Effects of Soil Microbiomes and Enzymatic Activities on *Glechoma longituba*

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Abstract. Soil microbes and enzymes play important roles in plant growth and metabolism. However, for *Glechoma longituba* (Nakai) Kupr., an important crop with edible and medicinal uses in China, their effects are not well elucidated. To explore their impacts on plant morphology and bioactive compounds, the plant samples and rhizosphere soil of five different *G. longituba* populations were collected and investigated in this study. After high-throughput sequencing combined with data analyses, high microbial diversity and richness in the rhizosphere soil of each *G. longituba* population were observed, and the variations on bacterial and fungal community compositions among these soil samples were also proved. The activities of urease, neutral phosphatase, sucrase, protease, and polyphenol oxidase were significantly different among the rhizosphere soils from different *G. longituba* populations. Among the major microbial communities and soil enzymes we studied, the genera of *Tomentella*, *Sebacina*, *Fusarium*, *Nitrospira*, and the activity of soil sucrase were remarkably correlative with both the morphological indices and the contents of bioactive compounds of *G. longituba* by redundancy analysis. These findings would help guide the scientific plantation of *G. longituba* to promote its medicinal quality.

Soil microbes are the most abundant biological organisms in soil. It is reported that the microbial biomass of surface soil could reach up to 10^3 – 10^4 kg·ha⁻¹ (Fierer et al., 2007). Soil microorganisms not only participate in the formation and evolution of soil, but also maintain the stability of the soil ecosystem. In the microecosystem of rhizosphere soil, these microorganisms also play important roles in the growth of plants. Previous work found that increasing the richness and diversity of certain microbial communities could have direct positive effects on plant growth (Maherali and Klironomos, 2007), and counteract the establishment of pathogens in a soil system (van Elsas et al., 2012). Specific plant-microbe interactions have also been verified to modulate plant metabolism (Glawischnig, 2007). But the mechanisms of soil microorganisms exerting influences on plants have not been unclosed so far.

Soil enzymes are indispensable components of soil ecology. They mainly come from the decay of microorganisms, plants, and an-

imals in the soil. It has been proved that soil enzymes are involved in many soil reactions, such as organic matter decomposition, carbohydrate transformation, and nutrient cycling (Adetunji et al., 2017). Therefore, these enzymes in soil could affect the growth of plants by changing soil chemical status. However, this current study mostly focused on the effects of field management practices or soil physicochemical properties on soil enzymatic activities (Shen et al., 2018); information about the direct correlations between soil enzymes and plants is still very limited.

Glechoma longituba (Nakai) Kupr. is a stoloniferous perennial plant widely used as a traditional herbal medicine, vegetable, beneficial tea, and ornamental groundcover in China. As recorded in the *Chinese Pharmacopoeia*, its aerial part has a broad spectrum of clinical uses for treatments of urinary calculus, hepatic calculus, cough, flu, diarrhea, and hysteresis, without any side effects (National Pharmacopoeia Committee, 2015). Because of its edible and medicinal applications, the current resource of *G. longituba* is not easily satisfying the increasing demand, and an effective cultivation system of the plant needs to be established as soon as possible. Therefore, to develop a scientific planting industry and further improve the quality of *G. longituba*, this work was going to do the following: 1) explore the effects of diverse soil microbiomes and soil enzymatic activities on *G. longituba*; 2) find the key microbial populations and enzymes that function in soil.

Study site and sampling. Yangtze-Huaihe's hilly region is the traditional habitat of *G. longituba*. The plant's long-term adaptability to the climatic and edaphic conditions of this region helps to breed a better *G. longituba* population with higher contents of bioactive substances (Liu et al., 2012). Therefore, five different *G. longituba* populations in Nanjing City, a representative area of the Yangtze-Huaihe hilly region, were chosen as the experimental materials in this study, and their location information was listed as follows: Jiangjunshan (JJS), lat. 31°55'32" N, long. 118°46'06" E; Zijinshan (ZJS), lat. 32°02'50" N, long. 118°50'22" E; Zhenzhuquan (ZZQ), lat. 32°07'16" N, long. 118°39'13" E; Shangfeng (SF), lat. 32°00'46" N, long. 119°02'44" E; and Qixiashan (QXS), lat. 32°09'18" N, long. 118°57'43" E.

All the plant and soil samples were collected on two consecutive sunny days in Sept. 2018. Five clusters of *G. longituba* from each sampling site were separately collected as five replicates for the determinations of bioactive compounds. At the same time, the rhizosphere soil of these *G. longituba* clusters was also sampled for the microbiome analysis and measurements of soil enzyme activities. Each soil replicate was mixed from the rhizosphere soil of at least five plants to ensure the weight was no less than 10 g. The operations for collecting rhizosphere soil were as follows: First, remove litter and debris on the ground. Excavate the roots of *G. longituba* with the surrounding soil carefully and shake slightly to let the easily falling soil drop from the roots. Collect the soil still adhering to the roots as the rhizosphere soil and store on a slurry of dry ice. Upon arrival to the laboratory, the rhizosphere soil samples were to be immediately frozen at -80 °C after removing impurities, whereas the samples for the determinations of soil enzyme activities were air-dried and stored at 4 °C.

Determination of morphological characters of *G. longituba*. Before collection, twenty *G. longituba* plants were randomly selected from each population to measure leaf length, leaf width, petiole length, and length of stem node. All the parameters were determined on the leaf attached at the third stem node from the stem base.

Determination of bioactive compounds of *G. longituba*. The aerial parts of the collected plants were washed and dried in a drying oven at 55 °C to a constant weight, then crushed and sifted through a 100-meshes sieve. The content of ethanol-soluble extract was determined by hot-dip method according to the general principle of 2201 in "Part Four" of the *Chinese Pharmacopoeia* (National Pharmacopoeia Committee, 2015). The content of total flavonoid (TF) was determined using an aluminum chloride colorimetric method; the contents of chlorogenic acid (ChA), caffeic acid (CaA), rosmarinic acid (RA), oleanolic acid (OA), and ursolic acid (UA) were determined using an Agilent 1100 high-performance

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liquid chromatography (Agilent Technologies, Santa Clara, CA), and all the detailed procedures referred to in the previous study we published (Jin et al., 2019b).

Soil DNA extraction and PCR amplification.

Soil DNA was extracted using a PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) according to the manufacturer's instructions; the purity and quality of genomic DNA were checked on 0.8% agarose gels.

The V3–4 hypervariable region of bacterial 16S rRNA gene was amplified with the primers 338F (5'-ACTCCTACGGGAGG-CAGCAG-3') and 806R (5'-GGACTACH-VGGGTWTCTAAT-3') (Munyaka et al., 2015), whereas the fungal ITS region was amplified with the primers of ITS1 (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 (5'-TGCCTTCTTCATCGATGC-3') (White et al., 1990). For each soil sample, the 5' ends of the both primers were tagged. The PCR was carried out on a Mastercycler Gradient (Eppendorf, Germany) with amplification procedures as follows: 94 °C for 5 min, followed by 28 cycles of 94 °C for 30 s, 57 °C for 30 s, 72 °C for 60 s, with a final extension at 72 °C for 7 min. But the PCR mixtures for the V3–4 and ITS regions were different. The PCR for the V3–4 region used 25 µL reaction volumes, containing 12.5 µL KAPA2G Robust HotStart ReadyMix (Merck KGaA, Darmstadt, Germany), 1 µL of each primer (5 µM), 5 µL template DNA (30 ng), and 5.5 µL ddH₂O. The PCR mixtures of ITS region were as follows: 4 µL 5 × FastPfu Buffer, 1 µL of each primer (5 µM), 2 µL of dNTP mixture (2.5 mM), 2 µL template DNA (30 ng), and 10 µL ddH₂O. Three separate reactions were conducted to account for potentially heterogeneous amplification from the environmental template for each sample.

High-throughput sequencing and data analysis. The PCR products were purified using QIAquick Gel Extraction Kit (QIAGEN, Germany) and quantified using Real Time PCR. An equimolar mix of all three amplicon libraries was used for sequencing on a Miseq platform at Allwegene Company, Beijing, China.

The raw sequences were screened out to let them meet all the three criteria: 1) the sequence with precise primers and barcodes; 2) a quality score greater than 20; 3) greater than 200 bp in length. Qualified reads were separated using the sample-specific barcode sequences and trimmed with Illumina Analysis Pipeline Version 2.6 (Illumina, San Diego, CA). Then the dataset was analyzed using QIIME platform (version 1.8). The sequences were clustered into operational taxonomic units (OTUs) at a similarity level of 97% to calculate the richness and diversity indices (Cole et al., 2009), and the Ribosomal Database Project (RDP) Classifier tool was used to classify all sequences into different taxonomic groups (Wang et al., 2007). The richness estimators (observed richness of OTUs and Chao1 estimator) and diversity indices (Shannon's index and Faith's phylogenetic diversity index) of each soil sample were calculated using the software Mothur. Principal component anal-

ysis (PCA) was performed to examine the dissimilarities among soil samples from different *G. longituba* populations, based on the OTU information using R. The evolution distances between soil microbial communities were calculated using the Bray-Curtis coefficient and represented as an unweighted pair group method with arithmetic mean clustering tree.

Measurements of soil enzyme activities.

The activities of soil sucrase, urease, protease, neutral phosphatase, catalase, and polyphenol oxidase were measured by colorimetric test (Guan et al., 1986). The activity of soil sucrase was determined by the 3,5-dinitrosalicylic acid colorimetric method, which was expressed as the number of milligrams of glucose produced in 1 g of dry soil after 24 h. The activities of soil urease and protease were determined by a phenol-sodium hypochlorite colorimetric method and modified ninhydrin colorimetric method, respectively, and they were both expressed as the number of milligrams of amino nitrogen produced in 1 g of dry soil after 24 h. The activity of soil neutral phosphatase was determined by a disodium phenyl phosphate colorimetric method, which was expressed in milligrams of phenol produced after 3 h per gram of dry soil. The activity of catalase was determined by the volumetric method, which was expressed in milliliters of potassium permanganate per unit weight of 0.1 M. The activity of soil polyphenol oxidase was determined by a pyrogallol method and expressed as the number of milligrams of purple gall released by pyrogallol hydrolysis per gram of dry soil for 2 h. The activity of soil β-glucosidase was determined by a p-nitrophenyl β-D-glucopyranoside colorimetric method and expressed as µg p-nitrophenyl released per gram of dry soil for 1 h (Gutiérrez et al., 2017).

Statistical analyses. SPSS Statistics (version 21.0; IBM, Armonk, NY) were used for descriptive statistics and one-way analysis of variance. The differences between means were determined by calculation of the least significant difference at the 5% level. Redundancy analysis (RDA) was performed using Canoco 5.0 (Petr & Majka Smilauer, Czech Republic) to analyze the effects of the main microbial genera and soil enzyme activities on *G. longituba*.

Results

Morphological variations of *G. longituba* populations. High variations on the four morphological characters among different *G. longituba* populations were observed (Table 1). The average values of leaf width, leaf length, petiole length, and stem node length of *G. longituba* were 3.88, 2.94, 4.35, and 6.77 cm, respectively. The highest values of all the morphological indices were shown in ZJS, whose leaf width, leaf length, petiole length, and stem node length were respectively 1.89-, 1.90-, 1.37-, and 1.70-fold higher than SF with the lowest values.

Differences in bioactive compounds of *G. longituba* populations. As shown in Table 2,

there were great differences in the contents of seven bioactive compounds among different *G. longituba* populations. The content of ethanol-soluble extract reached the highest content (29.22%) in ZZQ. The sample from JJS had the highest contents of the two triterpene acids and CaA, but it was lowest in the content of ethanol-soluble extract, which even could not satisfy the quantitative demand requested in the *Chinese Pharmacopoeia* (no lower than 25%). The highest contents of ChA and RA both shown in SF were 3.37 times and 5.52 times higher than their lowest contents, which were respectively observed in the samples from QXS and ZZQ. Meanwhile, the plants in SF showed the lowest contents of TF and CaA among all the *G. longituba* populations.

Soil microbiome analysis of different *G. longituba* populations. In total of 1,722,551 valid sequences were amplified using bacterial 16S rRNA primers, and 1,502,182 valid sequences were generated from fungal ITS primers in all the soil samples. With the increasing abscissa sequence data, the upward trends of observed species index of both bacterial and fungal communities slowed down and turned to level off (Supplemental Fig. 1), indicating that the amount of data obtained by sequencing was sufficient enough for the further identification of microbiomes. In addition, 6297 bacterial OTUs and 6290 fungal OTUs were clustered according to the above valid sequences at a 97% similarity level.

The richness of soil bacterial communities ranked as JJS > QXS > SF > ZJS > ZZQ, while the bacterial diversities of JJS, QXS, and ZZQ were higher than those of SF and ZJS (Table 3). For fungal communities, the rhizosphere soil from JJS also showed the highest richness and diversity among all the *G. longituba* populations, followed by the soil samples from ZZQ and ZJS, whereas the richness and diversity of QXS and SF stayed lower than those of the other populations (Table 3).

Through clustering analysis and PCA, 25 soil samples from five *G. longituba* populations were successfully distinguished and clustered into five groups based on both bacterial and fungal communities (Fig. 1), except for two soil samples of ZZQ and ZJS (Fig. 1A). Thus, higher variations on bacterial and fungal communities were shown among rhizosphere soil from different *G. longituba* populations.

Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Bacteroidetes, Planctomycetes, Latescibacteria, Verrucomicrobia, Nitrospirae, and Gemmatimonadetes were dominant bacterial phyla in the rhizosphere soil of *G. longituba* in Nanjing City (Fig. 2A), accounting for more than 96% of the total bacterial communities in each population. Actinobacteria had the highest relative abundance in ZJS. The relative abundance of Chloroflexi in SF was significantly higher than those in the other populations. The relative abundances of Bacteroidetes and Verrucomicrobia in JJS were at the highest level, while Nitrospirae and Proteobacteria reached

Table 1. Variations of morphological characters among different *G. longituba* populations.

Sample ID	Leaf width (cm)	Leaf length (cm)	Petiole length (cm)	Length of stem node (cm)
JJS	4.12 ± 0.28 b	3.18 ± 0.22 b	4.54 ± 0.37 ab	7.59 ± 0.51 b
QXS	3.87 ± 0.15 b	3.08 ± 0.11 b	4.32 ± 0.45 b	6.71 ± 0.17 c
SF	2.54 ± 0.23 c	1.82 ± 0.20 c	3.62 ± 0.27 c	4.78 ± 0.39 d
ZJS	4.80 ± 0.22 a	3.46 ± 0.18 a	4.95 ± 0.36 a	8.12 ± 0.14 a
ZZQ	4.09 ± 0.15 b	3.15 ± 0.10 b	4.32 ± 0.17 b	6.63 ± 0.34 c

Each value was represented as mean value ± SE of twenty individual determinations, and the different letters indicate the values are significantly different at $P < 0.05$.

Table 2. Variations of bioactive compounds among different *G. longituba* populations.

Sample ID	Extract (%)	TF (mg·g ⁻¹)	ChA (mg·g ⁻¹)	CaA (mg·g ⁻¹)	RA (mg·g ⁻¹)	OA (mg·g ⁻¹)	UA (mg·g ⁻¹)
JJS	24.68 ± 2.37 b	10.10 ± 0.64 a	0.44 ± 0.04 c	0.43 ± 0.06 a	1.11 ± 0.10 b	4.07 ± 0.34 a	4.15 ± 0.36 a
QXS	27.30 ± 0.84 a	10.47 ± 1.26 a	0.27 ± 0.05 e	0.41 ± 0.06 a	0.75 ± 0.12 c	4.01 ± 0.25 a	3.47 ± 0.13 b
SF	28.56 ± 0.85 a	7.86 ± 0.40 c	0.91 ± 0.03 a	0.29 ± 0.03 c	1.82 ± 0.50 a	2.80 ± 0.14 c	2.70 ± 0.13 c
ZJS	27.38 ± 0.65 a	9.51 ± 0.46 ab	0.37 ± 0.04 d	0.35 ± 0.02 bc	0.86 ± 0.07 bc	1.69 ± 0.13 d	3.32 ± 0.17 b
ZZQ	29.22 ± 2.29 a	8.71 ± 0.62 bc	0.55 ± 0.05 b	0.40 ± 0.06 ab	0.33 ± 0.07 d	3.58 ± 0.11 b	2.89 ± 0.21 c

Each value was represented as mean value ± SE of five independent determinations, and the different letters indicate significant differences ($P < 0.05$).

Extract = contents of ethanol-soluble extract; TF = total flavonoid; ChA = chlorogenic acid; CaA = caffeic acid; RA = rosmarinic acid; OA = oleanolic acid; UA = ursolic acid.

Table 3. Microbial community richness (S_{obs} , S_{chao}) and diversity (PD , H) of the soil samples from different *G. longituba* populations.

Sample ID	Bacterial community				Fungal community			
	S_{obs}	S_{chao}	PD	H	S_{obs}	S_{chao}	PD	H
JJS	1794	2702	366	8.159	2918	4032	261	9.891
QXS	1791	2665	358	8.157	2481	3394	225	9.375
SF	1629	2489	337	7.886	2489	3330	225	9.573
ZJS	1614	2426	341	7.635	2682	3714	239	9.646
ZZQ	1595	2209	347	8.169	2761	3672	241	9.780

S_{obs} = observed richness of OTUs; S_{chao} = Chao1 estimate; PD = Faith's phylogenetic diversity index; H = Shannon's diversity index.

their peaks in ZZQ, and the population of QXS had the highest abundances of Latescibacteria, Acidobacteria, Planctomycetes, and Gemmatimonadetes. In addition, *Haliangium*, *Acidibacter*, *Variibacter*, *Steroidobacter*, *H16*, *RB41*, *11-24*, *Flavobacterium*, *Nitrospira*, and *Gaiella* were predominant at the genus level in the rhizosphere soil of *G. longituba* populations (Supplemental Table 1).

Ascomycota, Basidiomycota, Mortierellomycota, Rozellomycota, and Glomeromycota were the main fungal phyla in the rhizosphere soil of *G. longituba* (Fig. 2B). In the soil samples of ZJS, JJS, ZZQ, QXS, and SF, the relative abundances of these five phyla summed up to 91.24%, 90.83%, 89.71%, 91.59%, and 88.76%, respectively. Interestingly, there were notable differences in the proportions of various phyla across every tested soil. The highest relative abundances of Ascomycota, Basidiomycota, and Mortierellomycota were respectively observed in JJS, QXS, and ZJS, whereas the rhizosphere soil from ZZQ had a significantly greater proportion of Rozellomycota than those of the other populations, and Glomeromycota was significantly higher in SF than the other groups. Down to the genus level, ≈45% of the fungal genera in each soil sample were unclassified, and the top ten fungal genera in the rhizosphere soil of *G. longituba* were *Mycorhizaria*, *Mortierella*, *Plectosphaerella*, *Inocybe*, *Archaeorhizomyces*, *Apiotrichum*, *Chaetomium*, *Tomentella*, *Fusarium*, and *Sebacina* (Supplemental Table 1).

Soil enzyme activities of G. longituba populations. The activities of urease and protease were the highest in the rhizosphere soil of QXS, but were the lowest in ZZQ and SF, respectively (Table 4). For sucrose and β-glucosidase, their minimum activities were both observed in SF, but their maximum activities were presented in QXS and ZJS, respectively. However, the highest activity of neutral phosphatase in the soil from SF was 50.9% higher than its lowest activity shown in ZZQ. The activities of catalase and polyphenol oxidase were the highest in the rhizosphere soil of SF and JJS, respectively, but were the lowest in the rhizosphere soil of ZZQ.

Correlations between soil microbial communities and G. longituba. The result of RDA analysis showed that all the major microorganism communities discovered in the rhizosphere soil of *G. longituba* could explain 95.84% of the total variability of the morphological indices, 91.75% of which was explained by axis 1 (RDA1) and 4.09% was explained by axis 2 (RDA2) (Fig. 3A). Twelve microorganism genera distributed in the left part of axis 1 were positively correlated with the morphological indices of *G. longituba*, especially the genera of *Tomentella*, *Sebacina*, *Fusarium*, and *11-24*. Negative correlations were observed between the relative abundances of *Flavobacterium*, *Variibacter*, *Nitrospira*, *H16* and the morphological indices. The relative abundances of *Mycorhizaria*, *Chaetomium*, and *Acidibacter* showed weaker relations with the plant mor-

phology of *G. longituba*. Among the twenty microbial communities, five genera were selected as the main explanatory parameters for the plant morphology of *G. longituba*, including *Tomentella* ($P = 0.001$), *Nitrospira* ($P = 0.001$), *11-24* ($P = 0.004$), *Sebacina* ($P = 0.002$), and *Fusarium* ($P = 0.022$) (Supplemental Table 2).

Through RDA analysis, 76.23% of the total variation of the bioactive compounds in *G. longituba* were explained by the first two RDA axes, which contributed 46.10% and 30.13%, respectively. The microbial genera of *Mycorhizaria*, *Inocybe*, *Archaeorhizomyces*, *Variibacter*, and *Gaiella* were positively related with the content of ethanol-soluble extract and were negatively related with the contents of UA and OA in *G. longituba* (Fig. 3C). Besides, strong positive relations existed among the relative abundances of *Acidibacter*, *Chaetomium*, *RB41*, *Steroidobacter*, *Apiotrichum*, *Plectosphaerella* and the contents of ChA and RA. Similarly, microbial genera of *H16*, *11-24*, *Sebacina*, *Tomentella*, *Fusarium* and the contents of CaA, TF, UA, and OA in the lower left quadrant were positively correlated. However, two bacterial genera of *Haliangium* and *Nitrospira* showed negative relationships with the contents of ChA and RA. According to the RDA model, *Steroidobacter*, *RB41*, *Apiotrichum*, *Chaetomium*, *Tomentella*, *Mycorhizaria*, and *Nitrospira* had highly significant effects on the bioactive compounds of *G. longituba* ($P < 0.01$), while the effects of *Fusarium*, *H16*, *Acidibacter*, *Sebacina*, and *Inocybe* were significant as well ($P < 0.05$) (Supplemental Table 2).

Correlations between soil enzyme activities and G. longituba. According to the RDA analysis between the soil enzyme activities and plant morphology of *G. longituba*, axis 1 and 2 explained 61.04% of the total variable distribution, which were 57.89% and 3.15%, respectively (Fig. 3B). The activities of β-glucosidase, sucrose, and protease showed positive correlations with the morphological indices of *G. longituba*, whereas the other enzymatic activities were

Fig. 1. Clustering tree and PCA plots of soil microbiomes based on OTU levels. (A) The clustering plot of bacteria communities. (B) The PCA plot of bacteria communities. (C) The clustering plot of fungi communities. (D) The PCA plot of fungi communities.

negatively related to the morphological indices of the plant. After a significance test for all the explanatory variables (soil enzyme activities), protease and sucrase were screened out as the most important enzymes in rhizosphere soil for contributing to 27.2% and 20.7% of the plant morphology at significant levels ($P < 0.05$) (Supplemental Table 3).

Correlations between the activity of sucrase and the content of CaA and between the activity of neutral phosphatase and the content of RA were proved to be significantly high (Fig. 3D). The activities of urease and protease were closely associated with the contents of TF, OA, and UA. The content of ChA was highly relevant with the activity of catalase, and it had a strong and negative relationship with the activity of sucrase in rhizosphere soil. The content of ethanol-soluble extract was positively related with the activity of β -glucosidase, but it was negatively related with the activity of urease. Besides, except for the β -glucosidase and urease, the activities of all the soil enzymes we investigated showed significant effects on the bioactive compounds of *G. longituba*, especially the activity of neutral phosphatase, which was the major explanatory parameter and explained 26% of the bioactive compounds we studied (Supplemental Table 3).

Discussion

Microbiome in rhizosphere soil of *G. longituba*. In this study, high-throughput sequencing technology was used to investigate the microbial diversity and structure of rhizosphere soil of different *G. longituba* populations, and the predominant microbial species were further screened. The results of diversity analyses suggested that microbial communities differentiated both within groups and between groups for the rhizosphere soil from five *G. longituba* populations. Moreover, though all were collected from Nanjing City, the soil samples were successfully clustered according to their origins, which validated the viewpoint that temporal surroundings would significantly affect the structure and composition of soil microbiomes (Hartmann et al., 2008).

Proteobacteria, Acidobacteria, and Actinobacteria were uncovered as the most dominant bacterial phyla in the rhizosphere soil of *G. longituba*; the same results were also reported in the rhizosphere soil of oak (Uroz et al., 2010), poplar (Gottel et al., 2011), and cultivated rice (Knief et al., 2011). We found that Ascomycota and Basidiomycota were predominant fungal phyla in the rhizosphere soil of *G. longituba*; the relative abundance of Ascomycota in each soil sample of *G. longituba* was significantly

higher than Basidiomycota, which were also reported in the rhizosphere soil of pea (Xu et al., 2012) and peanut (Li et al., 2014).

Effects of soil microbial communities on *G. longituba*. Among the major ten bacterial genera in the rhizosphere soil of *G. longituba*, *Steroidobacter*, *RB41*, *Nitrospira*, *H16*, and *Acidibacter* were screened out to have significant effects on the contents of bioactive compounds, while both *Nitrospira* and *H16* played significant roles in the plant morphology of *G. longituba*. Previous studies have revealed some characteristics and functions of these bacterial genera in soil. For example, a *Steroidobacter* strain isolated from soil could degrade agarose (Gong et al., 2016), while commamox *Nitrospira*, which belonged to *Nitrospira* in phylogenetic evolution, could completely oxidize ammonia to nitrate alone and promote the transformation of ammoniacal nitrogen into nitrate nitrogen in soil (Costa et al., 2006). Furthermore, a strain of *Acidibacter* had been reported to catalyze the reduction and dissolution of trivalent iron minerals in soil (Falagan and Johnson, 2014). *RB41* is a member of the family Blastocatellaceae, class Blastocatellales, and *H16* belongs to the family Desulfurellaceae, class Desulfurellales. Though their biological affiliations at the genus level have not been identified, the sibling genera from the same family were found to have functions in metabolic processes of some elements in soil, such as nitrogen, sulfur, and heavy metals (Huber et al., 2017; Sun et al., 2019). Therefore, influencing material circulations in rhizosphere soil and further changing the nutrient supply for plants would be the possible mechanisms involved in the effects of these bacterial genera on the growth and the contents of bioactive compounds of *G. longituba*.

It was found that *Apiotrichum*, *Chaetomium*, *Tomentella*, *Mycorhizis*, *Fusarium*, *Sebacina*, and *Inocybe* had significant effects on the bioactive compounds of *G. longituba*, while *Tomentella*, *Fusarium*, and *Sebacina* were also playing dominant roles on the morphological indices of the plant. Previous studies showed that *Chaetomium* could not only promote plant growth, but also act as a potential biological control agent for plant diseases, and *Chaetomium globosum* is the most reported species of *Chaetomium* (Xia et al., 2016; Yan et al., 2018). *Tomentella* is a common ectomycorrhizal genus forming symbiosis with orchids and other plants—so does *Sebacina* (Tedersoo et al., 2014). It has been reported that *Sebacina vermifera* inoculation could enhance the biomass production of switchgrass under drought conditions (Ghimire and Craven, 2011) and increase the number of umbels per plant, the dry weight of 1000 fruits, and the oil yield of *Foeniculum vulgare* (Dolatabadi et al., 2011). Members of *Inocybe* are mycorrhizal (Esteve-Raventós et al., 2018), and evidence has shown that the spores of some *Inocybe* species are

Fig. 2. Relative abundances (%) of the major bacterial (A) and fungal (B) phyla in the rhizosphere soil from different *G. longituba* populations.

Table 4. Enzyme activity variations of the rhizosphere soil from different *G. longituba* populations.

Sample ID	Urease (mg·g ⁻¹ ·24 h ⁻¹)	Protease (mg·g ⁻¹ ·24 h ⁻¹)	Neutral phosphatase (mg·g ⁻¹ ·3 h ⁻¹)	Sucrase (mg·g ⁻¹ ·24 h ⁻¹)	β-glucosidase (μmol·g ⁻¹ ·h ⁻¹)	Catalase (mL·g ⁻¹)	Polyphenol oxidase (mg·g ⁻¹ ·2 h ⁻¹)
JJS	0.77 ± 0.11 b	7.96 ± 1.17 a	69.48 ± 5.95 ab	2.00 ± 1.16 a	1.15 ± 0.64 ab	0.80 ± 0.45 ab	0.49 ± 0.29 a
QXS	1.01 ± 0.16 a	7.51 ± 1.16 a	52.48 ± 6.36 c	2.97 ± 0.94 a	1.02 ± 0.20 b	0.74 ± 0.43 b	0.20 ± 0.13 b
SF	0.81 ± 0.06 b	6.04 ± 0.46 b	74.16 ± 9.56 a	0.44 ± 0.38 b	0.86 ± 0.12 b	1.60 ± 0.55 a	0.34 ± 0.18 ab
ZJS	0.72 ± 0.07 bc	7.38 ± 0.59 a	63.45 ± 4.10 b	1.98 ± 1.32 a	1.52 ± 0.33 a	1.50 ± 1.00 ab	0.19 ± 0.07 b
ZZQ	0.63 ± 0.04 c	6.89 ± 0.21 ab	49.14 ± 5.24 c	2.32 ± 0.84 a	1.02 ± 0.28 b	0.70 ± 0.45 b	0.15 ± 0.06 b

Each value was represented as mean value ± SE of five independent determinations, and the different letters indicate significant differences ($P < 0.05$).

negatively related to soil carbon and nitrogen contents (Reverchon et al., 2012). *Fusarium* is a large genus of filamentous fungi; its members often are referred to as the pathogenic fungi of plants, such as *Fusarium mangiferae* (Usha et al., 2019), *Fusarium fujikuroi* (Hwang et al., 2013), *Fusarium oxysporum* (Dong et al., 2019), etc. Furthermore, a correlation was found between the quantity of *Fusarium* fungi and the textural feature of wheat kernel (Ropelewska et al., 2019). Consequently, forming symbiotic or pathogenic relationships with plants and modulating rhizospheric nutrient conditions might be the main influencing patterns of these fungal genera to *G. longituba*.

Effects of soil enzymatic activities on *G. longituba*. Soil enzyme is one of the sensitive biological indicators reflecting soil quality and maintaining crop yield. Researchers have suggested that determining the activity of a single enzyme could not reflect the comprehensive health status of soil (Nannipieri and Gianfreda, 1998). Thus, five hydrolases and two oxidoreductases were investigated in this study; and except for the activities of urease and β-glucosidase, the other enzyme activities we measured had significant effects on the contents of bioactive compounds in *G. longituba* through RDA analysis. Similar correlations between activities of soil enzymes and phytochemicals were also reported between the activity of polyphenol oxidase and the content of polyphenols in *Houttuynia cordata* (Wu et al., 2015). Additionally, positive relationships were also discovered between the activities of soil urease, phosphatase, and sucrase and the contents of artemisinin and arteannuinic acid in wild *Artemisia annua*

L. (Zhao and Luo, 2019). Such research supported our conclusion that activities of soil enzymes had an important role in the secondary metabolism and quality formation of *G. longituba*.

It should be noted that the activity of neutral phosphatase had the highest explanatory rate for the contents of bioactive compounds in *G. longituba*, suggesting that soil phosphorus might have great influences on these phytochemicals with pharmaceutical effects. In our previous investigation, soil phosphorus was also screened out as the most important factor of the same bioactive compounds of *G. longituba* (Jin et al., 2019a). Besides, the activities of urease and sucrase showed significant effects on the morphological indices of *G. longituba* in this study, which suggested that the nutritional status of carbon and nitrogen in soil would have great influences on the plant morphology of *G. longituba*.

Future perspectives for cultivation of *G. longituba*. In this study, significant correlations have been found between *G. longituba* and several microbial genera, which would mainly due to the genera's functions on regulating nutrient dynamics in soil and forming symbiotic relationships with plants. Therefore, using the interactions between *G. longituba* and these rhizosphere microorganisms would be an effective and economical strategy for its cultivation to achieve high quality and yield. Finding beneficial microbial strains from these genera and conducting their inoculation to *G. longituba* are urgent issues for future investigation, to further develop beneficial microbial inoculums for the plant. Also, *Tomentella*, *Sebacina*, and *Nitrospira* could be developed emphatically because of their essential roles

in both the plant morphology and the accumulations of bioactive compounds of *G. longituba*. Furthermore, the finding of common plant pathogens in the rhizosphere soil of *G. longituba*, such as *Fusarium*, can remind growers to prevent the invasion and improve the resistance of the plant in its practical cultivation.

In addition, paying more attention to the activities of soil enzymes, especially neutral phosphatase, is also an efficient way to improve the medicinal quality of *G. longituba* based on our results. Some studies demonstrated that supplementation of organic substances and inoculation of mycorrhizal microorganisms in soil increased the activity of phosphatase to degrade phosphorus in soil (Joner and Jakobsen, 1995; van Aarle and Plassard, 2010). Therefore, these measures are worthwhile attempts in the future planting practice to ensure the accumulation of bioactive compounds of *G. longituba*.

Conclusions

As a conclusion, high diversity of microbial communities in the rhizosphere soil of *G. longituba* was observed. The composition of soil microbial community and the activities of soil enzymes were quite different among five *G. longituba* populations. Five genera of bacteria (*Steroidobacter*, *RB41*, *Nitrospira*, *H16*, *Acidibacter*, and *Abiotrichum*), seven genera of fungi (*Chaetomium*, *Tomentella*, *Mycorarthris*, *Fusarium*, *Sebacina*, and *Inocybe*) and five enzymatic activities (neutral phosphatase, sucrase, catalase, protease, and polyphenol oxidase) had significant effects on the accumulations of bioactive compounds of *G. longituba*. *Tomentella*, *Nitrospira*, *11-24*, *Sebacina*, *Fusarium* and the

Fig. 3. Correlations between *G. longituba* and the microorganism community, enzyme activity in its rhizosphere soil. (A) RDA triplot of soil microorganism communities and morphological indices of *G. longituba*. (B) RDA triplot of soil enzyme activities and morphological indices of *G. longituba*. (C) RDA triplot of soil microorganism communities and bioactive compounds of *G. longituba*. (D) RDA triplot of soil enzyme activities and bioactive compounds of *G. longituba*. Note: The abbreviations of LW, LL, PL, and LSN represent the leaf width, leaf length, petiole length, and length of stem node of *G. longituba*, respectively. The abbreviations of Extract, TF, ChA, CaA, RA, OA, and UA represent the contents of ethanol soluble extract, total flavonoid, chlorogenic acid, caffeic acid, rosmarinic acid, oleanolic acid, and ursolic acid in *G. longituba*, respectively. The abbreviations of *Haliangi*, *Acidibac*, *Flavobac*, *Nitrospr*, *Variibac*, *Steroidb* represent the bacteria genera of *Haliangi*, *Acidibacter*, *Flavobacterium*, *Nitrospira*, *Variibacter*, and *Steroidobacter*, respectively. The abbreviations of *Apiotric*, *Chaetomi*, *Tomentel*, *Mycoarth*, *Plectosp*, *Mortierel*, and *Archaeor* represent the fungi genera of *Apiotrichum*, *Chaetomium*, *Tomentella*, *Mycoarthritis*, *Plectosphaerella*, *Mortierella*, and *Archaeorhizomyces*, respectively. The abbreviations of β -glucos, Phosphat, and PolyOxid represent the soil enzyme activities of β -glucosidase, neutral phosphatase, and polyphenol oxidase, respectively.

activities of sucrase and urease played significant roles in the plant morphology of *G. longituba*. To improve the medicinal quality of *G. longituba*, using beneficial strains from the above genera and modulating the activities of soil enzymes would be efficient strategies in future cultivation.

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Supplemental Fig. 1. Rarefaction curves of bacterial (A) and fungal (B) communities of the rhizosphere soil from different *G. longituba* populations.

Supplemental Table 1. Relative abundances (%) of major bacterial and fungal genera in the rhizosphere soil from different *G. longituba* populations.

	Genera	ZJS	JJS	ZZQ	QXS	SF
Bacteria	Unidentified	71.18 ± 9.37 ab	72.50 ± 2.27 ab	70.61 ± 8.93 ab	78.66 ± 3.19 a	68.42 ± 2.28 b
	<i>Haliangium</i>	1.31 ± 0.32 ab	0.96 ± 0.10 b	1.56 ± 0.45 a	1.64 ± 0.42 a	1.04 ± 0.27 b
	<i>Acidibacter</i>	1.27 ± 0.51 a	1.25 ± 0.19 a	0.98 ± 0.14 ab	0.87 ± 0.15 b	1.31 ± 0.19 a
	<i>RB41</i>	1.20 ± 0.61 bc	0.90 ± 0.21 c	0.92 ± 0.48 c	1.63 ± 0.34 b	3.45 ± 0.55 a
	<i>Flavobacterium</i>	1.08 ± 0.39 a	1.24 ± 0.26 a	1.01 ± 0.38 a	0.37 ± 0.14 b	0.63 ± 0.14 b
	<i>H16</i>	0.91 ± 0.24 a	1.09 ± 0.18 a	1.07 ± 0.16 a	0.88 ± 0.20 ab	0.64 ± 0.13 b
	<i>Nitrospira</i>	0.80 ± 0.20 b	0.61 ± 0.09 bc	1.29 ± 0.45 a	0.55 ± 0.15 bc	0.35 ± 0.13 c
	<i>Gaiella</i>	0.80 ± 0.46 a	0.58 ± 0.12 a	0.69 ± 0.42 a	0.63 ± 0.18 a	0.79 ± 0.25 a
	<i>Variibacter</i>	0.72 ± 0.40 a	0.54 ± 0.09 ab	0.68 ± 0.47 ab	0.31 ± 0.10 b	0.52 ± 0.08 ab
	<i>11-24</i>	0.62 ± 0.32 b	0.43 ± 0.06 b	0.36 ± 0.20 b	1.16 ± 0.50 a	0.54 ± 0.16 b
	<i>Steroidobacter</i>	0.56 ± 0.16 bc	0.62 ± 0.10 b	0.32 ± 0.04 c	0.48 ± 0.16 bc	1.30 ± 0.32 a
Fungi	Unidentified	46.46 ± 6.25 ab	46.84 ± 1.50 ab	49.97 ± 4.28 a	45.10 ± 1.86 b	49.15 ± 0.58 ab
	<i>Mycorhizis</i>	11.99 ± 3.53 a	4.08 ± 1.20 bc	6.12 ± 1.79 b	2.62 ± 0.93 cd	1.38 ± 0.57 d
	<i>Mortierella</i>	4.00 ± 1.86 a	2.91 ± 1.48 a	3.03 ± 0.65 a	3.34 ± 1.16 a	2.55 ± 1.04 a
	<i>Plectosphaerella</i>	3.02 ± 0.90 b	4.23 ± 0.83 a	2.54 ± 0.30 bc	1.81 ± 0.32 c	3.06 ± 0.72 b
	<i>Inocybe</i>	2.76 ± 1.76 a	0.21 ± 0.10 c	0.38 ± 0.16 bc	1.52 ± 1.29 ab	0.33 ± 0.17 bc
	<i>Archaeorhizomyces</i>	1.81 ± 1.30 a	0.87 ± 0.98 a	0.91 ± 0.79 a	1.07 ± 1.21 a	1.03 ± 1.10 a
	<i>Apiotrichum</i>	1.58 ± 0.78 c	1.38 ± 0.28 c	1.69 ± 0.90 c	2.97 ± 0.74 b	5.57 ± 1.41 a
	<i>Chaetomium</i>	1.08 ± 0.36 c	0.47 ± 0.08 d	1.20 ± 0.21 c	1.72 ± 0.11 b	3.17 ± 0.71 a
	<i>Tomentella</i>	1.04 ± 0.19 c	2.81 ± 0.90 b	0.29 ± 0.18 d	3.88 ± 0.73 a	0.69 ± 0.18 cd
	<i>Fusarium</i>	0.81 ± 0.53 b	2.74 ± 0.97 a	0.78 ± 0.39 b	2.89 ± 1.13 a	1.43 ± 0.85 b
	<i>Sebacina</i>	0.11 ± 0.14 b	0.36 ± 0.08 b	0.07 ± 0.05 b	5.33 ± 1.53 a	0.47 ± 0.26 b

Each value was represented as mean value ± SE of five independent samples, and the different letters indicate significant differences ($P < 0.05$).

Supplemental Table 2. Simple term effects of major microbial genera in rhizosphere soil on plant morphology and bioactive compounds of *G. longituba*.

	Genera	Plant morphology		Bioactive compounds	
		Explains (%)	<i>P</i>	Explains (%)	<i>P</i>
Bacteria	<i>Nitrospira</i>	37.1	0.001**	18.4	0.003**
	<i>11-24</i>	31.5	0.004**	4.4	0.382
	<i>RB41</i>	5.2	0.251	27.4	0.001**
	<i>Steroidobacter</i>	5.1	0.283	36.6	0.001**
	<i>Acidibacter</i>	0.5	0.846	12.9	0.018*
	<i>H16</i>	7.4	0.161	13.5	0.017*
	<i>Variibacter</i>	13.1	0.061	4.5	0.354
	<i>Flavobacterium</i>	13.6	0.057	2.0	0.741
	<i>Haliangium</i>	0.9	0.717	10.3	0.072
	<i>Gaiella</i>	0.5	0.845	4.2	0.367
Fungi	<i>Tomentella</i>	38.0	0.001**	21.0	0.002**
	<i>Sebacina</i>	31.2	0.002**	11.1	0.029*
	<i>Fusarium</i>	18.5	0.022*	13.6	0.016*
	<i>Apiotrichum</i>	4.9	0.283	25.2	0.001**
	<i>Mycorhizis</i>	1.4	0.610	18.7	0.003**
	<i>Chaetomium</i>	2.2	0.515	22.2	0.002**
	<i>Inocybe</i>	12.3	0.078	10.5	0.040*
	<i>Mortierella</i>	2.9	0.401	3.2	0.537
	<i>Archaeorhizomyces</i>	2.9	0.426	3.2	0.534
	<i>Plectosphaerella</i>	1.7	0.578	5.7	0.233

*indicates that the correlation is significant at the 0.05 level; ** indicates that the correlation is significant at the 0.01 level.

Supplemental Table 3. Simple term effects of soil enzyme activities in rhizosphere soil on plant morphology and bioactive compounds of *G. longituba*.

Soil enzymes	Plant morphology		Bioactive compounds	
	Explains (%)	<i>P</i>	Explains (%)	<i>P</i>
Sucrase	22.5	0.022*	17.5	0.001**
Urease	12.5	0.036*	6.2	0.211
Protease	11.5	0.052	11.9	0.036*
β-glucosidase	5.2	0.116	7.4	0.135
Neutral phosphatase	3.0	0.280	26.0	0.001**
Catalase	2.2	0.372	14.7	0.012*
Polyphenol oxidase	1.8	0.410	10.2	0.041*

*indicates that the correlation is significant at the 0.05 level; ** indicates that the correlation is significant at the 0.01 level.