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Soil microbial community and network changes after long-term use of plastic mulch and nitrogen fertilization on semiarid farmland

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ABSTRACT

Soil microbes are crucial for improving soil quality and productivity. Plastic film mulch (FM), in conjunction with fertilization, has significantly improved crop yields over vast areas of dryland production. However, how these practices affect soil microbial communities, especially as regards co-occurrence patterns within microbial taxa, is unclear. The objective of this study was to determine the effects of 10 years of FM and four nitrogen (N) fertilization rates [0 (N0), 100 (N100), 250 (N250), and 400 (N400) kg N ha^{-1}] on soil bacterial and fungal diversity, community structure, composition, and the co-occurrence network in a rainfed maize (*Zea mays* L.) field on the Loess Plateau of China. Results showed that N fertilization and FM did not affect soil microbial biomass carbon, but these practices changed the soil bacterial and fungal community structures. The bacterial community structure was dominantly affected by N fertilization, owing to the increased soil N content and decreased soil pH, which reduced bacterial community diversity and altered the relative abundance of some copiotrophic/oligotrophic taxa (e.g., Gemmatimonadetes, Acidobacteria, Rokubacteria, and Planctomycetes). Plastic mulch played a greater role in regulating the fungal community structure, primarily because FM increased soil moisture and promoted soil organic matter decomposition, thereby reducing fungal richness and altered the relative abundance of Chytridiomycota, Mortierellomycota, Glomeromycota, and Mucoromycota. Moreover, FM mediated the effects of N fertilization by reducing soil N content, and then increased the N threshold that caused changes in microbial structure. Network analysis indicated that FM caused an unstable co-occurrence network with fewer positive and negative links, while N fertilization increased both positive and negative (except N400) links, indicating enhanced cooperation and competition among microbes. These results indicate that long-term plastic mulch and high N fertilization could result in risk for soil quality in terms of soil microbial community structure and stability, suggesting that developing new management strategies is necessary to sustain dryland productivity.

1. Introduction

Dryland agriculture plays a critical role in global food supply, as drylands occupy ~45% of the earth's land surface (Prăvălie, 2016). Agricultural production in dryland areas is primarily constrained by low water availability and increasing soil degradation (e.g., declining soil fertility) (Prăvălie, 2016). Soil microbes are essential for soil biogeochemical processes and function, and play prominent roles in soil nutrient cycling and plant performance (Bardgett and van der Putten, 2014). Previous studies have reported that soil microbial community structure and function are sensitive to changes in soil microenvironment (Bandopadhyay et al., 2018; Dai et al., 2018). Therefore, management that aims to alleviate water and nutrient limitations in drylands could probably alter soil microbial diversity, community composition, and inter-species interaction, thereby affecting soil productivity and sustainability.

Plastic film mulch (FM) increases rainwater harvesting and reduces soil evaporation, thereby improving water-use efficiency and crop

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productivity in drylands (Liu et al., 2014). Due to its remarkable economic benefits, FM has been used in crop production worldwide (Steinmetz et al., 2016), with approximately 8.8 million tons of film mulch estimated to be used in 2024 (Transparency Market Research, 2016). Extensive and continuous use of FM changes the soil microclimate, and subsequently affects soil microbial communities (Bandopadhyay et al., 2018). Some studies have reported that FM increases the richness and diversity of soil bacteria and fungi (Dong et al., 2017; Huang et al., 2019a, b). Other studies have reported that FM changes the community composition and structure of soil bacteria and arbuscular mycorrhizal fungi (Liu et al., 2012a; Chen et al., 2014). Plastic film mulch significantly decreased the absolute abundance of soil microbes in a two-year study (Luo et al., 2019), but an eight-year FM study reported the enrichment of some fungal species (e.g., mycotoxigenic fungi) (Muñoz et al., 2015). How soil microbes respond to long-term mulching is not yet understood. A recent study reported that drought stress reduced the complexity and stability of the co-occurrence network of soil bacterial communities, which then affected soil function in a grassland ecosystem (de Vries et al., 2018). However, there have been no studies to date that evaluate the interactions between microbes or variations in keystone taxa in connection with the improved soil moisture conditions under FM. Bridging this knowledge gap will offer insight for accurately assessing the impacts of FM on soil quality.

Nitrogen (N) is the most essential nutrient for increasing crop yields, but most soils throughout the world are N deficient (Li et al., 2009). The use of N fertilizers increases crop yields, and N fertilizer inputs are projected to increase further due to the growing food demand (Springmann et al., 2018). Soil microbial communities and function are directly and indirectly affected by N application (Ramirez et al., 2010), which in turn influence soil nutrient cycling and plant nutrition (Jacoby et al., 2017). A recent study using meta-analysis showed that N fertilization had adverse effects on soil microbial biomass, composition, and function in all terrestrial ecosystems (Zhang et al., 2018). Similar results have been reported by others (Dai et al., 2018; Yao et al., 2014; Zhong et al., 2015), indicating that N fertilization decreases soil microbial diversity and simplifies the bacterial interaction network. In contrast, Mbuthia et al. (2015) reported that N fertilization had an insignificant effect on bacterial and saprophytic fungal community composition, and Geisseler and Scow (2014) reported increased microbial biomass under N fertilization in agroecosystems. In another study, the response of soil microbes to N fertilization varied depending on soil property, plant type, management practice, and other factors (Ramirez et al., 2010). Specifically, N application rate and duration of fertilizer use had pronounced effects on soil microbial composition and function (Zhang et al., 2018). A threshold N fertilization rate has been reported (Yao et al., 2014; Zhong et al., 2015), with changes in microbial diversity and community structure observed with N application rate above the threshold. The fate of fertilizer N is affected by mulching practice (Guo et al., 2019), and the improved soil moisture under FM could promote crop N uptake and reduce soil N accumulation (Liu et al., 2015), thereby probably mediating the potential impact of N fertilization on soil microbial communities. A short-term study showed that soil microbial abundance and community structure responded differently to N fertilization under plastic mulched and non-mulched fields (Luo et al., 2019). Understanding the changes in microbial diversity and community subjected to long-term N fertilization under FM conditions will enable the development of appropriate fertilization and mulching recommendations for dryland agriculture.

The Loess Plateau in northwestern China, which covers 64 million ha, is a typical and important dryland farming area. Polyethylene plastic film mulch, in conjunction with N fertilization, has been used extensively in the region, which not only substantially increased crop production but also affected soil properties (Liu et al., 2014, 2021; Luo et al., 2019). The overall objective of this study was to investigate the response of soil microbial communities to long-term (10-yr) FM and N fertilization. The specific objectives were to (i) assess the effects of long-term FM and N fertilization on soil microbial diversity, community composition, and structure, (ii) understand how bacterial and fungal communities interact, and identify keystone taxa following long-term FM and N fertilization, and (iii) detect the vital soil properties that affect microbial community structure and relate those properties to the keystone taxa.

2. Materials and methods

2.1. Site description

The field experiment was established in 2009 at the Changwu Agroecological Experimental Station (35.28°N, 107.88°E; 1200 m asl), located on the Loess Plateau of northwestern China. The area has a semiarid monsoon climate with a mean annual air temperature of 9.2 °C and average annual precipitation of 582 mm (with about 73% received between May and September). The dominant cropping system is rainfed and produces one harvest of maize or wheat (*Triticum aestivum* L.) per year. The experimental field belongs to one household and has been used for maize production for more than five years. The soil is a light silt loam (Heilutu series) derived from loess deposits. At the beginning of the experiment in 2009, the soil had a bulk density (BD) of 1.31 g cm^{-3}, pH of 8.35, soil organic carbon (SOC) of 9.01 g kg^{-1}, and total N (TN) of 0.95 g kg^{-1} in the 0–20 cm soil layer.

2.2. Experimental design

The field experiment was performed using a split-plot design, and the main plots were arranged in a randomized complete block with three replicates. The main plots consisted of four N fertilizer rates: 0 (N0), 100 (N100), 250 (N250), and 400 (N400) kg N ha^{-1}, while the subplots were assigned to two mulching methods: no mulch (NM) and FM. The size of each subplot was 28 m^2 (7 × 4 m). N fertilizer was applied three times as urea (46% N): 40% as a base application before planting, 30% at the 10th leaf stage, and 30% at the silking stage. All plots received 40 kg P ha^{-1} as calcium superphosphate (12% P_2O_5) and 80 kg K ha^{-1} as potassium sulfate (45% K_2O) before planting. The basal fertilizers were manually broadcast over the soil surface and then plowed into the subsurface after ridging treatment plots. The topdressing fertilizer was applied using a hole-sowing machine. The FM treatments involved placing a transparent non-biodegradable polyethylene plastic film (0.008 mm thick) over each plot. Hybrid maize ('Xianyu 335') was planted at 80,000 plants ha^{-1} in monoculture. Maize seeds were planted at a depth of 5 cm using a hand-powered hole-drilling machine at the end of April each year, with the cobs harvested in September. Maize straws in all plots were completely removed after harvest. During the growing season, insecticides were used to control pests, and weeds were periodically removed manually.

2.3. Soil sampling

In the early August 2018 (maize silking stage), in the 10th year of the field trial, nine individual bulk soil cores (5 cm diameter) per plot were randomly collected from the 0–15 cm soil layer between maize plants in rows and combined to form one composite sample per plot. The composite samples were passed through a 2-mm sieve to remove roots and other debris and then separated into two subsamples. One subsample was placed in a 50 ml centrifuge tube, transferred to the laboratory in an insulated cold container, and maintained at −80 °C until DNA extraction was performed. The other subsample was placed in a self-sealing plastic bag and transferred to the laboratory in an insulated cold container for determination of soil physicochemical properties.

2.4. Soil property measurements

Soil BD was measured in the field using the cutting-ring method. Soil

water content (SWC) was determined by oven-drying the samples to a constant weight at 105 °C. Soil pH was measured with a pH meter (Sartorius pH Meter PB-10, Germany) after shaking the soil in a water (1:2.5 w/v) suspension for 30 min. SOC content was determined using the dichromate oxidation method (Mebius, 1960). Soil TN content was determined using the Kjeldahl method (Bremner and Mulvaney, 1982). Soil NO_3^--N and NH_4^+-N content were determined by extracting samples with 1 mol L^{-1} KCl solution (1:10 w/v), and the extracts were analyzed with a continuous flow injection analyzer (FLOWSYS, Italy). Soil available phosphorus (AP) was measured using the $NaHCO_3$ extraction method (Olsen et al., 1954). Soil available potassium (AK) was extracted using ammonium acetate and analyzed with a Sherwood 410 flame photometer (Sherwood Scientific Ltd., England). Soil microbial biomass C (MBC) and N (MBN) were measured using the chloroform fumigation–extraction method (Brookes et al., 1985; Vance et al., 1987).

2.5. Soil DNA extraction, PCR amplification, and Illumina MiSeq sequencing

Soil DNA was extracted from 0.5 g soil using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) following the manufacturer's protocol. The extracted DNA was assayed for quality and quantity using a NanoDrop spectrophotometer (NanoDrop Technologies Inc, Wilmington, DE, USA).

The V3–V4 region of the bacterial 16S rRNA gene was amplified using the primer pair of 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Castrillo et al., 2017). For fungi, the primer pair of ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) was used to amplify the ITS1 region (Coleine et al., 2018). The primers were labeled with unique barcodes for each sample. The triplicate PCR reactions were performed in a total volume of 40 μl, containing 20 μl of 2 × Phμsion HF MM (New England Biolabs), 1 μl of each 10 μM primer, 10 μl of purified template DNA, and 8 μl ddH_2O. The thermal cycling was as follows: an initial denaturation at 98 °C for 30 s, followed by 10 cycles at 98 °C for 10 s, 65 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 7 min.

The obtained PCR products were verified by 1.8% agarose gel electrophoresis, purified with the Monarch® DNA Gel Extraction kit (New England Biolabs), and quantified with Quant-iT™ds DNA HS Reagent (Thermo Scientific). The purified PCR products were pooled in equimolar concentrations and paired-end sequenced (2 × 250) using an Illumina Hiseq 2500 platform at Biomarker Technologies Corporation, Beijing, China. The raw sequences have been deposited in the Genome Sequence Archive in the BIG Data Center, Chinese Academy of Sciences under accession number CRA002345 that are publicly accessible at https://bigd.big.ac.cn/gsa (GSA, 2017).

2.6. Bioinformatic analysis

Paired-end sequences were merged using FLASH V1.2.7 (Magoč and Salzberg, 2011) for each sample. To obtain high-quality sequences, quality filtering of the raw sequences was performed using QIIME V1.7.0 (Caporaso et al., 2010). After detecting and removing the chimeric sequences using UCHIME V4.2 (Edgar et al., 2011), the effective sequences were obtained, which were analyzed using UPARSE V7.0.1001 software (Edgar, 2013). Operational taxonomic units (OTUs) were clustered based on 97% sequence similarity. The taxonomic information of each sequence was annotated based on the SILVA (bacteria) and UNITE (fungi) database. To compare differences in microbial diversity between samples, the lowest number of sequences (43,359 for bacteria and 51,772 for fungi) in all samples was used to normalize the dataset.

2.7. Statistical analysis

Data analysis was performed using R V3.5.2 (R Core Team, 2018) unless otherwise indicated. Linear mixed modelling for a split-plot design was executed to determine the effects of N fertilization, mulching, and their interaction on soil properties and microbial abundance using the nlme package (Pinheiro et al., 2019). Pair-wise comparisons were conducted using the emmeans (Lenth, 2020) and multcomp (Hothorn et al., 2008) packages, and differences between treatments were detected using the Tukey's test ($P < 0.05$). The Chao1 and Shannon indices for alpha-diversity were calculated using QIIME software. Spearman's correlation coefficients were used to evaluate the associations of soil microbial richness and diversity with soil physicochemical properties. Cluster analysis, which used the unweighted pair-group method with arithmetic mean (UPGMA) based on the Bray-Curtis distance, was performed using the *hclust* function in the stats package. Principal coordinates analysis (PCoA), based on the Bray-Curtis distance, was performed to assess the similarities in microbial community composition between samples. Permutational multivariate ANOVA (PerMANOVA) was used to assess the effects of mulching and N fertilization on microbial communities. Redundancy analysis (RDA) was used to investigate the relationships between soil physicochemical properties and microbial communities. PCoA, PerMANOVA, and RDA was performed using the *cmdscale*, *adonis*, and *capscale* function in the vegan package (Oksanen et al., 2019), respectively.

Co-occurrence network analysis was performed to reveal correlations between microbial taxon using the vegan, Hmisc (Harrell Jr, 2019), and igraph (Csardi and Nepusz, 2006) packages. Bacterial and fungal genera with average relative abundances above 0.05% were selected to build networks for the different mulching and N fertilization treatments. Spearman's correlation coefficient (r) with an absolute value >0.6 and $P < 0.01$ was used for network building (Barberán et al., 2012). The topological features of the networks were calculated using the igraph package, and all networks were visualized by the interactive platform Cytoscape V3.7.1 (https://cytoscape.org) (Shannon et al., 2003). The real networks were compared with their randomized versions with equal nodes and edges, generated by the Network Randomizer plugin in Cytoscape. Genera with the highest betweenness centrality values were identified as keystone taxa (Vick-Majors et al., 2014).

3. Results

3.1. Soil physicochemical properties and microbial biomass

The measured soil physicochemical properties responded differently to the N fertilization and plastic mulch regimes (Table 1). Soil pH and AK significantly decreased, while TN, NO_3^--N, and NH_4^+-N content significantly increased with N fertilization (mean of the two mulching practices). N fertilization decreased SWC by 1.9–7.9%, and significantly lower value was found in the N250 treatment compared to the N0 treatment. Soil BD, SOC, AP, MBC, and MBN content did not differ between the N rates. Averaged over the four N rates, the FM treatments had significantly higher SWC (by 12.7%) and lower SOC (3.3%), TN (5.2%), NO_3^--N (45.5%), and NH_4^+-N (37.8%) content than the NM treatments, while the other indices did not differ between the two mulching treatments. A significant interaction effect of N fertilization and plastic mulch was detected for SWC and NO_3^--N content.

3.2. Microbial diversity and community structure

Averaged over the two mulching practices, the bacterial Shannon index was significantly lower with the N400 treatment than with the N0 treatment (Table 2). However, N fertilization affected neither the Chao1 nor the Shannon index for fungi. The FM treatments significantly increased the bacterial Shannon index and decreased the fungal Chao1 index in comparison with NM treatments. The correlation analysis showed that the bacterial Shannon index had a significant positive correlation with pH, and significant negative correlations with TN, NO_3^--N, and NH_4^+-N content (Fig. S1). For fungi, the Chao1 index had a

Table 1
Soil physicochemical and microbial properties in the top 15 cm as affected by N fertilization and plastic film mulch.

Treatments	pH	BD ($g\ cm^{-3}$)	SWC (%)	SOC ($g\ kg^{-1}$)	TN ($g\ kg^{-1}$)	NO_3^--N ($mg\ kg^{-1}$)	NH_4^+-N ($mg\ kg^{-1}$)	AP ($mg\ kg^{-1}$)	AK ($mg\ kg^{-1}$)	MBC ($mg\ kg^{-1}$)	MBN ($mg\ kg^{-1}$)
N fertilizer rates (N)											
N0	8.23 ± 0.06a	1.19 ± 0.07a	14.3 ± 1.72a	9.50 ± 0.43a	0.81 ± 0.05b	1.21 ± 0.25c	0.89 ± 0.26c	41.3 ± 5.04a	231 ± 33.3a	233 ± 26.2a	33.7 ± 6.02a
N100	8.09 ± 0.18ab	1.23 ± 0.06a	13.5 ± 1.63ab	10.0 ± 0.34a	0.83 ± 0.04ab	2.30 ± 0.57c	1.55 ± 0.47b	35.0 ± 3.29a	186 ± 30.8b	212 ± 24.2a	28.1 ± 4.40a
N250	8.16 ± 0.07ab	1.24 ± 0.11a	13.1 ± 0.73b	9.90 ± 0.42a	0.84 ± 0.05ab	18.1 ± 9.73b	1.70 ± 0.73b	33.3 ± 6.94a	185 ± 19.6b	216 ± 9.7a	30.1 ± 5.30a
N400	7.94 ± 0.24b	1.27 ± 0.10a	14.0 ± 0.55ab	9.70 ± 0.44a	0.87 ± 0.02a	39.1 ± 12.0a	2.55 ± 0.77a	35.0 ± 6.20a	171 ± 19.7b	201 ± 16.7a	27.0 ± 1.36a
Mulching practices (M)											
NM	8.12 ± 0.21a	1.22 ± 0.05a	12.9 ± 0.78b	9.94 ± 0.45a	0.86 ± 0.04a	19.7 ± 20.9a	2.06 ± 0.85a	38.1 ± 5.66a	194 ± 37.4a	218 ± 20.8a	31.5 ± 4.42a
FM	8.09 ± 0.16a	1.25 ± 0.11a	14.5 ± 1.13a	9.62 ± 0.37b	0.81 ± 0.04b	10.7 ± 11.9b	1.28 ± 0.59b	34.1 ± 5.92a	192 ± 31.7a	213 ± 24.4a	27.9 ± 5.15a
Source of variance											
N	0.0321	0.4136	0.0418	0.0977	0.0303	<0.0001	<0.0001	0.0924	0.0085	0.1237	0.0868
M	0.7004	0.2667	<0.0001	0.0397	0.0062	<0.0001	<0.0001	0.0833	0.8390	0.5876	0.0657
N × M	0.1867	0.1986	0.0171	0.2856	0.7895	0.0001	0.1179	0.5446	0.5376	0.7563	0.6533

Values (mean ± standard deviation) followed by different lowercase letters within a column for the same factor indicate significant differences by Tukey test ($P < 0.05$). N0–N400 represent the N application rates from 0 to 400 kg N ha^{-1}; NM, no mulching; FM, plastic film mulching. BD, soil bulk density; SWC, soil water content; SOC, soil organic carbon; TN, total nitrogen; NO_3^--N, soil nitrate nitrogen; NH_4^+-N, soil ammonium nitrogen; AP, available phosphorus; AK, available potassium; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen.

Table 2
Microbial richness and diversity indices at 97% sequence similarity as affected by N fertilization and plastic film mulch.

Treatments	Bacteria		Fungi	
	Chao1	Shannon	Chao1	Shannon
N fertilizer rates (N)				
N0	5300 ± 1878a	9.828 ± 0.071a	586 ± 187a	7.154 ± 0.188a
N100	4495 ± 165a	9.776 ± 0.052a	579 ± 147a	6.865 ± 0.855a
N250	4412 ± 187a	9.810 ± 0.117a	525 ± 162a	6.671 ± 0.555a
N400	4292 ± 144a	9.580 ± 0.198b	552 ± 131a	6.416 ± 0.873a
Mulching practices (M)				
NM	4752 ± 1387a	9.686 ± 0.172b	634 ± 163a	6.973 ± 0.402a
FM	4497 ± 153a	9.810 ± 0.105a	487 ± 92b	6.580 ± 0.865a
Source of variance				
N	0.2793	0.0053	0.8295	0.3233
M	0.5177	0.0148	0.0119	0.1780
N × M	0.3371	0.6744	0.2645	0.9936

Values (mean ± standard deviation) followed by different lowercase letters within a column for the same factor indicate significant differences by Tukey test ($P < 0.05$). N0–N400 represent the N application rates from 0 to 400 kg N ha^{-1}; NM, no mulching; FM, plastic film mulching.

significant negative correlation with SWC and positive correlations with TN and MBN content.

Cluster analyses showed that the N application rate affected the bacterial communities the most, with the N0 and N100 treatments clustered separately with the N400 treatment (Fig. 1A). At the same N rate, similarities in bacterial communities were dependent on the mulching method. In contrast, mulching had a greater influence than N fertilization on the fungal community structure (Fig. 1C). For the N250 treatment, it clustered with the N400 treatment under NM, but with the N0 and N100 treatments under FM for both the bacterial and fungal communities. The PCoA results also revealed a significant difference between treatments in the bacterial (Fig. 1B and Table S1) and fungal community structure (Fig. 1D and Table S1). Along the PCo1 axis, the bacterial communities were clearly separated based on N application rates, while the fungal communities were clearly separated according to mulching practices.

The RDA results showed that the bacterial and fungal community structures were strongly affected by soil properties. The first two axes explained 68.4 and 49.0% of the variation in the bacterial (Fig. 2A) and fungal communities (Fig. 2B), respectively. In particular, SWC, pH, NO_3^--N, TN, and NH_4^+-N had significant correlations with bacterial community structure, and SWC, TN, and NH_4^+-N had significant correlations with fungal community structure.

3.3. Relative abundance of major bacterial and fungal taxa

The dominant bacterial phyla in the treatments were Proteobacteria, Acidobacteria, Gemmatimonadetes, Actinobacteria, and Chloroflexi (Fig. 3A), with relative abundance averaging 36.5, 18.1, 10.5, 8.6, and 8.2%, respectively (Table S2). The subdominant phyla were Bacteroidetes, Rokubacteria, Planctomycetes, Nitrospirae, Verrucomicrobia, and Armatimonadetes. These 11 phyla accounted for 94.3% of the sequences. Nitrogen fertilization significantly increased the relative abundance of Gemmatimonadetes, and decreased the relative abundance of Acidobacteria, Rokubacteria, Planctomycetes, Armatimonadetes, and Latescibacteria. The relative abundance of Bacteroidetes, Nitrospirae, and Verrucomicrobia at first increased and then decreased with increasing N rate. Averaged over the four N rates, FM significantly increased the relative abundance of Planctomycetes and Latescibacteria, and decreased the relative abundance of Gemmatimonadetes, Actinobacteria, and Nitrospirae. The relative abundance of Actinobacteria and Verrucomicrobia was significantly affected by the interaction of N fertilization and plastic mulch.

The fungal composition was dominated by Ascomycota, Basidiomycota, Chytridiomycota, Mortierellomycota, Glomeromycota, and Mucoromycota (Fig. 3B). Nitrogen fertilization significantly decreased the relative abundance of Glomeromycota, while having insignificant effects on other phyla (Table S2). Averaged over the four N rates, FM significantly increased the relative abundance of Glomeromycota, and decreased the relative abundance of Chytridiomycota, Mortierellomycota, and Mucoromycota, compared with NM.

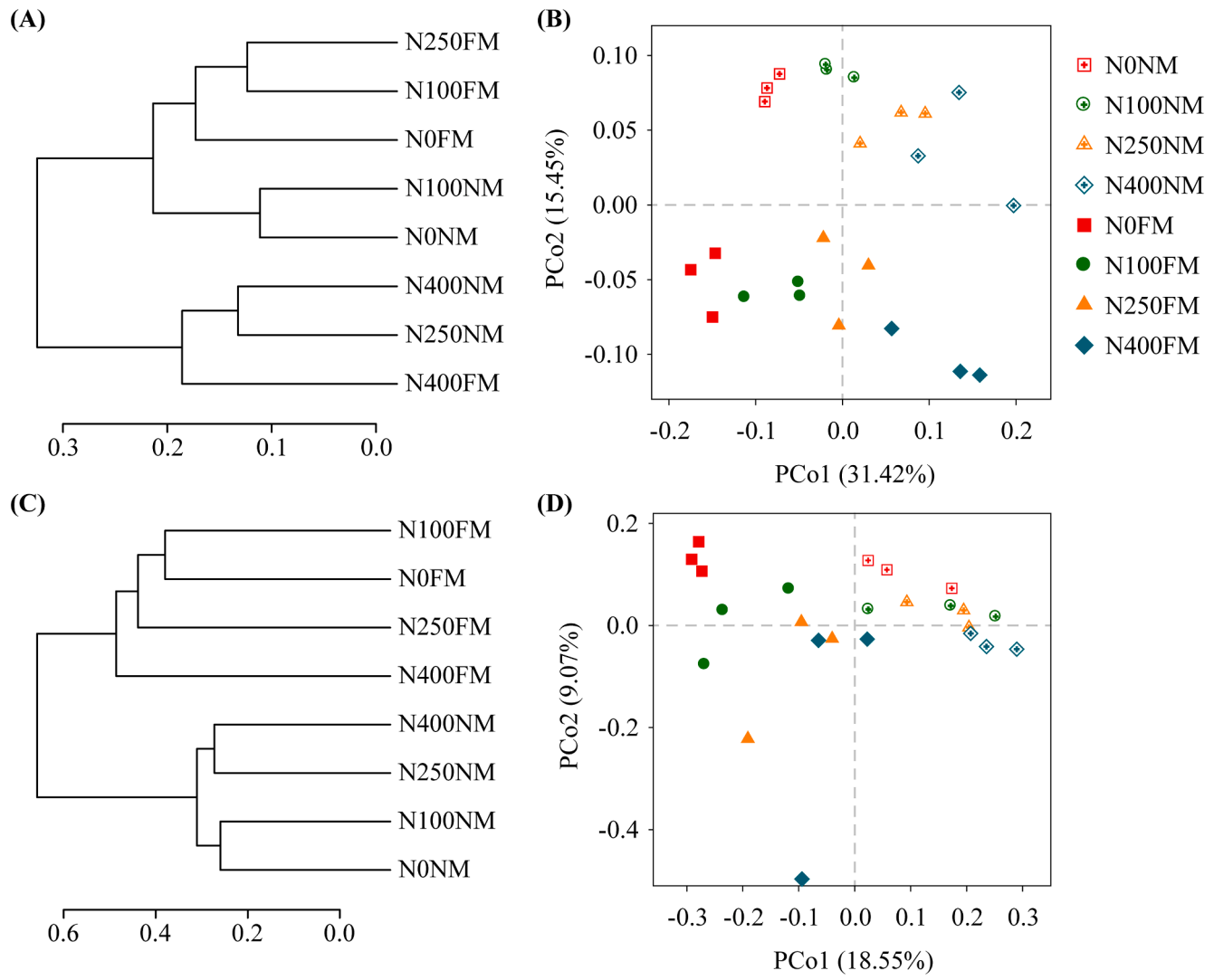


Fig. 1. Clustering tree and principal coordinates analysis (PCoA) based on Bray-Curtis distance at operational taxonomic units (OTU) levels for bacteria (A, B) and fungi (C, D). N0–N400 represent the N application rates from 0 to 400 kg N ha^{-1}; NM, no mulching; FM, plastic film mulching.

3.4. Topological properties and keystone taxa in the co-occurrence networks

The co-occurrence patterns of bacteria and fungi were strongly affected by the N fertilization (Fig. 4) and plastic mulch (Fig. 5) treatments. As the N rate increased, the clustering coefficient, average path length, network diameter, and modularity values followed an inverted parabolic-like changing trend (i.e., first decreasing and then increasing with increasing N rate, Table S3). Additionally, N fertilization increased the average degree and network density values, but the increment was relatively small with the N250 treatment. Relative to NM, FM increased the average path length and modularity values, and decreased the clustering coefficient, average degree, and network density values (Table S4). Compared with the corresponding random networks, the real networks had higher clustering coefficient, average path length, and modularity values.

Nitrogen fertilization increased the total links among microbial taxa by 8.7–14.7%, with increases in the order N400 > N100 > N250 (Fig. 4 and Table 3). Interestingly, the N100 and N250 treatments had greater increases in negative links (21.1 and 11.4%, respectively) than positive links (3.4 and 6.0%, respectively), while the N400 treatment had fewer negative links than the N0 treatment. In addition, the number of links between bacterial and fungal taxa increased with N fertilization, while the number of links declined within fungal taxa in N100 and within bacterial taxa in N250. Relative to NM, FM weakened the interactions among microbial taxa by 35.0, 40.8, and 26.6% for the numbers of total, positive, and negative links, respectively (Fig. 5 and Table 4). The weakened interactions under FM were mainly due to reduced links within bacterial taxa and between bacterial and fungal taxa.

The keystone taxa in the co-occurrence networks differed for the various N fertilization (Fig. 4) and plastic mulch treatments (Fig. 5). The correlation analysis showed that the keystone taxa had more significant correlations with soil N content than other soil factors (Fig. S2).

4. Discussion

This study showed that soil microbial biomass did not change after 10 years of N fertilization and plastic mulch, which is inconsistent with most previous studies (Farmer et al., 2016; Zhang et al., 2018). However, field experiments conducted in the same region also revealed little effects of chemical fertilization (Wang et al., 2018a) and mulching (Luo et al., 2015) on microbial biomass. This indicates that soil microbial biomass is relatively stable in the studied semiarid areas. Differently, microbial diversity, community structure, composition, and interspecies interaction were all sensitive to the various fertilization and mulching practices.

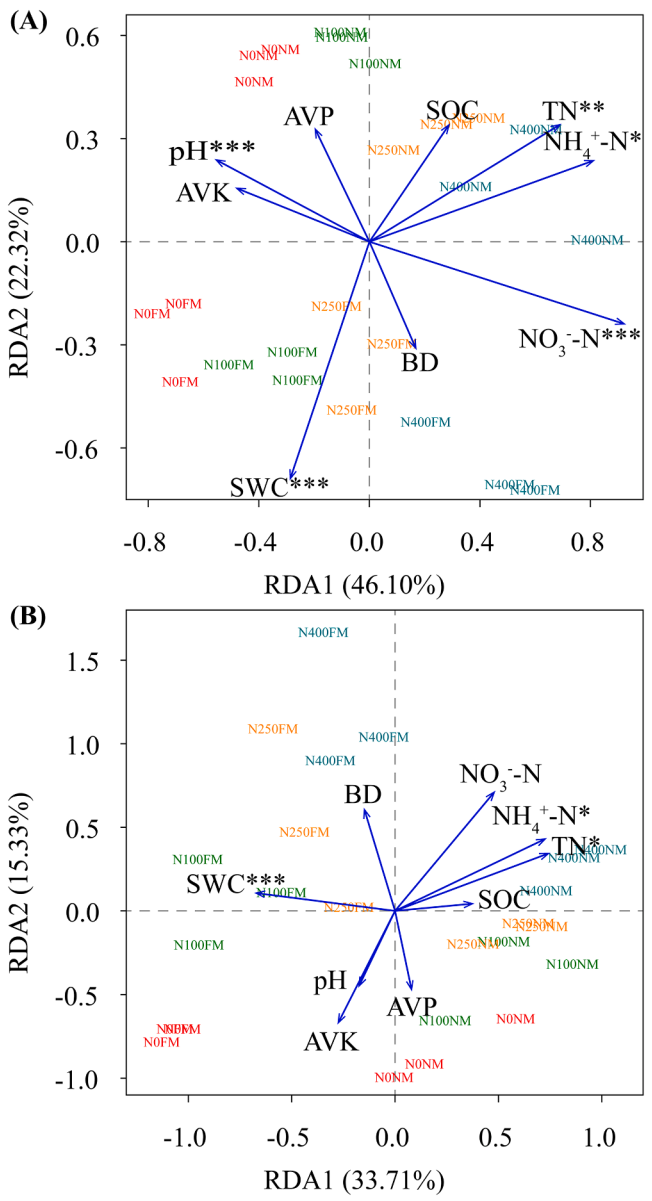


Fig. 2. Ordination plots of the results from the redundancy analysis (RDA) to investigate correlations between the microbial community and soil physicochemical properties for bacteria (A) and fungi (B). BD, soil bulk density; SWC, soil water content; SOC, soil organic carbon; TN, total nitrogen; NO_3^--N, soil nitrate nitrogen; NH_4^+-N, soil ammonium nitrogen; AP, available phosphorus; AK, available potassium. N0–N400 represent the N application rates from 0 to 400 kg N ha^{-1}; NM, no mulching; FM, plastic film mulching. *, **, and *** indicate significant correlations at $P < 0.05$, 0.01, and 0.001, respectively.

4.1. Microbial diversity and community structure respond to N fertilization and plastic film mulch

Nitrogen fertilization and plastic mulch changed soil microenvironment by altering soil acidity, moisture, and N content, thus inducing changes in the bacterial and fungal communities. Similar results have been reported elsewhere (Ramirez et al., 2012; Dong et al., 2017; Huang et al., 2019b). Nevertheless, the bacterial and fungal communities varied in their response to fertilization and mulching practices.

In this study, the bacterial community was dominantly affected by the N application rates. This is in agreement with other studies (Ramirez et al., 2010; Dai et al., 2018), which indicated that soil bacteria was sensitive to N fertilization in various ecosystems. Nitrogen fertilization can change the bacterial community directly by increasing soil N content and indirectly by reducing soil pH (Geisseler and Scow, 2014; Yao et al., 2014). Both of these mechanisms were confirmed in our study. Additionally, high N input resulted in loss in the bacterial diversity, thereby causing adverse effects on soil function (Ramirez et al., 2012). However, some studies have reported that N fertilization increased (Lupwayi et al., 2018) or had no effect (Eo and Park, 2016) on bacterial diversity, which may have been related to the application rate or duration of fertilizer use. Though plastic mulch played a less important role in regulating bacterial community, it significantly increased the bacterial diversity. This was most likely because FM promoted crop growth, which increased competition for soil N by soil microbes (Liu et al., 2015), and then alleviated the adverse effects of high soil N on bacterial growth.

Different from bacteria, the fungal community was affected more by mulching practice. It has been reported that most fungi prefer aerobic environments (Lennon et al., 2012), and the fungal community was closely correlated with soil moisture (Dong et al., 2017). Plastic mulch could create a localized anaerobic environment by restricting oxygen exchange and increasing soil water content. This probably caused changes in fungal community and decrease in fungal richness. Moreover, the decline in soil TN content with mulching further suppressed the growth of fungi. In agreement, the fungal richness was found to be positively correlated with soil N availability (Hu et al., 2017). Soil fungi have the ability to acquire heterogeneously distributed nutrient resources through specialized hyphae (Schmieder et al., 2019). This could partly explain why the fungal community was less affected by N fertilization.

As indicated by the cluster analysis results, the magnitude of N fertilization influence on the bacterial and fungal communities was regulated by the mulching practice. This result is in line with the findings of Dai et al. (2018), who reported that the response of soil bacterial diversity to N fertilization was closely related to the water management regime. Meanwhile, the results of this study suggest that the threshold of N application rate that induced changes in microbial community structure was higher under FM treatments (between 250 and 400 kg N ha^{-1}) than under NM treatments (between 100 and 250 kg N ha^{-1}). This is likely due to the greater plant N uptake under FM than NM (Liu et al., 2015), which reduced soil N residues. Similarly, a farmland ecosystem was observed to have a much higher N threshold for altering microbial community structure and activity (Zhong et al., 2015) than a grassland ecosystem (Yao et al., 2014; Zhang et al., 2019), which was most likely due to its greater productivity.

4.2. Microbial community composition responds to N fertilization and plastic film mulch

The relative abundance of Gemmatimonadetes increased significantly with increasing N rate, while the opposite was true for Acidobacteria, Rokubacteria, and Planctomycetes. The copiotrophic hypothesis can explain this phenomenon. Gemmatimonadetes are fast-growing copiotrophs that thrive in high-nutrient environments and are stimulated by N fertilization (Liu et al., 2019). In contrast, Acidobacteria, Rokubacteria, and Planctomycetes are slow-growing oligotrophs the prefer low-nutrient conditions and whose growth is inhibited by N inputs (Trivedi et al., 2017; Che, 2018). Latescibacteria have a saprophytic lifestyle and are responsible for the degradation of lipids and polysaccharides (Farag et al., 2017). In this study, the relative abundance of Latescibacteria declined with N fertilization, which agrees with the results of Yin et al. (2019), indicating that this phylum is probably oligotrophic bacteria. Bacteroidetes and Verrucomicrobia are important groups participating in soil C cycling (Dunfield et al., 2007; Wolińska et al., 2017). The relative abundance of these two phyla followed a parabolic pattern with increasing N rate, as did Nitrospirae, which is involved in soil N transformation (Ramirez et al., 2010). These results suggest that the growth of these three phyla is stimulated by low N inputs but inhibited by high N inputs. Similar findings have been

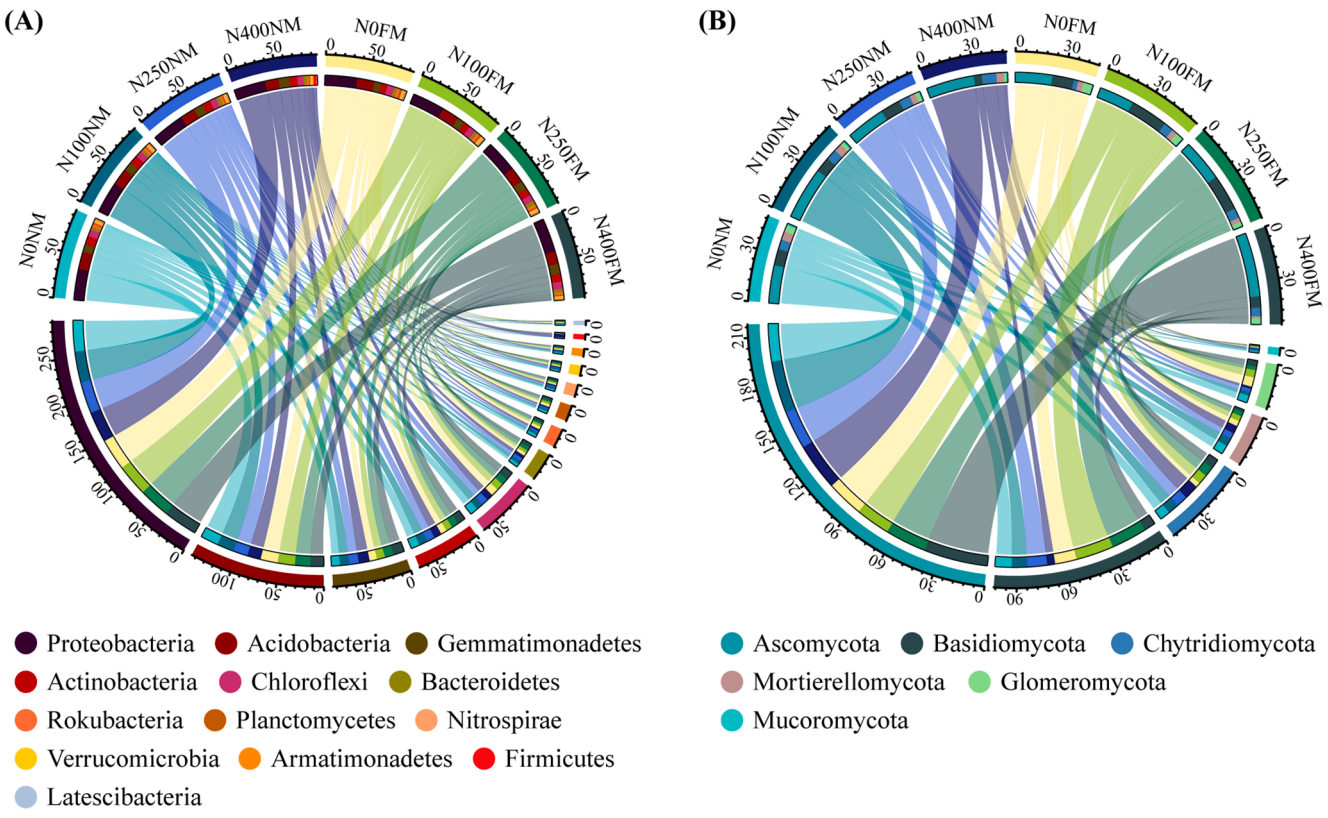


Fig. 3. The relative abundance of major taxonomic groups at the phylum level (relative abundance > 0.5%) for bacteria (A) and fungi (B). N0–N400 represent the N application rates from 0 to 400 kg N ha^{-1}; NM, no mulching; FM, plastic film mulching.

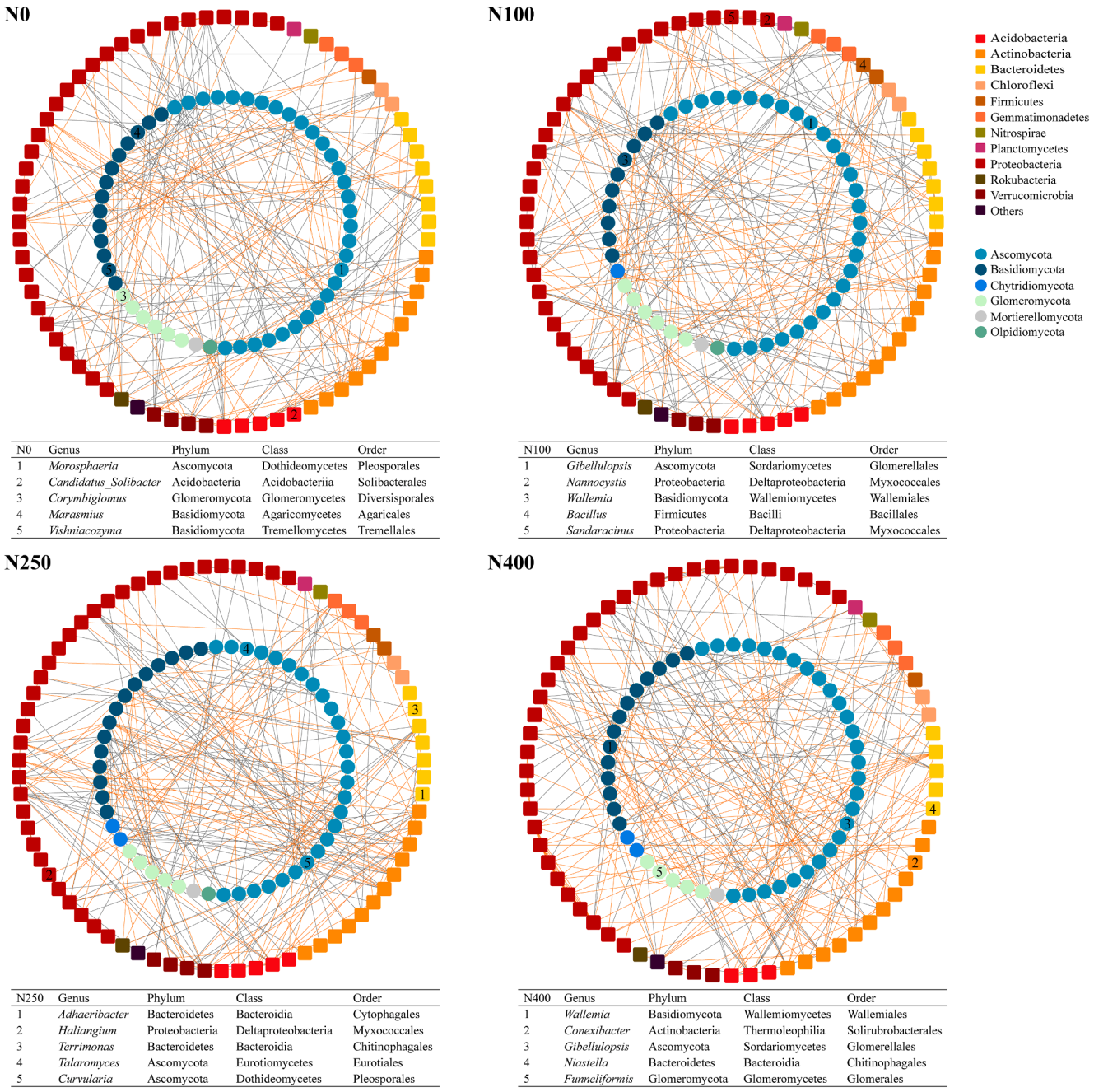
reported by Ling et al. (2017) and Wang et al. (2018b).

Plastic mulch significantly increased the relative abundance of Planctomycetes and Latescibacteria, while decreasing the relative abundance of Gemmatimonadetes, Actinobacteria, and Nitrospirae, mainly due to the higher soil moisture and lower oxygen conditions under FM (Shahzad et al., 2019). Members of Planctomycetes were identified as anammox bacteria (Mohamed et al., 2010), and Latescibacteria can survive anoxic conditions (Youssef et al., 2015); changes in the abundance of these two phyla could strongly affect soil N cycling and organic matter (SOM) decomposition. The phylum Glomeromycota includes arbuscular mycorrhizal fungi that form mutualistic associations with most plant roots (Liu et al., 2012b), and an increase in root biomass under plastic mulch (Gao et al., 2014) was expected to increase its abundance. Gemmatimonadetes and Actinobacteria are also involved in SOM degradation, and as aerobic heterotrophs that adapt to thrive in low soil moisture conditions (DeBruyn et al., 2011; Zhou et al., 2015). Decrease in the relative abundance of Nitrospirae might contribute to reducing N loss, because this phylum performs nitrite oxidation in the nitrification process (Daims et al., 2016). Similarly, the relative abundance of fungal phyla Chytridiomycota and Mortierellomycota both significantly decreased under FM. This is probably because plastic mulch increased soil temperature, and then promoted the decomposition of organic matter (Li et al., 2019; Luo et al., 2019).

4.3. Microbial co-occurrence patterns respond to N fertilization and plastic film mulch

Various soil microbes generally live together to form a complex system that determine soil functioning (Banerjee et al., 2016). The co-occurrence patterns of microbial communities change under changing environmental conditions, which can affect microbial network stability and related substance circulation and energy transformation processes (de Vries et al., 2018; Zheng et al., 2018). Nitrogen fertilization increased both the positive and negative links of microbial taxa, which agrees with the findings of Zheng et al. (2018) in an apple orchard, but is inconsistent with those of Yao et al. (2014) in a steppe ecosystem. Because the N requirement was relatively low in the steppe, N fertilization possibly led to N accumulation and nutrient imbalance in the soil, which subsequently had an adverse effect on the microbial communities (Geisseler and Scow, 2014; Yuan et al., 2019). Farm production systems, however, demand large amounts of N to sustain high productivity. Insufficient N input could exacerbate the competition between plants and microbes and between different microbial groups for soil N (Kuzyakov and Xu, 2013). So, in this study, the increase in negative links within bacterial taxa and between bacteria and fungi were more pronounced in the N100 or N250 treatments. Moreover, bacteria had greater richness and N demand than fungi (Zechmeister-Boltenstern et al., 2016), which may have inhibited fungal growth in the low N treatment, reducing the links within fungal taxa. The high N rate (400 kg N ha^{-1}) relieved the N shortage, improving crop growth and increasing root exudation of abundant labile and recalcitrant organic compounds (Lin et al., 2019), which promoted cooperation between microbes. The positive links between bacteria and fungi increased with N application, most likely because some bacteria can use the products released during organic matter decomposition by saprotrophic fungi (Ballhausen and de Boer, 2016).

Studies have reported that plastic mulch changed soil microbial activity and community structure (Dong et al., 2017; Bandopadhyay et al., 2018), but it is not clear how the co-occurrence network responds to the mulching practice. In this study, the modularity of the network increased under FM, suggesting that the microbes favoring similar soil conditions survived, and environmental filtering reshaped the community assembly. However, FM decreased the complexity of the co-occurrence network, as indicated by the reduced clustering coefficient, network density, total nodes, and links. This is primarily because plastic mulch created a more favorable soil environment (Luo et al., 2019).



N0	Genus	Phylum	Class	Order
1	*Morosphaeria*	Ascomycota	Dothideomycetes	Pleosporales
2	*Candidatus_Solibacter*	Acidobacteria	Acidobacteriia	Solibacterales
3	*Corymbiglomus*	Glomeromycota	Glomeromycetes	Diversisporales
4	*Marasmius*	Basidiomycota	Agaricomycetes	Agaricales
5	*Vishniacozyma*	Basidiomycota	Tremellomycetes	Tremellales

N100	Genus	Phylum	Class	Order
1	*Gibellulopsis*	Ascomycota	Sordariomycetes	Glomerellales
2	*Nannocystis*	Proteobacteria	Deltaproteobacteria	Myxococcales
3	*Wallemia*	Basidiomycota	Wallemiomycetes	Wallemiales
4	*Bacillus*	Firmicutes	Bacilli	Bacillales
5	*Sandaracinus*	Proteobacteria	Deltaproteobacteria	Myxococcales

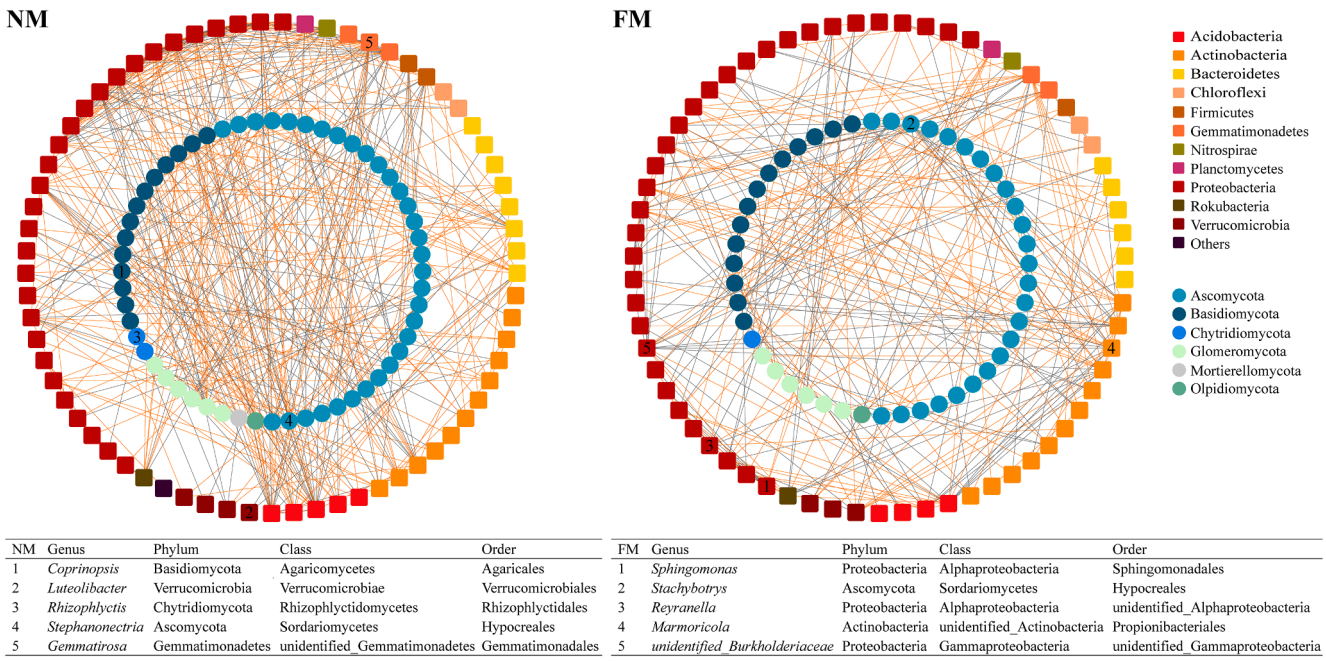
N250	Genus	Phylum	Class	Order
1	*Adhaeribacter*	Bacteroidetes	Bacteroidia	Cytophagales
2	*Haliangium*	Proteobacteria	Deltaproteobacteria	Myxococcales
3	*Terrimonas*	Bacteroidetes	Bacteroidia	Chitinophagales
4	*Talaromyces*	Ascomycota	Eurotiomycetes	Eurotiales
5	*Curvularia*	Ascomycota	Dothideomycetes	Pleosporales

N400	Genus	Phylum	Class	Order
1	*Wallemia*	Basidiomycota	Wallemiomycetes	Wallemiales
2	*Conexibacter*	Actinobacteria	Thermoleophilia	Solirubrobacterales
3	*Gibellulopsis*	Ascomycota	Sordariomycetes	Glomerellales
4	*Niastella*	Bacteroidetes	Bacteroidia	Chitinophagales
5	*Funneliformis*	Glomeromycota	Glomeromycetes	Glomerales

Fig. 4. Co-occurrence network analysis showing the interactions between bacterial and fungal communities under N application rates of 0 (N0), 100 (N100), 250 (N250), and 400 (N400) kg N ha^{-1} (all treatments containing the two mulching treatments). Orange lines represent significant positive relationships and grey lines represent significant negative relationships. The numbers inside the nodes denote the top five keystone species.

Similar findings were reported by Ma et al. (2018), where the cooperation and competition among microbial communities declined under favorable environments. Moreover, the reduced microbial richness under FM was also responsible for the simpler network, and the greater reduction ratios in fungal richness and links containing fungi confirmed this. In agreement, de Vries et al. (2018) reported markedly reduced links among bacterial taxa under simulated drought, most likely because the extremely low soil moisture and nutrient contents inhibited bacterial growth and survival, which significantly reduced bacterial richness. In particular, it is expected that FM could have a long-term impact on the microbial co-occurrence network as a result of the accumulation of plastic residues in soils (Ren et al., 2019). More seriously, plastic residues could lead to soil degradation and then restrict the sustainable development of agriculture (Steinmetz et al., 2016). Biodegradable plastic film has been demonstrated to be a promising alternative to polyethylene plastic film (Liu et al., 2021), and evaluation of the long-term effects of biodegradable film on soil quality is needed.

The keystone taxa always drive the structure and functioning of microbial communities (Banerjee et al., 2018). Some management practices, such as fertilization (Lin et al., 2019) and cover crops (Zheng et al., 2018) can alter the keystone taxa in farmland soils. In this study, N fertilization and FM changed the keystone taxa in the co-occurrence networks, which is consistent with previous findings. In the N fertilization and FM treatments, the co-occurrence networks were mostly dominated by keystone taxa belonging to bacteria, which is similar to the findings of Zheng et al. (2018). However, there were more fungal genera in the top five keystone taxa in the N0 and NM treatment, suggesting that fungal taxa mostly determined co-occurrence network



NM	Genus	Phylum	Class	Order
1	*Coprinopsis*	Basidiomycota	Agaricomycetes	Agaricales
2	*Luteolibacter*	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales
3	*Rhizophlyctis*	Chytridiomycota	Rhizophlyctidomycetes	Rhizophlyctidales
4	*Stephanonectria*	Ascomycota	Sordariomycetes	Hypocreales
5	*Gemmatirosa*	Gemmatimonadetes	unidentified_Gemmatimonadetes	Gemmatimonadales

FM	Genus	Phylum	Class	Order
1	*Sphingomonas*	Proteobacteria	Alphaproteobacteria	Sphingomonadales
2	*Stachybotrys*	Ascomycota	Sordariomycetes	Hypocreales
3	*Reyranella*	Proteobacteria	Alphaproteobacteria	unidentified_Alphaproteobacteria
4	*Marmoricola*	Actinobacteria	unidentified_Actinobacteria	Propionibacteriales
5	*unidentified_Burkholderiaceae*	Proteobacteria	Gammaproteobacteria	unidentified_Gammaproteobacteria

Fig. 5. Co-occurrence network analysis showing the interactions between bacterial and fungal communities in the no mulching (NM) and plastic film mulching (FM) treatments (both treatments containing the four N rates). Orange lines represent significant positive relationships and grey lines represent significant negative relationships. The numbers inside the nodes denote the top five keystone species.

Table 3
Numbers of nodes and links in the networks under N application rate of 0 (N0), 100 (N100), 250 (N250), and 400 (N400) kg N ha^{-1}.

	N0	N100	N250	N400
Number of nodes	125	120	125	118
Number of links	231	259	251	265
Positive links	117	121	124	161
Negative links	114	138	127	104
Bacteria to bacteria	107	112	93	115
Positive links	57	54	55	82
Negative links	50	58	38	33
Fungi to fungi	35	29	41	36
Positive links	18	17	23	20
Negative links	17	12	18	16
Bacteria to fungi	89	118	117	114
Positive links	42	50	46	59
Negative links	47	68	71	55

Table 4
Numbers of nodes and links in the co-occurrence networks under the no mulching (NM) and plastic film mulching (FM) treatments.

	NM	FM
Number of nodes	125	113
Number of links	437	284
Positive links	260	154
Negative links	177	130
Bacteria to bacteria	237	166
Positive links	149	99
Negative links	88	67
Fungi to fungi	54	29
Positive links	39	21
Negative links	15	8
Bacteria to fungi	146	89
Positive links	72	34
Negative links	74	55

stability and microbial community function under less favorable soil conditions. This is probably because fungi are well adapted to environmental stresses (Lennon et al., 2012) and better able to degrade recalcitrant soil carbon (Treseder et al., 2016). In addition, more keystone taxa were significantly correlated with soil N content, indicating that the co-occurrence networks were mainly affected by soil N availability, as reported by Zheng et al. (2018) in orchard soils.

5. Conclusions

In this study, 10 years of FM and N fertilization changed the soil physicochemical properties (i.e., SWC, SOC, TN, NO_3^--N and NH_4^+-N contents, and pH), which affected the soil bacterial and fungal communities as well as their co-occurrence network, though soil microbial biomass remained constant. Nitrogen fertilization and plastic mulch had inconsistent effects on soil bacterial diversity, which significantly increased under FM but tended to decrease with N application. Additionally, the FM treatment significantly reduced fungal richness compared with the NM treatment. The relative abundances of some microbial taxa changed under FM and N fertilization, thereby altering the soil bacterial and fungal community structure. Nitrogen fertilization played the leading role in modifying the bacterial community structure, while FM had a more significant role with the fungal community structure. There was an N threshold for changes in microbial structure, and FM increased this value by promoting crop N uptake. In the FM treatment, the positive and negative links among microbes both declined, suggesting that FM decreased the complexity and stability of the co-occurrence network. However, N fertilization generally promoted cooperation and competition within microbial taxa. The keystone taxa varied in the various N fertilization and mulching treatments, with more significant correlations with soil N content. The results of this study contribute to our understanding of the effects of long-term FM and N fertilization on soil microbial communities and provide valuable information for the sustainable development of dryland agriculture.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2021.115086.

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