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Communication

Comparative Analysis of Biodegradable Mulches on Soil Bacterial Community and Pepper Cultivation

Tuo Jin [1,2,*], Lin Li [3], Kewei Peng [3], Wei Li [3], Decai Jin [4], Wu Chen [3] and Jianwei Peng [1]

1 College of Resources, Hunan Agricultural University, Changsha 410128, China; jianweipenglab@hunau.edu.cn
2 Rural Energy and Environment Agency, Ministry of Agriculture and Rural Affairs, Beijing 100125, China
3 College of Plant Protection, Hunan Agricultural University, Changsha 410128, China; liling9855@126.com (L.L.); kewei75@hunau.edu.cn (K.P.); liwei350551@163.com (W.L.); wuchen77@hunau.edu.cn (W.C.)
4 Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China; dcjin@rcees.ac.cn
* Correspondence: jintuo273@126.com

Abstract: Biodegradable mulch films (BMFs) are becoming increasingly popular in agricultural practices. However, research on the ecological impact of biodegradable mulch films on pepper–soil systems is still scarce. To compare the differential effects of BMFs and polyethylene (PE) mulch on soil chemical properties, soil bacterial community composition, and pepper cultivation, a study was conducted encompassing eight distinct treatments. These treatments included three varieties of polybutylene adipate terephthalate (PBAT) combined with polylactic acid (PLA) mulches: PP-JL, PP-SD, and PP-SH; a black polypropylene carbonate mulch (PPC-BK); a brown PPC mulch (PPC-BR); a polyethylene (PE) mulch; straw mulching (NCK); and an uncovered control (PCK). After applying mulches for 129 days, most PPC and PBAT + PLA films had reached the rupture phase, whereas the PE film was still in the induction phase. Pepper yield was obviously higher in all mulched treatments (4830 kg hm^{-1}) than in the un-mulched control (3290 kg hm^{-1}), especially the BMF PP-JL treatment, which showed the most notable improvements in yield. Although BMF treatments maintained a lower soil temperature than the PE film mulch, they were still higher than the un-mulched control. Furthermore, the soil bacterial community composition and ecological network were not markedly affected by different mulching conditions. However, the PP-SH treatment significantly increased the abundance of *Pseudomonas*, *Nitrosomonas*, and *Streptomyces* genera. Moreover, *Lactobacillus* and *Gp16* were substantially more abundant in the PPC-black (BK) and PPC-brown (BR) treatments compared to the PE mulching treatment. This study could provide valuable insights into the ecological benefits of BMFs in pepper cultivation. However, as our experiments were conducted for only one season, it is imperative to undertake long-term experiments across consecutive seasons and years for a thorough understanding and comprehensive study.

Keywords: fully biodegradable mulches; pepper; soil properties; degradation rate; bacterial community

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1. Introduction

Plastic mulching is an agricultural strategy that can improve the soil microclimate and enhance crop yields [1–3]. The use of plastic mulch films has expanded globally, with over 1 million tons applied annually. Polyethylene (PE) films have been the predominant choice due to their durability, transparency, and low cost. However, the nondegradable characteristic of PE results in plastic accumulation in soil, which can negatively impact soil quality [4]. Biodegradable mulch films (BMFs) derived from materials such as polylactic acid (PLA) [5], polybutylene adipate terephthalate (PBAT) [6], and polyhydroxyalkanoates (PHA) [7] have emerged as alternatives. BMFs provide the same agricultural benefits as PE films. Furthermore, they are completely degraded over time to avoid plastic pollution [8,9].

Numerous studies have indicated that BMFs enhance growth and yields for crops such as cotton [10], tomato [11], peanut [12], and maize [13]. The microclimate modification provided by BMFs can increase soil temperature, conserve soil moisture, suppress weeds, and reduce soil erosion. The physicochemical changes in soil-applied BMFs, including temperature, moisture, salinity, and pH, can strongly influence microbial diversity and composition. In addition, potentially toxic substances associated with BMF degradation intermediates have been identified. However, there are concerns about the impacts of BMFs on soil health and crop yields compared to PE mulch. The stability and activity of the soil microbial community are vital for key ecosystem processes, such as nutrient cycling and organic matter decomposition [14]. Therefore, understanding the impacts of BMFs on native soil microbial communities is critical when evaluating their sustainability for agriculture.

The biodegradation of BMFs relies on microbial enzymatic activity, and their presence exerts strong selective pressure on soil's native microbial community. Previous studies have demonstrated that BMFs can significantly alter the soil bacterial community structure compared to bare soil [15]. Microbial diversity and biomass have been reported to decline under BMFs compared to bare soil [16,17]. Nevertheless, our understanding remains limited regarding how BMFs influence microbial community structure and diversity compared to traditional PE mulch. Furthermore, elucidating the ecological networks within soil influenced by BMFs could offer profound insights into how environmental changes, such as the biodegradation of BMFs, affect symbiotic relationships and ecosystem stability. Consequently, it is imperative to analyze the impact of BMFs on soil health and crop yields, in comparison to conventional PE mulch, to comprehensively assess the sustainability of BMFs.

BMFs provide several benefits in the growth and production of vegetables [18], yet scant attention is paid to their ecological impact on pepper (*Capsicum annuum* L.) cultivation. According to a report by the Chinese Ministry of Agriculture, China planted and harvested 8.27 million hectares of peppers, yielding 20.01 million tons, in 2021. Nowadays, China stands as one of the foremost producers of peppers globally. Notably, Hunan Province holds a particularly significant position in China's chili production. Furthermore, the application of mulching film on peppers in agricultural production possesses notable advantages and benefits, making it a cultivation method deserving of widespread adoption. Consequently, the objectives of this study are (1) to compare the degradation characteristics of various mulches used for pepper cultivation, (2) to investigate how the utilization of BMFs impacts soil chemical properties and pepper crop growth in comparison to PE mulch, and (3) to analyze the microbial community's response patterns to different mulches. This research will provide novel insights into soil microbial ecology under BMFs to support the expanded adoption of BMFs as an eco-friendly alternative to PE in agriculture.

2. Materials and Methods

2.1. Materials

2.1.1. Location, Climate, and Soil

The experimental station (113.07° E, 28.18° N) is located in farmland in Changsha City, China. The climate is subtropical monsoon, and the details of the weather during the experimental period (May to July 2021) are shown in Table 1. The chemical properties of the studied soil were as follows: pH 6.3, organic matter 27.1 g kg^{-1}, total nitrogen 2.43 g kg^{-1}, and total phosphorus 0.65 g kg^{-1}.

Table 1. The specific climatic trend during the experimental period.

	May	**June**	**July**
Monthly rainfall	173.8 mm	13 mm	57.9 mm
Minimum air temperature	15 °C	16 °C	25 °C
Maximum air temperature	34 °C	37 °C	37 °C
Average air temperature	23 °C	28 °C	30 °C

2.1.2. Experimental Design of Plastic Mulch and Plot Trials

In 2021, five types of BMFs produced by Baishan Xifeng, Shandong Qingtian, Shanghai Hongrui, and Hangzhou Jiale Honey, as well as one type of PE film, straw mulching, and an uncovered control were selected for the experimental mulching film treatment (Table S1). Each treatment was repeated three times. The plots were arranged in a randomized block design, with a plot area of 5 m^2. Soil was mulched on 21 April, and chili pepper (*Capsicum annuum* L.) (Zhangshugang, produced by Xiangying Country, Hunan, China) was transplanted on April 22nd. The fertilizer and water management measures were consistent for all treatments.

2.1.3. Soil Sample Collection

On the 19th day (10 May, T1), 49th day (10 June, T2), and 79th day (10 July, T3) following transplanting, soil samples were taken. Ten soil samples were taken randomly from the three plots for each treatment at a depth of 0–20 cm in the vicinity of the peppers. After removing stones and crop residues, the soil was mixed as one sample and placed in self-sealing bags, and six replicates were collected for each treatment in each period. Part of the soil samples was used for the extraction of total soil DNA and high-throughput sequencing analysis, while the other part was air-dried for the determination of soil chemical properties (organic matter, total nitrogen, and available phosphorus) and soil enzyme activities (β-glucosidase, acid phosphatase, urease, and sucrase).

2.2. Methods

2.2.1. Plastic Mulch Degradation and Soil Temperature

The visual observation method was used to survey the degradation rate and intensity of the plastic mulches. After mulching, they were investigated once every 5–10 days based on the degradation of mulches, being investigated a total of 11 times. The changes in the color, morphology, and surface integrity of the plastic mulch were recorded, with a focus on the appearance date of the induction period. Reference standards for plastic mulch degradation levels were as follows: Level 0: no cracks observed; Level 1: cracks begin to appear (induction period); Level 2: small cracks appear on 2% of the mulch in the field; Level 3: cracks of 2.0–2.5 cm appear; Level 4: cracks of more than 10 cm appear, and the mulch starts to thin; Level 5: the mulch decomposes into fragments below 4 cm × 4 cm and becomes thinner; Level 6: 25% of the ground has no visible mulch; Level 7: 50% of the ground has no visible mulch; Level 8: 75% of the ground has no visible mulch; Level 9: 100% of the ground has no visible mulch [19]. The temperature changes in the soil at a depth of 10 cm under the film were recorded using the Jingchuang GSP-6 temperature and humidity meter (Jiangsu Jingchuang Electronics Co., Ltd., Xuzhou, China), and data were automatically collected every 30 min. During the experiment, one of the instruments for NCK malfunctioned, and the remaining seven treatments operated normally.

2.2.2. Measurement of Agronomic Traits of Chili Pepper

After soil tilling, organic fertilizer (400 kg/667 m^2) and compound fertilizer (N:P_2O_5:K_2O = 25:10:10, 80 kg/667 m^2) were applied in strips, and then ridded and mulched. After transplanting, 5 kg/667 m^2 urea was applied to chili pepper before flowering, and 25–30 kg/667 m^2 compound fertilizer was applied during the full bearing period. Water was added by drip irrigation when the soil water content was below 50%. During the flowering and fruiting period of chili peppers, the plant height and stem diameter were measured. The chili peppers were harvested in batches when the fruit length exceeded 4 cm. In the later stage, the peppers were harvested every 10 days until no peppers were produced. Each plot was harvested separately and weighed using an electronic balance YH-T3 (Yingheng Electronic Scale Company, Shanghai, China) to calculate the chili pepper yield for each treatment.

2.2.3. Measurement of Soil Enzyme Activity

Five grams of soil was taken and air-dried in a cool place, then sieved through a 30–50 mesh sieve (Harbin Yongxu Company, Harbin, China). The enzyme activity of soil urease, β-glucosidase, acid phosphatase, and sucrase was determined using a test kit (Shanghai Enzyme Linked Soil Enzyme Activity Kit, Shanghai, China), following the methods provided in the instruction manual.

2.2.4. Soil Chemical Property Testing

The testing and analysis of soil chemical properties were performed in the Resource and Environment Testing Center of Hunan Agricultural University (Changsha, China). The testing methods for pH, soil organic matter, total nitrogen, and available phosphorus are referenced from the following standards: "Determination of Soil Organic Matter" NY/T 1121.6-2006, "Determination of Available Phosphorus in Soil" NY/T 1121.7-2014, and "Determination of Nitrogen in Forest Soil" LY/T 1228/-2015.

2.2.5. Soil Total DNA Extraction and High-Throughput Sequencing and Analysis

High-throughput sequencing was employed to investigate the effects of different mulches on bacterial community structure. Briefly, after vacuum freeze-drying the soil, 0.5 g was weighed to extract the total DNA using the FastDNA SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA). The extracted DNA was then tested by 1.0% agarose gel electrophoresis and stored at −20 °C for later use. The universal primers 515F and 806R were used to amplify the V4 region of the 16S rRNA gene, and sequencing adapters were added to the ends of the primers for PCR amplification, purification, quantification, and homogenization to form sequencing libraries. Sequencing was conducted using the NovaSeq 600 platform by Guangdong Meige Gene Technology Co., Ltd. (Guangzhou, China).

2.2.6. Statistical Analysis

Sequencing data were analyzed according to the pipelines presented in Men et al. [20]. The differences among groups were analyzed by one-way ANOVA post hoc tests using IBM SPSS Statistics 28. Based on the sequencing data of the 16S rRNA gene, a molecular ecological network (pMENs) was constructed. The network construction, hub, and connector OTU identification were performed using the random matrix theory (RMT) method (https://dmap.denglab.org.cn/ accessed on 23 April 2024), and the topological characteristics were determined using a similar threshold (0.960). OTUs that appeared in all 6 replicates were used for network analysis to ensure the reliability of correlations. The indices describing the network, such as average degree, average path distance, average clustering coefficient, and modularity, were generated using the fast greedy modularity optimization method. The network graph was visualized using Cytoscape 3.6.1 software (http://cytoscape.org/ accessed on 23 April 2024). Based on the sequencing data from 8 treatments, molecular ecological networks were constructed to reveal the interactions of soil microorganisms after mulching.

3. Results

3.1. Degradation Characteristics of Plastic Mulch

According to the survey, PPC-BR, as the first one, started the induction period on the 21st day after mulching, while PP-JL, PPC-BK, PP-SD, and PP-SH began the induction period on the 40th, 50th, 55th, and 64th day after mulching, respectively (Table S2). After 129 days of mulching, the degradation levels of these five types of plastic mulch films from high to low were PPC-BR (level 8), PPC-BK (level 7), PP-SD (level 5), PP-SH (level 4), and PP-JL (level 3), indicating that the degradation speed and level of PPC material-based plastic mulch films were faster and higher than PBAT + PLA material-based plastic mulch films. The PE film remained intact with only minor cracks, and no obvious degradation was found.

3.2. The Influence of Plastic Mulch on the Agronomic Traits and Yield of Pepper

There were no significant differences in pepper plant height and stem diameter among the treatments, but large differences in pepper yield were observed (Table S3). Among the treatments, the pepper yield of the PE film-covered treatment was 5140 kg hm^{-1}, and the PP-JL-covered treatment increased the yield by 14.59% compared to the PE film-covered treatment. The differences between PE and degradation film treatments in plant height and stem diameter were relatively small. Still, the plant height of degradation film treatments and NCK treatment was 2 to 3.6 cm higher than the PCK treatment, and the stem diameter was 0.5 to 1.4 cm larger. The pepper yield of the NCK and PCK treatments was lower than that of the fully biodegradable film-covered treatment. The pepper yield of treatments in descending order was PP-JL, PPC-BR, PPC-BK, PP-SH, PP-SD, NCK, and PCK.

3.3. The Influence of Plastic Mulch on Soil Temperature

The soil daily average temperatures of each treatment were analyzed, and the results showed that the PE film-covered soil was the highest at PE (29.55 °C), followed by PP-SD (29.37 °C), PP-SH (29.17 °C), PP-JL (29.09 °C), PPC-BK (28.95 °C), PPC-BR (28.35 °C), and PCK (28.26 °C) (Figure 1).

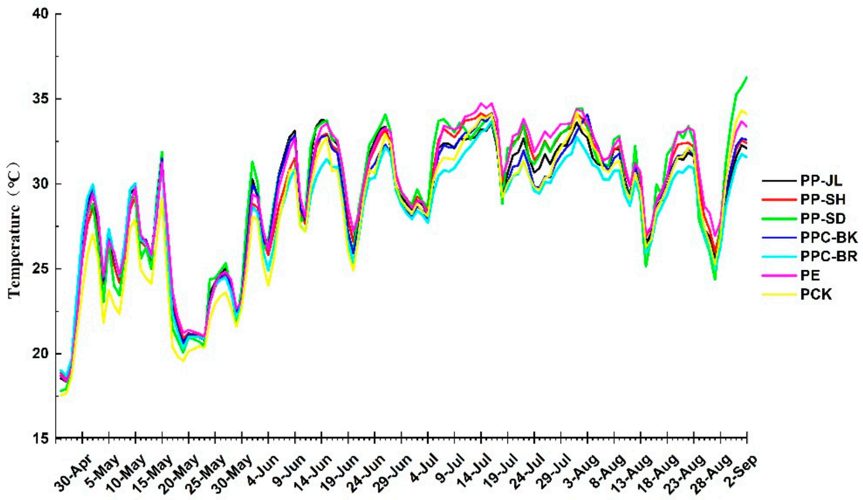

Figure 1. Daily average temperature variation in 10 cm of soil under different mulches. Biodegradable mulch films: PP-JL, PP-SD, PP-SH, PPC-BK, and PPC-BR. Polyethylene mulch: PE. Uncovered control: PCK.

Further analysis revealed that the influence of BMFs on soil temperature in the early stage (T1) of film covering was similar to that of PE film. In the middle stage of film covering, the differences in soil temperature among treatments were increased due to the variation in BMF degradation degree. Among BMFs, the PP-SD cover treatment showed the most apparent warming effect, with a soil temperature only 0.08 °C lower than that of the PE treatment, while the soil temperatures of PP-JL, PP-SD, PPC-BK, PPC-BR, and PCK treatments were 0.40 °C to 1.64 °C lower than that of the PE treatment. In the later stage of film covering, because the degradation of BMFs resulted in many cracks and voids without mulch, the soil temperature gradually approached the situation without mulch. However, the PE film showed the strongest warming ability with the highest soil temperature at 31.23 °C, which was due to no degradation of the PE film.

3.4. The Impact of Plastic Mulch on Soil Enzyme Activity

The soil enzyme activity in each treatment was detected. As shown in Figure 2A, there was no significant difference in sucrose enzyme activity between BMFs and PE film treatments in the T1 period, while the sucrose enzyme activity of PCK and NCK was significantly lower than that of the mulch treatments. In the T2 period, the sucrose enzyme activity of PPC-BK was significantly higher than that of PE, while there was no significant difference in sucrose enzyme activity between the other four degradation film treatments and PE (Figure 2A). Similarly, the sucrose enzyme activity of PCK and NCK was significantly lower than that of the mulch treatments. In the T3 period, there was no significant difference in soil sucrose enzyme activity between PP-JL and PE treatments, and that in PP-JL was significantly higher than that of the other four degradation film treatments (Figure 2A). The soil acid phosphatase activity in each treatment was also determined. As shown in Figure 2B, the acid phosphatase activity of PP-SH was significantly lower than PP-SD, PCK, and NCK in the T1 period. There was no significant difference in acid phosphatase activity between the degradation film treatments and the PE treatment during the T2 to T3 period.

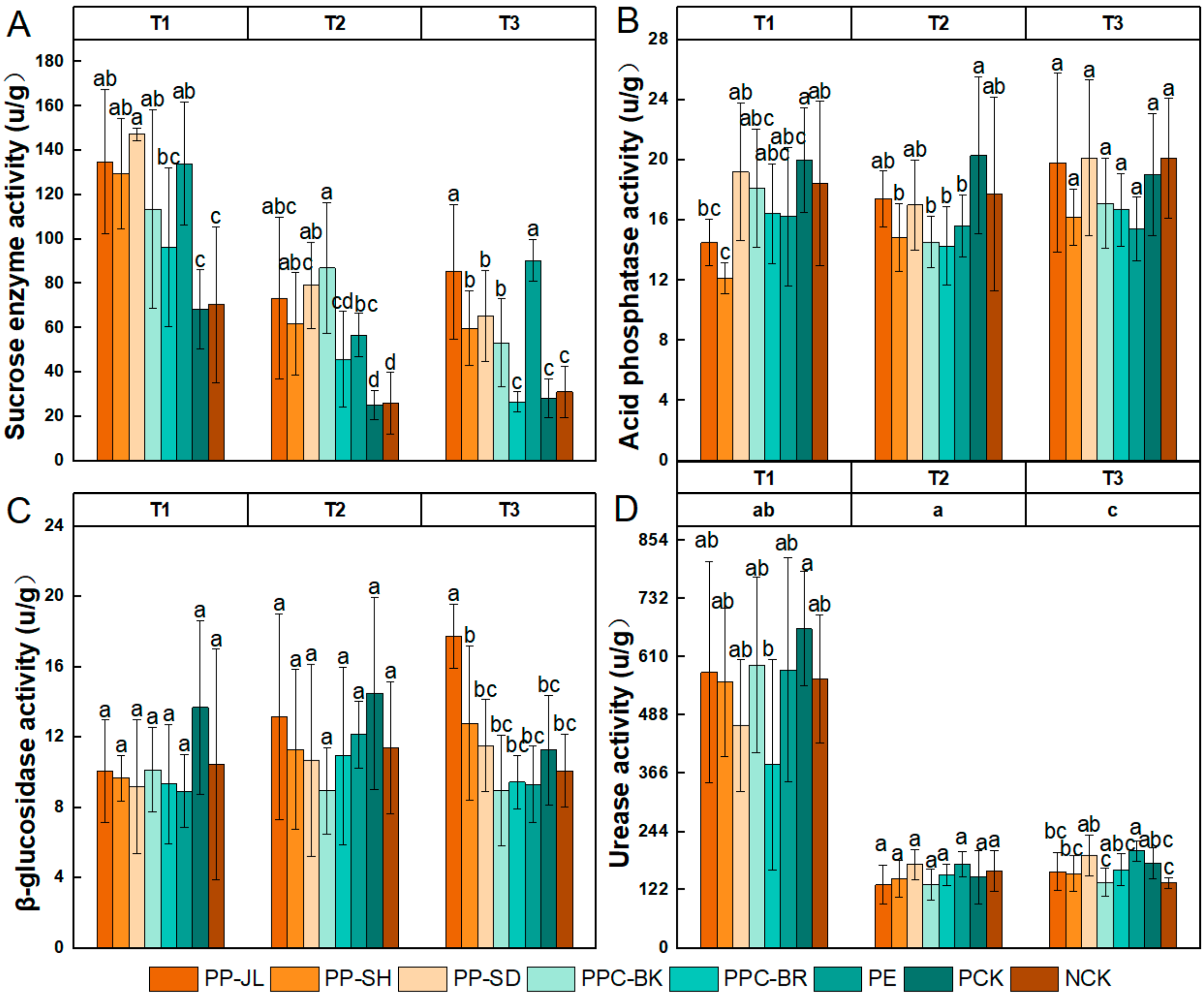

Figure 2. Dynamics of soil enzyme activity with different mulches. Bars represent the standard errors calculated from three replicates. Bars that do not share the same letter represent significant differences among treatments ($p < 0.05$) according to one-way ANOVA. (**A**) Soil sucrose enzyme activity; (**B**) Soil acid phosphatase activity; (**C**) Soil β-glucosidase activity; (**D**) Soil urease activity. T1: 10 May. T2: 10 June. T3: 10 July. Biodegradable mulch films: PP-JL, PP-SD, PP-SH, PPC-BK, and PPC-BR. Polyethylene mulch: PE. Straw mulching: NCK. Uncovered control: PCK.

In addition, the analysis revealed no significant difference in soil β-glucosidase activity among treatments in the T1 and T2 periods except the T3 period (Figure 2C). The soil β-glucosidase activity in the PP-JL treatment was significantly higher than in the other treatments, while there was no significant difference between the other four BMF treatments and PE treatment (Figure 2C).

Lastly, the soil urease activity of the above treatments was detected during the T1 toT3 periods. The results showed that the soil urease activity of all treatments in T1 was significantly higher than that of T2 and T3 (Figure 2D). In the T1 and T2 periods, there was no significant difference among the eight treatments. In the T3 period, there was no significant difference in soil urease activity between the PE treatment and the PP-SD, PPC-BK, PPC-BR, and PCK treatments, but there was a significant difference between the NCK treatment and the PP-SD, PPC-BR, and PE treatments.

3.5. The Impact of Plastic Mulch Covering on Soil Bacterial Community Diversity

The biological classification of soil bacteria from the eight treatments was analyzed. At the phylum level, the soil bacteria in each treatment included 43 phyla, but only 11 of them were dominant phyla with a relative abundance > 1% in three periods (Figure 3). The bacteria composition of the eight treatments was similar, including Proteobacteria, Acidobacteria, Actinobacteria, Thaumarchaeota, Bacteroidetes, Chloroflexi, Firmicutes, Gemmatimonadetes, Planctomycetes, Verrucomicrobia, and Nitrospirae, which accounted for 86.7–95.2% of the total bacterial community. From the T1 to T3 period, the population abundance of Thaumarchaeota and Firmicutes showed an increasing trend followed by a decreasing trend with the extension of mulching time, while the population abundance of Actinobacteria showed no significant change. Comparing the abundance of microbial populations at the phylum level among the treatments, the abundance of Proteobacteria in the PE treatment was significantly higher than in the other treatments. Compared with T1, the abundance of Thaumarchaeota was increased significantly in T2, with an increase of 10.0% in the PE treatment and an increase ranging from 5.9% to 10.0% in the other treatments. In addition, the abundance of Firmicutes increased by 6.6–12.9% in T2, with the highest increase in the PPC-BK treatment (12.9%).

At the genus level, the bacterial composition and abundance changes in each treatment are visualized in Figure 4. We focused on the bacterial species whose population abundance was significantly higher in the PP and PPC treatments than in the PE treatments, which were thought to be possibly caused by different mulching film types. After mulching, there were three trends: the abundance of *Pseudaethrobacter*, *Gp4*, and *Nitrosospira* was significantly increased; the abundance of *Nitrososphaera* and *Nocardioides* showed an increasing trend followed by a decreasing trend; the abundance of *Gp6* and *Gp16* showed a decreasing trend followed by a rising trend. In the T2 period, the abundance of *Nitrospira* in the PE treatment was significantly higher than in other treatments, and there was no significant difference in the abundance of *Nitrospira*, *Nitrososphaera*, and *Flavobacterium* in the degradation film. In the T3 period, the abundance of *Gaiella* and *Gp16* in the PPC-BK and PPC-BR treatments was significantly higher than in the PE and PP series treatments. Both duration time and types of mulch can cause changes in soil chemical properties.

Multi-response permutation procedure (MRPP) analysis was used to determine whether there were significant differences between the intergroup data and to compare the effects of biodegradable plastic mulch on soil bacterial community composition. According to Table S4, there were significant differences between PE and PPC-BK/PPC-BR in T1 but no significant differences between PE and PP series films. The intergroup differences in community composition were significant in T2, with significant differences between PE and PPC-BR, PPC-BR and PP-JL, and PP-SD and PPC-BK, but no significant differences among the PP series treatments. In T3, there were significant differences between PE and PP-JL and PP-SH and significant differences between PP-JL, PP-SH, and PPC series, as well as significant differences between the PPC-BK and PP treatments.

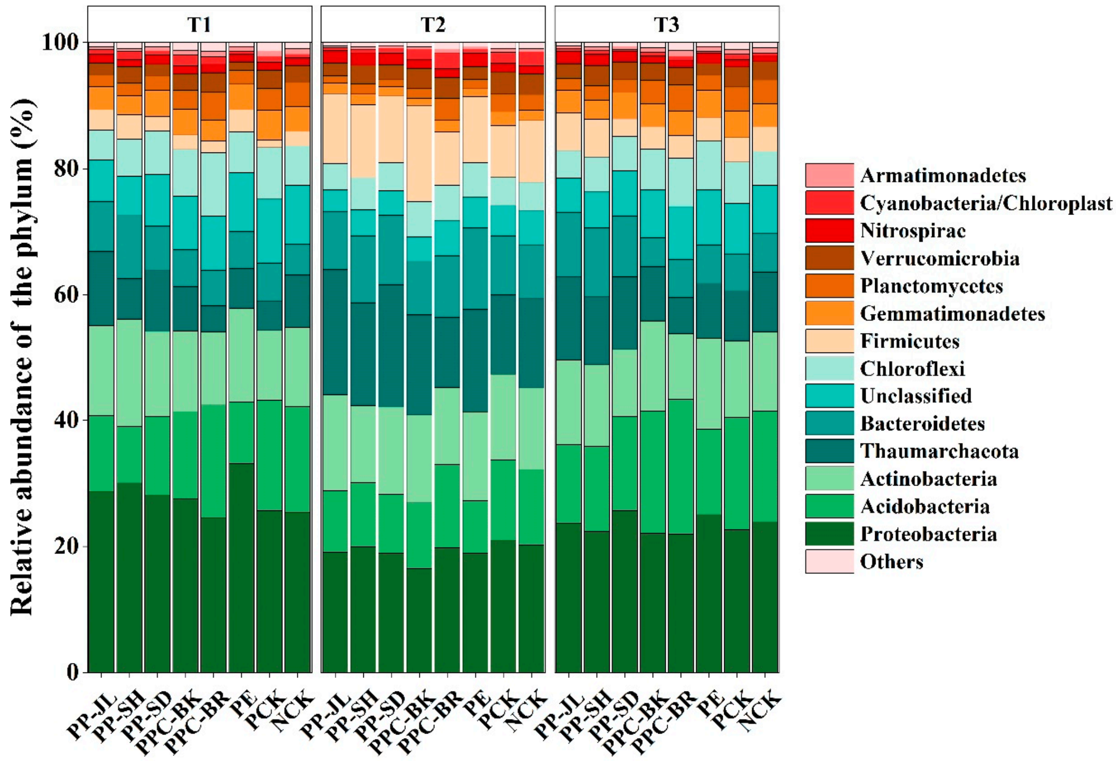

Figure 3. Relative abundance of soil bacterial communities at the phylum level in different mulches. T1: 10 May. T2: 10 June. T3: 10 July. Biodegradable mulch films: PP-JL, PP-SD, PP-SH, PPC-BK, and PPC-BR. Polyethylene mulch: PE. Straw mulching: NCK. Uncovered control: PCK.

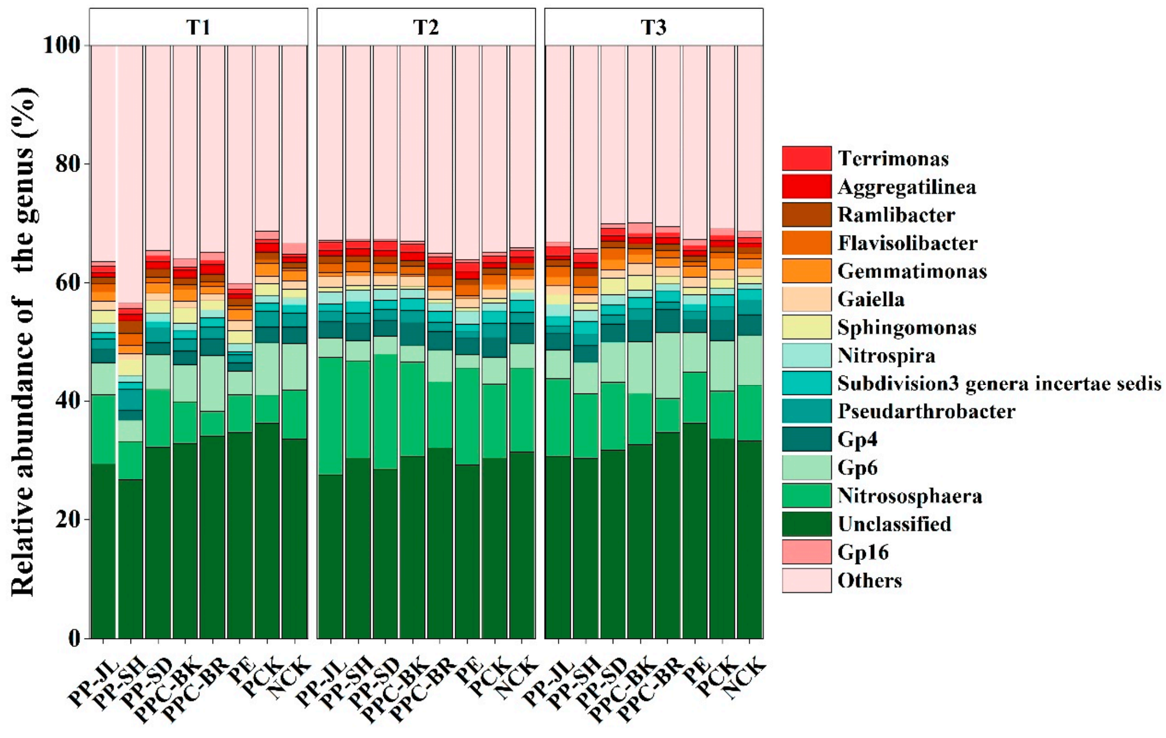

Figure 4. Relative abundance of soil bacterial communities at the genus level in different mulches. T1: 10 May. T2: 10 June. T3: 10 July. Biodegradable mulch films: PP-JL, PP-SD, PP-SH, PPC-BK, and PPC-BR. Polyethylene mulch: PE. Straw mulching: NCK. Uncovered control: PCK.

The α-diversity of bacterial communities, including the Shannon index, Simpson index, and Chao1 index, was analyzed (Table S5). There were significant differences in Shannon index and Simpson index between the PP and PPC treatments, with the PP treatments having lower values for both indices. There were no significant differences in Shannon index between BMFs and PE in the T1 period. And in the T2 period, the Shannon index of PPC-BR was significantly higher than PE, PP-JL, PP-SH, PP-SD, and PPC-BK, while there were no significant differences between PE and PP-JL, PP-SH, PP-SD, PPC-BK; there were also no significant differences among the BMF treatment groups. Similarly, in the T3 period, PP-JL was significantly lower than PP-SD and PP-JL, and there were significant differences between PP-JL and PP-SH and PPC-BK. Comparing the Simpson index of treatments, there was a significant difference between PPC-BR and PE in the T1 period, as well as between the PP series and PPC series; there were significant differences between PPC-BR and PE in the T2 period but no significant difference between PE and other BMF treatments; in the T3 period, there was a significant difference between PP-JL and PPC-BR and no significant differences between other six treatments. Moreover, the Shannon index and Simpson index of the three PP treatments showed decreased variation from the T1 to T3 period.

On the other hand, the Chao1 index, representing species richness in the sample, was analyzed. Overall, the Chao1 index of the five BMF treatments showed a decreasing trend from the T1 to T3 period. In addition, PPC-BR was higher than the other four BMF treatments, and all of them were lower than that of PE (Table S5). In the T1 period, the Chao1 index of PE was significantly higher than PP-SH, PP-SD, and NCK. And also, in the T2 period, the Chao1 index of PE was higher than that of PP-JL, PP-SD, and PPC-BK. Moreover, the Chao1 index of PE was significantly higher than that of PP-SD and PPC-BK in the T3 period. These results indicated significant differences in Chao1 index between PE and PP-SD in three periods.

Non-metric multidimensional scaling (NMDS) was used to analyze the β-diversity of soil bacterial communities in each treatment. In NMDS analysis, the closer the distance between samples, the more similar their species composition structure is. In the T1 period, PE treatment was distributed widely and could not be well separated from other treatments, indicating that the structural difference in species composition was small between PE and other treatments (Figure 5). However, in the T2 period, the PE was distributed compactly, so the overlap between PE and other treatments was decreased, indicating an increasing differentiation in structural composition. Furthermore, the PE distribution was significantly clustered in the T3 period, and it did not overlap with PP-JL and PP-SH, indicating their significant difference in species composition. Overall, the difference between the BMF treatments and the PE treatment became more pronounced from the T1 to T3 period. In each treatment, the distance between samples had a tendency to cluster together, and the coverage type affected the composition of the bacterial community, but the clustering trend of T1, T2, and T3 was more prominent, indicating that the climatic conditions had a more significant impact on the bacterial community.

The molecular ecological network structure of soil bacterial communities was constructed by the theory of random matrix (RMT) based on soil bacteria 16S rRNA gene high-throughput sequencing data at the T1, T2, and T3 time points for each treatment. The results showed that the positive and negative interactions accounted for about 50% each in the T1 and T2 periods, but the difference in positive and negative interaction in the molecular ecological network of T3 tended to increase: the positive interaction in the PP-SH treatment decreased from 56.0% to 17.7%, and the negative interaction increased to 82.2%; the positive interaction in the PP-SD treatment decreased from 55.4% to 12.1%, and the negative interaction increased to 87.2%; the positive interaction in the PPC-BR treatment decreased from 54.9% to 26.9%, and the negative interaction increased to 73.0% (Figure S1). These results indicate that in these four BMFs, the positive interactions such as symbiosis and mutual promotion between different species are transformed into competition or antagonism in the later stage after mulching, reducing beneficial relationships and increasing antagonistic relationships. In contrast to the three aforementioned treatments, the positive interaction in PPC-BK, PE, PCK, and NCK treatments increased by 10.8%, 21.9%, 1.8%, and 7.9%, respectively. This indicates that the soil bacterial communities in these four treatments form a more closely connected network through synergistic and mutual promotion interaction between different species.

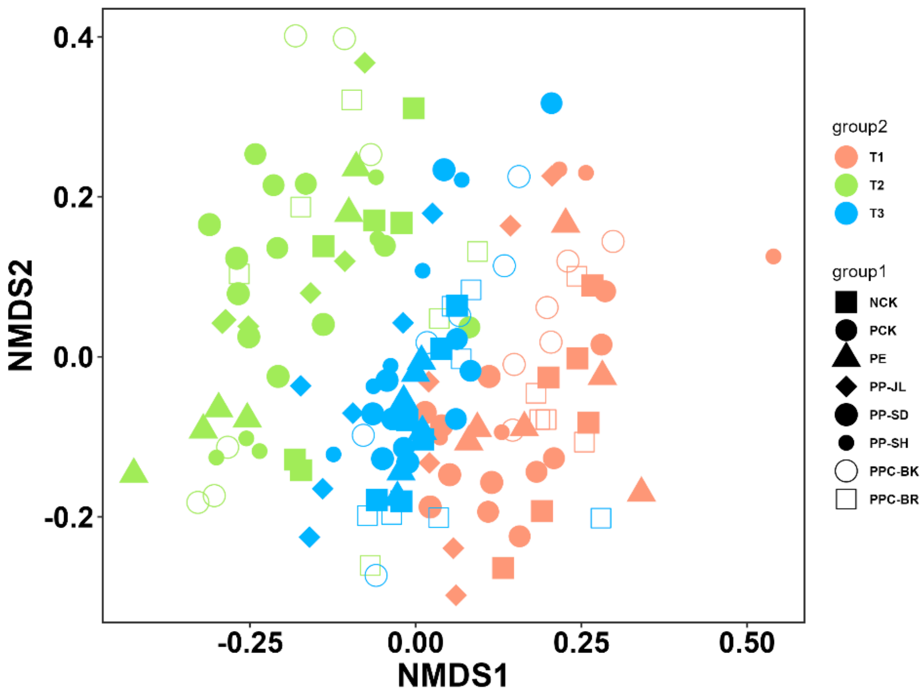

Figure 5. NMDS analysis of soil bacterial communities under different mulches. T1: 10 May. T2: 10 June. T3: 10 July. Biodegradable mulch films: PP−JL, PP−SD, PP−SH, PPC−BK, and PPC−BR. Polyethylene mulch: PE. Straw mulching: NCK. Uncovered control: PCK.

The network topology properties and network topology structure of each treatment varied considerably (Table S6). Under the same threshold of 0.960, the PP-JL, PP-SH, and PP-SD treatments exhibited a pattern of low, high, and low numbers of nodes and connections across three periods, whereas the PPC-BK and PE treatments displayed an opposing trend; the PPC-BR showed a significant downward trend, while the PCK treatment had a small change range and remained stable overall.

Moreover, in the T1 period, only the PP-SD and PPC-BK treatments had lower connection than the PE treatment, while the other treatments had higher connection than the PE treatment, for example, PPC-BR, which was higher than PE; and in the T2 period, the PP-SH, PP-SD, and PPC-BR treatments were higher than PE.

Under the same threshold, the ecological network parameters of the BMF treatments and the PE treatment were different. The topological properties of the bacterial community network are shown in Table S7. In the T1 period, the average degree (avgK) of PE was higher than PP-SH and PPC-BK; in the T2 period, the average degree of all treatments was higher than PE; in the T3 period, the average degree of PE was higher than PPC-BK, indicating that the BMF treatment network had more connection between nodes and was more complex. In terms of the average geodesic distance (avgGD) of the network nodes, the average clustering coefficient of PE in T1 was lower than PP-SH and PPC-BR; in T2, only the average clustering coefficient of PPC-BK was lower than PE; the average clustering coefficient of each treatment in T3 was similar, indicating that the higher degree of connection between adjacent nodes, the easier it is for nodes to cluster together. Overall, in T2, the OTUs in the PP series degradation film treatment were more closely connected, with a denser and more complex structure.

In ecological networks, nodes act as connectors, module hubs, and network hubs, and play a crucial role in facilitating energy, information, and material exchange among different species. The analysis of key microorganisms in the molecular ecological networks of soil bacterial communities revealed that most of the node connections in terms of module intra-connectivity (Zi) and module inter-connectivity (Pi) were distributed within the modules. As shown in Table S8, all the key OUTs in this study were found in the networks of the PP-JL treatment, with 11 module hubs, including 3 in the T1 period, 2 in the T2 period, and 6 in the T3 period, and 34 connectors, including 12, 18, and 4 in the T1 to T3 period, respectively. However, there was an overlap between the connector nodes and the hub nodes of the modules. Overall, the networks of the PP-JL and PPC-BR treatments in the T1 and T2 period showed higher module connectivity. The PP-JL, PP-SH, and NCK treatments had more nodes connecting within their own modules and between modules, forming more stable, organized, and efficient networks.

3.6. Analysis of the Correlation between Environmental Factors and Microbial Community

Soil chemical properties were also obtained (Table S9). And Mantel tests were carried out to identify the correlations between environmental factors and soil bacterial communities (Table S10). It was found that soil pH significantly influenced the soil microbial community structure, with p-values < 0.001. In addition, urease and sucrose also had a significant impact on the community structure of soil microorganisms. Other environmental factors, such as acid phosphatase, glucosidase, organic matter, available phosphorus, and total nitrogen content, did not show a significant impact on the soil microbial community structure.

4. Discussion

There were significant differences in the degradation characteristics of different BMFs. The different integrity of the films led to significant differences in soil temperature and directly affected soil physicochemical properties, soil enzyme activity, and microbial diversity. These differences ultimately manifested in substantial variations in the agronomic traits and yield of pepper. The PPC-BR film cracked prematurely, which resulted in a decrease in its warming and moisture-retaining effect, and consequently, the lower agronomic traits of pepper. The PPC film started rapid degradation after 55 days of coverage, with a degradation intensity of 7–8 levels at the time of crop harvest, which was close to the no-film state. The PP series treatments had a degradation intensity of 3–5 levels on the soil surface at the time of crop harvest, and the buried part of the film had completely degraded, while the PE film only showed slight degradation. The pepper yield of the BMF and PE-covered treatments was higher than that of the PCK treatment, which is similar to the conclusions of the previous study [21]. The soil enzyme system is the most active part of the physiological activity in soil, and soil enzymes are indispensable bioactive substances for energy flow and material transformation in the soil ecosystem [22]. It is reported that the activity of soil urease and β-glucosidase is enhanced with an increase in soil temperature in winter. On the contrary, the rise in soil temperature in summer decreases soil enzyme activity [23]. This finding can explain the results of this study, which show that the enzyme activity of the PE mulched soil was lower than that of the BMFs in summer. The different ability to control soil temperature by BMFs is reflected in the mulched soil enzyme activity. The different enzyme activity in response to temperature changes depends on the types of enzyme, and further systematic research is needed to elucidate the differences in soil enzyme activity under various BMF coverage.

The differences in pepper’s agronomic traits (Table S3), the composition of soil bacteria community (Table S4), and its correlation with soil chemical properties (Table S9) among BMFs and PE treatments were analyzed in this study. At the phylum level, the dominant bacteria groups in the soil of each treatment were Proteobacteria, Acidobacteria, Actinobacteria, Thaumarchaeota, Bacteroidetes, and Chloroflexi. Acidobacteria is a kind of oligotrophic bacteria that can grow in nutrient-poor or barren environments [24]. Previous studies have shown that Acidobacteria is particularly abundant in soil habitats [25], which is consistent with the findings of this study. Proteobacteria belong to saprotrophic bacteria and have a higher growth rate in nutrient-rich environments. The abundance of Proteobacteria in the soil under the PE film cover treatment was higher than that under the BMF treatments. At the genus level, the dominant bacteria in the soil of each treatment were *Nitrosospira*, *Gp6*, *Gp4*, and *Sphingomonas*. Previous research has shown that *Sphingomonas* is mainly involved in the degradation of polycyclic aromatic hydrocarbons in the soil [26], while *Sphingomonas* plays a positive role in inducing systemic resistance and promoting plant growth [27]. Further analysis revealed that among BMF treatments, the abundance of *Pseudomonas*, *Nitrospira*, and *Streptomyces* showed a significant increase in the PP-SH treatment, while the abundance of *Gaiella* and *Gp16* significantly was increased in the PPC-BK and PPC-BR treatments. Meanwhile, soil bacteria with the potential to degrade polymers, such as *Streptomyces* [27], *Pseudaethrobacter* [28], and *Nitrosospira*, had no significant differences in the relative abundance among different degradation films in the later stage of covering; the increase in these genera may have been due to changes in agronomic management, temperature, humidity, and other conditions in the plot [29,30]. In addition, the soil microbial community structure of the PE treatment was significantly different from the PP-JL and PP-SH treatments, and the PP-JL and PP-SH treatments were also different from the PPC-BK, PPC-BR, PCK, and NCK treatments. These results indicate that different BMF treatments significantly affect the composition of the soil bacterial community, which is consistent with the findings of Zhao et al. [12]. Regarding the analysis of environmental factors on soil microbial communities, soil pH was an important factor affecting the structure and composition of bacterial communities (Table S10).

A higher Shannon index and Simpson value indicate greater richness of bacterial diversity. Throughout the mulching process, the PP series treatments exhibited varying degrees of decline in both the Shannon index and Simpson index. The Chao1 index of the BMF treatments showed a decreasing trend and was smaller than that in the PE treatment. Soil bacterial diversity is an important indicator of soil bacterial functions and is greatly influenced by human activities [31]. In this study, the α diversity index of the BMF treatments showed a decreasing trend. However, the changes in each treatment over time combined with their yield proportions indicate that the PP series, when covered until June, increased the interaction among soil microorganisms and greatly promoted yield. The β diversity analysis of soil bacteria in each treatment showed that the BMF treatments and the PE sample were not well separated, and their intergroup difference was not significant. However, with the passage of time, the PE treatment gradually clustered and gradually distinguished itself from the degradation film treatments, indicating the difference in species composition structure. By constructing soil bacterial ecological networks under different mulching conditions to explore the interaction and symbiotic patterns of communities, the negative interaction relationships between network nodes and connections of the PP-SH and PP-SD treatments were significantly increased, indicating that the interactions between bacterial nodes are mostly competitive and antagonistic. On the other hand, the positive interaction between network nodes and connections of the PE cover condition was increased.

In the networks of the eight treatments, the key nodes were all dominant genera including *Deinococcus*, *Phycomycetes*, *Actinobacteria*, *Pseudomonas*, and *Rhizobium*. Among them, it was found that *Pseudomonas*, *Bacillus*, *Pseudomonas*, *Streptomyces*, and *Nocardia*, which were key nodes in the constructed network in this work. *Pseudomonas* has a strong ability to metabolize and clean soil toxins and promote crop growth. It appears to be a key point in the BMF treatments and NCK treatment, playing a role in soil remediation and fixing soil nitrogen. *Bacillus* plays an important role in decomposing complex polysaccharides and water-soluble organic matter. It functions as a key connector node in the PP-JL treatment network, promoting the connection between nodes within the module. It was reported that *Streptomyces*, *Nocardia*, and *Actinobacillus* could degrade clustered compounds [32,33]. Different strains of *Nocardia* have outstanding abilities of decomposition and metabolization for aliphatic and aromatic hydrocarbons, various nitrile polymers, and so on. *Nitrosomonas* and *Nitrospira* have multiple enzyme-catalyzed metabolic pathways, accompanied by complex substance and energy transformations [34]. They function as key points in the PP series treatments. These genera have different mechanisms of action, degrading the components of plastic mulch, reducing degradation materials that cause damage to soil, and promoting the interaction between soil microorganisms and crop growth.

In conclusion, our findings revealed that the degradation characteristics of various mulches exhibit significant variations, and distinct mulching treatments have diverse impacts on soil chemistry, enzymatic activities, and microbial communities. Ultimately, they may affect the agronomic traits and yield of pepper. However, since our experiments were conducted at only one site, different climates, soil types, etc., may have influenced the conclusions, and similar studies at larger scales are needed.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy14050905/s1. Table S1: Details of the tested mulch films; Table S2: Degradation strength of BMF and PE films; Table S3: Agronomic characters and yield of pepper treated with plastic films; Table S4: MRPP analysis of bacterial community in different treatments; Table S5: Alpha diversity of bacterial community in different mulches; Table S6: Number of nodes and connections in the building network; Table S7: Molecular ecological network index of soil bacterial communities covered by different mulches; Table S8: Number of critical nodes present in each network; Table S9: Soil chemical properties in different mulches; Table S10: Mantel test analysis between soil environmental factors and bacterial community; Figure S1: Molecular ecological network of bacterial communities in different mulches.

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