

# Bacterial and fungal diversity in the rhizosphere of buckwheat under different mulching techniques

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**The present research aimed to assess the effects of plastic film mulch on microbial diversity and community in the Tartary buckwheat rhizosphere. Treatments included regular cultivation, polyethylene film mulch on the whole ground and furrow-ridge plastic film mulch (FR). We found that FR prominently reduced the relative abundance (RA) of the members of phylum Mortierellomycota while increasing the RA of the members of phylum Ascomycota, especially *Fusarium* and *Dokmaia*. FR also reduced the predicted sequences related to mannan degradation and the biosynthesis of phospholipases, phosphatidylglycerol and ubiquinol. This study suggests that it is necessary to evaluate the effect of mulch techniques on pathogenic and mycotoxin-producing species before application.**

**Keywords:** Buckwheat, microbial diversity, mulching, relative abundance, rhizosphere.

As a member of the dicotyledonous family Polygonaceae, Tartary buckwheat (*Fagopyrum tataricum*) is a traditional pseudocereal crop with medicinal value. Its gluten-free products contain high levels of essential nutrients, including ideal proteins, vitamins, lipids, dietary fibre and minerals<sup>1</sup>. In terms of medicinal value, Tartary buckwheat has a high content of bioactive flavonoids and polyphenols, which provide beneficial health effects of antioxidant, anti-obesity, anti-inflammatory and anti-hypercholesterolemia potential<sup>2</sup>. Tartary buckwheat originated in southwest China and is consumed mainly in Europe and Asian countries<sup>3</sup>. Due to its attractive nutritional and health benefits, it has attracted worldwide attention in recent years.

The rhizosphere refers to the root surface and soil surrounding the root. It is the most active region of plant–microbe interactions in the soil. The rhizosphere microbiota contributes to plant health and productivity by activating soil nutrients, decomposing soil organic matter and resisting plant diseases. On the other hand, plant root activities secretions and environmental changes around the root can influence the microbial community and diversity in the rhizosphere<sup>4–6</sup>. Farming activities could disturb the farmland soil environ-

ment, thus altering the microbial communities associated with the crop rhizosphere<sup>7</sup>.

Polyethylene film mulch is extensively used to conserve water, suppress weeds and improve productivity in agriculture. In recent years, several studies have explored the effect of plastic film mulch on microbial community and diversity, as well as microbial biomass, colony-forming units and microbial functionality<sup>8</sup>. However, the effect was varied with microorganism types and agricultural systems. Polyethylene film mulch altered the richness, alpha diversity and ecological functions of soil microorganisms. Mulching treatments showed a greater effect on the fungal community than the bacterial community. Soil nutrients, especially carbon and nitrogen fractions were shown to regulate the bacterial diversity and fungal richness<sup>9</sup>. Polyethylene mulch could modulate soil moisture and root biomass to maximize crop production<sup>10</sup>.

In China, Tartary buckwheat is mostly planted in hilly areas with low temperatures, barren land and severe drought<sup>11</sup>. In these areas, water scarcity seriously limits crop production. To attenuate the hazard of drought, several cultivating strategies have been applied to reduce evaporation and improve rainwater use efficiency, like plastic film mulching and furrow-ridge (FR) mulching systems. In the FR mulching system, mulched ridges are used to harvest rainwater while unmulched furrows are used to collect rainwater and also grow plants<sup>12</sup>. However, the effect of these two mulching techniques on crop rhizosphere microbial communities remains poorly understood. The objective of the present study was to assess the microbial diversity and community in the Tartary buckwheat rhizosphere under three cultivating strategies: regular cultivation, plastic film mulch and FR mulch.

## Materials and methods

### Soil sampling

This study was carried out at Shanxi Agricultural University Experimental Station (37°43'N, 112°59'E), Mengjiazhuang, Jinzhong City, Shanxi Province, China. The field trial was performed by a randomized complete block design and one factor variable of three cultivating methods, which included regular cultivation (R), plastic film mulch on the

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whole ground (F) and FR. Buckwheat Jinqiao 5 was cultivated in late May at the rate of 900,000 plants per hectare and harvested at the beginning of October. Fertilizers were applied when sowing at a rate of 120 kg CO(NH<sub>2</sub>)<sub>2</sub>, 90 kg Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> and 90 kg KCl per hectare. Soil samples were collected on the day of harvest in October 2020. Five different points were sampled randomly on every block and pooled to form a homogenous sample. The samples were put in aseptic bags and preserved at -80°C for future use.

### Soil DNA isolation and sequencing

Metagenome DNA was isolated from the mixed samples using the TIANamp Soil DNA Isolation kit (DP 336; TIANGEN, Beijing, China), following the manufacturer's instructions. The concentration of extracted DNA in each soil sample was assessed by a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). Primers 338F + 806R and ITS5F + ITS2R were selected to PCR amplify the V3–V4 region of bacterial 16S rRNA and fungal ITS1 genes respectively. The amplicons were purified and sequenced by HiSeq 2500 System (Illumina, CA, USA) in the Shanghai Personal Biotechnology Co, Ltd, China. All sequences are available in NCBI under accession no. PRJNA-797228.

### Bioinformatic analyses

The raw sequencing data were trimmed, denoised, merged and chimera-removed by DADA2 workflow<sup>13</sup> in the QIIME2 software<sup>14</sup>. The quality-checked sequences were de-replicated and defined as amplicon sequence variants (ASVs). After removing the singletons, ASVs were assigned taxonomic annotations based on the Greengenes<sup>15</sup> and UNITE taxonomy databases<sup>16</sup>. To identify the specific taxa among different cultivation methods, LEfSe analysis was performed using R software 4.0.2 (ref. 17).

The alpha diversity was measured by estimating Chao1, observed species, Shannon–Weaver, Simpson, Pielou's evenness and Goods coverage using QIIME2. The data were visualized using box plots, and statistical analysis was performed using Kruskal–Wallis test and Dunn test. Beta diversity was analysed using R software 4.0.2. Principle coordinates analysis (PCoA)<sup>18</sup> and nonmetric multidimensional scaling (NMDS) were conducted by the R packages 'ape' and 'vegan' respectively.

### Metabolic function analysis

PICRUSt 2 software was used to analyse the potential function of the buckwheat rhizosphere microbiota based on MetaCyc, KEGG and COG databases<sup>19</sup>. The difference in metabolic function among groups was analysed using the R package 'metagenomeSeq'.

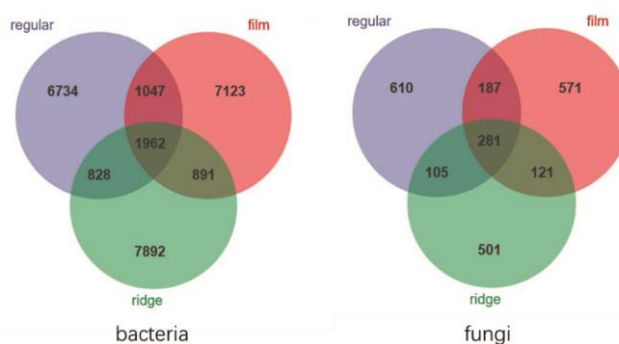
## Results

### Microbial composition in the rhizosphere of Tartary buckwheat

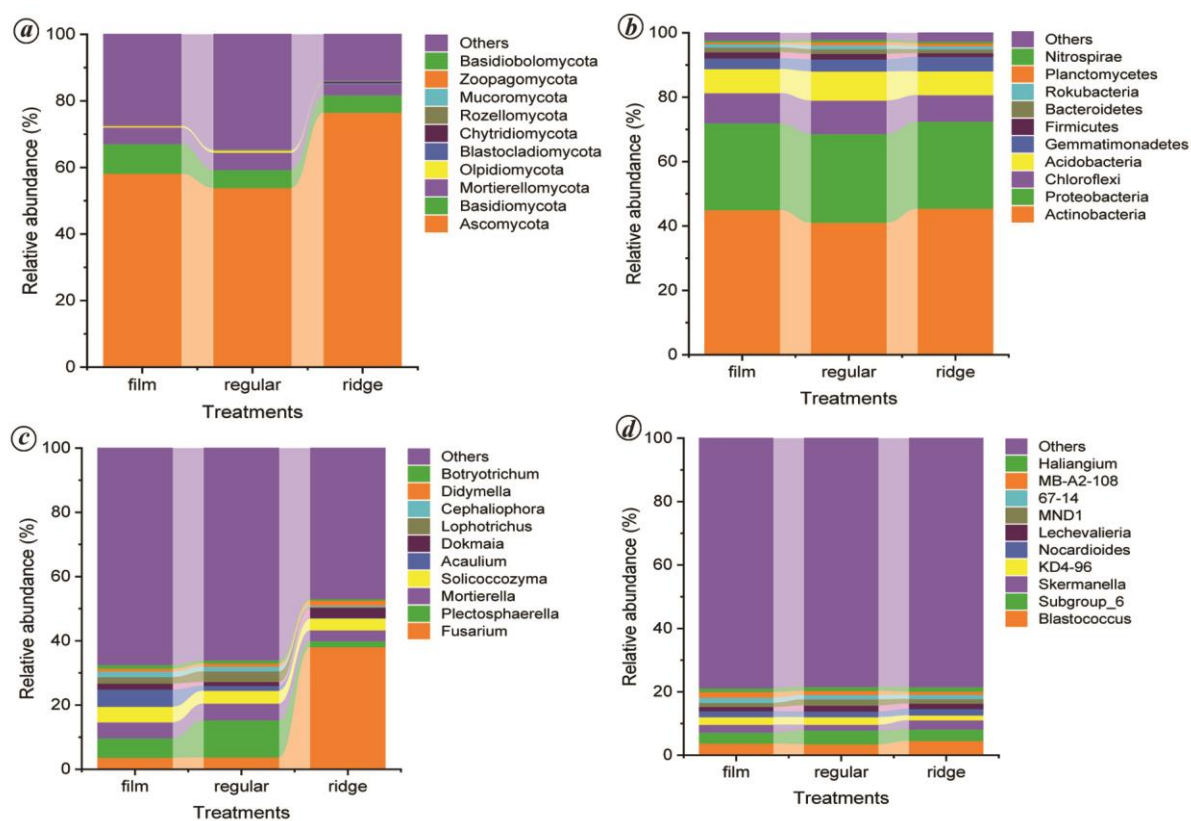
In the tested samples, the quality-filtered bacterial 16S rRNA and fungal ITS1 sequences ranged from 29,369 to 50,674 and 96,326 to 119,390 respectively (Supplementary Table 1). ASVs were used to analyse the quality-filtered sequences. The classification of bacterial and fungal ASVs was done by comparing them to the Greengenes and UNITE databases respectively. A total of 1962 bacterial and 281 fungal ASVs were common for all groups, while 6734, 7123, 7892 bacterial ASVs and 610, 571, 501 fungal ASVs were only detected in the R group, F group and FR group respectively (Figure 1). Compared to the R group, bacterial 16S rRNA ASVs were 12.6% and 13.2% lower, while fungal ITS ASVs were 2.8% and 10.6% higher with F and FR treatments respectively.

Across treatments, the dominant bacterial phyla identified were Actinobacteria (41.1–45.4%), Proteobacteria (27.0–27.6%), Chloroflexi (8.2–10.3%), Acidobacteria (7.4–9.1%) and Gemmatimonadetes (3.3–4.5%). The phyla Ascomycota (53.9–76.5%), Basidiomycota (5.2–8.9%) and Mortierellomycota (3.4–5.2%) were most abundant for fungi. The abundance of dominant bacteria phyla showed no significant difference among groups ( $P > 0.05$ ). For dominant fungi phyla, Ascomycota was prominently more abundant in the FR group, while Mortierellomycota was less abundant in the FR group, in comparison with those in the F and R groups ( $P < 0.05$ ) (Figure 2).

At the genus level, the composition of bacteria and fungi was mostly identical in all soil samples (Figure 2). The top 10 dominant bacterial genera were *Blastococcus*, Subgroup\_6, *Skermanella*, KD4-96, *Nocardioidea*, MB-A2-108, 67-14, *Lechevalieria*, MND1 and *Haliangium*. The dominant fungal genera were *Plectosphaerella*, *Mortierella*, *Solicocozyma*, *Fusarium*, *Lophotrichus*, *Acaulium* and *Dokmaia*. The abundances of *Fusarium* and *Dokmaia* were prominently higher, while that of *Plectosphaerella*



**Figure 1.** Venn diagrams of the total bacterial and fungal amplicon sequence variants (ASVs) in the rhizosphere of Tartary buckwheat under three treatments.



**Figure 2.** Microbial community at the phylum and genus levels in different samples. (a–d) The top ten (a) fungi phyla; (b) bacteria phyla; (c) fungi genera; (d) bacteria genera.

was prominently lower in the FR group compared to the F and R groups ( $P < 0.05$ ).

#### Effects of mulching technique on soil microbial diversity

Six indices were estimated to measure the alpha diversity of buckwheat rhizosphere microbiota: Chao1, observed species, Shannon–Weaver, Simpson, Pielou’s evenness and Goods coverage. Generally, the cultivation method showed no prominent effect on microbial alpha diversity (Figure 3). The fungal alpha diversity was lower in the FR group than in the F and R groups, although the difference was non-prominent ( $P > 0.05$ ). The bacterial alpha diversity had no prominent difference among all groups ( $P > 0.05$ ).

To assess the relatedness among microbiota structures in different groups, the beta diversity of buckwheat rhizosphere microbiota was analysed by PCoA and NMDS. As shown in Figure 4 a and b, 55.6% and 31.3% of the overall variance was explained by PCo1 and PCo2 for fungi and bacteria respectively. The FR group in the PCoA plots was obviously distinguished from the R and F groups, between which no significant separation was found. A similar trend was observed in the NMDS plots (Figure 4 c and d). These results indicate that FR mulching has a greater impact on buckwheat rhizosphere microbiota diversity compared to F mulching.

LefSe was used for analysing the feature taxa markedly influenced by tested cultivation methods (Figure 5). Based on the LDA method ( $\log_{10} > 2$ ), 11 fungal and 5 bacterial ASVs were prominently associated with the tested treatments. Fungal families Gymnoascaceae and Thelebolaceae were prominently enriched in the R group, Nectriaceae in the FR group and Magnaporthaceae in the F group. At the genus level, *Gymnoascus* and *Thelebolus* were prominently richer in the R group than the other groups, whereas *Metarhizium* and *Gaeumannomyces* were most abundant in the F group, while *Ctenomyces* was significantly enriched in the FR group. For bacteria, the family Saprospiraceae and the genera *Planomicrobium* and *Romboutsia* were prominently enriched in the F group, while the genera *Gemmatirosa* and *Sphingoaureantiacus* were most abundant in the FR group. No bacterial taxon was significantly enriched in the R group.

#### Effects of mulching technique on microbial metabolic function

The function of microbial metabolism could be predicted by comparison of sequencing data with the genome database. In this study, the potential function of microbial metabolism was analysed by PICRUSt 2 in MetaCyc, KEGG and COG databases. The most abundant functional categories at subsystem level 1 in the tested samples were as

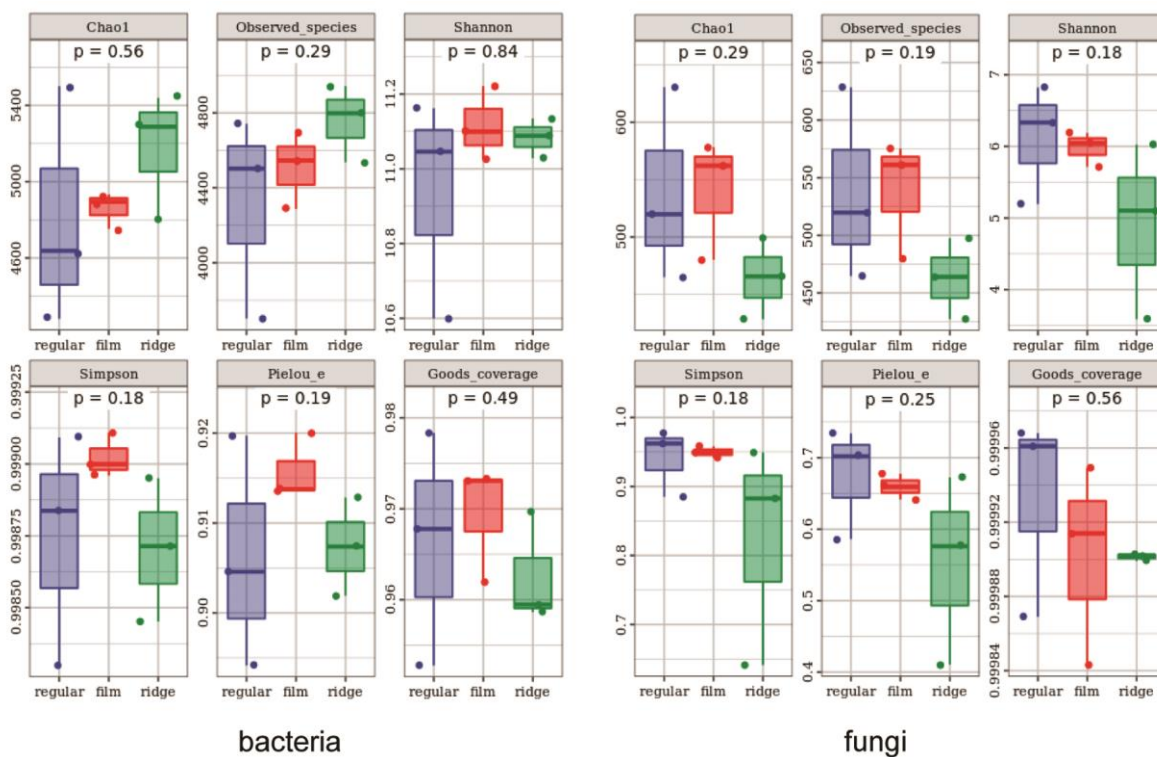


Figure 3. Alpha diversity indices of bacteria and fungi in the samples.

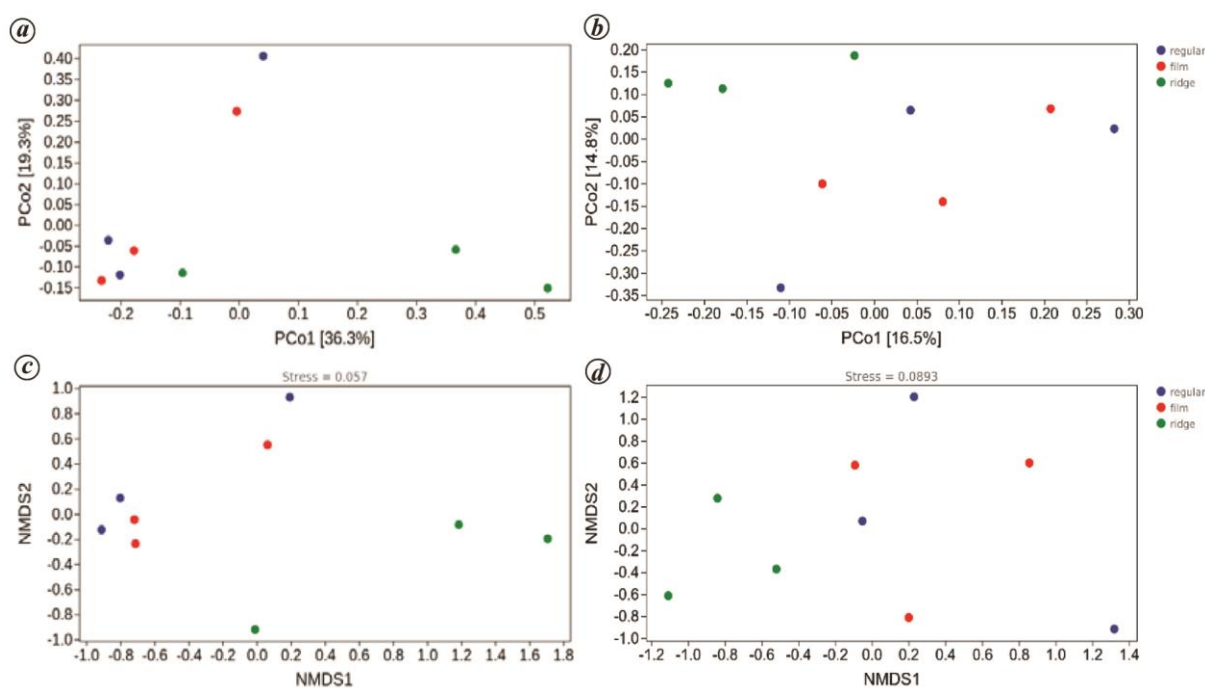
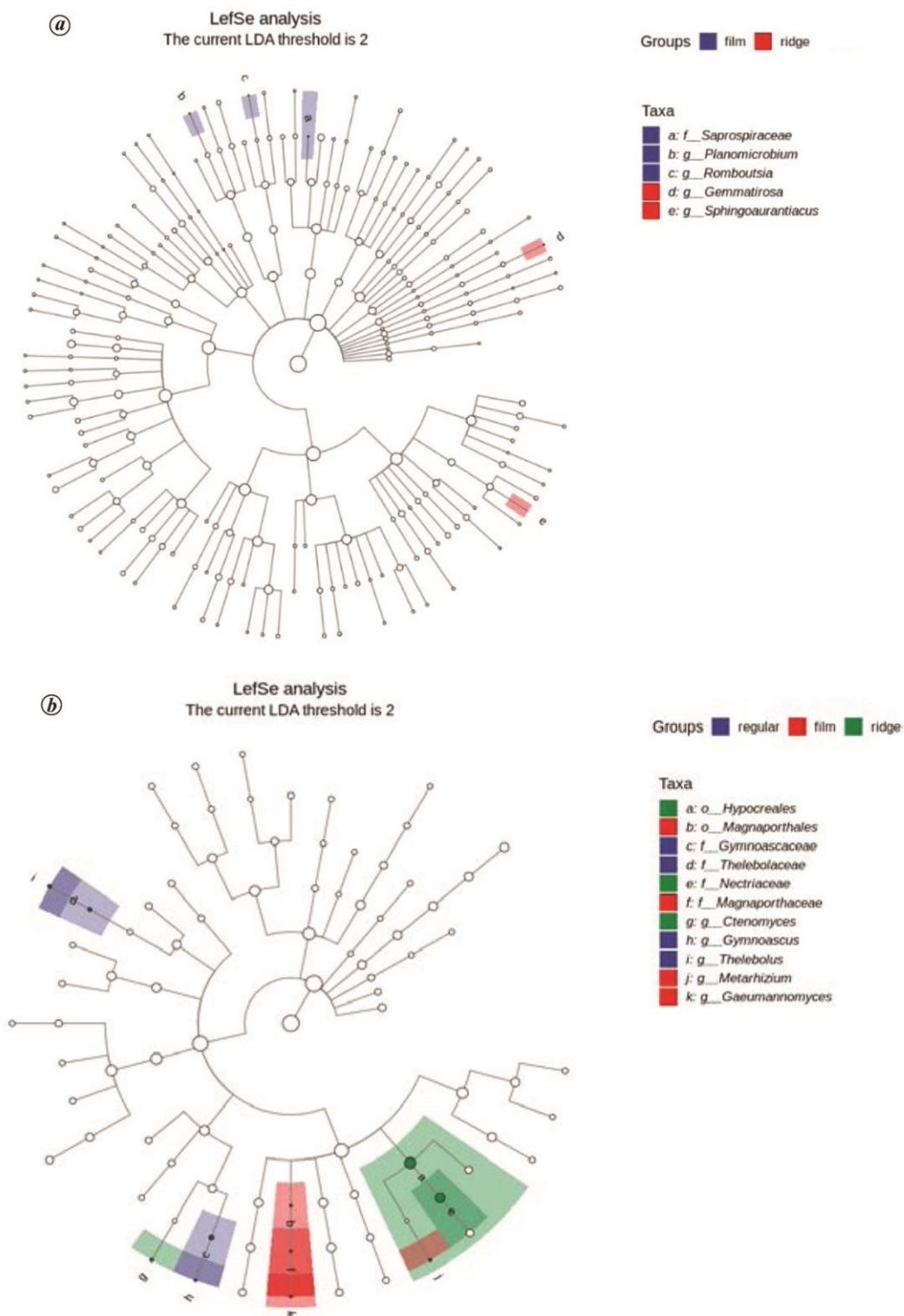


Figure 4. Plots of PCoA and NMDS analysis based on the (a, c) fungal; (b, d) bacterial communities.

follows: biosynthesis, degradation/utilization/assimilation, generation of precursor metabolite and energy, glycan pathways, detoxification, macromolecule modification and metabolic clusters.

As shown in Table 1, the profile of metabolic pathways in the FR group differed from that of the F and R groups. Levels of pathways related to mannan degradation, and biosynthesis of phospholipases, phosphatidylglycerol and



**Figure 5.** LefSe comparison of (a) bacterial and (b) fungal taxa from the rhizosphere of three groups. The node size corresponds to the taxon with the highest relative abundance ( $P < 0.05$ ).

ubiquinol in the FR group were prominently reduced in comparison with the F group and R group ( $P < 0.05$ ). The decline of relative abundance (RA) of *Flavobacte-*

*rium* and unclassified fungi was related to the decrease of mannan degradation and phospholipase levels respectively. The microbial composition related to pathways of

**Table 1.** Different metabolic pathways among treatments

Microbes	Comparison	Pathway	Description	log FC
Bacteria	FR versus R	PWY-7456	Mannan degradation	-1.2
	FR versus F	PWY-7456	Mannan degradation	-1.1
	R versus F	–	–	–
Fungi	FR versus R	LIPASYN-PWY	Phospholipases	-1.5
		PWY4FS-7	Phosphatidylglycerol biosynthesis I (plastidic)	-1.1
		PWY4FS-8	Phosphatidylglycerol biosynthesis II (non-plastidic)	-1.1
	FR versus F	PWY-5871	Ubiquinol-9 biosynthesis (eukaryotic)	-1.1
		PWY-5873	Ubiquinol-7 biosynthesis (eukaryotic)	-1.1
	R versus F	–	–	–

phosphatidylglycerol biosynthesis and ubiquinol biosynthesis was not analysed for they were performed by various types of microorganisms.

## Discussion

Microorganisms play an important role in the soil ecosystem and their diversity is one of the factors that characterize the stability of the soil community structure. In arid and semiarid areas, mulching techniques are widely used to increase soil moisture and temperature conditions, which can substantially affect the soil microbial diversity and community. Mulch occurs in various styles, textures and colours. Numerous mulches can be classified into organic, inorganic, mixed and special mulch by texture<sup>20</sup>. According to the feedstock inorganic mulch mainly has two categories, undegradable and degradable. Polyethylene mulch has been a widely used undegradable of inorganic mulch in agriculture since the middle of the 20th century<sup>21</sup>.

According to previous studies, polyethylene mulch played various roles in regulating the microbial structure in different agricultural systems. Generally, compared to bacteria, plastic mulch had a greater effect on the fungal population, mainly by increasing the soil water content and promoting the degradation of soil organic matter<sup>9</sup>. In northeastern China, plastic film mulch prominently reduced the RA of Chloroflexi, Planctomycetes and Verrucomicrobia, while it improved the RA of Proteobacteria in black soil<sup>22</sup>. Plastic film mulch enriched proteobacteria and actinobacteria in brown soil in northeastern China<sup>23</sup>. In the Loess Plateau of China, plastic film mulch reduced the richness of fungi and regulated the RA of Chytridiomycota, Mortierellomycota, Glomeromycota and Mucoromycota in the farmlands<sup>9</sup>, while it prominently improved fungal abundance and alpha diversity in an orchard system<sup>24</sup>. In this study, the F and FR treatments showed a non-prominent influence on microbial diversity but altered the microbial community composition in the rhizosphere of Tartary buckwheat in the Loess Plateau of China. For bacteria, the FR treatment improved the RA of Gemmatimonadetes, Cyanobacteria, Armatimonadetes and Fibrobacteres while reducing that of Firmicutes and Patescibacteria. The F treatment improved the RA of Acidobacteria, Planctomycetes and Latescibacteria while

reducing that of Armatimonadetes. For fungi, the FR treatment improved the RA of Ascomycota, Blastocladiomycota, Chytridiomycota and Rozellomycota while reducing that of Mortierellomycota, Zoopagomycota, Mucoromycota and Olpidiomycota. The F treatment improved the RA of Basidiomycota and Zoopagomycota while reducing that of Blastocladiomycota, Chytridiomycota and Mucoromycota.

Plastic film mulch provided a higher soil temperature and moisture profile, which benefitted fungal growth and mycotoxin production<sup>25</sup>. Plastic mulch increased pathogenic and mycotoxin-producing fungal taxa in luvisol soil with asparagus crops in Germany<sup>26</sup>. In the present study, the tested mulching techniques, especially FR, also enriched pathogenic fungi. Compared to R, the FR treatment increased the RA of six pathogenic genera, *Fusarium*, *Dokmaia*, *Mycosphaerella*, *Ilyonectria*, *Alternaria* and *Didymella*, while decreasing that of two pathogenic genera, *Olpidium* and *Plectosphaerella*. The F treatment increased the RA of pathogenic genera *Dokmaia* and *Alternaria* while decreasing that of pathogenic genera *Plectosphaerella* and *Ilyonectria*. Therefore, with more and more plastic film applied in agriculture, it is necessary to evaluate the effect of mulch techniques on pathogenetic and mycotoxigenic microorganisms before application.

Plastic film mulch also showed different effects on microbial metabolism pathways in different agricultural systems. Plastic film mulching increased the genes associated with the metabolism of cofactors, vitamins, amino acids, terpenoids and polyketides in croplands of subtropical China<sup>27</sup>, while it had a negative effect on the carbon and nitrogen cycle and the genes associated with the amino acid metabolism pathway in orchards in the Loess Plateau of China<sup>28</sup>. This study revealed that the FR treatment effectively decreased the genes related to mannan degradation and biosynthesis of phospholipases, phosphatidylglycerol and ubiquinol. The degradation of mannan and phospholipids could provide nutrients for plants. Ubiquinol is primarily distributed on the mitochondrial internal membrane of eukaryotic microorganisms and on the membrane of Gram-negative bacteria, participating in aerobic and nitrate respiration processes. Thus, the impact of plastic mulch techniques on microflora, including pathogenic and mycotoxin-producing species, needs evaluation before application.

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