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Stage-Dependent Dynamics of the Rhizosphere Bacterial Community in Cultivated *Morchella sextelata*

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Abstract

As a high-value edible mushroom, *Morchella sextelata* faces several cultivation challenges, including unstable yields, continuous cropping constraints, and soil-borne diseases. The rhizosphere soil microbial community plays a crucial role in morel growth, yet its dynamic changes across different developmental stages remain poorly understood. In this study, *M. sextelata* cultivated in the Huangshan City was selected as the study system. Rhizosphere soil physicochemical properties and bacterial community structure were analyzed across six stages: the primordium stage, needle tip stage, mulberry stage, young mushroom stage, fruiting stage, and blank soil before planting. High-throughput sequencing, combined with soil physicochemical analyses, was used to characterize the dynamic rhizosphere bacterial community changes and their associations with soil factors. The results showed that rhizosphere soil remained weakly acidic throughout the growth period. The soil C/N ratio decreased significantly, indicating dynamic changes in carbon and nitrogen use efficiency. Bacterial diversity gradually declined during development, while a relatively stable community structure was established. The dominant phyla were Proteobacteria, Acidobacteria, Bacteroidetes, Actinobacteria, and Firmicutes, with relative abundances varying among growth stages. At the genus level, *Candidatus Koribacter*, *Massilia*, *Mucilaginibacter*, *Janthinobacterium*, and *Sphingomonas* predominated. Notably, *Mucilaginibacter* was progressively enriched during growth and showed a positive correlation with total carbon, whereas *Massilia* was significantly negatively correlated with the C/N ratio. This study clarifies the stage-dependent dynamics of the rhizosphere bacterial community in *M. sextelata* and provides a theoretical basis for improving cultivation stability through soil microbiome regulation.

Keywords: morel; *Morchella sextelata*; growth stage; high-throughput sequencing; rhizosphere soil bacterial community

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Academic Editors: Ming Gong, Yuhua Gong and Henan Zhang

Received: 14 January 2026
Revised: 5 February 2026
Accepted: 6 February 2026
Published: 9 February 2026

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1. Introduction

Morel is a macrofungus belonging to the phylum Ascomycota, order Pezizales, and genus *Morchella* [1]. It is a rare and highly valued edible mushroom with substantial nutritional and medicinal importance [2,3]. Morels are rich in polysaccharides, amino acids, fatty acids, organic acids, and minerals [4–8]. Previous studies indicate that morels exhibit diverse biological activities, including anticancer, immunomodulatory, antioxidant, antiproliferative, antitumor, and anti-inflammatory effects [9–13]. These attributes highlight their strong potential for applications in functional foods and biomedicine.

Morels have gained increasing global attention because of their nutritional value. Although artificial cultivation was first achieved in the 1980s, stable domestication and large-scale cultivation were not established in China until the early 21st century [14,15]. The annual production of fresh morels reached 15,000 tons in 2020 [16]. In recent years, rising market demand has accelerated the expansion of commercial morel production. The literature reports that the annual cultivation area of morels was stable at 16,000–20,000 ha, and the yield of morels in paddy fields can reach 504 kg/km^2 in China [17,18]. However, several challenges continue to limit sustainable field cultivation, including unstable yields, high susceptibility to pathogenic infections, and strong sensitivity to environmental variation. For example, continuous cropping can significantly reduce yields [19]. Pathogenic fungi such as *Penicillium*, *Trichoderma*, and *Aspergillus* further constrain production [20]. In addition, morels are highly sensitive to heavy metal accumulation, posing risks to both yield and product safety [21]. Many of these constraints are closely associated with the soil microbiome, which plays a key role in morel growth and development.

Soil parameters affect the rhizosphere microorganisms of edible mushroom, such as pH, water, and organic matter, etc. During cultivation, morel mycelia primarily obtain nutrients from soil and nutrient bags to support growth [22]. Consequently, the soil environment is a critical factor regulating mycelial development and fruiting body formation. Specifically, temperature, humidity, pH, and nutrition affect the mycelial development and fruiting body formation of morels [23]. Moisture is an important medium for soil bacterial transmission. A wet environment is conducive to the growth of morels, and the optimal soil moisture should be controlled between 85 and 90% [24]. High-throughput sequencing has been widely used to characterize rhizospheric soil microbiomes. For example, Benucci et al. analyzed fungal and prokaryotic communities in soils associated with *M. sextelata* grown in outdoor greenhouses using 16S rRNA and ITS amplicon sequencing [25]. An increasing number of studies have examined rhizospheric microbiomes of morels, revealing that microbial composition varies with cultivar, cultivation region, and rotation mode [14,22,26–28]. Accordingly, this study analyzed rhizospheric soil physicochemical properties and bacterial community diversity and composition of *M. sextelata* at different growth stages, providing a theoretical basis for daily cultivation and post-harvest soil management in Huangshan City.

2. Materials and Methods

2.1. The Cultivation of the Morel

The experiment was carried out with the *M. sextelata* HSUM10001 cultivar. The spawn and exogenous nutrient bags were provided by Huangshan Qijun Agricultural Technology Co., Ltd (Huangshan, China). The weight of each exogenous nutrient bag was 400 g, including 89% wheat grain, 10% rice husk, and 1% quicklime. The cultivation of *M. sextelata* was followed by a technical protocol [29]. The following conditions were maintained in the greenhouse throughout the cultivation process: air temperature below 20 °C; soil temperature between 8 °C and 12 °C; relative air humidity at 85–90%; and the use of a shading net providing 60–70% shade.

2.2. Sample Collection

Rhizosphere soil samples of *M. sextelata* at different cultivation stages were collected from December 2023 to March 2024 at the base of Huangshan University (29°40′ N, 118°16′ E, Huangshan, China) using a three-point sampling method. Six stages were sampled: primordium stage (YJ), needle tip stage (ZJ), mulberry stage (SS), young mushroom stage (YG), fruiting stage (CG), and blank soil before planting (XS). At each sampling point along the ridge, spaced 2–3 m apart, the top 2 cm of soil was removed. Inter-root soil from a depth of 2–8 cm was then collected using a 10 cm soil auger. Soils from the

three points at each stage were thoroughly homogenized to obtain one composite sample. Each composite sample was divided into three equal subsamples, placed in sterile plastic bags, and transported to the laboratory under cooled conditions [30]. All samples were stored at −80 °C for subsequent analyses of soil physicochemical properties and high-throughput sequencing.

2.3. Soil Physiochemical Analysis

Soil pH was measured using a glass electrode. Briefly, 10 g of soil was suspended in distilled water at a soil-to-liquid ratio of 1:2.5 (*w*/*v*), following the method described by Zhao et al. [31]. Soil total carbon (TC) and total nitrogen (TN) were determined using an elemental analyzer (Vario EL III, Elementar, Hanau, Germany). Carbon and nitrogen contents and the soil C/N ratio were calculated based on these measurements [32].

2.4. DNA Extraction, PCR Amplification and Sequencing

Total genomic DNA was extracted from rhizosphere soil using the E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek, Norcross, USA) according to the manufacturer's protocols. The bacterial 16S rRNA gene was amplified by PCR using primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-AAGTCGTAACAAGGTARCCGTA-3′). The PCR reaction system and cycling conditions followed previously reported protocols [33]. PCR products were verified by 2% agarose gel electrophoresis, purified, and sequenced on the PacBio Sequel platform. Amplicon sequencing was conducted by Shanghai Biozeron Biotechnology Co., Ltd. (Shanghai, China). The raw data are available in the NCBI Short Reads Archive (BioProject PRJNA1398512).

2.5. Bioinformatics Analysis

Raw reads were processed using SMRT Link Analysis software v9.0 to generate high-quality sequences. These sequences were clustered into operational taxonomic units (OTUs) at 98.65% similarity using UPARSE v7.1, and chimeric sequences were identified and removed with UCHIME. Species annotation was performed using the RDP classifier v2.2 against the Silva 16S rRNA database with a confidence threshold of 80%. Based on OTU representative sequences, community composition was analyzed at multiple taxonomic levels, including domain, phylum, class, order, family, genus, and species [34].

2.6. Statistical Analyses

Rarefaction analysis was conducted using Mothur v1.21.1 to assess alpha diversity, including the Chao, ACE, and Shannon indices [35]. R v4.0 software was used to generate comparative taxonomic bar charts for sample groups at different levels and community distribution bar charts for individual samples. Beta diversity was evaluated using UniFrac, and results were visualized by principal coordinates analysis (PCoA) [36]. Sparse curves were generated based on OTUs, and Venn diagrams were used to compare core OTUs among samples. The LefSe analysis (screening criteria: $p < 0.05$, LDA score > 2) was used to obtain the indicator species between groups based on the OTU abundance matrix. The co-occurrence network was built using Spearman rank correlation analysis for studying sample microbial communities. To characterize microbial functions, functional annotation and metabolic pathway analyses were performed using the KEGG database. Finally, relative abundance data of bacterial communities at the genus level were used as species variables, soil physicochemical properties were used as environmental variables, and redundancy analysis (RDA) was performed in Canoco to examine correlations between bacterial taxa and soil factors. All data are presented as means ± standard error (SE), and the differential abundance analysis was conducted using analysis of compositions of microbiomes (ANCOM) [37].

3. Results

3.1. Soil Physicochemical Properties

The physicochemical properties of rhizosphere soil associated with *M. sextelata* varied significantly across growth stages (Table 1). All soil samples were slightly acidic, with pH values ranging from 5.05 to 6.19. The lowest pH was observed at the ZJ stage (5.05), whereas the highest pH occurred at the SS stage (6.19), followed by a gradual decline to 5.70. The soil carbon to nitrogen (C/N) ratio decreased continuously across stages, dropping sharply from 21.90 at the XS stage to 5.86 at the YJ stage and further to 0.87 at the CG stage. Total sulfur (TS) content peaked at 3.67% during the YJ stage and then declined steadily to 0.12% at the CG stage, which was comparable to the XS stage (0.16%). These results indicate substantial shifts in rhizosphere nutrient cycling during *M. sextelata* development.

Table 1. Physicochemical properties of the soil samples of *Morchella sextelata* at different growth stages.

Sample	TN	TC	TS	C/N	pH
XS	0.06 ± 0.03^{c}	0.96 ± 0.08^{b}	0.16 ± 0.03^{c}	21.90 ± 14.78^{b}	5.53 ± 0.49^{ab}
YJ	0.18 ± 0.03^{c}	1.04 ± 0.14^{b}	3.67 ± 0.50^{a}	5.86 ± 0.31^{a}	5.70 ± 0.03^{ab}
ZJ	0.33 ± 0.05^{c}	1.07 ± 0.15^{b}	3.57 ± 1.12^{a}	3.35 ± 0.83^{a}	5.05 ± 0.06^{b}
SS	0.45 ± 0.07^{c}	1.15 ± 0.13^{b}	2.26 ± 0.38^{ab}	2.60 ± 0.43^{a}	6.19 ± 0.36^{a}
YG	0.96 ± 0.27^{b}	1.59 ± 0.09^{a}	0.81 ± 1.11^{bc}	1.75 ± 0.49^{a}	6.00 ± 0.68^{ab}
CG	1.87 ± 0.35^{a}	1.62 ± 0.19^{a}	0.12 ± 0.02^{c}	0.87 ± 0.09^{a}	5.70 ± 0.29^{ab}

Note: the same letter on the same index indicates that there is no significant difference between the groups at $p < 0.05$.

3.2. Bacterial Community Structure and Relative Abundance

Third-generation amplicon high-throughput sequencing was used to analyze rhizosphere soil samples of *M. sextelata* at different growth stages. A total of 503,427 raw reads were generated, of which 83,471 high-quality sequences were retained after quality filtering. Sequence lengths ranged from 1401 to 1500 bp (Figure S1). Rarefaction curves for all samples reached saturation, confirming sufficient sequencing depth and reliable representation of bacterial community composition (Figure S2).

Operational taxonomic unit (OTU) clustering identified 37,872 bacterial OTUs across all growth stages, including 736 core OTUs shared among stages (Figure 1). The XS stage contained the highest number of unique OTUs (9780). In contrast, unique OTU numbers at subsequent stages (YJ, SS, ZJ, YG, CG) remained at moderate to low levels and were consistently lower than those at the XS stage. Unlike the pronounced decline observed after the primordium stage, OTU numbers showed only minor fluctuations during later stages. Overall, the rhizospheric bacterial community of *M. sextelata* exhibited a relatively stable structure throughout growth, suggesting adaptation to the developmental requirements of *M. sextelata*.

3.3. Microbial Community Diversity

Alpha diversity analysis was conducted to characterize changes in richness and evenness of rhizosphere microbial communities associated with *M. sextelata* from pre-cultivation to maturity. Significant differences in alpha diversity were detected among growth stages (Table 2). For species richness indices, Chao1 and ACE reached their maximum values in XS samples at 7690 and 8179, respectively, indicating the highest species richness in pre-cultivation soils. In contrast, the lowest Chao1 value (4150) and ACE value (4395) were observed in CG samples, reflecting a progressive decline in total rhizosphere bacterial abundance during *M. sextelata* growth.

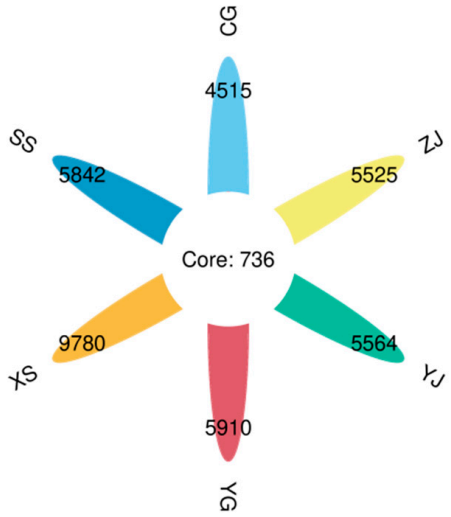


Figure 1. Venn distribution of rhizosphere soil bacteria in *Morchella sextelata* at different growth stages.

Table 2. Statistics of sequencing output across sample groups and alpha diversity indices of the bacterial community.

Sample	Chao1	ACE	Shannon	Simpson	Pielou
XS	7690 ± 617 ^{a}	8179 ± 577 ^{a}	8.33 ± 0.23 ^{a}	0.9993 ± 0.00 ^{a}	0.9376 ± 0.01 ^{a}
YJ	5203 ± 205 ^{b}	5459 ± 208 ^{b}	7.48 ± 0.08 ^{bc}	0.9973 ± 0.00 ^{a}	0.8792 ± 0.01 ^{bc}
ZJ	4657 ± 686 ^{bc}	4857 ± 747 ^{bc}	7.12 ± 0.01 ^{cd}	0.9937 ± 0.00 ^{b}	0.8472 ± 0.01 ^{cd}
SS	5391 ± 690 ^{b}	5697 ± 708 ^{b}	7.61 ± 0.21 ^{b}	0.9976 ± 0.00 ^{a}	0.8909 ± 0.02 ^{b}
YG	5184 ± 231 ^{b}	5478 ± 293 ^{b}	7.56 ± 0.16 ^{b}	0.9979 ± 0.00 ^{a}	0.8878 ± 0.02 ^{b}
CG	4150 ± 600 ^{c}	4395 ± 570 ^{c}	6.83 ± 0.45 ^{d}	0.9922 ± 0.00 ^{b}	0.8271 ± 0.03 ^{d}

Note: the same letter on the same index indicates that there is no significant difference between the groups at $p < 0.05$.

Similar patterns were observed for species diversity (Shannon index) and community evenness (Pielou index). The highest Shannon index (8.33) and Pielou index (0.9993) occurred in XS samples, indicating high diversity and near-uniform species distribution prior to cultivation. However, both indices declined with plant development, reaching their lowest values at the CG stage (Shannon index: 6.83; Pielou index: 0.8271).

3.4. Principal Coordinate Analysis

PCoA based on Bray–Curtis distance revealed clear separation of soil bacterial communities across *M. sextelata* growth stages (Figure 2). Growth stage significantly influenced community composition, explaining 30.81% of the total variation. The first two principal coordinates accounted for 16.22% (PC1) and 14.59% (PC2) of the variance, respectively. During early growth stages (XS, YJ, ZJ), rhizosphere bacterial communities were clearly separated, indicating pronounced structural differences. In contrast, communities from later stages (SS, YG, CG) clustered closely, suggesting increasingly similar community compositions. Additionally, the XS stage has a smaller intragroup distance, suggesting less variability within its group. Conversely, YJ, YG and CG stages exhibit a wide intragroup distance, indicating significant variability within the group.

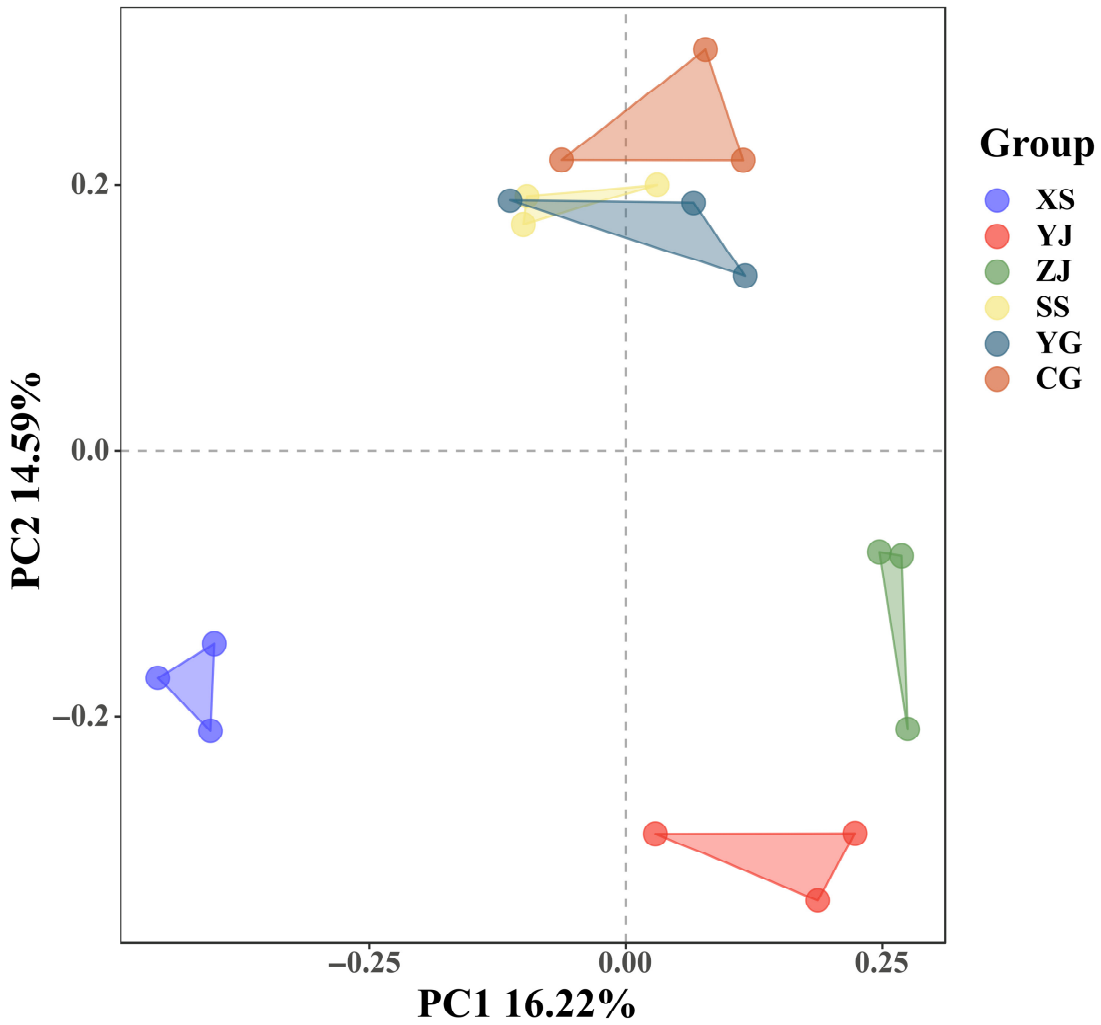


Figure 2. Venn distribution of rhizosphere soil bacteria in *Morchella sextelata* at different growth stages.

3.5. Soil Bacterial Community Composition Analysis

A total of 45 phyla, 75 classes, 166 orders, 356 families, and 1275 genera of bacteria were detected in the rhizosphere soil of *M. sextelata* across different growth stages. Bacterial community compositions were further analyzed at the phylum and genus levels. The phylum-level distribution across growth stages is shown in Figure 3A. The dominant phyla were Proteobacteria, Acidobacteria, Bacteroidetes, Actinobacteria, and Firmicutes, with mean relative abundances of 44.2%, 13.0%, 11.3%, 7.8%, and 6.7%, respectively, together accounting for nearly 83.0% of total bacterial abundance. Overall, bacterial taxa were largely consistent among the six stages, although stage-dependent differences in community composition were evident. Proteobacteria showed an increase–decrease–increase pattern with plant growth, whereas Acidobacteria exhibited a declining trend, with the highest abundance at the YJ stage and the lowest at the CG stage. Bacteroidetes reached its maximum relative abundance at the CG stage. Actinobacteria and Firmicutes peaked at the SS and ZJ stages, respectively. In contrast, Chloroflexi and Gemmatimonadetes remained relatively stable across all growth stages.

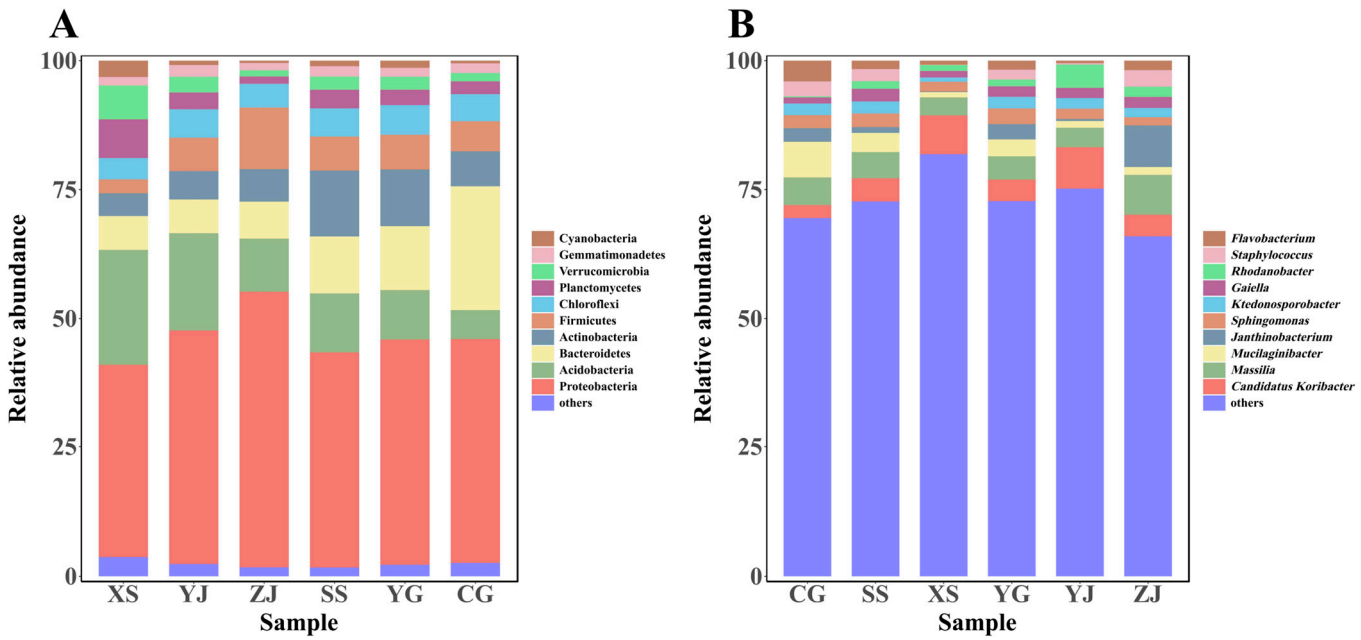


Figure 3. The relative abundances of rhizosphere soil bacteria in different growth stages of *Morchella sextelata*: (**A**) phylum level; (**B**) genus level.

At the genus level, the top 10 bacterial genera were selected for comparative analysis (Figure 3B). The dominant genera were *Candidatus Koribacter*, *Massilia*, *Mucilaginibacter*, *Janthinobacterium*, and *Sphingomonas*, accounting for 5.1%, 5.0%, 2.9%, 2.6%, and 2.3% of total abundance, respectively. The relative abundance of *Candidatus Koribacter* decreased over time, with the highest abundance at the YJ stage and the lowest at the CG stage. *Massilia* and *Janthinobacterium* both showed peak abundances at the ZJ stage and minimum levels at the YJ stage. In contrast, *Mucilaginibacter* displayed a gradual increasing trend during growth stages, reaching its highest relative abundance at the CG stage.

3.6. Differences in Soil Bacterial Communities

The LefSe analysis was based on the rhizosphere soil bacterial community composition of *M. sextelata* across different growth stages (Figure 4). There was a significant difference in the indicator species of soil samples from the *M. sextelata*. Specifically, there were 18 bacterial indicator species in the YJ stage, including Acidobacteriaceae, Acidobacteriales, Holophagales, Holophagae, Aphanizomenonaceae, Micropepsales, Sterolibacteriaceae, Kofleriaceae, Labilitrichaceae, Syntrophobacterales, Ectothiorhodospiraceae, Thioalkalispiraceae, Steroidobacteraceae, Nevskiales, Xanthomonadales, Candidatus_Babeliaceae, Candidatus_Babeliales, and Candidatus_Babeliae. Furthermore, there were 4 bacterial indicator species in the CG stage, including Cytophagia, Brucellaceae, Bacteriovoracaceae, and Bacteriovoracales. These indicator species play a very important role in affecting the differences in bacterial community composition in the rhizosphere soil of *M. sextelata*. Moreover, the microbial co-occurrence network of soil bacterial communities in all samples was constructed using Spearman rank correlation analysis (Figure S3). Proteobacteria, Acidobacteria, and Bacteroidetes occupied the main nodes.

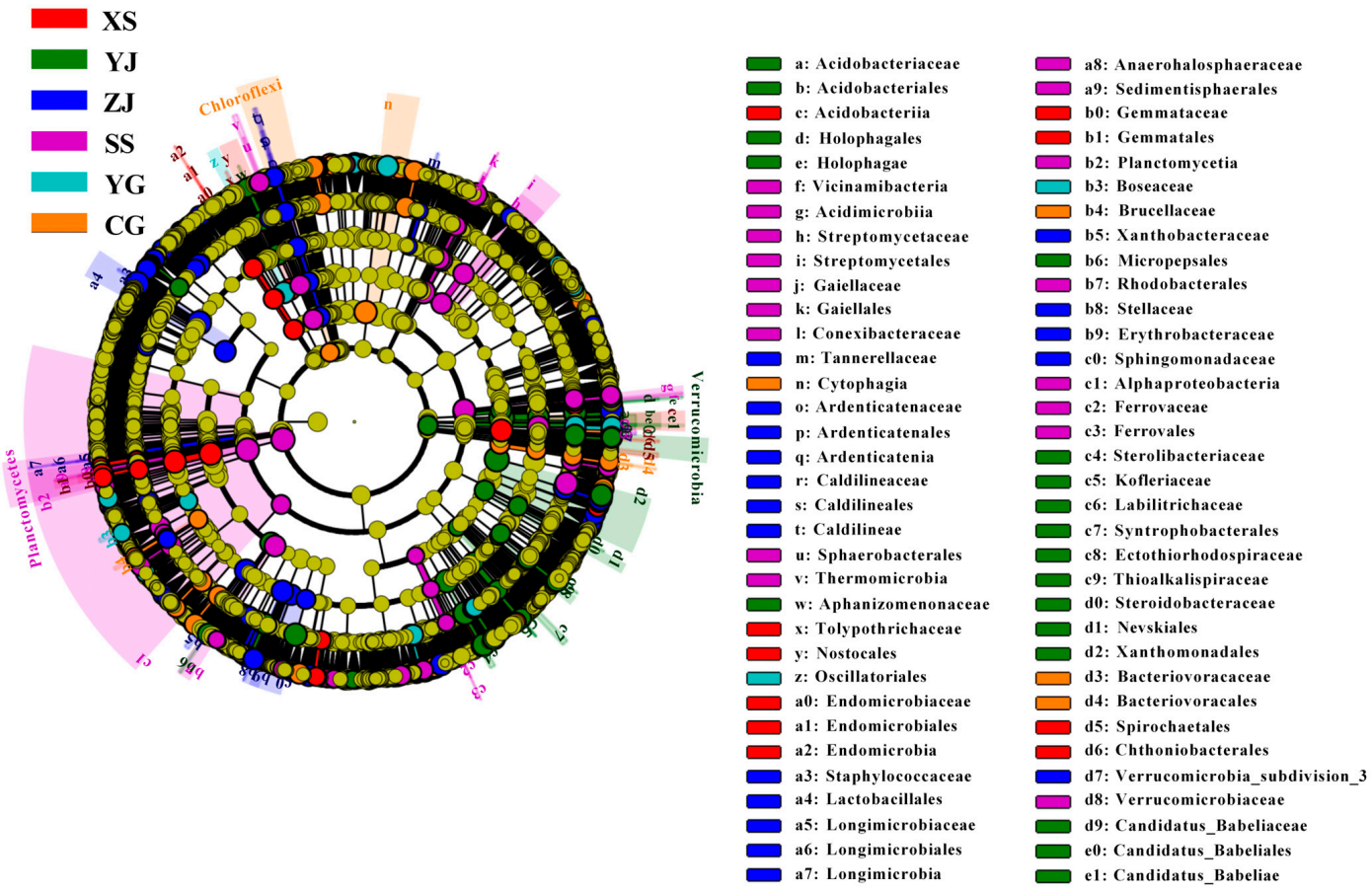


Figure 4. Taxonomic cladogram of LEfSe at a logarithmic LDA score > 2.0 of the rhizosphere soil bacterial community composition of *Morchella sextelata* across different growth stages.

3.7. Correlation Analysis Between Soil Bacterial Community and Mineral Elements

To explore correlations between the rhizosphere soil bacterial community of *M. sextelata* and soil environmental factors, ten dominant bacterial genera and six soil variables were selected for RDA (Figure 5). The results showed significant associations between soil environmental factors and the dominant bacterial community. The first and second RDA axes explained 41.6% and 12.4% of the variance, respectively, accounting for 54.0% in total. Specifically, *Mucilaginibacter* showed a significant positive correlation with TC and TN. In contrast, *Massilia* was significantly negatively correlated with the C/N ratio but positively correlated with TC and TN, indicating a preference for readily decomposable organic carbon sources. Moreover, *Candidatus Koribacter* was significantly positively correlated with the C/N ratio. *Janthinobacterium* and *Rhodanobacter* were significantly positively correlated with the C/N ratio. These results suggest a potential mechanism by which *M. sextelata* regulates the proliferation of specific microbial taxa through modifications of rhizosphere soil physicochemical properties.

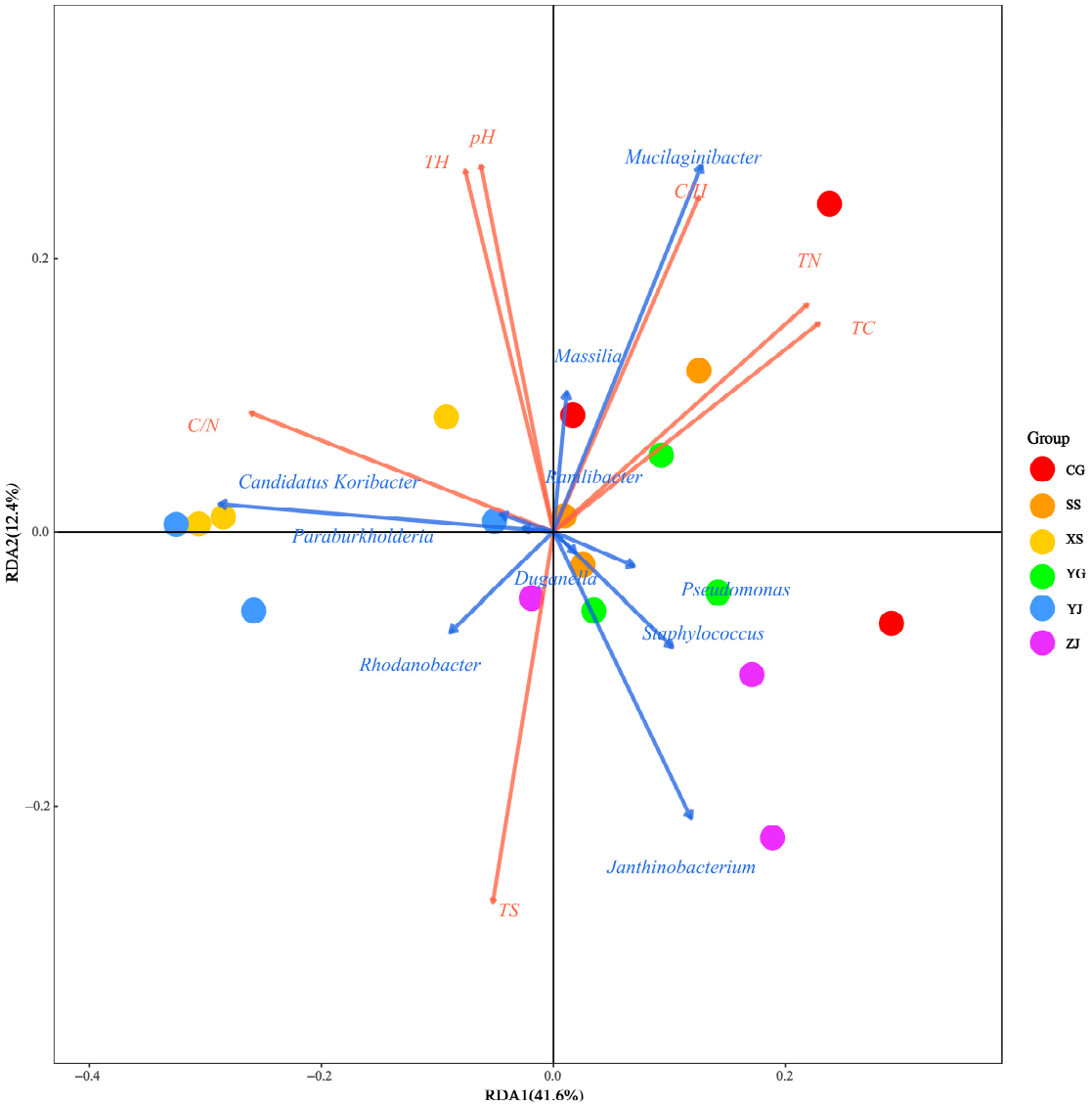


Figure 5. Relationship between the top ten dominant bacterial genera RDA and soil environmental factors. Note: Each point represented one sample. Red arrows represented environmental factors, and blue arrows represented dominant bacteria. The strength of the correlation with bacterial communities was indicated by arrow length, with longer arrows denoting stronger correlations. The correlation between environmental factors and dominant bacteria was reflected by the angle between their arrows: an acute angle indicated a positive correlation, a right angle indicated no correlation, and an obtuse angle indicated a negative correlation.

3.8. Functional Analysis

To explore functions of the rhizosphere soil bacterial community, we performed a functional analysis of *M. sextelata* across different growth stages based on the KEGG orthology database (Figure 6). The results showed that the majority of enriched KEGG pathways were related to metabolism (72.00%), environmental information processing (11.68%), and cellular processes (8.37%). Specifically, pathways related to cysteine and methionine metabolism were enriched at the YJ stage. At the ZJ stage, the rhizosphere soil bacteria were enriched in two-component system, bacterial secretion system, and flagellar assembly pathways. The pathways related to propanoate metabolism, starch, and sucrose metabolism were enriched at the SS and CG stages, respectively. At the late growth stage of *M. sextelata*, the soil microbiome was enriched in nitrogen metabolism, ABC transporters, and arginine and proline metabolism pathways.

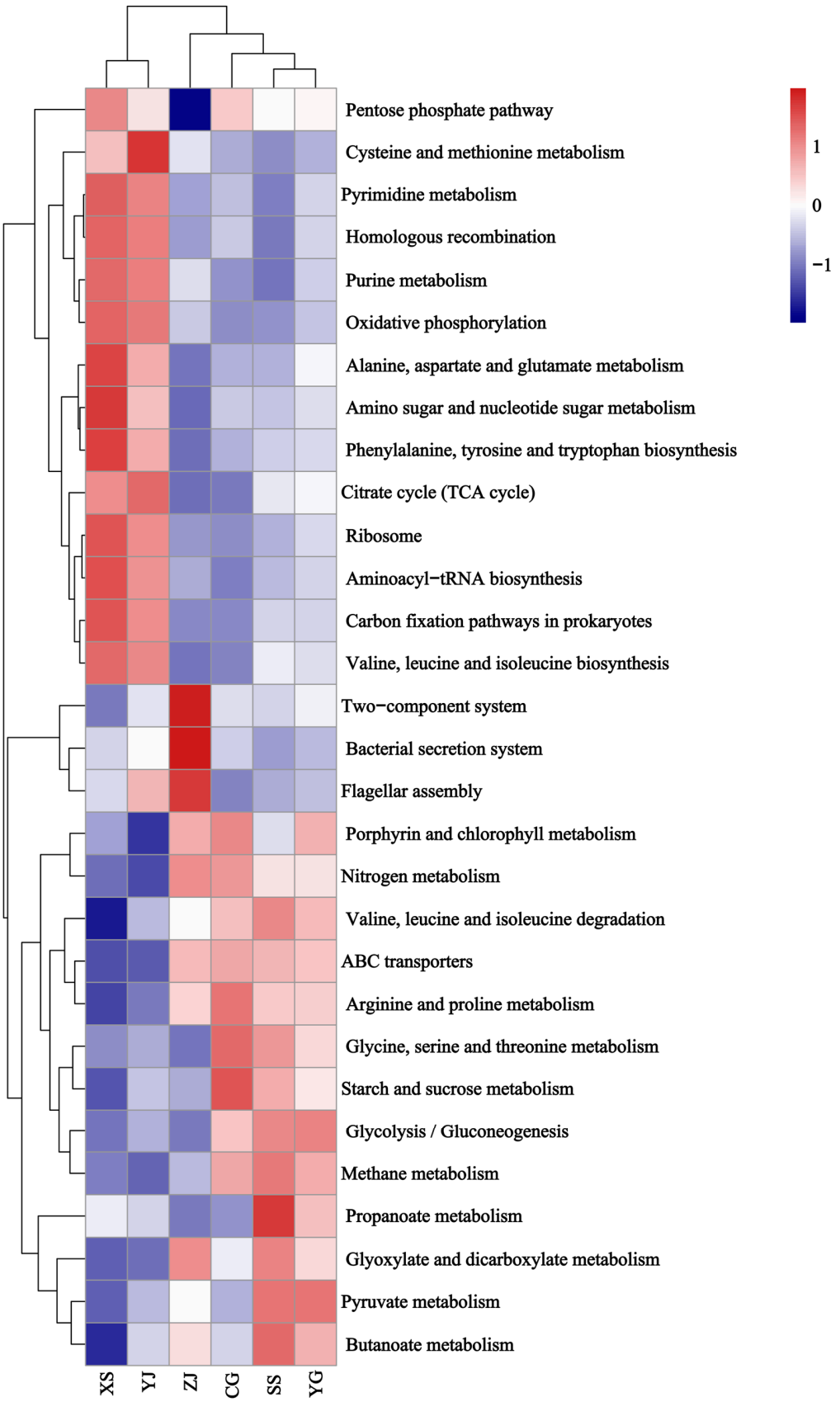


Figure 6. Variations in KEGG functions of the soil microbiome in different growth stages of *Morchella sextelata*.

4. Discussion

Morels are widely recognized as edible and medicinal fungi with high economic and nutritional value and have been increasingly cultivated worldwide in recent years. However, morel cultivation remains unstable, often resulting in severe yield losses and, in extreme cases, complete crop failure. This instability has markedly constrained the sustainable development of the morel industry. Major limiting factors include continuous cropping barriers, soil physicochemical properties, and soil-borne diseases [19,27,38]. The soil microbial community plays an important role in the growth of *M. sextelata*. The study on the rhizosphere fungal community of *M. sextelata* can provide a reference for disease prevention [24,39]. However, compared with the rhizosphere fungal community, most of the studies focused on the rhizosphere bacterial community. Therefore, this study aimed to elucidate the relationships between dynamic changes in rhizospheric soil bacterial community structure and physicochemical properties of *M. sextelata* across different growth stages in Huangshan City. The findings are expected to provide a theoretical basis for optimizing morel cultivation and management practices.

A weakly acidic soil is conducive to the growth of bacteria and fungi [30]. In this study, *M. sextelata* grew under acidic soil conditions throughout its entire growth period, with the highest acidity observed at the needle stage. However, this result is inconsistent with previous reports and may be attributable to regional differences in cultivation conditions [40,41]. In addition, the rhizospheric soil C/N ratio showed a decreasing trend during *M. sextelata* growth, indicating dynamic shifts in carbon and nitrogen utilization efficiency. This pattern likely reflects the saprophytic nature of *M. sextelata*, as soil organic carbon and nitrogen are progressively enriched during its growth [28]. Furthermore, carbon and nitrogen sources affected the interaction between *Morchella* spp. and bacteria dispersed along the hypha [42]. Accordingly, sodium nitrate has been reported as an optimal nitrogen source for morel cultivation [43]. Besides the C, N, and pH factors of soil, potassium, phosphorus, sucrose, and starch may be the soil factors determining the growth of *Morchella* [30]. Among these, potassium was the key nutrient for mycelial growth and primordium formation of morels [44]. The nitrogen, potassium loss, and phosphorus accumulation were the main reasons for the decline in morel yield under continuous cropping [28]. Therefore, potassium can be supplemented during the cultivation of morels. Moreover, Mn significantly promoted mycelia growth rates of morels, and trace elements such as Fe, Zn, and Mn may also affect yield outcomes of morels [23].

Rhizosphere microorganisms are considered important for improving host health and soil fertility [45]. Previous studies have shown that the community composition and relative abundance of rhizosphere microorganisms can be significantly altered by some edible mushrooms across different growth environments [46,47]. In this study, Proteobacteria, Acidobacteria, and Bacteroidetes were the dominant bacterial phyla in the rhizosphere soil of *M. sextelata*. Proteobacteria showed the highest relative abundance across all growth stages, consistent with a previous report [48]. These three phyla have also been identified as major rhizosphere microbial components in *M. galilaea* and *M. esculenta* [27,49]. However, marked variations in the relative abundances of these phyla were observed across the growth stages of *M. sextelata*. Specifically, the relative abundance of Bacteroidetes increased steadily from 6.5% at the primordium stage to 24.0% at the fruiting stage. Additionally, the relative abundance of Actinobacteria first increased and then decreased, reaching its maximum at the mulberry stage. This pattern differs from previous findings [27], possibly due to differences in cultivation regions.

The influence of microbial communities on *M. sextelata* growth was further examined. The top 10 dominant bacterial genera were selected for comparative analysis. Among them, *Candidatus Koribacter* and *Rhodanobacter* were most abundant at the primordium stage.

The relative abundance of *Candidatus Koribacter* declined sharply from the primordium stage to the needle tip stage, which may resemble the role of *Candida nitrosocosmicus* in nutrient transformation and supply [49]. *Massilia*, *Janthinobacterium*, *Sphingomonas*, and *Flavobacterium* were identified as key microbial biomarkers associated with the growth stages of *M. sextelata* [27]. Among which, *Sphingomonas* has the ability to decompose lignocellulose, which helps to elongate mycelia and metabolize nutrients [50]. Notably, *Massilia* appears to play an important role in promoting growth and development. *Sphingomonas* has been linked to yield in *Agaricus bisporus* [51], and *Janthinobacterium* has been detected in both rhizosphere soil and the stipe of *M. sextelata* [25]. The relative abundance of *Flavobacterium* increased progressively with maturation and became a core member of the ascocarp microbiome [25]. Similarly, *Mucilaginibacter* showed a gradual increase during the growth of *M. sextelata*. This genus has been reported to promote plant growth [52], and in this study it was positively correlated with total carbon, suggesting a potential role in carbon transformation [53]. Moreover, the increase in Proteobacteria bacteria may help to improve the ascocarp yield of morels, such as *Pseudomonas*, which was stimulated by hyphal growth [23]. *Bacillus* has been found to have a significant effect on the growth and development of *Morchella* fruiting body [20].

5. Conclusions

This study revealed dynamic changes in the rhizosphere soil bacterial community of *M. sextelata* in the Huangshan City and identified microbial groups closely associated with its growth stages. The results showed that *M. sextelata* growth altered the rhizosphere microenvironment, leading to a continuous decline in the soil C/N ratio and a gradual reduction in bacterial diversity. Additionally, Proteobacteria, Acidobacteria, and Bacteroidetes dominated the rhizosphere bacterial community. Key taxa were closely associated with soil carbon and nitrogen components, including *Massilia* and *Mucilaginibacter*. This study provides an important scientific basis for improving cultivation through microbial management. Future research should integrate metagenomics and metabonomics to elucidate the functional pathways of key bacterial communities and the molecular mechanisms underlying their interactions with *M. sextelata*, thereby supporting the sustainable development of morel cultivation.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae12020211/s1; Figure S1: High-quality sample sequence length distribution; Figure S2: Rarefaction curves of rhizosphere soil bacteria in *Morchella sextelata* at different growth stages; Figure S3: Microbial co-occurrence network analysis. Each point represents an OTU, and the line connecting two nodes represents a significant correlation in the abundance of the two OTUs. The color of the node represents the phylum level taxonomy to which the OTU belongs, and the size of the node is its degree in the network.

Author Contributions: Conceptualization and methodology, X.S.; formal analysis and data curation, P.C., T.Y., R.Y., R.L., G.G. and Z.G.; investigation and writing, P.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the natural science major project of Anhui province's education department (2023AH040174), the open research project of the rural revitalization collaborative technical service center of Anhui province (Huangshan University) (XCZXZD2302). We would like to thank the program for excellent scitech innovation teams of universities in Anhui province (2023AH010054). We would also like to thank the scientific and technological innovation research project of Huangshan University (XSKY202401), the university synergy innovation program of Anhui province (GXXT2023054), the Huizhou mushroom industry and microbial technology innovation center of Huangshan university (kypt202001) and the first level discipline of Huangshan University (ylxk202101).

Data Availability Statement: The raw data supporting the conclusions of this manuscript will be made available to the authors, without undue reservation, to any qualified researcher. Publicly available datasets were analyzed in this study. The original data have been deposited into the NCBI SRA database with the accession number PRJNA1398512.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

YJ	Primordium stage
ZJ	Needle tip stage
SS	Mulberry stage
YG	Young mushroom stage
CG	Fruiting stage
XS	Blank soil before planting
PCR	Polymerase chain reaction
RDP	Ribosomal database project
OTU	Operational Taxonomic Units
ACE	Abundance-based Coverage Estimator
KEGG	Kyoto Encyclopedia of Genes and Genomes
RDA	Redundancy analysis
PCoA	Principal coordinates analysis
SE	Standard error
LDA	Linear discriminant analysis
ANCOM	Analysis of compositions of microbiomes

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