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Differences in Soil Microbiota of Continuous Cultivation of *Ganoderma leucocontextum*

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Abstract: The tendency of microbiota changes in the soil of *Ganoderma leucocontextum* continuous cultivation is investigated by high-throughput sequencing technology. Medium bag cultivation of *G. leucocontextum* with uncultivated soil significantly increased the organic matter (OM), hydrolyzable nitrogen, available phosphorus, and available potassium content of soil. The relative abundance of the dominant beneficial bacteria (*Sphingomonas* spp., *Mucilaginibacter* spp., *Bryobacter* spp., and *Bradyrhizobium* spp.) for *G. leucocontextum* continuous cultivation, was decreased in the soil. *Mortierella* spp. and *Pyrenochaeta* spp. were the dominant fungi with negative effects on *G. leucocontextum* cultivation in the soil. The correlations between the microbiota and soil physicochemical properties indicated that continuous cultivation not only caused changes in the soil physicochemical factors but also affected the structure of dominant microbial communities, especially bacteria and environmental factors.

Keywords: *Ganoderma leucocontextum*; casing cultivation; continuous cultivation obstacle; microbiota

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1. Introduction

Ganoderma leucocontextum is a fungus of Basidiomycetes, with a cap of white tissue [1,2]. It is suitable for planting at an altitude of 1900–3000 m under a medium–low temperature [3]. It has been widely cultivated as a high-quality *Ganoderma* species for its medicinal values with high contents of polysaccharides, triterpenes, and ganoderic acid [4–6].

Medium bag cultivation and log cultivation are the two main cultivation methods of edible fungi. Different edible fungi can choose different cultivation methods according to the requirements of the growth substrate and environment. *G. leucocontextum*'s cultivation is mostly in soil with a casing layer [7]. The soil can stabilize the water content in the medium, and provides plentiful probiotics and further mineral elements. By this cultivation method, *G. leucocontextum* fruiting bodies have higher contents of medicinal components. Moreover, this cultivation method can be of high yield. However, cultivation can be restricted by its continuous cultivation obstacles [8], including slow growth, infection by *Penicillium*, *Trichoderma*, and *Neurospora*, and its yield decreasing after 3–5 years of continuous cultivation. Some soil-borne pathogens were isolated from the continuous cultivation soil in our previous researches [9].

The researches on the decline in yield and quality caused by the continuous cultivation of mushrooms mainly focused on the cultivation technology with casing materials, and cultivation substrates [10–12]. Some microorganisms, such as *Trichoderma* sp. [13], *Clostridium* sp., *Alkaligenes* sp., and *Bacillus* sp. with strong allelopathic effects on *G. lucidum* growth with continuous cultivation obstacles had=ve been researched [14]. It was found that *Penicillium*, *Sphingomonas*, *Anaeromyxobacter*, *Bradyrhizobium*, and *Dehalococcoides* were related to

the continuous cultivation obstacles of *G. lucidum* [15,16]. A strong correlation between the continuous cultivation obstacles and the changes in microbiota in the *G. lucidum* cultivation soil was found [17]. However, the effect of the continuous cultivation of *G. leucocontextum* on soil physical and chemical properties and soil microbiota is unknown.

In our study, the differences in the soil microbiota after two cultivation methods, medium bag cultivation and log cultivation, were investigated, and the relationships between the microbiota changes and the continuous cultivation obstacles were analyzed. This study has promoted our understanding of the impact of the continuous cultivation of *G. leucocontextum* on micro-ecology, and provided a reference for the development of the cultivation technology of *G. leucocontextum*.

2. Materials and Methods

2.1. Cultivation

The *G. leucocontextum* (YBLZ4) strain was obtained from the Key Laboratory of Southwestern Crop Gene Resources and Germplasm Innovation, Ministry of Agriculture, Yunnan, China.

For medium bag cultivation, the sawdust/bran fruiting formula for cultivating *G. leucocontextum* was sawdust (78%), wheat bran (13%), corn flour (7%), sucrose (1%), and gypsum (1%), and the water content of the medium was 55–60% after soaking. The polypropylene bags (17 cm × 35 cm × 0.06 mm) were filled with the 1200 g medium (wet weight), and then the bags were autoclaved at 126 °C for 2 h, and inoculated with *G. leucocontextum* spawn after cooling to room temperature. Bags were incubated in the dark at 23 °C until substrate was filled with hyphae. The bags were transferred into arched shed after incubating another 30 days. The bags were opened and covered with 6.5 ± 0.5 cm of soil.

With log cultivation, wood logs (20 cm in diameter × 25 cm long) were bagged in polypropylene bags (35 cm × 50 cm × 0.005 mm), sterilized with steam at 95 °C for 15 h, and inoculated with actively growing mycelia of *G. leucocontextum* spawn at one side of each log. After incubating at 23 °C for 50–60 days, the fully colonized logs were taken out and transferred into arched shed to induce fruiting. Logs were put on the soil plot (1.5 m wide × 25 cm deep) vertically and covered with 8 cm thickness of soil.

Moreover, the continuous cultivated soil was used for the cultivation of *G. leucocontextum*. There were three treatments for the continuous cultivation in March 2021. Group I was medium bag cultivation with uncultivated soil in No.1 arched shed, Group II was log cultivation with continuous cultivated soil after 2-year log cultivation in No.2 arch shed, Group III was log cultivation with continuous cultivated soil after 4-year log cultivation in No.3 arch shed. The soil water content of all treatment groups was kept at 50%, and different treatment groups adopted unified farming management. A number of 1000 bags/logs were prepared for each treatment.

2.2. Collection of Soil Samples

All soil samples were collected at the end of *G. leucocontextum* harvest in October 2021 and were divided into four groups (Table 1).

In each group, four randomly placed sampling quadrates (1 m × 1 m) were selected. A 5-point sampling method (centre and four corners) was used, and 300 g soil was extracted by sterilized shovel around 3–7 cm in depth. The soil samples were collected and mixed together in sterile ziplock bags, which were immediately transported to the laboratory and stored at −80 °C for further analyses.

Each group contained four replicates resulting a total of 16 samples. The 200 g from each sample was homogenized for the analyses of soil physicochemical properties, another 20 g was used for microbial culture, another 50 g was used for total genomic DNA analyses.

Table 1. Soil sampling information and sample IDs.

Sampling Information	Sample ID	Sample Group
Medium bag cultivation ① soil of Group I (after 1 year)	B1a.1	B1a
Medium bag cultivation ② soil of Group I (after 1 year)	B1a.2	
Medium bag cultivation ③ soil of Group I (after 1 year)	B1a.3	
Medium bag cultivation ④ soil of Group I (after 1 year)	B1a.4	
CK ① uncultivated soil	CK.1	CK
CK ② uncultivated soil	CK.2	
CK ③ uncultivated soil	CK.3	
CK ④ uncultivated soil	CK.4	
Log cultivation ① Group II (after 3 year)	W3a.1	W3a
Log cultivation ② Group II (after 3 year)	W3a.2	
Log cultivation ③ Group II (after 3 year)	W3a.3	
Log cultivation ④ Group II (after 3 year)	W3a.4	
Log cultivation ① Group III (after 5 year)	W5a.1	W5a
Log cultivation ② Group III (after 5 year)	W5a.2	
Log cultivation ③ Group III (after 5 year)	W5a.3	
Log cultivation ④ Group III (after 5 year)	W5a.4	

2.3. Soil Physicochemical Properties

The homogenized soil samples were all air-dried and sieved. Soil pH was measured using a calibrated pH meter INESA PHS-3E in a 1:2.5 soil–water suspension. The organic matter (OM) in the soil samples was measured using the chromic acid titration method [18]. The hydrolyzable nitrogen (N) was measured using the alkaline solution diffusion method [19]. The available phosphorus (P) was determined by UV—colorimetric analysis [20]. The potassium (K) was determined by flame atomic absorption spectrometry (FAAS) [21].

2.4. The Number of Cultivatable Microorganisms

Samples meant for microbial culture were subjected to gradient dilution, and the number of cultivable soil microorganisms was determined by the dilution plating method [22]. The medium for the cultivation of bacteria, fungus, and actinomycetes were beef extract peptone, potato dextrose agar (PDA), and Gauze's Medium No.1, respectively. Bacteria, fungi, and actinomycetes were cultured for 72 h in incubators set at 35 °C, 28 °C, and 30 °C respectively. The microorganisms were spread onto the plate, the number of colonies on each plate was counted, and finally the number of colonies in each group of samples was calculated. In order to verify the microorganisms we isolated, we selected some representative isolates from bacteria, fungi, and actinomycetes for 16S rRNA Sanger sequencing.

2.5. Sequencing for Microbial Diversity Analysis

The total genomic DNA of the soil sample was extracted using a Mag Pure Soil DNA LQ Kit (Magen), then it was diluted to 1 ng/μL with sterile water, and stored at −80 °C.

The soil microbial DNA was amplified by PCR using barcoded primers. The corresponding regions of the primers were: (i) 343F 5′-TACGGRAGGCAGCAG-3′, and 798R 5′-AGGGTATCTAATCCT-3′ for the 16S V3-V4 region. (ii) ITS1F-ITS2R for the ITS region, ITS1F 5′-CTTGGTCATTTAGAGGAAGTAA-3′, ITS2R 5′-GCTGCGTTCTTCATCGATGC-3′. The PCR reaction system (30 μL) included 2 × Gflex PCR Buffer 15 μL, Tks Gflex DNA Polymerase (1.25 U/μL) 0.6 μL, Primer F/R (5 μM) 1 μL, gDNA 1 μL (50 ng), and added H_2O to 30 μL. The PCR program was 94 °C pre-denaturation for 5 min, followed by 26 cycles of (94 °C for 30 s, 56 °C for 30 s, and 72 °C for 20 s), then 72 °C for 5 min.

PCR products were detected by electrophoresis and purified using magnetic beads of the Qubit dsDNA Assay Kit (Life Technologies) after detection. Then, they were subjected to two rounds of PCR amplification, detection, and purification, and then quantified by Qubit. Equal amounts of PCR products were mixed according to their concentrations, and

the products were sent to Shanghai Ouyi Industry and Trade Co., Ltd. for sequencing using the Illumina MiSeq high-throughput sequencing platform.

2.6. Bioinformatics Analysis

Following sequencing, the generation of 250 bp paired-end reads ensued. These reads were then assigned to their respective samples based on their unique barcode and truncated by removing the barcode and primer sequence. FLASH V1.2.7 [23] was utilized to merge the paired-end reads. To obtain high quality clean tags, raw tags were filtered under specific filtering conditions in accordance with the QIIME 2 [24] quality-controlled process. The tags were then compared to the reference Silva database [25] to detect any chimera sequences, which were subsequently removed.

Operational taxonomy units (OTUs) were established by grouping sequences with a similarity of $\geq$97% using Uparse v7.0.1001 [26]. A representative sequence was selected for each OTU and subjected to further annotation [27]. Taxonomic information was assigned to each representative sequence using the Mothur algorithm and the Silva database [25]. Analyses on microbial community structure, and alpha and beta diversity, and multivariate were further carried out according to previous studies [28].

2.7. Yield Measurement

The above-mentioned continuous cultivated soil was used for the two cultivation methods of *G. leucocontextum* in March 2022 (adopting the same cultivation method as in Section 2.1), medium bag cultivation (B-CK and B1a) and log cultivation (W-CK, W3a, W5a). A number of 100 bags/logs were prepared for each treatment, and the fruit bodies were harvested when there was spore on the pileus surface and the white margins changed to brown. The yields of fresh and dry mushrooms (g/bag or g/log) were determined in August–September. Biological efficiency and conversion rate were estimated by Formulas (1) and (2), respectively [29].

$$\text{Biological efficiency } (\%) = \frac{\text{Weight of fresh fruit bodies}}{\text{Weight of dry substrate}} \times 100\% \quad (1)$$

$$\text{Conversion rate } (\%) = \frac{\text{Weight of dry fruit bodies}}{\text{Weight of dry substrate}} \times 100\% \quad (2)$$

2.8. Statistical Analysis

The sequencing data were analyzed and processed using SPSS 17.0 statistical software. The t-test was used for comparison between groups, and $p < 0.05$ indicated significant difference, and $p < 0.01$ indicated very significant difference.

3. Results

3.1. Comparison of Soil Physicochemical Properties

The soil physicochemical properties are shown in Table 2. Compared with the background control, the OM of the casing soil (273.58%) in B1a treatment significantly increased, and the contents of hydrolyzable nitrogen, available phosphorus, and available potassium increased by 266.67%, 45.45%, and 414.81%, respectively. The pH increased by 2.5 units. The OM in the W3a and W5a treatments had significantly increased N and K contents, just not as significant as in the medium bag treatment; the P content slightly decreased, and little effect on the pH was seen.

Table 2. Soil physicochemical analysis.

Treatment	pH	OM (g/kg)	N (mg/kg)	P (mg/kg)	K (mg/kg)
B1a	8.0 ^{a}	19.8 ^{a}	44 ^{a}	19.2 ^{a}	973 ^{a}
CK	5.5 ^{b}	5.3 ^{c}	12 ^{c}	13.2 ^{b}	189 ^{c}
W3a	5.1 ^{b}	10.6 ^{b}	29 ^{b}	10.5 ^{b}	437 ^{b}
W5a	5.4 ^{b}	8.3 ^{b}	26 ^{b}	10.2 ^{b}	638 ^{b}

Note: The different letters in a row indicate significant differences between treatments at $p < 0.05$.

3.2. The Number of Culturable Microorganisms

Among the four studied groups, the total count of soil microorganisms in B1a was the largest compared with the CK, the former was increased by 2.1-fold, and the counts of the three major microbial groups (bacterium, actinomycete, and fungus) were increased significantly (Table 3). There were significant differences in the numbers of culturable bacteria, actinomycetes, and fungi in the casing soil with different continuous cultivation years of the log mode. Different from the medium bag mode, the number of microorganisms in W3a and W5a were both smaller than that in the CK treatment, and the number of microorganisms decreased with the increase in cultivation years. In particular, the decrease in actinomycetes was the largest and the decrease in bacteria was small, whereas the number of fungi increased.

Table 3. The number of soil culturable microorganisms.

Treatment	Total Count		Bacterium Count		Actinomycete Count		Fungus Count	
	(10^4 CFU/g)	%	(10^4 CFU/g)	%	(10^4 CFU/g)	%	(10^4 CFU/g)	%
B1a	1553.23 ^{a}	212.05	842.81 ^{a}	571.56	697.15 ^{a}	89.03	13.276 ^{a}	284.59
CK	497.75 ^{b}	/	125.50 ^{b}	/	368.80 ^{b}	/	3.452 ^{b}	/
W3a	174.33 ^{c}	−64.98	87.38 ^{c}	−30.37	84.43 ^{c}	−77.11	2.513 ^{b}	−27.20
W5a	63.35 ^{c}	−87.27	60.37 ^{c}	−51.90	*	*	2.985 ^{b}	−13.53

Note: The different lowercase letters in a row indicate significant differences between treatments at $p < 0.05$. * indicates not detectable.

3.3. Microbial Community Structure

The ASV sequences obtained from high-throughput technology were used to evaluate the changes in soil bacteria and fungi in different cultivation modes with different continuous cultivation years. After processing the sequences, the final number of bacterial reads was between 55,062 and 64,624, and the number of OTUs of the samples varied from 800 to 1574. On the other hand, the final number of fungal reads was in the range of 56,927–71,173, and the number of OTUs of the samples was in the range of 167–529.

The samples were annotated and summarized at various taxonomic levels such as phylum, class, order, family, genus, and species. Then the absolute abundances and relative abundances were sorted at each taxonomic level. The analysis results at the phylum and genus levels showed that the community structure and diversity of the soil bacteria and fungi were changed significantly after the continuous cultivation of *G. leucocontextum*.

3.3.1. Bacterial Community Composition

There were significant differences in the abundances of the top ten phyla in the soil bacterial community composition from different cultivation modes of *G. leucocontextum*. However, their abundance in different years of continuous cultivation varied slightly but not the composition (Figure 1). A total of approximately 40 bacterial phyla were found, the average abundance of the top 10 bacterial phyla accounted for >90% of the abundance of all the 16 samples, and the top five phyla accounted for >70%. In log cultivated soil, with the increase in years of continuous cultivation, the abundances of *Actinobacteria*, *Bacteroidetes*, *Gemmatimonadetes*, and *Firmicutes* decreased, and, conversely, the abundances of *Acidobac-*

teria, *Myxococcota*, and *Nitrospirae* increased, while in the medium bag cultivation, the abundance of *Proteobacteria* was >50%, and the abundances of *Actinobacteria*, *Bacteroidetes*, and *Gemmatimonadetes* were also higher than those in the CK and log cultivation to varying degrees, while *Acidobacteria*, *Myxococcota*, and *Firmicutes* were sparsely distributed.

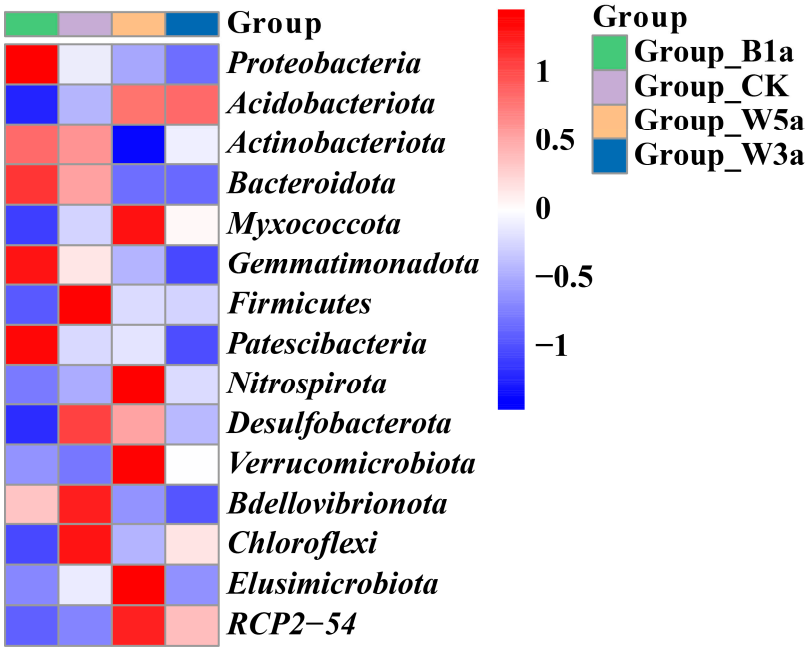

Figure 1. The composition of bacterial communities at the phylum level in the casing soil of different treatments of *G. leucocontextum* cultivation.

In the present study, most bacterial genera showed decreased abundances in the soil bacterial community composition from various cultivation modes, among which the abundances of *Sphingomonas* spp., *Bryobacter* spp., *Bradyrhizobium* spp., *Mucilaginibacter* spp., and *Granulicella* spp. were decreased with the increase in years of continuous cultivation (Figure 2). The abundances of *Massilia* spp., *Brevundimonas* spp., and *Pedobacter* spp. were also increased in the medium bag cultivation, and became the dominant bacteria for medium bag cultivation.

3.3.2. Fungal Community Composition

Not much difference was found in the dominant fungal phyla in the rhizosphere of the medium bag cultivation and log cultivation treatments. The average abundance of *Ascomycota*, *Basidiomycota*, *Zygomycota*, *Rozellomycota*, *Chytridiomycota*, *Cercozoa*, and *Glomeromycota* (top 7 phyla) was about 70% of the total and thus regarded as dominant (Figure 3).

However, at the generic level, big differences were found (Figure 4), where the abundances of *Ganoderma* spp., *Mortierella* spp., *Hypomyces* spp., *Geminibasidium* spp., and *Pyrenochaeta* spp. were increased with the increase in years of continuous cultivation. However, in this trend, the abundances of *Spirosphaera* spp. and *Russula* spp. were decreased. *Phaeoacremonium* spp. and *Protocrea* spp. were dominant fungi in the treatment B1a. While the abundances of *Psathyrella* spp. and *Psilocybe* spp. were slightly increased in the fungal community of the log cultivation W3a.

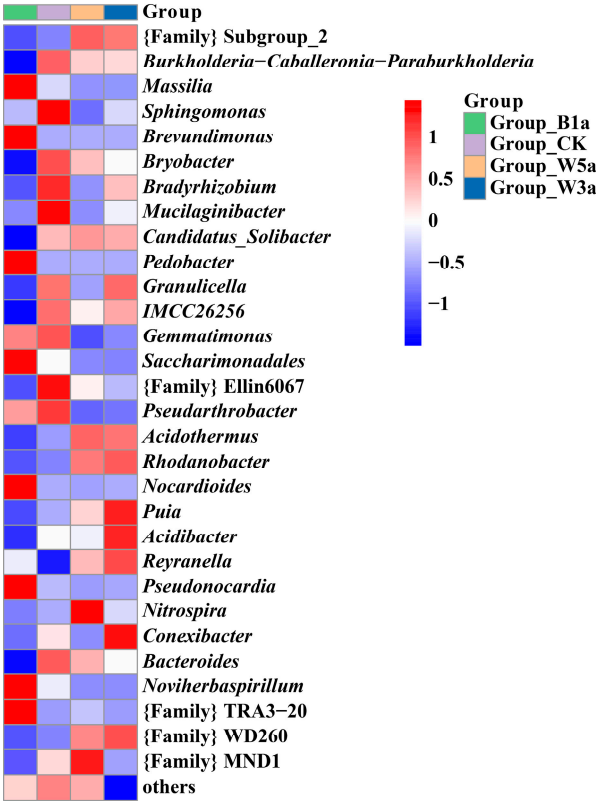

Figure 2. The composition of bacterial communities at the genus level in the casing soil of different treatments of *G. leucocontextum* cultivation.

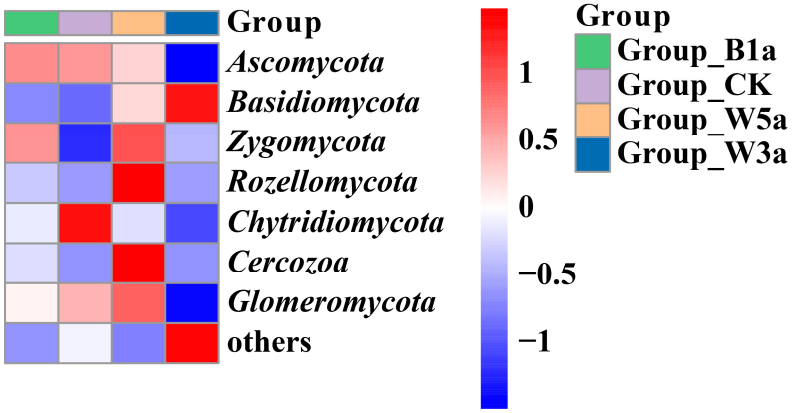

Figure 3. The composition of fungal communities at the phylum level in the casing soil of different treatments of *G. leucocontextum* cultivation.

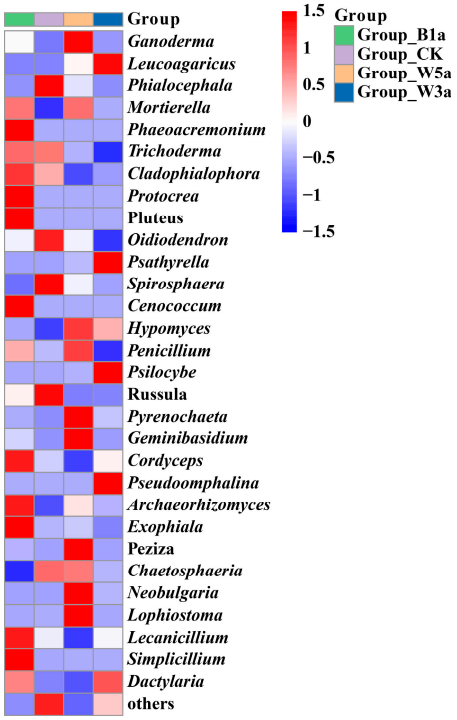

Figure 4. The composition of fungal communities at the genus level in the casing soil of different treatments of *G. leucocontextum* cultivation.

3.4. Microbial Alpha Diversity Analysis

The indexes such as Chao1, goods coverage, and observed species were used to exhibit community richness, and the Shannon index, Simpson index, and phylogenetic diversity (PD) were used to display community diversity. To investigate the significance of differences in the diversity indices in different groups, Violin plot analysis (using the Wilcoxon algorithm) was performed by calculating the number of operational taxonomic units (OTUs) and multiple alpha diversity indices.

The alpha diversity represented the measure of the species richness in the community ecology according to the Shannon index (Figure 5). It demonstrated the comprehensive index of species richness and evenness and the relationship was direct. The index degree and relationship intensity could be explained in various ways. In the soil bacterial community of the CK treatment, the index was the highest, and it was significantly different from B1a (p = 0.029 by Wilcoxon rank sum test) but not W3a and W5a. Treatments B1a and W5a showed significantly different values of the index. However, for the soil fungal community, it was the highest in the B1a treatment, which was significantly different from W5a (p = 0.029 by Wilcoxon rank sum test) but not the other treatments. The index values were significantly different between the W3a and W5a treatments. From this result, it can be seen that continuous cultivation reduced the diversity of bacterial communities in the casing soil. With the increase in years of continuous cultivation, the diversity of bacterial communities decreased, whereas fungi behaved differently. The diversity of fungal communities was enhanced, and it only declined after 5 years of continuous cultivation.

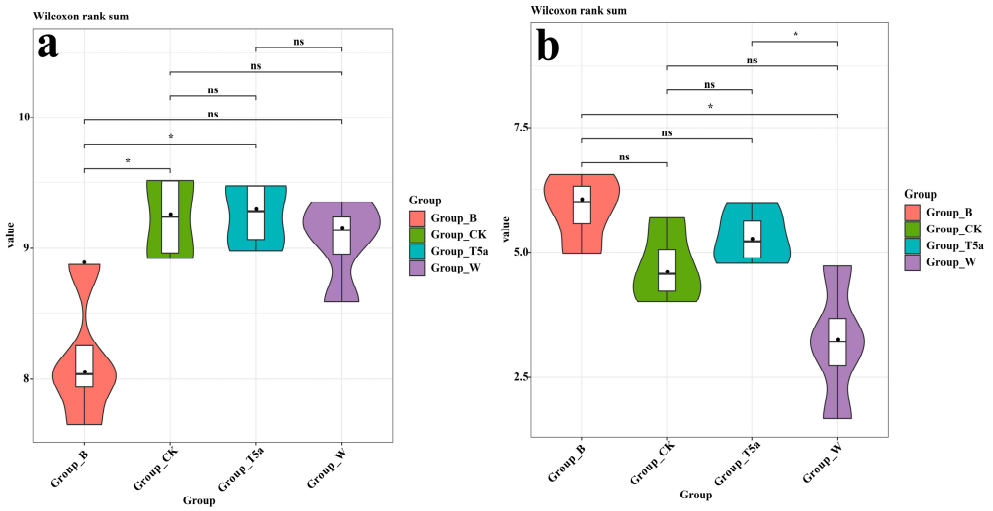

Figure 5. Alpha diversity of soil bacterial (**a**) and fungal (**b**) communities in the casing soil of different treatments of *G. leucocontextum* cultivation. * indicates significant differences between treatments at $p < 0.05$. ns indicates there is no significant difference between different treatments.

3.5. Microbial Beta Diversity Analysis

In this study, beta diversity analysis was carried out via non-metric multidimensional scaling (NMDS) plots. It shows the NMDS clustering results of the communities for bacteria (stress = 0.014) and fungi (stress = 0.062) in the continuous cultivated soil of *G. leucocontextum* cultivation (Figure 6). NMDS analysis is a type of beta diversity. Through dimensionality reduction, nonlinear models were adopted, and the distance between points represented the level of difference between samples. Usually, NMDS can exhibit the relationship between objects with less distortion than PCoA, and intuitively represents the clustering or difference between samples.

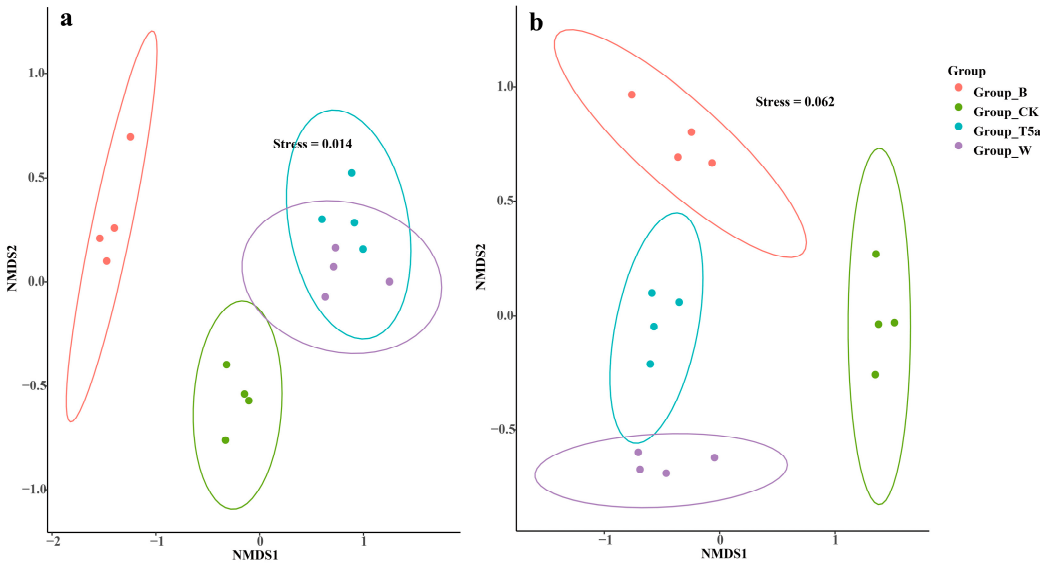

Figure 6. NMDS plots of bacterial (**a**) and fungal (**b**) communities in continuous cropping soil for *G. leucocontextum* cultivation. Note: The horizontal (NMDS1) and the vertical (NMDS2) are two sorting axes. Each point in the plot represents a sample. Each group was represented by one color. Similar samples are clustered together. A long distance between points means big difference between samples. The confidence ellipses are shown in the plot.

3.6. Multivariate Analysis of Microorganisms

The samples were statistically analysed at each level of the phylum, class, order, family, genus, and species. The results of the analysis of variance (ANOVA) showed 1147, 235 and 22 different bacterial OTUs, genera, and phyla, respectively. It also revealed 233 different fungal ASV, and 67, 32, and 3 fungal genera, orders, and phyla, respectively.

According to the relative abundance of the differential species, ANOVA was employed to conduct multivariate analysis on soil microorganisms to reveal the differential bacteria and fungi. Statistical analysis was performed at the genus level, and each of the top 10 abundant differential genera was subjected to relative abundance boxplot analysis to demonstrate the abundance of dominant differential genera within a group and for between group comparisons. It listed the group comparisons of the abundance of some dominant bacterial and fungal genera (Figure 7). The results showed significant changes in the bacterial and fungal community structures of *G. leucocontextum* caused by continuous cultivation.

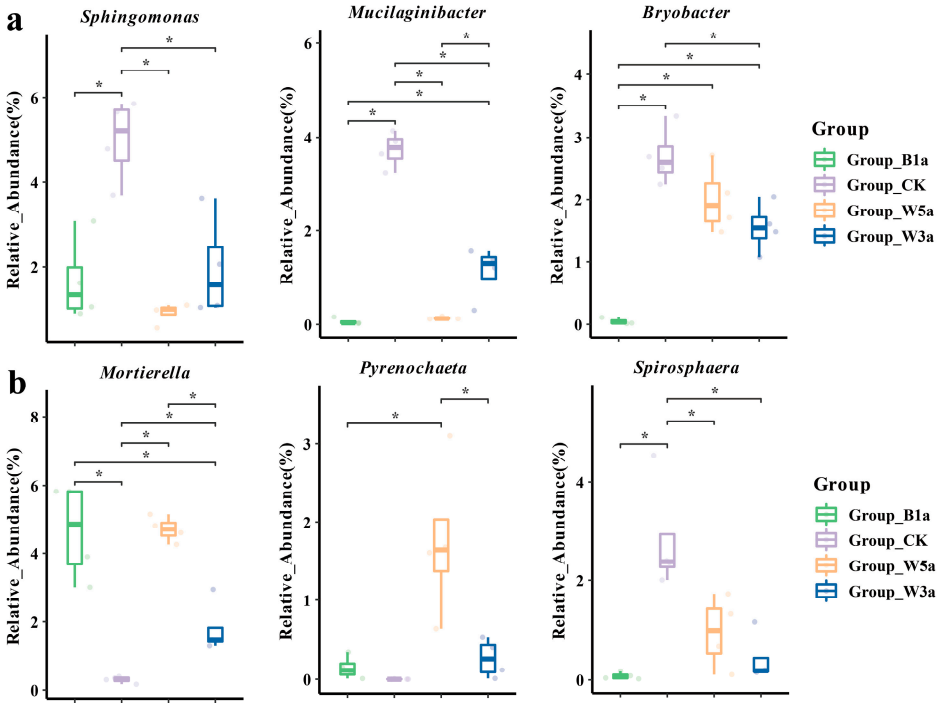

Figure 7. Comparison of differences between different bacterial (**a**) and fungal (**b**) dominant genera. * indicates significant differences between treatments at $p < 0.05$.

In different cultivation modes, most of the bacterial differential genera reduced their relative abundances. In the group comparison, the dominant bacterial genera *Sphingomonas* spp. showed a decrease in abundance in continuous cultivated soil, which clearly decreased with the increase in cultivation years, reaching the lowest abundance in W5a treatment. The abundance of *Mucilaginibacter* spp. declined even more sharply, showing an extremely low abundance in B1a and W5a treatments. Combining the results of the fruit body growth and yield of *G. leucocontextum*, it is not hard to find that the abundances of *Sphingomonas* spp. and *Mucilaginibacter* spp. were positively correlated with the growth of *G. leucocontextum*.

For the fungal community composition in the continuous cultivated soil of *G. leucocontextum* cultivation, the relative abundances of differential genera such as *Ganoderma* spp., *Mortierella* spp., and *Pyrenochaeta* spp. increased significantly. In particular, *Mortierella* spp. had the highest abundance in the B1a treatment, showing a clear pattern of increase with the increase in years of continuous cultivation. It was the dominant genus in continuous cultivated soil and had a negative effect on the growth of *G. leucocontextum*.

3.7. Correlation between Microbial Flora and Soil Physicochemistry

Combining the changes in soil microorganisms and soil physicochemical properties of different samples, their relationships were analysed through redundancy analyses (RDA) (Figure 8).

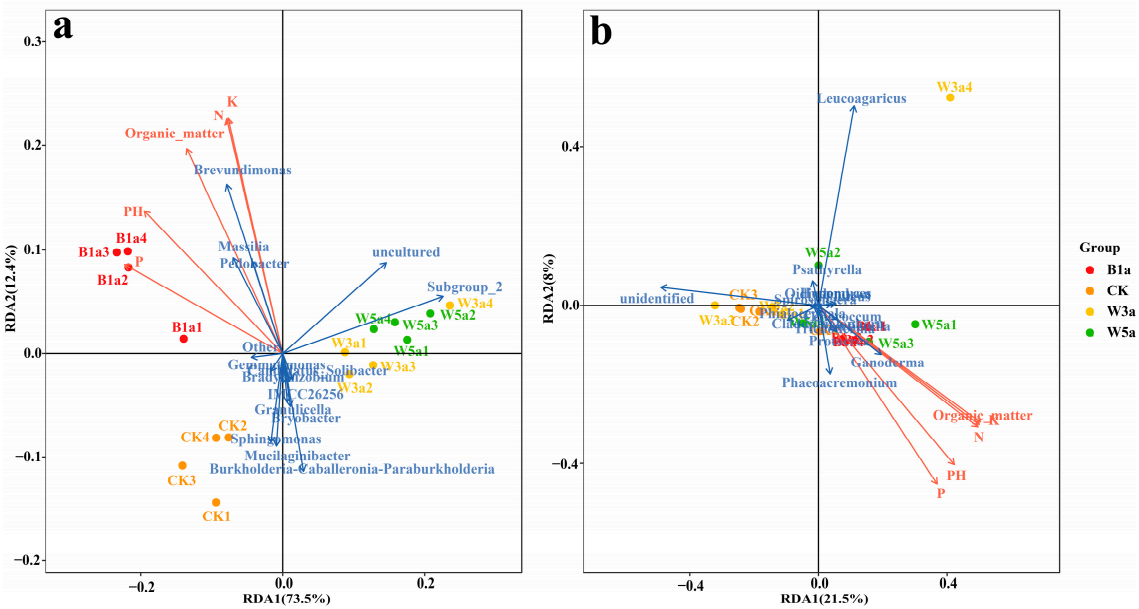

Figure 8. RDA relationship between soil bacteria (**a**) and fungi (**b**) with soil physicochemistry.

Soil physicochemical factors were positively correlated with each other, but no correlation was found between dominant genera. However, they correlated positively with *Brevundimonas* spp., *Massilia* spp., and *Pedobacter* spp., and negatively with *Burkholderia-Caballeronia-Paraburkholderia*, *Mucilaginibacter* spp., *Sphingomonas* spp., *Granulicella* spp., and *Bryobacter* spp. The longer rays of the soil physicochemical factors also indicated the stronger impacts of these factors. The results demonstrated that continuous cultivation not only caused changes in soil physicochemical factors, such as soil pH, organic matter content, hydrolysable nitrogen, available phosphorus, and available potassium, but also affected the structure of dominant bacterial communities. Regarding fungi, the soil physicochemical properties were negatively correlated with *Leucoagaricus* spp., and positively correlated with *Phaeoacremonium* spp., *Ganoderma* spp., and *Protocrea* spp. to a certain extent, but the degree of influence was less than that of bacteria.

3.8. Effects on Yield with Continuous Cultivated Soil

The continuous cultivation yield of *G. leucocontextum* shows that the medium bag method had a lower value than the log (Table 4). However, it had a remarkably high biological efficiency and conversion rate. Regarding the impact of continuous cultivated soil on cultivation, medium bag cultivation was more affected, and the B1a treatment had a dramatically reduced yield. Log cultivation had a reduced yield in the W3a treatment, and the yield was decreased with the increase in years of continuous cultivation.

Table 4. Comparison of yield of *G. leucocontextum*.

Yield	Medium Bag		Log		
	B-CK	**B1a**	**W-CK**	**W3a**	**W5a**
fresh mushroom (g/bag·log)	148.8 ± 12.37	91.2 ± 7.91	294.1 ± 25.32	108.3 ± 8.14	25.5 ± 2.13
dry mushroom (g/bag·log)	41.2 ± 5.67	25.3 ± 3.79	108.9 ± 9.11	40.0 ± 6.29	9.4 ± 1.71
biological efficiency (%)	24.8%	15.2%	9.8%	3.6%	0.85%
conversion rate (%)	6.87%	4.22%	3.63%	1.33%	0.31%

4. Discussion

With respect to the medium bag cultivation, the sharp increase in pH, organic matter, N, and K led to an increase in the number of bacteria, actinomycetes, and fungi in the soil, and induced a significant decline in the yield of *G. leucocontextum*. Presumably, it was related to the in-bag high-temperature and high-humidity microenvironment. It also confirmed the cause of stronger continuous cultivation obstacles for bag-cultivated *G. leucocontextum*. Regarding the log cultivation, the contents of organic matter, N, and K were slightly increased, the number of bacteria and actinomycetes showed a decreasing pattern but fungi was increased, and pH was slightly affected. However, the yield still decreased each year, indicating that the continuous cultivation of *G. leucocontextum* caused decreased metabolism such as nutrient decomposition and transformation that the bacteria participated in in the soil. On the other side, this also helped to increase substances such as secondary metabolites produced by fungi, which are one of the major members participating in the decomposition and synthesis of organic matter in soil [30]. They also play an essential role in soil carbon metabolism and the nitrogen cycle, and directly affect soil fertility [11]. However, many species of fungi are pathogenic to crops, and are closely related to the occurrence of soil-borne diseases in crops [31]. *G. leucocontextum* is a fungus, so an increase in the number of other fungi across years of continuous cultivation decreases the effective nutrition for it. As a result, the pests and diseases increase, and the pathogenic fungi in the soil compete with *G. leucocontextum* for nutrients. This condition affects the yield and quality of *G. leucocontextum*, consistent with the symptoms of continuous cultivation obstacles.

The changes in soil microbiota and their quantity are usually an index to evaluate soil quality [32]. Nowadays, the development of high-throughput sequencing technology has enabled a deeper understanding of the diversity of soil microbial communities [33–35]. Using this technology on the microbial diversity, rich species differences were obtained in the present study, and the declined abundance of *Proteobacteria* and *Actinobacteria* in the bacterial taxa in the continuous cultivated soil of log cultivation seems worthy of consideration. As we all know, actinomycetes are one of the largest soil microbial groups, and they play an important role in the biodegradation and recycling of organic matter; the groups that generate antibiotics are also widely applied in medicine and agriculture to inhibit a variety of pathogenic bacteria [36,37]. Beneficial bacteria in the continuous cultivated soil such as *Bacteroidetes*, *Gemmatimonadetes*, and *Firmicutes* decreased compared with the control (CK). Earlier researches revealed that some members of the phyla of *Alphaproteobacteria*, *Firmicutes*, and *Cyanobacteria* as well as some members of the genera *Pseudomonas* and *Bacillus* have an inhibition effect on *Ralstonia solanacearum* disease [38]. A microbiome with a high abundance of functional genes encoding antimicrobial and antibiotic compounds was thought to be able to provide protection to plants [39]. In the fungal taxa, it was found that the fungi *Ganoderma*, *Mortierella*, and *Pyrenochaeta* were increased in the continuous cultivated soil, affecting the normal growth of the bag-grown *G. leucocontextum*. It is thus speculated that continuous cultivation obstacles resulted from the increase in the metabolites of the cultivated species itself, and the possible distribution of some soil-borne pathogens from the cultivation of edible fungi and potential soil-borne pathogen species. It is similar to the results that researchers achieved by applying the Illumina platform ITS1 amplicon technology to analyze the rhizosphere fungal structure in the continuous cropping of watermelon [40] and *Panax notoginseng* [41].

Since the differences at the genus level can better inform the changes in taxa in continuous cultivation, so as to correspond to the effects on the fruiting body growth of *G. leucocontextum*, the present study focused on the differences at the genus level based on the high-throughput sequencing results of this study. The results provided two potential differences: (i) It was found that among the dominant genera with positive effects on the growth of *G. leucocontextum*, *Sphingomonas* spp., and *Mucilaginibacter* spp., there were decreased relative abundances in continuous cultivated soil, and the declines were significant with the increase in cultivation years. This was consistent with the findings of Ren (2020)

et al. [17]. *Sphingomonas* spp. happens to be a rich new microbial resource, which may be used for the biodegradation of aromatic compounds. It has great application potential in environmental protection and industrial production for its high metabolic capacity and multifunctional physiological characteristics [42]. The species has the ability to degrade polyphenols and tributylphosphates (TBP) [43,44]. Members of *Mucilaginibacter* spp. were present in various soil samples [45], and they may play an important role in carbon processing [46]. In the present research, it was considered that they may promote the growth of fruiting bodies of *G. leucocontextum* to a certain extent, or may synergize with various factors in the microenvironment. However, the continuous cultivating style sabotages this positive effect. (ii) Among the dominant genera with a negative effect on the growth of *G. leucocontextum*, *Ganoderma* spp., *Mortierella* spp., and *Pyrenochaeta* were dominant in the continuous cultivated soil. Their relative abundance increased significantly with the increase in cultivation years. *Mortierella* spp. happens to be a key microbial member of transferring soil carbon and nutrients and could produce arachidonic acids and single-cell oil (SCO) [47]. Some species of this genus can produce antibiotics, a few isolates of which have proved to be potential antagonists against various plant pathogens. However, with respect to the negative effects on the growth of the fruiting bodies of the *G. leucocontextum*, it can be seen that the secondary metabolites produced by *Mortierella* spp. had certain inhibitory effects.

Combined with the soil, growth, and structural characteristics of the dominant microbiota in the continuous cultivation of *G. leucocontextum*, it can be found that the factors relevant to bacterial flora have a greater degree of effects. However, this result differed from the findings obtained in the continuous cultivation obstacles of higher plant crops. In the study, soil physicochemical factors correlated negatively with bacteria *Mucilaginibacter*, *Sphingomonas*, and *Bryobacter*, which is consistent with the effects of dominant bacteria in continuous cultivated soil on the growth of *G. leucocontextum*. Soil pH has been considered as one of the important abiotic elements affecting the diversity and richness of soil microbiota [48,49]. The acidic soil in the cultivation and production of this experiment favored the growth of *G. leucocontextum*, consistent with the previous research results on the pathogenic *G. boninense* of the same genus [39]. It is believed that conditions of acidic soil and low pH favored the growth of *G. boninense* compared to neutral soil with pH 6–7 [50]. In the bagged continuous cultivated soil, the abundances of some acid-producing bacteria, *Acidobacteria*, *Myxococcota*, and *Nitrospirae*, were decreased, making the soil pH shift toward alkaline. Compared to acidic soils (pH 4), the bacterial diversity and relative abundance were bigger in alkaline soils (pH 8) [51]. Differently, some researchers believed that the composition of soil microorganisms changed over time, and the changes in fungal species richness and the relative abundance of several major species were greater than bacteria [52,53]. In this study, in the continuous cultivated soil for *G. leucocontextum* cultivation, the contents of N and P decreased, while soil pH and Fe content increased. Therefore, targeting a further follow-up of the research, the change in the soil physicochemical parameters in the continuous cultivation of *G. leucocontextum* seems valuable and we need to find the dynamic change patterns of microorganisms and environmental factors during the cultivation period.

Finally, it can be seen that the continuous cultivation changed the soil microbial community structure elaborately, shaping a special microenvironment together with changes in soil biological and physicochemical properties. Therefore, analyzing the changes in microbial communities in continuous cultivated soil has important guiding significance for the prevention and control of continuous cultivation obstacles.

5. Conclusions

The continuous cultivation of *G. leucocontextum* affected the micro-ecology of the soil in the area, resulting in soil physicochemical imbalance and reduced yield. The relative abundance of the dominant beneficial bacteria for *G. leucocontextum*, e.g., *Sphingomonas* spp., *Mucilaginibacter* spp., *Bryobacter* spp., and *Bradyrhizobium* spp., decreased in the continuous cultivated soil and with the increased number of years of cultivation. It was speculated

that they could promote the growth of the fruiting bodies of *G. leucocontextum*. From the soil fungal community, *Mortierella* spp. and *Pyrenochaeta* spp. were dominant in the composition of continuous cultivated soil. Their relative abundances showed significant direct relationships with the increasing cultivation years and thus had negative effects on the growth of *G. leucocontextum*. Changes in the bacterial community structure were inconsistent between the two modes of cultivation, namely, medium bag and log cultivated soil, whereas the changes in the fungal community structure were consistent. It was observed that the changes in the structure of soil microbiota communities and species abundance were potential factors responsible for the continuous cultivation obstacles of *G. leucocontextum*.

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References

1. Li, T.H.; Hu, H.P.; Deng, W.Q.; Wu, S.H.; Wang, D.M.; Tsering, T. *Ganoderma leucocontextum*, a new member of the *G. lucidum* complex from Southwestern China. *Mycoscience* **2015**, *56*, 81–85. [CrossRef]
2. Wu, F.; Zhou, L.W.; Yang, Z.L.; Yang, Z.L.; Bau, T.; Li, T.H.; Dai, Y.C. Resource diversity of Chinese macrofungi: Edible, medicinal and poisonous species. *Fungal Divers* **2019**, *98*, 1–76. [CrossRef]
3. Yao, C.X.; Tian, G.T.; Wang, H. Three new cultivars of *Ganoderma leucocontextum*. *Mycosystema* **2022**, *41*, 2095–2097. [CrossRef]
4. Chen, H.Y.; Zhang, J.J.; Ren, J.W.; Wang, W.Z.; Xiong, W.P.; Zhang, Y.D.; Bao, L.; Liu, H.M. Triterpenes and meroterpenes with neuroprotective effects from *Ganoderma leucocontextum*. *Chem. Biodivers.* **2018**, *15*, 177. [CrossRef]
5. Zhang, J.J.; Ma, K.; Han, J.J.; Wang, K.; Chen, H.Y.; Bao, L.; Liu, L.; Xiong, W.P.; Zhang, Y.D.; Huang, Y.; et al. Eight new triterpenoids with inhibitory activity against HMG -CoA reductase from the medical mushroom *Ganoderma leucocontextum* collected in Tibetan plateau. *Fitoterapia* **2018**, *130*, 79–88. [CrossRef]
6. Wang, K.; Bao, L.; Xiong, W.P.; Ma, K.; Han, J.J.; Wang, W.Z.; Yin, W.B.; Liu, H.M. Lanostane triterpenes from the Tibetan medicinal mushroom *Ganoderma leucocontextum* and their inhibitory effects on HMG-CoA reductase and α-glucosidase. *J. Nat. Prod.* **2015**, *78*, 1977–1989. [CrossRef]
7. Van Griendsven, L.J.L.D. *Science and Cultivation of Edible Fungi 2000*; Taylor and Francis: London, UK, 2012; ISBN 9781466539075/9058091457/978-9058091451.
8. Buckley, D.H.; Schmidt, T.M. The structure of microbial communities in soil and the lasting impact of cultivation. *Microb. Ecol.* **2001**, *42*, 11–21. [CrossRef]
9. Yao, C.X.; Liang, M.T.; Ma, Y.H.; Liu, J.X.; Zhang, S.S.; Chen, X.; Tian, G.T. Isolation and Identification of Soil-borne Pathogenic Microorganisms in *Ganoderma leucocontextum*. S.W. *Chin. J. Agric. Sci.* **2022**, *35*, 2811–2818. [CrossRef]
10. Longley, R.; Benucci, G.M.N.; Mills, G.; Bonito, G. Fungal and bacterial community dynamics in substrates during the cultivation of morels (*Morchella rufobrunnea*) indoors. *FEMS Microbiol. Lett.* **2019**, *366*, fnz215. [CrossRef]
11. Carrasco, J.; Preston, G.M. Growing edible mushrooms: A conversation between bacteria and fungi. *Environ. Microbiol.* **2020**, *22*, 858–872. [CrossRef]
12. Liu, D.; Wang, Y.L.; Zhang, P.; Yu, F.Q.; Jesús, P. Macrofungi cultivation inshady forest areas signifificantly increases microbiome diversity, abundance and functional capacity in soil furrows. *J. Fungi* **2021**, *7*, 775. [CrossRef]
13. Kang, H.; Chang, W.; Yun, S.; Lee, Y. Roles of ascospores and arthroconidia of *Xylogone ganodermophthora* in development of yellow rot in cultivated mushroom, *Ganoderma lucidum*. *Plant Pathol. J.* **2011**, *27*, 138–147. [CrossRef]
14. Ma, H.M.; Chen, D.X.; Chen, Y.G. Alelopathic effect of dominant microflora on its mycelium of continuous cropping obstacles *Ganoderma lucidum* in field cultivation. *Chin. J. Trop. Crops* **2016**, *37*, 372–375. [CrossRef]
15. Yuan, Y.; Li, L.; Huang, H.C.; Liu, G.H.; Xie, F.Q.; Fu, J.S.; Wu, X.P. Analysis of bacterial community in *Ganoderma lingzhi* continuous cropping soil based on 16S rDNA amplicon sequencing. *Chin. Agric. Sci. Bull.* **2021**, *37*, 116–123. [CrossRef]
16. Yuan, Y.; Huang, H.C.; Ye, L.Y.; Fu, J.S.; Wu, X.P. Analysis of fungal community in continuous cropping soil of *Ganoderma lingzhi*. *Mycosystema* **2019**, *38*, 2112–2121. [CrossRef]

17. Ren, F.; Zhang, Y.G.; Yu, H.; Zhang, Y.A. *Ganoderma lucidum* cultivation affect microbial community structure of soil, wood segments and tree roots. *Sci. Rep.* **2020**, *10*, 3435. [CrossRef]
18. Walkley, A.; Black, I.A. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci.* **1934**, *37*, 29–38. [CrossRef]
19. Mulvaney, R.L.; Khan, S.A. Diffusion methods to determine different forms of nitrogen in soil hydrolysates. *Soil Sci. Soc. Am. J.* **2001**, *65*, 1284–1292. [CrossRef]
20. Truog, E. The determination of the readily availably phosphorus soils. *J. Am. Soc. Agron.* **1930**, *22*, 874. [CrossRef]
21. Ieggli, C.V.S.; Bohrer, D.; Do Nascimento, P.C.; De Carvalho, L.M. Determination of sodium, potassium, calcium, magnesium, zinc and iron in emulsified chocolate samples by flame atomic absorption spectrometry. *Food Chem.* **2011**, *124*, 1189–1193. [CrossRef]
22. He, J.Z.; Zheng, Y.; Chen, C.R.; He, Y.Q.; Zhang, L.M. Microbial composition and diversity of an upland red soil under long-term fertilization treatments as revealed by culture-dependent and culture-independent approaches. *J. Soils Sediments* **2008**, *8*, 349–358. [CrossRef]
23. Magoc, T.; Salzberg, S.L. FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **2011**, *27*, 2957–2963. [CrossRef]
24. Bolyen, E.; Rideout, J.R.; Dillon, M.R.; Bokulich, N.A.; Abnet, C.C.; Al-Ghalith, G.A.; Caporaso, J.G. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **2019**, *37*, 852–857. [CrossRef] [PubMed]
25. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F.O. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* **2013**, *41*, D590–D596. [CrossRef]
26. Edgar, R.C. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nat. Meth.* **2013**, *10*, 996–998. [CrossRef]
27. Li, Q.; Xiang, P.; Zhang, T.; Wu, Q.; Bao, Z.; Tu, W.; Li, L.; Zhao, C. The effect of phosphate mining activities on rhizosphere bacterial communities of surrounding vegetables and crops. *Sci. Total Environ.* **2022**, *821*, 153479. [CrossRef]
28. Li, Q.; Wu, Q.; Zhang, T.; Xiang, P.; Bao, Z.; Tu, W.; Li, L.; Wang, Q. Phosphate mining activities affect crop rhizosphere fungal communities. *Sci. Total Environment.* **2022**, *838 Pt 2*, 156196. [CrossRef]
29. Sudheer, S.; Alzorqi, I.; Ali, A.; Cheng, P.G.; Siddiqui, Y.; Manickam, S. Determination of the biological efficiency and antioxidant potential of *Lingzhi* or *Reishi* medicinal mushroom, *Ganoderma lucidum* (*Agaricomycetes*), cultivated using different agro-wastes in Malaysia. *Int. J. Med. Mushrooms* **2018**, *20*, 89–100. [CrossRef]
30. Gea, F.J.; Navarro, M.J.; Santos, M.; Diánez, F.; Carrasco, J. Control of Fungal Diseases in Mushroom Crops while Dealing with Fungicide Resistance: A Review. *Microorganisms* **2021**, *9*, 585. [CrossRef] [PubMed]
31. Tang, S.; Fan, T.; Jin, L.; Lei, P.; Shao, C.; Wu, S.; Yang, Y.; He, Y.; Ren, R.; Xu, J. Soil microbial diversity and functional capacity associated with the production of edible mushroom *Stropharia rugosoannulata* in croplands. *PeerJ* **2022**, *10*, e14130. [CrossRef]
32. Lauber, C.L.; Ramirez, K.S.; Aanderud, Z.; Lennon, J.; Fierer, N. Temporal variability in soil microbial communities across landuse types. *ISME J.* **2013**, *7*, 1641–1650. [CrossRef]
33. Bulgarelli, D.; Rott, M.; Schlaeppi, K.; Themaat, E.V.L.V.; Ahmadinejad, N.; Assenza, F.; Schulze-Lefert, P. Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature* **2012**, *488*, 91–95. [CrossRef]
34. Muller, D.B.; Vogel, C.; Bai, Y.; Vorholt, J.A. The plant microbiota: Systems-level insights and perspectives. *Annu. Rev. Genet.* **2016**, *50*, 211–234. [CrossRef]
35. Frindte, K.; Pape, R.; Werner, K.; Löfer, J.; Knief, C. Temperature and soil moisture control microbial community composition in an arctic-alpine ecosystem along elevational and micro-topographic gradients. *ISME J.* **2019**, *13*, 2031–2043. [CrossRef]
36. Plassart, P.; Prévost-Bouré, N.C.; Uroz, S.; Dequiedt, S.; Stone, D.; Creamer, R.; Lemanceau, P. Soil parameters, land use, and geographical distance drive soil bacterial communities along a European transect. *Sci. Rep.* **2019**, *9*, e605. [CrossRef]
37. Delgado-Baquerizo, M.; Oliverio, A.M.; Brewer, T.E.; Benavent-González, A.; Eldridge, D.J.; Bardgett, R.D.; Fierer, N. A global atlas of the dominant bacteria found in soil. *Science* **2018**, *359*, 320–325. [CrossRef]
38. Wei, Z.; Gu, Y.; Friman, V.P.; Kowalchuk, G.A.; Xu, Y.C.; Shen, Q.R.; Jousset, A. Initial soil microbiome composition and functioning predetermine future plant health. *Sci. Adv.* **2019**, *5*, eaaw0759. [CrossRef]
39. Goh, Y.K.; Zoqratt, M.Z.H.M.; Goh, Y.K.; Ayub, Q.; Ting, A.S.Y. Determining soil microbial communities and their influence on *Ganoderma* disease incidences in oil palm (*Elaeis guineensis*) via high-throughput sequencing. *Biology* **2020**, *9*, 424. [CrossRef]
40. Wang, T.T.; Hao, Y.W.; Zhu, M.Z.; Yu, S.T.; Ran, W.; Xue, C.; Ling, N.; Shen, Q.R. Characterizing diferences in microbial community composition and function between *Fusarium* wilt diseased and healthy soils under watermelon cultivation. *Plant Soil* **2019**, *438*, 421–433. [CrossRef]
41. Tan, Y.; Cui, Y.S.; Li, H.Y.; Kuang, A.X.; Li, X.R.; Wei, Y.L.; Ji, X.L. Rhizospheric soil and root endogenous fungal diversity and composition in response to continuous *Panaxnoto ginseng* cropping practices. *Microbiol. Res.* **2016**, *194*, 10–19. [CrossRef]
42. Bao, Z.; Feng, H.; Tu, W.; Li, L.; Li, Q. Method and mechanism of chromium removal from soil: A systematic review. *Environ. Sci. Pollut. Res.* **2022**, *29*, 35501–35517. [CrossRef]
43. Feng, Y.; Yuan, Q.; Yang, Z.; Zhang, T. Effect of *Sphingomonas* sp. strain on degradation of polyphenols in redried tobacco leaves. *Acta Tab. Sin.* **2019**, *25*, 19–24. [CrossRef]
44. Liu, J.; Lin, H.; Dong, Y.; Li, B. Elucidating the biodegradation mechanism of tributyl phosphate (TBP) by *Sphingomonas* sp. isolated from TBP-contaminated mine-tailings. *Environ. Pollut.* **2019**, *250*, 284–291. [CrossRef] [PubMed]
45. Huq, M.A.; Akter, S.; Lee, S.Y. *Mucilaginibacter formosus* sp. nov., a bacterium isolated from road-side soil. *Antonie Van Leeuwenhoek* **2019**, *112*, 513–521. [CrossRef]

46. Gadkari, P.; Haggblom, M.; Barkay, T.; Kerkhof, L.; Mannisto, M. *Ecology and Physiological Potential of Tundra Soil Bacteria*; Rutgers University, School of Graduate Studies: New Brunswick, NJ, USA, 2019; pp. 1–31. [CrossRef]
47. Papanikolaou, S.; Komaitis, M.; Aggelis, G. Single cell oil (SCO) production by *Mortierella isabellina* grown on high-sugar content media. *Bioresour. Technol.* **2004**, *95*, 287–291. [CrossRef]
48. Rousk, J.; Brookes, P.C.; Bååth, E. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Appl. Environ. Microbiol.* **2009**, *75*, 1589. [CrossRef] [PubMed]
49. Bao, Z.; Wang, X.; Wang, Q.; Zou, L.; Peng, L.; Li, L.; Tu, W.; Li, Q. A novel method of domestication combined with ARTP to improve the reduction ability of *Bacillus velezensis* to Cr(VI). *J. Environ. Chem. Eng.* **2023**, *11*, 109091. [CrossRef]
50. Rahman, K.; Othman, R. Influence of pH levels on disease development in oil palm seedling roots infected with *Ganoderma boninense*. *Rhizosphere* **2020**, *13*, 100181. [CrossRef]
51. Rousk, J.; Bååth, E.; Brookes, P.C.; Lauber, C.L.; Lozupone, C.; Caporaso, J.G.; Knight, R.; Fierer, N. Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J.* **2010**, *4*, 1340–1351. [CrossRef]
52. Li, Q.; Xiang, P.; Li, L.; Zhang, T.; Wu, Q.; Bao, Z.; Tu, W. Phosphorus mining activities alter endophytic bacterial communities and metabolic functions of surrounding vegetables and crops. *Plant Soil* **2023**, *485*, 1–20. [CrossRef]
53. Ke, L.Q.; Li, P.D.; Xu, J.P.; Wang, Q.S.; Wang, L.L.; Wen, H.P. Microbial communities and soil chemical features associated with commercial production of the medicinal mushroom *Ganoderma lingzhi* in soil. *Sci. Rep.* **2019**, *9*, 15839. [CrossRef]