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Effects of Film Mulching on Soil Microbial Diversity and Community Structure in the Maize Root Zone under Drip Irrigation in Northwest China

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Abstract: Mulching is a widely used agricultural water conservation measure in the semiarid regions of Northwest China. In order to explore the response process of different film mulching methods to soil microorganisms, we characterized the effect of different film mulching methods on soil microbial diversity and community structure characteristics in the root zone of drip-irrigated maize during the heading and maturity stages using high-throughput sequencing of 16SrDNA and ITS amplicons combined with bioinformatics analysis. Full mulching (FM) was contrasted to controls of no mulching (NM) and half-mulching (HM), yielding an order of microbial diversity, abundance, and evenness scores of HM > FM > NM. The HM and FM treatments reduced the relative abundance of Proteobacteria and Actinobacteria (the most abundant bacteria) in the bacterial community structure but increased that of *Acidobacteria* and *Chloroflexi*. In the fungal community structure, HM decreased the abundance of *Sordariomycetes* but increased that of *Eurotiomycetes* (the most abundant fungi). The abundance and community structure of bacteria were significantly correlated with soil temperature and those of fungi with pH. HM improved network complexity and competitive relationships among bacteria, while FM increased the relationship between fungal groups and the symbiosis of fungal communities. HM significantly increased maize yield (20.37% and 6.01% above NM and FM, respectively). In summary, full mulching was more favorable than no mulching for soil microbial diversity and community structure composition, but soil microbial diversity and yield responded better to half-mulching. These results provide a background for improving the yield of drip-irrigated maize and protecting the microbial ecosystems of farmland soils.

Keywords: mulching method; bacteria; fungi; microbial diversity; community structure; drip-irrigated maize

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1. Introduction

Maize (*Zea mays* L.) is one of the three major food crops in the world, which is of great significance to global food supply and economic development. It has a wide range of uses, such as food, biofuels, and industrial raw materials. In recent years, maize production and demand have continued to grow, with global production of maize accounting for more than 35% of total grain production each year [1]. In China, maize is the third-largest food crop after rice and wheat. According to statistics for 2022, China's maize planting area is about 42 million hectares, accounting for nearly 30% of the global maize

planting area [2]. In semi-arid areas, mulch can significantly increase soil moisture content, improve the growth conditions of maize, and thus increase the yield of maize [3]. The application of film mulching has substantially increased crop yields by up to 20–30% [4]. In recent years, the rate of ground cover application in China has been increasing on an average annual basis of 12.7% [5]. However, while this has helped to ensure the high quality and yield of crops, the increase in the area of mulch cover and the amount of mulch input per unit area has engendered a series of environmental problems and affected the sustainable development of agricultural production [6]. These issues include the destruction of farmland soil structure, decline in arable land quality, obstruction of agricultural farming, and degradation of soil fertility [7]. Causes include the poor air permeability and thermal conductivity of the film mulching. Prolonged mulching causes different degrees of premature senescence in the middle and late stages of crop growth and development, thereby affecting crop yield and quality [8]. It is therefore important that mulching be carried out at an appropriate time. At present, there are relatively few studies on the changes in microbial soil microbial diversity and community structure characteristics on maize rhizosphere soil under film mulching; the vast number of soil rhizosphere microorganisms and numerous flora necessitate urgent investigations.

Soil microorganisms are an indispensable part of the soil ecosystem, influencing soil formation, development, material cycling, and soil fertility evolution [9]. Soil root zone microorganisms constitute the link between crops and soil and play an important role in crop growth and yield [10]. Soil microbial communities are sensitive to changes in environmental conditions, and physicochemical properties, such as temperature, moisture, pH, and nutrients, are closely related to microbial growth and metabolism [11–13]. Film mulching can change the physicochemical properties of soil, the microbial living environment, and nutrient supply and demand, resulting in changes in the growth and activity of microbiota, which can further cause a change in the microbial community in crop rhizosphere soil [14,15]. Film mulching can significantly increase soil microbial diversity and bacterial abundance [16]; it can also significantly increase the α-diversity of soil bacteria and have a crucial effect on shaping the soil bacterial community [17]. Bacterial networks in film-mulched soils are more complex and have a higher index of microbial community diversity than those in non-mulched soils [18]. It has also been shown that film mulching agricultural soils accelerates the succession of microbial communities in the soil and affects the stability and diversity of the soil microbial community structure [19]. Long-term mulching significantly increased the degree of variability among the treatments and the instability of the microbial communities [20]. However, short-term mulching can significantly increase the structural diversity of fungal communities in agricultural soils [21]. Long-term mulching was found to have a significant negative effect on soil bacterial abundance in maize and led to a decrease in β-diversity due to the reduction of soil aggregate stability and microbial interactions [22]. At the same time, long-term mulching causes an increase in microplastic content in the soil and the enrichment of specific microbial flora on plastic residual films, which negatively impacts microbial community diversity [23].

While recent studies on soil microorganisms in agricultural fields have focused on the favorable aspects of full mulching [24,25], the changes in soil microorganisms in the root zone caused by different mulching methods remain unclear. In our study, we used high-throughput sequencing (based on 16S rRNA and ITS gene analysis) to elucidate the effects of mulching methods on microorganisms and yield changes in the root zones of drip-irrigated maize setups using three mulching methods, i.e., no mulching (NM), half-mulching (HM), and full mulching (FM). We assumed that: different film mulching methods will directly change soil properties, thus changing soil microbial composition; soil microbial composition will have different responses to different mulching methods. The purpose of this study was to study the response of different treatments to soil structure and composition, microbial diversity, and community; to analyze the important soil properties that affect soil microorganisms; and to determine how these soil properties will lead to changes in community structure to provide a theoretical basis for evaluating the effects

of different mulching methods on soil microorganisms. The results of this study provide a theoretical basis for the rational application of mulch film for drip irrigation maize in Northwest China.

2. Materials and Methods

2.1. Experimental Location

The field experiment was conducted in 2021 at the Crop Water Use Experiment Station of the Ministry of Agriculture in Shihezi City, northern China (86°09′ E, 45°38′ N) (Figure 1). The study area is located on an alluvial fan plain at the northern foot of Tianshan Mountain, which has a typical temperate continental climate. During the growing season, maximum temperature was 34.11 °C, minimum temperature was 13.60 °C, total rainfall was 66.2 mm, and the depth of the water table was 1.2–1.8 m. Annual evaporation was 1850–2100 mm. The experimental soil was a gray desert soil [26] with a bulk weight of 1.67 g·cm^{-3}, a saturated water content of 28.8%, and a water holding capacity of 17.45% in the field.

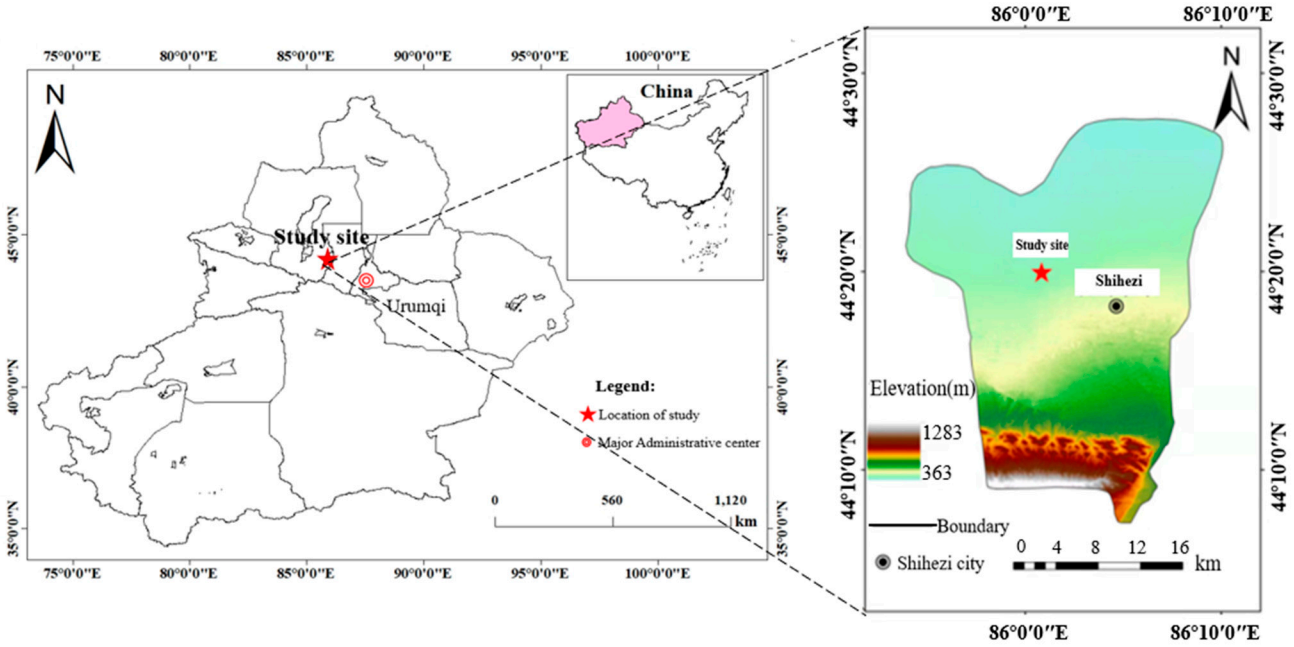


Figure 1. Geographical location of the study area.

2.2. Materials

The maize variety "ZD958", which is commonly planted in northern China, was used as the experimental variety. Zhengdan 958 is the offspring of inbred Zheng 58 and Chang 7–2 (deposition number 20000009), which have been approved in China. Zhengdan 958 seeds were provided by Beijing Denong Seed Technology Co., Ltd. The experimental research and field studies on plants complied with relevant institutional, national, and international guidelines and legislation. The urea (N ≥ 46.4%, granules) used in the experiment was produced by Xinlianxin Co., Ltd. (Xinjiang, China). Monoammonium phosphate (N ≥ 12%, P_2O_5 ≥ 61%, powder) was produced by Guizhou Kai Phosphorus Group Co., Ltd. (Guiyang, China). Potassium sulfate was obtained from Luobupo Potassium Salt Co., Ltd. (Xinjiang, China). The source of the irrigation water was a deep well with a depth of 100 m; the salinity of the water was 0.2–0.3 g·L^{-1}. We employed a single-wing labyrinth drip irrigation belt (WDF16/2.6–100) produced by the Xinjiang Tianye Company (Shihezi, China). The wall thickness was 0.18 mm, the inner diameter was 16 mm, the drip hole spacing was 300 mm, the rated flow was 2.0 L·h^{-1}, and the working pressure was 0.1–0.15 MPa.

The test film used was transparent, with a thickness of 0.01 mm and a width of 70 cm, and was produced by Xinjiang Tianye Company (Shihezi, China). The film is polyethylene material, and the degradation intensity is general.

2.3. Experimental Design

The field experimental design consisted of three treatments (no mulching (NM), half-mulching (HM), and full mulching (FM)) and three replicates of each treatment. NM treatment represents no plastic film mulching during the whole growth period; HM treatment indicated that the film was covered before heading stage but not covered after heading stage; and FM treatment represented the whole growth period of maize. The maize planting date was 5 May, and the harvest date was 15 September. For HM, the period of half-mulching was from planting to 19 July, while the remainder of the time was used as a non-mulched control period (Figure 2). A joint planter was used to lay the drip tape and plastic film and to sow. A planting density of 1.26×10^5 ·ha^{-1} was used in the experiment. The plants were sown in alternating wide and narrow rows of, respectively, 0.7 m and 0.4 m in width. The spacing between plants within a row was 14.4 cm, and that between drip tapes was 110 cm. Conventional pest and weed control practices for the area were followed. Specific fertilizer rates, irrigation application times, and irrigation amounts were allocated according to the reproductive period of the maize (Table 1).

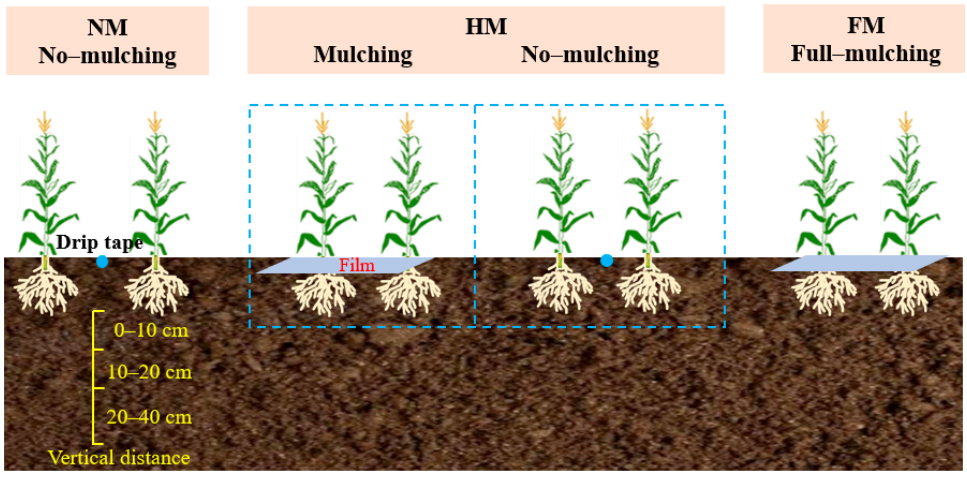


Figure 2. Experimental treatments and a soil sampling map.

Table 1. Fertilization and irrigation used in different periods of maize development.

Growth Period	Seedling Period	Jointing Period	Small Bell Mouth Period	Big Bell-Mouth Period	Heading Period	Flowering Period	Silking Period	Grain Formation Period	Milk-Ripe Period	Total
Irrigation and Fertilization Date	**5/13**	**6/15**	**6/28**	**7/7**	**7/15**	**7/27**	**8/6**	**8/14**	**8/25**	
Irrigation amount (m^3·ha^{-1})	163.6	600	600	600	600	600	600	563.6	472.8	4800.0
Urea (kg·ha^{-1})	0	81.8	81.8	90.9	81.8	81.8	72.7	54.7	0	545.5
Monoammonium phosphate (kg·ha^{-1})	36.4	36.4	45.5	45.5	45.5	27.3	18.2	18.2	0	273.0
Potassium sulphate (kg·ha^{-1})	0	18.2	27.3	27.3	36.4	22.7	18.2	13.6	0	163.7

2.4. Analysis of Soil Properties

Soil samples were collected at the heading stage (16 July) and maturity stage (10 September) of maize. Soil samples were collected from within 0–10 (D1), 10–20 (D2), and 20–40 cm (D3) of the root zone of the maize plants (Figure 2). The soil samples were decontaminated, and half of the soil was transferred to a refrigerator and stored at −80 °C for the extraction of soil microbial DNA. The other half were air-dried and sieved (<1 mm) to determine their physical and chemical properties.

Soil pH was measured using a pH meter (Mettler Toledo FE28-Standard, Switzerland) using a potentiometric method (water:soil = 2.5:1). Total nitrogen (TN) was adopted by the Kjeldahl digestion process (Kjeldahl nitrogen analyzer, KDN-08C, China). Soil available potassium (AK) was measured with a CH_3COONH_4 solution (Kelamaer, China) (soil:CH_3COONH_4 solution = 1:10) and an HCl-NH_4F solution (Kanuosi, Nancun, China) (soil:HCl-NH_4F solution = 1:10), respectively [27]. Soil-available *p* was determined by spectrophotometry (Shimadzu UV-1780, Japan). Soil organic matter (SOM) was applied by potassium dichromate (NIST, Gaithersburg, MA, USA) external heating. The soil moisture (SM) and soil temperature (ST) were determined using an ET-60/100 soil moisture meter (Klein Tools, Lincolnshire, IL, USA).

2.5. High-Throughput Sequencing of Soil Microorganisms

The samples were tested by Guangzhou Kidio Biotechnology Co. (https://www.omicsmart.com, accessed on 15 January 2024). DNA was extracted using a HiPure Soil DNA Mini Kit (#3412; Magen, Guangzhou, China). The quality of the collected DNA was determined by 1% agarose gel electrophoresis. The concentration and purity of DNA were determined by a NanoDrop 2000 spectrophotometer. (Thermo Fisher Scientific, Waltham, MA, USA). The 341F (5′-CCTACGGGNGGCWGCAG-3′) and 806R (5′-GGACTACHVGGGTATCTAAT-3′) binding adapters and barcode sequences in the V3-V4 region of the bacterial 16S rRNA gene were amplified using universal primers. ITS1 (5′-CTGTCATTAGGGAGAGAGA-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGA-3′) were amplified by fungal ITS1 primers and the combination of adaptor sequence and barcode sequence [28]. The amplification product was connected to the sequencing connector, the product was processed to form a sequencing library, and the sequencing qualified libraries using Illumina Hi Seq 2500 (Illumina, San Diego, CA, USA). All the original sequence data sets were uploaded to the NCBI Sequence Read Archive (SAR) to obtain the login number. PRJNA1061271.

2.6. Analysis of the Yield of Maize

During the maize maturation period, random sampling was performed on each plot. Twenty maize plants were selected at each sampling time, the length of the panicles, the number of rows, and the length of baldness were measured, and the ears of the maize were threshed. The grain was air-dried, weighed (1000-grain mass and total grain mass), and then converted into yield per hectare. Grain yield and kernel weight were expressed at 14% moisture content [29] using the following formula:

Yield ($kg \cdot ha^{-1}$) = 20-grain weight (g)/20 panicles × 126,000/1000 × [1 − grain moisture content (%)]/(1–14%)

2.7. Statistical and Bioinformatics Analysis

Soil physicochemical, bacterial, or fungal diversity and community structure differences were statistically analyzed using one-way analysis of variance (ANOVA), which was determined by the Duncan test to determine whether the levels were significantly different between the control and full-mulching treatments. The α-diversity indices (Chao1, Shannon, Ace, and Sobs) of the microorganisms in the phylogenetic tree were calculated using the "vegan" package (version 2.5.6) in R (version 4.0.2). Greater Chao1 and Ace indices indicate greater species richness. A greater Shannon index indicates greater community diversity. The Sobs index reflects changes in the number of species. Multivariate analysis of variance

based on Bray-Curtis distance was used to test whether there were significant differences in β-diversity among treatments. Dilution curves and box plots were plotted in R. Linear discriminant analysis (LDA) was performed using LefSe (https://www.omicsmart.com/, accessed on 15 January 2024) for bacteria and fungi at the genus level, retaining species with LDA scores > 3 and $p < 0.05$ [30]. We constructed an ecological network of bacteria and fungi and used the "igrath" package (version 1.6.0) to calculate network topology parameters in R. In order to reduce the complexity of the database, only the average relative abundance > 0.1% (bacteria) and > 0.05% (fungi) were selected for microbial analysis [31]. Calculate the Pearson's correlation matrix between OTUs using the "psych" (version 2.4.1) and "wgcna" packages (version 1.72) in R and construct the network using thresholds with Pearson's correlation coefficients > 0.6 and $p < 0.05$. Finally, Gephi software (version 0.9.2) is used to calculate the topological parameters of the network, including nodes, edges, clustering coefficients, modularity, and average degree [32].

3. Results

3.1. Effect of Film Mulching Treatments on Soil Characteristics

The HM treatment significantly affected SM, ST, SOM, and TN content; however, it had no significant influence on AP, AK, or pH (Table 2). The HM treatment significantly improved the properties of the soil compared to the NM and FM treatments. With increasing soil depth, soil moisture, soil temperature, and SOM gradually increased during the same period. The TN, AP, and AK levels first increased and then decreased. The soil pH gradually decreased with soil depth. There were no significant differences between the treatments during the heading and maturity periods. Compared with NM, the HM and FM treatments, respectively, showed significant increases in SOM by 32.39% and 26.38%, in soil temperature by 10.63% and 10.33%, and in soil TN content by 11.38% and 9.05%.

Table 2. Changes in soil physical and chemical properties of drip irrigation maize under different film mulching methods.

Depth (cm)	Reproductive Period	Treatment	SM (%)	ST (°C)	SOM (g·kg^{-1})	TN (g·kg^{-1})	AP (mg·kg^{-1})	AK (mg·kg^{-1})	pH
0–10	Heading period	NM	14.31 ± 0.16 c	21.02 ± 0.19 b	12.19 ± 0.29 c	1.29 ± 0.07 b	27.20 ± 0.50 a	416.56 ± 0.43 a	8.61
		HM	15.72 ± 0.29 b	22.27 ± 0.36 a	17.79 ± 0.25 a	1.41 ± 0.11 ab	27.35 ± 0.64 a	418.07 ± 0.69 a	8.97
		FM	18.03 ± 0.33 a	18.03 ± 0.33 c	16.73 ± 0.32 b	1.76 ± 0.23 a	26.84 ± 0.23 a	417.79 ± 0.44 a	8.99
	Maturity period	NM	8.61 ± 0.48 c	19.31 ± 0.26 c	14.30 ± 0.11 c	1.16 ± 0.22 b	27.61 ± 0.65 a	417.62 ± 0.77 a	8.47
		HM	16.62 ± 0.32 b	20.14 ± 0.10 b	18.31 ± 0.27 a	1.39 ± 0.03 a	27.70 ± 0.62 a	419.00 ± 0.59 a	8.79
		FM	21.93 ± 0.13 a	21.43 ± 0.28 a	17.24 ± 0.29 b	1.34 ± 0.15 a	27.70 ± 0.65 a	418.64 ± 0.65 a	8.41
10–20	Heading period	NM	22.33 ± 0.23 c	21.07 ± 0.09 c	13.17 ± 0.34 b	1.43 ± 0.07 b	27.49 ± 0.63 a	417.26 ± 0.36 b	8.37
		HM	25.45 ± 0.30 b	23.14 ± 0.36 b	17.81 ± 0.39 a	1.64 ± 0.12 a	27.22 ± 0.39 a	418.89 ± 0.58 a	8.68
		FM	26.59 ± 0.31 a	24.39 ± 0.28 a	16.88 ± 0.35 a	1.51 ± 0.25 ab	27.23 ± 0.42 a	418.85 ± 0.56 a	8.43
	Maturity period	NM	8.72 ± 0.25 c	19.44 ± 0.18 c	14.34 ± 0.52 c	1.51 ± 0.11 a	27.50 ± 0.33 a	417.79 ± 0.47 a	8.06
		HM	18.63 ± 0.33 b	23.65 ± 0.21 a	19.56 ± 0.24 a	1.55 ± 0.11 a	27.68 ± 0.65 a	420.82 ± 0.43 a	8.28
		FM	22.07 ± 0.28 a	22.54 ± 0.35 b	17.26 ± 0.34 b	1.49 ± 0.03 a	27.95 ± 0.62 a	418.93 ± 0.64 a	8.20

Table 2. *Cont.*

Depth (cm)	Reproductive Period	Treatment	SM (%)	ST (°C)	SOM (g·kg^{-1})	TN (g·kg^{-1})	AP (mg·kg^{-1})	AK (mg·kg^{-1})	pH
20–40	Heading period	NM	32.51 ± 0.50 b	21.43 ± 0.11 b	13.23 ± 0.23 c	1.20 ± 0.12 a	24.79 ± 0.35 b	296.88 ± 0.40 c	8.18
		HM	32.74 ± 0.38 ab	23.56 ± 0.23 ab	18.31 ± 0.39 a	1.44 ± 0.02 a	25.97 ± 0.29 ab	308.31 ± 0.54 a	8.56
		FM	33.93 ± 0.70 a	24.61 ± 0.27 a	17.11 ± 0.27 b	1.35 ± 0.14 a	26.38 ± 0.38 a	301.35 ± 0.61 b	8.36
	Maturity period	NM	11.33 ± 0.44 c	20.15 ± 0.11 c	14.85 ± 0.21 c	1.35 ± 0.09 b	24.77 ± 0.69 a	300.89 ± 0.58 c	7.88
		HM	20.62 ± 0.44 ab	24.22 ± 0.24 b	19.71 ± 0.17 a	1.53 ± 0.07 a	25.65 ± 0.70 a	309.14 ± 0.36 a	8.26
		FM	22.12 ± 0.23 a	25.53 ± 0.27 a	17.28 ± 0.37 b	1.28 ± 0.03 b	25.26 ± 0.53 a	305.16 ± 0.35 b	8.07

Note: Different lowercase letters indicate that there are significant differences between different treatments in the same soil depth and the same period ($p < 0.05$).

3.2. Response of Different Film Mulching Methods to Microbial α- and β-Diversity

The Sobs, Chao1, Ace, and Shannon indexes were calculated as the criteria for judging the richness and diversity of microorganisms. (Figure 3). Soil bacterial α-diversity at the heading stage (S1) was significantly increased by the HM treatment (Shannon's index) compared to the NM and FM. At maturity (S2), the NM treatment significantly increased bacterial abundance (Sobs index) compared with the FM treatment, and NM significantly increased bacterial diversity (Shannon index) compared with the HM and FM treatments. Bacterial abundance and diversity increased with soil depth. During the heading period, depths D2 and D3 showed significantly increased bacterial abundance (Sobs, Ace, and Chao1 indices) compared to that at D1, but there were no significant differences in bacterial diversity (Shannon index). Both bacterial abundance and diversity were significantly higher at depths D2 and D3 than at depth D1 at maturity (all indices).

Fungal richness or diversity was not significantly affected by any treatments during the heading stage. At the maturity stage, there was no significant effect on fungal richness (Sobs, Ace, and Chao1 indices), but HM showed significantly increased fungal diversity (Shannon index) compared to the FM treatment. There were also no significant effects of soil layer depth on either fungal richness or diversity. Overall, the mulching method had a greater impact on the degree of bacterial diversity than on the degree of fungal diversity.

To further measure the difference in microbial community structure, Bray-Curtis dissimilarity was used to analyze the β-diversity of bacteria and fungi (Figure 4). The two principal component axes (PCoA1 and PCoA2), respectively, contributed 22.13% and 10.68% (cumulative 32.81%) to bacterial β-diversity at a depth of 0–40 cm. There was a significant divergence in the bacterial community composition between treatments, indicating that the mulching method significantly altered the community structure of soil bacteria ($R = 0.5119$, $p = 0.001$), with the greatest degree of variability in the 0–10 cm soil layer ($R = 0.6856$). Fungal β-diversity at 0–40 cm was 13.46% and 11.52% (cumulative 24.98%) for the two axes, respectively. Fungal communities showed clear overlaps among treatments and no significant differentiation between different periods, indicating that different mulching methods and the two fertility periods had less effect on fungal community structure ($R = 0.189$, $p = 0.001$). The greatest degree of differentiation was found in the 20–40 cm soil layer ($R = 0.2634$). Overall, there were significant divergences in bacteria and fungi in the 0–10, 10–20, and 20–40 cm soil layers in the NM treatment compared with the HM and FM treatments, suggesting that the β-diversity of both types of microorganisms substantially differed between the absence and presence of mulching among different soil layers and that these effects were significantly stronger for bacteria than for fungi.

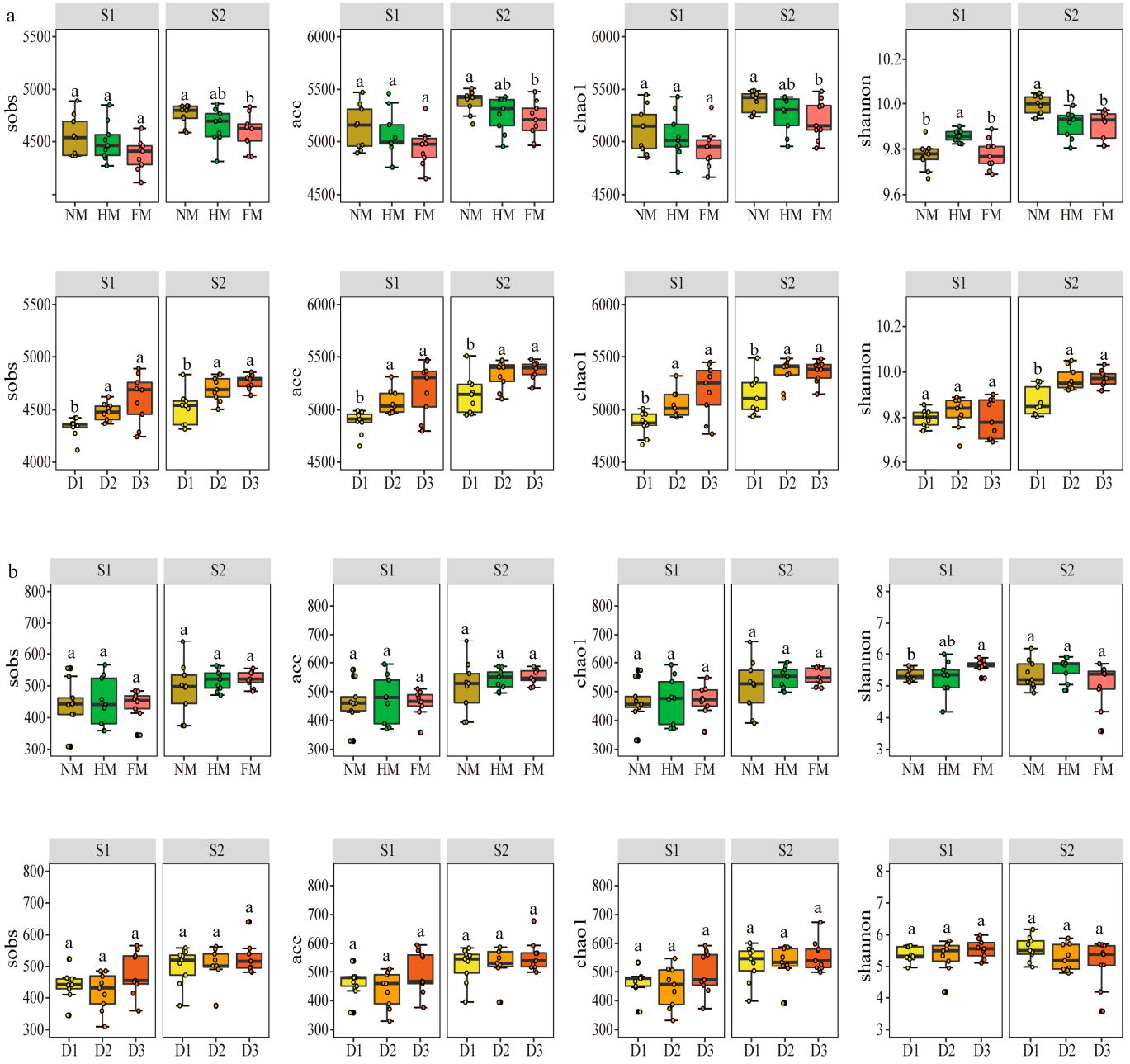


Figure 3. Changes in soil bacterial and fungi α-diversity under different film mulching methods for evaluated α-diversity indices (Chao1, Shannon, Ace, and Sobs). S1, heading stage; S2, maturing stage. (**a**) Bacteria; (**b**) fungi. Different letters indicated that there were significant differences between different treatments or different depths in the same stage ($p < 0.05$).

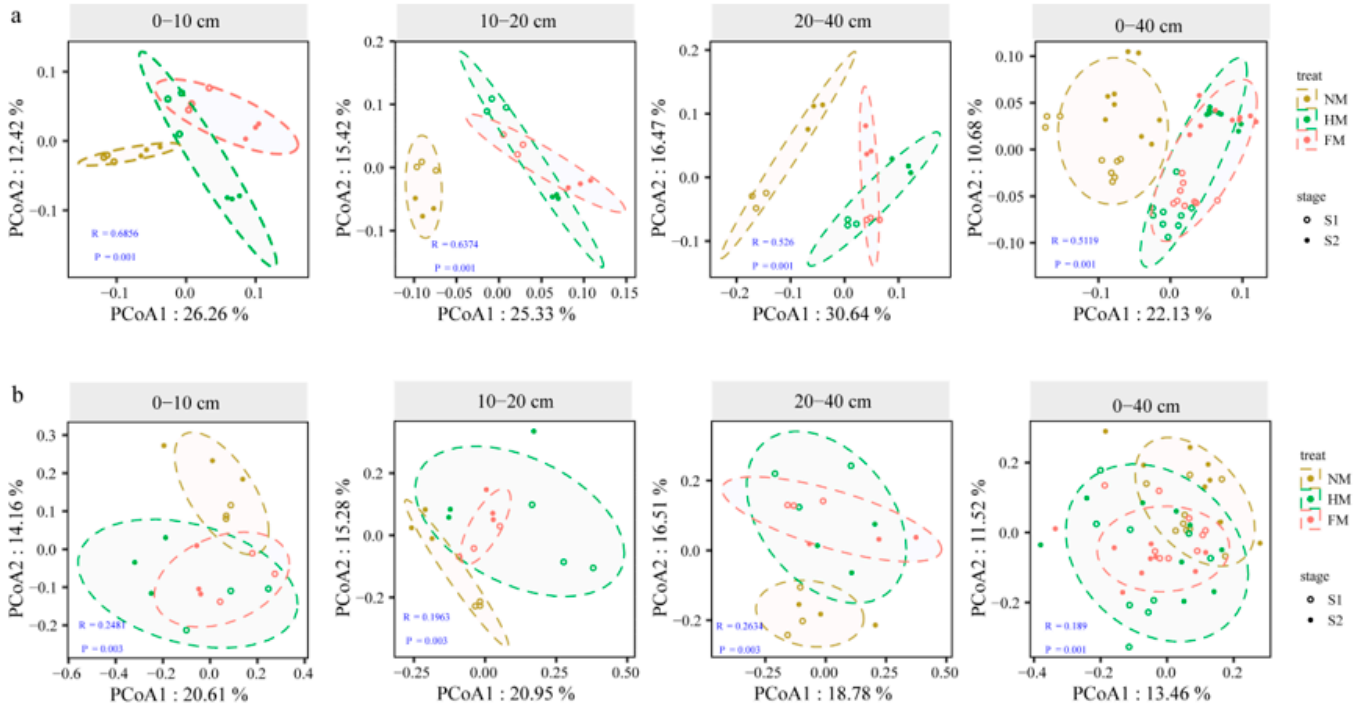


Figure 4. Principal component analysis of changes in soil bacterial and fungi β-diversity under different film mulching methods. S1, heading stage; S2, maturing stage. (**a**) Bacteria; (**b**) fungi.

3.3. Changes in Microbial Community Composition under Different Film Mulching Methods

Different film mulching methods influenced the bacterial community composition (Figure 5). The structure of the soil bacterial communities varied among the different mulching methods. The top 10 most abundant phyla of bacteria were *Actinobacteria*, *Proteobacteria*, *Acidobacteria*, *Chloroflexi*, *Planctomycetes*, *Gemmatomonadetes*, *Firmicutes*, *Verrucomicrobia*, *Bacteroidetes*, and *Rokubacteria*. *Actinobacteria* and *Proteobacteria* were dominant overall, with an average abundance of 50%. At the heading stage, the relative abundances in the NM, HM, and FM treatments were respectively 28.26%, 23.74%, and 23.10% for *Actinobacteria*; 22.47%, 23.39%, and 22.54% for *Proteobacteria*; 12.95%, 15.08%, and 15.69% for *Acidobacteria*; and 12.06%, 13.64%, and 13.47% for *Chloroflexi*. The rest of the relative abundances were minor, at 0.94–9.89%. At maturity, the relative abundances of NM, HM, and FM treatments were 24.28%, 20.81%, and 20.11%, respectively. The relative abundances of *Proteobacteria* were 22.60%, 21.60%, and 20.94%, respectively. The relative abundances in the NM, HM, and FM treatments were respectively 13.98%, 16.52%, and 17.13% for *Acidobacteria*, and 12.57%, 14.72%, and 14.11% for *Chloroflexi*, while the rest were minor at 0.95–9.66%. Compared with the NM treatment, the HM and FM treatments significantly reduced the relative abundances of *Actinobacteria* (by 15.19% and 17.75%, respectively) and *Proteobacteria* (by 0.19% and 3.52%, respectively). The FM treatment significantly increased the relative abundance of *Acidobacteria* (by 17.97%), whereas the HM treatment significantly increased the relative abundance of *Chloroflexi* (by 10.67%). There were no significant differences in the composition of the bacterial community structure among the different soil layers, although visual trends were present.

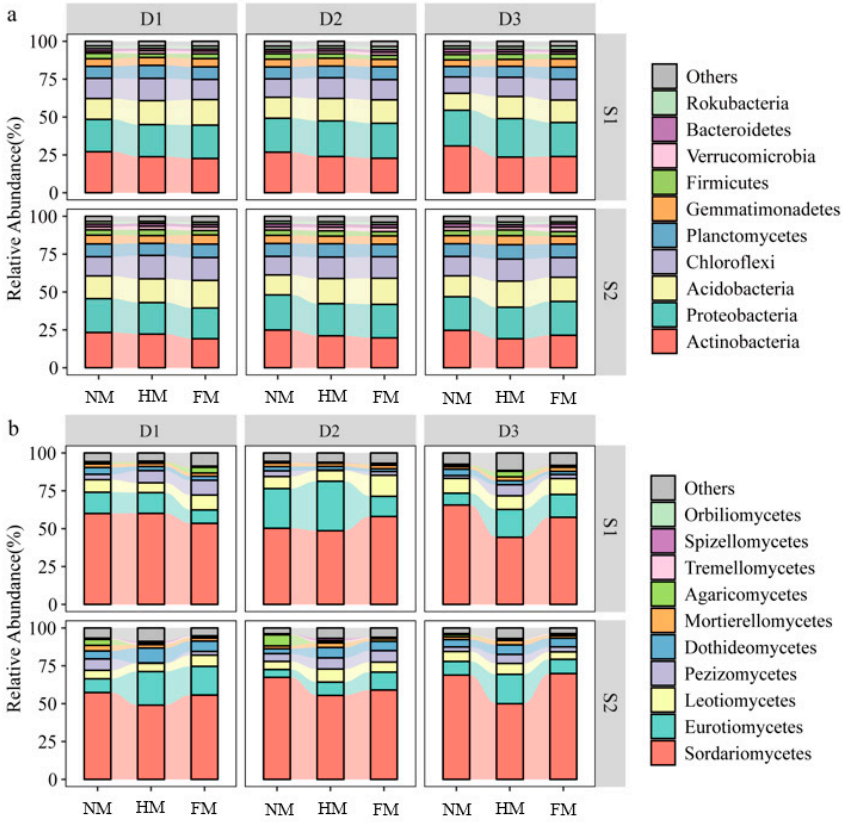


Figure 5. Effects of different film mulching methods on the composition of bacterial and fungal communities. D1, D2, and D3 indicate soil depths of 0–10, 10–20, and 20–40 cm, respectively; S1, heading stage; S2, maturity stage. Phylum-level composition of bacterial and class-level composition of fungal communities. (**a**) Bacteria; (**b**) fungi.

The fungal community composition was also altered by the different film mulching methods (Figure 5). The top 10 most abundant fungal classes were *Sordariomycetes*, *Eurotiomycetes*, *Leotiomycetes*, *Pezizomycetes*, *Dothideomycetes*, *Mortierellomycetes*, *Agaricomycetes*, *Tremellomycetes*, *Spizellomycetes*, and *Orbiliomycetes*. Among these, *Sordariomycetes* and *Eurotiomycetes* were the dominant classes, with an average abundance > 65%. The HM and FM treatments significantly reduced the relative abundance of *Sordariomycetes* (by 16.81% and 5.73%) as compared to the NM treatment (Figure 6). The HM treatment significantly increased the abundance of *Eurotiomycetes* by 38.06% and 31.27% as compared with the NM and FM treatments, respectively. Different treatments thus significantly affected the soil fungal community structure, while different soil layers had little effect.

Differential taxa of bacteria and fungi for the NM, HM, and FM treatments were assessed by LDA, focusing on the top fifty species in terms of relative species abundance (LDA score > 3, $p < 0.05$) (Figure 7). LDA showed that of the 40 assessed bacterial taxa, 17 were detected in the NM treatment, 10 in the HM treatment, and 13 in the FM treatment. The number of bacterial taxa that were significantly enriched in the soil decreased with increasing mulching time. The NM mainly consisted of *Actinobacteria* (phylum), *Alphaproteobacteria* (class), *Micrococcales* (order), *Micrococcaceae* (family), and *Propionibacteriales* (genus), of which *Actinobacteria* was the most abundant. The NM treatment had higher bacterial species complexity and a more stable fine community structure than the HM and FM treatments. Of the 46 assessed fungal communities, 13 were detected in NM, 21 in HM, and 12 in FM. *Sordariomycetes* constituted the most enriched taxon in the NM treatment, *Bionectriaceae* in the FM, and *Eurotiales* in the HM. Overall, the HM treatment presented higher fungal taxon complexity than the NM and FM treatments and a more stable fungal community structure.

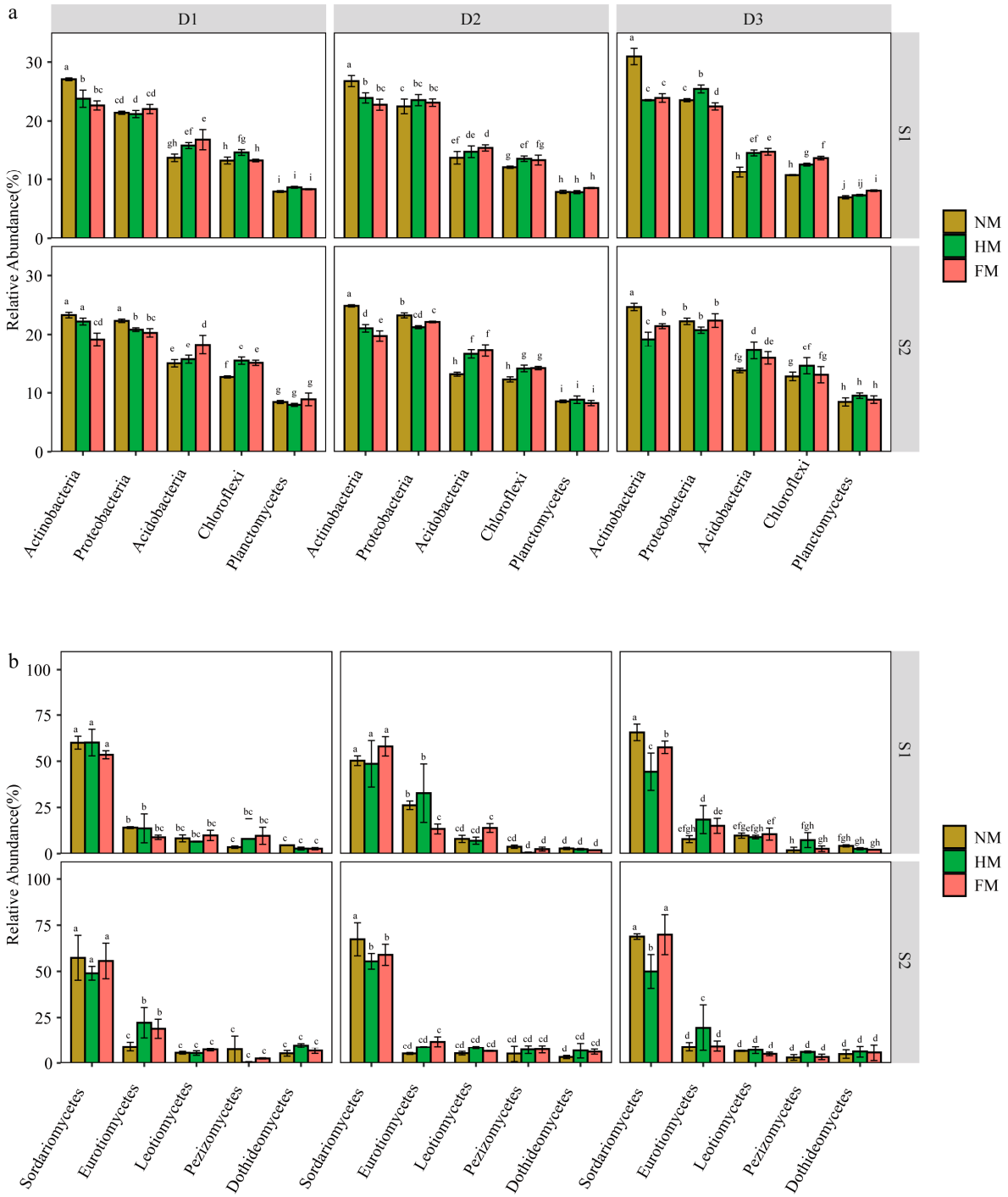
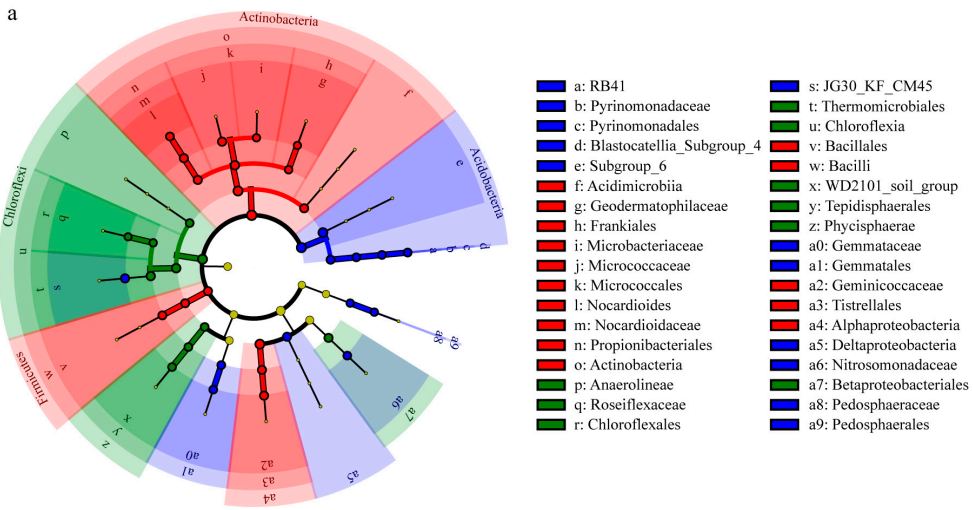


Figure 6. Multiple comparisons of the top five most abundant bacteria and fungi under different film mulching treatments. (**a**) Bacteria; (**b**) fungi. Different letters indicated that there were significant differences between different treatments or different depths in the same stage ($p < 0.05$).



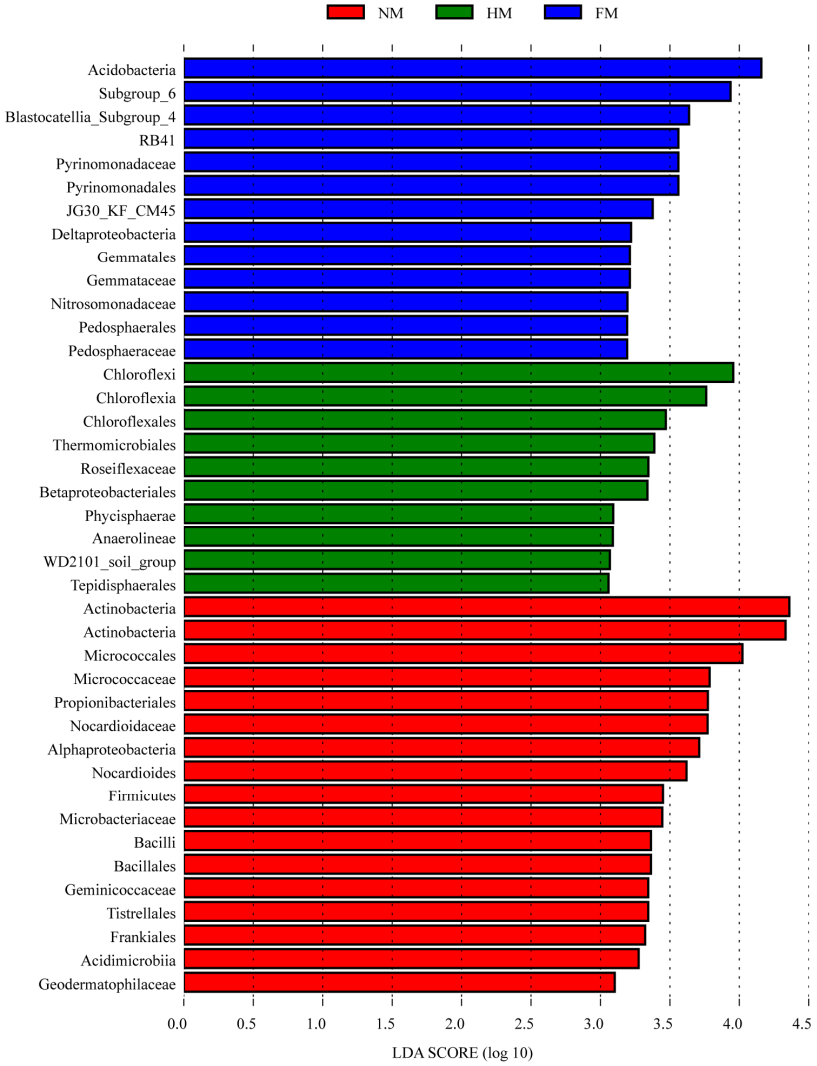


Figure 7. *Cont.*

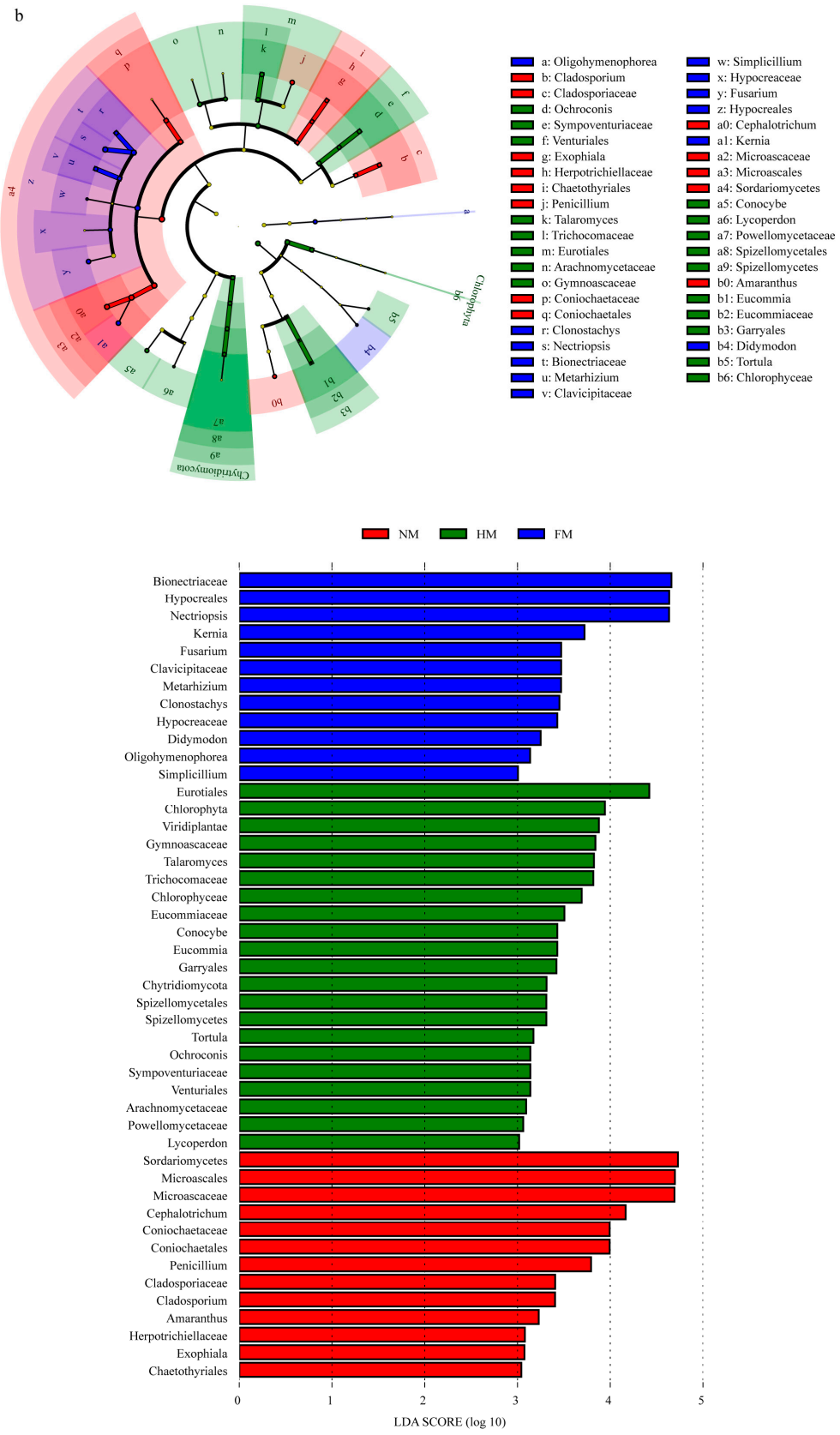


Figure 7. Linear discriminant analysis of bacterial and fungi community structure under different film mulching methods (NM, HM, and FM) at the domain–genus level. (**a**) Bacteria; (**b**) fungi.

3.4. Correlation Analysis between Microbial Community Structure and Soil Properties

Film mulching mainly improves soil nutrient status by changing the soil water, gas, and thermal environments. In our study, bacterial richness and bacterial diversity were significantly negatively correlated with ST (Figure 8). Bacterial community structure was extremely significantly positively correlated with the content of ST, TN, and AP. There was a significant positive correlation between fungal community structure and pH, fungal richness significantly with TN, and fungal diversity with AK.

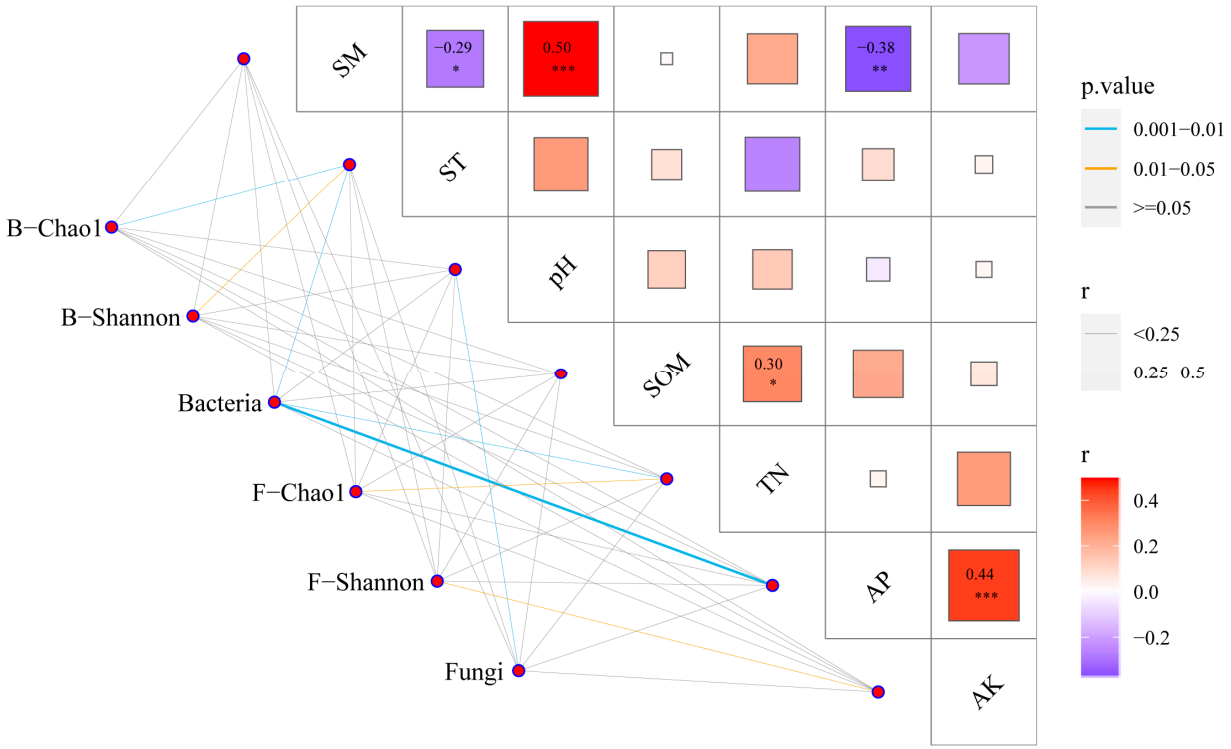


Figure 8. Correlation analysis between soil properties and the microbial community. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.5. Analysis of the Soil Microbial Symbiotic Network by Different Film Mulching Methods

Microorganisms often form complex network groups to accommodate specific soil compositions. In a co-occurrence pattern analysis, the clustering of nodes in a specific module of the network suggests that the corresponding microorganisms share similar ecological niches and/or functional interdependencies [33]. In our study, the network complexities of the bacterial and fungal communities were significantly affected by the different film mulching methods, which increased topological parameters, including node number, edge number, and average degree (Figure 9). The highest values of these indicators appeared at HM. In the bacterial topology, the number of nodes, edges, and average degree of HM treatment increased by 538, 2417 and 6.201, respectively, compared with NM treatment. Compared with FM treatment, HM increased by 720, 2860 and 4.431, respectively. In the fungal topology, the number of nodes, the number of edges, and the average degree of FM treatment increased by 11, 95 and 0.959, respectively, compared with NM treatment. Compared with HM treatment, FM increased by 4101 and 1.098, respectively. The clustering coefficient increased with increasing film-covering time, whereas modularity exhibited the opposite tendency (Table 3). These results indicate that HM treatment improved network complexity and competitive relationships among bacteria. For fungi, the number of nodes, number of edges, average degree, clustering coefficient, and modularity were significantly higher in the FM treatment than in the NM treatment, similarly indicating an increase in the relationship between fungal groups and the symbiosis mode of fungal communities.

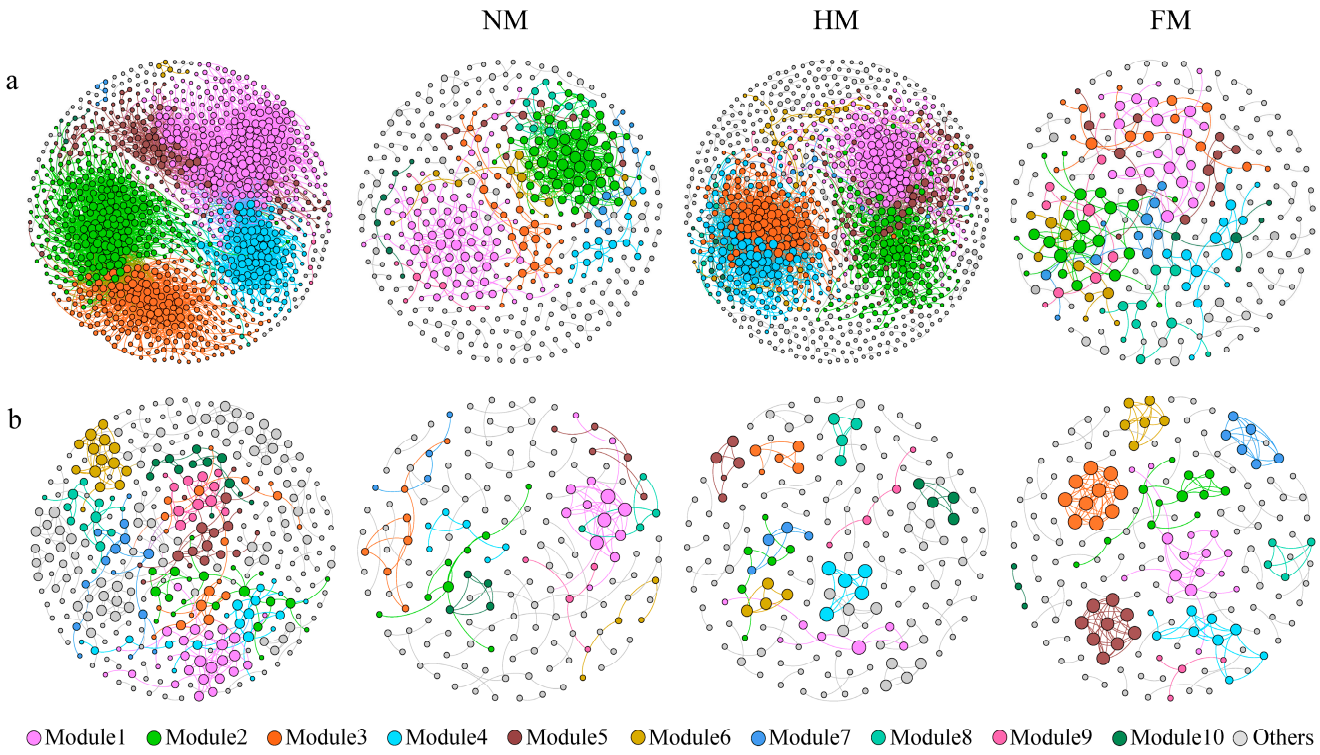


Figure 9. Diagram of the microbial co-occurrence network under different film mulching methods (NM, HM, and FM). Nodes represent individual operational taxonomic units. The margin represents a significant Spearman correlation ($R > 0.6$, $p < 0.01$). The main module is represented by different colors, and the smaller module is represented by gray. The size of each node is proportional to its degree. (**a**) Bacteria; (**b**) fungi.

Table 3. Soil microbial symbiosis network data under different film mulching treatments.

Network Properties	Treatment	Nodes	Edges	Average Degree	Clustering Coefficient	Modularity
Bacteria	NM	418	689	0.297	0.249	0.840
	HM	956	3106	6.498	0.298	0.685
	FM	236	246	2.085	0.336	0.609
Fungus	NM	167	147	1.760	0.458	0.906
	HM	174	141	1.621	0.703	0.909
	FM	178	242	2.719	0.806	0.970

3.6. Effect of Mulching Treatments on the Yield of Drip-Irrigated Maize

The yield of drip-irrigated maize differed under the different film mulching methods (Figure 10). The HM treatment significantly increased yield by 20.37% and 6.01%, respectively, over that of NM (12,603.63 kg·ha^{-1}) and that of FM (14,876.15 kg·ha^{-1}). This shows that whole-film mulching only moderately improved the yield, while an appropriately chosen partial period of film mulching can help in maximizing yield.

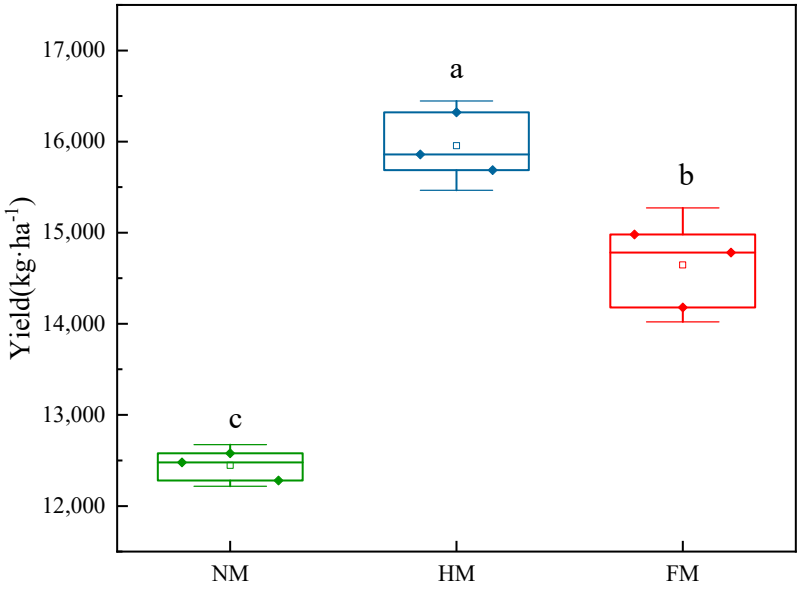


Figure 10. Effects of different film mulching methods (NM, HM, and FM) on maize yield. Different letters showed significant differences between different treatments ($p < 0.05$).

4. Discussion

Film mulching can alter the soil environment, thereby affecting the composition of microbial communities [34]. It has been determined that changes in soil temperature and soil moisture produced by mulching have a significant effect on microorganisms as they affect the survival and activity of microorganisms [35]. Film mulching has a profound impact on soil microbial community structure, soil nutrient cycling, and crop yield by changing the microenvironment. Therefore, it is a comprehensive study of its changes, which is of great significance. The soil microbial diversity and community structure under different plastic film mulching methods and their influencing factors. Overall, half-mulching treatments are more conducive to soil microbial diversity and community structure composition in drip irrigation maize root zones than full-film mulching and no-film mulching treatments. Film mulching has no significant effect on soil microbial fungi but directly affects the structure of bacterial communities. Mulching indirectly affects bacterial community structure through available phosphorus, total nitrogen, and soil temperature, while soil pH is an important factor affecting fungal community structure. Film mulching affected bacterial community structure by changing available phosphorus, total nitrogen, soil temperature, and other indicators, while pH was an important factor affecting fungal community structure.

4.1. Effects of Different Film Mulching Methods on Soil Microbial Diversity

In our research, each treatment had little effect on soil fungal α- and β-diversity. HM and FM treatments significantly affected soil bacterial diversity compared with NM treatments. This is similar to the results of previous studies on soil microbial diversity and the community of cotton [36]. However, film mulching reduced soil microbial diversity [37,38]. This is different from the results obtained in this study [39]. First, this may be due to the different mulching materials; the sensitivity of soil microorganisms to changes in the external environment is different [40]. Second, this may be because this study is a short-term experiment and has little effect on soil fungal diversity [41]. Long-term mulching has been reported to have no significant effect on fungal diversity but to lead to a decrease in soil bacterial diversity. Further, the sensitivity of bacterial diversity to the different treatments was higher than that of fungi. The results of this study are similar to those of previous studies [42,43]. Previous studies have shown that plastic film mulching improves soil nutrients, which in turn affects the composition of soil microbial communities in maize

fields. In the study, the film mulching method was also a short-term experiment, so the film mulching had no significant effect on fungi. Third, in this study, semi-mulching and full-mulching had no significant difference on pH, which could also explain why HM and FM significantly affected soil bacterial diversity compared with NM, while HM and FM treatments had no significant difference on soil microbial diversity. Many studies have shown that soil pH is the main predictor of soil microbial diversity [44]. Studies have shown that as soil pH increases, microbial diversity increases, and vice versa. When the pH value was not affected, the soil microbial diversity did not change [45].

Furthermore, previous studies have found that long-term mulching can significantly reduce soil microbial diversity, especially that of Proteobacteria and Ascomycota, whereas short-term mulching can increase soil microbial diversity [46]. Some studies have also shown that long-term mulching significantly reduces the α-diversity of soil microorganisms, while an appropriately chosen shorter mulching time may increase it. Soil microorganisms are greatly affected by various abiotic and biotic factors and changes in soil moisture, temperature, and gas conditions caused by the removal of the plastic film [47]. The results of the present study show that the FM treatment tends to reduce the α-diversity of soil microorganisms compared to the HM treatment. Overall, there was no significant difference in the α-diversity of soil microbial bacteria and fungi between HM and FM treatments, although there were increasing trends in all four indices with increasing soil depth. While the growth of maize reduces the amount of sunlight reaching the ground, the temperature and moisture difference between HM and FM treatments was small, and microbial diversity consequently did not change much in response. However, regional and environmental differences in previous studies have shown that there are corresponding differences in the sensitivity of soil microorganisms to environmental factors.

In our study, Actinobacteria and Proteobacteria were the main bacterial communities under different film mulching methods, which are similar to those obtained in organic mulching [48]. Actinobacteria are mostly saprophytes in the soil. They are functionally diverse and contribute to the decomposition of organic matter. They can break down complex substrates, giving them a competitive advantage over other bacteria. Proteobacteria are important phyla in the microbial community. They are capable of exploiting labile carbon sources and have higher relative abundances in nutrient-rich environments. It is worth noting that the relative abundance of Actinobacteria in the NM treatment reached 28.26% and 24.28% at the soil bacterial heading stage and maturity stage in this study, which were significantly higher than those in the HM and FM treatments. This differs from previous research results; this is mainly due to the different covering materials and covering time [49]. Studies have shown that in farmland ecosystems, the abundance of Actinobacteria is higher when the content of organic matter and nitrogen in soil nutrients is lower [50]. The organic matter and total nitrogen in the NM were significantly lower than those in the HM and FM; therefore, they were consistent with the above.

Mulching has been reported to significantly increase the diversity and abundance of soil microorganisms [31]. Some studies have shown that mulching can lead to a gradual increase in the amount of mulch residue in the soil, exacerbate the speed of succession of bacterial communities, and reduce the stability of the soil bacterial community structure [17]. A stable microbial community structure is important for the realization of ecological functions and plays an important role in ensuring the functioning of farmland soil. In this study, LDA analysis showed that the community complexity of the fungi treated was greater under the HM treatment than that under the FM treatment, implying commensurately better structural stability and resistance to stress. The HM treatment also improved network complexity and interactions among bacteria. The FM treatment increased the relationship between fungal groups and the symbiosis of the fungal communities. These outcomes may be due to two connected causes. Mulch cover acidifies the soil in the maize root zone, inhibiting the activity of the soil bacterial community and leading to a decrease in microbial complexity [30].

4.2. Effects of Different Film Mulching Methods on Soil Microbial Community Structure

Changes in the soil microbial community composition are usually related to environmental conditions, and mulching practices have a significant influence on microbial communities. Our analysis of the correlations between soil bacteria, fungi, and environmental factors shows that bacterial diversity, abundance, and community structure were significantly and negatively correlated with soil temperature. Previous studies have shown that the effect of temperature on the community structure and activity of soil microorganisms is prominent because increased temperature enhances the dominance of fungus, improves the production efficiency of the soil through the presence of some fungal enzymes, and overall greatly affects the community structure of microorganisms [51]. We found that microbial diversity was significantly correlated with soil physicochemical properties; however, community structure was slightly affected by soil moisture. Half-mulching not only increases maize yield but also keeps the microbial community structure in the soil more stable, which is determined by several factors. First, the warming and moisture-conservation effect of the film during the maize pre-reproductive stage slows down heat loss and drought in early spring, reduces stress inflicted on plants, and prolongs the reproductive period and photosynthesis time [52,53]. Second, the early stages of the Xinjiang drip irrigation maize planting process generally do not include irrigation, corresponding to a greater need for soil water retention, which is provided by the film. Third, the real-time irrigation connected to water and fertilizer integration reduces the need for moisture conservation. The late half-mulching makes the soil more permeable, increases the soil O_2/CO_2 ratio [8], and enhances soil microbial diversity and community structure, which in turn strengthens root respiratory metabolism, improves root vigor, and increases yield. Finally, mulching can greatly increase soil carbon dioxide (CO_2) emissions due to the increase in soil temperature, which is not favorable to maize growth. However, aeration significantly improved gas exchange and soil oxygen content, thereby increasing root activity. Roots affect the microbial community structure in soil by releasing secretions. Therefore, HM treatment exerted the advantages of plastic film in the early stage and improved root activity and soil microbial community structure through appropriate ventilation in the later stage [54].

5. Conclusions

Our study fills the gap in our understanding of the effects of different mulching techniques on soil bacterial and fungal community composition under maize. Mulching treatments, especially HM, played an important role in improving SM and ST conditions throughout the growing season while increasing soil nutrients (e.g., SOM and TN) and pH. Film mulching treatment had little effect on soil fungal α- and β-diversity. HM and FM treatments significantly affected soil bacterial diversity compared with NM treatments. Film mulching directly affected bacterial community structure and indirectly affected bacterial community structure through AP, TN, and ST, while soil pH was an important factor affecting fungal community structure. The HM treatment significantly increased maize yield (20.37% and 6.01% higher than NM and FM, respectively). In summary, HM treatment is a good practice for maintaining microbial diversity and changing microbial composition. The challenge in the future will be to better understand the changes in microbial patterns over time under different mulching treatments and to elucidate the expression of functional genes within each community. Further research is required to verify the effects of different film mulching regimes in other regions and under different environmental conditions.

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