

## Article

# Preliminary Findings of Polypropylene Carbonate (PPC) Plastic Film Mulching Effects on the Soil Microbial Community

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**Abstract:** The farmland residual film pollution caused by traditional PE film has an adverse impact on crops and the environment. Polypropylene carbonate (PPC) film is a fully biodegradable film that can alleviate “white pollution”. In this study, the soil physicochemical properties and the composition and function of the soil community of FM (PPC film mulching) and CK (no film) treatments were determined to explore the effect of PPC film mulching on soil and the soil microbial community. Furthermore, the microorganisms at different time periods during the degradation of PPC mulch film were also analyzed. The results showed that film mulching increased soil pH but decreased the contents of EC and SOC, and there was no significant difference in the contents of AP and AK. The relative abundance of the phyla *Acidobacteria* was increased with film mulching, and the relative abundance of the genera *MB\_A2\_108* also increased in the film mulched soil. Among the soil physicochemical properties, pH and SOC were the most important factors leading to changes in the composition of the bacterial and fungal communities. PPC film mulching had no significant effect on soil microbial community abundance and diversity. In addition, *Pseudomonas*, *Flavobacterium*, and *Rhizobacter* were dominant in the degradation of PPC film. Our research results provide a scientific theoretical basis for soil safety and the large-scale use of PPC biodegradable mulching films and a research foundation for the degradation of PPC plastics.

**Keywords:** PPC film mulching; soil microbial community; soil organic carbon; soil biological safety



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## 1. Introduction

Film mulching technology for farmland is an important strategy to maintain soil moisture and increase the soil temperature and crop yield [1,2]. It can effectively change the growth environment of crops, promote early maturity of crops, and increase crop yields and water use efficiency [3,4]. Usually, the plastic film material used for mulch is polyethylene (PE) plastic, and 48.7% of polyethylene film products are used for farmland film covering. However, traditional PE film causes serious farmland pollution [5]. At present, the annual residual film in the field is as high as 350,000 tons, and the residual rate of film is 42%. The residual film gradually accumulates over the mulching years [6]. Plastic film residues pose a huge threat to the soil, soil microbial community, and crop growth and yield [7–9]. It was reported that the residual plastic mulch film can destroy the structure of soil aggregates and reduce soil ventilation and water permeability, and the residual film can also affect the absorption of water and nutrients by plant roots and reduce root growth and overall plant productivity [10–12]. Another study showed that soil microbial carbon, nitrogen, enzyme activity and microbial diversity gradually decreased with the increase in residual film [13]. In order to reduce the pollution arising from traditional film, biodegradable film may serve as a suitable ecologically sustainable substitute.

Biodegradable film can be completely degraded under the action of soil microorganisms; thus, it can be directly plowed into the soil after use without residual film recovery and processing [14–17]. Polypropylene carbonate (PPC) is a new type of biodegradable plastic, and is the product of the “alternating” copolymerization of carbon dioxide and propylene oxide (PO), of which the CO<sub>2</sub> content accounts for more than 42% [18–20]. According to the “2020–2024 China Polypropylene Carbonate (PPC) Product Market Analysis Feasibility Study Report”, PPC plastics account for about 6% of the biodegradable plastics industry scale. In 2019, the output of PPC was about 20,000 tons, an increase of about 20% compared with 2015. PPC film has good barrier properties, and its water vapor barrier capacity is the most similar to PE among the biodegradable plastics, such that PPC film can achieve an agronomic performance similar to that of polyethylene in terms of heat preservation and moisture retention. Yang et al. explored the degradation of PPC film in farmland soil, and found that the PPC film buried in the soil lost 39.67% of its weight in 150 days [21]. To explore the application of PPC mulch in agricultural production, Bi et al. conducted experiments in mulching sorghum with PPC film and PE film, and found that neither film had any effect on sorghum yield [22]. However, the biological safety of PPC film and its influence on the soil microbial community structure, and soil physical and chemical properties, have not yet been sufficiently studied. In addition, the soil microbiome is crucial for plant growth and adaptability [23]. The effects of soil physical and chemical properties and microbial communities on crop growth are related to the crop yield and economic benefits. Therefore, whether the physical and chemical properties of the soil and microbial communities change due to PPC film mulching is worth investigation. Research on the response of soil microbial community to the PPC film will provide a reference for the safety and large-scale use of biodegradable film.

In this study, the physical and chemical properties of the soil after PPC film mulching were determined, and 16S rRNA gene/Internal Transcribed Spacer (ITS) high-throughput sequencing analysis was performed to explore the microbial community structure. The main objectives of the current study were: (i) to understand how PPC film mulching affects the composition and structure of the soil bacterial and fungal communities; (ii) to elucidate the coupling relationship between the changes in the soil physical and chemical properties and the changes in soil microbial communities caused by PPC film mulching; and (iii) to explore the related communities of degrading PPC film and whether it has the degradation ability.

## 2. Materials and Methods

### 2.1. Sampling

In November 2020, a PPC-covered soil sample (FM) and the uncovered soil sample (CK) were collected from the experimental field (125°406'554" E, 44°000'361" N) of the Northeast Institute of Geography and Agricultural Ecology, Chinese Academy of Sciences, Changchun City, Jilin Province, China. The altitude of this area in the northeast is 250 m, the annual average temperature is 6.5 °C, the frost-free period is about 150 days, the annual precipitation is 800–1000 mm, and the soil type is dark brown soil [24]. The experiment was set up in May 2020. A PPC mulch film of 0.008 mm × 45 cm × 500 cm covered the soil surface, the uncovered soil was used as a control, and there was a 25 cm gap between the covered soil and the uncovered soil. The soils (0–10 cm from the top) were collected using a soil borehole sampler (4 cm diameter) using an “S”-shaped sampling method, and soils from five locations were fully homogenized and treated as one biological replicate, and three biological replicates were collected for each treatment (FM and CK). The samples were then sieved through a 2 mm sieve to remove visible impurities such as roots. All samples were divided into two sub-samples; one sub-sample was air-dried at room temperature for soil property analysis, and the other sub-sample was stored at –80 °C for DNA extraction.

## 2.2. Soil Physicochemical Analysis

Soil pH and electrical conductivity (EC) were measured following the methods of Ji Li et al. [24]. Soil organic matter (SOM) was evaluated as previously described by Shasha et al. [25]. Soil available phosphorus (AP) and available potassium (AK) were evaluated using the methods described in the previous study of Shi Shaohua et al. [26].

## 2.3. DNA Extraction, PCR and Sequencing

The V4 segment of the bacterial 16S rRNA gene and the fungal ITS1 segment was amplified with the relevant primers with a barcode. The bacterial 16S rDNA gene V4 segment amplification primers were 515F: 5'-GTGCCAGCMGCCGCGGTAA-3' and 806R: 5'-GGACTACHVGGGTWTCTAAT-3'; the fungal ITS1 segment amplification primers were ITS5F: 5'-GGAAGTAAAAGTCGTAACAAGG-3' and ITS2R: 5'-GCTGCGTTCTTCATCGATGC-3'. The PCR reaction system (50  $\mu$ L) was as follows: Forward and reverse primers (10  $\mu$ mol/L) 2  $\mu$ L each, dNTPs (2.5 mmol/L) 4  $\mu$ L, 10  $\times$  Pyrobest buffer 5  $\mu$ L, Pyrobest DNA polymerase (2 U/ $\mu$ L) 0.3  $\mu$ L, ddH<sub>2</sub>O dissolved DNA 36.7  $\mu$ L (1 ng/ $\mu$ L). The PCR reaction conditions were: 95  $^{\circ}$ C 5 min; 95  $^{\circ}$ C 30 s, 56  $^{\circ}$ C 30 s, 72  $^{\circ}$ C 40 s, a total of 25 cycles; 72  $^{\circ}$ C 10 min [27]. The Illumina-MiSeq platform was used for paired-end sequencing, and the raw data obtained was filtered to cut off the end sequence of reads with a quality value of less than 20, remove reads from linker contamination, remove reads containing N, and remove low complexity reads. Then, we used FLASH (Fast Length Adjustment of Short reads, v1.2.11) to assemble the paired reads obtained by paired reads sequencing into a sequence using the overlap relationship to obtain tags in the hypervariable region (the minimum matching length was 15 bp, and the overlapping region allowed mismatches; the rate was 0.1, removing the reads without overlap relationships). The classify-sklearn algorithm of QIIME2 (<https://github.com/QIIME2/q2-feature-classifier>, accessed on 10 November 2021) was used. For the characteristic sequence of each ASV, the pre-trained Naive Bayes classifier was used for species annotation in QIIME2 software with default parameters. For bacterial 16S rRNA sequences, species taxonomic annotation was performed using the Greengenes database (Release 13.8, <http://greengenes.secondgenome.com/>, accessed on 10 November 2021); and for fungal ITS sequences, species taxonomic annotation was performed using the UNITE database (Release 8.0, <https://unite.ut.ee/>, accessed on 10 November 2021). The confidence threshold was set to 0.6.

## 2.4. Bioinformatics and Statistical Analysis

QIIME2 was used for sparse curve and taxonomic composition mapping. Alpha diversity indices including Chao1 and Shannon indices were derived using the "phyloseq" package in Microbiome Analyst1. The "VennDiagram" package in R (v3.6.1) was used to visualize the number of shared and unique OTUs between different samples. Principal coordinate analysis (PCoA) based on Bray–Curtis distances from the "vegan" package was used. Relative abundance differences at the phylum and class levels were calculated using the ANOVA test. All data with differences at  $p < 0.05$  were considered statistically significant. Genus-level co-occurrence analyses were performed using pairwise Spearman correlation coefficients (Spearman's  $|RHO| > 0.9$ ,  $p < 0.01$ ), and network topological features were calculated using Gephi (v0.9.2). In addition, nodes and correlations were computed based on the robustness of the co-occurrence scores. Redundancy analysis (CCA/RDA) was performed using the "vegan" package in R (v3.6.1) to determine whether changes in microbial communities were related to soil environment. A Welch's t-test and one-way analysis of variance (Duncan's multiple test) were used to compare mean differences between samples using SPSS (v20.0). All data with differences at  $p < 0.05$  were considered statistically significant.

### 3. Results

#### 3.1. Soil Physicochemical Properties

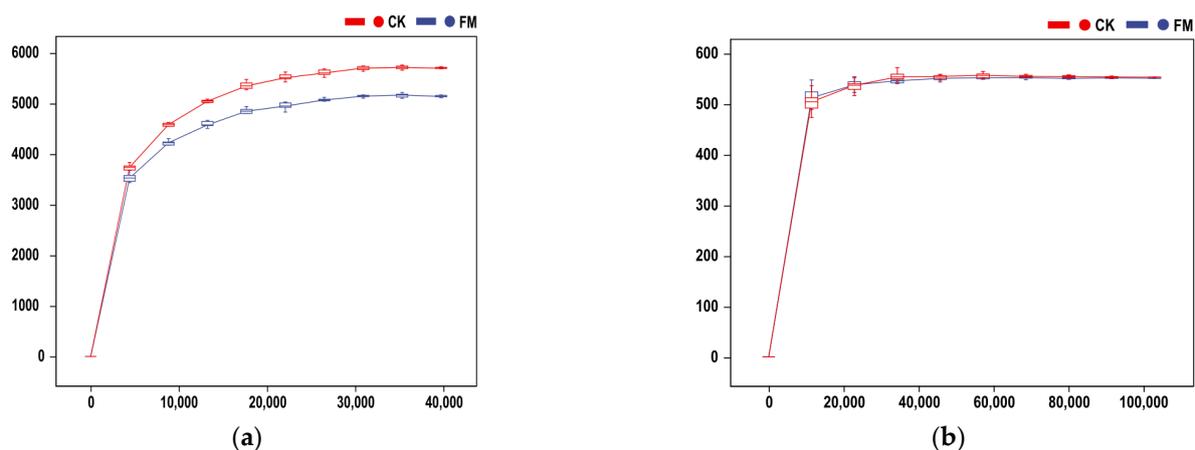
The results showed that compared with CK, the pH value of FM was significantly increased ( $p < 0.05$ ), the electrical conductivity and organic matter of FM were significantly reduced ( $p < 0.05$ ), and there was no significant difference between available phosphorus (AP) and available potassium (AK) ( $p > 0.05$ ) (Table 1).

**Table 1.** Physical and chemical data of soil under different treatments. And “a” and “b” represent the significance of the difference between the data.

Treat	pH	EC ( $\mu\text{s}/\text{cm}$ )	SOC (g/kg)	AP (mg/kg)	AK (mg/kg)
FM	$7.75 \pm 0.11$ a	$45.93 \pm 5.85$ b	$18.93 \pm 1.15$ b	$10.94 \pm 4.78$ a	$157.67 \pm 23.54$ a
CK	$7.15 \pm 0.07$ b	$70.80 \pm 4.07$ a	$24.61 \pm 1.51$ a	$14.05 \pm 2.72$ a	$171.0 \pm 20.42$ a

#### 3.2. Illumina MiSeq Sequencing Data

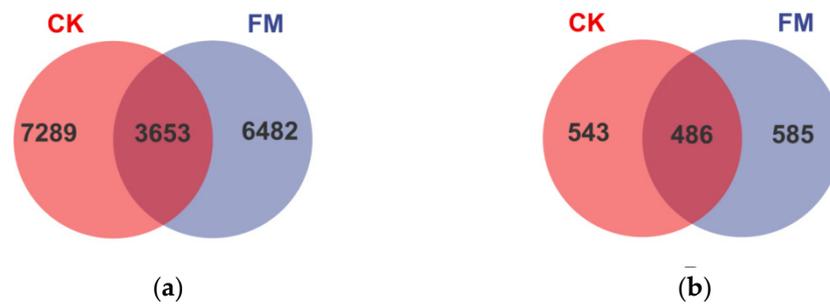
After screening the 16S RNA gene amplicon sequences using QIIME2 (<http://QIIME.org/>, accessed on 10 November 2021), 0.49 M reads remained. For the ITS amplicons, 0.72 M clean reads were obtained from the filtered raw sequence data. The dilution curve showed that the curves of all samples basically reached a steady state, indicating that the sequence reached the saturation representing the total OTUs (Figure 1).



**Figure 1.** Thinning curves of bacterial (a) and fungal (b) communities in the FM and CK groups. OTU, operating classification unit.

#### 3.3. Soil Bacterial and Fungal Community Diversity

The total number of bacterial OTUs of FM and CK were 10,135 and 10,942, respectively, of which 3653 OTUs were common to both groups. The total number of fungal OTUs of FM and CK were 1071 and 1029, respectively, of which 486 OTUs were common to both groups (Figure 2). The microbial alpha diversity of bacteria and fungi showed that Chao1 and Shannon indices in the FM and CK were not significantly different ( $p > 0.05$ ) (Table 2). Therefore, PPC film mulching had no significant effect on the abundance and diversity of microbial communities.



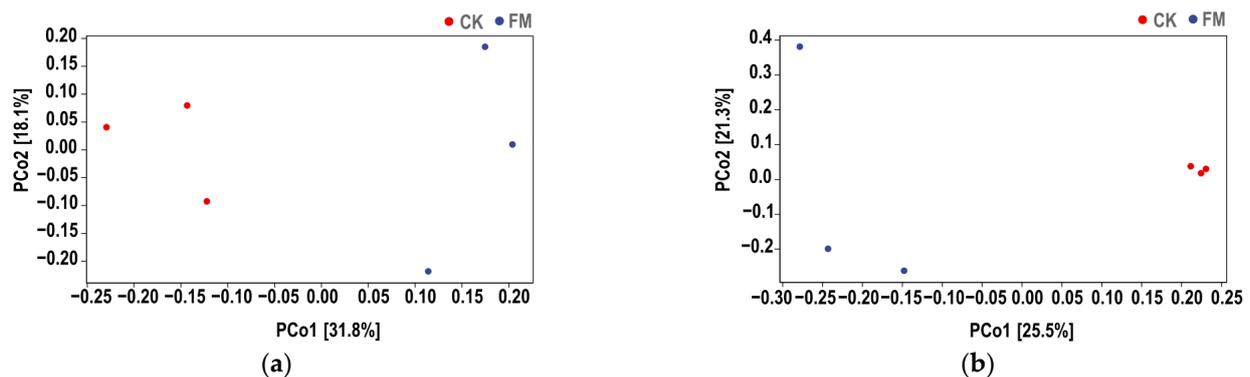
**Figure 2.** Venn diagrams of bacteria (a) and fungi (b) of OTUs in FM and CK samples.

**Table 2.** Microbial diversity index. The different letters after the numbers represent the significance of the difference between the data.

Sample	Bacteria		Fungus	
	Chao1	Shannon	Chao1	Shannon
FM	5149.6 ± 1140.5 a	10.88 ± 0.24 a	551.3 ± 58.7 a	5.85 ± 0.36 a
CK	5701.9 ± 152.9 a	11.04 ± 0.02 a	553.7 ± 58.2 a	5.75 ± 0.18 a

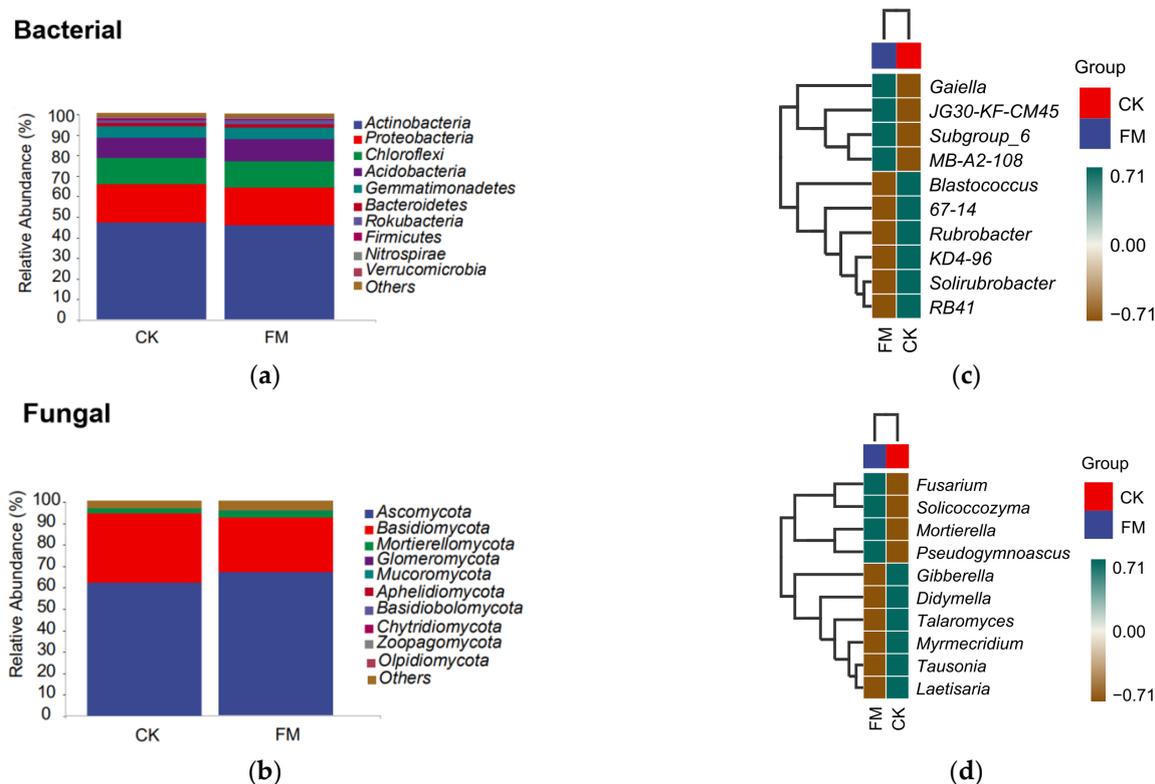
### 3.4. Composition of the Bacterial and Fungal Communities

The PCoA based on the Bray–Curtis distance matrix was used to evaluate the overall pattern of bacterial and fungal communities of FM and CK (Figure 3). For the bacterial community, the first principal coordinate (PCo 1) and the second principal coordinate (PCo 2) explained 31.8% and 18.1% of all variables, respectively, and the cumulative contribution rate of the variance of the two axes reached 49.9% (Figure 3a). For the fungal community, the first principal coordinate (PCo 1) and the second principal coordinate (PCo 2) explained 25.5% and 21.3% of all variables, respectively, and the cumulative contribution rate of the variance of the two axes reached 46.8% (Figure 3a). Taken together, the compositions of the microbial community of the FM and CK were different (Figure 3).



**Figure 3.** Principal coordinate analysis (PCoA) of bacterial (a) and fungal (b) communities; PCoA analysis was performed based on the weighted UniFrac distance of the bacterial and fungal communities.

In order to explore the response of the soil microbial groups to PPC film mulching, the relative abundance changes of bacteria and fungi at the phylum levels (top 10) were analyzed (Figure 4a,b). The results showed that the relative abundance of *Actinobacteria* at the bacterial phylum level in FM and CK treatments was 46.05% and 47.06%; of *Proteobacteria* was 18.24% and 18.48%; of *Chloroflexi* was 12.64% and 12.71%; of *Acidobacteria* was 11.05% and 9.76%; and of *Gemmatimonadetes* was 5.27% and 5.87%, respectively. The relative abundance of *Ascomycota* at the fungal phylum level was 66.99% and 62.06%; of *Basidiomycota* was 25.25% and 32.05%; and of *Mortierellomycota* was 3.13% and 2.40%, respectively.



**Figure 4.** The relative abundance of dominant bacteria (a) and fungi (b) at the level of the phylum; the abscissa is the name of each sample, and the ordinate is the relative abundance of each dominant group at the phylum level (top 10). Heat map of species composition of bacterial (c) and fungal (d) communities; the samples were clustered by UPGMA according to the Pearson correlation coefficient matrix, and arranged according to the clustering results. The abundance data of the top 10 genera of average abundance were used to draw the heat map.

A heatmap analysis based on genus level was also performed, and the top 10 species with significant differences in relative abundance were analyzed (Figure 4c,d). For fungal community of FM and CK, *Tausonia* was 20.61%, 21.98%; *Fusarium* was 8.02%, 6.97%; *Talaromyces* was 1.11%, 7.59%; *Didymella* was 3.27%, 3.75%; *Gibberella* was 3.31%, 3.45%; *Solicoccozyma* was 3.42%, 2.88%; *Mortierella* was 3.13%, 2.40%; *Pseudogymnoascus* was 3.31%, 2.04%; *Laetisaria* was 0.00%, 3.08%; *Myrmecridium* was 0.42%, 2.18% respectively. In bacterial FM and CK soil, *Subgroup\_6* was 5.91%, 4.89%; *KD4-96* was 4.43%, 4.79%; *Rubrobacter* was 4.19%, 4.57%; *Blastococcus* was 3.80%, 3.81%; *67-14* was 3.62%, 3.64%; *MB-A2-108* was 4.08%, 2.70%; *JG30-KF-CM45* was 2.91%, 2.61%; *Gaiella* was 2.57%, 2.40%; *Solirubrobacter* was 2.19%, 2.63%; *RB41* was 1.59% and 1.68%, respectively. The abundance of *Gaiella*, *Fusarium*, *Solicoccozyma*, *Mortierella*, and *Pseudogymnoascus* in FM soil was higher than that in CK soil, while the abundance of *Blastococcus*, *Rubrobacter*, *Solirubrobacter*, *Gibberella*, *Didymella*, *Talaromyces*, *Myrmecridium*, *Tausonia*, and *Laetisaria* in CK was higher than that in FM (Table A2).

### 3.5. Co-Occurrence Network of the Soil Bacterial and Soil Fungal Communities

Fungal and bacterial networks based on genus level were constructed to identify changes in the correlation of microbial communities with and without mulching. The data showed that the number of nodes of the soil bacterial community network of FM and CK was 571 and 563, respectively, the number of edges was 25,974 and 27,250, the positive correlation ratios were 60.30% and 55.52%, and the negative correlation ratios were 39.70% and 44.48%. The number of nodes of the soil fungus community network of FM and CK was 233 and 211, respectively, the number of edges was 4752 and 4506, the positive

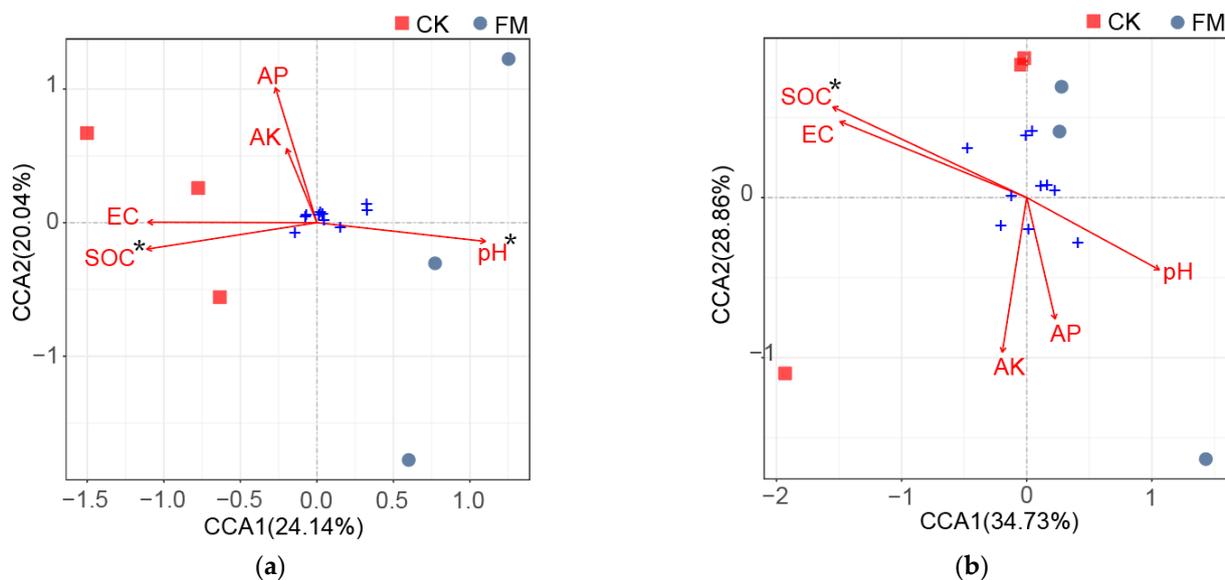
correlation ratios were 60.344% and 62.16%, and the negative correlation ratios were 36.56% and 37.84% (Table 3).

**Table 3.** Analysis of the co-occurrence network of bacterial and fungal communities.

Sample	Bacteria				Fungus			
	Nodes	Edges	Positive	Negative	Nodes	Edges	Positive	Negative
FM	571	25,974	60.30%	39.70%	233	4752	63.44%	36.56%
CK	563	27,250	55.52%	44.48%	211	4506	62.16%	37.84%

### 3.6. Soil Physicochemical Properties and Their Relationship with the Soil Microbial Community

CCA was used to study whether the change in community was affected by the soil physical and chemical properties. The research results showed that the CCA axis of the bacteria and fungi explained 44.18% and 63.59% of the total variation of the bacterial and fungal community structure, respectively. The pH and SOC in the soil were the most important factors affecting the bacterial community, and SOC was the most important factor affecting the fungal community (Figure 5).

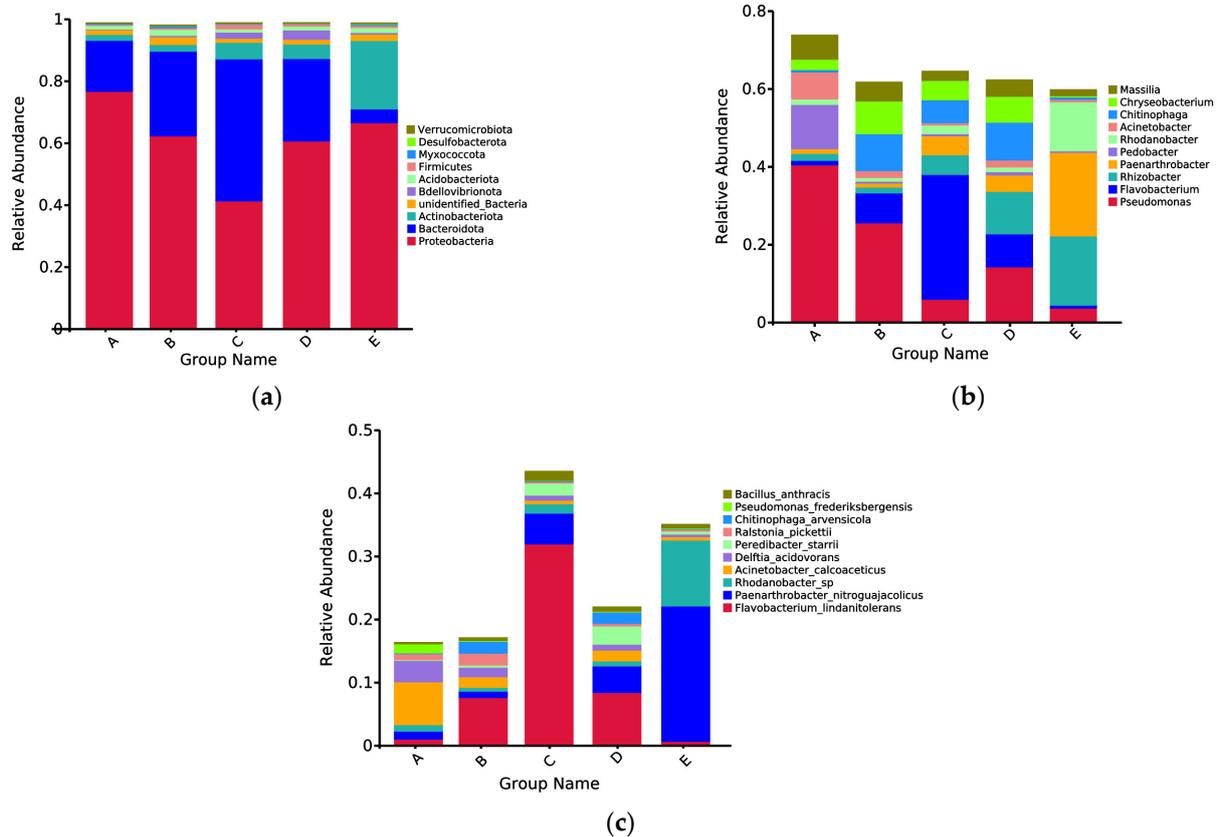


**Figure 5.** Correlation analysis of the soil physical and chemical properties and bacterial (a) and fungal (b) communities based on OTUs. The arrow length is directly proportional to the influence of soil physical and chemical properties on the microbial community structure. Three repetitions per group (n = 3). pH: acid-base; EC: electrical conductivity. SOC: soil organic carbon; AP: available phosphorus; AK: available potassium. "\*" indicates that this factor has an important influence on the community. "+" indicates the distribution of microbial communities in relation to environmental factors.

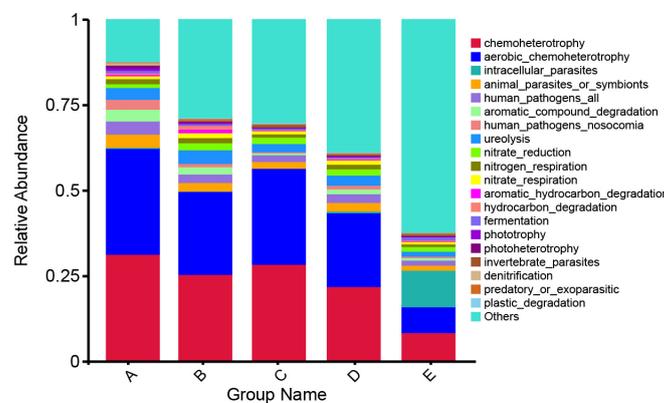
### 3.7. Bacterial Community and Metabolic Prediction during PPC Film Degradation

We also made soil suspension using PPC film mulching soil samples, and then co-cultivated with PPC film, so that the bacteria that degraded the PPC film could be enriched on the surface of the film. We collected the bacterial community on the membrane surface to conduct degradation experiments, and found that these bacterial communities have good degradation ability. We analyzed these bacterial groups and found that *Proteobacteria*, Bacteroidota, and Actinobacteriota were dominant at the phylum level; *Pseudomonas*, *Flavobacterium*, and *Rhizobacter* were dominant at the genus level; and the dominant species were *Flavobacterium lindanitolerans*, *Paenarthrobacter nitroguajacolicus*, and *Rhodanobacter sp* (Figure 6). FAPROTAX was used to dissect microbial community functions and to classify 90 functional groups based on 16S rRNA gene data [28]. Then, we identified the top 20

major bacterial metabolic activities during PPC film degradation. The results showed that Chemoheterotrophy and Aerobic\_Chemoheterotrophy were the most dominant metabolic functions among all PPC film degradation processes, which may be a common property of heterotrophic bacterial communities [29]. We also found that the metabolic activities related to the degradation function were Aromatic\_hydrocarbon\_degradation, Hydrocarbon\_degradation, and Plastic\_degradation (Figure 7).



**Figure 6.** The relative abundance of dominant bacteria at phylum (a), genus (b), and species (c) levels; the abscissa is the plastic degradation process at five time points, and the ordinate is the relative abundance of each dominant taxa at the phylum level.



**Figure 7.** Metabolic community analysis in bacterial communities during plastic degradation of PPC mulch based on FAPROTAX. Degradation stage: A, in the stage of degradation adaptation, the community is enriched; B, in the early stage of degradation, the plastic begins to degrade; C, in the middle stage of degradation, the plastic begins to break; D, in the late stage of degradation, the plastic is completely fragmented; E, in the final stage of degradation, the plastic basically degrades.

#### 4. Discussion

Film mulching leads to changes in many factors that affect the structure and abundance of microbial communities, including changes in the soil microclimate (temperature, humidity, etc.) [30,31], changes in soil physical and chemical properties [32], stress of different materials to soil microorganisms [33], the introduction of additives in the film soil [34], and the enrichment of functions related to the degradation of the mulch film [35]. Biodegradable plastic film potentially influences soil microbial communities in two ways: first, as a surface barrier prior to soil incorporation, indirectly affecting soil microclimate and atmosphere (similar to PE films); and, second, as a direct input of physical fragments after soil incorporation [16]. Studies have showed that the process of water evaporation can bring salt to the soil surface [36], and mulching can reduce water evaporation, so that the salinity of the mulched soil is lower than that of the unmulched soil, which may be the reason why the EC in the mulched soil is lower than that of the unmulched soil. Rainfall causes the leaching of alkaline salts such as calcium and magnesium in the soil, resulting in a decrease in soil pH, and film mulching prevents the leaching of alkaline salts, which may be a factor that causes the pH in the mulched soil to be higher than that of the unmulched soil. All studies on the effect of mulching on soil have shown that mulching increases soil temperature [37,38], so we speculated that the temperature of PPC film mulched soil was also higher than that of unmulched soil. Soil temperature increases significantly during mulching, and higher temperature is beneficial to the enhancement of metabolic processes. Thus, film mulching may lead to enhanced degradation of SOC and lower SOC content in soil [39]. This is consistent with our findings, which showed that PPC film mulching significantly increased soil pH and decreased soil EC and SOC. According to some studies, the degradable membrane enters the soil as a carbon source after being broken, and although the total carbon input is small, it may also affect soil organic matter dynamics [16]. Bacteria and fungi are the drivers of soil carbon and nutrient cycles, and their abundance and activity affect organic matter dynamics and nutrient availability [17]. In addition to SOC, pH is another main factor that accounts for the change in bacterial community abundance (Figure 5a,b). Some studies have shown that changes in soil pH affect the microbial community, especially the bacterial community [40], which is also consistent with our results that showed that SOC was the most important factor for bacterial and fungal changes (Figure 5a,b) and pH was the most important factor for bacterial changes (Figure 5a).

We analyzed the alpha diversity analysis and between-group significance of PPC mulched and unmulched soil. It is worth noting that there was no significant difference in the abundance and diversity of the communities of PPC mulched and unmulched soil, and the difference between the groups was also not significant (Table A1). Therefore, we believe that the short-term one-year PPC film mulching has little effect on the overall soil microorganisms. The study by Sreejata et al. also did not detect any significant effect of plastic mulches on bacterial community structure, richness, or diversity [41]. We explored the effect of the PPC film mulching on the microbial community in the overall large space of the soil, rather than the microorganisms in the soil next to the plastic film, ignoring the microbial changes on the local spatial scale; thus, we believe that mulching has little effect on the overall system of soil microorganisms. The reasons for this may also be that the PPC film mulching period is short, the difference between the one-year mulched soil and the unmulched soil is not obvious, and long-term mulching is required.

Soil microbes constitute a network of complex relationships, and different species have different interactions [42]. In this study, the microbial network analysis showed that negative correlations between the microbial species in the mulched soil decreased while positive correlations increased compared with the unmulched soil, suggesting that PPC film mulching reduces the competition between species and increases the cooperation between species (Table 3), which affects the structure of the microbial community. CCA also proved that soil organic carbon is the most important factor affecting the microbial

community. Thus, it may be affected by the SOC in the soil, so that the correlation between microorganisms changes, and the microbial composition produces a new balance.

We performed significant difference analysis on soil microbes at the phylum and genus levels with the top 10 relative abundances, and then found significant differences in the relative abundances of *Acidobacteria* and *MB-A2-108*. As reported previously, the accumulation of *Acidobacteria* is negatively correlated with the incidence of disease [43], which reduces the potential risk of pathogens to a certain extent [44]. The change in the abundance of *MB-A2-108* is negatively correlated with the carbon in the soil, so it is dominant in a low-carbon environment [45,46].

At present, the most common reports are about the degradation of polyester plastics, such as polyethylene terephthalate (PET), poly butyleneadipate-co-terephthalate (PBAT), and polyester polyurethane (PU), by *Pseudomonas* [28,47–49]. Another study found that *Pseudomonas* can also degrade low-density polyethylene (LDPE) [50]. *Flavobacterium* is often found to aggregate on the surface of polyethylene plastic films to form biofilms and has the ability to degrade phthalic acid plasticizers [51–54]. Some studies suggest that *Rhizobacter* may enhance the degradation of petroleum hydrocarbons as a biosurfactant [55]. Based on the existing research and our analysis of microbial changes in different periods during the degradation of PPC film, we speculate that *Paenarthrobacter\_nitroguajacolicus* and *Rhodanobacter\_sp* may play a key role in the degradation of PPC film. Regarding the reason why the metabolism of chemoheterotrophy first decreased, then increased, and finally decreased, we speculated that the microorganisms were adapting to the PPC plastic film as a carbon source during the period from when the microbial community was first added to the medium to the early stage of degradation, so the metabolism decreased. In the middle stage of degradation, the PPC mulching film was obviously damaged. At this time, the metabolism of chemoheterotrophy increased again. In the later stage of degradation and at the end of degradation, the PPC mulching film was completely fragmented and the content of the carbon source gradually decreased, causing the metabolism of the chemoheterotroph to decline continuously. In addition, the metabolism of Aromatic\_hydrocarbon\_degradation, Hydrocarbon\_degradation, and Plastic\_degradation also increased first and then decreased during the degradation of the PPC mulching film. Therefore, we speculate that the metabolisms of Aromatic\_hydrocarbon\_degradation, Hydrocarbon\_degradation, and Plastic\_degradation were also involved in the degradation of PPC film, because these three metabolisms all increased significantly in the process of PPC film degradation. It is worth noting that the metabolism of Hydrocarbon\_degradation and Aromatic\_hydrocarbon\_degradation reached the highest level in the early stage of degradation and then began to decline. This may be involved in the degradation of the PPC mulching film during the early stage of degradation. The metabolism of Plastic\_degradation began to increase continuously after the microbial community was added to the medium, and it did not decrease significantly until the end of degradation. Therefore, we speculate that the metabolism of Plastic\_degradation was involved in the degradation of PPC mulching film during the whole process. We also speculate that the degradation of the PPC mulching film is not caused by a single metabolism, but involves multiple metabolisms.

## 5. Conclusions

In conclusion, this study explored the effects of PPC film mulching on the diversity, composition, and interaction characteristics of soil microbial communities and changes in soil physicochemical properties. Moreover, the microorganisms at different time periods during the degradation of PPC mulch film were also analyzed. We found that PPC film mulching increased soil pH and decreased soil EC and SOC values, but had no significant effect on soil microbial community abundance and diversity, and the PPC film mulching promoted the enrichment of *Acidobacteria* and other functional disease-suppressing strains. This study not only provides a scientific theoretical basis for soil safety and the large-scale use of PPC biodegradable film, but also lays a research foundation for the degradation of PPC plastics.

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## Appendix A

**Table A1.** Statistical table of difference analysis between groups.

Sample	Group 1	Group 2	Sample Size	Permutations	pseudoF	p Value	q Value
Bacteria	all	-	6	999	1.792229	0.091	-
	FM	CK	6	999	1.792229	0.097	0.097
Fungus	all	-	6	999	1.484782	0.224	-
	FM	CK	6	999	1.484782	0.205	0.205

**Table A2.** Relative abundance table of bacteria and fungi based on heatmap analysis.

	Bacteria		Fungus		
	FM	CK	FM	CK	
Subgroup_6	0.0591	0.0489	Tausonia	0.2061	0.2198
KD4-96	0.0443	0.0479	Fusarium	0.0802	0.0697
Rubrobacter	0.0419	0.0457	Talaromyces	0.0111	0.0759
Blastococcus	0.0380	0.0381	Didymella	0.0327	0.0375
67-14	0.0362	0.0364	Gibberella	0.0331	0.0345
MB-A2-108	0.0408	0.0270	Solicoccozyma	0.0342	0.0288
JG30-KF-CM45	0.0291	0.0261	Mortierella	0.0313	0.0240
Gaiella	0.0257	0.0240	Pseudogymnoascus	0.0331	0.0204
Solirubrobacter	0.0219	0.0263	Laetisaria	0.0000	0.0308
RB41	0.0159	0.0168	Myrmecridium	0.0042	0.0218

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