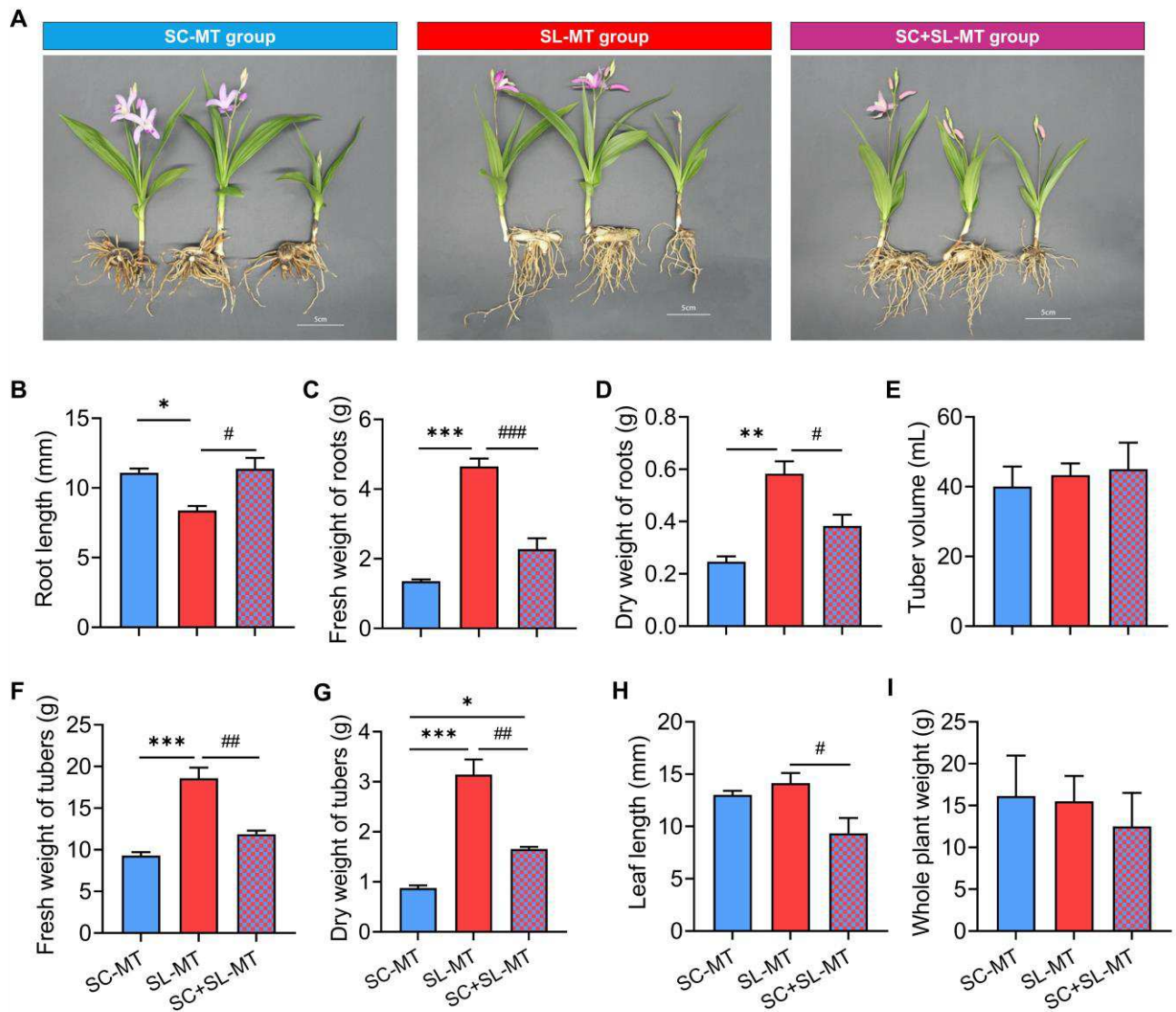


Figure 7. Influence of microbiota from sandy clay or loam soil on *B. striata* growth. *B. striata* was cultivated under the three microbiota transplant conditions in Figure 2A.

(A) Representative photographs of *B. striata*. (B-I) Assessment of various indicators of plant growth. Data are mean $\pm$ 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.

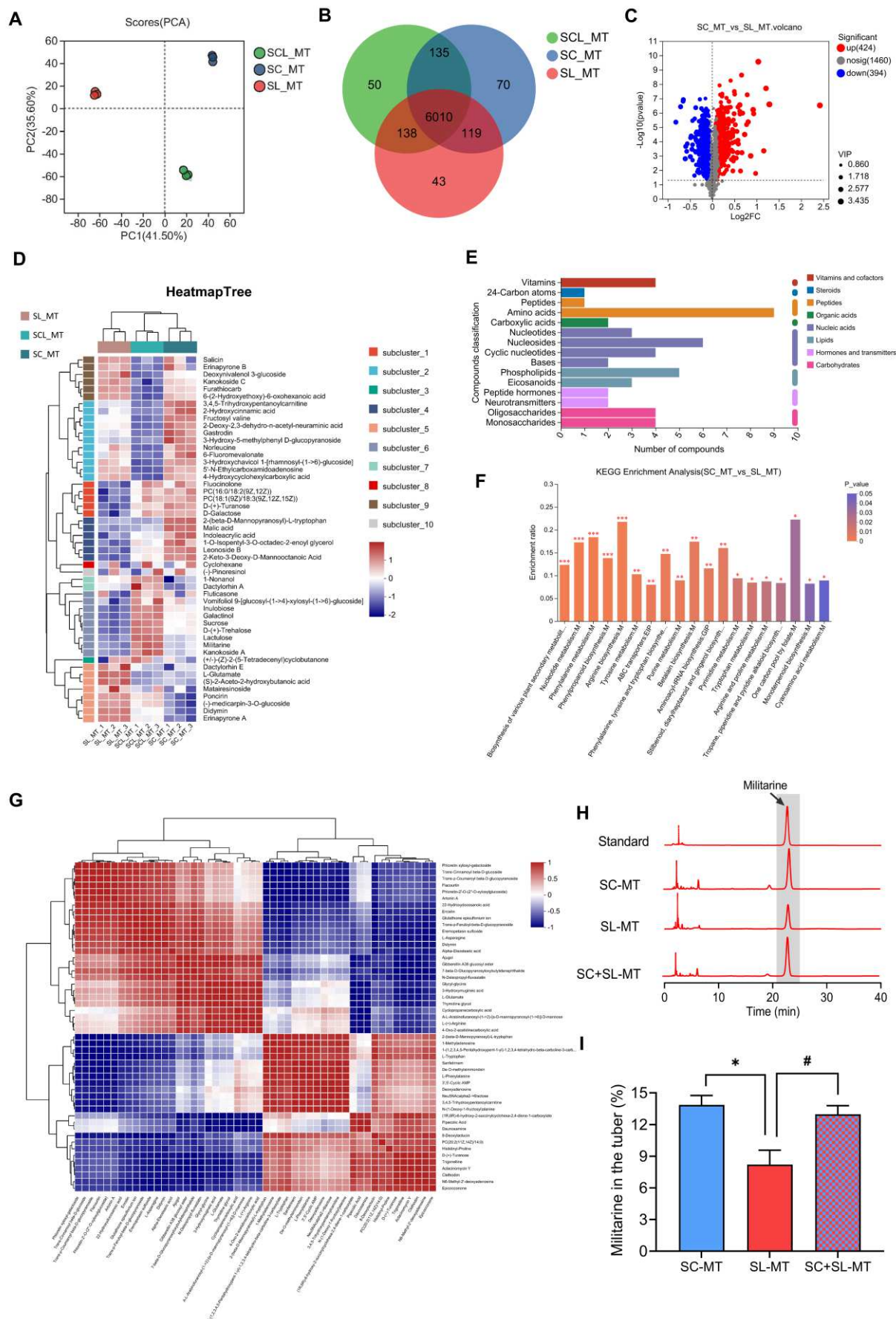


Figure 8: Metabolic profiling of *B. striata* by microbial community transplantation: A comparative analysis. *B. striata* was cultivated under the three microbiota transplant conditions in Figure 2A. (A) Plots of principal component analysis (PCA) scores for plant samples, with PC1 representing the 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 Pvalue 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 $\pm$ 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.

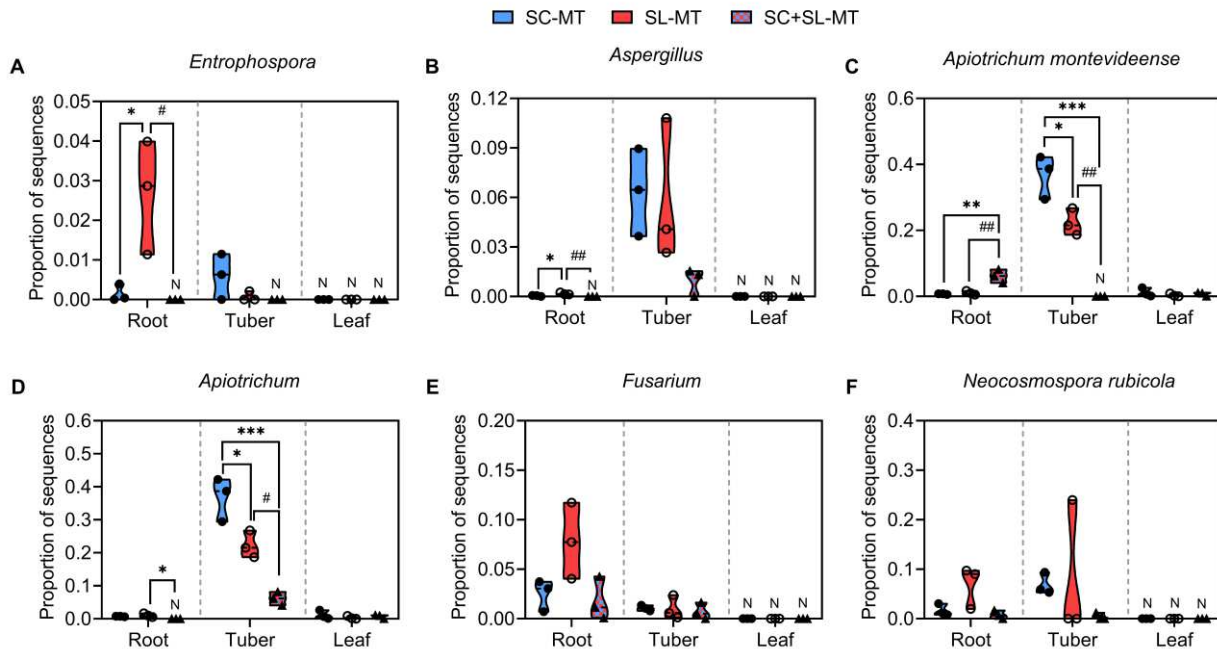


Figure 9. Relative abundances of fungi in different *B. striata* tissues. Roots, tubers, and leaves of *B. striata* were cultivated under the three microbiota transplant conditions in Figure 2A, then analyzed using microbial metagenomics.

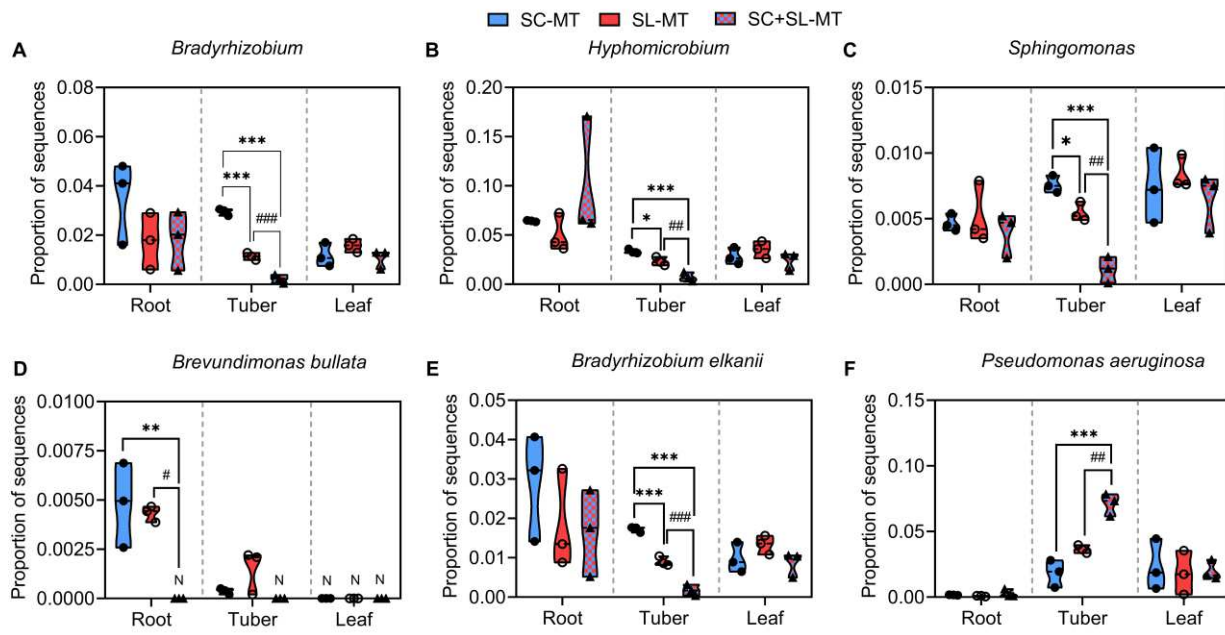


Figure 10. Relative abundances of bacterial in different *B. striata* tissues. Roots, tubers, and leaves of *B. striata* were cultivated under the three microbiota transplant conditions in Figure 2A, then analyzed using microbial metagenomics.

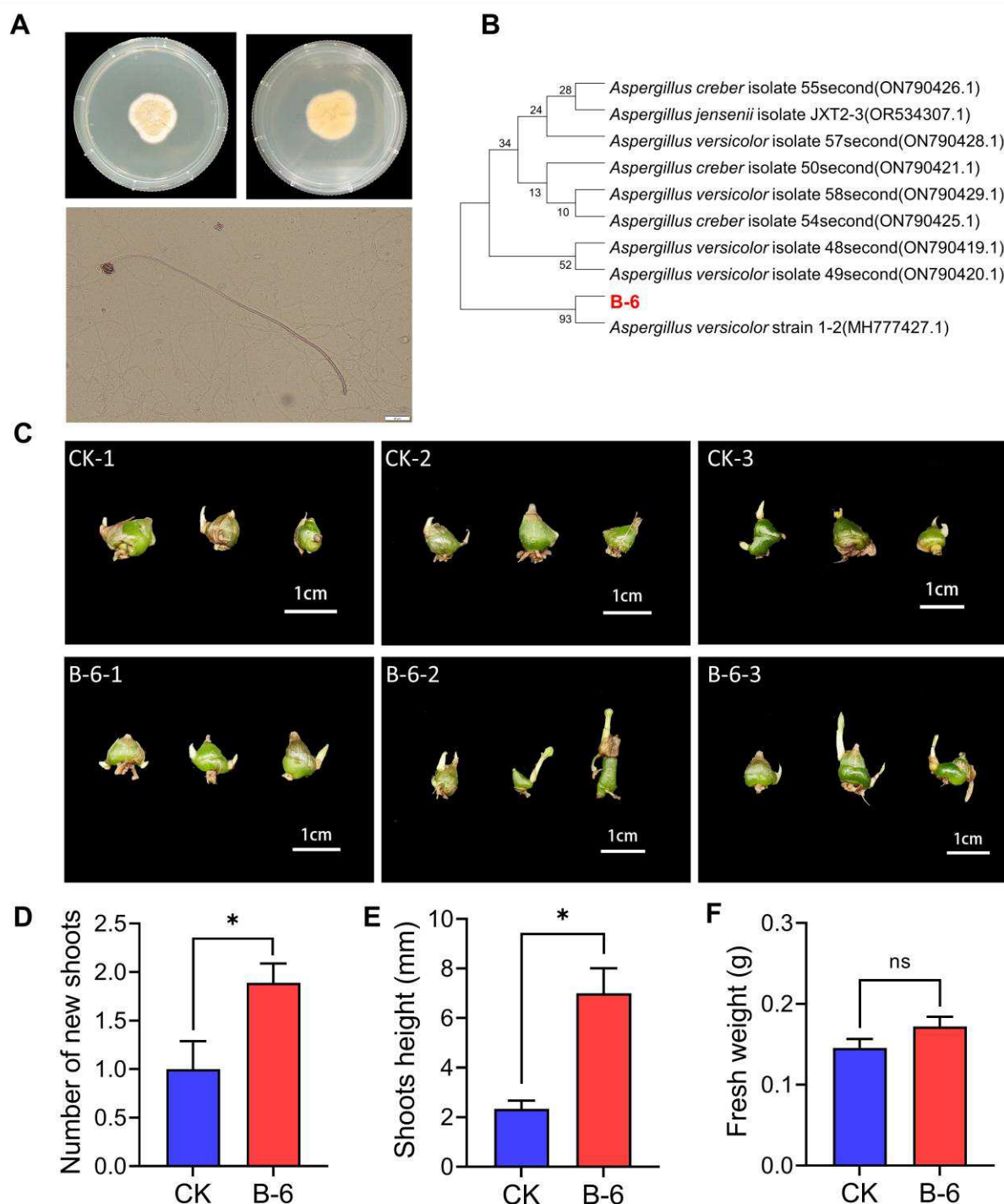


Figure 11: Isolation of B-6 and its effect on *B. striata* sterile seedlings growth and development.

(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 60mm. (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

346 of plant growth. Data are mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. All tests were one-way ANOVA with
347 Tukey's multiple-comparisons test.
348

350 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	<i>Sorangium</i> , <i>Phaselicystis</i> , <i>Rubellimicrobium</i> , <i>Reyranella</i> , <i>Lechevalieria</i> , <i>Microbacterium</i> , <i>Haliangium</i> , <i>Pseudorhodoplanes</i> , <i>Sphingomonas</i> , <i>Actinoplanes</i> , <i>Acidibacter</i> , <i>Devosia</i> <i>Pseudomonas</i> , <i>Hyphomicrobium</i>		<i>Gaiella</i> , <i>Nocardia</i> , <i>Pseudarthrobacter</i> , <i>Arthrobacter</i> , <i>Ramlibacter</i> , <i>Streptomyces</i>
	SL-MT group	<i>Sorangium</i> , <i>Nannocystis</i> , <i>Caldicoprobacter</i> , <i>Caldicoprobacter</i> , <i>Tumebacillus</i> , <i>Tumebacillus</i> , <i>Phaselicystis</i> , <i>Actinoplanes</i> <i>Arthrobacter</i> , <i>Chryseobacterium</i>		<i>Sporosarcina</i> , <i>Candidatus_Solibacter</i> , <i>Gaiella</i> , <i>Ureibacillus</i> , <i>Microvirga</i> , <i>Paenibacillus</i> , <i>Lysinibacillus</i> , <i>Rhodoplanes</i> , <i>Fictibacillus</i> , <i>Bacillus</i>
	SC+SL-MT group	<i>Tahibacter</i> , <i>Comamonas</i> , <i>Sutterella</i> , <i>Pseudorhodoplanes</i> , <i>Lechevalieria</i> , <i>Parabacteroides</i> , <i>Aquabacterium</i> , <i>Rhizobacter</i> , <i>Branchiibius</i> , <i>Pedobacter</i> <i>Hyphomicrobium</i>		<i>Nakamurella</i> , <i>Cutibacterium</i> , <i>Microvirga</i> , <i>Calditerricola</i> , <i>Solirubrobacter</i> , <i>Alkaliphilus</i> , <i>Propioniceella</i> , <i>Prevotella</i> , <i>Laceyella</i>
Species level	SC-MT group	<i>Lactobacillus johnsonii</i> , <i>marine</i> <i>Cutibacterium acnes</i> , <i>metagenome</i> , <i>metagenome</i> , <i>Microbacterium oxydans</i> , <i>Bdellovibrio bacteriovorus</i> , <i>Anaeromyxobacter dehalogenans</i> , <i>Rhizocola hellebori</i> , <i>Hyphomicrobium</i> <i>aestuarii</i> , <i>Cryptosporangium</i> <i>japonicum</i> , <i>Pedomicrobium</i> <i>manganicum</i>		<i>Lactobacillus brevis</i> , <i>Nocardia cyriacigeorgica</i> , <i>Aneurinibacillus danicus</i> , <i>Bacillus</i> <i>thermolactis</i> , <i>Arthrobacter globiformis</i> , <i>Streptomyces scabiei</i> , <i>Methylobacterium</i> <i>adhaesivum</i>
	SL-MT group	<i>Pseudonocardia halophobica</i> , <i>Clostridium subterminale</i> , <i>Hyphomicrobium aestuarii</i> , <i>Thermobifida fusca</i> YX , <i>Chthonobacter albigriseus</i> , <i>Tumebacillus avium</i> , <i>Anaeromyxobacter dehalogenans</i> , <i>Parabacteroides distasonis</i> ,		<i>Solibacillus silvestris</i> , <i>Bacillus acidiceler</i> , <i>Ensifer adhaerens</i> , <i>Fictibacillus barbaricus</i> ,

Rhizocola hellebori , *Pedomicrobium* *Gordonia sputi* , *Ureibacillus thermosphaericus* , *Fictibacillus manganicum*, *Variovorax paradoxus*, *Arthrobacter globiformis* *arsenicus*
Bradyrhizobium elkanii

**SC+SL-MT
group**

Roseateles depolymerans , *Bacteroides eggerthii* , *Bacillus smithii* , *Paenibacillus ihbetae* ,
Pararhizobium giardinii, *Sphingobium* *Pseudomonas aeruginosa* *Tepidimicrobium ferriphilum*, *Symbiobacterium*
xenophagum, *Comamonas aquatica*, *ostreiconchae* , *Rhodospirillaceae bacterium* ,
Lentzea albidocapillata , *Variovorax* *Bacillus thermoamylovorans* , *Lactobacillus*
paradoxus *brevis* , *Laceyella sacchari* , *Brevibacillus*
formosus, *Methylobacterium adhaesivum*

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352

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 (%)	
	<i>R</i> -value	<i>P</i> -value	<i>R</i> -value	<i>P</i> -value	<i>R</i> -value	<i>P</i> -value	<i>R</i> -value	<i>P</i> -value
<i>g_Cercophora</i>	0.2777	0.5942	0.2639	0.6133	0.0273	0.9591	0.1729	0.7433
<i>g_Plectosphaerella</i>	0.0499	0.9251	0.0785	0.8825	0.4058	0.4247	0.0467	0.9300
<i>g_Paramyrothecium</i>	0.1935	0.7130	0.5412	0.2675	0.2647	0.6121	0.2736	0.5998
<i>g_Pyrenochaeta</i>	0.3465	0.5009	0.3610	0.4821	0.4614	0.3570	0.6164	0.1924
<i>g_Myrmecridium</i>	0.5459	0.2625	0.7186	0.1076	0.3851	0.4510	0.3016	0.5613
<i>g_Apiotrichum</i>	0.6340	0.1765	0.3596	0.4839	0.6986	0.1225	0.9080	0.0123
<i>g_Entrophospora</i>	0.6870	0.1316	0.8806	0.0205	0.4423	0.4423	0.2782	0.5935
<i>g_Mortierella</i>	0.4147	0.4135	0.4320	0.3924	0.5636	0.7355	0.3857	0.4500
<i>g_Aspergillus</i>	0.9392	0.0054	0.7826	0.0658	0.0699	0.8953	0.3226	0.5328
<i>g_Fusarium</i>	0.8900	0.0175	0.6855	0.1328	0.1170	0.8253	0.0982	0.8532
<i>s_Apiotrichum montevidense</i>	0.6404	0.1707	0.3709	0.4692	0.6987	0.1225	0.9508	0.0036
<i>s_Neocosmospora rubicola</i>	0.8267	0.0424	0.5834	0.2241	0.0180	0.9730	0.2699	0.6050

<i>s_Entrophospora sp</i>	0.5165	0.2941	0.6173	0.1917	0.3359	0.5152	0.2637	0.6137
<i>s_Rozellomycota sp</i>	0.3860	0.4496	0.1233	0.8159	0.01846	0.9723	0.1994	0.7049

Note: Data are based on analyses of three *B. striata* samples from each soil type described in Figure 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 $P < 0.05$.

358 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 indicators	metabolite
	Root length (mm)		Dry weight of roots (g)		Dry weight of tubers (g)		Militarine in the tuber (%)	
	<i>R</i> -value	<i>P</i> -value	<i>R</i> -value	<i>P</i> -value	<i>R</i> -value	<i>P</i> -value	<i>R</i> -value	<i>P</i> -value
<i>g_Reyranella</i>	0.3024	0.5602	0.6660	0.1487	0.1229	0.8165	0.1615	0.7599
<i>g_Rhizobacter</i>	0.2966	0.5681	0.2665	0.6121	0.1389	0.7931	0.0905	0.8647
<i>g_Microbacterium</i>	0.6237	0.1858	0.3497	0.4969	0.3742	0.4649	0.1504	0.7760
<i>g_Sphingomonas</i>	0.4401	0.3824	0.1438	0.7858	0.9077	0.0124	0.9890	0.0002
<i>g_Bradyrhizobium</i>	0.4386	0.3842	0.5645	0.2431	0.9750	0.0009	0.8462	0.0336
<i>g_Ralstonia</i>	0.1932	0.7138	0.1766	0.7379	0.3216	0.5343	0.2732	0.6004
<i>g_Brevundimonas</i>	0.4124	0.4164	0.4849	0.3297	0.4900	0.3238	0.8125	0.0494
<i>g_Aquabacterium</i>	0.7724	0.0718	0.5399	0.2688	0.3622	0.4805	0.3206	0.5355
<i>g_Chryseobacterium</i>	0.5098	0.3015	0.6212	0.1880	0.3839	0.4523	0.3037	0.5585
<i>s_Comamonas aquatica</i>	0.4683	0.3489	0.1776	0.7299	0.5684	0.2392	0.7329	0.0974
<i>g_Hyphomicrobium</i>	0.5568	0.2512	0.4918	0.3217	0.8145	0.0484	0.9916	0.0001
<i>s_Bradyrhizobium elkanii</i>	0.4002	0.4317	0.4069	0.4233	0.9177	0.0099	0.9410	0.0051

<i>s_Pseudomonas aeruginosa</i>	0.6660	0.1487	0.9255	0.0081	0.8040	0.0538	0.7480	0.0873
<i>s_Brevundimonas bullata</i>	0.0710	0.8937	0.4436	0.3782	0.6256	0.1840	0.8822	0.0200
<i>s_Rhizobium phaseoli</i>	0.4429	0.3791	0.3895	0.4453	0.8039	0.0539	0.7622	0.0781

Note: Data are based on analyses of three *B. striata* samples from each soil type described in Figure 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 $P < 0.05$.

362 5 Materials and methods

363 5.1 Materials

364 *B. striata* sterile seedlings from one-year-old tissue cultures were obtained from Guizhou Fusheng Tianhong
365 Ecological Industry Development (Guiyang, China), while sandy clay soil and sandy loam soil were obtained from the *B.*
366 *striata* Experimental Base of Shibing County (Guizhou, China; 26° 57' 52" N, 108° 1' 33" E). The reference standard for
367 militarine was obtained from Le Meitian Pharmaceutical/De Site Biological (batch DSTDP00250; Chengdu, Sichuan,
368 China).

369 5.2 Methods

370 5.2.1 Transplantation of soil microorganisms and planting of *B. striata*

371 Prior to microbial transplantation, the entire *B. striata* was collected using a "double diagonal" (five-point sampling)
372 technique, including the soil around the *B. striata* roots, which involves taking one sample at the diagonal's midpoint and
373 four additional samples at evenly spaced intervals along the diagonal[22]. The collected samples were transferred to the
374 laboratory at 4 ° C, and the large pieces of soil around the *B. striata* roots were gently knocked out, only soil particles firmly
375 attached to the *B. striata* tubers and roots (at a distance of approximately 2 mm within the soil layer) are considered[23].
376 Finally, the five collected samples of soil were evenly mixed to form a single sample.

377 Sandy clay soil and sandy loam soil were divided into two parts, one of which was irradiated for 3 days with 25 kGy
378 of γ -rays from cobalt-60, while the other was not. In parallel, mother soil was prepared by mixing nutrient soil, loam,
379 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
380 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
381 pots we added irradiated sandy loam soil (200 mL) and non-irradiated sandy clay soil (200 mL), corresponding to
382 microbiota transplantation from sandy clay. (2) We added the same volumes of non-irradiated sandy loam soil (200 mL)
383 and irradiated sandy clay soil (200 mL) to another set of pots, which corresponded to microbiota transplantation from sandy
384 loam soil. (3) To the third set of pots we added the same volumes of both types of non-irradiated soil (each 200 mL),
385 corresponding to microbiota transplantation from both types of soil.

386 Carefully selected *B. striata* seedlings were soaked in a 0.1% mercury disinfectant for 10 minutes, rinsed with sterilized
387 water, and planted at an equal depth in the center of each pot with approximately 2 cm of soil covering the tubers. Ten
388 sterile seedlings were planted per pot and grown under plastic film coverage in a greenhouse at Guizhou University of
389 Traditional Chinese Medicine (26°23'17.68"N,106°37'43.18"E) to maintain consistency. Maintain the ambient temperature
390 between 23-25°C and humidity levels between 40-50%. Provide 16 hours of light daily from March to November. From
391 December to February of the following year, set the temperature at 4-10°C, maintain humidity between 50-60%, and expose
392 *B. striata* tubers to 12 hours of light per day. Administer weekly sterile water irrigation according to specific growth
393 requirements (heating tap water to 121°C for 20 minutes and then cooling). Implement manual control measures for pest
394 and disease management.

395 5.2.2 Biomass of *B. striata*

396 In April 2022, we quantified the growth indices of each plant under varying treatment conditions. The total weight of
397 *B. striata* plants, root length, fresh root weight, dry root weight, tuber volume, fresh tuber weight, dry tuber weight, and
398 leaf length were all quantified as growth indicators. The dry weights of *B. striata* tissues were determined using an electronic
399 scale (Mettler Toledo, Shanghai, China), following oven drying at 60°C to achieve a constant weight[24].

5.2.3 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°C. *B. striata* should undergo laboratory processing to remove any lumpy soil through gentle shaking and tapping [23]. 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-minute 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-minute soak in 75% alcohol. This was followed by another 3-4 rinses with sterile water, and a 7-minute 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[25]. The segments or pieces were meticulously placed in sterile, self-sealing bags and securely stored at -80°C. 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'- GCTGCGTTCTTCATCGATGC-3'). Additionally, the hypervariable region V3-V4 of the bacterial 16S rRNA gene was amplified through the use of primer pairs, such as 799F (5'-AACMGGATTAGATACCCKG-3') and 1193R (5'- ACGTCATCCCCACCTTCC-3')[26]. 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>.

5.2.4 The analysis of metabolites in *B. striata* tuber

The tubers from each group of *B. striata* were dried at 50°C, 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 m, 1.8 µm; Waters, Milford, USA) [27]. 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 μ L, and the column temperature was maintained at 40°C. 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 μ m 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[28]. A 1.63 mg/mL solution in 50% (v/v) ethanol in water was made as the militarine reference solution.

5.2.5 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 minutes. Subsequently, 30 μ L of soil diluent was carefully aspirated and evenly coated on a potato dextrose agar (PDA) plate[29]. After a continuous cultivation period of 3-7 days at a temperature of 27°C 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[25]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[30].

5.2.6 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)[29]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)[31]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[32]. 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 hours 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.

5.3 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 [33].

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 $p<0.001$.

6 Discussion

Here we demonstrate that, as for other plants of economic or medicinal interest[34-36], 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* and *Streptomyces*. 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 [21]. Consistent with our findings, *Fusarium oxysporum* can promote the germination of *B. striata* seeds[37]. *Sphingomonas sanguinis* promote degradation of organic metal compounds as well as plant growth[38, 39]. *Trichoderma virens* can inhibit growth of plant pathogens in soil, while promoting plant growth and resistance to disease and stress[40-42]. *Streptomyces* microorganisms can promote uptake and transport of phosphorus by plants[43].

Our work identified several microbial taxa that differentially colonized *B. striata* tissues. For example, *Fusarium*, *Myrmecridium*, *Paramyrothecium*, and *Entrophospora* from sandy clay or sandy loam soil colonized the roots; *Sphingomonas*, *Sporosarcina*, and *Aspergillus* from sandy clay soil colonized tubers; and *Teleomorph*, *Streptomyces*, *Epicoccum*, *Paramyrothecium*, *Fictibacillus* from sandy clay soil colonized leaves. Different microorganisms selectively

colonize different parts of plants, which likely reflects the different pathways through which microbes can reach the tissues. For example, *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[44]. *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[45]. *Streptomyces* strain AgN23 colonizes the leaves of plants with terminated filaments in vesicular structures, penetrating the stomata[6]. Bacterial communities in the roots of reeds are significantly more diverse than the bacterial communities in leaves[46]. Similarly, the abundance and species richness of endophytic bacteria and fungi in *Stellera chamaejasme* L. are significantly higher in the root system than in the leaves or stems[47, 48].

In the present study, we identified some microorganisms highly abundant in sandy loam that can specifically colonize roots of *B. striata* and correlation analysis showed that these microorganisms may have positive effects on roots growth, such as *Entrophospora*, *Fusarium*, *Aspergillus*, *Neocosmospora rubicola*, *Pseudomonas aeruginosa*. It has been shown to improve the survival and growth of *Echinacea pallida*, but our report appears to be the first to suggest that this microbe can promote the growth of *B. striata*[49]. *Fusarium oxysporum* can promote the germination of *B. striata* seeds[37]. 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*. We also identified microorganisms highly abundant in sandy loam that can specifically colonize tubers of *B. striata* and correlation analysis showed that these microorganisms may have positive effects on tubers growth, such as *Sphingomonas*, *Bradyrhizobium*, *Hyphomicrobium*. Inoculation with *Sphingomonas* sp. and *Micrococcus luteus* can promote the growth of willows (*Salix viminalis* x *caprea*) [50]. Inoculation with *Bradyrhizobium japonicum* can increase soybean yield and increase levels of nitrogen and phosphorus available in the soil[51]. We identified some microorganisms highly abundant in sandy loam that can specifically colonize tubers of *B. striata* and correlation analysis showed that these microorganisms may have positive effects on secondary metabolites accumulate, such as *Apiotrichum*, *Brevundimonas*, *Sphingomonas*, *Bradyrhizobium*, *Bradyrhizobium elkanii*, and *Hyphomicrobium*. *Bradyrhizobium elkanii* is a common Rhizobia known to promote nitrogen fixation by plants[52], and ours appears to be the first report that this taxon can promote the synthesis and accumulation of secondary metabolites. We analyzed the metabolic components in *B. striata* after microbial transplantation, and found that there were significant differences in metabolic components under the three conditions. Microbial transplantation of sandy clay resulted in a significant upregulation of 424 differential metabolites in *B. striata* compared to microbial transplantation of sandy loam, and the expression of glycosides and organic acids was higher, which significantly enriched Nucleotide metabolism, Metabolites of Phenylalanine metabolism, Phenylpropanoid biosynthesis and other pathways.

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 [53, 54], soil physical and chemical properties[55, 56] and age at planting[57] modulate the beneficial effects of microbes, since these factors are known to affect the composition and diversity of endophytic microbes in plants.

7 Conclusion

Employing a systematic investigation, it was determined that notable disparities exist in the microbial community structure between sandy clay and sandy loam soils. We also discovered that the transplanted microorganisms can colonize various parts of *B. striata*, including its roots, tubers, and leaves, influencing the growth and secondary metabolite

556 production of the host plant. The transplantation of microorganisms from sandy clay soil can enhance the accumulation of
557 *B. striata* secondary metabolites, such as militarine, whereas the transplantation of sandy loam microbial communities can
558 promote the growth of sandy loam. We confirmed that *Aspergillus versicolor* promotes the growth and development of *B.*
559 *striata*. Metabolomic analysis revealed that the soil microorganisms from sandy clay and sandy loam soil can result in
560 variations in metabolites within the tuber of *B. striata*. The study provides a foundation for colonizing different *B. striata*
561 tissues with certain microbes in order to improve *B. striata* yield and quality.

562 **8 Abbreviations**

563 SC, sandy clay soil.

564 SL, sandy loam soil.

565 MT, Microbiota transplantation.

566 SC-MT, microbiota transplantation from sandy clay soil.

567 SL-MT, microbiota transplantation from sandy loam soil.

568 SC+SL-MT, microbiota transplantation from both types of soil.

569 PCA, Principal Component Analysis.

570 PCoA, Principal Coordinate Analysis.

571 LDA, Linear Discriminant Analysis.

572 LefSe, LDA Effect Size.

573 PDA, potato dextrose agar medium.

574 MS, Murashige and Skoog medium.

575 **9 Ethics approval and consent to participate**

576 This study did not involve animal or human trials.

577 **10 Consent for publication**

578 Not applicable.

579 **11 Availability of data and materials**

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

582 **12 Competing interests**

583 The authors declare that they have no competing interests.

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590 **14 Authors' contributions**

591 Conceptualization, Q.X. and Y.F.; Investigation, C.Y.X.; Methodology, J.Z. and C.H.X.; Project administration,
592 C.H.X. and T.Z.; Resources, Q.Y.; Supervision, J.X.; Validation, W.J.; Visualization, C.Y. and Y.Z.; Writing-original draft,
593 Q.X.; Writing-review & editing, C.H.X.

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