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Effects of Conventional Non-Biodegradable Film-Derived Microplastics and New Biodegradable Film-Derived Microplastics on Soil Properties and Microorganisms after Entering Sub-Surface Soil

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Abstract: Plastic film mulching, widely used in agriculture, leads to microplastic (MP) pollution in soils. While biodegradable polybutylene adipate terephthalate (PBAT) films may offer a solution, their impacts on subsurface soils and microorganisms remain unclear. To investigate the effects of conventional non-biodegradable polyethylene (PE) and biodegradable PBAT MPs on the properties of sub-surface soils and microbial communities, MPs were added at varying doses in a field experiment and incubated for 160 days. Physicochemical characteristics, nutrient dynamics, and microbial composition, diversity, and networks of soils were analyzed using standard techniques and 16S rRNA/ITS gene sequencing. Correlations between soil properties and microbes were assessed. Both MP types significantly altered soil characteristics, with PBAT-MP elevating pH and the levels of available phosphorus and potassium more than PE-MP. Microbial composition shifts occurred, with low-addition PBAT-MP promoting plastic-degrading genera. The assessment of α/β-diversity indicated that PBAT-MP predominantly influenced fungi while PE-MP impacted bacteria. An examination of microbial co-occurrence networks highlighted that PE-MP primarily disrupted fungal interactions, whereas PBAT-MP streamlined network complexity. Correlation analyses revealed that PBAT-MP promoted fungal diversity/network resilience correlating to nutrients. PE-MP and PBAT-MP significantly altered native soil/microbe relationships. PBAT-MP may exert greater, yet unknown, impacts over time through its biodegradation into newer and smaller fragments. Future research needs to integrate multi-omics and stable isotope science to elucidate the deep mechanistic impacts of degraded film-derived MPs on microbial ecological functions and biogeochemical cycles. Attention should also be paid to the long-term accumulation/transport of MPs in agricultural soils. Overall, this work deepens the impact and understanding of MPs from plastic film on sub-surface soil ecology. Furthermore, it provides a theoretical foundation for managing 'white pollution' in the film-covered farmlands of arid and semi-arid regions in China.

Keywords: plastic film residual pollution; microbial community structure and stability; film-derived microplastics

Citation: Liu, X.; Wei, W.; Liu, G.; Zhu, B.; Cui, J.; Yin, T. Effects of Conventional Non-Biodegradable Film-Derived Microplastics and New Biodegradable Film-Derived Microplastics on Soil Properties and Microorganisms after Entering Sub-Surface Soil. *Agronomy* **2024**, *14*, 753. https://doi.org/10.3390/agronomy14040753

Academic Editor: Francesc Xavier Prenafeta Boldú

Received: 18 March 2024
Revised: 29 March 2024
Accepted: 4 April 2024
Published: 5 April 2024

1. Introduction

In response to the challenges posed by population growth and food security, China initiated the adoption of plastic film mulching technology in agricultural production during the 1980s [1]. This innovation significantly transformed farming practices in the arid and semi-arid regions of China, resulting in substantial enhancements in crop yields [2,3]. It has since evolved into a crucial component of modern agricultural production. Nevertheless, the extensive and continuous use of plastic film has led to significant environmental consequences, particularly the accumulation of plastic film debris in the soil, resulting in severe microplastic (MP) pollution [4–7]. These MP remnants undermine soil health and present a potential risk to human health via the crop/food pathway [8].

MPs exert significant effects on soil health, plant growth, and groundwater quality. These effects include a compromised soil structure, reduced nutrient availability, altered microbial communities, and an increased potential for groundwater contamination [9]. Specifically, MPs diminish soil porosity and stability, adversely affect water retention and microbial activity [10], and reduce the availability of critical nutrients such as nitrogen and phosphorus, impeding plant growth [11]. They further alter soil microbial communities, resulting in an increased presence of pathogenic microbes that negatively impact plant health [12]. Additionally, MPs pose a risk of contaminating groundwater with hazardous chemicals, leading to broader environmental and health concerns [13,14]. These studies underscore the urgent need to develop and implement strategies to mitigate MP pollution in agricultural systems [15].

The development of polybutylene adipate terephthalate (PBAT) as a biodegradable alternative to traditional polyethylene (PE) films has been explored to address environmental concerns [16]. However, evidence indicates that the degradation rate of PBAT under field conditions is slower than anticipated, casting doubts on its environmental advantages over PE films [17]. Liu et al. (2022) [18] discovered that PBAT films fail to decompose completely in colder climates within a single growing season. Additionally, Yang et al. (2023) [19] observed that soil microbial activity, crucial for PBAT decomposition, is less effective with PBAT than with natural polymers, indicating the need for improved microbial adaptation or genetic engineering to accelerate PBAT biodegradation. Sreejata et al. (2020) [20] reported differential impacts of PBAT films on soil microbial communities compared to PE, indicating variable environmental effects. Furthermore, preliminary findings by Qiu et al. (2023) [21] highlight the risk of MPs from both PBAT and PE films penetrating deeper soil layers, potentially threatening ground water and subsoil ecosystem integrity. However, much of the current research on film-derived microplastics concentrates on topsoil or tillage soils, highlighting significant research gaps regarding the impact of agricultural film-derived MPs on deeper soil properties and microbial ecosystems [22]. Given the potential permanence of MPs in deeper soil layers, further research is imperative to understand the distinct adverse impacts of PE and PBAT film MPs [23]. Such investigations are crucial for establishing a theoretical basis for the development and application of biodegradable film MPs.

The aim of this study is to assess the effects of MPs, originating from various film materials such as PE and PBAT, on the properties of sub-surface soil and the configuration of microbial communities. This research hypothesizes that these MPs significantly affect the characteristics of sub-surface soil and the composition of microbial communities. To test this hypothesis, MPs derived from PBAT and PE films were introduced into the sub-surface soil layer to (i) investigate the impact of different MPs on soil properties (nutrients and physicochemical properties), and microbial community structure and stability; and (ii) explore the correlation between soil properties (nutrients and physicochemical properties), and microbial community structure and stability under the influence of different MPs.

2. Materials and Methods

2.1. Experimental Design

A randomized block design field experiment focusing on MP addition was conducted in a flat area in Chengyang, Qingdao, China (36°29′ N, 120°36′ E, 70 m asl). This region experiences a temperate semi-humid climate, characterized by an average annual precipitation of 630 mm and a mean annual temperature of 12.9 °C, based on a 25-year record over the last three decades. The selected experimental plots had been under continuous cultivation with non-plastic film mulching for over 20 years, resulting in significantly low levels of film-derived MP contamination before the start of the experiment.

In this study, MPs were sourced from PE films provided by Kingfa Company (Guangzhou, China) and PBAT films supplied by BASF (Ludwigshafen, Germany). These materials were processed into MPs using a RETSCH MM 400 frozen mixing ball mill (Retsch, Haan, Germany) with pre-freezing in liquid nitrogen. Particle size measurements for PE and PBAT MPs were conducted using a laser particle size analyzer (Microtrac S3500, Microtrac Inc., Montgomeryville, PA, USA), revealing average sizes of 420 μm (range: 350–450 μm) for PE and 320 μm (range: 220–400 μm) for PBAT MPs [24]. The applied doses of MPs were 0.01, 0.1, and 0.5% (*w*/*w*) of wet soil weight (14.5%, soil mass water content). The doses of MPs were selected based on the current understanding of MP concentrations in soil [25,26].

For this experiment, soil was collected from a depth of 40–60 cm and subsequently mixed with varying quantities of MPs, as detailed previously. This mixture was then introduced into ceramic columns, which were sealed at both ends with nylon netting (100 mesh size) and reinserted into the soil at the original collection depth. A control setup without added MPs was also established for comparison. Each experimental condition was replicated thrice, and the incubation period was set at 160 days. Prior to incubation, the soil characteristics measured at the 40–60 cm depth included a pH of 5.84, soil organic carbon (SOC) of 7.59 $g \cdot kg^{-1}$, total nitrogen (TN) of 0.68 $g \cdot kg^{-1}$, available potassium (AK) of 170.5 $mg \cdot kg^{-1}$ and available phosphorus (AP) of 13.4 $mg \cdot kg^{-1}$.

2.2. Soil Sampling and Analysis

Upon completion of the incubation period, the ceramic columns were carefully extracted from the soil. Subsequently, selected soil samples from these columns were analyzed for nutrient content and physicochemical properties. Soil designated for aggregate analysis was transported to the laboratory in a plastic container. Furthermore, a fraction of the soil was wrapped in tinfoil and placed in a dry ice insulated box to be brought back to the laboratory at −80 °C for subsequent DNA extraction.

The selection of soil for nutrient and physicochemical property index analysis was executed with meticulous care to exclude MPs. SOC concentration was determined using the K_2CrO_7-H_2SO_4 oxidation method with external heating. TN content was quantified via the Kjeldahl technique. AP and AK in the soil were measured using the Olsen extraction and ammonium acetate leaching-flame photometer methods, respectively [27]. The pH was determined at a soil-to-water ratio of 1:2.5. Bulk density (BD) was assessed through the core method [28], while field water-holding capacity (WHC) followed Hillel's (1998) [29] guidelines. Soil samples were physically fractionated by wet sieving, following Elliott's (1986) [30] protocol. The mean weight diameter (MWD) of soil aggregates was calculated according to Bavel et al. (1950) [31]:

$$\mathrm{MWD} = \sum_{i=1}^{n} X_i W_i \quad (1)$$

where X_i is the mean diameter of the size fraction and W_i is the proportion of each size fraction over the total sample weight.

2.3. DNA Extraction, Sequencing, and Analysis

At the end of the incubation, three replicates of each treatment were individually high-throughput sequenced. DNA was extracted from 0.5 g of frozen soil samples using the Fast DNA Spin Kit for Soil (MP Biomedicals, Solon, OH, USA). The quality of the extracted DNA was assessed with a NanoDrop ND-1000 spectrophotometer (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and verified for integrity via 2% agarose gel electrophoresis. PCR amplification targeting the V3–V4 variable regions of the bacterial 16S rRNA was performed with the 338 F/806 R primers, which included barcode sequences. The amplification protocol included an initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 55 °C for 60 s, extension at 72 °C for 90 s, and a final extension at 72 °C for 7 min. Similarly, for the ITS1 region of fungi, amplification was conducted using ITS1 F/ITS1 R primers, with a protocol of 95 °C for 5 min for denaturation, 30 cycles of 94 °C for 30 s, 54 °C for 40 s, 72 °C for 30 s, and a final extension at 72 °C for 8 min. The PCR products underwent agarose gel electrophoresis (1.5%) for mixing, followed by purification using the QIAquick Gel Extraction Kit (Qiagen Inc., Valencia, CA, USA). Sequencing was performed on an Illumina MiSeq PE 250 platform (Illumina, Inc., San Diego, CA, USA), with the sequencing data processed using fastq-join and UPARSE within the QIIME software package (version 1.8.0).

2.4. Statistical Analysis

Statistical analyses were performed using R software, version 4.3.2, with data visualization facilitated by the ggplot2 package [32]. One-way ANOVA was used to determine significant differences between different additions of the same MP, while two-way ANOVA evaluated the effects of MP treatment types, quantities, and their interactions, adopting a significance threshold of $p < 0.05$. *t*-tests were utilized to identify significant differences between MP treatments, without control. The microbial community structure was depicted through stacked histograms, highlighting the ten most abundant bacterial and fungal genera. Principal Coordinate Analysis (PCoA), leveraging Bray–Curtis dissimilarity, was conducted with the vegan package to elucidate sample relationships [33]. Network analysis, incorporating igraph, ggClusterNet, and phyloseq packages, mapped co-occurrences within the microbial community [34]. The Mantel test, conducted using the vegan package, and the linkET package were used to assess microbial community correlations with soil properties [35]. Lastly, Pearson's correlation analysis elucidated the relationships between microbial properties and specific soil nutrients [36].

3. Results

3.1. Impact of MPs on Soil Physicochemical Properties and Nutrient Dynamics

This study elucidates the differential impacts of PBAT-MP and PE-MP on the physicochemical properties and nutrient dynamics of soil. Regarding the effects of MPs on soil physicochemical properties, a statistical analysis utilizing *t*-tests to compare different MP treatments revealed that PBAT-MP significantly elevated soil pH compared to PE-MP, without significantly affecting MWD, BD, or WHC (Figure 1a–d). One-way ANOVA results for various concentrations of the same MP indicated that both PE-MP and PBAT-MP treatments notably enhanced MWD and WHC and reduced pH and BD, although these changes were not statistically significant (Figure 1a–d). Furthermore, two-way ANOVA findings indicated that the MP treatment parameter significantly influenced soil pH alone, while the addition parameter substantially affected MWD and WHC, with the interaction between MP type and addition showing no significant impact on any of the tested soil physicochemical properties (Figure 1a–d).

Regarding soil nutrient effects, *t*-tests across different MP treatments demonstrated that PBAT-MP increased the levels of AP and AK when compared to PE-MP, yet exerted no significant effect on SOC and TN (Figure 1e–h). One-way ANOVA, analyzing varying additions of the same MP, showed that increased MP additions initially caused a decrease followed by an increase in SOC and TN levels, whereas AP demonstrated an opposite trend under both PE-MP and PBAT-MP treatments compared to the control (Figure 1e–h). Conversely, AK declined with rising additions under PE-MP treatment, whereas PBAT-MP did not significantly influence AK (Figure 1h). Two-way ANOVA results indicated that MP treatment significantly affected TN, AP, and AK, but not SOC, with the addition parameter significantly impacting all nutrient indices. Furthermore, the interaction between MP type and addition level significantly influenced AP and AK (Figure 1e–h).

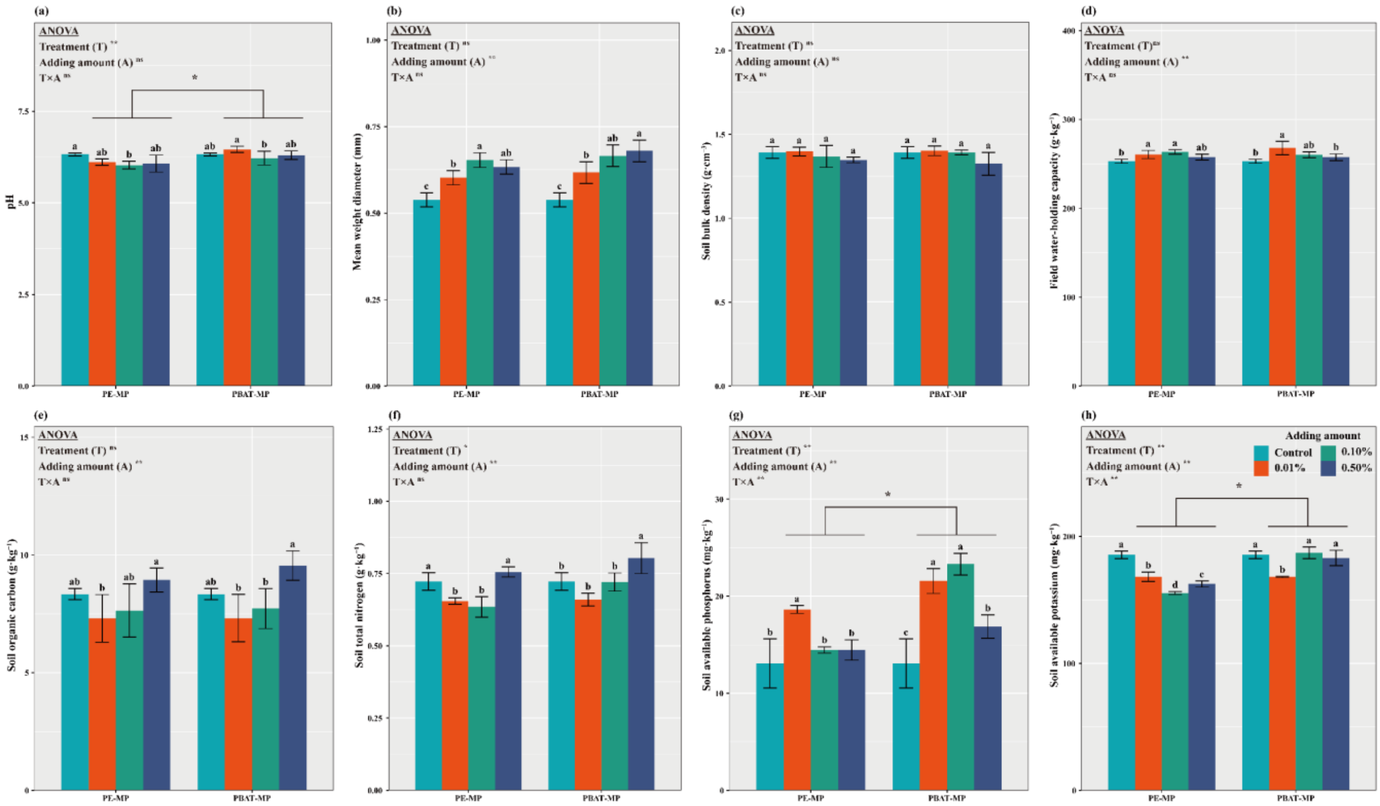

Figure 1. The effects of various mulching film-derived microplastics and added amounts on deep soil physicochemical properties (**a**–**d**) and soil nutrients (**e**–**h**). Control, without microplastic addition; PE-MP, microplastics derived from polyethylene film; PBAT-MP, microplastics derived from polybutylene adipate-co-terephthalate film. *t*-test was used to test the difference between the mean values of soil properties of PE-MP and PBAT-MP, without control. One-way ANOVA determined significant differences between different additions of the same microplastics. Two-way ANOVA was used to test the effects of treatment, adding amount, and their interactions on soil properties. Different lowercase letters indicate significant differences among treatments within each soil depth ($p < 0.05$). * and ** indicate statistically significant differences at $p < 0.05$ and $p < 0.001$, respectively; ns indicates no significant difference.

3.2. Impact of MPs on the Community Composition of Bacteria and Fungi

At the bacterial genus level, an analysis of community abundance revealed that *Lysobacter* showed the largest increase under both PE-MP and PBAT-MP treatments compared to the control (Figure 2a,b). Specifically, an increase in *Lysobacter* abundance correlated positively with increased PE-MP concentrations (Figure 2a). Conversely, PBAT-MP exposure led to an initial surge followed by a decline in *Lysobacter* numbers with higher additions (Figure 2b). *Methylophaga* showed the greatest reduction across both treatments, with its abundance decreasing as the MP dosage increased (Figure 2a,b). Additionally, a significant portion of the microbial community was unclassified or unidentified, with the control, PE-MP, and PBAT-MP treatments showing unidentified proportions of 25.4%, 28.4%, and 25.9%, respectively (Figure 2a,b).

At the fungal genus level, *Stachybotrys* and *Phlyctochytrium* were identified as the genera most significantly increased under PE-MP and PBAT-MP treatments, respectively, compared to the control (Figure 2c,d). The abundance of *Stachybotrys* increased with rising PE-MP additions (Figure 2c), while *Phlyctochytrium* decreased under progressive PBAT-MP treatments (Figure 2d). Regarding declines, *Spizellomyces* and *Didymella* were the most adversely affected under PE-MP and PBAT-MP treatments, respectively, both showing a negative correlation with the amount of MP added (Figure 2c,d). Moreover, a significant portion of the fungal community remained unclassified or unidentified, with the control, PE-MP, and PBAT-MP treatments showing unidentified proportions of 76.0%, 73.9%, and 73.6%, respectively (Figure 2c,d).

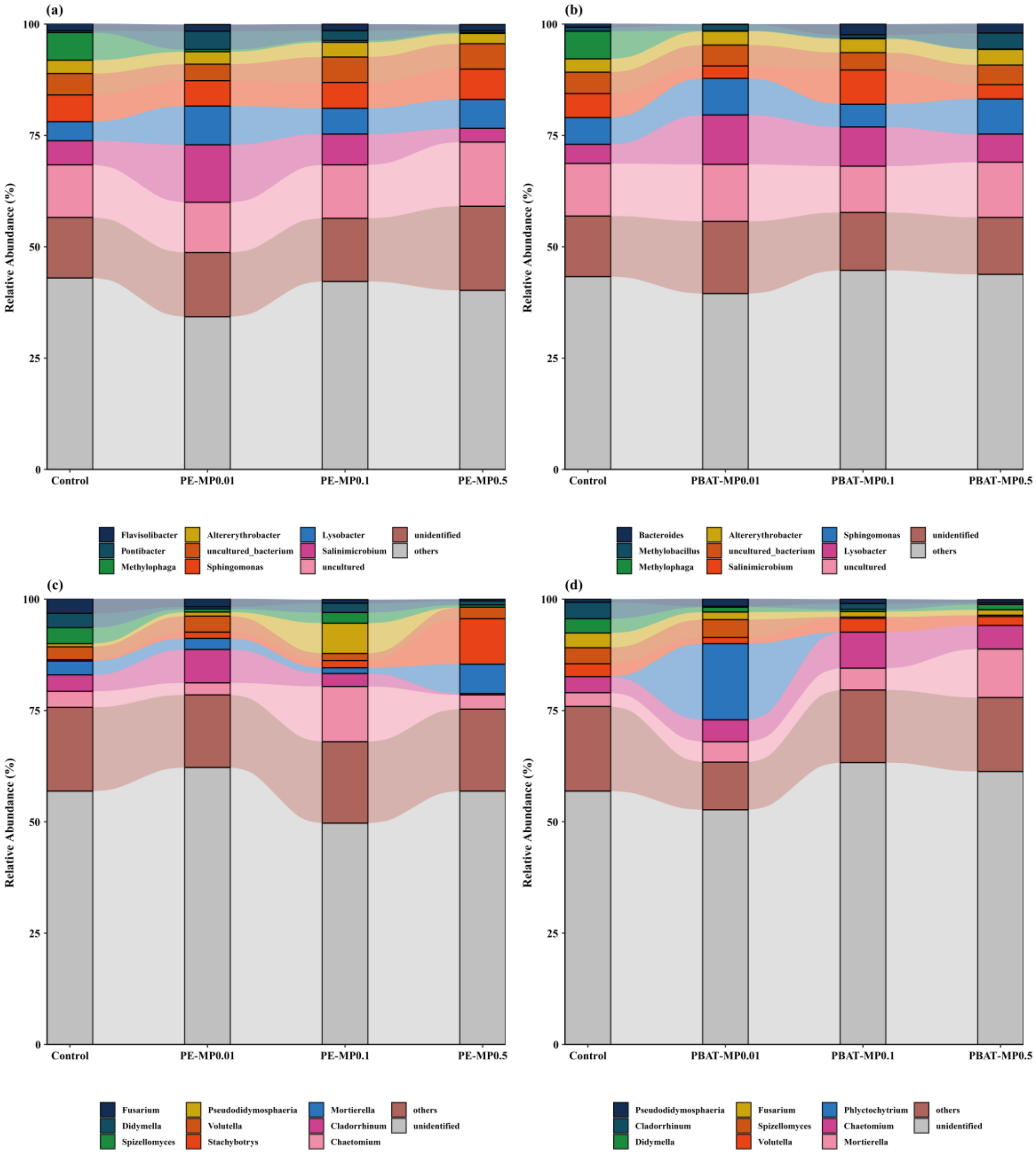

Figure 2. The effects of various mulching film-derived microplastics and added amount on the community composition of deep soil bacteria (**a**,**b**) and fungi (**c**,**d**) at the genus level. Control, without microplastic addition; PE-MP, microplastics derived from polyethylene film; PBAT-MP, microplastics derived from polybutylene adipate-co-terephthalate film. While 0.01, 0.1, and 0.5 represent additions of 0.01, 0.1, and 0.5% (*w*/*w*), respectively.

3.3. Impact of MPs on the Diversity of Bacterial and Fungal

In the assessment of bacterial α-diversity, a *t*-test analysis of MP treatments showed no significant differences between PE-MP and PBAT-MP treatments (Figure 3a,b). Subsequent one-way ANOVA comparisons, relative to the control, indicated that the Chao1 and Shannon diversity indices for the PE-MP treatment initially decreased, followed by an increase with escalating MP concentrations. In contrast, the PBAT-MP treatment consistently led to a marked decline in both indices. Two-way ANOVA analysis highlighted that the type of

MP treatment had no significant effect; however, the amount of addition and its interaction with MP type significantly influenced the Chao1 and Shannon indices (Figure 3a,b).

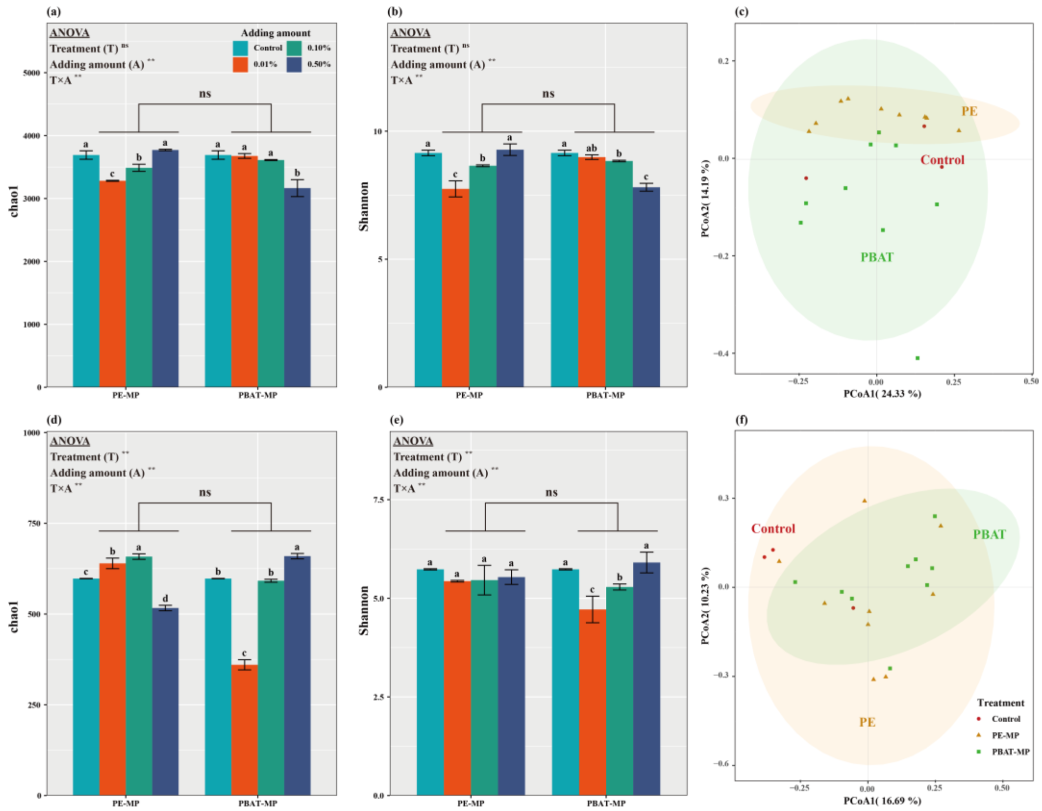

Figure 3. The effects of various mulching film-derived microplastics and added amounts on deep soil bacterial and fungal abundance (**a**,**d**), diversity (**b**,**e**), and principal coordinate analysis based on the Bray–Curtis distance, indicating variations between distinct treatment groups (**c**,**f**). PE-MP, microplastics derived from polyethylene film; PBAT-MP, microplastics derived from polybutylene adipate-co-terephthalate film. Control, without microplastic addition. T-test was used to test the difference between the mean values of soil properties of PE-MP and PBAT-MP, without a control. One-way ANOVA determined significant differences between different additions of the same microplastics. Two-way ANOVA was used to test the effects of treatment, adding amount, and their interactions on soil properties. Different lowercase letters indicate significant differences among treatments within each soil depth ($p < 0.05$). ** indicate statistically significant differences at $p < 0.001$; ns indicates no significant difference.

Regarding fungal α-diversity, *t*-test analyses of MP treatments revealed no significant differences between PE-MP and PBAT-MP treatments (Figure 3d,e). One-way ANOVA findings, when compared to the control, demonstrated that the Chao1 index under PE-MP treatment initially increased and then significantly decreased as the amount of addition was elevated, whereas the Shannon index consistently decreased, though not significantly. In contrast, for the PBAT-MP treatment, both indices initially showed a decline, followed by a significant increase (Figure 3d,e).

In examining β-diversity, Principal Coordinates Analysis (PCoA), utilizing Bray–Curtis distances for bacterial communities, demonstrated that PCoA1 and PCoA2 explained 38.52% of the total variance (Figure 3e,f). In fungi, PCoA analysis demonstrated that PCoA1 and PCoA2 accounted for 27.22% of the variance. Different treatments significantly altered the structures of both bacterial and fungal communities, highlighting the imperative for additional research.

3.4. Impact of MPs on the Co-Occurrence Networks of Soil Bacteria and Fungi

Spearman's correlation coefficient (r > 0.6, $p < 0.05$) was employed to elucidate the relationships within microbial communities, and co-occurrence networks for both bacterial and fungal taxa were generated (Figure 4). In the bacterial co-occurrence network analysis, compared to the control, treatments with PE-MP and PBAT-MP demonstrated a decrease in community complexity. This reduction was evidenced by a decrease in the number of nodes and edges, with reductions of 49.4% and 85.3% for PE-MP, and 46.6% and 87.5% for PBAT-MP, respectively. This was also reflected in the reductions of the average network degree, which decreased by 70.9% for PE-MP and 76.6% for PBAT-MP. Despite these alterations, the interaction dynamics within the community remained relatively unchanged across all treatments (Figure 4a).

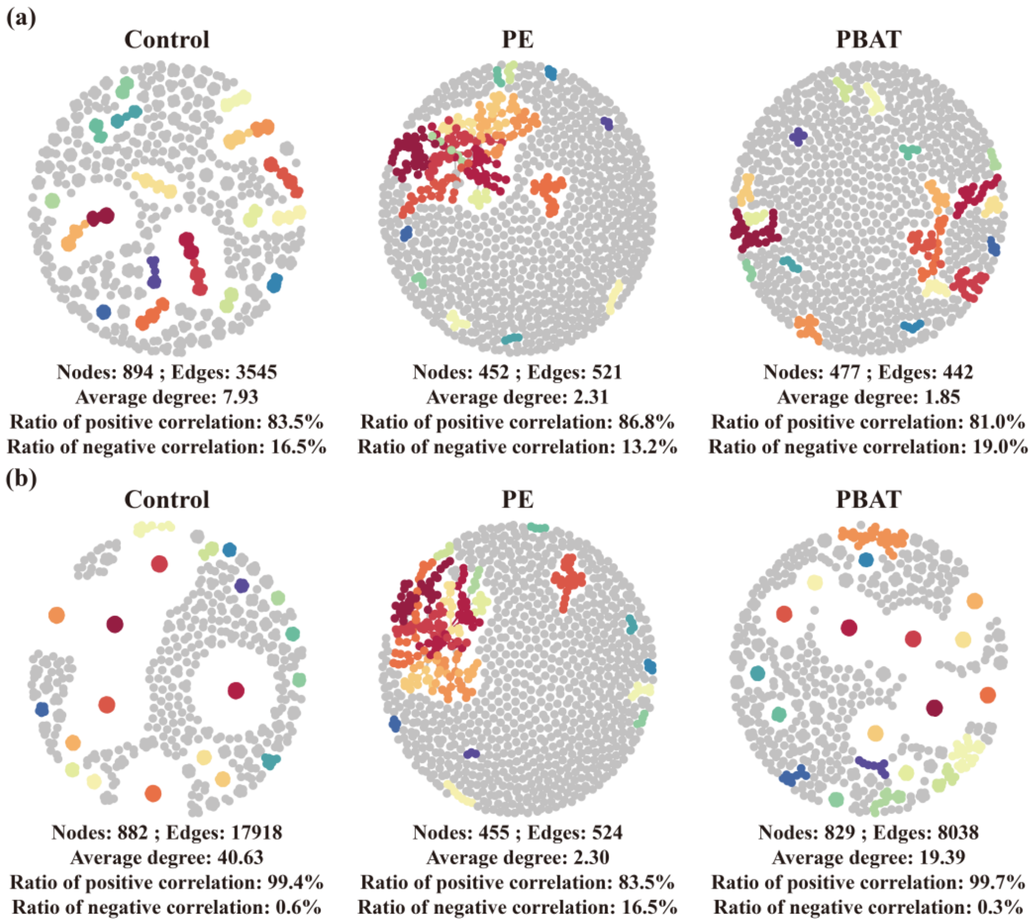

Figure 4. Co-occurrence network analysis of bacterial (**a**) and fungal (**b**) communities under various mulching film-derived microplastics and added amounts. Nodes of the same color belong to the same module. Control, without microplastic addition. PE-MP, microplastics derived from polyethylene film; PBAT-MP, microplastics derived from polybutylene adipate-co-terephthalate film. Spearman's correlation coefficient $r > 0.6$ and $p < 0.05$ were used for network construction.

For the fungal co-occurrence networks, a comparison with the control highlighted that both PE-MP and PBAT-MP treatments similarly diminished community complexity. Specifically, for PE-MP, the reductions were 48.4% in nodes and 97.1% in edges, with a 94.3% decrease in average degree. Conversely, PBAT-MP exhibited decreases of 6.0% in nodes, 55.1% in edges, and 52.3% in average degree. Notably, in contrast to the control and PBAT-MP treatments, the PE-MP treatment led to a reduction in mutualistic interactions and an increase in competitive dynamics within the fungal community (Figure 4b).

3.5. Relationship between Soil Properties and Microbial Communities

Figure 5 depicts the influence of MPs on taxonomic community composition via multivariate analysis, using Mantel tests to determine the statistical significance of environmental factors. It contrasts three distinct treatments, using a color-coded matrix and network diagrams to illustrate the magnitude and significance of Pearson correlation coefficients between community composition and diverse soil properties.

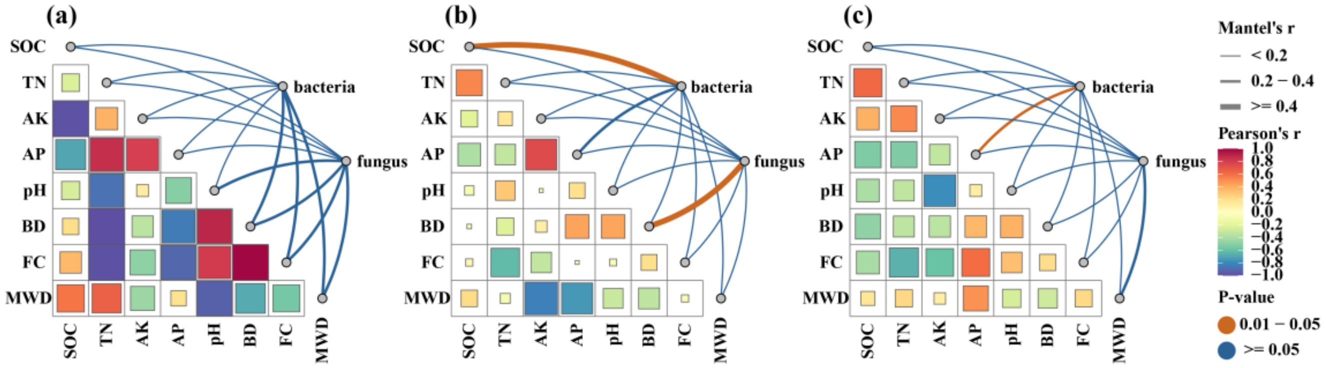

Figure 5. Taxonomic community composition was related to each environmental factor Mantel test. Pairwise comparisons of environmental factors are shown, with a color gradient denoting Pearson's correlation coefficient. (**a**) Control (without microplastics addition); (**b**) PE-MP (microplastics derived from polyethylene film); (**c**) PBAT-MP (microplastics derived from polybutylene adipate-co-terephthalate film).

The analysis reveals that the introduction of MPs significantly alters the correlation patterns between community composition and environmental factors, deviating from those observed in the control treatment. Specifically, within the context of the PE-MP treatment, significant correlations were observed between the bacterial community and SOC, and between the fungal community and BD. Under the PBAT-MP treatment, a notable correlation was exclusively identified between the bacterial community and AP, suggesting the potential of MPs to influence soil ecosystem functions and interactions. These findings highlight the necessity of further research to elucidate the ecological implications of MP pollution.

3.6. Relationship between Soil Properties and Microbial Diversity

Pearson correlation analysis revealed distinct patterns of interaction between soil characteristics and microbial diversity across different treatments (Figure 6). Significantly, within the control treatment, a strong positive correlation was observed between the fungal Shannon diversity index and SOC, as well as between both fungal and bacterial Chao1 indices and SOC. This underscores the critical importance of SOC in sustaining microbial richness. In contrast, the PE-MP treatment was characterized by a pronounced negative correlation of fungal diversity indices with AK and AP, notably, the Chao1 index demonstrated a particularly strong negative association with AK. Conversely, the PBAT-MP treatment revealed a markedly distinct interaction pattern, with strong positive correlations observed for the fungal Shannon diversity index with SOC, TN, and AP. Similarly, bacterial diversity under the PBAT-MP treatment showed positive associations with SOC, TN, AK, and AP, suggesting that the PBAT-MPs might bolster microbial diversity in relation to these soil nutrients. These results suggest that the introduction of specific MPs can selectively influence soil/microbe interactions, with implications for soil ecosystem functionality. The observed divergent correlations across treatments highlight the complex role of MPs in influencing soil microbial community dynamics.

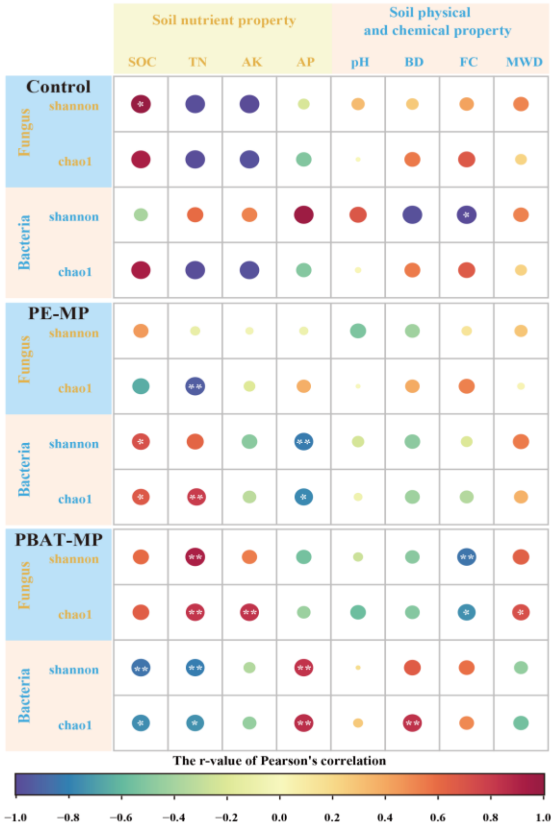

Figure 6. Pearson correlation analysis between soil factors and soil bacteria and fungi. The size of the circle represents the size of the absolute value of the r-value. Control, without microplastic addition. PE-MP, microplastics derived from polyethylene film; PBAT-MP, microplastics derived from polybutylene adipate-co-terephthalate film. * and ** indicate statistically significant differences at $p < 0.05$ and $p < 0.001$, respectively. No symbols are shown for $p \geq 0.05$.

3.7. Relationship between Soil Properties and Network Characteristics

Under the control treatment, fungal networks exhibit significantly positive correlations with SOC and TN, demonstrating a strong interaction network. Conversely, bacteria in control treatment soils show a pronounced positive correlation with SOC and an inverse correlation with BD, highlighting the diversity of microbial responses to soil physical properties (Figure 7).

In the presence of PE-MP treatment, fungal networks display a negative correlation with soil pH, suggesting that increased acidity may impair fungal network complexity. Additionally, bacterial networks in the PE-MP treatment demonstrate a strong positive association with SOC and a negative correlation with BD, reflecting the control condition but with intensified effects (Figure 7).

The PBAT-MP treatment reveals a complex interaction: fungal networks exhibit positive correlations with SOC, TN, and AP, while showing a negative correlation with BD. This suggests that PBAT-MP treatment may bolster microbial network resilience in nutrient-enriched conditions, yet potentially diminish network complexity under soil compaction. Bacterial networks under PBAT-MP treatment exhibit positive correlations with SOC, TN, and AK, suggesting that PBAT-MP may promote enhanced bacterial interactions within nutrient-rich environments (Figure 7).

Collectively, these treatments induce distinct microbial network dynamics, with PBAT-MP treatment exerting a significant positive effect on both bacterial and fungal network properties, particularly in terms of soil nutrient availability. The observation of significant correlations between various soil properties and microbial network metrics highlights the complex influence of MPs on soil microbial ecosystems (Figure 7).

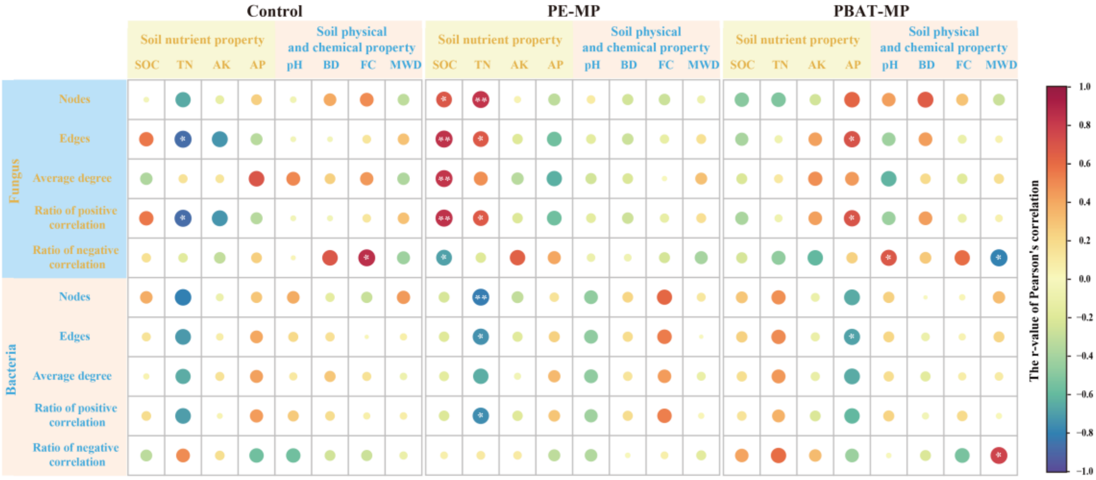

Figure 7. Pearson correlation analysis between soil factors and network characteristics. The size of the circle represents the size of the absolute value of the r-value. Control, without microplastic addition. PE-MP, microplastics derived from polyethylene film; PBAT-MP, microplastics derived from polybutylene adipate-co-terephthalate film. * and ** indicate statistically significant differences at $p < 0.05$ and $p < 0.001$, respectively. No symbols are shown for $p \geq 0.05$.

4. Discussion

4.1. Effect of Different MPs on Soil Properties

This research delves into the impact of PE-MP and PBAT-MP on the crucial properties of sub-surface soils. The findings reveal that both PE-MP and PBAT-MP significantly alter soil pH, AP, AK, and WHC, with PBAT-MP exhibiting relatively less adverse effects compared to PE-MP. This indicates variability in the impact of different MP types on soil physicochemical properties and nutrient cycling. The existing literature corroborates these observations to a degree, albeit with some distinctions. For instance, Palansooriya et al. (2023) [37] demonstrated that while low-density polyethylene MP did not markedly influence soil pH at lower concentrations, an increase in soil electrical conductivity was observed at higher concentrations, which could be attributed to increased microbial activity. Qi et al. (2022) [12] found that MPs alter soil nutrient dynamics through the formation of a plasticsphere. Moreover, Li et al. (2022) [38] reported that incorporating 0.03% and 0.3% PBAT-MPs into grassland and agricultural soils resulted in a reduction in SOC content. Overall, the influence of PBAT-MP on soil demonstrates a more intricate pattern when compared to PE-MP. This complexity arises primarily because PBAT-MP, being a biodegradable plastic, impacts soil characteristics through its decomposition process. In contrast, PE-MP, characterized as a stable, non-degradable plastic, predominantly exerts its effect on soil through physical impediment [25,39].

4.2. Effects of Different MPs on Soil Microbial Community Composition and Diversity

The investigations reveal distinct impacts of MPs on microbial communities at both bacterial and fungal levels. For bacteria, *Lysobacter* showed a notable increase in abundance with PE-MP and an initial increase followed by a decrease with PBAT-MP exposure. *Methylophaga* levels consistently declined across both MP treatments. At the fungal level, *Stachybotrys* and *Phlyctochytrium* were significantly enhanced under PE-MP and PBAT-MP treatments, respectively. Conversely, *Spizellomyces* (PE-MP) and *Didymella* (PBAT-MP) abundances decreased with increasing MP concentrations. This disparity underscores the pivotal role of MP composition in influencing microbial community dynamics. Aligning with observations that PBAT-MPs exert a more pronounced influence on microbial community structures than PE-MPs, Cao et al. (2022) [40] observed that PBAT-MPs significantly increase the relative abundance of MP-degrading bacteria, including *Lysomycetes*, *Pseudomonas*, and *Amylomycetes*, within soil environments compared to PE-MPs. The observed differences in microbial composition may stem from experimental differences such as MP concentration, exposure duration, and environmental context, thus elucidating the complexity of MP/microbe interactions [41]. In addition, a significant proportion of unidentified genera were observed commonly in previous studies. For example, Zhang et al. (2021) [42] and Xiao et al. (2023) [43] had a large proportion of uncultured and undefined genera in their studies on the effects of microplastics on soil microorganisms, which is consistent with the present study.

In this research, the impact of PE-MP and PBAT-MP treatments on microbial α-diversity was further explored. Contrary to initial hypotheses, the findings indicated no significant difference in bacterial and fungal α-diversity between treatments with PE-MPs and PBAT-MPs. Nevertheless, a biphasic pattern of decline and subsequent rise in bacterial α-diversity was observed under PE-MP treatments, contrasted with a consistent decline in bacterial α-diversity with increasing PBAT-MP concentration. Fungal α-diversity exhibited divergent trends between PE-MP and PBAT-MP treatments, mirroring differences observed in bacterial α-diversity. Principal Component Analysis (PCA) corroborated these findings, suggesting a predominant effect of PE-MPs on bacterial communities, whereas PBAT-MPs primarily influenced fungal populations. This is consistent with the findings of Li et al. (2024) [44] and Ruthi et al. (2020) [45], who reported a reduction in fungal α-diversity and an enrichment of functional communities involved in film degradation due to PBAT.

4.3. Effects of MP Addition on Soil Microbial Co-occurrence Networks

Within the bacterial network, treatments with PE-MP and PBAT-MP simplified the network structure relative to the control without significantly altering levels of competition and cooperation. Conversely, the fungal network exhibited a decrease in reciprocal interactions and an increase in competition under PE-MP treatment, while PBAT-MP's impact on fungal community dynamics was more subdued. These observations indicate that PE-MP primarily suppresses microbial activity, whereas PBAT-MP promotes fungal participation in the decomposition process. This is consistent with the findings of Li et al. (2023) [46] and Li et al. (2024) [44], who examined the impact of MPs derived from film-mulched farmland on bacterial and fungal communities. Despite the consensus that PBAT-MPs have a more pronounced influence on soil microbial symbiotic networks, this study distinguishes itself by focusing on sub-surface soil layers rather than the soil surface. Therefore, further research is essential, especially in sub-surface soils, to elucidate the interactions between PBAT-MPs and soil properties and the mechanisms by which various MPs influence the ecological roles of soil microorganisms.

4.4. Correlation between Soil Properties and Microbial Properties under MP Addition Conditions

This study reveals significant alterations in the association patterns among soil parameters, microbial community structures, biodiversity, and network interactions subsequent to the introduction of MPs. PE-MP treatments manifested correlations between bacterial and fungal communities with specific soil properties, notably SOC and BD. Conversely, fungal diversity was inversely correlated with AK and AP. PBAT-MP treatments, however, primarily linked bacterial communities with AP and showed positive associations with SOC, TN, and fungal diversity indices. Notably, PE-MP treatments negatively impacted the complexity of fungal networks relative to soil pH, suggesting potential disruption in less alkaline environments. In contrast, PBAT-MP treatments enhanced microbial network resilience in correlation with SOC, TN, and AP. These results indicate PBAT-MP's capacity to modulate soil microbial community dynamics by modifying nutrient profiles. This is consistent with several findings that PBAT-MPs can influence the structure and function of microbial communities in soil by altering the content and distribution of nutrients in the soil. For example, PBAT degradation in soil directly increases soil organic matter (e.g., POM, DOM, and MAOM) [47,48] and also promotes some enzymes related to carbon and nitrogen cycling [49,50]. In addition to increasing SOC content, PBAT-MPs can also affect the content and distribution of other nutrients; for example, low doses of PBAT-MPs resulted in a slight increase in TN and total phosphorus in the soil, which could be attributed to the fact that the input of PBAT-MPs provides an additional source of organic matter [51], and its degradation products can promote the proliferation of some nitrogen-fixing bacteria [38]. However, high doses of PBAT-MPs significantly decreased the phosphorus content in the soil. This may be due to the fact that high concentrations of PBAT-MPs promote the growth of oligotrophic soil bacteria such as Chlorophyceae and Archaea, which can utilize soil phosphorus for metabolism [52]. Therefore, further studies should construct a direct link between the effects of PBAT film-derived MPs on soil properties and microbial communities through stable isotopes combined with a multi-omics approach to reveal the effects of PBAT film-derived MPs on soil microbial ecology [53].

5. Conclusions

This study provided novel insights into the impacts of conventional non-biodegradable PE-MP and biodegradable PBAT-MP on sub-surface soil properties and microbial communities. It was found that both MP types can significantly alter soil physicochemical characteristics and nutrient cycling dynamics. However, PBAT-MP treatment resulted in an increase in soil pH, AP, and AK compared to PE-MP treatment.

Regarding microbial communities, both PE-MP and PBAT-MP induced shifts in the composition and diversity of bacterial and fungal populations. Notably, PBAT-MP initially promoted the abundance of certain genera involved in plastic degradation, such as *Lysobacter*, before levels declined at higher concentrations. Similarly, PE-MP also selectively enriched specific taxa but did not demonstrate this biphasic response. An assessment of α- and β-diversity revealed that PBAT-MP treatment predominantly influenced fungal communities, while PE-MP treatment exerted stronger impacts on bacterial communities.

An examination of microbial co-occurrence networks highlighted how PE-MP primarily disrupted fungal interactions and competition. In contrast, PBAT-MP did not significantly alter interaction dynamics but streamlined network complexity. Correlation analyses between soil properties, microbial properties, and network interactions revealed that PBAT-MP treatment promoted fungal diversity and enhanced network resilience.

Both types of MPs, PE and PBAT, significantly alter the native soil/microbe relationships. These findings suggest that PBAT-MPs exhibit differential effects compared to PE-MPs, influencing microbial community structure and function. Moreover, the biodegradation potential of PBAT-MPs over time remains underexplored. This study primarily focused on correlating MPs with soil properties and microbial relationships, thereby presenting a limitation due to the lack of direct experimental evidence to support these observations. Future research should utilize multi-omics approaches and isotope tracing techniques to elucidate the mechanisms by which MPs influence microbial ecological functions and biogeochemical cycles. Additionally, the long-term effects of MPs, including their accumulation and migration in agricultural soils, present a critical challenge that requires further investigation. Addressing these limitations and challenges is essential for a comprehensive understanding of MPs' impacts on the subsurface environment and the sustainability of agricultural practices in regions utilizing plastic mulch films. Overall, this work advances our understanding of the impacts of MPs on the subsurface environment and the sustainability of agricultural practices in regions utilizing plastic mulch films.

Author Contributions: Writing—original draft preparation, funding acquisition, X.L.; data curation, methodology, writing—original draft preparation, funding acquisition, W.W.; data curation, methodology, writing—original draft preparation, G.L.; visualization, methodology, writing—original draft preparation, B.Z. and J.C.; writing—review and editing, funding acquisition, T.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Xinjiang Uygur Autonomous Natural Science Foundation of China (2023D01A14), Natural Science Foundation of Shandong Province, China (ZR2020QD117), the Open Foundation of the Key Laboratory of Seaweed Fertilizers, Ministry of Agriculture and

Rural Affairs (KLSF-2023-006), the Shandong Provincial College Youth Innovation Team Program (2023KJ169), and the State Key Laboratory of Cotton Biology Open Fund (CB2022A14).

Data Availability Statement: Data will be made available on reasonable request.

Conflicts of Interest: Authors Bo Zhu and Jie Cui were employed by Qingdao Brightmoon Seaweed Group Co., Ltd. All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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