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Soil fungi respond more violently to both polyethylene and PBAT biodegradable mulch film residues than bacteria do

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Biodegradable mulch film (BDM) residues in farmland have attracted extensive concern due to their low degradation rate in soil after the service period. However, different reactions of the bacterial and fungal communities to LDPEM and BDM residues have been confusing. A pot experiment was implemented to explore the influences of 0.5% and 2.0% (w/w) LDPEM and BDM residues on soil physicochemical properties and bacterial and fungal communities in the present study. The results indicated that BDM residues significantly increased soil pH and SOC to an increasing degree under the treatment with a higher mulch film residue amount, while LDPEM residues did not. The dissimilarities of the bacterial community between the treatment groups and the control ranged from 0.24 to 0.27, while the dissimilarities of the fungal community were higher, with the variation ranging from 0.43 to 0.46. Higher variations in the internal correlation coefficient were observed in the fungal community than in the bacterial community under the treatment groups. In addition, the modules of the bacterial community network increased from 2 to 3 under the BDM 0.5% and BDM 2.0%. Comparatively, the treatments with BDM residues doubled the modules of the fungal community network from 2 to 4. Structural equation modelling indicated that mulch film residues had a higher negative direct effect on fungal community structure (-0.752) than on bacterial community structure (-0.600). However, soil physicochemical properties had no significant influences on either bacterial or fungal communities. Overall, soil fungi respond more violently to mulch film residues than bacteria do.

KEYWORDS

mulch film residues, soil fungi, soil bacteria, low-density polyethylene mulch film, biodegradable mulch film

1 Introduction

Plastic film mulching technology is widely promoted worldwide due to its superiority in maintaining soil temperature and moisture (Zhang et al., 2016). Plastic mulch film usage was approximately 0.3 million tons in 1991 in China, and the number rapidly increased to 1.30 million by 2021 (Ren et al., 2023), and it will continue to grow in the future (Yang et al., 2021). However, plastic mulch film residues in farmland have been a global concern due to their long application history and low degradation rate (Mak-Mensah et al., 2021). Numerous studies have demonstrated the significant harm of plastic film residues to

soil environment and crops (Hua et al., 2024; Li et al., 2023a; Li et al., 2023b). To alleviate residual pollution from plastic mulch, researchers have developed biodegradable mulch film (BDM), which can be degraded easily by microorganisms under composting conditions (Song et al., 2021; Giordano et al., 2020). Currently, the main component of BDM with the largest promotion area currently is poly (butylene adipate-co-butylene terephthalate) (PBAT). Although the degradation rate of commercially available BDM derived from PBAT at present is faster than that of polyethylene mulch films (Anunciado et al., 2021; Wang et al., 2022). The soil-biodegradable plastic films do not decompose in a lake sediment over 9 months of incubation (Van and Carsten, 2023). The investigation of Deirdre et al. (2022) found that up to 58 months were required to realize a degradation rate of approximately 90% in soil according to modelling data forecasting. Therefore, BDM can also cause residues in farmland before being degraded completely.

Soil harbours highly diverse microorganisms including bacteria, fungi, archaea, viruses, protozoa, and microalgae, which are involved in processes such as nitrogen fixation, decomposition of organic matter, and the breakdown of toxic substances, helping to maintain ecosystem functions and plant productivity (Bardgett and Van, 2014; De Vries et al., 2018). Plastic mulch residues can negatively impact soil bacterial communities by several ways. Firstly, they physically block soil pores, reducing aeration and water infiltration, which favors anaerobic bacteria and inhibits aerobic bacteria (Cao et al., 2022). The slow breakdown of plastics also releases harmful chemicals, such as plasticizers and stabilizers, which can leach into the soil and disrupt bacterial growth, potentially harming microbial diversity (Cheng et al., 2024; Souza et al., 2019). In addition, bacteria have been proven to actively participate in the geochemical processes of low-density polyethylene mulch film (LDPEM) residues, including migration, fragmentation and degradation (Yuan et al., 2020; Dong et al., 2024). Soil bacteria and their relationships with environmental factors in different soil types contaminated with LDPEM residues have also been studied (Hu et al., 2022; Fan et al., 2022; Dong et al., 2024). In addition to various concentrations and sizes of LDPEM residues, pH and soil organic carbon have been demonstrated to be the main environmental factors influencing the structure of the bacterial community (Song et al., 2023; Wilhelm et al., 2023). Besides some bacterial groups that are sensitive to LDPEM residues, potential LDPEM microplastic-degrading bacteria were also identified in the study of Wu et al. (2022). Despite great achievements brought about during the years of efforts regarding the bacterial community in LDPEM residue-contaminated soil, the responses of the bacterial community to BDM residues remain unclear.

Fungi are indispensable soil microbes and perform crucial functions in the transformation of soil nutrients (Khan et al., 2024; Li et al., 2023). However, our knowledge about how soil fungi respond to mulch film residues is extremely limited compared with that of bacteria (Romano et al., 2024; Jiao et al., 2024). Some toxic substances present in plastic materials may inhibit fungal metabolic processes, thereby reducing fungal activity and altering community composition (Francioni et al., 2024). Additionally, the presence of plastic residues may affect the decomposition rates of organic matter, as fungi play a crucial role in this process (Qi et al., 2022). It has been demonstrated that 14% polyethylene and

polyvinyl chloride mulch film residues addition in aquic soil can significantly decrease the diversity of the soil fungal community (Fan et al., 2022). The relative abundance of *Ascomycota*, which is a crucial decomposition agent in farmland, significantly increased by 20.1% with 14% LDPEM residues addition compared with control (Fan et al., 2022). Mulch film residues could alter the abundance of *arbuscular mycorrhizal*, which is highly dependent on the mulch film material types, dose, and cultivation environment (De Souza et al., 2019). Moreover, endophytic fungi could provide protection for host plants in mulch film residue-contaminated soil (Fan et al., 2022). Despite the studies mentioned above, fungal communities in soil with LDPEM and BDM residues are far less studied.

Despite the involvement of both bacteria and fungi in the degradation of organic matter, bacteria are generally regarded as significant regulators of the rapid carbon metabolism pathways of soils, whereas fungi are well known for their performance in the decomposition of refractory and highly polymeric organic matter (Wengel et al., 2006; Perkins et al., 2019; Fierer et al., 2007). Despite the distinct roles of bacteria and fungi in soil functions, they regulate soil biogeochemical processes jointly and cooperate closely (Zhang et al., 2021a). This is because the different contributions and close cooperation of bacteria and fungi on nutrient cycling and energy flow of the soil food web guarantee the stability of the soil nutrient supply (Rashid, et al., 2016; Li et al., 2016). Multiple studies have demonstrated that the optimum environmental conditions for the reproduction of fungi and bacteria are different (Xu et al., 2023; Fan et al., 2022), and their sensitivities to environmental disturbances are also different. For example, soil bacteria are more susceptible to long-term throughfall reduction than fungal communities in warmtemperate forests (Zhang et al., 2021b). While, the study of Chen et al. (2020) revealed that soil fungal networks are more sensitive to grazing exclusion than bacterial networks. Variations in the soil micro-environment induced by LDPEM and BDM residues (Gao et al., 2021) could stimulate differential responses of bacteria and fungi, which have not been studied to our knowledge.

To this end, a well-controlled pot experiment planting Chinese cabbage with different levels of LDPEM and BDM residues was conducted to examine the influences of mulch film residues on soil bacterial and fungal communities.

2 Materials and methods

2.1 Experimental design and sampling

The soil applied in the present study was collected in farmland in the plough layer (0–20 cm) with a alluvial soil texture in Beijing, China, which had never been mulched by LDPEM or BDM. The collected soil was air-dried and passed through a 2-mm sieve for experiment. The main component of the LDPEM low density polyethylene. The color of the LDPEM is white with the thickness of 0.014 mm. The applied BDM was the most widely promoted mulch film in China, with the main component being poly (butylene adipate-*co*-butylene terephthalate) (Su et al., 2020). There are also Poly lactic acid (PLA) and inorganic materials such as calcium carbonate. The color of the mulch is black with the thickness of 0.01 mm. Both the LDPEM and BDM were cut into three sizes by scissors, namely, 10 mm \times 10 mm, 5 mm \times 5 mm and 1 mm \times 1 mm,

to imitate residues in farmland according to the literature (Huang et al., 2020; Liu et al., 2018; Zhou et al., 2020). The three particle sizes were mixed in equal proportions. Five treatments with three replicates planting Chinese cabbage were conducted with ceramic planting containers (L: 45 cm × W: 23 cm × H: 17 cm), namely: 1) CK, no mulch film fragment addition; 2) LDPEM 0.5%, 0.5% (w/w) of LDPEM fragments added to soil; 3) LDPEM 2.0%, 2.0% (w/w) of LDPEM fragments added to soil; 4) BDM 0.5%, 0.5% (w/w) of BDM fragments added to soil; and 5) BDM 2.0%, 2.0% (w/w) of BDM fragments added to the soil. The pots were installed the smart greenhouse at Beijing Academy of Agriculture and Forestry. The temperature and humidity during the experiment were recorded every 10 min, as shown in the Supplementary Figure S1. It lasted for 54 days from sowing to harvesting. The irrigation amount was 500 mL for each time with a frequency of twice a week. No fertilizers were applied in the present study to avoid the interference of fertilization on soil microbial community. Other management measures for all treatments were consistent. Soil samples were collected and divided into two parts in parallel after crop harvesting. One part was applied to determine the soil's physicochemical properties with air drying. The other part was stored at -80°C before the measurements of soil microbes.

2.2 Analysis of soil physicochemical properties and enzymes activities

The soil property indices determined in this study included pH, soil electrical conductivity (EC), soil organic carbon (SOC), available nitrogen (AN), available phosphorus (AP), and available potassium (AK) following the description of Bao, (1999). In briefly, pH and EC were determined by electrode method with the soil to water ratio of 1: 2.5 and 1:5 respectively. SOC was determined by oxidizing organic C with potassium dichromate. AN, AP and AK were determined by Kjeldahl digestion, Olsen method and flame photometry respectively.

Benzene disodium phosphate colorimetric method was applied to determined the activity of phosphatase (AKP). The AKP activity is expressed in micromolar (µmol) of phenols in 1.0 g of soil after 24 h (Peng et al., 2022). Urease (UE) activity was determined using the sodium phenate-sodium hypochlorite colorimetric method. The UE activity is expressed in microgram (µg) of NH₄ ⁺-N in 1.0 g of soil after 24 h (Lu and Chen, 2022). The method of pyrogallol oxidation was used to determine the activity of peroxidase (POD). The POD activity is expressed in milligram (mg) of quinone in 1.0 g of soil after 24 h (Li et al., 2022).

2.3 Analysis of soil bacterial and fungal communities

According the instructions of the soil DNA kit (Omega Bio tek, Norcross, GA, U.S.) to extract the total genomic DNA, whose quality was detected by 1% agarose gel electrophoresis and spectrophotometry (optical density at 260 nm/280 nm ratio). Bacterial 16S rRNA and fungal ITS1 gene were sequenced for bacterial and fungal communities with the bacterial primer set 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') and fungal primer set ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 (5'- GCTGCGTTCTTCATCGATGC-3') Wang et al. (2022). The PCR reaction system were performed in triplicate 25 mixtures including 4 μ L of 5 × TransStart FastPfu buffer, 2 μ L 2.5 mM dNTPs, 0.8 μ L upstream primer (5uM), 0.8 μ L downstream primer (5uM), 0.4 μ L TransStart FastPfu DNA polymerase. The amplification procedure is as follows: pre denaturation at 95°C for 3 min, 27 cycles (denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s), followed by stable extension at 72°C for 10 min, and finally stored at 4°C. The 2% agarose gel and DNA gel recovery and purification kit (PCR Clean Up Kit, China Yuhua) was applied to recover PCR products and purify the recovered products. The detection and quantify of the recovered products was by Qubit 4.0 (Thermo Fisher Scientific, USA). The library of purified PCR products was build by NEXTFLEX Rapid DNA Seq Kit. Illumina PE300 platform was applied for sequencing (Majorbio Bio-pharm Technology Co., Ltd., Shanghai, China).

2.4 Data analysis

To obtain the species classification for each operational taxonomic unit (OTU), the Bayesian RDP classifier was used for taxonomic analyses at a 97% similarity level. One-way analysis of variance (ANOVA) was applied to analyse soil physicochemical properties, composition of bacterial and fungal communities on phyla level. The structure of bacterial and fungal communities at the OTU level was calculated by principal component analysis (PCA) by the software of R. The correlations of bacterial and fungal communities at OTU level was calculated by by "hmisc" in R. The distribution of correlation coefficients was analyzed to clarity the distribution patterns of bacterial community and fungal community respectively. Absolute value of all correlation coefficients was calculated to clarity the absolute distribution patterns of bacterial community and fungal community respectively. The networks of bacterial and fungal communities were performed by "igraph" in R (R > 0.80, P < 0.01). The significant differences between groups were determined based on network topological indices were determined by Kolmogorov-Smirnov test (Banerjee et al., 2019). The Z score analysis among mulch film residues, physicochemical properties and microbial community structure was carried out by Origin 2021. Furthermore, the relationships among mulch film residues, physicochemical properties and microbial community structure were calculated by a structural equation model (SEM) by the software of R (Zhang et al., 2022). The Jaccard dissimilarity index is calculated using the following formula, where a is the number of species shared between the two samples, and b and c are the numbers of species that appear only in the first and second samples, respectively.

$$J_{\rm dis} = 1 - \frac{a}{a+b+c}$$

3 Results and discussion

3.1 Soil physicochemical properties and enzymes activities

Variations in pH, EC, SOC and available nutrients under the stress of different concentrations and types of mulch film residues are shown in Figure 1. Soil pH showed no significant responses to



fragments added to the soil. Different letters (a-f) denote significant differences

LDPEM residues, which was in accordance with the result of Wu et al. (2022). Comparatively, the BDM residues increased the soil pH significantly, and the enhancement degree increased with the increasing residual amount (Figure 1a). The degradation of biodegradable polymers in solution could generate acidic monomers and oligomers, which accounted for the decrease in solution pH (Wei et al., 2022). It would be taken for granted that the soil pH would decrease due to the release of organic acids similar to the solution. Unexpectedly, no decrease but a substantial increase in soil pH was determined under 0.5% BDM and 2.0% BDM. The discrepant responses of pH in soil and solution might be induced by differences in degradation progress and mechanisms (Wei et al., 2022; Deirdre et al., 2022). Compared with CK, there was no significant difference in EC in the treatment groups (Figure 1b). Studies have demonstrated that soil EC is mainly affected by ion concentration and charge, including Cl⁻, Na⁺, and K⁺ (Yan and Petra, 2013; Yasenjiang et al., 2019). The main elements contained in LDPEM and BDM were carbon, hydrogen and carbon, and hydrogen and oxygen (Pei et al., 2020; Zhang et al., 2021), and these elements made little contribution to soil EC. The two treatments with BDM residues significantly increased SOC by 7.66% and 12.45%, while LDPEM residues did not (Figure 1c). No significant influence of LDPEM residues on SOC was also proven in a previous study (Wu et al., 2022); rather, the influence was induced mainly by the inertness of LDPE hindering the release of carbon (Restrepo-Flórez et al., 2014). There have also been studies indicating that LDPEM residues decrease SOC (Dong et al., 2015). Different influences of LDPEM residues on SOC might be induced by the various soil types and culturing durations. Different from LDPEM, the carbon in BDM could be easily metabolized by microorganisms, which could replenish the soil carbon pool.

Overall, all three types of available nutrients showed a downtrend in the four treatment groups (Figures 1d-f). In detail, LDPEM 2.0%, BDM 0.5% and BDM 2.0% decreased soil AN by 4.50%, 7.42%, and 8.14%, respectively, compared with CK (Figure 1d). The four treatment groups significantly decreased the soil AP content by 6.29%, 7.17%, 8.14% and 7.99% compared with the control, as shown in Figure 1e. In terms of AK, a significant difference was found between the control and BDM 2.0% treatments, with an 11.49% reduction in the BDM 2.0% treatment (Figure 1f). The downtrend of available nutrients, especially for AN and AP, under the treatments with mulch film residues was in line with the findings of Dong et al. (2015) and Koskei et al. (2021). There could be several reasons accounting for these results. First, there was almost no nitrogen, phosphorus or potassium in either LDPEM or BDM (Zhang et al., 2021; Pei et al., 2020). Moreover, film residues had adverse effects on soil physical properties, including bulk density and water holding capacity, which are closely related to soil available nutrients (De Souza et al., 2018). On the other hand, studies have indicated that soil enzyme activities,



which act as mediators and catalysts to realize the biochemical processes of soil ecosystems, would be inhibited by mulch film residues, resulting in a decrease in nutrient conversion efficiency (Yu et al., 2020).

The variations of alkaline phosphatase (AKP), urease (UE), and peroxidase (POD) was determined as shown in the Figure 2. The addition of BDM residues significantly decreased soil AKP activities by 11.96% and 15.99% under the treatments of BDM 0.5% and BDM 2.0% respectively, which was in accordance with the study of Awet et al. (2018). On the contrary, the addition of mulch film residues for both LDPEM and BDM significantly increased the activities of UE and POD. Compared with CK, the activities of UE significant increased by 4.43%-6.37% under the four treatments with mulch film residues as shown in the Figure 2b. Studies have demonstrated the mulch film residues can stimulate soil UE activity and affect soil carbon and nitrogen content (Huang et al., 2019; Judy et al., 2019). While, the activities of POD increased by 9.37%-12.5% under the four treatments with mulch films residues. Researches have confirmed that soil POD is significantly positively correlated with soil aerobic microorganisms (Huang et al., 2019; Liu et al., 2017a). Mulch film residues could increase the abundance of soil aerobic microorganisms by increasing soil porosity, further enhancing POD activity (Qi et al., 2020b; Gao et al., 2021).

3.2 Structure and composition of bacterial and fungal communities

The structures of both bacterial and fungal communities were strongly, but differently, affected by mulch film residues (Figure 3). The PCA at the OTU level of the bacterial and fungal communities indicated significant differences among the different treatments (Figures 3a, e). For bacteria and fungi, the five treatments were distributed in the whole quadrants, with the sum explanation proportions of 67.62% and 86.61% for the first two principal components, respectively. The results of LDPEM 2.0% and BDM 2.0% were farther from CK compared with those of LDPEM 0.5% and BDM 0.5% for bacteria and fungi, in line with previous studies that indicated greater influences in the groups with higher concentrations of residues on the microbial community (Fan et al., 2022), which highlights the dose-dependent nature of mulch film residue impacts on soil microbial ecosystems.

LDPEM 0.5%, BDM 0.5% and BDM 2.0% significantly decreased microbial community richness and evenness compared with the control, as shown in Figures 2f, 3b. The richness in BDM 2.0% was the lowest, with reductions of 6.25% and 4.75% for the bacterial community and fungal community, respectively. Compared with the control, BDM 2.0% decreased the evenness of the bacterial and fungal communities by 1.15% and 7.34%, respectively, as shown in Figures 3c, g, indicating that a high concentration of BDM residues significantly influenced the structure of the microbial community, which has also been demonstrated by Hu et al. (2022). However, no significant difference was observed in the richness and evenness between the control and 0.5% LDPEM for both bacteria and fungi, which was attributed to the certain buffer capacity of the soil (Malczyk et al., 2008). In addition, the same concentration of BDM residues had a stronger effect on soil microorganisms than that of LDPEM residues, which was mainly induced by the different compositions of the two types of mulch film (Liu et al., 2022). BDM can be degraded by microorganisms, while LDPEM is rarely utilized by microbes (Tribedi and Samrat, 2017).

To further clarify the influences of mulch film residues on soil bacteria and fungi, Jaccard dissimilarities between the control and each treatment for the bacterial and fungal communities were calculated, as shown in Figures 3d, h. The dissimilarities of the bacterial community between the treatment groups and the control ranged from 0.24 to 0.27. The dissimilarities of the fungal community varied from 0.43 to 0.46 (Figure 3h), and the dissimilarities under the 0.5% LDPE treatment were significantly lower than those under the other three treatments. The dissimilarities of the fungal community were higher under the same treatment than the bacterial community, as shown in Figures 3d, h, which indicated that fungi were more sensitive to



FIGURE 3

The response of the bacterial and fungal communities to mulch film residues. PCA of bacterial (a) and fungal (e) communities. Richness of bacterial (b) and fungal (f) communities. Evenness of bacterial (c) and fungal (g) communities. Jaccard dissimilarity of bacterial (d) and fungal (h) communities between the control and treatments. Note: LDPEM 0.5%, 0.5% (w/w) of LDPEM fragments added to soil; LDPEM 2.0%, 2.0% (w/w) of LDPEM fragments added to soil; BDM 0.5%, 0.5% (w/w) of BDM fragments added to soil; BDM 2.0%, 2.0% (w/w) of BDM fragments added to the soil.



mulch film residues. Previous studies have demonstrated that the influences of long-term application of nitrogen fertilizer on the UniFrac distance of the fungal community were greater than those of the bacterial community (Wang et al., 2019), which was attributed to the higher resistance to interference of bacteria from its higher growth rate and unicellular properties (Powell et al., 2015). Moreover, the lower habitat niche breadths of fungi than bacteria resulted in a lower tolerance of fungi to environmental variation (Zhao et al., 2019). At lower concentrations (0.5%), LDPEM is more stable and resistant to microbial degradation, so its impact on soil microorganisms is relatively small. Bacterial communities are generally better able to adapt to this stable environment, with their metabolic activity being less affected by the mulch film residues. While fungal communities also show some adaptability, they are more sensitive to environmental changes compared to bacteria. Therefore, under the 0.5% LDPEM treatment, the response of the fungal community is more subtle compared to that of the bacterial community. However, at the higher concentration of BDM 2.0%, the differences in the responses of bacteria and fungi become more pronounced. This is primarily due to the biodegradability of BDM. The degradation of BDM residues in the soil releases more organic matter, providing rich carbon sources for microorganisms. However, the degradation process of BDM may also promote the rapid growth of specific microbial groups. For fungal communities, although the degradation products of BDM provide additional nutrients, the excessive stimulation may disrupt the balance of the fungal community. As a result, the impact of BDM 2.0% on bacteria is milder compared to fungi, with a significant decrease in the evenness of the fungal community, demonstrating stronger niche competition and changes in community structure.

As the dominant strains of soil bacteria (Delgado-Baquerizo et al., 2018), the bacterial phyla *Actinobacteriota*, *Proteobacteria*,

Acidobacteriota, Chloroflexi, and Firmicutes were the top five bacterial phyla, accounting for 81.75%-86.12% of the total soil bacterial community (Figure 4a; Supplementary Table S1). Actinobacteriota and Proteobacteria, which are capable of decomposing microplastics (Wu et al., 2022), increased by 24.38% and 24.32% in BDM 2.0% compared with the control, accelerating the degradation of BDM suggesting that BDM may stimulate the growth of specific microbial communities that facilitate its degradation. The increased abundance of Actinobacteria and Proteobacteria could be associated with their enhanced metabolic activity, particularly in degrading complex organic compounds such as plastic polymers (Liu al., 2022). However, the relative abundance of et Acidobacteriota decreased significantly by 40.88% in BDM 2.0%. The increased soil pH under the 2.0% BDM treatment could explain the decrease in the relative abundance of Acidobacteria. Acidobacteria are generally more competitive in low pH environments (Liu et al., 2020), and their reduced abundance suggests that soil pH changes have a profound effect on microbial community structure. Moreover, LDPE 2.0%, BDM 0.5%, and BDM 2.0% significantly decreased the relative abundance of Gemmatimonadota and Myxococcota, which is in line with the previous work of Liu et al. (2021). Gemmatimonadota and Myxococcota are typically involved in organic matter decomposition and carbon and nitrogen cycling in the soil ecosystem (Ning et al., 2024). The changes in the relative abundance of Gemmatimonadota and Myxococcota may be due to changes in soil organic matter content or microbial interaction patterns induced by the presence of these plastic materials, which in turn affect the growth and metabolic activity of these bacterial groups.

Regarding the fungal community, the top five determined phyla were *Basidiomycota*, *Ascomycota*, *Mortierellomycota*,

Olpidiomycota, and Chytridiomycota, making up 87.98%-99.35% of the fungal sequences (Figure 4b; Supplementary Table S2), which was consistent with the findings of the majority of studies (Paungfoo-Lonhienne et al., 2015; Feng et al., 2019; Pan et al., 2020), indicating the governing position of these phyla in soil fungal communities. The relative abundances of Basidiomycota were 18.33%, 24.99% and 24.21% lower in the LDPEM 0.5%, LDPEM 2.0% and BDM 2.0% treatments than in the control, but no significant difference was observed in the relative abundance of Basidiomycota between the BDM 0.5% and CK treatments (Figure 4b). The reduction in *Basidiomycota* in the treatments with plastic residues might be due to the inability of these fungi to degrade the plastic film residues, which highlights the potential inhibitory effects of plastic materials on key decomposition agents in agricultural soils, affecting microbial diversity and functioning. Studies have indicated that Basidiomycota are key decomposition agents in agricultural soil for phenolic compounds (Martínková et al., 2016). The combination of its inability to degrade mulch film residues and the enhancement of the relative abundance of fungal phyla enabling the degradation of mulch film residues resulted in a decrease in Basidiomycota.

The relative abundances of Ascomycota in LDPEM 2.0% and BDM 2.0% were 34.25% and 32.86%, respectively. However, the LDPEM 0.5% and BDM 0.5% treatments had no significant effect on the relative abundance of Ascomycota. The significant increase in Ascomycota in the higher concentration treatments may reflect the role of this microbe in breaking down plastic film residues. A similar finding was recorded by Fan et al. (2022), whose study indicated that high concentrations of mulch residues could stimulate the abundance of Ascomycota because Ascomycota can produce hydrolase and promote mulch film degradation (Phosri et al., 2012). This supports the idea that the presence of higher concentrations of mulch residues encourages the growth of specific fungal groups, which are involved in the degradation process, providing further insight into the microbial adaptation to plastic pollution. Apart from BDM 2.0%, which increased Mortierellomycota significantly by 86.08%, the other three treatments with residues showed no significant influence on Mortierellomycota compared with the control. Yuan et al. (2020) indicated that diseased soils harboured a higher relative abundance of Mortierellomycota, which has a strong decomposition ability (Wu et al., 2021). The significant increase in Mortierellomycota in the BDM 2.0% treatment may be linked to its specific metabolic pathways or the ability to adapt to the presence of BDM, enhancing its growth and activity in degrading organic matter. The four treatment groups had no significant influence on the abundance of Olpidiomycota, in agreement with the works of Zhang et al. (2020) and Liu et al. (2020), which revealed that Olpidiomycota was not sensitive to external disturbances. LDPEM 2.0% and BDM 2.0% significantly increased the relative abundance of Chytridiomycota, which was approximately five and four times that of the control, respectively (Figure 4b; Supplementary Table S2). Studies have demonstrated that most Chytridiomycota are complex compounds-degrading including cellulose- and chitin (Letcher et al., 2008). Therefor, Chytridiomycota might be responsible for mulch film residue degradation.

3.3 Networks of bacterial and fungal communities

To clarify the internal relationships of the bacterial and fungal communities under different treatments, the correlations of the bacterial community and fungal community at the genus level were calculated. Similar to the study of De Vries et al. (2018), the distribution of the correlation coefficients for both bacteria and fungi presented a trend similar to the normal distribution for all correlations, as shown in Supplementary Figure S1A. When considering correlation coefficients greater than 0.6, which was regarded as the cut-off of significant correlation, the negative correlation coefficients of the bacterial community showed an increasing trend in the treatments with mulch film residues, especially for the treatment of BDM 2.0%, which significantly increased the negative correlation by 45.68% (two side x^2 - test of proportion; P < 0.05). The competitive and antagonistic interactions among bacterial communities may be enhanced with the increase of mulch film residues due to the increase of the negative correlation. In particular, the treatment of BDM 2.0% significantly intensified this antagonism, likely due to the chemical composition of BDM, which bacteria might find less adaptable. This phenomenon suggested that ecological niche overlap between bacteria may decrease, resulting in stronger resource competition within the bacterial community. In contrast, the treatments of LDPEM 0.5%, LDPEM 2.0%, BDM 0.5% and BDM 2.0% significantly (two-sided x^2 - test of proportion; P < 0.05) decreased the positive correlation more than 0.6 for the bacterial community by 13.92%, 19.46%, 22.52% and 38.49%, respectively, compared with the control, which indicated that BDM residues had a greater impact on bacterial internal relationships than LDPEM residues (Supplementary Figure S1A). This finding was supported by Liu et al. (2022), revealing that microorganisms were more perceptive of the chemical compositions of BDM than of LDPEM. On the other hand, the decrease in the positive correlation and increase in the negative correlation indicated that mulch film residues enhanced the antagonism among bacteria. The presence of mulch film residues likely altered the soil microenvironment, intensifying competition among bacteria. Particularly in the BDM treatment, this competitive relationship became more pronounced. Due to the environmental stress posed by the mulch film residues, bacteria may have been forced to increase negative interactions as a strategy to outcompete one another. Compared with the control, the negative correlation coefficients for the fungal community were significantly increased by 40.71% under BDM 2.0% (two-sided x^2 - test of proportion; P < 0.05). However, no significant difference was observed among LDPEM 0.5%, LDPE 2.0%, BDM 0.5% and the control (twosided x^2 - test of proportion; P > 0.05) suggesting that BDM residues have a more significant effect on the fungal community. The BDM likely exerted a strong influence on fungal communities, promoting increased antagonism between fungal species.

For the positive correlation coefficients, the LDPEM 0.5%, LDPEM 2.0%, BDM 0.5% and BDM 2.0% treatments significantly increased the positive correlation coefficients by 43.06%, 47.59%, 47.91% and 49.15%, respectively, compared with the control (two-sided x^2 - test of proportion; P < 0.05), which suggesting that mulch film residues enhanced cooperative relationships within the fungal community. Notably, higher



variations in the correlation coefficients of fungi under the treatments with mulch film residues were observed compared with bacteria, indicating greater changes in internal relations in the fungal community than in the bacterial community. This could be explained by the lower habitat niche breadths of fungi than bacteria, which resulted in a lower tolerance of fungi to environmental variation induced by interference than bacteria (Zhao et al., 2019).

Networks at the genus level for different treatments of the bacterial community and fungal community are shown in Figure 5. Overall, the network density of bacterial networks was significantly higher than that of fungal networks under the same treatment, indicating stronger interactions in the bacterial community, which was supported by the study by De Vries et al. (2018). To deeply determine network structure variations, the

distribution patterns of the bacterial community and fungal community under different treatments were analysed. As shown in Figure 5, the module numbers of the bacterial community and fungal community also responded to mulch film residues. In detail, there were 2 modules in the networks of the bacterial community for the CK, LDPEM 0.5% and LDPEM 2.0% treatments. However, there were 3 modules of the bacterial community in BDM 0.5% and BDM 2.0%, and they increased by a ratio of 50.0% compared with the control, indicating that BDM residues could disturb the soil bacterial community doubled from 2 to 4 in BDM 0.5% and BDM 2.0% compared with the control. Stronger module variation for the fungal community network probably resulted from the stronger structural variation of the fungal community than that of the bacterial community, as shown in Figures 3, 4.



Z scores of the pH, EC, SOM, AN, AP, AK, richness and evenness for the bacterial community (a) and fungal community (b) Partial least squares path model for the bacterial community (c) and fungal community (d) Path coefficients were calculated after 1000 bootstrap replicates and are reflected by the width of the arrow, with blue and red indicating positive and negative effects, respectively. Dashed arrows show that the coefficients did not significantly differ from 0 (P > 0.05).

3.4 The relationship among mulch film residues, physicochemical properties, enzyme activities, and microbial community structure

To synthetically evaluate how mulch film residues influenced physicochemical properties, enzyme activities, and microbial community structure, Z score analysis was carried out. The Z scores of richness, evenness, AK, AP, AN, and AKP decreased for bacterial communities in the treatment groups compared with CK. However, pH, EC, SOC, UE and POD showed adverse trends, as shown in Figure 6a. The Z-score of soil physicochemical properties in fungal community structure is different from bacterial communities. Specifically, the Z scores of evenness, AK, AP, AN and AKP decreased in the treatments with mulch films compared with CK, especially for BDM 2.0% (Figure 6b). However, the richness, pH, EC, SOC UE and POD scores increased in the treatment groups. Differences in the results of the Z scores for the bacterial and fungal communities also indicated the various reactions of the bacterial and fungal communities to mulch film residues. To further determine the complex interrelationships of mulch film residues on soil bacterial and fungal community structures, SEMs were established, as shown in Figures 6c, d, respectively. The results indicated that mulch film residues significantly influenced soil physicochemical properties and enzymes activities (P < 0.05), which are closely related to the growth and development process of crops (Mandal et al., 2003), in line with the study of Wang et al. (2020). This suggests that the degradation and accumulation of mulch residues can alter the basic soil environment, potentially hindering plant growth by affecting nutrient availability and soil structure, similar to how previous studies have pointed out the role of soil properties in crop performance. Consequently, urgent actions are needed to tackle film mulch pollution to ensure global food security, whether for LDPEM residues or BDM residues. Mulch film residues had a significantly negative direct influence on the structure of the bacterial community (-0.600) and fungal community structure (-0.752), which indicated that mulch film residues had a greater impact on soil fungal communities. Fungi often play more specialized roles in decomposition and nutrient cycling, may be more sensitive to the presence of mulch film residues, leading to a more pronounced alteration in their community composition compared to bacteria. The influences of soil enzymes activities on bacterial community and fungal community structure were coefficient significant with path of -0.724 and -0.765 respectively. The close relationship between enzyme activities and microbial structure suggests that enzymatic processes are key drivers of microbial community composition. However, the soil physicochemical properties had no significant influences on the bacterial and fungal communities (P > 0.05), which indicates that while physical and chemical soil properties are important, mulch film residues appear to exert a more direct and pronounced effect on microbial community structures, overshadowing the influence of other soil characteristics. This finding was supported by the study of Wu et al. (2021), who also revealed that residues of mulch film significantly influenced soil microbial communities but did not alter soil physicochemical properties. Therefore, mulch film residues directly affected soil microbial communities, though not by influencing soil physicochemical properties.

3.5 The significance of soil bacterial ad fungal community for agricultural environmental protection and sustainable development

Promote the circulation of soil organic matter: Soil bacteria and fungi decompose organic matter, converting it into nutrients that can be absorbed and utilized by plants, which could improve soil fertility and provides a continuous supply of nutrients for agricultural production (Puškarić et al., 2021; Chen et al., 2023). Improve soil structure: Bacteria and fungi promote aggregation of soil particles, and form a good soil structure, which improves soil aeration and water retention and provides a good soil environment for plant growth (Navas et al., 2021; Rashid et al., 2016). Promote plant growth: Some soil bacteria and fungi can form symbiotic relationships with plants, helping them absorb nutrients, resist diseases and pests, and promote plant growth, which improve plant stress resistance and allow plants to grow normally under adverse conditions (Feng et al., 2023). Decompose residual plastic film: Soil bacteria and fungi can decompose residual plastic film, converting it into small molecular substances, thereby reducing its negative impact on the soil. The microplastics produced by the residual film after degradation will increase the soil microbial biomass carbon (Liu et al., 2022; Xiang et al., 2024).

4 Conclusion

The present study investigated the different reactions of bacterial and fungal communities as well as soil physicochemical properties to LDPEM and BDM residues. The results indicated that BDM residues significantly increased soil pH and SOC, with greater enhancement observed in higher residue treatments, while LDPEM residues did not. Both film types residues reduced AN, AP, and AK levels. The dissimilarities of the fungal community between the treatment groups and the control were greater than those of the bacterial community. Higher variations in the internal correlation coefficient were observed in the fungal community than in the bacterial community under the treatments with mulch film residues compared with CK. Modules of the bacterial community network increased from 2 to 3 under the BDM 0.5% and BDM 2.0%. Comparatively, BDM 0.5% and BDM 2.0% doubled the modules of the fungal community network from 2 to 4. Notably, SEM indicated that residues of mulch film and soil enzymes had a higher negative direct effect on fungal community structure than on bacterial community structure. The soil physicochemical properties had no significant influences on the bacterial and fungal communities (P >0.05), suggesting that the effects of mulch residues on soil bacterial and fungal communities were not produced through the changes of soil physicochemical properties. In summary, our findings underscore the differential microbial responses to mulch residues, particularly emphasizing the heightened respond of soil fungi to mulch film residues compared to bacteria, thus enhancing our understanding of the broader environmental impacts of mulch

residues on soil ecosystems. Future research could focus on elucidating the long-term effects of mulch residues on soil microbial communities across different agricultural systems.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

LyL: Conceptualization, Methodology, Writing – original draft. LuL: Formal Analysis, Writing – original draft. GZ: Conceptualization, Software, Writing – review and editing. JG: Methodology, Supervision, Writing – review and editing. QZ: Formal Analysis, Validation, Writing – original draft. XZ: Writing-review and editing. LD: Methodology, Validation, Writing – original draft, Writing – review and editing. DL: Funding acquisition, Writing–original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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