# Leaf-Associated Shifts in Bacterial and Fungal Communities in Response to Chicken Rearing Under Moso Bamboo Forests in Subtropical China

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**Abstract:** Integrated bamboo-chicken farming (BCF) systems are a traditional agroforestry pattern with large economic benefits in subtropical China. However, little is known regarding the effect of this integration on the bamboo leaf-associated microbiome, which can be very important for disease control and nutrient turnover. In the present study, we compared the leaf-associated bacterial and fungal communities of moso bamboo (*Phyllostachys edulis*) in a BCF system and an adjacent moso bamboo forest (MBF). The results showed that Cyanobacteria and Ascomycota were the predominant microbial phyla associated with bamboo leaves. Chicken farming under the bamboo forest significantly increased the bacterial and fungal alpha diversity (observed operational taxonomic units (OTUs) and Simpson's index) associated with bamboo leaves. Principal components analysis (PCoA) further confirmed the shifts in the bacterial and fungal communities caused by chicken farming. Based on the observed relative abundances, the phyla Bacteroidetes, Actinobacteria, TM7, and Basidiomycota were significantly increased on BCF-associated leaves compared with MBF leaves, while Acidobacteria and Ascomycota were significantly decreased. An ecological function prediction analysis based on metabolic processes indicated that BCF could accelerate nutrient (C, N, and S) cycling but may increase the risk of fungal-associated diseases. Our findings suggest that shifts in leaf-associated bacterial and fungal communities can be important indicators for the scientific management of BCF systems.

**Keywords:** bamboo-chicken system; bacterial community; fungal community; leaf-associated microbiome; ecological niche shift

## 1. Introduction

Bamboo forests are an important type of forest in tropical and subtropical areas, covering a total area of 31.5 million ha in 2010 and accounting for approximately 0.8% of the world's total forest area [1]. Moso bamboo (*Phyllostachys edulis* (Carrière) J. Houzeau, synonym *Phyllostachys heterocycla* (Carrière) is currently the most important source of woody bamboo [2]. The shoot of moso bamboo can grow from 0–20 m in 45–60 days under suitable spring conditions [3]. Moso bamboo plays an important role in ecological and environmental protection [4,5], as well as in rural economic development in China [6,7]. Bamboo belongs to the subfamily Bambusoideae in the family Gramineae, which includes approximately 1500 species from approximately 90 genera worldwide [1]. China has more than 6 million ha of bamboo forest, 70% of which are moso bamboo forests [8]. However, because

of increasing labor operational costs in China that have occurred in the last decade, the traditional management of bamboo forests has lost a great deal of economic attraction. Therefore, how to rationally utilize vast bamboo resources has become an important issue for scientific study.

Agroforestry systems are land-use systems and technologies where woody perennials are deliberately used on the same land management units as agricultural crops and/or animals [9]. Because bamboo forests are characterized by their short rotation and strong regeneration ability, they can typically be used to establish agroforestry systems as a potential strategy for providing food and nutritional security and for contributing to the economic development of developing countries in the tropics [10]. The use of an agroforestry pattern consisting of moso bamboo and chicken farming has been reported in China [11,12]. The results of a previous study showed that chicken farming in a bamboo forest increased soil C, total N, total P, total K, and the soil water-holding capacity [12]. However, because of the continuous input of chicken manure on the soil surface, there was a high risk of surface erosion and manure seepage [12], which may cause water pollution. Therefore, chicken rearing density is the most crucial aspect of this agroforestry practice [11]. There is also increasing concern that the introduction of chickens into a bamboo forest ecosystem may cause niche displacement of native microbiomes [12], which will further influence their functionality.

The surfaces and inner tissues of all land plants are colonized by diverse microorganisms, including bacteria, fungi, oomycetes, viruses, archaea, and protists [13,14]. Lorenz Hiltner defined the term "rhizosphere" in 1904 [15], and plant-associated microorganisms with root systems have received substantial attention in recent years [13,14,16]. Moso bamboo-associated bacteria and fungi have been isolated using culture-dependent methods [17,18] and high-throughput sequencing [19], but the latter study was primarily focused on the bamboo rhizome and pole-bacterial communities. The leaves of plants are ubiquitous global habitats that are inhabited by a diverse community of microorganisms [20,21]. These microbial communities have the potential to influence plant biogeography and ecosystem function through their influence on the fitness and function of their hosts and on soil nutrient turnover [22]. Additionally, the most important fungal and bacterial bamboo diseases are foliar, such as leaf spot disease on *Bambusa polymorpha* Munro., *Dendrocalamus longispathus* (Kurz) Kurz, and *Dendrocalamus strictus* (Roxb.) Nees [23]. However, the shifts in the leaf-associated microbiota (epiphytes and endophytes) in a bamboo-chicken farming (BCF) ecosystem have not been explored.

In this study, we comparatively investigated the leaf-associated bacterial and fungal communities of moso bamboo with and without chicken farming. The aim of this study was to determine how the leaf-associated microbiota of moso bamboo responds to the introduction of chickens into a bamboo forest and the functional profiles associated with these shifts in the microbiota.

## 2. Materials and Methods

### 2.1. Experimental Site and Design

The study area (30°38′14.92″ N, 119°21′1.05″ E) was located in the town of Hanggai, western Anji County, Zhejiang Province. The area has a mid-latitudinal subtropical monsoon climate, with an average annual precipitation of approximately 1854 mm, a mean annual temperature of approximately 17 °C, and an altitude of 400–450 m above sea level. The average annual sunshine duration in this region is 1946 h, and the frost-free period is 230 days. In addition, the soils in the region are classified as Ferric Luvisols, derived from silty sand and fine sand-mixed rock [24]. The investigated BCF system was established in 2006. To maintain a rational bamboo density, a selective harvest practice was adopted into this system. The stand density was approximately 2100 stems ha$^{-1}$, with an average plant height and diameter at breast of 9.0 m and 8.9 cm, respectively. A previous study showed that chicken farming resulted in significant increases in soil organic C and the total N, P, and K contents [12]. Therefore, the chicken rearing density was maintained within 2500–3000 ha$^{-1}$ to avoid the risk of over-application of manure. The adjacent bamboo forest was selected as a control, because it had very

similar site conditions with respect to soil type, position of slope, slope gradient, and other aspects that were the same as in the BCF system. The adjacent moso bamboo forest (MBF) without chicken farming was the control group. The MBF was selectively harvested to maintain a specific density. The growth status of the MBF forest was very close to that of the BCF system, namely, the stand density was approximately 2300 stems ha$^{-1}$ and the average plant height and diameter at breast were 8.6 m and 8.5 cm, respectively.

## 2.2. Sampling

Leaf samples were collected in late September 2018. The paired-site sampling method [25] was adopted for sampling, namely, three sites along the border between BCF and MBF forests were chosen. The distance between each site was greater than 100 m. Each site included two sampling subplots (10 m × 10 m) belonging to BCF and MBF, respectively. Originally, both forests (BCF and MBF) in each site had very similar soil conditions, slope positions, and management systems. One bamboo plant with an average size was chosen in each subplot for sampling. The healthy leaves were randomly collected from the fifth to the seventh branches (from the bottom up) from both the shaded and unshaded parts of the plant. Approximately 10 g of leaves were pooled as a composite sample, these were immediately frozen in liquid nitrogen and stored at −80 °C for further analysis.

## 2.3. Illumina High-Throughput Sequencing

DNA was extracted from the leaf samples using a Magnetic Soil and Stool DNA kit (Tiangen, Beijing, China) according to the manufacturer's instructions. The bacterial 16S rRNA and fungal ITS2 genes were amplified using the primer pairs 338F (5′-ACTCCTACGGGAGGCAGCAG-3′)/806R (5′-GGACTACHVGGGTWTCTAAT-3′) and ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′)/ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′), respectively. PCR amplification and MiSeq sequencing were performed by Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China). Raw sequences have been deposited in NCBI under Bioproject PRJNA514420 and PRJNA514422.

## 2.4. Data Analyses

Paired reads were assembled using FLASH [26], and the assembled sequences from each sample were combined into one file using the add_qiime_labels.py script in QIIME [27]. The chimeric 16S and ITS sequences were removed with Vsearch v2.8.0 [28] using the RDP "Gold" database (https://drive5.com/uchime/gold.fa) and the UNITE/INSD ITS2-only UCHIME reference dataset v. 7.2 [29] as references, respectively. Next, the nonchimeric sequences with 97% similarity were clustered into operational taxonomic units (OTUs) using the open-reference OTU picking workflow pipelines in QIIME [27]. The representative OTU sequences were assigned using the RDP classifier to identify bacterial taxa against the Greengenes reference database released in August 2013 [30], while the representative sequences of fungi were identified using the UNITE database [31].
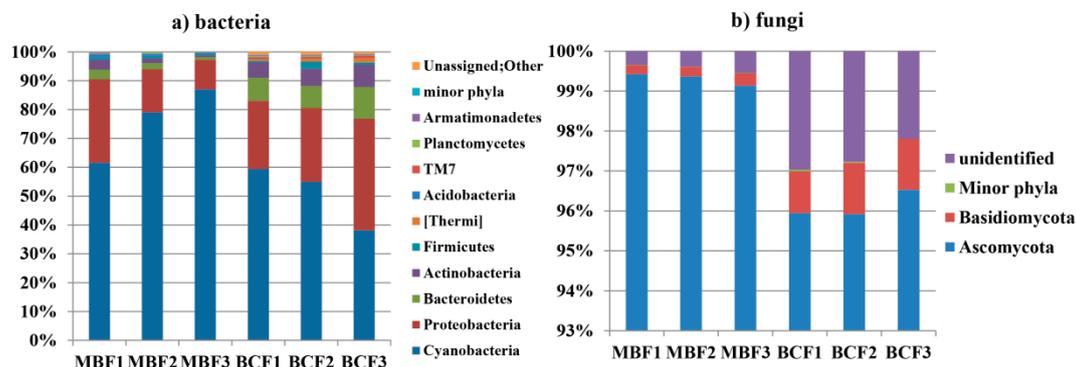
The bacterial and fungal OTU tables were rarefied to the lowest number of sequences using QIIME [27]. Alpha diversity indices (observed OTUs, Shannon, and Simpson's index) were calculated using the alpha_rarefaction.py workflow in QIIME. Principal coordinates analysis (PCoA) based on the Bray–Curtis distance was conducted and visualized in R using the phyloseq package [32] and ggplot2 [33]. The Sorenson Similarity Index (Cs) was used to measure the similarity between MBF and BCF based on the presence and absence of OTUs. The index was calculated as: Cs=2C/(A + B), where A and B are the number of OTUs in MBF and BCF, respectively, and C is the number of OTUs shared by the two groups. METAGENassist [34] and FUNGuild [35] were used to assess putative functional profiles based on the bacterial and fungal community composition of the leaf samples from moso bamboo. The differences in microbial composition and alpha diversity were analysed using an independent samples t-test using IBM-SPSS (version 22.0; Chicago IL, USA).

## 3. Results

### 3.1. Compositions of Bacterial and Fungal Communities

After filtering out the low-quality reads and chimeric sequences, 328,471 and 377,590 bacterial and fungal sequences were obtained from the moso bamboo leaf samples of both MBF and BCF, which clustered into 2056 and 798 OTUs, respectively, at the 97% similarity cut-off level.

The most abundant bacterial phylum in the moso bamboo leaves was Cyanobacteria, accounting for an average of 63.38% of the total sequences, followed by Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutes, Thermi, Acidobacteria, TM7, Planctomycetes, and Armatimonadetes, which accounted for 23.65, 5.53, 4.13, 0.93, 0.51, 0.45, 0.40, 0.25, and 0.19% of the total sequences, respectively (Figure 1a). In contrast, only two fungal phyla with an average relative abundance of >0.1% were detected in the bamboo leaves, with the dominant phylum being Ascomycota, accounting for an average of 97.72% of the total sequences (Figure 1b), followed by Basidiomycota, which accounted for 0.72% of the total sequences. At the genus level, fifteen bacterial genera with an average relative abundance >0.1% were detected (Table 1), i.e., *Methylobacterium* (7.73%), *Hymenobacter* (4.74%), *Sphingomonas* (4.70%), *Deinococcus* (0.51%), *Kineococcus* (0.48%), *Spirosoma* (0.41%), *Beijerinckia* (0.35%), *Curtobacterium* (0.19%), *Friedmanniella* (0.17%), *Microbacterium* (0.16%) and *Ralstonia* (0.16%). Based on the classifiable sequences, the fungal reads were primarily assigned to fourteen genera with an average relative abundance of >0.1%, and these genera were ranked in order of relative abundance as follows (Table 1): *Alatosessilispora* (12.66%), *Strelitziana* (12.10%), *Shiraia* (1.75%), *Cladosporium* (1.01%), *Camptophora* (0.75%), *Geastrumia* (0.39%), *Mycosphaerella* (0.36%), *Ramularia* (0.35%), *Bacidina* (0.35%), *Trichomerium* (0.27%), *Hortaea* (0.20%), *Hygrocybe* (0.14%), and *Arthrinium* (0.14%).
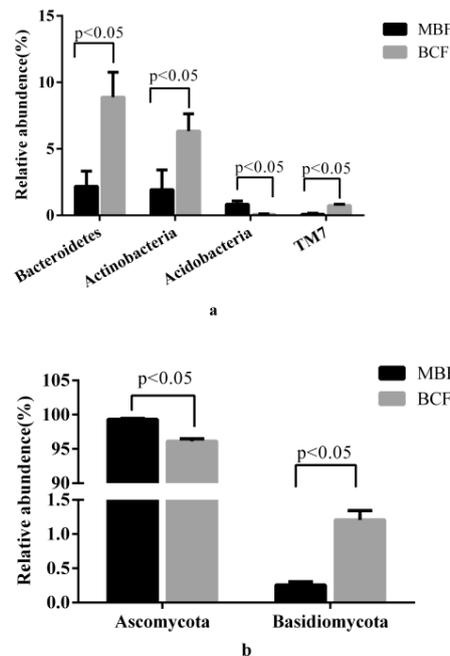


**Figure 1.** Bacterial and fungal compositions at the phylum level in the moso bamboo leaves. (**a**) Bacterial phyla with an average relative abundance of greater than 0.1%; (**b**) Fungal phyla relative abundance. MBF, adjacent moso bamboo forest; BCF, moso bamboo-chicken farming agroforestry.

At the phylum level, the abundances of the bacterial phyla Bacteroidetes, Actinobacteria, and TM7 were significantly increased ($p < 0.05$) and that of Acidobacteria was significantly decreased ($p < 0.05$) in the BCF samples compared with those of the MBF (Figure 2a). The bacterial genera *Methylobacterium*, *Hymenobacter*, *Kineococcus*, *Spirosoma*, *Friedmanniella*, *Bdellovibrio*, and *Arthrobacter* were significantly increased in the BCF samples ($p < 0.05$), while that of *Terriglobus* was significantly decreased ($p < 0.05$) relative to the MBF samples (Table 1). For the fungal communities (Figure 2b, Table 1), the relative abundances of the taxa Ascomycota, *Shiraia*, *Geastrumia*, *Mycosphaerella*, *Bacidina*, and *Hortaea* were significantly lower ($p < 0.05$) in the BCF samples than in the MBF samples, while the relative abundances of the taxa Basidiomycota, *Alatosessilispora*, *Strelitziana*, *Cladosporium*, *Camptophora*, *Ramularia*, *Trichomerium*, and *Arthrinium* in the BCF samples were significantly increased ($p < 0.05$) compared to those observed in the MBF samples.

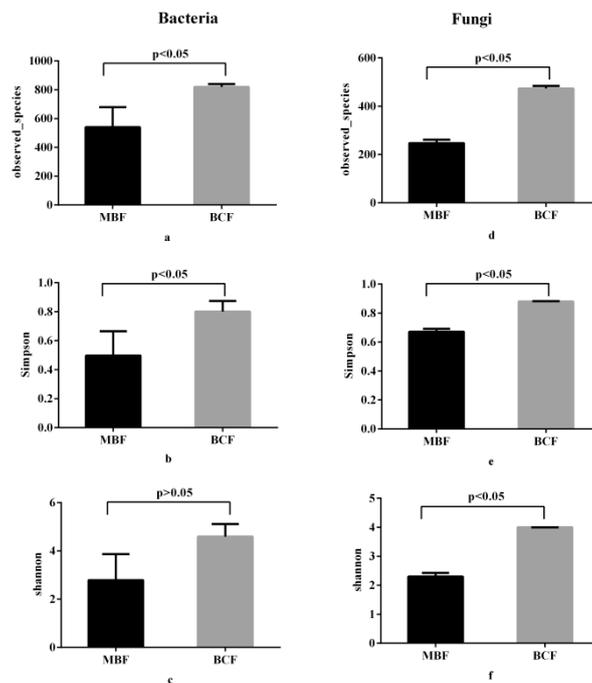**Table 1.** Relative abundances of the dominant bacterial and fungal genera in moso bamboo leaves.

| | Phylum | Genus | MBF | BCF | *p* Value |
|---|---|---|---|---|---|
| Bacteria | Proteobacteria | *Methylobacterium* | 2.05% ± 1.46% | 13.40% ± 3.96% | 0.010 |
| | Bacteroidetes | *Hymenobacter* | 1.84% ± 0.88% | 7.64% ± 2.14% | 0.012 |
| | Proteobacteria | *Sphingomonas* | 3.57% ± 2.41% | 5.84% ± 2.20% | 0.297 |
| | [Thermi] | *Deinococcus* | 0.00% ± 0.01% | 1.02% ± 0.42% | 0.052 |
| | Actinobacteria | *Kineococcus* | 0.08% ± 0.05% | 0.88% ± 0.12% | 0.003 |
| | Bacteroidetes | *Spirosoma* | 0.02% ± 0.01% | 0.79% ± 0.23% | 0.027 |
| | Proteobacteria | *Beijerinckia* | 0.58% ± 0.46% | 0.11% ± 0.07% | 0.158 |
| | Actinobacteria | *Curtobacterium* | 0.11% ± 0.09% | 0.26% ± 0.15% | 0.203 |
| | Actinobacteria | *Friedmanniella* | 0.00% ± 0.00% | 0.33% ± 0.06% | 0.001 |
| | Actinobacteria | *Microbacterium* | 0.22% ± 0.22% | 0.10% ± 0.04% | 0.446 |
| | Proteobacteria | *Ralstonia* | 0.18% ± 0.20% | 0.14% ± 0.11% | 0.790 |
| | Proteobacteria | *Burkholderia* | 0.25% ± 0.20% | 0.03% ± 0.01% | 0.197 |
| | Proteobacteria | *Bdellovibrio* | 0.08% ± 0.05% | 0.17% ± 0.03% | 0.039 |
| | Acidobacteria | *Terriglobus* | 0.20% ± 0.07% | 0.04% ± 0.02% | 0.019 |
| | Actinobacteria | *Arthrobacter* | 0.01% ± 0.01% | 0.22% ± 0.08% | 0.012 |
| Fungi | Ascomycota | *Alatosessilispora* | 0.02% ± 0.02% | 25.30% ± 2.14% | 0.002 |
| | Ascomycota | *Strelitziana* | 0.03% ± 0.02% | 24.17% ± 0.84% | 0.000 |
| | Ascomycota | *Shiraia* | 3.11% ± 0.69% | 0.40% ± 0.10% | 0.003 |
| | Ascomycota | *Cladosporium* | 0.11% ± 0.05% | 1.91% ± 0.35% | 0.001 |
| | Ascomycota | *Camptophora* | 0.05% ± 0.01% | 1.46% ± 0.13% | 0.000 |
| | Ascomycota | *Geastrumia* | 0.76% ± 0.30% | 0.02% ± 0.02% | 0.013 |
| | Ascomycota | *Mycosphaerella* | 0.54% ± 0.04% | 0.18% ± 0.03% | 0.000 |
| | Ascomycota | *Ramularia* | 0.01% ± 0.01% | 0.70% ± 0.15% | 0.001 |
| | Ascomycota | *Bacidina* | 0.69% ± 0.18% | 0.01% ± 0.01% | 0.003 |
| | Ascomycota | *Trichomerium* | 0.00% ± 0.00% | 0.53% ± 0.05% | 0.003 |
| | Ascomycota | *Hortaea* | 0.40% ± 0.09% | 0.00% ± 0.00% | 0.015 |
| | Basidiomycota | *Hygrocybe* | 0.00% ± 0.00% | 0.29% ± 0.19% | 0.118 |
| | Ascomycota | *Arthrinium* | 0.07% ± 0.03% | 0.20% ± 0.07% | 0.043 |
| | Ascomycota | *Didymella* | 0.06% ± 0.01% | 0.14% ± 0.02% | 0.006 |



**Figure 2.** Differences in the dominant phyla of the moso bamboo leaf-associated microbiome in the BCF and MBF samples. (**a**) Bacteria; (**b**) fungi.
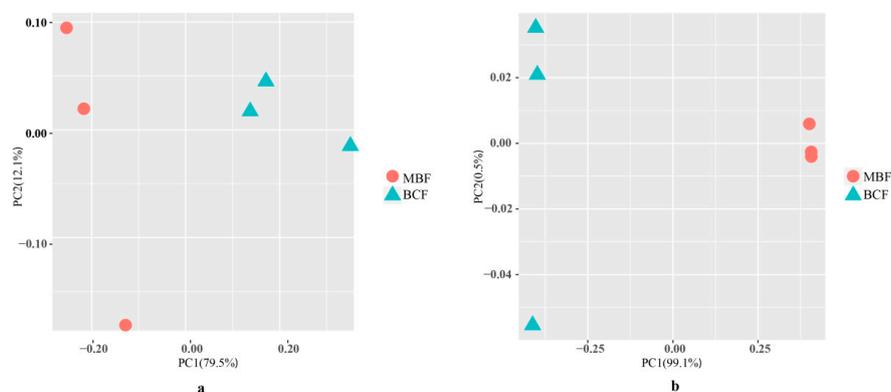
## 3.2. Bacterial and Fungal Community Diversities

The observed OTUs and Simpson's indices were calculated to estimate the alpha-diversity characteristics of each sample. As shown in Figure 3, compared with the MBF, the BCF treatment significantly increased the number of observed OTUs and the Simpson's indices of the bacterial and fungal communities associated with bamboo leaves. For the fungal communities, the Shannon index was significantly higher in BCF than that in MBF ($p < 0.05$), while no difference ($p > 0.05$) was observed between MBF and BCF within the bacterial communities.



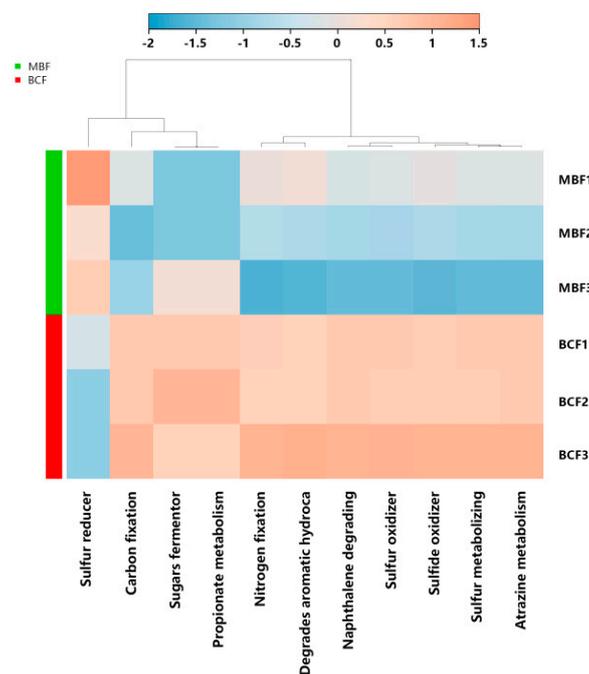**Figure 3.** Alpha diversity indices of bacterial and fungal taxa for the BCF and MBF samples from moso bamboo.

The PCoA based on Bray–Curtis distances revealed that the first principal component (PCoA 1) explained 79.50% and 99.10% of the variability in the bacterial and fungal community, respectively (Figure 4a,b). Two different clusters were formed for the MBF and BCF samples with respect to the bacterial (Sorenson index = 0.51) and fungal (Sorenson index = 0.55) community compositions, indicating that chicken farming in the bamboo forest caused bacterial and fungal community shifts associated with the bamboo leaves.
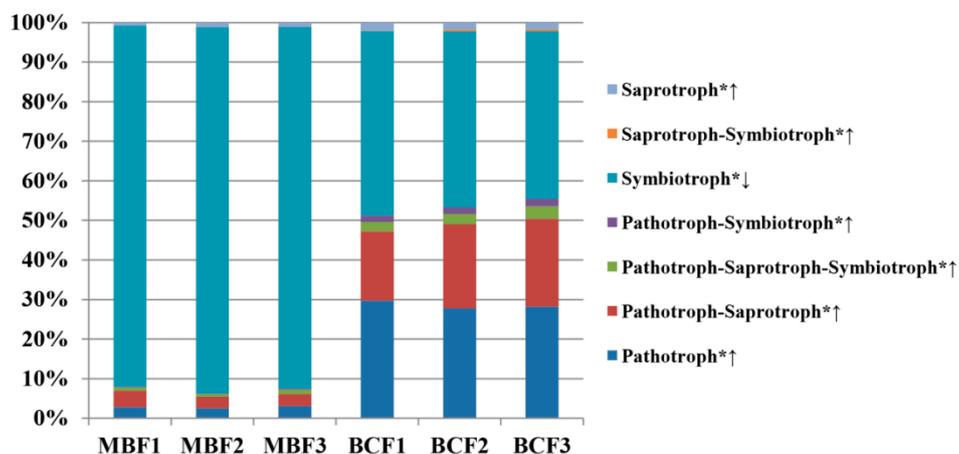


**Figure 4.** Principal coordinates analysis based on the Bray–Curtis distance for the moso bamboo leaf-associated bacterial (**a**) and fungal (**b**) community structures in the MBF and moso bamboo-chicken farming agroforestry system samples.

### 3.3. Ecological Function Evaluation of the Niche Shift of Leaf-Associated Microbiome

The functional potential of the bacterial and fungal communities associated with moso leaves was evaluated based on their metabolic processes using METAGENassist [33] and FUNGuild [34], respectively. The results (Figure 5) showed that the shift in the bacterial niche in the BCF treatment significantly increased the nutrient cycling pathways, including nitrogen (nitrogen fixation), carbon (carbon fixation), sulfur (sulfur and sulfide oxidation), and other metabolic processes (propionate metabolism, aromatic hydrocarbon degradation, naphthalene degradation, and atrazine metabolism). In contrast, the results showed that the shift in the fungal niche in the BCF treatment significantly increased the proportion of trophic types of fungi (pathotrophic, pathotrophic–saprotrophic, saprotrophic–symbiotrophic, pathotrophic–symbiotrophic and saprotrophic taxa), but significantly decreased the proportion of symbiotrophic fungi in comparison to the MBF treatment (Figure 6).



**Figure 5.** Taxonomic to phenotypic mapping based on the metabolism of bacterial communities associated with the leaves of moso bamboo.



**Figure 6.** Taxonomy-based functional profiling of fungal communities from the MBF and BCF samples. *↑, significantly increased in the BCF samples relative to the MBF samples; *↓, significantly decreased in the BCF samples relative to the MBF samples.

## 4. Discussion

The worldwide biomass of moso bamboo leaves is 2.89–3.65 Mg ha$^{-1}$, which accounts for 3–5% of the total aboveground bamboo biomass and changes every two years [36]. Bamboo leaves play an important role in bamboo forest carbon sequestration, soil nutrient development, and adaptation to environmental change [37,38]. To the best of our knowledge, the moso bamboo leaf-associated bacterial and fungal communities have not been well investigated. In this study, the use of high-throughput sequencing revealed that Cyanobacteria was the dominant bacterial phylum in the leaves of moso bamboo. A previous study showed that the bamboo species *Merostachys neesii* had a large number of cyanobacterial 16S rRNA genes per cm$^2$ of leaf [39]. Cyanobacteria have been suggested to be capable of both nitrogen and carbon fixation [40,41]. We also observed that Ascomycota was the most abundant fungal phylum associated with moso bamboo leaves, followed by Basidiomycota. This finding is in line with that of a previous study in which the endophytic fungi isolated from moso bamboo seeds belonged to the phyla Ascomycota and Basidiomycota [18], the members of which are important decomposers that break down dead leaves in forest ecosystems [42,43]. As mentioned above, the dominant leaf-associated bacterial and fungal phyla may contribute to C and N cycling in moso bamboo plantation ecosystems. The results of this study also identified fifteen bacterial genera with a relative abundance greater than 0.1% in the moso bamboo leaves (Table 1), which differs from the findings obtained using traditional bacterial isolation and culture methods. Yuan et al. [17] isolated five genera (*Pseudomonas*, *Micrococcus*, *Dermacoccus*, *Pantoea*, and *Streptomyces*) from the leaves of moso bamboo stands in the Wuyi Mountain, Jiangle, and Changting regions of China. However, traditional culture-based bacterial isolation methods cannot sufficiently identify the bacteria associated with leaves, especially for the cyanobacteria.

The results of the current study indicated that the introduction of chicken farming into a moso bamboo forest markedly influenced the moso bamboo leaf-associated microbiome, by increasing the relative abundance of the taxa Bacteroidetes, Actinobacteria, TM7, *Arthrobacter*, *Kineococcus*, *Friedmanniella*, *Hymenobacter*, *Methylobacterium*, *Bdellovibrio*, and *Spirosoma* compared with that observed in the MBF. Members of the bacterial phylum Bacteroidetes are important contributors to nutrient turnover because they harbour genes for denitrification, indicating a possible role in nitrogen cycling [44]. Members of the genus *Hymenobacter* can be used as plant growth-promoting bacteria to enhance plant nutritive properties [45]. *Methylobacterium* and *Spirosoma* belong to the class Alphaproteobacteria within the phylum Proteobacteria. Alphaproteobacteria participate in a variety of metabolic strategies including photosynthesis, nitrogen fixation, ammonia oxidation, and methylotrophy [46]. The results of previous studies showed that moderate chicken farming in bamboo forests can significantly improve the soil properties, increasing soil N, organic matter, total P, available P, total K, and available K [12]. As mentioned above, the shifts in bacterial abundances in moso bamboo leaves caused by chicken farming may contribute to nutrient cycling. Actinobacteria species may contribute to their host plants by promoting growth and enhancing their ability to withstand environmental stresses [47]. Arthrobacter species can promote legume growth by solubilizing iron, which is then taken up by the plants [48]. Members of the genus *Kineococcus* are reported to play roles in the immediate response to stress and/or the recovery from stress [49]. Species of the genus *Methylobacterium* are able to interact symbiotically with different plant species, and these interactions can promote plant growth or induce systemic resistance, increasing plant fitness [50]. Zhu et al. [12] also observed that chicken farming in bamboo forests may increase the risk of excess phosphorus input. Thus, the presence of different bacteria may help bamboo trees respond to the excessive nutrient stress caused by chicken farming. Fries et al. [51] isolated a *Spirosoma* strain from Zn- and Cd-accumulating *Salix caprea*, and Lee et al. [52] reported a novel *Spirosoma* strain with high gamma and UVC radiation resistance. In addition, some studies have reported that moso bamboo has a high metal tolerance [53,54]. Thus, the genus *Spirosoma* may be associated with moso bamboo with a high metal tolerance.

Fungal endophytes have been reported to promote plant growth [55], affect plant resistance to abiotic and biotic stresses [55–57], and decompose plant litter [58]. Rodriguez and Redman [59] noted that fungal symbiosis represents significant ecological plasticity, e.g. *Colletotrichum* species can express either parasitic or mutualistic lifestyles depending on the host genotype colonized. Similar to endophytic fungi, epiphytic fungal colonization on the surfaces of plant tissues can protect host plants from disease [60,61], and can participate in the decomposition of plant litter [62]. In the current study, BCF was observed to increase the relative abundance of Basidiomycota and decrease that of Ascomycota associated with moso bamboo leaves compared to that observed in MBF. Previous studies have shown that species of the phyla Ascomycota and Basidiomycota are important soil fungal decomposers [63,64]. Members of the phylum Basidiomycota are able to degrade lignocellulose organic matter [65,66], while members of the phylum Ascomycota have a limited ability to degrade recalcitrant lignin-containing litter material [67]. The results also indicated that all of the different genera belong to the phylum Ascomycota, such as *Alatosessilispora*, *Strelitziana*, *Cladosporium*, *Camptophora*, *Ramularia*, *Trichomerium*, and *Arthrinium*. Thus, these fungal phyla and genera may contribute to the carbon cycle in bamboo plantations.

Previous studies observed that animal manure in forest-livestock systems can improve the soil nutrient status by accelerating N cycling [68,69], although excess manure may result in soil nutrient erosion and imbalance [70,71]. Zhu et al. [12] observed that chicken trampling can significantly increase soil bulk density, which causes further environmental stress that impacts plant growth. The niche shift of leaf-associated bacterial communities in BCF also showed higher potential for nutrient cycling, which may be related to change in environmental stress. Chicken guts are colonized by diverse microbial classes including archaea, bacteria, and fungi [72]. Those microbial communities also contain pathogens [73], and the gastrointestinal pathogens can enter the food chain through defecation in the farm environment or by fertilization of crops with manure [74]. In this study, we also found that moso bamboo leaves from the BCF treatment exhibited an increased proportion of pathotrophic, pathotrophic–saprotrophic and saprotrophic fungi, indicating that chicken farming in bamboo plantations may lead to a higher risk of fungal-associated diseases. Thus, BCF systems should include certain management practices such as controlling chicken density, and good sterilization practices should be undertaken during chicken farming.

## 5. Conclusions

In summary, introducing chicken farming to a moso bamboo forest markedly changed the leaf-associated bacterial and fungal communities. The observed bacterial and fungal shifts may have dual effects. On the one hand, BCF systems may contribute to increased nutrient cycling in moso bamboo forests and enhance the adaption ability of trees to environmental stresses caused by shifts in bacterial communities. On the other hand, BCF may increase the risk of fungal-associated diseases. Therefore, increased attention is required in terms of soil nutrient management and leaf disease control in BCF systems.

**Author Contributions:** Z.Z. and X.Z. designed the experiment. X.Z., X.G., J.Y., W.L., and X.D. performed the study data collection. X.Z., C.Y., F.B., and X.G. analyzed the results. Z.Z. contributed to discussing content and reviewing the article. X.Z. wrote the final article. All authors approved the final version of the manuscript.

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

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