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Exploring the Influence of Culture Environment on the Yield of *Volvariella volvacea* Based on Microbiomics

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Abstract: As one of the most nutritious and delicious mushroom varieties, *Volvariella volvacea* has always been popular among people around the world. Different from other artificially cultivated mushrooms, *Volvariella volvacea* is mostly planted on non-sterile substrates. As the cultivation time increases, the yield of mushroom houses continues to decrease. In our experiment, we selected two groups of samples from the old and new mushroom houses, environmental samples and substrate samples. The results showed that the diversity and abundance of microorganisms in the culture environment of the old mushroom room were consistent with that of the new mushroom room, but the proportion of actinomycetes in the former was significantly higher than that of the new mushroom room. The metabolic activity of microorganisms in the old mushroom room was enhanced compared with the new mushroom room. The microbial diversity in the growth substrate was investigated, and it was found that the structure and diversity of the microbial community in the substrate had changed. In conclusion, the interplay between mushrooms, the culture environment, and the host bacterial community may be the cause of the changes in the yield of *Volvariella volvacea*.

Keywords: *Volvariella volvacea*; edible mushroom; metagenome; microbial community

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1. Introduction

Mushrooms, traditionally harvested from forests but now more often artificially planted, have recently become a product of China's fifth-largest agricultural sector [1]. Straw mushroom (*Volvariella volvacea*) belongs to *Pluteaceae*, *Agaricales*, *Agaricomycetidae*, *Agaricomycetes*, *Agaricomycotina*, *Basidiomycota*, Fungi [2]. It is also known as a Chinese mushroom and is a nutritious edible mushroom [3]. *Volvariella volvacea* cultivation originated in China and has a history of more than 300 years [4]. *Volvariella volvacea* is not only nutritious but can also be used as medicine, making them one of the most famous mushrooms both domestically and internationally [5]. *Volvariella volvacea* vitamin C content is high, can promote human metabolism, improve body immunity, and enhance disease resistance. It also has a detoxification effect, where substances such as lead, arsenic, and benzene in the human body can be combined with the formation of ascorbic elements and excreted with urine [6]. The main reason for the popularity of *Volvariella volvacea* is its short growing period and low energy consumption [7].

Although *Volvariella volvacea* has a good taste and is beneficial to human health, it has high requirements for the cultivation environment, requiring a temperature of 28–35 °C and a pH value of 6–8 [8]. *Volvariella volvacea* uses the humus from the fermentation of crop straw (such as wheat stalk, corn stalk, etc.) as the main source of nutrition [9]. In the past three years, China's *Volvariella volvacea* production has increased year by year, and the

output of China's *Volvariella volvacea* in 2021 was about 256,000 tons [10]. It is estimated that more than 25 million farmers in China are currently engaged in collecting, growing, processing, and selling mushrooms [1]. As an important edible fungus with high flavor and nutritional value, its annual production in China is estimated at 330,000 tons, accounting for more than 80% of global production, but erratic yields have posed serious challenges to its cultivation and propagation [11,12].

Planting methods of *Volvariella volvacea* in the Guangdong region are unlike those of most edible fungi, which are grown in fungi bags, as they are cultured directly on a soil bed, and their mycelial growth and results are influenced by interacting soil-resident microbial community [13]. Some studies have shown that the possible reasons for the decline in mushroom production include unstable bacterial quality, improper field management methods, adverse environmental factors, pathogenic bacteria, soil characteristics, and soil microbial community dynamics [14–17]. Several studies have shown the obstacles associated with continuous cropping effects of conjunctions on mushroom yield [18]. The root cause of the obstacles associated with continuous cropping is a decrease in mushroom production due to changes in its microbial community [13]. The composition of the microbial population in the soil ecosystem is one of the key factors affecting the cultivation of edible mushrooms [19]. Simultaneous environmental factors can affect the growth and development of edible mushrooms through different metabolic pathways [20]. The microbiota selected by the fungus exists on the surface of and near the mycelium [21]. The environment in and around the fungal mycelium influences and is strongly influenced by the bacterial community [22,23]. Various interactions between bacteria and cultivated mushrooms have been described as leading to both positive and negative outcomes for mushrooms, depending on the stage of development of the bacterial isolate and the fungus [24]. The production of edible mushrooms is inseparable from the compost substrate, which is mainly composed of lignocellulose [25]. Macromolecular substances such as lignocellulose are not easily decomposed and absorbed by edible fungi when used directly without degradation, but microorganisms can change the lignin structure to a certain extent and become water-soluble products [19]. Studies have shown that the distribution of four main bacterial phyla [Firmicutes, Actinomyces, Proteobacteria, and Chlorobacteria] affects mushroom yield [26].

At present, there have been a large number of reports on the relevant research on the microorganisms of edible mushrooms, but there has been no relevant report on the phenomenon of the yield of *Volvariella volvacea* decreasing with long cultivation time in the same culture house. Moreover, there are few reports on the effect of mushroom culture environment on bacterial community [27]. Therefore, exploring the reasons for this phenomenon will have a very important impact on the increase of the yield of *Volvariella volvacea* in China. Based on the microbiome, we comprehensively investigated the microbial changes in different culture environments and the diversity of substrate microorganisms during the growth period. Two sets of different yield environments and received culture samples were collected from the culture chamber. The results of our study were designed to investigate (i) the changes in the microbial community of the culture environment and substrate after cultivation; (ii) the changes in the microbial metabolism of the culture environment after cultivation; (iii) the changes in the microbial function of the culture environment and substrate after cultivation.

2. Materials and Methods

2.1. Strains, Compost Substrate Formula, Cultivation Method, and Sampling

This study was conducted in Guangzhou City, Guangdong Province, China (112°94′ E, 23°30′ N). The *Volvariella volvacea* variety used was V26, stored in the Edible Fungi Culture Collection Center of the Vegetable Research Institute, Guangdong Academy of Agricultural Sciences. The formula and production method of the compost substrate of *Volvariella volvacea* are as follows: using waste cotton residue as the base material, soaking it with lime water, picking it up, stacking it for 3 days, turning the pile once during the period, adding

lime (5% of the dry weight of waste cotton residue) before cultivation, and mixing it fully. After the pile is turned and mixed evenly, the mushroom room is spread on the culture bed frame, and a coal stove is heated for pasteurization. The temperature reaches 65 °C for 6~8 h and then drops naturally. When the temperature in the mushroom room drops to about 45 °C, the doors and windows are opened to ventilate. When the temperature drops to about 36 °C, the seeds are sown evenly on the surface of the culture material. After sowing, the management should be carried out according to the conventional cultivation methods: the substrate is covered with film to moisturize, the temperature in the mushroom house is controlled at 30–33 °C, the film is removed after 3 days, water is sprayed after 4 days and 6 days, and the appropriate temperature, humidity, and ventilation in the mushroom house are maintained during the emergence of mushrooms. During this period in our experiment, the artificial management and harvesting were consistent.

The method of multipoint sampling was used in this experiment. In the middle of the mushroom growing period, substrates of the new/old mushroom house were taken and denoted as SNMH/SOMH. These samples were defined as substrate samples. Biological repetition was three. The 6 samples were sequenced by 16S rDNA. After harvesting, the walls, beds, and windows of the old and new mushroom houses were wiped with sterile cloth and marked as NMH1, NMH2, and NMH3 (new mushroom house), and OMH1, OMH2, and OMH3 (old mushroom house), respectively. These samples were defined as environmental samples. All samples were stored at −80 degrees and subsequently used for metagenomic sequencing.

2.2. Metagenomic Sequencing

Metagenomic sequencing was performed on environmental samples. The DNA in each sample was extracted using OMEGA's E.Z.N.A.® Soil DNA Kit (R6731-00S, Omega Bio-tek, Norcross, GA, USA). The method was magnetic bead extraction, cleavage digestion under the action of lysate and protease, adsorption of pure DNA by binding solution and magnetic bead, washing, and elution recovery. More than 1 μg of DNA was extracted from each sample. All the extracted DNA was subjected to gel electrophoresis (0.8% agarose gel) to show the quality and size of the DNA; the bands were clear, indicating no degradation or slight degradation. It was then used for metagenomic sequencing.

The extracted DNA (250 ng) was sonicated in a sonicator (JY92-IIN, Xinzhi, Ningbo, Zhejiang, China) to a fragment size ranging from 200 to 500 bp. Sequencing libraries were prepared using the VAHTS Universal DNA Library Prep Kit for Illumina (ND607-01, Vazyme Biotech Co., Ltd., Nanjing, China).

PCR was conducted (Catalog NO. ND607, Vazyme Biotech Co., Ltd., Nanjing, China) following the manufacturer's instructions. PCR products corresponding to 200–500 bp were enriched, quantified, and finally sequenced using a NovaSeq 6000 sequencer (Illumina Inc., San Diego, CA, USA).

2.3. 16S rDNA Sequencing

A substrate sample was taken for 16S rDNA sequencing. After genomic DNA was extracted according to the instructions of the corresponding DNA extraction kit for each sample, the integrity and purity of DNA were detected by 1% agarose gel electrophoresis, while the concentration and purity of DNA were detected by NanoDropOne. PCR amplification and product electrophoresis were performed using genomic DNA as a template, using primers with barcode and PremixTaq (TaKaRa, Kusatsu, Japan) according to the selection of sequencing region. Using GeneToolsAnalysisSoftware (Version 4.03.05.0, SynGene, Bangalore, India) concentration of PCR products after contrast was calculated in accordance with the principle of quality such as the sample size required for each PCR product. E.Z.N.A. GelExtractionKit (D2500-01, Omega Bio-tek, Norcross, GA, USA) gel recovery kit was used to recover PCR mixed products, and TE buffer was eluted to recover target DNA fragments. After creating a library according to the NEB Next Ultra TMDNA Library

Prep Kit for lllumina standard process for building operation, sequencing is completed by high-throughput sequencing platform Hiseq or Miseq sequence on the computer.

2.4. Metagenomic Analysis

Data evaluation and quality control: The original sequencing data was evaluated by FastQC and filtered by Trimmomatic to obtain relatively accurate and effective data.

Splicing assembly: IDBA_UD was used to splice and assemble the growth sequence contig for each sample of clean reads. Contigs were obtained according to the overlap between reads. The assembly results of multiple KMers were comprehensively evaluated, and the best Kmer assembly results were selected.

Gene prediction: ORF prediction was performed on splicing results by Prodigal, and genes with a length greater than or equal to 100 bp were selected and translated into amino acid sequences.

Gene set construction: For the gene prediction results of each sample, CD-HIT Version 4.6 software was used to remove redundancy to obtain non-redundant gene sets.

Gene set abundance: Bowtie2 was used to compare clean reads to non-redundant gene set sequences, and SAMtools Version 0.1.18 software was used to obtain the corresponding reads. Combined with gene length, gene abundance information in each sample was calculated.

Gene set functional annotation: The gene set was compared with NR, KEGG, ARDB, and CAZy to obtain the species annotation information and functional annotation information of the genes. Functional abundance and species abundance were obtained according to gene set abundance.

Abundance analysis: Based on gene set abundance information and annotation information, species abundance and functional abundance were obtained, and statistical analysis and exploration in multiple directions, such as species and functional composition analysis, species and functional difference analysis, and sample comparison analysis were carried out, and the results were visually displayed.

2.5. 16S rDNA Analysis

After sequencing, based on the quality of the single nucleotides, the raw data was filtered primarily by trimmomatic (version 0.33). Identification and removal of primer sequences were performed by Cutadapt (version 1.9.1). The PE reads obtained from the previous step were assembled by usearch (10th edition) and then the chimeras were removed using UCHIME (8.1 edition). High-quality reads generated from the above steps were used for the analysis below. Sequences with similarity $\geq$97% were clustered into the same operational taxon (OTU) using software (v10.0), and relative abundances were evaluated. Apply a filter to retain only 0.005% of the OTUs. Based on the naive Bayes classifier in QIIME2e, using the SILVA database (132nd edition), the confidence threshold was 70%. The alpha diversity was calculated and displayed using QIIME2 Version 2021.11 and R Version 3.3 software. QIIME was used to measure beta diversity and evaluate the similarity of microbial communities in different samples. Principal coordinate analysis (PCoA), heat maps, UPGMA, and non-metric multidimensional scaling (NMDS) were used to analyze beta diversity. In addition, we used linear discriminant analysis (LDA) effect sizes (LEfSe) to examine significant taxonomic differences between groups. The logarithmic LDA score of 4.0 was used as the threshold for identification features.

3. Results

3.1. Illumina Sequencing of Culture Environment and Substrate Microbiota of Volvariella volvacea

Volvariella volvacea is an important edible mushroom, whose yield is directly related to the development of the straw mushroom industry. However, we found that the yield of *Volvariella volvacea* in the mushroom house often declined significantly after a period of cultivation. In order to ensure the stability of straw mushroom yield and the development of the straw mushroom industry, we decided to study the causes of this phenomenon. We

first collected the yield of *Volvariella volvacea* per square meter from three groups in a new mushroom house (NMH) and an old mushroom house (OMH) that had been cultivated for five years. The data from all three groups showed that the yield of the NMH was significantly higher than that of the OMH (Figure 1a–e). This indicates that the usage time of the mushroom house indeed affects the yield of the straw mushroom.

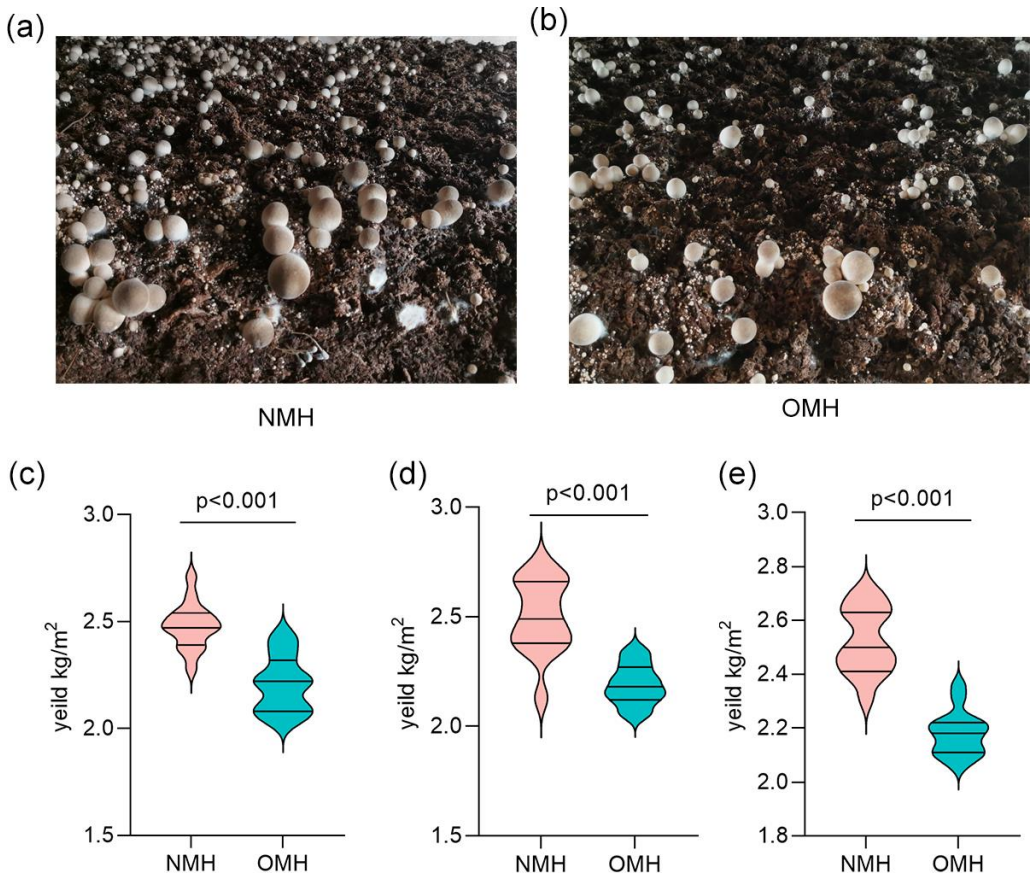


Figure 1. The *Volvariella volvacea* growth in the new mushroom house (NMH) and the old mushroom house (OMH). (**a**) The new mushroom house; (**b**) the old mushroom house. (**c**–**e**) NMH and OMH yield comparison. The mushroom beds were divided into 1 square meter sections, and the yield per square meter was recorded. Each group consisted of 20 square meters of data. The result was analyzed using a *t*-test.

To investigate the impact of the culture environment on the yield of *Volvariella volvacea*, Illumina sequencing was conducted on the microorganisms within this environment. Metagenomic sequencing yielded 3,898,494 assembly sequences (contigs); the average length of a single sample contig ranged from 709.5 to 896.3 pb, with an N50 from 756 to 1172 bp (Table 1). Following this, we performed ORF prediction on the assembly results. Genes with nucleic acid lengths of 100 bp or greater were selected and translated into amino acid sequences. This led to the clustering of the predicted gene sequences from all samples (Figure 2a), revealing that most amino acid sequence lengths clustered within the 0–250 bp range. These findings affirmed the reliability of the metagenomic data for further analysis.

Additionally, the Pan and Core analysis plots of the 16S rDNA sequencing demonstrated that the dilution curve had plateaued (Figure 2b). This suggests that with an increasing number of sequencing samples, both the total species count and the core species count tend to stabilize. Thus, our sample size was adequate, and the data were suitable for subsequent analysis.

Table 1. Summary of de novo assembly results of metagenomic DNA samples.

Sample	Contig				
	Contig Number	N50 (bp)	Min (bp)	Max (bp)	Average Length (bp)
NMH1	632,326	1172	200	547,145	896.3
NMH2	507,465	756	200	190,352	709.5
NMH3	710,665	876	200	308,874	764.6
OMH1	541,423	1004	200	185,247	847.8
OMH2	692,502	758	200	281,280	718.6
OMH3	814,113	799	200	307,081	742.2

NMH, samples for new mushroom house; OMH, samples for old mushroom house; contig number, number of assembled contigs; N50 (bp), length of contig N50; min (bp), minimum contig length; max (bp), maximum length of contig; average length (bp), average length of contig.

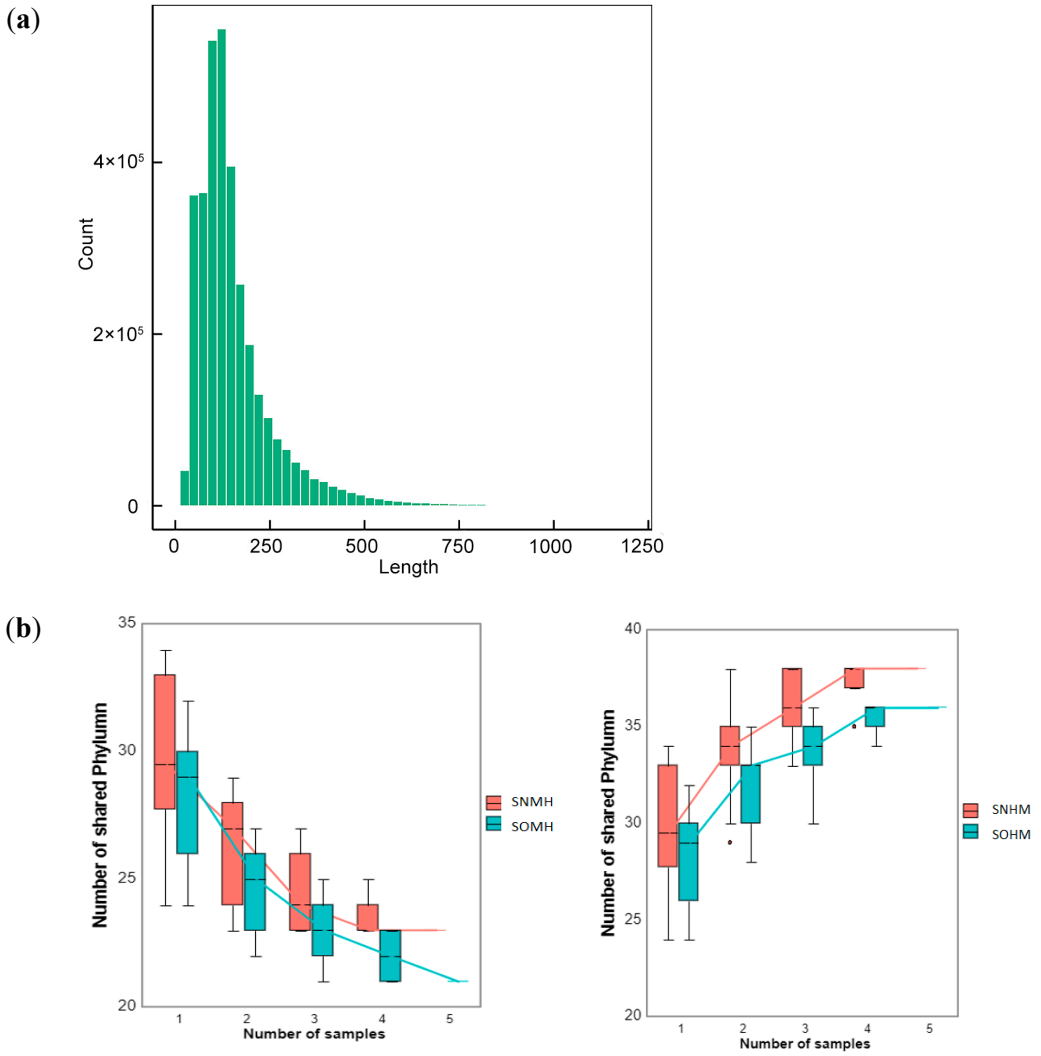


Figure 2. Sequencing Quality Control. (**a**) Amino acid sequence distribution of the non-redundant gene set. The horizontal axis represents the length intervals of the gene amino acid sequences, whereas the vertical axis indicates the number of sequences within each interval. (**b**) Pan–Core species analysis derived from 16S rDNA sequencing. The left and right panels depict the Pan and Core analyses, respectively. In these charts, the horizontal axis signifies the count of sampled specimens, and the vertical axis denotes the statistical quantity obtained from random sampling (set to 100 iterations) within this layer. SNMH stands for the substrate from the new mushroom house, and SOMH represents the substrate from the old mushroom house.

3.2. Microbial Community Structure and Diversity in Different Culture Environments

The samples of the *Volvariella volvacea* culture environment were analyzed by metagenomic sequencing technology to detect the genetic information of its microflora. Excluding the unidentified species, the abundance of dominant phyla in the *Volvariella volvacea* culture environment samples were *Pseudomonadota*, *Bacillota*, *Actinomycetota*, *Ascomycota*, *Planctomycetota*, *Bacteroidota*, *Basidiomycota*, *Verrucomicrobiota*, and *Deinococcota*. Compared with NMH samples, the abundance of *Actinomycetota* in the OMH significantly increased, while the abundance of *Bacillota* decreased. Other TOP20 phyla abundances did not change significantly in all samples (Figure 3a). Excluding the unidentified species, the abundance of dominant genera in the *Volvariella volvacea* culture environment samples were *Pseudonocardia*, *Paenibacillus*, *Geobacillus*, *Paecilomyces*, and *Bacillus*. Compared with NMH samples, the abundances of *Pseudonocardia* and *Rhodococcus* in the OMH were significantly increased. Other TOP20 genus abundances did not change significantly in all samples (Figure 3b). Linear discriminant analysis (LDA) effect size (LEfSe) was used to find the dominant indicator species. The LEfSe analysis revealed that the dominant taxa within the OMH samples were primarily from the orders *Mycohacteriales* and *Actinomycetales*, with the latter focusing on three main members. Specifically, within *Actinomycetales*, the families *Nocardiaceae*, *Mycobacteriaceae*, and *Gordoniaceae* were identified as the top contributors. The TOP 2 dominant species of NMH are *Moraxellales* and *Moraxellaceae*, belonging to the genus *Moraxella* (Figure 3c). The results showed that the composition and structure of microorganisms in different culture environments were different.

Alpha diversity refers to the diversity within a specific region or ecosystem and is a comprehensive indicator of richness and evenness. Chao1 index, observed index, and ACE index represent community richness, Shannon index and Simpson index represent community diversity, and the higher the value, the higher the species diversity. The Alpha diversity of each sample was calculated at the genus classification level (Figure 3d). There was no significant difference in microbial diversity and abundance between the old and new mushroom culture environments, as shown in Figure 3d (p-values were 0.7, 0.7, 0.7, 1, and 1, respectively, which were all greater than 0.05). The results showed that the richness and diversity of microbial community structure in the old mushroom houses were almost the same as in the new ones.

3.3. Microbial Metabolism in Different Culture Environments

In order to further explore the influence of the culture environment on the growth of mushrooms, the changes in microbial metabolism in the culture environment were studied. KEGG pathway annotation of the predicted gene sequences obtained from six samples enabled the identification of a total of 16,556 KOs, which were organized into six pathways. There were more enriched metabolic pathways in the OMH (Figure 4a). The identified metabolic pathways were associated with 12 basic metabolic systems (Figure 4b). Among them, the top three metabolic pathways that were most enriched were the global and overview map, carbohydrate metabolism, and amino acid metabolism. The enriched metabolic pathways in the OMH were higher than those in the NMH (Figure 4b). Next, we examined the global and overview map pathway in more depth and found that the differences between the metabolic pathways of the OMH and the NMH were centered on metabolic pathways and microbial metabolism in diverse environments (Figure 4c). It can be seen that the metabolic activity of the OMH was higher than that of the NMH.

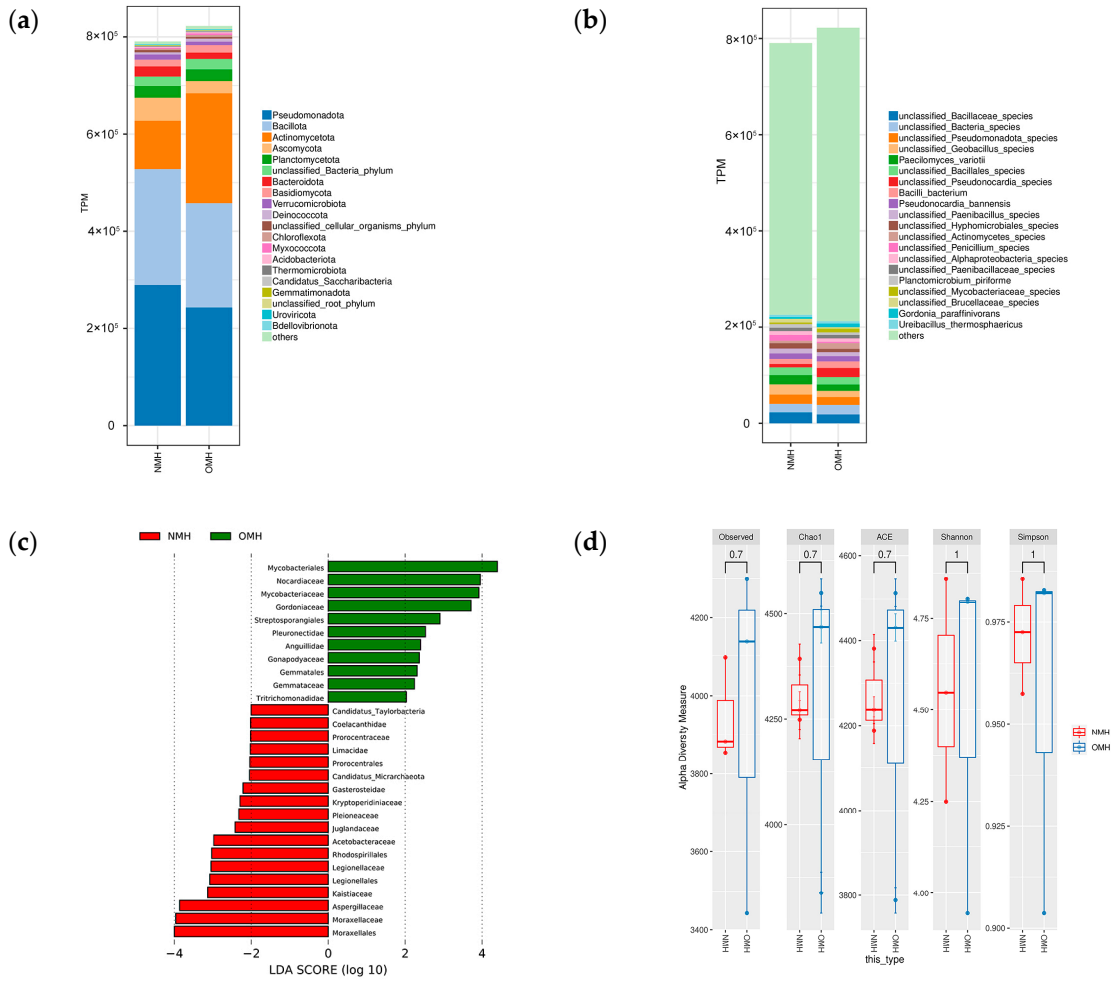


Figure 3. Microbial community composition and diversity in different culture environments. The relative abundance of TOP20 species at phylum and genus level in different culture environments (**a**). LEfSe analysis diagram of different culture environments (**b**). (**c**) Box diagram of diversity index in different culture environments. (**d**) Box diagram of diversity index in different culture environments. NMH, samples for the new mushroom house; OMH, samples for the old mushroom house. TPM, relative abundance (%).

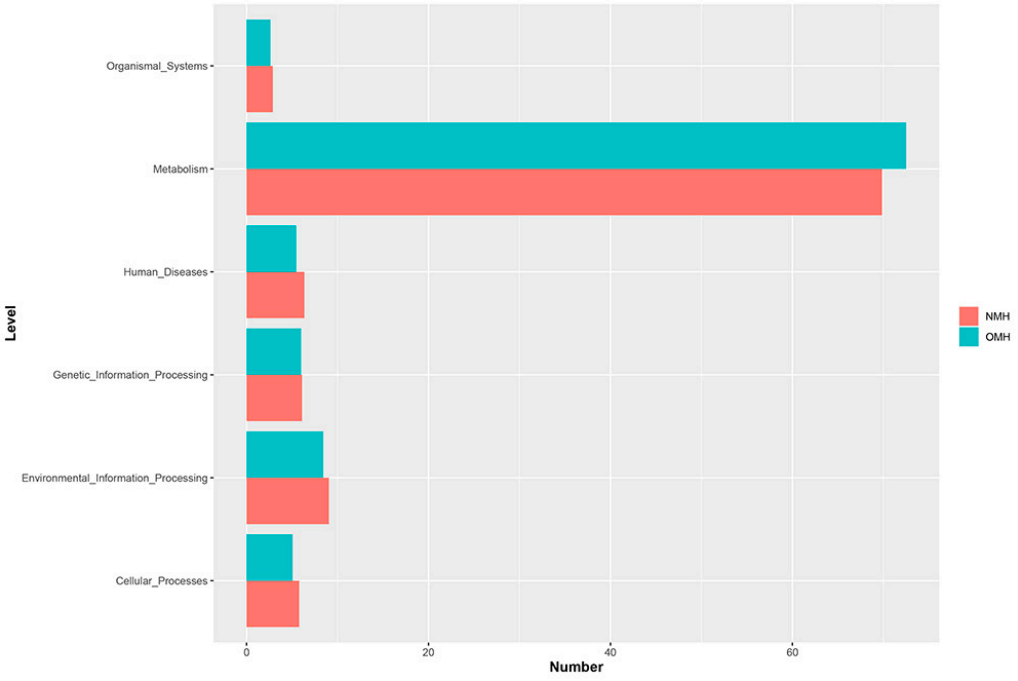
3.4. Microbial Community Function in Different Culture Environments

We compared the genes of the cultivation environment with the Pfam database and obtained a GO annotation. The TOP3 GO terms are obsolete oxidation–reduction process (GO:0055114), which belongs to biological processes; and ATP binding (GO:0005524) and DNA binding (G0:0003677), which belong to molecular function. The GO term of TOP 10 in all samples was analyzed by a circle diagram. As shown, the expression pattern of the OMH was not significantly different from that of the NMH (Figure 5a). GO enrichment analysis showed that the microbial functions of the OMH and NMH were not significantly different.

We annotated the genes to the CAZy database, a database resource for enzymes that can synthesize or break down complex carbohydrate and sugar complexes. Based on amino acid sequence similarity in the protein domain, active carbohydrate enzymes from different species can be divided into glycoside hydrolases (GHs), glycosyl transferases (GTs), carbohydrate esterases (CEs), carbohydrate-binding modules (CBMs), polysaccharide lyases (PLs), auxiliary oxidoreductases (AAs), and six other protein families. The circle diagram shows that the functional expression patterns of OMH and NMH are not different (Figure 5b). CAZy functional annotation analysis showed no significant difference between the OMH and the NMH.

In conclusion, by analyzing the microbial functions of the culture environments of the old and new mushroom houses, we found that there was no significant difference between them.

(**a**)



(**b**)

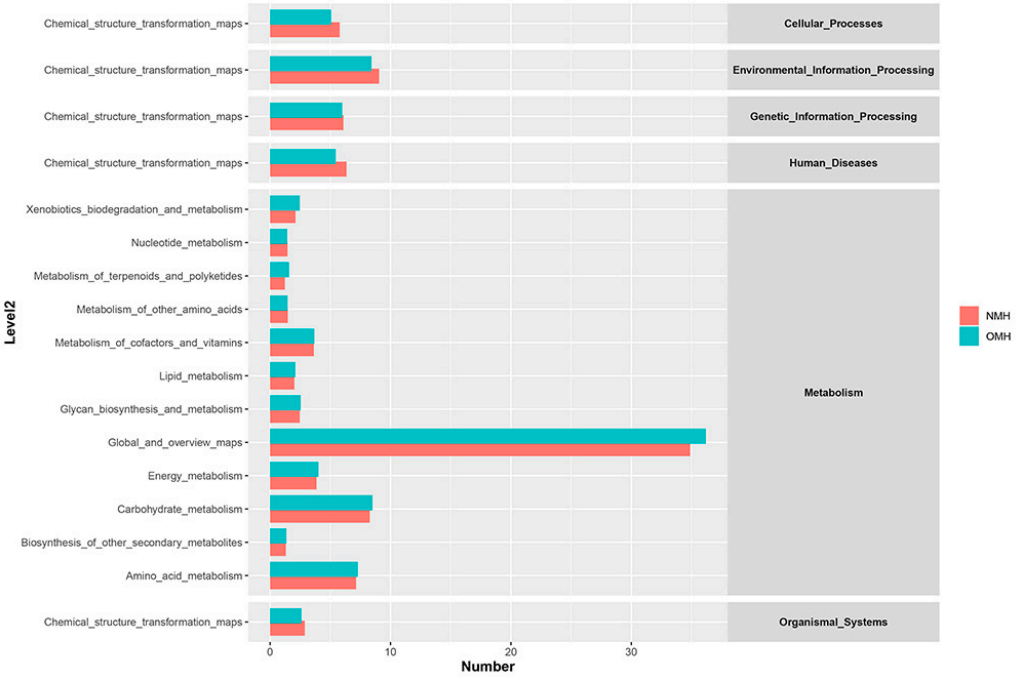


Figure 4. *Cont*.

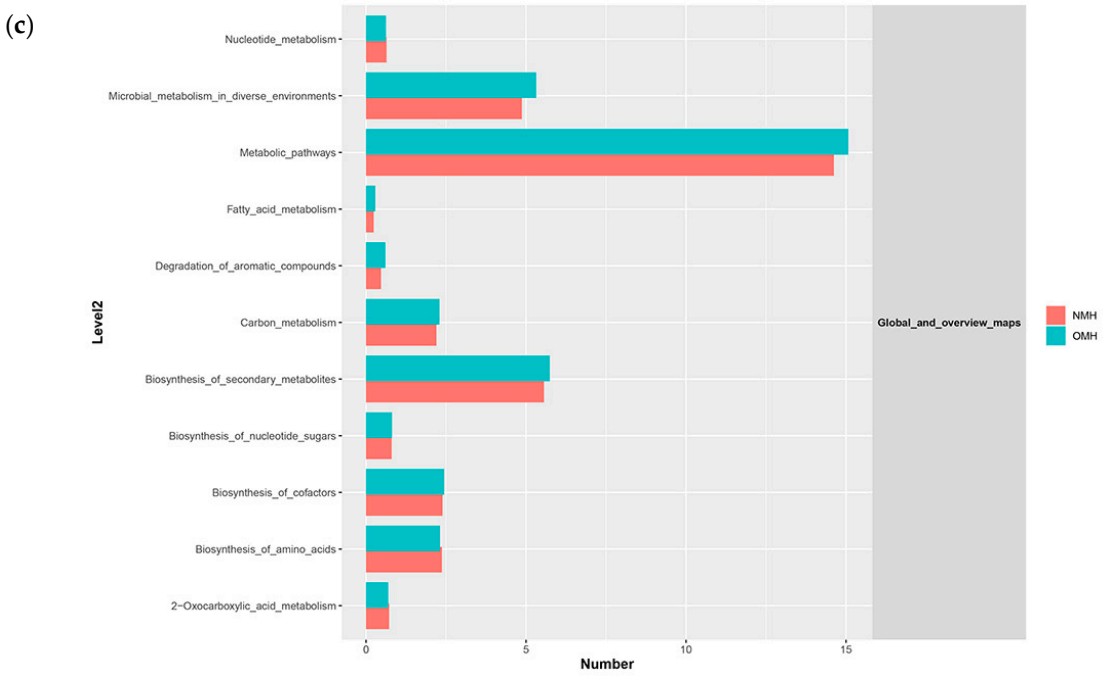


Figure 4. Analysis of KEGG functional enrichment of microorganisms in cultivation environment (**a**–**c**). NMH, samples from the new mushroom house; OMH, samples from the old mushroom house.

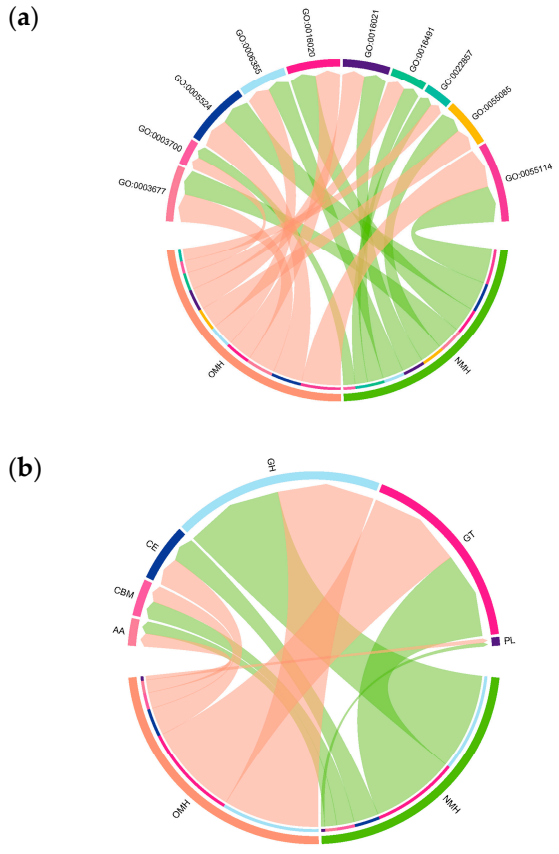


Figure 5. Microbial function results from culture environment. Circle diagram of TOP10 GO term (**a**). CAZy annotation results of TOP10 CAZy family of culture environment (**b**). NMH, samples from new mushroom house; OMH, samples from old mushroom house.

3.5. The Difference of Microbial Composition and Structure in the Substrate of Volvariella volvacea in Different Culture Environments

The above results showed that the composition and metabolism of the microbial community in different culture environments changed. In order to explore whether the culture environment affected the matrix, 16S rDNA sequencing was performed on the matrix samples during the growth period of *Volvariella volvacea*. 16S rDNA sequencing has become a technique to accurately identify bacteria [28], so it is used to explore the bacterial community and structure in the substrate.

Based on the principle that 97% of similar species make up an OTU, there were 1342 OTUs in the two sets of samples. Species composition of a substrate Venn diagram show that 1208 OTUs were the same in the substrate of the old and new mushroom houses, 94 OTUs were unique to the new mushroom house, and 40 OTUs were unique to the old mushroom house (Figure 6a). LDA was used to find species with significant abundance differences between different groups (LDA score > 2). The significant difference species in the new mushroom house matrix were Prosthecobacter, Aeribacillus, and Burkholderiaceae; the significant difference species in the new mushroom house matrix were Paenibacillaceae, Bacillales, Bacilli, and Firmicutes (Figure 6b). This showed that the microbial community of the substrate changed with the growth of the *Volvariella volvacea* in different culture environments. The richness diversity index of the substrate of old mushroom houses was higher than that of new mushroom houses (Figure 6c). The 16S rDNA sequencing results indicated that the composition and structure of the substrate microorganisms in the old and new mushrooms during the growth period changed.

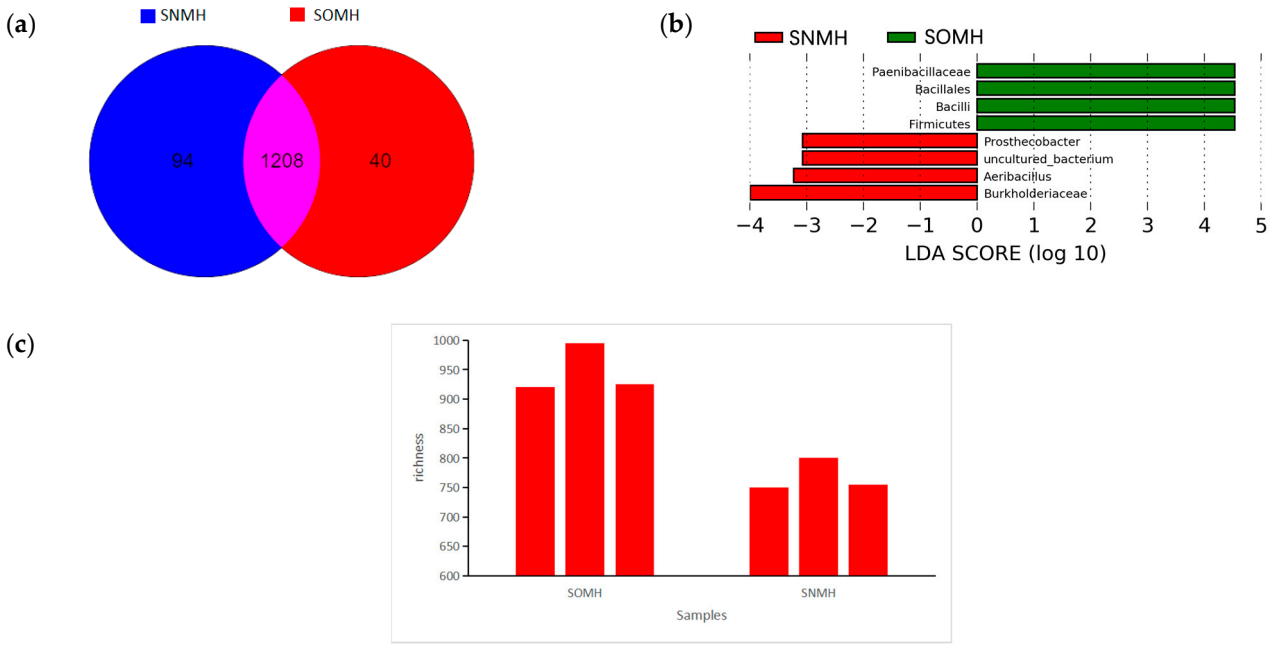


Figure 6. The results of 16S rDNA sequencing of the substrate in the new and old mushroom houses. A Venn diagram of substrate species in the new and old mushroom houses (**a**); an LDA diagram of substrate species in new and old mushroom houses (**b**). For species with significantly different abundance in different groups under conditions greater than the set LDA value (set to 2 by default), the length of the bar chart represents the size of the impact of the different species (i.e., the LDA Score). An Alpha Diversity Index graph (**c**). SNMH, the substrate from the new mushroom house; SOMH, the substrate from the old mushroom house.

4. Discussion

Previous studies have reported that mushroom bed cultivation is superior to bottle or plastic bag cultivation [29]. The main cultivation methods of *Volvariella volvacea* are greenhouse cultivation and indoor cultivation after primary fermentation or secondary

fermentation treatment, and the cultivation period is 20–23 days [30]. Microorganisms in the compost and culture environments coexist and interact with the mushrooms, and this ecological relationship has been described as an important driver of mycelium growth and fruited body development [31]. As shown in Figure 3d, the richness and diversity of the microbial community in the old mushroom house were greater than those in the new mushroom house at the genus level, which also verified that the microbial community in the culture environment would gradually enrich and diversify over time [20]. The symbiotic bacterial community associated with mushrooms exhibited variations in its structure across different stages of growth and development. [32]. In our experiment, the substrate used in the old and new mushroom houses was the same, but the bacterial community changed in the middle of the mushroom cultivation period (Figure 6). The beneficial microorganisms in the fermentation feed can promote the macromolecular degradation, transformation, and utilization of bioheat energy of the culture feed and inhibit the breeding of diseases, insects, and bacteria [33]. In the reported studies on the cultivation of *Volvariella volvacea* with straw, waste cotton, or bacterial residue as the main culture material, it was found that the microbial dominant population in the fermentation material would change in different fermentation periods, and the microbial community structure and dominant population were significantly different with different culture material composition [34]. In our study, it was found that there was a difference in the microbial community of the substrate in the old and new mushroom houses at the phylum level (Figure 6a).

Pseudomonas is widely distributed in nature in soil, water, food, and air. There are capsular, flagella, and fimbriae. Its nutritional requirements are not high, and there are many types [35]. There are also reports that *Pseudomonas* is the most abundant in the air of oyster mushroom breeding facilities [36]. This is consistent with our experimental results (Figure 2). The abundance ratio of this genus in the new mushroom house was higher than that in the old mushroom house (Figure 2b). *Pseudomonas* has been shown to be capable of defeating or fighting plant pathogens and can thus be used for biological control [37]. *Paecilomyces* has also been found to be highly abundant in NMH1 (Figure 2a) and it has been shown to be effective in controlling the emergence of plant pathogens [38]. This may be one of the reasons for the high yield of new mushroom houses. Actinomycetes are important antibiotic-producing bacteria, which are related to soil humus content, play a certain role in material transformation in soil, and can significantly inhibit fungi causing higher plant diseases [39]. In our experiment, we found that the proportion of actinomyces in the old mushroom house was higher than that in the new mushroom house (Figure 2).

Invertase is closely related to plant growth, and invertase activity can be used as an indicator of metabolism and growth intensity [40]. In our experiment, the functional activities of invertase produced by microorganisms in the old mushroom house were intensified (Figure 5). Studies have shown that the interaction of beneficial bacteria and fungi can lead to a decrease in the metabolic activity of the two [41]. Compared with the new mushroom house, the metabolic pathways involved by microorganisms in the old mushroom house increased (Figure 3). It can be seen that the intensification of microbial metabolic activities in the old mushroom house may also be one of the reasons for the decrease in *Volvariella volvacea* yield.

The old mushroom house was more involved in the obsolete oxidation-reduction process and DNA binding than the new mushroom house (Figure 3b). The new mushroom house also had more microbial carbohydrate-binding modules (CBMs) than those of the old mushroom house (Figure 5). CBMs have been reported to promote the degradation of lignocellulose [42]. Lignocellulose is an important natural polymer compound that exists in plant cell walls and is the main component of plant structure. Lignocellulose has many important functions and plays an important role in plant growth, structural support, and environmental adaptation [43]. Lignocellulose also contributes to the decay of the substrate, helping the plant to better absorb nutrients; lignocellulosic degradation is therefore key to rapid substrate production at scale [44]. Liang et al. [45] found that ascomycota played a dominant role in the degradation process of lignocellulose. In our experiment, we found

that the proportion of *Ascomycota* abundance in the new mushroom house was higher than that in the old mushroom house (Figure 2b). This may be one of the reasons for the high yield in the new mushroom house.

Studies have shown that some bacteria and fungi can improve the productivity of fermentation materials, inhibit the spread of pathogenic bacteria, and also promote the growth of edible fungi mycelia and induce the formation of fruiting bodies [46]. As technology develops, biological inoculants of new bacteria that promote mushroom growth and yield may serve as a potential eco-friendly alternative [47]. Burkholderia can be used for biodegradation and biological control in agriculture and as endosphere microorganisms that promote plant growth [48]. At the same time, our experiment also proved that *Burkholderiaceae* could be used as markers of high and low yield in the substrate (Figure 6b). In summary, the interaction between mushrooms, culture environment, and substrate bacterial community affects the degradation of nutrients in the substrate, which may be the cause of the change in the mushroom yield.

In conclusion, we observed significant yield differences between the new and old mushroom houses, likely due to changes in the microbial community with ongoing cultivation, leading to a gradual decrease in beneficial microbial populations. Microorganisms such as *Pseudomonas* (Figure 2), *Paecilomyces* (Figure 2b), and *Ascomycota* were notably more abundant in the new mushroom houses than in the old ones (Figure 2b), as mentioned earlier. These microbes are closely associated with the metabolism and stress responses of *Volvariella volvacea*. It can be anticipated that the reduction of these beneficial microbes, as the usage time of mushroom houses increases, could worsen the growth environment for *Volvariella volvacea*, consequently leading to decreased yields. Similarly, we also discovered that an increase in *Actinomycetes* could affect the yield of *Volvariella volvacea*, which is an intriguing finding. While several studies have highlighted the importance of abiotic factors on the growth of fungi, our research corroborated this information through omic techniques, enhancing our understanding of the relationship between the production of fruiting bodies and environmental factors. Here, through bioinformatics analysis, we identified microorganisms beneficial and detrimental to the yield of *Volvariella volvacea*, laying the groundwork for future research on regulating mushroom production through microbial interaction. Further studies may include physiological and biochemical experiments to verify whether these microbes directly influence the growth and development of straw mushrooms. This paper may also have a positive impact on production, suggesting that the cultivation of *Volvariella volvacea* can be guided by artificially increasing beneficial microbes and reducing harmful ones.

5. Conclusions

In this study, high-throughput sequencing technology was used to analyze the change characteristics of substrate and culture environment biological communities after cultivation of high- and low-yielding *Volvariella volvacea*, compare and analyze their composition differences, and explore the differences in the metabolism and function of their microbial communities. It was found that the proportion of microbial actinomycetes groups increased, microbial diversity increased, and the oxidation–reduction process intensified in the culture environment with a long cultivation time. In addition, the microbial community structure of the substrate changed during culture, which may be the reason for the lower yield of *Volvariella volvacea*. This study has laid a foundation for analyzing the effects of the growing environment on mushroom yield.

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