Metagenomic Analysis of Bacterial and Fungal Communities Inhabiting Shiro Dominant Soils of Two Production Regions of Tricholoma Matsutake S. Ito & S. Imai in Korea

Gi-Hong An, Jae-Han Cho, Ok-Tae Kim and Jae-Gu Han *

Abstract: Tricholoma matsutake is an ectomycorrhizal fungus that has obligate symbiotic relationships with Pinus densiflora. Its fruiting body has a distinctive flavor and is traded at a high price. Thus, it has been a significant source of income for rural communities in Korea. We hypothesized that biotic factors considerably influence the formation of the T. matsutake mushroom, and the soils producing T. matsutake share similar microbial characteristics. Therefore, the present study aimed to detect the specific fungal and bacterial groups in T. matsutake production soils (shiro+) and nonproduction soils (shiro−) of the Bonghwa and Yanyang regions via next-generation sequencing. In a total of 15 phyla, 36 classes, 234 genera of bacteria, six phyla, 29 classes, and 164 genera of fungi were detected from four samples at both sites. The species diversity of shiro+ soils was lower than the shiro− samples in both the fungal and bacterial groups. In addition, we did not find high similarities in the microbial communities between the shiro+ soils of the two regions. However, in the resulting differences between the fungal communities categorized by their trophic assembly, we found a distinguishable compositional pattern in the fungal communities from the shiro+ soils and the shiro− soils of the two sites. Thus, the similarity among the microbial communities in the forest soils may be due to the fact that the microbial communities in the T. matsutake dominant soils are closely associated with biotic factors and abiotic factors such as soil properties.

Keywords: ectomycorrhizae; bacterial communities; fungal communities; metagenomics; miseq; shiro dominant soil; Tricholoma matsutake

1. Introduction

Tricholoma matsutake (S. Ito & S. Imai) that forms a symbiotic association with the root tips of Pinus densiflora (Siebold & Zucc.) provides attractive commercial benefits to rural communities in Korea [1,2]. The annual yields of this mushroom are highly limited and unpredictable. Since it has not yet been successfully artificially cultivated, the entire production of T. matsutake still depends upon natural harvesting from forests. In recent decades, many researchers have strived to succeed in the artificial production of T. matsutake [2–6]. However, the artificial cultivation of this fungus has not been established. As an obligated symbiont, the biology of this mycorrhizal fungus must be considered from the perspective of the ecological interaction with the surrounding biotic factors, especially microbial groups.

Soil ecosystems have a wide variety of microbial communities. Microorganisms in the soil can have positive or negative effects on the growth of ectomycorrhizal fungus [7]. Many studies have been conducted on the microbial communities in the soils adjacent to T. matsutake [6,8–11]. The influence of the diverse microbial communities in soil on the life cycle of T. matsutake, such as the development of mycelia and the formation of fruiting bodies in various ways, has been investigated [4,12,13]. In particular, some soil bacteria, which are called mycorrhizal helper bacteria (MHB), have beneficial effects on the
mycorrhizal symbiosis by mobilizing nutrients in nutrient-deficient soils [14]. In addition, some fungi that have frequently been detected from the fruiting body and fairy ring of *T. matsutake* co-exist in the hyphal dominant environment as a potential mycorrhizal helper fungus [11]. Therefore, the various microbial communities associated with *T. matsutake* could potentially contribute to the growth of hyphae and the formation of the fruiting body of *T. matsutake* [7,15–17].

Recent advances in metagenomics contribute to unveiling the microbial communities in various environmental samples [11,13,18]. Next-generation sequencing (NGS) based on metagenomics is a culture-independent method that enables the identification of uncultured microbes. The recent application of NGS sequencing methods, such as pyrosequencing 454 and Illumina, may provide a more direct way to detect microbial taxa, especially those with a low level of species changes [19,20]. In addition, Illumina sequencing is cost-effective and could obtain tenfold or more sequences per sample than pyrosequencing 454, thereby allowing the analysis of a high number of detailed taxonomic profiles from samples [21].

According to the report by Korean Statistical Information Service (KOSIS), the annual yields of *T. matsutake* in Korea dramatically decreased from 480 tons in 2007 to 140 tons in 2017. Previously, Gyeongsangbuk-do and Gangwon-do provinces were well-known as the representative *T. matsutake* producing regions that occupied the largest (61.3%) and second (18.0%) largest total yields of *T. matsutake* in Korea. However, the yields of *T. matsutake* in Gyeongsangbuk-do province have been gradually decreasing, and it was reported by KOSIS to be 340 tons in 2007, further decreasing to 75 tons in 2017. Thus, we speculated that the reduction in the yields of *T. matsutake* is due to various environmental factors, and we focused on the changes in microbial communities in the production of soils for *T. matsutake*. We hypothesized that the microbial communities in the production of soils for *T. matsutake* would be quite similar to one another, regardless of geographical characteristics, if there are microorganisms that have beneficial effects on the growth of mycelium and the formation of the fruit body of *T. matsutake*. Therefore, in this study, to determine the differences between the microbial communities in soils where *T. matsutake* occurs in Gyeongsangbuk-do and Gangwon-do provinces, we conducted sampling of the *T. matsutake* production soil (shiro+ soil) and nonproduction soil (shiro− soil) in two main *T. matsutake* production regions (Bonghwa and Yangyang) in Korea and investigated the soil bacterial and fungal communities from each sample using the Illumina Miseq sequencing platform.

2. Materials and Methods

2.1. Sampling Sites

Two sampling sites were mountains located near Seo-myeon, Yangyang-gun, Gangwon-do (110 m ELV, 38°03′23.8″ N, 128°38′38.7″ E), and Beopjeon-myeon, Bonghwa-gun, Gyeongsangbuk-do (360 m ELV, 36°55′14.2″ N, 128°56′47.2″ E) in south-eastern parts of the Korean peninsula. The climate and major vegetation at these sites are summarized in Table 1. At the Yangyang site, in 2019, the annual mean temperature was 13.9 °C, and annual precipitation was 1517.3 mm. The major canopy vegetation was *P. densiflora* at more than 80%, and understory vegetation comprised *Rhododendron schlippen* (Maxim.), *R. mucronulatum* (Turcz.), *Smilax nipponica* (Miq.), and *Carex fernaldiana* (H.Lév. & Vaniot). The site belongs to the public research forest under the management of the Yangyang-gun Agricultural Technology Center. At the Bonghwa site, in 2019, the annual mean temperature was 11.5 °C, and annual precipitation was 970.5 mm. The major canopy vegetation was *P. densiflora* at more than 80%, and understory vegetation comprised *R. schlippen*, *R. mucronulatum*, *Melampyrum roseum* (Maxim.), *Pteridium aquilinum* (Underw. ex A. Heller), *S. nipponica*, and *C. fernaldiana*. The site in Bonghwa is privately owned. The sampling sites are geographically remote, located approximately 120 km from each other (Figure 1). In September 2019, soil sampling was conducted immediately after *T. matsutake* fruiting bodies were harvested. By using a soil sampler, soils containing shiro (shiro+) were collected at 10 cm depth from the soil surface at the three spots of the zone beneath the *T. matsutake*, and soils without
shiro (shiro−) were collected at approximately 3–4 m intervals from the spots with the shiro+ soils. Each soil sample was placed into a polyvinyl bag and mixed well. Shiro can be distinguished by its features of whitish-gray-colored soil, in which fungal hyphae are aggregated [11]. Soil samples taken from each sampling site were transported on ice and stored at −4 °C before DNA extraction.

Table 1. Climates and vegetation of the experimental sites (http://www.nongsaro.go.kr/, (accessed on 26 April 2021)).

| Sites      | Location          | Temp./Precipitation (Elevation) | Major Vegetation (Canopy/Understory)                                                                 |
|------------|-------------------|---------------------------------|------------------------------------------------------------------------------------------------------|
| Yangyang   | Gangwon-do        | 13.9 °C/1517.5 mm               | *Pinus densiflora* Siebold & Zucc. / *Rhododendron schlippen* Maxim. / *Smilax nipponica* Miq. / *Carex fernaldiana* H.Lév & Vaniot |
|            |                   | *(160 m ELV.)*                  |                                                                                                       |
| Bonghwa    | Gyeongsangbuk-do  | 11.5 °C/970.5 mm                | *Pinus densiflora* / *Rhododendron schlippen* / *R. mucronulatum* / *Melampyrum roseum* Maxim. / *Pteridium aquilinum* Underw. ex A. Heller / *Smilax nipponica* / *Carex fernaldiana* |
|            |                   | *(360 m ELV.)*                  |                                                                                                       |

Figure 1. Sampling sites were located in Yangyang-gun, Gangwon-do (A), and Bonghwa-gun, Gyeongsangbuk-do (B) in Korea. The map was downloaded from National Geographic Information Institute (NGII, https://www.ngii.go.kr/ (accessed on 11 June 2020)).

2.2. DNA Extraction, Library Construction, and Illumina Miseq Sequencing

For each sample, microbial DNA was extracted from 0.5–1 g per soil using a DNeasy Power Soil Kit (Qiagen, Hilder, Germany) according to the manufacturer’s instructions. The extracted DNA was quantified using Quant-IT PicoGreen (Invitrogen). The sequencing libraries were prepared according to the Illumina 16S Metagenomic sequencing library protocols for the V3–V4 region for bacteria, and 5.8S and ITS2 regions for fungi. For bacteria,
the input 2 uL (10 ng uL$^{-1}$) was PCR amplified with 1 x reaction buffer, 1 nM dNTP mix, 500 nM concentrations of the universal F/R PCR primers, and 2.5 U of Herculase II fusion DNA polymerase (Agilent Technologies, Santa Clara, CA, USA). The cycle condition for the 1st PCR was 3 min at 95 °C for heat activation, and 25 cycles of 30 s at 95 °C, 30 s at 55 °C, and 30 s at 72 °C, followed by a 5 min final extension at 72 °C. The 1st PCR product was purified with AMPure beads (Agencourt Bioscience, Beverly, MA, USA). Following purification, the 2 uL of 1st PCR product was PCR amplified for final library construction containing the index using the NexteraXT Indexed Primer. The cycle condition for the 2nd PCR was the same as the 1st PCR condition except for 10 cycles. The PCR product was purified with AMPure beads. The resulting PCR products were pooled, and the fragment sizes were checked using agarose gel electrophoresis and the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The final purified product was then quantified using qPCR according to the qPCR Quantification Protocol Guide (KAPA Library Quantification Kits for Illumina Sequencing platforms) and qualified using the TapeStation D1000 ScreenTape (Agilent Technologies, Waldbronn, Germany). The paired-end (2 x 300 bp) sequencing was performed by the Macrogen using the MiSeq™ platform (Illumina, San Diego, CA, USA).

2.3. Processing and Analyzing of Sequencing Data

The bacterial and fungal sequence reads were assembled using FLASH 1.2.11 (Fast Length Adjustment of Short reads, http://ccb.jhu.edu/software/FLASH/ (accessed on 17 December 2019)). After assembly, pre-processing and clustering were carried out using the CD-HIT-OTU program (http://weizhongli-lab.org/cd-hit-otu/ (accessed on 17 December 2019)) that performed the OTU (Operational Taxonomic Units) finding. CD-HIT-OTU comprises the following steps: (1) Low-quality reads are filtered out and extra-long tails are trimmed. (2) Filtered reads are clustered at 100% identity using CD-HIT-DUP. (3) Chimeric reads are identified. (4) Secondary clusters are recruited into primary clusters. (5) Noise sequences in clusters of size x or below are removed. Size x is statistically calculated. (6) Remaining representative reads from non-chimeric clusters are clustered into OTUs at a user-specified OTU cutoff (e.g., 97% identity at species level) [22]. Representative sequences for each OUT were selected and assigned to taxonomic data at RDP for bacteria [23] and UNITE for fungi [24] databases using the Quantitative Insights Into Microbial Ecology (QIIME) which is an open-source bioinformatics pipeline for performing microbiome analysis from raw DNA sequencing data [25]. Alpha diversity indices, such as the Shannon index, Chao 1, Simpson index, and Good’s coverage were also calculated using QIIME [25]. Sorensen’s classic similarity analysis that is based on the probability between two randomly chosen individuals, one from each of the two samples, was performed using EstimateS 9.1.0 [26].

3. Results

3.1. Statistical Data Analysis for Bacterial Communities in Sampling Sites

A total of 473,975 reads and 1315 OTUs were detected in four sampling spots (Table 2). The number of total reads ranged from 112,489 to 129,607. In all, 221 to 379 OTUs per sampling spot were obtained at a 99% similarity level. The result of the Chao1 estimation showed that the species richness of the shiro− soil samples of the Bonghwa and Yangyang sites was lower than that of the shiro+ soils. The diversity of species of bacterial communities for each sampling spot indicated that the shiro− soils included more bacterial communities than shiro+ soils at both sampling sites. The shiro− soil at the Bonghwa site represented the most diverse bacterial community (Shannon index = 6.777), while the shiro+ soil of Bonghwa had the lowest bacterial diversity (4.628). The Good’s coverage of all sampling spots ranged from 0.997 to 0.999, indicating that the sequencing depth appropriately represented the bacterial diversity. The rarefaction curves, which reveal the species richness of each sample, also showed that the shiro− soil samples of both of the sampling sites have a greater bacterial community than the shiro+ soil samples (Figure 2a).
Table 2. Summary of Illumina sequencing and statistical analysis of bacterial communities inhabiting the *Tricholoma matsutake* production (shiro+) and nonproduction (shiro−) soils of Bonghwa (B) and Yangyang (Y) sampling sites.

|                  | B_Shiro+ | B_Shiro− | Y_Shiro+ | Y_Shiro− |
|------------------|----------|----------|----------|----------|
| Number of total reads | 115,236  | 116,643  | 112,489  | 129,607  |
| Number of OTUs    | 340      | 375      | 221      | 379      |
| Chao1 estimation  | 349.5    | 385.1    | 230.0    | 400.7    |
| Shannon index     | 4.628    | 6.777    | 5.187    | 6.212    |
| Inverse Simpson index | 0.885  | 0.977    | 0.937    | 0.969    |
| Good’s coverage   | 0.999    | 0.997    | 0.999    | 0.997    |

3.3. Relative Abundance of Bacterial Communities

Taxonomic composition analysis of bacteria for four sampling spots was conducted at the phylum level (Figure 3a). In total, 15 phyla were identified: 12 phyla in the shiro+ soil of Bonghwa, 10 phyla in the shiro− soil of Bonghwa, 12 phyla in the shiro+ soil of Yangyang, and 13 phyla in the shiro− soil of Yangyang. At the Bonghwa site, Bacteroidetes and Proteobacteria in the shiro+ soil were relatively abundant (46.01% and 45.08%), compared with the shiro− soil (14.56% and 38.16%). Acidobacteria was the dominant phylum in the shiro− soil (37.92%) compared with the shiro+ soil (3.44%). At the Yangyang site, the highest abundance of phyla in the shiro+ soil was shown by Actinobacteria, and Chloroflexi compared with those of the shiro− soil, whereas Acidobacteria, Proteobacteria, and Verrucomicrobia were the dominant phyla in the shiro− soil.

The relative abundance at the class level for Acidobacteria, Actinobacteria, Bacteroidetes, and Proteobacteria, the most frequently observed phyla in the four soil samples, showed distinct differences between the soil samples and the sampling sites. Of the total 36 classes, four classes of Acidobacteria phylum, four classes of Actinobacteria phylum, four classes of Bacteroidetes phylum, and five classes of Proteobacteria phylum were mainly observed. Acidobacteria, Acidobacteriia, and Vicinamibacteria were commonly detected in all soil samples (Figure 3b). Within the phylum Actinobacteria, the most abundant class in all samples was Actinobacteria (Figure 3c). Chitinophagia and Sphingobacteriia commonly showed Bacteroidetes in all soil samples (Figure 3d). Five bacterial classes of Proteobacteria phylum, i.e., Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Gammaproteobacteria, and Oligoflexia, were commonly detected in all samples (Figure 3e). The Alphaproteobacteria class was highly detected in the shiro− soils of both sites, while the Betaproteobacteria class was more abundant in the shiro+ soils than the shiro− soils at both sites.

3.2. Statistical Data Analysis for Fungal Communities in Sampling Sites

A total of 496,994 reads and 781 OTUs were detected at four sampling spots (Table 3). For the number of OTUs, the shiro− soil sample of Bonghwa was the highest (364 OTUs), followed by the shiro− soil of Yangyang (244 OTUs), the shiro+ soil of Yangyang (107 OTUs), and the shiro+ soil of Bonghwa (66 OTUs). All indices of Chao1, Shannon diversity, and inverse Simpson were lower in the shiro− soil sample than the shiro+ soil samples in both sampling sites. In particular, the fungal diversity of the shiro− soil sample of Bonghwa (Chao1 estimation = 364; Shannon index = 5.846; Inverse Simpson index = 0.948) was the highest among the soil samples. All samples showed Good’s coverage that indicated sufficient sequencing depth for characterizing fungal diversity. The rarefaction curves also showed that the shiro− soil samples of both of the sampling sites have a higher number of OTUs than the shiro+ soil samples of both sites (Figure 2b).

Figure 2. Rarefaction curves for chao1 of bacteria (a) and fungi (b) from soil samples inhabiting the *Tricholoma matsutake* production (shiro+) and nonproduction (shiro−) soils of Bonghwa and Yangyang sampling sites.
Forests 2021, 12, x FOR PEER REVIEW 7 of 17

Table 3. Summary of Illumina sequencing and statistical analysis of fungal communities inhabiting the Tricholoma matsutake production (shiro+) and nonproduction (shiro−) soils of Bonghwa (B) and Yangyang (Y) sampling sites.

|                     | B_Shiro+   | B_Shiro−   | Y_Shiro+   | Y_Shiro−   |
|---------------------|------------|------------|------------|------------|
| Number of total reads | 142,261    | 113,784    | 127,090    | 113,859    |
| Number of OTUs       | 66         | 364        | 107        | 244        |
| Chao1 estimation     | 66.5       | 364        | 111.7      | 246.1      |
| Shannon index        | 1.355      | 5.846      | 1.873      | 3.845      |
| Inverse simpson index| 0.498      | 0.948      | 0.505      | 0.840      |
| Good’s coverage      | 1.000      | 1.000      | 1.000      | 1.000      |

3.3. Relative Abundance of Bacterial Communities

Taxonomic composition analysis of bacteria for four sampling spots was conducted at the phylum level (Figure 3a). In total, 15 phyla were identified: 12 phyla in the shiro+ soil of Bonghwa, 10 phyla in the shiro− soil of Bonghwa, 12 phyla in the shiro+ soil of Yangyang, and 13 phyla in the shiro− soil of Yangyang. At the Bonghwa site, Bacteroidetes and Proteobacteria in the shiro+ soil were relatively abundant (46.01% and 45.08%), compared with the shiro− soil (14.56% and 38.16%). Acidobacteria was the dominant phylum in the shiro− soil (37.92%) compared with the shiro+ soil (3.44%). At the Yangyang site, the highest abundance of phyla in the shiro+ soil was shown by Actinobacteria, and Chloroflexi compared with those of the shiro− soil, whereas Acidobacteria, Proteobacteria, and Verrucomicrobia were the dominant phyla in the shiro− soil.

Figure 3. Taxonomic composition analysis of bacterial communities at the phylum level (a) and class level (b–e) inhibiting the Tricholoma matsutake production (shiro+) and nonproduction (shiro−) soils of Bonghwa and Yangyang sampling sites. (b) four bacterial classes of Acidobacteria phylum, (c) 4 bacterial classes of Actinobacteria phylum, (d) 4 bacterial classes of Bacteroidetes phylum, and (e) 5 bacterial classes of Proteobacteria phylum.

The relative abundance at the class level for Acidobacteria, Actinobacteria, Bacteroidetes, and Proteobacteria, the most frequently observed phyla in the four soil samples, showed distinct differences between the soil samples and the sampling sites. Of the total...
36 classes, four classes of Acidobacteria phylum, four classes of Actinobacteria phylum, four classes of Bacteroidetes phylum, and five classes of Proteobacteria phylum were mainly observed. Acidobacteria, Acidobacteriia, and Vicinamibacteria were commonly detected in all soil samples (Figure 3b). Within the phylum Actinobacteria, the most abundant class in all samples was Actinobacteria (Figure 3c). Chitinophagia and Sphingobacteriia commonly showed Bacteroidetes in all soil samples (Figure 3d). Five bacterial classes of Proteobacteria phylum, i.e., Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Gammaproteobacteria, and Oligoflexia, were commonly detected in all samples (Figure 3e). The Alphaproteobacteria class was highly detected in the shiro− soils of both sites, while the Betaproteobacteria class was more abundant in the shiro+ soils than the shiro− soils at both sites.

In the total 234 genera of bacteria, 42 genera were observed in more than 1% of relative abundance in the shiro+ soils and shiro− soils of the two sampling sites (Table 4). Of these genera, *Mucilaginibacter* (26.19%) from Bacteriodetes phylum, *Acidobacterium* (11.46%) from Acidobacteria phylum, *Actinoallomurus* (18.17%) from Actinobacteria phylum, and *Mycobacterium* (14.50%) from Proteobacteria phylum were the most dominant genera in the shiro+ soil of Bonghwa, the shiro− soil of Bonghwa, the shiro+ soil of Yangyang, and the shiro− soil at the Yangyang site, respectively. Seven genera, *Flavobacterium* (4.44%), *Pedobacter* (2.92%), *Sphingobacterium* (8.56%), *Novosphingobium* (1.60%), *Janthinobacterium* (21.94%), *Pseudononas* (6.73%), and *Stenotrophomonas* (1.76%), were only observed in the shiro+ soil at the Bonghwa site. In addition, the genera of *Acidipila* (2.98%), *Silicibacterium* (1.28%), *Labedaes* (13.37%), *Pseudonocardia* (1.16%), *Sinosporangium* (1.95%), *Actinoallomurus* (18.17%), *Actinocorallia* (1.79%), *Actinomadura* (3.91%), *Dictyobacter* (8.63%), *Rhodopila* (3.58%), *Alidongia* (1.98%), and *Caballeronia* (2.18%) were more abundantly detected in the shiro+ soil at the Yangyang site among the four samples. The only two genera that were observed with high abundances in the shiro+ soils of the two sites were *Caballeronia* and *Paraburkholderia*, while eight genera, *Acidobacterium*, *Edaphobacter*, *Granulicella*, *Paludibaculum*, *Phenylobacterium*, *Rhizomicrobium*, *Sulfuriflexus*, and *Povalibacter*, have high proportions in the shiro− soils of the two sites.

| Table 4. List of bacterial genera inhabiting the *Tricholoma matsutake* production (shiro+) and nonproduction (shiro−) soils of Bonghwa and Yangyang sampling sites. The bacterial genera present more than 1% of at least one sample among the four soil samples. |
|---|---|---|---|---|---|
| Phylum | Family | Genus | B_Shiro+ | B_Shiro− | Y_Shiro+ | Y_Shiro− |
| Acidobacteria | Acidobacteriaceae | Acidipila | 0.09% | 0.52% | 2.98% | 2.90% |
| | Acidobacteriaceae | Acidobacterium | 1.97% | 11.46% | 0.32% | 4.82% |
| | | Edaphobacter | 0.12% | 5.06% | 1.55% | 3.66% |
| | | Granulicella | 0.07% | 1.29% | 0.06% | 0.29% |
| | | Silicibacterium | 0.05% | 0.96% | 1.28% | 1.13% |
| | | Terriglobus | 0.01% | 1.27% | 0% | 0.07% |
| | Bryobacteriaceae | Paludibaculum | 0.54% | 7.19% | 0.18% | 2.23% |
| Actionobacteria | Thermoaerobacteriaceae | Thermoaerobacterium | 0% | 1.14% | 0% | 0% |
| | Mycobacteriaceae | Mycobacterium | 1.57% | 1.21% | 13.48% | 14.50% |
| | | Mycolicibacterium | 0.33% | 0.33% | 0.47% | 1.02% |
| | Pseudonocardaceae | Labedaes | 0.01% | 0% | 13.37% | 0.45% |
| | | Pseudonocardia | 0.01% | 0.01% | 1.16% | 0.35% |
| | Streptosporangiaceae | Sinosporangium | 0.03% | 0.01% | 1.95% | 0.52% |
| | Thermomonosporaceae | Actinoallomurus | 0.44% | 0.86% | 18.17% | 4.61% |
| | | Actinocorallia | 0.09% | 0.22% | 1.79% | 1.24% |
| | | Actinomadura | 0.12% | 0.47% | 3.91% | 2.06% |
| Bacteroidetes | Chitinophagaceae | Flavitalae | 0.02% | 3.27% | 0.01% | 0.11% |
| | | Niiasterla | 0% | 3.57% | 0% | 0% |
| | | Puia | 0.44% | 1.54% | 0.43% | 2.13% |
| | Flavobacteriaceae | Flavobacterium | 4.44% | 0% | 0% | 0% |
| | Sphingobacteriaceae | Mucilaginibacter | 26.19% | 1.79% | 0.33% | 0.91% |
| | | Pedobacter | 2.92% | 0% | 0% | 0% |
| | | Sphingobacterium | 8.56% | 0% | 0% | 0% |
3.4. Relative Abundance of Fungal Communities

Fungal taxonomic composition analysis for four sample soils was conducted at the phylum level (Figure 4a). A total of six phyla were detected in all samples. Ascomycota, Basidiomycota, Mortierellomycota, and Mucoromycota were commonly observed in all soil samples. Basidiomycota and Mucoromycota phyla were more dominant in the shiro+ soils than the shiro− soils of both sites. In contrast, Ascomycota and Mortierellomycota had a higher abundance in the shiro− soils compared with the shiro+ soils of both sites.

Table 4. Cont.

| Phylum                  | Family               | Genus          | B_Shiro+ | B_Shiro− | Y_Shiro+ | Y_Shiro− |
|-------------------------|----------------------|----------------|----------|----------|----------|----------|
| Chloroflexi             | Dictyobacteraceae    | Dictyobacter    | 0.34%    | 0.15%    | 8.63%    | 2.88%    |
| Thermotogasprichetes    | Thermoplasmyra       | Thermoplasmyra  | 0%       | 0%       | 0.61%    | 2.57%    |
| Planctomycetes          | Tepidissphaeracae    | Tepidissphaera  | 0.17%    | 1.01%    | 0%       | 0.20%    |
| Proteobacteria          | Caulobacteracae      | Caulobacter     | 0.61%    | 0.67%    | 0.09%    | 1.43%    |
| Micropepsaeae           | Phyllobacter         | Phyllobacter     | 0.26%    | 1.39%    | 0.40%    | 1.82%    |
| Bradyrhizobiaae         | Rizickyobacter       | Rizickyobacter   | 0.56%    | 7.49%    | 0.30%    | 1.48%    |
| Acetobacteraceae        | Rhodopilo            | Rhodopilo       | 0.93%    | 6.75%    | 2.88%    | 2.78%    |
| Rhodospirillaceae       | Aliidongia           | Aliidongia      | 0.14%    | 0.90%    | 3.58%    | 0.70%    |
| Sphingomonadaceae       | Novosphingobium      | Novosphingobium  | 1.60%    | 0%       | 0%       | 0%       |
| Burkholderiaceae        | Caballeronia         | Caballeronia    | 0.50%    | 0.03%    | 2.18%    | 0.61%    |
| Oxalobacteraceae        | Janthinobacter       | Janthinobacter   | 21.94%   | 0%       | 0%       | 0%       |
| Granulosococaceae       | Sulfuriflexus        | Sulfuriflexus   | 0.40%    | 1.01%    | 0.79%    | 2.47%    |
| Sinobacteraceae         | Povalibacter         | Povalibacter     | 0.86%    | 1.15%    | 0.31%    | 1.38%    |
| Pseudomonadaceae        | Pseudomonas           | Pseudomonas      | 6.73%    | 0%       | 0%       | 0%       |
| Xanthomonadaceae        | Stenotrophomonas     | Stenotrophomonas | 1.76%    | 0%       | 0%       | 0%       |
| Terrimicrobacter        |                     | Terrimicrobacter | 0.30%    | 0.25%    | 3.81%    | 7.29%    |

Figure 4. Taxonomic composition analysis of fungal communities at phylum level (a) and class level (b–e) inhibiting the Tricholoma matsutake production (shiro+) and nonproduction (shiro−) soils of Bonghwa and Yangyang sampling sites. (b) ten fungal classes of Ascomycota phylum, (c) 8 fungal classes of Basidiomycota phylum, (d) 4 fungal classes of Mucoromycota phylum, and (e) fungal classes from the phyla Chytridiomycota, Rozellomycota, and Mortierellomycota.
The relative abundance of the class level for Ascomycota and Basidiomycota, the most frequently detected phyla in all samples, showed distinct differences between the production/nonproduction soils of *T. matsutake*. Of the total 29 classes, 10 classes from Ascomycota were observed. The four classes that were commonly detected in all soil samples were Dothideomycetes, Eurotinomycetes, Leotiomycetes, and Sordariomycetes (Figure 4b). A total of nine classes from phylum Basidiomycota were observed in all samples, and three classes were only commonly detected: Agaricomycetes, Geminibasidiomycetes, and Tremellomycetes (Figure 4c). The class that occurred the most frequently was Agaricomycetes in all the samples, and this class had high relative abundance in the shiro+ soils compared with the shiro− soils of both sites. At the level of phylum Mucoromycota, only Umbelopsidomycetes was observed in all soil samples (Figure 4d). In addition, the classes of unidentified Chytridiomycota of phylum Chytridiomycota, and Mortierellomycetes of phylum Mortierellomycota were detected in very low proportions (Figure 4e).

In the total 168 genera of fungi, 24 genera were observed at a higher than 1% relative abundance (Table 5). Especially one genus, *Tricholoma* was highly detected in the shiro+ soils (Bonghwa, 63.73%; Yangyang, 68.86%) compared with the shiro− soils (Bonghwa, 0.08%; Yangyang 29.46%) at the two sampling sites. In the *Tricholoma* genus of the shiro− soil at the Yangyang site, it was revealed that *T. saponaceum* was detected at 29.45% relative abundance, while *T. matsutake* was only detected at 0.01% (Table S1). In addition, *Umbelopsis* from Mucoromycota phylum was commonly observed in all soil samples. It showed relatively high proportions in the shiro+ soil (31.16%) compared with the shiro− soil (1.21%) at the Bonghwa site, while it was more abundantly detected in the shiro− soil (33.51%) than the shiro+ soil (19.62%) of the Yangyang site. The genera *Amphinema* (16.99%), *Astraeus* (1.46%), and *Sobacina* (4.59%) from Basidiomycota phylum were only detected in the shiro+ soil at Yangyang site. The genera of *Hydnum, Cladophialophora*, unidentified Herpotrichiellaceae, unidentified Chaetothyriales, *Penicillium*, unidentified Hyaloscyphaceae, *Oidiodendron*, unidentified Helotiales, unidentified Ascomycota, and *Mortierella* were more abundantly detected in the shiro− soils than the shiro+ soils at the two sites. In the fungal genus, ectomycorrhizal (ECM) assemblages and other fungi assemblage were separated. The assemblages of the seven genera of ECM, and one genus of the symbiotic fungal genera, *Tricholoma, Tylospora, Astraeus, Sistotrema, Russula, Sebacina, Tomentella*, and *Oidiodendron* were more abundantly observed in the shiro− soils than the shiro+ soils at the two sites (B_shiro+, 65.91%; B_shiro−, 20.15%; Y_shiro+, 74.43%; Y_shiro−, 37.62%). The relative abundances of other fungal genera assemblages including unknown genera were higher in the shiro− soils than the shiro+ soils at the two sites (B_shiro+, 34.00%; B_shiro−, 74.20%; Y_shiro+, 25.42%; Y_shiro−, 61.74%).

### 3.5. Similarity of Bacterial and Fungal Communities within/across Sampling Sites

The similarity in the bacterial and fungal communities within/across the sampling sites is shown in Table 6. The highest similarity index of the bacterial community was observed between the shiro+ soil in Bonghwa and the shiro− soil in Yangyang (0.769), and the lowest value was observed between the shiro− soil in Bonghwa and the shiro+ soil in Yangyang (0.565), or the shiro− soils in Bonghwa and Yangyang (0.565). In the similarity indices of the fungal community, the highest value was observed between the shiro− soils in Bonghwa and Yangyang (0.666), or between the shiro+ soil and the shiro− soil in Yangyang (0.666). The lowest value was observed between the shiro+ soil in Bonghwa and the shiro+ soil in Yangyang (0.578). In fungal communities separated from ECM and other fungi assemblages except for unknown fungi, the similarity of ECM communities was shown to be the highest value in the shiro− soils of the two sites (0.857), whereas low similarity indices were observed between the shiro+ soil and the shiro− soil at the Bonghwa site, the shiro+ soils of two sites, or the shiro+ soil at the Bonghwa site and the shiro− soil at the Yangyang site (Table S2). For other fungal assemblages, the highest
similarity was observed between the shiro− soils of the two sites (0.889), and between the shiro− soil at the Bonghwa site and the shiro+ soil at the Yangyang site (0.889).

| Table 5. List of fungal genera inhabiting the *Tricholoma matsutake* production (shiro+) and nonproduction (shiro−) soils of Bonghwa and Yangyang sampling sites. The fungal genera present more than 1% of at least one sample among the four soil samples. |
|-----------------------------------------------|
| **Phylum** | **Family** | **Genus** | **B_Shiro+** | **B_Shiro−** | **Y_Shiro+** | **Y_Shiro−** |
| Basidiomycota | Tricholomataceae | Tricholoma | 63.73% | 0.08% | 68.86% | 29.46% |
| Atheliales | | Amphinema | 0% | 16.99% | 0% | 0% |
| | | Tylospora | 0.01% | 10.55% | 0% | 0% |
| Astraeaceae | | Astraeus | 0% | 1.46% | 0% | 0% |
| Cantharellales | | Sistotrema | 0% | 0% | 2.36% | 0.11% |
| Hydnaceae | | Hydnum | 0.01% | 0.18% | 0% | 1.58% |
| Russulaceae | | Russula | 2.04% | 0.30% | 0% | 0.25% |
| Sebacinae | | Sebacina | 0% | 4.59% | 0% | 0% |
| Thelephiraceae | | Tomentella | 0.02% | 1.54% | 0% | 0% |
| unidentified | | unidentified | 0.03% | 1.43% | 0.26% | 0.23% |
| Trimorphomycetaceae | | Saitozyma | 0% | 2.10% | 0.01% | 0.02% |
| Ascomycota | Herpotrichiellaceae | Cladophialophora | 0.08% | 7.26% | 0.08% | 0.85% |
| unidentified | | unidentified | 0.04% | 3.40% | 0.01% | 1.73% |
| Aspergillaceae | | Penicillium | 0% | 1.34% | 0.17% | 3.84% |
| unidentified | | unidentified | 0% | 0.98% | 0.01% | 0.21% |
| Hyaloscyphaceae | | unidentified | 0.04% | 1.49% | 0.18% | 1.13% |
| Myxotrichaceae | | Oudiodendron | 0.10% | 1.64% | 2.38% | 7.79% |
| unidentified | | unidentified | 0.03% | 1.52% | 0.64% | 1.14% |
| unidentified | | unidentified | 0.03% | 1.81% | 0% | 0.16% |
| Mortierellomycota | Mortierellaceae | Mortierella | 0.01% | 5.48% | 0.11% | 4.11% |
| Mortierellomycota | | Bifiguratia | 0.13% | 0% | 0.70% | 1.65% |
| mucoromycota | | Umbelopsis | 31.16% | 1.21% | 19.62% | 33.51% |
| unidentified | | unidentified | 0.04% | 18.19% | 0.30% | 2.74% |

| Table 6. The results of Sorensen’s classic similarity index of bacterial and fungal communities within/across sampling sites. |
|-----------------------------------------------|
| **Phylum** | **Family** | **Genus** | **B_Shiro+** | **B_Shiro−** | **Y_Shiro+** | **Y_Shiro−** |
| Bacterial community | | | 1 | 0.702 | 0.6 | 0.769 |
| | | | 1 | 0.565 | 0.565 |
| | | | 1 | 0.625 |
| | | | 1 |
| Fungal community | | | 1 | 0.648 | 0.578 | 0.615 |
| | | | 1 | 0.628 | 0.666 |
| | | | 1 | 0.666 |
| | | | 1 |

4. Discussion

*Tricholoma matsutake* forms in the shiro, as unique and massive aggregates of mycorrhizal hyphae, host plant roots, and soil particles [4,27]. As fruiting bodies form in natural conditions, an understanding of the environment near the fairy ring is crucial to understanding the ecology of *T. matsutake* [28]. Many studies have shown that the biological and physiochemical characteristics are different between the fairy ring and the adjacent soil, and between the positions within fairy rings likely due to the effects of *T. matsutake* hyphae [8,13,29–31]. As a biotic environment in fairy rings, various co-existing microbial communities may influence *T. matsutake* occurrence in different ways [8,11,13].
4.1. Distinct Bacterial Community Structure

In the work reported here, we compared the difference in the microbial diversity and community between the presence and absence of the shiro (fairy rings) soils, and between two main production regions (Bonghwa and Yangyang) of *T. matsutake* in Korea. We found that both bacterial and fungal diversity was lower in the shiro+ soil than in the shiro− soil at both sites (Tables 2 and 3). For microbial diversity inhabiting the fairy ring, bacterial and fungal diversity was significantly lower in the *T. matsutake*–dominant soil compared with the *T. matsutake* minor soil [11]. The bacterial communities in the active mycorrhizal zone of *T. matsutake* were much simpler than those at locations far away from the shiro [29]. Moreover, the results of metagenomics analysis showed that the fairy ring zone of *T. matsutake* had the lowest OTUs, bacterial diversity, and evenness in all sampling zones [10]. In the taxonomic analysis of the bacterial community, our results are in part consistent with a previous study by Oh et al. [11], who documented that Acidobacteria and Proteobacteria were significantly higher in the Tm-minor soil. These phyla are abundant in soil environments, but low richness in the Tm-dominant soil could have a negative effect on *T. matsutake*, such as the competition for resources or secretion of antibiotics to exclude bacteria [32–34]. We found that Acidobacteria was more abundant in the shiro− soil than in the shiro+ soil of both sites. However, there is a large proportion of the phylum of Proteobacteria in the shiro+ soil at the Bonghwa site compared with the shiro− soil, whereas, at the Yangyang site, the phylum showed a higher abundance in the shiro− soil than in the shiro+ soil. Moreover, the Actinobacteria community showed the highest abundance in the shiro+ soil compared with the shiro− soil at the Yangyang site, whereas there was no difference between the shiro+ and shiro− soils at the Bonghwa site. Kim et al. [13] showed that Actinobacteria have a high abundance beneath the fairy ring, while some studies suggested that this community was negatively correlated with the activity of *T. matsutake* [6,8].

4.2. Distinct Fungal Community Structure

In this study, the total amounts of fungal OTUs richness of the shiro− soils were, respectively, approximately six (Bonghwa site) and two times (Yangyang site) higher than those of shiro+ soils (Table 3). Our results corroborate previous studies that investigated the decrease in fungal populations in the fairy ring zone of *T. matsutake*. The total numbers of OTUs and fungal taxa inside and outside the fairy ring zone were higher than those of the fairy ring zone, and the Tm-dominant soil had low fungal richness [9–11]. There are some observations that the sites of occurrence of *T. matsutake* have low fungal diversity, suggesting that the mycelia of *T. matsutake* form fruiting bodies under little competition with other microorganisms, and/or *T. matsutake* can secrete antifungal compounds to exclude other fungal species, promoting its own fitness by reducing competitors [10–12,35]. In our results of the fungal composition at the phylum level, Basidiomycota showed the greatest proportions in all samples, and it also showed higher relative abundances in the shiro+ soils than in the shiro− soils of both sites, whereas Ascomycota showed high abundance in the shiro− soils compared with the shiro+ soils at both sites. There are two previous studies that are consistent with our results about the dominant class in the fairy ring zone. In one study, Lian et al. [12], who compared fungal communities inside, beneath, and outside the fairy ring zone of *T. matsutake*, also frequently observed Agricomycetes from phylum Basidiomycota beneath the fairy ring zone. In another study, Buée et al. [36] found that Agaricomycetes was the dominant fungal class in forest soil. However, Oh et al. [11] found that Basidiomycota in OTU richness was significantly higher in the Tm-minor soils than in the Tm-dominant soils. They suggested that the reduction in fungal richness in the Tm-dominant soils may be caused by the dominance of *T. matsutake*. The results of the differentiation of the phylum Ascomycota community in all samples were consistent with the previous reports by Kim et al. [10] showing that the classes of Dothideomycetes, Leotiomycetes, and Sordariomycetes from phyla Ascomycota showed higher proportions inside and outside the fairy ring than those in the fairy ring zone. In addition to phylum
Ascomycota, the phylum with the highest abundance was Mucoromycota in all samples. In the phylum Mucoromycota, *Umbelopsis* was the most abundantly detected from the shiro+ soil at the Bonghwa site as well as the shiro− soil at the Yangyang site. In the previous study, *Umbelopsis* was frequently detected from the fruiting body and the fairy ring of *T. matsutake* [28,37], and Oh et al. [11] suggested that *Umbelopsis* may have positive interactions with *T. matsutake*. However, there were some differences between the results of the previous study and those obtained in our study because it was also highly detected in the shiro− soil at the Yangyang site. From our results, it can be assumed that *Umbelopsis* is not necessarily positively correlated with the occurrence of *T. matsutake* in the shiro− present in soils.

In our results of the fungal communities, we separated two main assemblages, the ECM and symbiotic fungal assemblages, and another fungal assemblage. The ECM fungi of genera *Tylospora, Astraeus, Sistotrema, Russula, Sebacina*, and *Tomentella* that were also detected in a coastal pine forest in the eastern region of Korea, were abundantly detected in the shiro− soil at the Bonghwa site [38–41]. In other fungal assemblages, there are a few fungal genera in the form of saprotrophic fungi groups such as *Cladophialophora, penicillium*, unidentified Hyaloscyphaceae, and *Mortierella* [42]. The fungal communities, except for *Tricholoma* and other ECMs, have lower proportions in the shiro+ soils than those in the shiro− soils at the two sites (B_shiro+, 34.00%; B_shiro−, 74.20%; Y_shiro+, 25.42%; Y_shiro−, 61.74%). According to the report by Kujawska et al. [42], the fungal communities in the soil of forests could be divided into ECM, saprotrophic, pathotrophic, and other fungi assemblages from different trophic groups. They suggested that the fungal communities in forest soils were closely related to different trophic groups, and were similar in abundance and diversity. Consequently, we found a distinguishable compositional pattern in ECM, and other fungi from the shiro+ soils and the shiro− soils at the two sites.

4.3. Differences between Microbial Communities

We observed that the similarities of bacterial and fungal communities were relatively low within/across the sampling sites (Table 6). Before we started this study, it was initially expected that the microbial communities between the shiro+ soils of the two sampling sites would be similar to each other. It is well known that *T. matsutake* had inhibitory effects on soil bacteria and fungi, and it could eliminate the competition for their colonization, the growth of hyphae, and the formation of fruiting bodies. Unexpectedly, our results indicated that the similarity in the bacterial communities between the shiro+ soil of Bonghwa and the shiro− soil of Yangyang showed the highest similarity, whereas the lowest similarity was observed between the shiro− soil of Bonghwa and the shiro+ soil, or the shiro− soil of Yangyang. In addition, the fungal community also differed within/across the samples and sites. The fungal communities between the shiro+ soils at the Bonghwa and Yangyang sites have the lowest community similarity among the samples. However, this could be divided into two main fungal assemblages. For one assemblage of ECM except for unknown fungi, the similarity of ECM communities showed the highest value in the shiro− soils at the two sites (0.857). In a previous study by Kujawska et al. [42], the re-assembly of the soil fungal community at the trophic level could be a strong stochastic component to overcome a general reduction in the similarity of the community composition between different regions.

Overall, the simplest explanations for our results showing low similarity between microbial communities within/across the site are as follows. First, in this study, soil samples containing shiro+ and shiro− were only collected once from each site, which was insufficient to compare microbial communities between each site. This was different from sampling many points without favorable permission from farmers. In Korea, a stranger entering a mountain has generally been taboo among villagers of rural communities due to the fear it will result in a bad harvest for *T. matsutake* that year. Second, the annual mean temperature and annual precipitation of the Yangyang site were slightly higher than those of the Bonghwa site. The changes in climate related to temperature and precipita-
tion influence the changes of microbial communities because the climate changes lead to consequences for the changes of plant communities [43,44]. Ectomycorrhizal fungal species, which are host-dependent symbiotic fungi, are also closely related to changes in climate [44,45]. T. matsutake and truffle, which are ectomycorrhizal mushrooms with high ecological and economic values, are the most sensitive to changes in environmental conditions. Yang et al. [46] reported that high temperature and high precipitation in August were correlated with the high productivity of fruiting bodies. Cejka et al. [44] demonstrated that precipitation is the most important factor for truffle production with drought events reducing truffle yields. We assumed that if climate changes caused changes in vegetation and microbial communities, and changes in vegetation and microbial communities are directly or indirectly affected by the formation of fruiting bodies of T. matsutake, the microbial community may also differ even if there are the same shiro+ soils in different regions. Third, an important environmental factor—soil properties—influence the microbial community. It is well known that the soil environment adjacent to T. matsutake had a lower organic content, and a higher CEC content [10,47], but there is still a large amount of controversy about whether T. matsutake prefers soils containing a low organic matter content or an organic soil content changed by T. matsutake [10]. Although the relationship between the formation of fruiting bodies of T. matsutake and soil properties is still unknown, the impact on microbial communities, diversity, and relative abundance is positively correlated with soil properties [48–50]. Moreover, the report by Kujawska et al. [42] showed that fungal communities differed from trophic groups, soil pH significantly influenced ectomycorrhizal fungal communities, and the volume of coarse woody debris and soil nitrate concentration influenced the saprotrophic fungi community [42]. However, the climate factors and soil properties of the two sites were not measured in this study. Fourth, this study investigated overall bacterial and fungal communities in the production (shiro+) and nonproduction (shiro−) soils of T. matsutake using the Illumina sequencing method. This technology has the advantage of acquiring high-throughput data more quickly for the assessment of microbial communities although there was a limitation in that it produced short read lengths of sequence [10,11,13]. To achieve the accurate assessment of microbial communities, it may be necessary to combine the massive throughput of the next-generation sequencer with the long read lengths by electrophoresis-based methods in Sanger sequencing [10,51]. Nevertheless, it is clear that our results may provide important information to contribute toward an expanding foundation for knowledge on the microbial community in habitats of T. matsutake. Based on the results of this study, further studies of both the microbial communities and abiotic factors in various sites and regions are needed. Furthermore, we expect that it will be the cornerstone for identifying differences in microbial community structure among T. matsutake production soils within/ across sites.

5. Conclusions

We compared bacterial and fungal communities in T. matsutake production (shiro+) and nonproduction (shiro−) soils in two different regions using the Illumina MiSeq sequencing platform. The shiro+ soils showed less OTUs and lower bacterial and fungal diversity than the shiro− soils. The similarity within the microbial communities of shiro+ samples was not significant. However, the similarity of fungal communities was affected by their trophic assembly. This suggested that abiotic and biotic factors are important factors that can determine not only the richness of the microbial community but also the quality of the microbial community structure. Further studies are needed to incorporate more diverse samples collected from multiple sites in different seasons. In addition, abiotic factors, such as soil characters, temperature, and humidity should also be synthetically considered. Therefore, the similarity between microbial communities may be due to the fact that the microbial communities in the T. matsutake-dominant soils are closely associated with abiotic factors and biotic factors. Our study may contribute to future studies where the number of study sites is sufficient for understanding the traits of microbial community structures in T. matsutake production soils.
Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/.../TableS1: List of fungal species inhibiting the Tricholoma matsutake production (shiro+) and nonproduction (shiro−) soils of Bonghwa and Yangyang sampling sites. The fungal species present more than 1% of at least one sample among the four soil samples, Table S2: The results of Sorensen classic similarity index of fungal communities separating ECM and other fungi assemblages within/ across sampling sites.

Author Contributions: Conceptualization, G.-H.A., J.-G.H. and J.-H.C.; methodology, G.-H.A., J.-G.H. and O.-T.K.; software, G.-H.A.; validation, G.-H.A., J.-G.H. and J.-H.C.; formal analysis, G.-H.A. and J.-G.H.; investigation, G.-H.A., J.-G.H. and J.-H.C.; resources, G.-H.A.; data curation, G.-H.A.; writing—original draft preparation, G.-H.A. and, J.-G.H.; writing—review and editing, J.-G.H. and O.-T.K.; visualization, G.-H.A. and J.-G.H.; supervision, O.-T.K.; project administration, J.-G.H. and J.-H.C.; funding acquisition, J.-G.H. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Rural Development Administration, Republic of Korea (grant number PJ01466012021).

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors are grateful for the support provided by the National Institute of Horticultural and Herbal Science, Rural Development Administration, Republic of Korea. We also express our sincere gratitude to the officials of Yangyang-gun Agricultural Technology Center and Jae-Mo Sung for their support of our sampling site surveys.

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

References

1. Ogawa, M. The Biology of Matsutake Mushroom; Tshkiji Shokkan: Tokyo, Japan, 1978; p. 326.
2. Yamada, A.; Maeda, K.; Ohmusa, M. Ectomycorrhiza formation of Tricholoma matsutake isolates on seedlings of Pinus densiflora in vitro. Mycosenes 1999, 40, 455–463. [CrossRef]
3. Kawai, M.; Terada, O. Artificial reproduction of Tricholoma matsutake (S. Ito et Imai) Sing. II. Effects of vitamins, nucleic acid-related substances, phytohormones and metal ions in the media on the vegetative growth of T. matsutake. Trans. Mycol. Soc. Jpn. 1976, 17, 168–174.
4. Ogawa, M.; Umehara, T.; Kontani, S.; Yamaji, K. Cultivating method of the mycorrhizal fungus, Tricholoma matsutake (Ito et Imai) Sing. (1) Growing method of pine saplings infected with T. matsutake in the field. J. Jpn. For. Soc. 1978, 60, 119–128. [CrossRef]
5. Kawagishi, H.; Hamajima, K.; Takanami, R.; Nakamura, T.; Sato, Y.; Akiyama, Y.; Akiyama, Y.; Sano, M.; Tanaka, O. Growth promotion of mycelia of the matsutake mushroom Tricholoma matsutake by D-isoleucine. Biosci. Biotechnol. Biochem. 2004, 68, 2405–2407. [CrossRef]
6. Vaario, L.M.; Fritze, H.; Spetz, P.; Heinonsalo, J.; Hanajik, P.; Pennanen, T. Tricholoma matsutake dominates diverse microbial communities in different forest soils. Appl. Environ. Microbiol. 2011, 77, 8523–8531. [CrossRef] [PubMed]
7. Smith, S.E.; Read, D.J. Mycorrhizal Symbiosis, 2nd ed.; Academic Press: London, UK, 1997.
8. Ohara, H.; Hamada, M. Disappearance of bacteria from the zone of active mycorrhizas in Tricholoma matsutake (S. Ito et Imai) Singer. Nature 1967, 213, 528–529. [CrossRef]
9. Song, H.; Min, K. Microfungal flora of Tricholoma matsutake producing and nonproducing sites in the forest of Pinus densiflora. Korean J. Mycol. 1991, 19, 109–119.
10. Kim, M.; Yoon, H.J.; You, Y.H.; Kim, Y.E.; Woo, J.R.; Seo, Y.G.; Lee, G.M.; Kim, Y.J.; Kong, W.S.; Kim, J.G. Metagenomic analysis of fungal communities inhabiting the fairy ring zone of Tricholoma matsutake. J. Microbiol. Biotechnol. 2013, 23, 1347–1356. [CrossRef]
11. Oh, S.Y.; Fong, J.J.; Park, M.S.; Lim, Y.W. Distinctive feature of microbial communities and bacterial functional profiles in Tricholoma matsutake dominant soil. PLoS ONE. 2016, 11, e0168573. [CrossRef] [PubMed]
12. Lian, C.; Narimatsu, M.; Nara, K.; Hogetsu, T. Tricholoma matsutake in a natural Pinus densiflora forest: Correspondence between above- and below-ground genets, association with multiple host trees and alteration of existing ectomycorrhizal communities. New Phytol. 2006, 171, 825–836. [CrossRef]
13. Kim, M.; Yoon, H.; Kim, Y.E.; Kim, Y.J.; Kong, W.S.; Kim, J.G. Comparative analysis of bacterial diversity and communities inhabiting the fairy ring of Tricholoma matsutake by barcoded pyrosequencing. J. Appl. Microbiol. 2014, 117, 699–710. [CrossRef]
14. Frey-Klett, P.; Garbaye, J.; Tarkka, M. The mycorrhiza helper bacteria revisited. New Phytol. 2007, 176, 22–36. [CrossRef] [PubMed]
15. Linderman, R.G. Mycorrhizal interactions with the rhizosphere microflora: The mycorrhizosphere effect. Phytopathology 1988, 78, 366–371.
16. Garbaye, J. Helper bacteria: A new dimension to the mycorrhizal symbiosis. New Phytol. 1994, 128, 197–210. [CrossRef]
17. Vivas, A.; Azon, R.; Biro, B.; Barea, J.M.; Ruiz-Lozano, J.M. Influence of bacterial strains isolated from lead-polluted soil and their interactions with arbuscular mycorrhizae on the growth of *Trifolium pretense* L. under lead toxicity. *Can. J. Microbiol.* **2003**, *49*, 577–588. [CrossRef] [PubMed]

18. Kim, C.S.; Nam, J.W.; Jo, J.W.; Kim, S.Y.; Han, J.G.; Hyun, M.W.; Sung, G.H.; Han, S.K. Studies on seasonal dynamics of soil-higher fungal communities in Mongolian oak-dominant Gwangneung forest in Korea. *J. Microbiol.* **2016**, *54*, 14–22. [CrossRef]

19. Oberauner, L.; Zachow, C.; Lackner, S.; Hogenauer, C.; Smolle, K.H.; Berg, G. The ignored diversity: Complex bacterial communities in intensive care units revealed by 16S pyrosequencing. *Sci. Rep.* **2013**, *3*, 1413. [CrossRef]

20. Uroz, S.; Ioannidis, P.; Lengelle, J.; Cebron, A.; Morin, E.; Buee, M.; Martin, F. Functional assay and metagenomics analyses reveals differences between the microbial communities inhabiting the soil horizons of a Norway spruce plantation. *PLoS ONE* **2013**, *8*, e55925. [CrossRef] [PubMed]

21. Kozich, J.J.; Westcott, S.L.; Baxter, N.T.; Highlander, S.K.; Schloss, P.D. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the Miseq Illumina sequencing platform. *Appl. Environ. Microbiol.* **2013**, *79*, 5112–5120. [CrossRef]

22. Li, W.; Fu, L.; Niu, B.; Wu, S.; Wooley, J. Ultrafast clustering algorithms for metagenomics sequence analysis. *Brief. Bioinform.* **2012**, *13*, 656–668. [CrossRef]

23. Cole, J.R.; Wang, Q.; Fish, J.A.; Chai, B.; McGarrell, D.M.; Sun, Y.; Brown, C.T.; Porras-Alfaro, A.; Kuske, C.R.; Tiedje, J.M. Ribosomal Database Project: Data and tools for high throughput rRNA analysis. *Nucleic Acids Res.* **2014**, *42*, 633–642. [CrossRef]

24. Nilsson, R.H.; Larsson, K.H.; Taylor, A.F.S.; Bengtsson-Palme, J.; Jeppesen, T.S.; Schige, L.D.; Kennedy, P.; Picard, K.; Glöckner, F.O.; Tedersoo, L.; et al. The UNITE database for molecular identification of fungi: Handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Res.* **2018**, *46*.[CrossRef] [PubMed]

25. Caporaso, J.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; Fierer, N.; Pena, A.G.; Goodrich, J.K.; Gordon, J.I.; et al. QIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **2010**, *7*, 335–336. [CrossRef]

26. Chao, A.; Chazdon, R.L.; Colwell, R.K.; Shen, T.J. A new statistical approach for assessing compositional similarity based on incidence and abundance data. *Ecol. Lett.* **2005**, *8*, 148–159. [CrossRef]

27. Hosford, D.; Pliz, D.; Molina, R.; Amaranthus, M. *Ecology and Management of the Commercially Harvested American Matsutake*; General Technical Report PNW-GTR-412; USDA, Forest Service, Pacific Northwest Research Station: Portland, OR, USA, 1997; p. 66.

28. Oh, S.Y.; Park, M.S.; Cho, H.J.; Lim, Y.W. Diversity and effect of Trichoderma isolated from the roots of *Pinus densiflora* within the fairy ring of pine mushroom (*Tricholoma matsutake*). *PLoS ONE* **2015**, *10*, e0205900. [CrossRef] [PubMed]

29. Kataoka, K.; Shiro, M.; Okura, R.; Oizumi, K.; Fujita, T.; Sasamori, T.; Tokitoh, N.; Yamada, A.; Tanaka, C.; Yamaguchi, M.; et al. The (oxalate) aluminate complex as an antimicrobial substance protecting the “Shiro” of eastern coastal pine forests of Korea. *Mycorrhiza* **2009**, *20*, 39–49. [CrossRef] [PubMed]

30. Hur, T.C.; Park, H. Dynamics of soil microflora and soil enzymes around the fairy-rings of *Tricholoma matsutake*. *J. Korean For. Soc.* **2004**, *90*, 767–773.

31. Hur, T.C.; Park, H.; Joo, S.H.; Ka, K.H. Dynamic changes of soil physicochemical properties in the fairy-rings of *Tricholoma matsutake*. *J. Korean For. Soc.* **2004**, *93*, 36–44. [CrossRef]

32. Yun, W.; Hall, I.R.; Evans, L.A. Ectomycorrhizal fungi with edible fruiting bodies. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 102–111. [CrossRef]

33. Janssen, P.H. Identifying the dominant soil bacterial taxa in libraries of 16S rDNA and 16S rRNA genes. *Appl. Environ. Microbiol.* **2006**, *72*, 1719–1728. [CrossRef]

34. Fierer, N.; Leff, J.W.; Adams, B.J.; Nielsen, U.N.; Bates, S.T.; Lauber, C.L.; Owens, S.; Gilbert, J.A.; Wall, D.H.; Caporaso, G. Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 21390–21395. [CrossRef]

35. Takakura, Y. *Tricholoma matsutake* fruit bodies secrete hydrogen peroxide as a potent inhibitor of fungal growth. *Can. J. Microbiol.* **2015**, *61*, 1–4. [CrossRef] [PubMed]

36. Buée, M.; Reich, M.; Murat, C.; Morin, E.; Nilsson, R.H.; Uroz, S.; Martin, F. 454 pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. *New Phytol.* **2009**, *184*, 449–456. [CrossRef] [PubMed]

37. Tominaga, Y. Studies on the life history of Japanese pine mushroom, *Armillaria matsutake* Ito et Imai. *Bull. Hiroshima Agric. Coll.* **1963**, *2*, 105–145.

38. Marino, E.D.; Scattolin, L.; Bodenstein, P.; Agerer, R. *Sistotrema* is a genus with ectomycorrhizal species confirmation of what sequence studies already suggested. *Mycol. Prog.* **2008**, *7*, 169–176. [CrossRef]

39. Wang, P.; Zhang, Y.; Mi, F.; Tang, X.; He, X.; Cao, Y.; Liu, C.; Yang, D.; Dong, J.; Zhang, K.; et al. Recent advances in population genetics of ectomycorrhizal mushrooms *Russula*. *Mycology* **2015**, *6*, 110–120. [CrossRef] [PubMed]

40. Obase, K.; Cha, J.Y.; Lee, J.K.; Lee, S.Y.; Lee, J.H.; Chun, K.W. Ectomycorrhizal fungal communities associated with Pinus thunbergii in the eastern coastal pine forests of Korea. *Myco Ara* **2009**, *20*, 39–49. [CrossRef] [PubMed]
42. Kujawska, M.B.; Rudawska, M.; Wilgan, R.; Leski, T. Similarities and differences among soil fungal assemblages in managed forests and formerly managed forest reserves. *Forests* 2021, 12, 353. [CrossRef]
43. Waldrop, M.P.; Firestone, M.K. Response of microbial community composition and function to soil climate change. *Microb. Ecol.* 2005, 52, 716–724. [CrossRef]
44. Guo, Y.; Li, X.; Zhao, Z.; Wei, H.; Gao, B.; Gu, W. Prediction of the potential geographic distribution of the ectomycorrhizal mushroom *Tricholoma matsutake* under multiple climate change scenarios. *Sci. Rep.* 2017, 7, 46221. [CrossRef]
45. Čejka, T.; Trnka, M.; Krusic, P.J.; Stobbe, U.; Oliach, D.; Václavík, T.; Tegel, W.; Büntgen, U. Predicted climate change will increase the truffle cultivation potential in central Europe. *Sci. Rep.* 2020, 10, 21281. [CrossRef] [PubMed]
46. Yang, X.; Luedeling, E.; Chen, G.; Hyde, K.D.; Yang, Y.; Zhou, D.; Xu, J.; Yang, Y. Climate change effects fruiting of the prize matsutake mushroom in China. *Fungal Divers.* 2012, 56, 189–198. [CrossRef]
47. Stucki, J.W.; Gan, H.; Wilkinson, H.T. Effects of microorganisms on phyllosilicate properties and behavior. In *Interacting Processes in Soil Science*; Wagenet, R.J., Baveye, P., Stewart, B.A., Eds.; Advances in Soil Science; CRC Press: Boca Raton, FL, USA, 1992; pp. 227–251.
48. Jones, R.T.; Robeson, M.S.; Lauber, C.L.; Hamady, M.; Knight, R.; Fierer, N. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. *ISME J.* 2009, 3, 442–453. [CrossRef] [PubMed]
49. Lauber, C.L.; Hamady, M.; Knight, R.; Fierer, N. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl. Environ. Microbiol.* 2009, 75, 5111–5120. [CrossRef] [PubMed]
50. Rousk, J.; Baath, E.; Brookes, P.C.; Lauber, C.L.; Lozupone, C.; Capraoaso, 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]
51. Hert, D.G.; Fredlake, C.P.; Barron, A.E. Advantages and limitations of next-generation sequencing technologies: A comparison of electrophoresis and non-electrophoresis methods. *Electrophoresis* 2008, 29, 4618–4626. [CrossRef]