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Mulching films affecting soil bacterial and fungal communities in a drip-irrigated potato soil

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ABSTRACT: Film mulching is an effective water-saving and yield-increasing measure for potato production in Northwest China. However, the response mechanism of microbial communities to mulching films in the soil is still unclear. In this study, polyethylene film mulching (PM), biodegradable film mulching (BM), liquid film mulching (LM), and non-mulching (NM) were applied on the drip-irrigated soil to investigate the effects of mulching films on soil bacterial and fungal communities through DNA sequencing, Pearson correlation analysis, and redundancy analysis. The results showed that LM treatment significantly increased the contents of soil mineral N (SMN), dissolved organic carbon (DOC), and dissolved organic nitrogen (DON) ($p<0.05$) in comparison with NM. The soil treated with LM presented high bacterial OTUs (operational taxonomic units), Chao1, ACE, and Shannon indices; however, the same indexes of fungi were low in LM and BM treatments. At the phylum level, *Proteobacteria*, *Actinobacteria,* and *Chloroflexi* were dominant bacterial communities. The LM treatment increased the OTUs of *Proteobacteria*; PM treatment increased the OTUs of *Actinobacteria* and *Chloroflexi*. *Ascomycota* was the dominant fungal community, which were decreased in soil under mulching films. In terms of soil properties, DON was closely correlated ($p<0.05$) with the microbial OTUs, Chao1, and ACE indices. The DOC and SWC (soil water content) contributed 51.2 % to the change of bacterial structure; however, the fungal structure was less sensitive to the variation of soil properties. Our results indicate that liquid film mulching favors increasing the diversity and abundance of dominant bacterial species, which were associated with the variation of soil properties.

Keywords: microbial community, carbon and nitrogen contents, DNA sequencing, potato field, plastic film.

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INTRODUCTION

The combination of drip irrigation and film mulching is an effective water-saving and yield-increasing measure for potato production in Northwest China (Hou et al., 2010; Zhang et al., 2017; Gao et al., 2019). Previous studies showed that film mulching was able to inhibit evaporation of water on the soil surface (Adhikari et al., 2016; Ding et al., 2018), increase soil surface temperature (Wu et al., 2017; Zhang et al., 2017), accelerate the mineralization of C and N nutrients (Luo et al., 2015; Ma et al., 2018; Fu et al., 2019), and decrease the soil pH (Wang et al., 2017a) and C/N ratio (Wang et al., 2021). Some studies pointed out that the alteration of soil environment induced the change of bacterial and fungal communities (Liu et al., 2016; Ren et al., 2018; Walker et al., 2018; Preusser et al., 2019). Several studies reported that plastic film mulching improved soil microbial population (Zhu et al., 2018) and altered the structure of bacteria (Chen et al., 2014) and fungi (Wang et al., 2020).

Currently, the mulching films used for potato planting are mainly made of polyethylene, which is difficult to degrade under natural conditions but easy to accumulate in the soil, thus destroying soil structure and impairing soil quality (Steinmetz et al., 2016; Jiang et al., 2017; Sintim and Flury, 2017). In recent years, the application area of environment-friendly mulching films (e.g., degradable plastic film, liquid plastic film) in potato production has been gradually expanding in Northwest China. Some studies reported that environment-friendly mulching films were conducive to improving soil environments (Xue et al., 2019; Yin et al., 2019), such as regulating hydrothermal conditions of soil (Gu et al., 2017; Wang et al., 2019a) and maintaining C and N availability (Li et al., 2014; Hou et al., 2019; Chen et al., 2020a). Bandopadhyay et al. (2018) reported that degradable plastic films stimulated the microbial activity in the soil, which affected the variation of soil organic matters. However, so far, the response mechanism of microbial communities to film mulching in soil has not been sufficiently researched.

As a contrast to bare land without mulching, in our study three mulching materials were applied on a drip-irrigated potato field. It is hypothesized that the diversity and structure of soil bacterial and fungal communities under environment-friendly mulching films might be different from that under polyethylene mulching film and in bare land, which might be associated with the alteration of soil physicochemical properties. This study aimed to (1) clear the alterations of soil physicochemical properties in different treatments; (2) ascertain the variations in diversity and structure of soil bacterial and fungal communities; and (3) reveal the response mechanism of soil microorganisms to soil properties. Our results will provide some theoretical knowledge for evaluating the environmental effects of degradable film and liquid film on drip-irrigated soil.

MATERIALS AND METHODS

Site description

The field experiment was conducted at the Modern Agricultural Science and Technology Demonstration Park, Yuyang District, Yulin City, Shaanxi Province (38° 23’ N, 109° 43’ E, 1080 m a.s.l.). The site belongs to a semi-arid continental climate with an annual mean temperature, precipitation, and evaporation of 8.6 °C, 410 mm, and 1900 mm, respectively (Wang et al., 2021). The annual accumulated temperature (≥10 °C) is 3300 °C. The soil is aeolian sandy type, with pH($CaCl_2$) value of 7.6, available nitrogen (AN) content of 38.76 mg kg^{-1}, organic carbon (SOC) content of 12.50 g kg^{-1}, total nitrogen (TN) content of 1.41 g kg^{-1}.

Experimental design

The experiment was set up with four treatments, i.e., polyethylene film mulching (PM), biodegradable film mulching (BM, made of 70 % butylene adipate-co-terephthalate

and 30 % polylactic acid), liquid film mulching (LM, made of humic acid, soluble starch, surfactant, and polymer compounds), and non-mulching (NM). Each treatment was set up with three replicates, and each replicate was a plot with an area of 5.4 × 7 m. Potatoes (variety "Zihuabai") were sown on May 5, 2018, and harvested on September 23, with 141 days of growth. One row of potatoes was buried 0.08-0.10 m deep in each ridge, and six ridges constituted a plot, where the ridge height, row spacing, and plant spacing were 0.30, 0.90, and 0.20 m, respectively. After that, drip irrigation hoses were placed in the ridges. Finally, mulching films were covered on the ridges. For PM and BM treatments, the films had a width of 1.2 m and thickness of 0.008 mm. The liquid film was made for LM treatment by mixing 60 kg of water with 10 kg of dry powder (powder dosage was 150 kg ha^{-1}) and evenly sprayed by an agricultural sprayer (spraying pressure: 2 kg cm^{-2}) on the ridge surface after potatoes being planted. The plants were fertilized with water-soluble fertilizer five times, with the dosage of 150 kg ha^{-1} each time (N : P : K = 14 : 6 : 15). The irrigation amount for each treatment was 2095 m^3 ha^{-1} during the entire growth period. After the potatoes were harvested, their yield in each plot was measured.

Collection and analysis of soil samples

Soil samples were collected before the last potato harvest on September 15, 2018. Five soil samples (0.00-020 m soil layer) were randomly collected from each plot using a soil auger and mixed. A 2 mm screen sieved one part of the fresh soil samples to analyze the contents of mineral nitrogen (SMN), dissolved organic carbon (DOC), and dissolved organic nitrogen (DON). Another part of the samples was air-dried, ground, and passed through a 0.25 mm sieve to determine the contents of SOC and TN. The remaining samples were stored at -80 ℃ for measuring the contents of soil microbial biomass carbon and nitrogen (MBC and MBN) and for the analyses of soil microbial communities.

The organic carbon content was analyzed using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) according to the oxidation method with $K_2Cr_2O_7$ (Bao, 2000). The total nitrogen content was determined using a Kjeldahl analyzer (K9860, Hanon, Jinan, China) with the semi-micro Kjeldahl method (Bao, 2000). The NH_4^+-N and NO_3^--N contents were determined by the indophenol-blue colorimetric and phenol-disulfonic acid colorimetric methods, respectively (Rayment and Higginson, 1992). Dissolved organic carbon was analyzed by the dichromate oxidation method (Jenkinson and Powlson, 1976). Dissolved organic nitrogen was obtained from the difference of total dissolved nitrogen (TDN) minus the dissolved inorganic nitrogen (DIN). The TDN content was measured according to the method of Hagedorn and Schleppi (2000), and the DIN content was measured using the UV spectrophotometer (Mulvaney, 1996). The MBC and MBN were determined by the chloroform fumigation-K_2SO_4 extraction method (Brookes, 1985; Vance et al., 1987). The pH value was determined by a pH meter (PHS-25, REX, Shanghai, China) with 1:2.5 soil:$CaCl_2$ extractant (w/v). The soil C/N ratio was the ratio of SOC to TN.

Soil water content (SWC, %) and soil temperature (TS, %) was measured, in the 0.00-0.20 m soil layer, by the oven-drying method and the L-shaped thermometers, respectively.

Analysis of microbial communities in soil

Soil DNA was extracted using the E.Z.N.A.® Soil DNA Kit (MO BIO Laboratories, Inc., USA) from 0.5 g of soil samples; each sample was repeated three times. Polymerase chain reaction (PCR) amplification system was 25 μL mixture containing 12.5 μL 2×Taq Plus Master Mix, 3 μL BSA, 1 μL Forward Primer, 1 μL Reverse Primer, 2 μL DNA, and 5.5 μL dd H_2O. The V3-V4 region of the bacterial 16S rRNA gene was amplified by primers 336F (5′-GTACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GTGGACTACHVGGGTWTCTAAT-3′). The reaction conditions were pre-denaturation at 94 °C for 5min, denaturation at

94 °C for 30 s, annealing at 50 °C for 30 s, extension at 72 °C for 60 s, 30 cycles (the above four steps constitute a cycle), and extension at 72 ℃ for 10 min. The fungal ITS1 region was amplified by primers ITS1-F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2 (5′-TGCGTTCTTCATCGATGC-3′). The reaction conditions were pre-denaturation at 94 °C for 5 min, denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 60 s, 34 cycles (the above four steps constitute a cycle), and extension at 72 °C for 10 min.

The PCR product was detected by 1 % agarose gel electrophoresis and quantified with a fluorometer (QuantiFluor™-ST, Promega, Madison, Wisconsin, USA). The Illumina MiSeq PE300 high throughput sequencing platform (Illumina, San Diego, CA, United States) was used for paired-end sequencing. The original sequencing data were uploaded to NCBI SRA database (http://www.ncbi.nlm.nih. gov/bioproject/611029).

Bioinformatics and statistical analysis

Raw sequencing data were filtered and spliced using Pear software (version 0.9.6). After splicing, VSearch software (version 2.7.1) was used to remove sequences shorter than 230 bp, and the Uchime method (Edgar et al., 2011) was used to remove chimeric sequences. The high-quality sequences were used to performed OTU (Operational Taxonomic Units) clustering at 97 % identity threshold using the Uparse algorithm (Edgar, 2013). Taxonomy was assigned at 70 % confidence level using the RDP (Ribosomal Database Project) classifier (Wang et al., 2007). The alpha diversity indices (including Chao1, ACE, Shannon, and Simpson) were calculated using QIIME1 software (version 1.8.0) (Caporaso et al., 2010). Intergroup differences were analyzed using Mothur software (version 1.30.1) (Schloss et al., 2009), and LEfSe (LDA Effect Size) analysis was performed using Python software (version 2.7) (Segata et al., 2011).

The measured data was tested by one-way ANOVA using SPSS software (version 22.0). Duncan's New Multiple Range Test at 0.05 significance level was applied for multiple comparisons. Pearson correlation coefficient between soil microbial diversities (including observed OTUs, Chao1, ACE, Shannon, and Simpson) and soil physiochemical properties were calculated by SPSS. Response of soil microbial communities (the relative abundance of bacteria and fungi at the phylum level ≥1 %) to soil physicochemical properties was determined by redundancy analysis (RDA) using Canoco software (version 5.0) (ter Braak and Šmilauer, 2012).

RESULTS

Changes in soil properties

The carbon and nitrogen contents (except for SOC content) of the soil treated with three mulching films were higher than that of the NM (Table 1). Regarding TN, SMN, DON, and DOC contents, the treatments followed the order LM > BM > PM > NM. Compared with NM, the LM and BM treatments significantly increased the contents of DON and DOC by 22.49 to 31.89 % (p-value from 0.0003 to 0.0248), and the LM and PM treatments significantly increased the contents of MBC and MBN by 16.51 to 32.09 % (p-value from 0.0030 to 0.0176). However, the three film mulching treatments reduced the values of SOC, C/N, and pH. In terms of soil temperature and water content, the treatments were PM > BM > LM > NM. The TS (20.45 ℃) and SWC (13.31 %) in PM treatment were significantly higher than that in LM and NM treatments (p-value from 0.0009 to 0.0228).

Diversity of bacterial and fungal communities

After removing the unqualified data, a total of 1,558,782 bacterial 16S rRNA gene sequences, and 1,115,200 fungal ITS sequences were obtained from the 12 soil samples.

The sequences were grouped into 32,622 bacterial OTUs and 9,219 fungal OTUs at the 97 % similarity level. The degrees of coverage were higher than 96 % for bacteria and 99 % for fungi, indicating that the data had enough representativeness to express the diversity of bacteria and fungi under the sequencing depth in this study.

For the bacterial community, LM and BM treatments increased the OTU abundance (2912 and 2754) and the Shannon index (9.56 and 9.57), but decreased the Simpsons index (0.0045 and 0.0042) (Table 2). In terms of Chao1 and ACE indices, the treatments were LM > PM > BM > NM; compared with NM, the LM treatment significantly increased the index of Chao1 (3907.18, $p = 0.0431$) and ACE (4021, $p = 0.0361$). For the fungal community, the abundance of OTU (869, $p = 0.0195$), the indices of Chao1 (1125.65, $p = 0.0315$), and ACE (1147.68, $p = 0.0166$) in NM were significantly higher than that in LM. The LM treatment decreased the Shannon index (5.24) but significantly increased the Simpsons index (0.0957, p-value from 0.0304 to 0.0382).

Structure of bacterial and fungal communities

At the phylum level, the relative abundance of *Proteobacteria*, *Actinobacteria,* and *Chloroflexi* accounted for 68.24-74.28 % in all bacterial communities (Figure 1a). The LM

Table 1. Changes in soil properties at 0.00-0.20 m layer under different treatments in a drip-irrigated potato soil

Soil properties	PM	BM	LM	NM
SOC (g kg^{-1})	10.46 ± 0.96 b	11.76 ± 0.87 ab	11.57 ± 0.86 ab	12.80 ± 0.55 a
TN (g kg^{-1})	1.43 ± 0.05 bc	1.49 ± 0.04 b	1.59 ± 0.06 a	1.40 ± 0.03 c
SMN (mg kg^{-1})	18.43 ± 1.77 b	19.16 ± 0.87 ab	21.23 ± 1.14 a	17.02 ± 1.23 b
DOC (mg kg^{-1})	203.86 ± 22.45 b	246.11 ± 10.09 a	268.00 ± 11.90 a	187.85 ± 18.56 b
DON (mg kg^{-1})	54.78 ± 4.59 a	56.78 ± 3.48 a	64.62 ± 6.70 a	44.01 ± 7.13 b
MBC (mg kg^{-1})	313.14 ± 8.70 a	291.40 ± 19.86 ab	327.32 ± 38.78 a	243.30 ± 36.42 b
MBN (mg kg^{-1})	60.37 ± 5.24 a	51.93 ± 5.73 ab	62.86 ± 7.09 a	42.68 ± 5.35 b
C/N ratio	7.33 ± 0.44 b	7.87 ± 0.40 b	7.26 ± 0.25 b	9.16 ± 0.20 a
pH value	7.51 ± 0.04 c	7.70 ± 0.03 b	7.59 ± 0.04 bc	7.87 ± 0.10 a
TS (°C)	20.45 ± 1.38 a	19.39 ± 0.57 ab	18.34 ± 0.78 bc	17.49 ± 0.74 c
SWC (%)	13.31 ± 0.85 a	12.07 ± 0.37 ab	11.36 ± 0.86 bc	10.35 ± 0.62 c

The soil physicochemical properties shown are SOC (soil organic carbon), TN (total soil nitrogen), SMN (soil mineral N), DOC (dissolved organic C), DON (dissolved organic N), MBC (microbial biomass C), MBN (microbial biomass N), C/N ratio (SOC/TN ratio), pH($CaCl_2$) (pH value), TS (soil temperature), and SWC (soil water content). The values are the means ± SE (standard error) of three replicates, and the same lowercase letters in a row are not significantly different at 0.05 level. PM: polyethylene film mulching; BM: biodegradable film mulching; LM: liquid film mulching; NM: non-mulching.

Table 2. Changes in OTU abundance and alpha diversity of bacteria and fungi under different treatments in a drip-irrigated potato soil

Species	Treatment	OTUs	Chao1	ACE	Shannon	Simpson
Bacteria	PM	2715 ± 81.38 ab	3591.82 ± 184.60 ab	3668.67 ± 176.52 ab	9.43 ± 0.03 a	0.0048 ± 0.0003 a
	BM	2754 ± 258.43 ab	3525.15 ± 340.31 ab	3605.19 ± 367.17 ab	9.57 ± 0.15 a	0.0042 ± 0.0009 a
	LM	2912 ± 167.15 a	3907.18 ± 107.40 a	4021.93 ± 95.98 a	9.56 ± 0.15 a	0.0045 ± 0.0012 a
	NM	2494 ± 61.22 b	3213.03 ± 76.13 b	3252.63 ± 108.94 b	9.28 ± 0.19 a	0.0064 ± 0.0015 a
Fungi	PM	795 ± 110.53 ab	1056.74 ± 90.98 a	1162.56 ± 64.61 a	6.04 ± 0.25 a	0.0437 ± 0.0092 b
	BM	750 ± 55.76 ab	956.96 ± 30.54 ab	1006.33 ± 31.96 ab	5.93 ± 0.17 a	0.0433 ± 0.0085 b
	LM	659 ± 109.29 b	844.88 ± 108.97 b	854.77 ± 101.69 b	5.24 ± 0.34 a	0.0957 ± 0.0234 a
	NM	869 ± 61.10 a	1125.65 ± 45.90 a	1147.68 ± 57.30 a	6.03 ± 0.36 a	0.0462 ± 0.0096 b

The values are the means ± SE (standard error) of three replicates, and the same lowercase letters in a column are not significantly different at 0.05 level. PM: polyethylene film mulching; BM: biodegradable film mulching; LM: liquid film mulching; NM: non-mulching.

treatment increased the OTUs of *Proteobacteria*. The PM treatment increased the OTUs of *Actinobacteria* and *Chloroflexi* but significantly decrease the OTUs of *Proteobacteria* (p-value ranging from 0.0017 to 0.0282). The LM and BM treatments significantly increased the OTUs of *Gemmatimonadetes* (p-value from 0.0001 to 0.0001). For the fungal communities, *Ascomycota* and *Basidiomycota* predominated in the fungal phyla; the relative abundance accounted for 55.89-71.19 % and 6.14-13.21 %, respectively (Figure 1b). The NM showed the highest relative abundance of *Ascomycota* (71.19 %) and the lowest relative abundance of *Basidiomycota* (6.14 %). The *Kickxellomycota* OTUs in BM treatment were 23- to 129-fold higher than that in other treatments, but the difference was not significant.

The LEfSe analysis showed that 41 taxonomic biomarkers of 7 bacterial phyla (Figure 2a) and 38 taxonomic biomarkers of five fungal phyla (Figure 2b) were sensitive to different mulching films ($p<0.05$; LDA score >3). Those biomarkers were equal to 2.03 % of all bacterial taxa retrieved and 4.62 % of all fungal taxa. The maximum bacterial

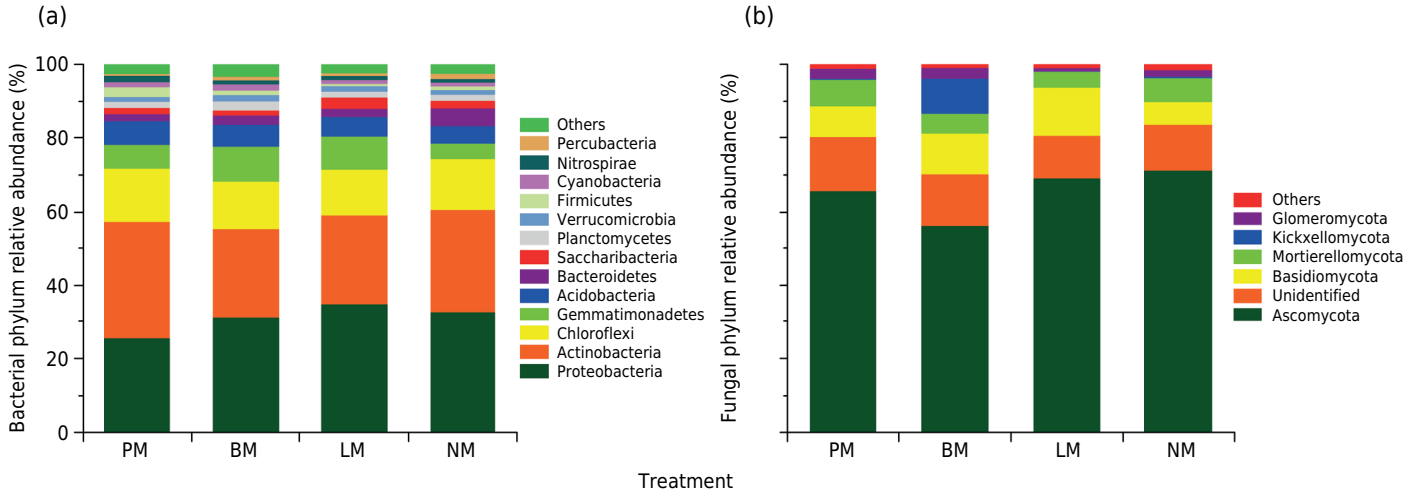

Figure 1. Relative abundance of soil bacteria (a) and fungi (b) at phylum level in different treatments. PM: polyethylene film mulching, BM: degradable film mulching, LM: liquid film mulching, NM: non-mulching.

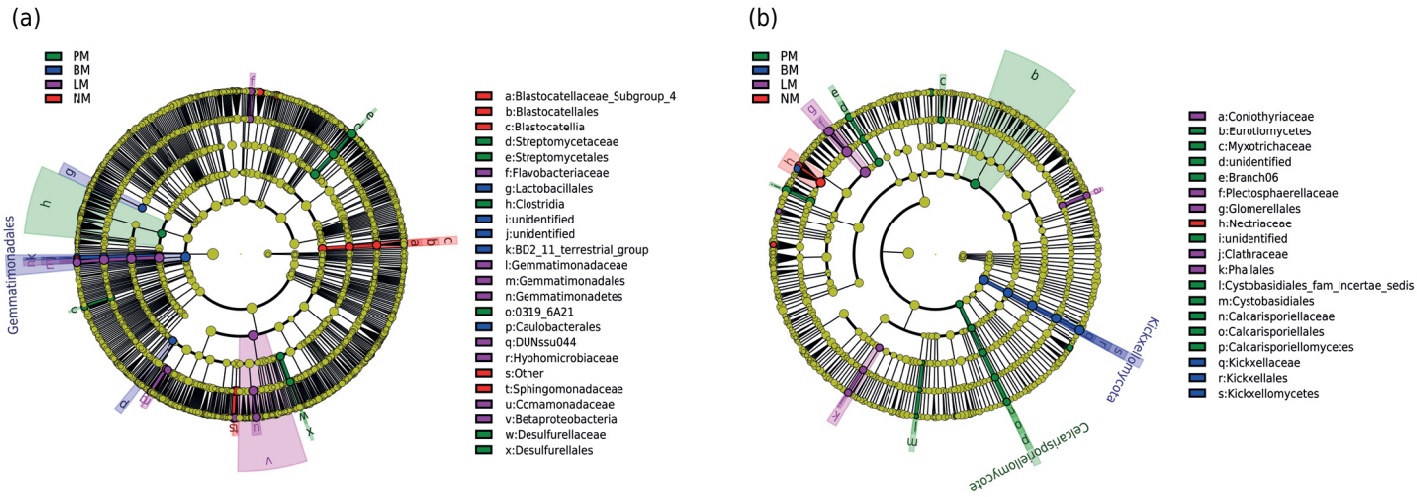

Figure 2. Bacterial (a) and fungal (b) LEfSe analysis results in different treatments. PM: polyethylene film mulching; BM: biodegradable film mulching; LM: liquid film mulching; and NM: non-mulching. There are five circular rings in the cladogram; each circular ring deposits all taxa within a taxonomic level; the circular rings from inside to outside represent kingdom, phylum, class, order, and family, respectively. The node on the circular ring represents a taxon affiliating at the taxonomic level. Taxa that had significantly higher relative abundance in a certain treatment within each soil type were color-coded within the cladogram according to the Protist Ribosomal Reference taxonomy. _X represents unidentified lower taxonomic ranks within the corresponding category.

biomarkers appeared in LM treatment, involving 14 biomarkers of 3 bacterial phyla. The maximum fungal biomarkers appeared in PM treatment, involving 19 biomarkers of 4 fungal phyla.

Response of bacteria and fungi to soil properties

Pearson analysis (Figure 3) showed that in 0.00-0.20 m soil layer, soil temperature and moisture, carbon (except for SOC), and nitrogen nutrients were positively correlated with the bacterial OTUs and alpha diversity (except for Simpson). The SOC, C/N ratio, and pH($CaCl_2$) were negatively correlated with the above diversity indices. The correlation between diversity and soil properties for fungi was contrary to that for bacteria. The DON was closely correlated with the OTUs, Chao1, and ACE indices (P from 0.0060 to 0.0411) of bacterial and fungal communities. Redundancy analysis (RDA) showed that DOC and SWC explained 29.2 and 22 % changes in bacterial structure, respectively (Figure 4a and Table 3), and they also explained 13.2 and 20.7 % changes of fungal structure, respectively (Figure 4b, Table 3).

DISCUSSION

Effects of mulching films on soil properties

Film mulching increased temperature and water content in 0.00-0.20 m soil layer (Table 1) which was conducive to accelerating the mineralization rate of organic matter (Curtin et al., 2012), thus increasing SMN (NH_4^+-N and NO_3^--N) contents (Wang et al., 2017a; Chen et al., 2020a). However, mulching films obstructed the entrance of plant residues in the soil, reduced SOC content, eventually resulting in a relatively low C/N ratio and pH value (Table 1). In particular, SMN content in PM treatment was lower than

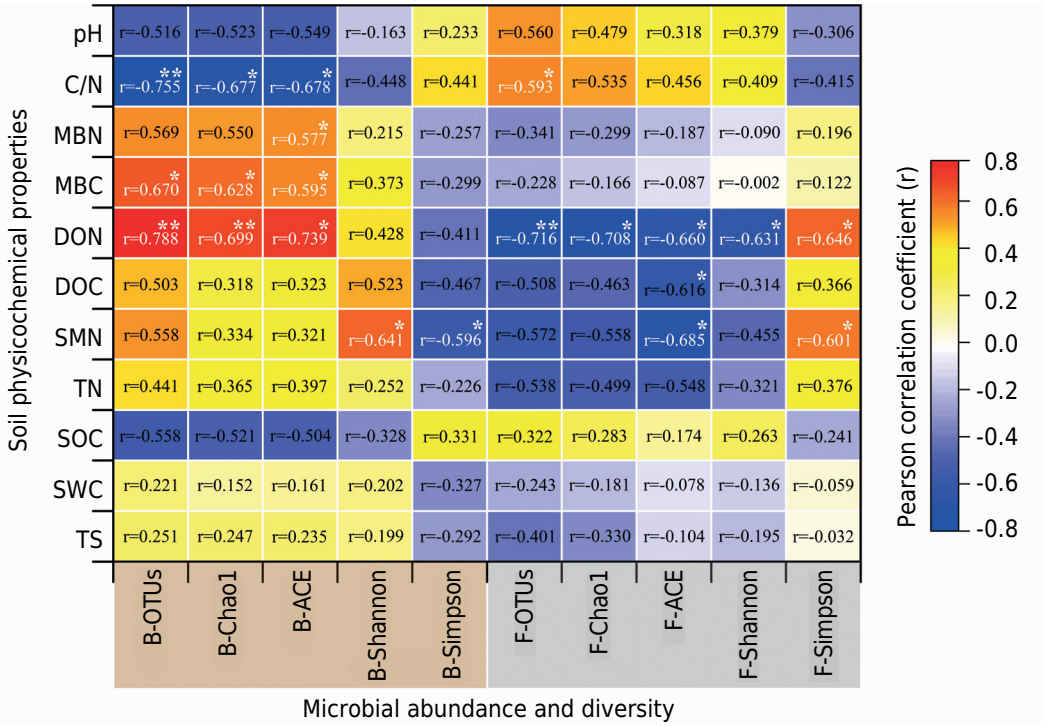

Figure 3. Pearson correlations between microbial abundance and diversity vs. soil physicochemical properties. Soil properties include TS (soil temperature), SWC (soil water content), SOC (soil organic carbon), TN (soil total nitrogen), SMN (soil mineral N), DOC (dissolved organic C), DON (dissolved organic N), MBC (microbial biomass C), MBN (microbial biomass N), C/N (SOC/TN), and pH($CaCl_2$) (pH value); bacterial (B-) and fungal (F-) abundance and diversity include OTUs, Chao1, ACE, Shannon, and Simpson indices; r is the Pearson correlation coefficient; * and ** indicate a significant correlation at the 0.05 and 0.01 level, respectively.

Table 3. Results of redundancy analysis (RDA) between microbial communities and soil properties in a drip-irrigated potato soil

	Bacterial community vs soil properties			Fungal community vs soil properties		
Name	**Explains %**	**Pseudo-F**	**p-value**	**Explains %**	**Pseudo-F**	**p-value**
SOC	4.2	0.8	0.482	4.4	0.5	0.664
TN	2.5	<0.1	0.952	10.1	<0.1	0.967
MSN	7.7	3.1	0.274	5.6	0.6	0.560
DOC	29.2	4.1	0.026	13.2	1.5	0.226
DON	3.4	0.8	0.496	4.7	0.6	0.601
MBC	12.5	2.8	0.086	6.4	0.8	0.532
MBN	4.3	0.9	0.418	10.7	1.4	0.248
C/N	5.6	1.3	0.288	5.6	0.7	0.556
pH	5.4	1.2	0.266	11.0	1.4	0.294
SWC	22.0	4.1	0.020	20.7	2.7	0.080
TS	3.3	0.7	0.540	7.5	0.7	0.486

Soil properties include SOC (soil organic carbon), TN (soil total nitrogen), SMN (soil mineral N), DOC (dissolved organic C), DON (dissolved organic N), MBC (microbial biomass C), MBN (microbial biomass N), C/N (SOC/TN), pH($CaCl_2$) (pH value), SWC (soil water content), and TS (soil temperature). Explanatory variables account for 100 % of microbial communities.

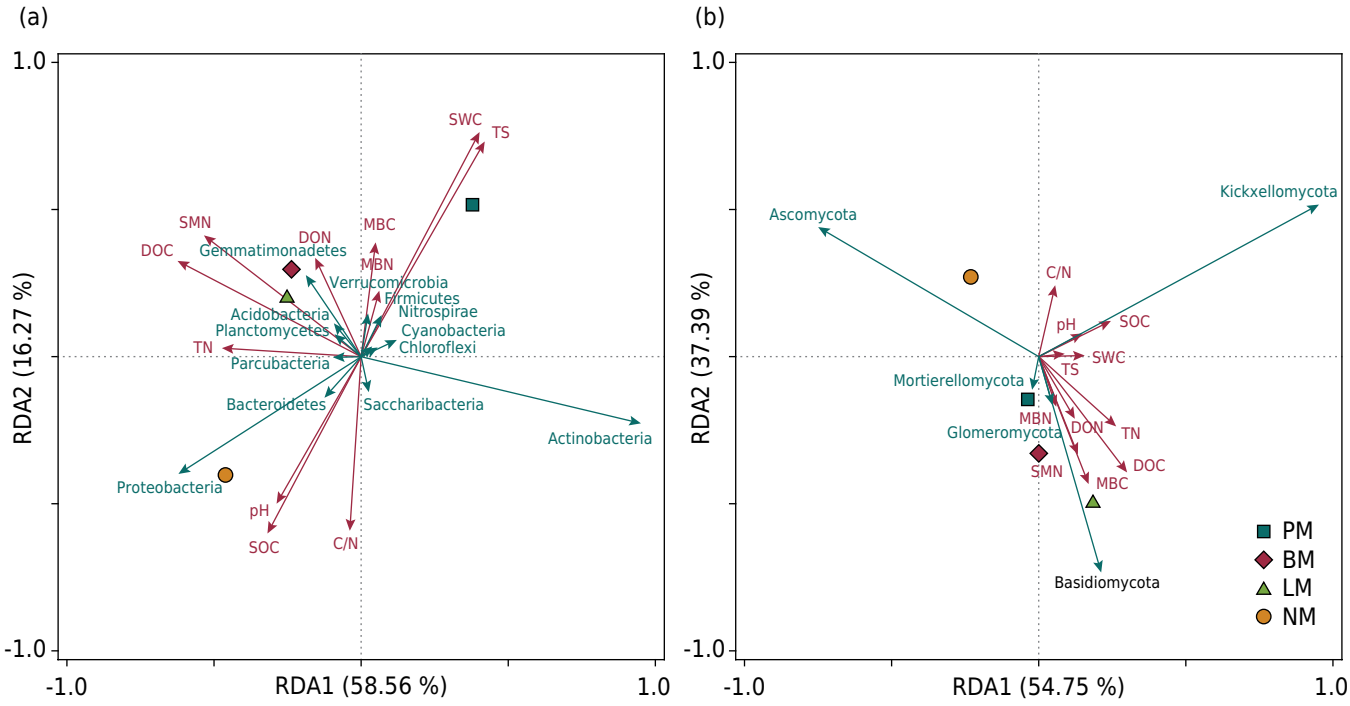

Figure 4. Redundancy analysis of (a) bacterial phyla vs. soil properties and (b) fungal phyla vs. soil properties. The relative abundance of bacteria and fungi ≥1 % in each treatment. At the RDA biplots, the position, angle, and length of arrows indicate the direction, degree, and scope of the response of the microbial communities to soil properties. Soil properties include TS (soil temperature), SWC (soil water content), SOC (soil organic carbon), TN (soil total nitrogen), SMN (soil mineral N), DOC (dissolved organic C), DON (dissolved organic N), MBC (microbial biomass C), MBN (microbial biomass N), C/N (SOC/TN), pH (pH value); PM: polyethylene film mulching; BM: biodegradable film mulching; LM: liquid film mulching; NM: non-mulching.

in LM and BM treatments (Table 1). It may be because polyethylene film kept the highest water content and aggravated the risk of mineral nitrogen leaching to the deep soil layer (Steinmetz et al., 2016; Ma et al., 2018; Gao et al., 2019). In the experiment, humic acid was added to the liquid plastic film, which contained functional groups such as carboxyl groups with strong adsorption capacity to N nutrients (Di et al., 2019; Liu et al., 2019), thus increasing the DON content. Also, LM treatment showed the maximum DOC content (Table 1), and that may be because there was soluble starch in the LM film (Xue et al.,

2019) and sufficient organic substrates (Ai et al., 2018; Walker et al., 2018) contributed to the increase of microbial biomass C and N.

Effect of mulching films on bacterial community

Bacterial OTU abundance and alpha diversity (except for Simpson index) in film mulching soil were higher than those in bare soil (Table 2). Chen et al. (2014) and Wang et al. (2020) also reported that mulching practice improved the abundance and diversity of soil bacteria. Film mulching enhanced nutrient availability in topsoil, and abundant labile organic C and N provided labile substrates for bacteria (Waring et al., 2013; Ai et al., 2018), which was conducive to thriving bacteria.

This study found that the DON content was closely related to the bacterial OTUs, Chao1, and ACE indices ($p<0.05$; Figure 3); meanwhile, DOC content contributed 29.2 % changes of bacterial structure (Figure 4a and Table 3), topsoil under LM treatment contained the highest DON and DOC contents (Table 1), and that partly explained why the LM treatment had the highest bacterial diversity indices (Table 2) as well as the most specific biomarkers (Figure 2a). The structuring effect of polymer materials on bacterial communities was demonstrated by Zhang et al. (2019) and Tian et al. (2020). Specifically, *Gemmatimonadetes* was sensitive to the change of DON and DOC contents (Figure 4a); thus, there was abundant *Gemmatimonadetes* in the soil under BM and LM treatments (Figure 1a). He et al. (2020) observed that *Gemmatimonadetes* was closely related to the labile organic matter in arable land. In this study, the C/N ratio was closely negatively associated with the bacterial OTUs, Chao1and ACE indices (Figure 3). The C/N ratio is a predictor of the decomposition degree of organic matter in the soil (Liang et al., 2017; Novair et al., 2020), and the low C/N ratio in film mulching soil indicated the increase of bacterial diversity. The pH value represents the cumulative effects of physiochemical properties (Ostrowska and Porębska, 2015). The pH value varied only 0.36 in the drip irrigated soil (Table 1), with a very slight influence on bacterial diversity. Bottrill et al. (2020) and Li et al. (2020) discovered that the small changes in soil pH had no apparent influence on bacterial diversity. Interestingly, the positive relationship of *Proteobacteria* to pH and C/N ratio was observed in this study (Figure 4a), indicating that the soil under PM treatment with low pH and C/N ratio was unfavorable for *Proteobacteria*.

Contrary to Ren et al. (2018), the RDA biplot showed the SOC was negatively correlated to the bacterial diversity (except for Simpson index; Figure 4a). The bare soil contained the highest SOC, but the lowest DOC, implying the recalcitrant organic C in NM might be higher than that in film mulching treatments. Zhong et al. (2018), Preusser et al. (2019), and Wang et al. (2020) verified that the bacteria presented the poor utilization of recalcitrant C directly from plant residues. This study noted that the SWC had a contribution rate of 22.0 % to the change of bacterial structure (Figure 4a and Table 3). *Chloroflexi* and *Actinobacteria* were significantly positively associated with the soil moisture, and both were abundant in the moist soil environment under PM treatment (Figure 1a). Previous research suggested that soil moisture was the main factor driving the alteration of soil bacterial community structure (Araujo et al., 2020; Dong et al., 2021).

Effect of mulching films on fungal community

In the potato field, fungal OTUs and alpha diversity indices (except for Simpson index) in LM and BM treatments were lower than those in NM; meanwhile, such values of the bacterial community were 3-5 times higher than those in the fungal community (Table 2). At the phylum level, this study found no apparent difference in fungal structure (Figure 1b). This phenomenon indicated that the bacterial activities might be higher than that of fungi in the agricultural soil. However, in the forest (Ren et al., 2019) and grassland (French et al., 2017; Chen et al., 2020b), fungi were often reported to be more active than bacteria. The niche segregation between soil fungi and bacteria

was confirmed by Bahram et al. (2018). They reported that fungi had a competitive advantage over bacteria in topsoil because the soil environment with a high C/N ratio induces fungal metabolism to produce antimicrobial substances, which is conducive to the enrichment of fungi. However, in agricultural soil with high N input, the eutrophic soil environment, accompanied by the lower C/N ratio, may be more suitable for the growth of bacteria.

Pearson analysis (Figure 3) showed that the fungal OTU abundance and alpha diversity decreased with the increase of N content (TN, DON, MBN, MSN); especially, DON was significantly negatively correlated to the abundance and diversity of the fungal community ($p<0.05$), suggesting that although high N availability in the drip-irrigated soil under LM and BM treatments was a key factor enhancing bacterial diversity, it also reduced the fungal diversity. A similar phenomenon had been reported by French et al. (2017). In addition, we found the fungal OTUs and alpha diversity increased with the increase of SOC and C/N ratio (Figure 3), previous literature reported that in the environment of high SOC (Li et al., 2015; Wang et al., 2017b; Li et al., 2020) and high C/N ratio (Montiel-Rozas et al., 2018; Wang et al., 2019b), fungi tended to decompose recalcitrant organic C in soil. *Ascomycota* and *Basidiomycota* in the drip-irrigated soil were dominant phyla (Figure 1b), and RDA biplot showed that the *Ascomycota* OTUs were negatively associated with C and N contents. In contrast, the *Basidiomycota* OTUs had a positive association with C and N nutrients (Figure 4b). The nutritional niches between *Ascomycota* and *Basidiomycota* were described in the researches of Bastida et al. (2016) and Li et al. (2017). Porras-Alfaro et al. (2007) and Liu et al. (2016) argued that *Ascomycota* contained mycorrhizal fungi, enriched under conditions of low N content. Therefore, the soil in NM had the highest *Ascomycota* abundance but the lowest *Basidiomycota* abundance. Moreover, this study observed that the PM treatment displayed the most fungal biomarkers (Figure 2b).

The results suggest that BM and LM treatments may increase the bacterial diversity but decrease the fungal diversity. This study found that fungal structure is less sensitive to soil properties than bacterial structure. This finding is partly inconsistent with our initial hypothesis (the diversity and structure of soil bacteria and fungi under environment-friendly mulching films were different from other treatments) since structuring effects were only observed for the bacterial community under the LM and BM treatments.

CONCLUSIONS

Compared with bare land, the soil under film mulching treatment increased the carbon (except for SOC) and nitrogen contents , which were highest under the liquid film. Environment-friendly mulching films increased soil bacterial OTUs abundance and diversity but decreased fungal abundance and diversity. The liquid plastic film contributed more to the alteration of bacterial diversity and structure than the degradable film. The change of bacterial structure was closely associated with dissolved organic carbon and soil moisture, whereas the fungal structure was less sensitive to the variation of the soil environment. These findings indicate bacteria community occupied important ecological niches, since bacteria are more active than fungi in the drip-irrigated soil. The long-term response mechanism of mulching films to key soil microorganisms should be further studied in the future.

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