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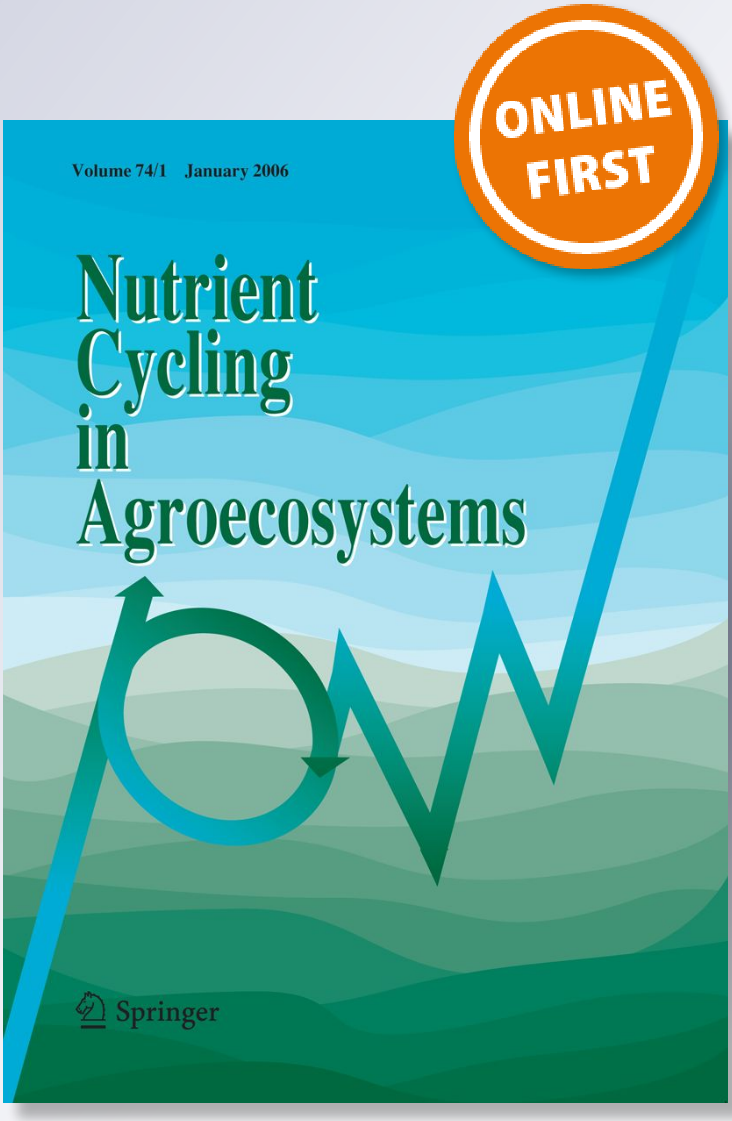

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ORIGINAL ARTICLE

Soil microbial communities under film mulching and N fertilization in semiarid farmland

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Abstract Film mulching and N fertilization can affect soil physicochemical properties, thereby improving plant growth, and may in turn affect soil microbial communities. Therefore, a 2-year field experiment was conducted to research the effects of film mulching and N fertilization on soil microbial communities. The four main treatments were N0F0, N0F1, N1F0, and N1F1, combining two N fertilizer rates (N0, 0 kg N ha^{-1}; N1, 225 kg N ha^{-1}) and two mulching methods (F0, no mulching; F1, film mulching) in the absence and presence of plants. The film mulching treatments significantly increased the mean temperature by 0.2 °C and decreased the soil organic carbon (SOC), mineral N and water soluble organic C by 5.6%, 35.5% and 24.0%, respectively. The N fertilization treatments significantly increased the mineral N, water soluble organic N and $KMnO_4$-oxidizable C by 117.9%, 256.4% and 55.3%, respectively. Additionally, the phospholipid fatty acid (PLFA) analysis of the soil microbial community revealed that the film mulching treatments significantly decreased the total PLFAs by 21.5% and the absolute abundance of fungi (F), bacteria (B), and actinomycetes by 26.7%, 23.1% and 24.6%, respectively. N fertilization significantly decreased the Gram-positive B/Gram-negative B ratio by 9.8%. Film mulching combining N fertilization significantly decreased the F/B ratio by 10.0%. Temperature ($P < 0.001$) and SOC/total P ($P < 0.001$) were confirmed to play significant roles in shaping the soil microbial community. Accordingly, short-term film mulching increases soil

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organic matter decomposition in the top soil and decreases the total soil microbial biomass and most microbial communities.

Keywords Plastic film mulching · Maize cultivation · Microbial community abundance · Microbial community structure · Labile soil organic matter

Introduction

Plastic film mulching is a worldwide agricultural technique due to its immediate economic and environmental benefits, such as crop productivity enhancement and quality improvement, increasing water-use efficiency, improving soil quality, and reducing greenhouse gas emissions and nitrate leaching (Wang et al. 2017; Liu et al. 2016, 2015a, b). However, the risks and other harmful effects associated with plastic film mulching are not yet fully understood. In particular, most of plastic films are inherently inert. Plastic film mulching modifies soil microclimatic conditions which may lead to shift of microbial communities (i.e., towards mycotoxigenic fungi) (Muñoz et al. 2015). Plastic film mulching also has the potential to expedite C and N metabolism processes, thereby exhausting soil organic matter (SOM) stocks (Steinmetz et al. 2016). Additionally, large rates of mineral fertilizers have been used on farmlands to increase crop yields and to meet food demand in recent decades (Savci 2012). Previous studies have shown that N fertilization can increase crop yields and biomass with or without film mulching (Liu et al. 2014b; Yao et al. 2017), accompanied by enhanced root-derived C. Meanwhile, short-term plastic film mulching increased the SOM mineralization induced by enhancing soil biological activity (Moreno and Moreno 2008; Zhang et al. 2015). However, few studies have shown that plastic film mulching and N fertilization work together to affect SOM and microbial communities.

Soil microorganisms play important roles in adjusting changes in SOM via mineralization-immobilization (Breulmann et al. 2014). Plastic film mulching provides a raised soil moisture and temperature and sufficient nutrient supply (i.e., more root-derived C input) for rhizosphere microorganisms, thereby enhancing their activity and metabolism (Subrahmaniyan et al. 2006; Maul et al. 2014; Liu et al. 2015a). Previous studies have shown that plastic film mulching slightly increased the diversity of arbuscular mycorrhizal fungi (AMF) and bacteria (Liu et al. 2012; Chen et al. 2014) and had no effect on the diversity of ammonia-oxidizing bacteria (Kapanen et al. 2008). A 28-year study demonstrated that plastic film mulching increased the relative abundances of Proteobacteria and Actinobacteria (Farmer et al. 2017). However, higher crop productivity should not be achieved by plastic film mulching at the expense of long-term soil degradation (Steinmetz et al. 2016). Until now, few reports have demonstrated the effect of film mulching on the soil microbial community as well as its influence on SOM changes. Moreover, N fertilization affected the SOM decomposition and nutrient supply for rhizosphere microorganisms (Zang et al. 2016; Li et al. 2017). Previous studies have shown that N fertilization did not significantly affect the soil total microbial biomass and fungal biomass but significantly decreased the soil bacterial biomass (Li et al. 2015; Liu et al. 2015b); meanwhile, a threshold of N fertilization at 180 kg ha^{-1} year^{-1} caused a decline in microbial activity (Zhong et al. 2015). Conversely, Stagnari et al. (2014) demonstrated that the application of green manure containing abundant N caused a higher fungal biomass. However, few studies have shown how N fertilization combined with plastic film mulching affects soil biochemical properties and microbial communities. Farmer et al. (2017) revealed that long-term N application induced a significant decline in soil bacterial richness and diversity, regardless of plastic film mulching. In addition, plant cultivation typically enhances root development and increases root exudation. Meanwhile, plant cultivation can change the effects of plastic film mulching and N fertilization on soil properties by roots absorbing water and nutrients. Therefore, it is essential to understand the mutual effects of soil microclimate and biochemical properties under film mulching and N fertilization in both the absence and presence of plants, which is necessary to evaluate the possible risks to soil quality.

Previous studies have shown that plastic film mulching increased the soil temperature and moisture (Bu et al. 2013; Liu et al. 2014a). The higher soil moisture and temperature induced by film mulching generally reduced soil fungal and bacterial richness due to favoring anaerobic and thermophilic species (Bonanomi et al. 2008; Simmons et al. 2014). In addition, both N fertilization and film mulching led to a decline in soil pH (Guo et al. 2010; Wang et al. 2017) and altered soil nutrient conditions, i.e., mineral N (NH_4^+–N + NO_3^-–N), total N (TN), and soil organic C (SOC) (Liu et al. 2015a; Hai et al. 2015; Luo et al. 2015b). Our previous studies indicated that water soluble organic C (WSOC), water soluble organic N (WSON), and $KMnO_4$-oxidizable C ($KMnO_4$-C) were more sensitive to film mulching application among the labile SOM fractions (Luo et al. 2015b, 2016). The top soil parameters are affected by film mulching and N fertilization and are closely related to the growth of soil microorganisms, which may differ in the absence or presence of plants. Accordingly, based on previous studies, we tested the hypothesis that short-term film mulching and N fertilization would decrease the soil microbial communities, which resulted from SOM decreases in both the absence and presence of plants.

Materials and methods

Site description

This experiment was conducted from 2014 to 2015 at the Changwu Agricultural and Ecological Experimental Station (35.28°N, 107.88°E, 1200 m altitude; Fig. 1). The study site is in a semiarid area on the Loess Plateau of northwest China. The annual mean air temperature is 9.7 °C (averaged for the previous 50 yr), and the average air temperature is 19 °C during the maize (*Zea mays* L.) growing season (MS) from May to September. The average annual precipitation is 579 mm (averaged for the previous 50 years), with 73% of this falls during the MS. In 2014 and 2015, the annual mean air temperature was 10.1 °C and 10.2 °C, respectively, and the precipitation amount was 573 mm and 556 mm. The daily precipitation and mean air temperatures (1.5 m above the ground) were obtained from the Changwu Meteorological Monitoring Station, which is located within 50 m of the experimental site. The soil at the study site developed from loess and had a silt loam texture according to the USDA texture classification system. Before the start of this experiment, maize had long been grown on the experimental field by local farmers. Additionally, field management practices were consistent such as fertilization, irrigation, and pesticide. Therefore, soil initial properties of each plot are basically uniform. The soil properties in the 0–0.2 m depth were as follows: a bulk density of 1.33 t m^{-3}, sand of 37.9%, silt of 40.6%, clay of 21.5%, pH of 8.15, organic C of 8.27 g kg^{-1}, total N of 1.05 g kg^{-1}, available phosphorus (Olsen-P) of 21.5 mg kg^{-1}, available potassium (NH_4OAc-K) of 147.8 mg kg^{-1}, and mineral N (NH_4^+–N + NO_3^-–N) of 21.6 mg kg^{-1} at the start of the experiment in April 2014.

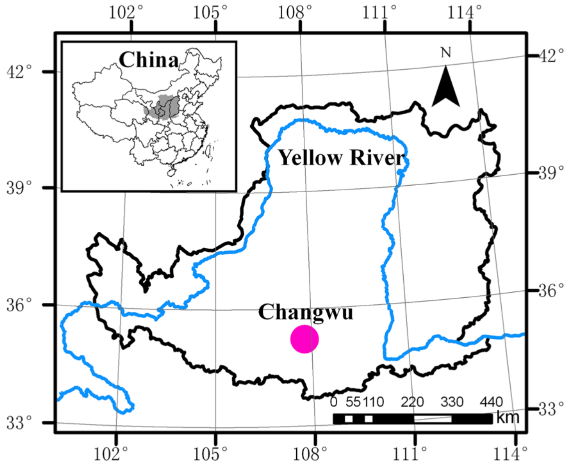

Fig. 1 The location of Changwu Agricultural and Ecological Experimental Station (35.28°N, 107.88°E, 1200 m altitude) on the Loess Plateau of China

Experimental design and field management

The eight treatments were arranged in a split-plot design with three replicates applied to 56 m^2 (8 m × 7 m) treatments. Four main treatments were N0F0, N0F1, N1F0, and N1F1 combined with two N fertilizer rates (N0, 0 kg N ha^{-1}; N1, 225 kg N ha^{-1}) and two mulching methods (F0, no mulching; F1, film mulching), as well as two maize cultivations (P0, unplanted; P1, maize-planted) as the sub-plot treatments. The chemical fertilizers were manually spread over the soil surface at rates of 75 kg N ha^{-1} (urea, 46% N), 40 kg P ha^{-1} (calcium super phosphate, 12% P_2O_5), and 80 kg K ha^{-1} (potassium sulfate, 45% K_2O) before plowing; then, the soil was plowed to mix the fertilizer into the subsoil. The rain-fed maize was sown (April 30, 2014 and April 26, 2015) to 50 mm deep at a density of 65,000 plants ha^{-1} and was harvested at the end of September. The distances between adjacent rows and hills were 0.5 m and 0.3 m, respectively. Top-dressed N fertilizer was added with 150 kg N ha^{-1} (urea, 46% N) during the maize jointing stage (July 5, 2014 and July 3, 2015) using the same handheld machine as that used for sowing. Chemical N fertilizer was used in the N fertilization treatments. Plastic film (1.2 m wide, 0.008 mm thick and transparent) was applied to cover the soil in the film mulching treatments. Plastic film was annually removed before spring plowing and was placed on the soil again as new film. Weeds were removed by hand during the MS. Irrigation and pesticides were not used in this experiment. Maize residues were cut and removed from the soil surface after harvest.

Soil sampling and measurements

Soil samples were collected from the 0–0.2 m layer on September 27, 2015 after harvesting. The soil sample for each plot was a mixture of five cores, which were randomly drilled ("S" distribution between two plants in the maize-planted plots) using a T sampler (40 mm in diameter). The samples were then stored in airtight polypropylene bags, placed in a cooler box at approximately 4 °C and transported immediately to the laboratory. After the removal of fresh litter material and visible roots, the composite samples were passed through a 2-mm sieve. The fresh sub-samples (Set 1) were immediately analyzed for soil moisture, mineral N, WSOC and WSON. The sub-samples used for phospholipid fatty acid (PLFA) analysis (Set 2) were refrigerated (− 80 °C) until the samples were freeze-dried with a lyophilizer, and the freeze-dried samples were kept in a desiccator before extraction. The remaining sub-samples (Set 3) were air-dried for the measurement of pH and electric conductivity (EC) and then ground to pass through a 0.15-mm sieve for the assessment of SOC, TN, total P (TP) and $KMnO_4$-C.

Soil temperature was measured as described by Bu et al. (2013) during the MS. The daily soil temperature was the mean of two readings at 7:00–7:30 h and 14:30–15:00 h, which were recorded manually using portable digital thermometers (JM624, Jinming Instrument Ltd., Tianjin, China) with the accuracy of 0.01 °C. The thermometer sensors were installed in the soil at the 0.1 m depth in each plot and were placed between the maize rows for the maize-planted treatments. The measurements of soil particle size, bulk density, mineral N, Olsen-P, and NH_4OAc-K were assessed as described by Yao et al. (2017). Soil moisture (gravimetric water content) was determined by the drying method after 24 h at 105 °C. Soil pH and EC were measured in a soil:water suspension (1:5) after 3 min of shaking at 25 °C. The pH was determined with a pH meter (PHS-3C, Shanghai Leici, China) with the accuracy of 0.01. The EC was determined with a conductivity meter (DDS-307, Shanghai Leici, China) with the accuracy of 1μS m^{-1}. The measurements of SOC ($K_2Cr_2O_7$–H_2SO_4 oxidation method), TN (Kjeldahl method), WSOC (soil/solution ratio of 1:2 w/v), WSON (soil/solution ratio of 1:2 w/v), and $KMnO_4$-C ($KMnO_4$ oxidation method) were determined as described by Luo et al. (2015a) and Luo et al. (2015b). The TP ($HClO_4$-H_2SO_4 digestion method) was measured as described by Luo et al. (2017). PLFA extraction was conducted as described by Bossio and Scow (1998). Briefly, lipids were extracted in a single-phase chloroform–methanol–citrate buffer system; phospholipids were separated from neutral lipids and glycolipids on solidphase extraction columns (Supelco, Inc., Bellefonte, PA, USA) (Zhang et al. 2014). After methylation of the polar lipids, the quantitative analysis of PLFA methyl esters was performed with a GC-FID (Agilent 7890B, Agilent Technologies, Santa Clara, CA). Nonadecanoic acid methyl ester (19:0, Sigma) was added as internal standard and used to convert fatty acid peak areas to absolute abundance. The MIDI Sherlock Microbial Identification System (Microbial ID Inc., Newark, NJ, USA) was used to identify fatty acids. Thirty-three individual PLFAs (C14–C20) consistently present in the samples were used to characterize the total microbial biomass. The response of used PLFAs (C14–C20) was 85% of total response in this study. The community indicators of Gram-positive bacteria (Gm+), Gram-negative bacteria (Gm−), saprotrophic fungi (SF), AMF, actinomycetes, and protozoa were completed as described by Luo et al. (2017). The sum of i14:0, a15:0, i15:0, i16:0, a17:0 and i17:0 was used to indicate Gm+, and the sum of 16:12OH, 16:1ω7c, 16:1ω9c, cy17:0, 17:1ω8c, 18:1ω7c and cy19:0 was used to indicate Gm−. The sum of 18:2ω6,9c and 18:1w9c was used to indicate SF, and 16:1w5c was used to indicate AMF. The sum of 10Me16:0, 10Me17:0 and 10Me18:0 was used to indicate actinomycetes, and 20:4ω6,9,12,15c was used to indicate protozoa. 'Bacteria' category includes Gm+ and Gm−, and 'Fungi' category includes AMF and SF. The indicated PLFAs accounted for 95% of total microbial biomass in this study.

Statistical analysis

The effect of the factors "film mulching", "N fertilization" and "maize cultivation", and their interactions on the obtained parameters were assessed using the mixed-design ANOVA. The factors "film mulching" and "N fertilization" were the within-subject factors and the factor "maize cultivation" was the between-subject factor. The least significant difference (LSD) test was further used when the F-values were significant to compare the means of different treatments with three biological repeats. In all cases, the differences were significant at $P < 0.05$ and analyses were performed using SPSS version 20.0. Detrended correspondence analysis (DCA) was used to check the PLFAs of the soil microbial community and to decide whether canonical correspondence analysis (CCA) or redundancy analysis (RDA) should be further applied to investigate the association between microbial communities and soil physico-chemical properties using the species-sample data-set (N = 24). For the soil microbial community, the DCA ordination gradient was less than 3 (i.e., 0.13), suggesting that the RDA associated with the linear model was more suitable for describing the association (Ter Braak and Prentice 1988). The 'rda' function in the package vegan v2.5-4 (Oksanen et al. 2019) of the R version 3.5.3 was used to perform the RDA. The proportion of explanatory variables was calculated using adjusted R-squared values (Peres-Neto et al. 2006). Permutation tests were conducted to check the significance of explained variances using the 'anova.cca' function.

Results

Soil physical properties

After two growing seasons, soil moisture was lower under N fertilization (N1F0 vs. N0F0), which decreased by 12.2% and 9.3% in the maize-planted (P1) and non-planted (P0) treatments, respectively (Fig. 2a). Moreover, soil moisture was higher under film mulching (N1F1 vs. N1F0), which increased by 11.1% and 13.0% in the P1 and P0 treatments, respectively (Fig. 2a). Across the 2 years' soil temperatures of maize growing season (MS) at the 0.1 m depth, film mulching (N1F1 vs. N1F0) significantly increased the soil mean temperature by 0.9 °C both in the P1 and P0 treatments (Fig. 2b). Across the P1 and P0 treatments, film mulching (N0F1 vs. N0F0, N1F1 vs. N1F0) significantly increased the soil mean temperature (Fig. 2b; $P < 0.001$, Online Resource 1). Additionally, maize cultivation (P1 vs. P0) significantly decreased the soil mean temperature of the MS at the 0.1 m depth (Fig. 2b; $P < 0.001$, Online Resource 1).

Soil chemical properties

After two growing seasons, film mulching (N0F1 vs. N0F0, N1F1 vs. N1F0) significantly decreased the SOC concentration in the maize-planted (P1) treatments, while N fertilization (N1F0 vs. N0F0, N1F1 vs. N0F1) significantly increased the SOC concentration in the non-planted (P0) treatments (Fig. 2c). In addition, maize cultivation (P1 vs. P0) significantly increased the SOC concentration under the N0F0 treatment (Fig. 2c). Across the P1 and P0 treatments, film mulching (N0F1 vs. N0F0, N1F1 vs. N1F0) significantly decreased the SOC concentration, whereas N fertilization (N1F1 vs. N0F1) significantly increased the SOC concentration (Fig. 2c; $P < 0.05$, Online Resource 1).

In the P1 treatments, compared with the N0F0 treatment (control), the N0F1, N1F0, and N1F1 treatments significantly decreased the soil pH by 0.20, 0.19, and 0.20, respectively (Fig. 2d). In the P0 treatments, compared with the control, the N1F1 treatment significantly decreased the soil pH by 0.13 (Fig. 2d). Across the P1 and P0 treatments, film mulching ($P < 0.001$, Online Resource 1) and N

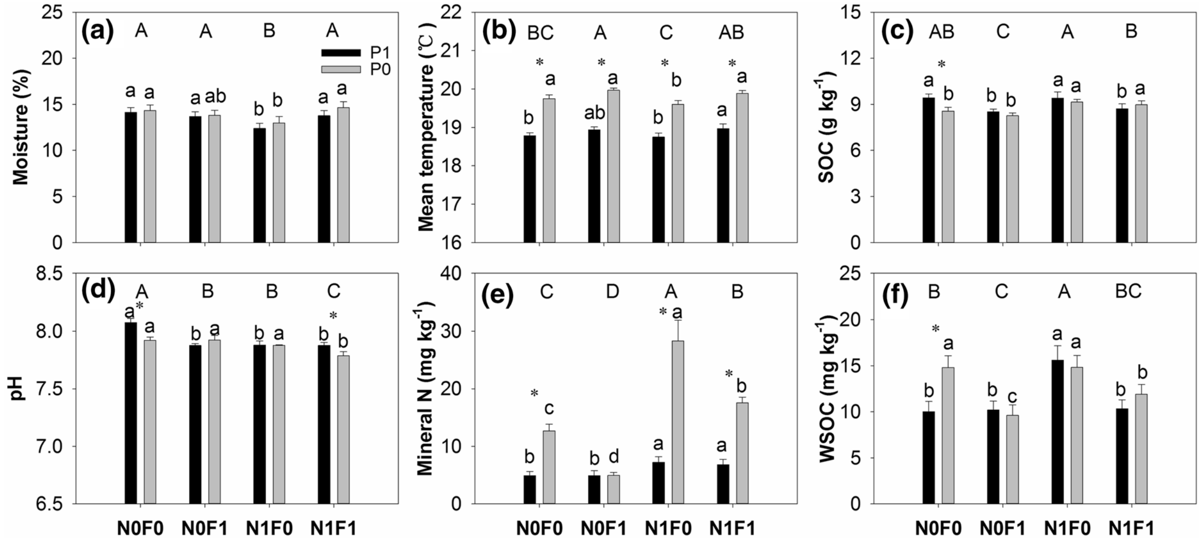

Fig. 2 Changes in soil moisture (**a**), mean temperature (**b**), organic C (**c**), pH (**d**), mineral N (**e**), and water soluble organic C (**f**) in the 0–0.2 m depth under different film mulching, N fertilization, and maize cultivation effects. Error bars are standard errors (n = 3). Different lowercase letters among the treatments within maize-planted (P1) or non-planted (P0) treatments indicate significant differences at $P < 0.05$. Different uppercase letters among the main treatments indicate significant differences at $P < 0.05$. N0, without N fertilization; N1, N fertilization with 225 kg N ha^{-1}; F0, no mulching; F1, film mulching. An asterisk (*) denotes significantly different means between the P1 and P0 treatments at $P < 0.05$. SOC, soil organic C; WSOC, water soluble organic C

fertilization ($P < 0.05$, Online Resource 1) had significantly negative effects on the soil pH (Fig. 2d).

After two growing seasons, film mulching and N fertilization had different effects on the mineral N concentration. N fertilization (N1F0 vs. N0F0, N1F1 vs. N0F1) significantly increased the mineral N concentration in the P0 and P1 treatments, while film mulching (N0F1 vs. N0F0, N1F1 vs. N1F0) significantly decreased the mineral N concentration in the P0 treatments (Fig. 2e). Moreover, maize cultivation (P1 vs. P0) significantly decreased the mineral N concentration under the N0F1, N1F0, and N1F1 treatments (Fig. 2e; $P < 0.001$, Online Resource 1). Across the P1 and P0 treatments, N fertilization significantly increased the mineral N concentration, whereas film mulching significantly decreased the mineral N concentration (Fig. 2e; $P < 0.001$, Online Resource 1). The shifts in soil NO_3^-–N concentration appeared to drive the changes of soil mineral N concentration, of which the NO_3^-–N concentrations followed the same pattern with the mineral N concentrations (Online Resource 2).

Labile soil organic matter fractions

Film mulching (N1F1 vs. N1F0) significantly decreased the WSOC concentration by 34%, while N fertilization (N1F0 vs. N0F0) significantly increased the WSOC concentration by 56% in the maize-planted (P1) treatments (Fig. 2f). In the non-planted (P0) treatments, film mulching (N0F1 vs. N0F0, N1F1 vs. N1F0) significantly decreased the WSOC concentration, while N fertilization (N1F1 vs. N0F1) significantly increased the WSOC concentration by 24% (Fig. 2f). Across the P1 and P0 treatments, film mulching (N0F1 vs. N0F0, N1F1 vs. N1F0; $P < 0.001$, Online Resource 1) significantly decreased the WSOC concentration, whereas N fertilization (N1F0 vs. N0F0; $P < 0.05$, Online Resource 1) significantly increased the WSOC concentration (Fig. 2f). After two growing seasons, N fertilization (N1F0 vs. N0F0, N1F1 vs. N0F1) significantly increased the WSON concentration, while film mulching had no significant effect on the WSON concentration in either P1 or P0 treatments (Online Resource 2). Across the P1 and P0 treatments, N fertilization (N1F0 vs. N0F0, N1F1 vs. N0F1) significantly increased the WSON concentration ($P < 0.001$, Online Resource 1).

In the P1 treatments, N fertilization (N1F0 vs. N0F0, N1F1 vs. N0F1) significantly increased the $KMnO_4$-C concentration; meanwhile, film mulching (N1F1 vs. N1F0) significantly increased the $KMnO_4$-C concentration (Online Resource 2). In the P0 treatments, compared with the control, the N0F1, N1F0, and N1F1 treatments significantly increased the $KMnO_4$-C concentration by 79%, 179%, and 76%, respectively (Online Resource 2). Across the P1 and P0 treatments, compared with the control, the N0F1, N1F0, and N1F1 treatments significantly increased the $KMnO_4$-C concentration by 45%, 95%, and 85%, respectively (Online Resource 2).

Soil microbial community composition

In maize-planted treatments (P1), film mulching (N0F1 vs. N0F0, N1F1 vs. N1F0) significantly decreased the total PLFAs (Fig. 3a). Similarly, changes in microbial biomass induced by film mulching were equally spread across different microbial communities, except for protozoa without N fertilization (Fig. 3). Additionally, film mulching significantly decreased the proportional abundance of SF, fungi, Gm−, and bacteria under N fertilization (Table 1). In non-planted treatments (P0), compared to the control (N0F0 treatment), film mulching and N fertilization significantly decreased the total PLFAs (Fig. 3a). Similarly, changes in microbial biomass induced by film mulching and N fertilization were equally spread across different microbial communities, except for protozoa (Fig. 3). Moreover, film mulching significantly decreased the proportional abundance of fungi without N fertilization (Table 1). Across the P1 and P0 treatments, film mulching (N0F1 vs. N0F0, N1F1 vs. N1F0) significantly decreased the absolute abundance of different microbial communities, except for protozoa (Fig. 3; $P < 0.001$, Online Resource 3). Furthermore, film mulching significantly decreased the proportional abundance of fungi without N fertilization and that of SF, fungi, and actinomycetes under N fertilization (Table 1).

Soil microbial community structure

After two growing seasons, compared with the N0F0 treatment (control), the N1F1 treatment (film mulching × fertilization) significantly decreased the F/B ratio in both the maize-planted (P1) and non-planted (P0) treatments (Fig. 3j). Across the P1 and P0 treatments, the N0F1, N1F0, and N1F1 treatments significantly decreased the F/B ratio compared with the control (Fig. 3j). In the P0 treatments, film mulching (N0F1 vs. N0F0) significantly increased the Gm+/Gm− ratio; however, N fertilization (N1F1 vs. N0F1) significantly decreased the Gm+/Gm− ratio in both the P1 and P0 treatments (Fig. 3l). Across the P1 and P0 treatments, compared with the control, the N0F1 treatment (film mulching) increased the Gm+/Gm− ratio, whereas the N1F0 and N1F1 treatments (N fertilization) decreased the Gm+/Gm− ratio (Fig. 3l).

Redundancy analysis

The redundancy analysis (RDA) of the PLFA data revealed that the first and second ordination axes explained 75.64% and 15.62% of the total variance, respectively (Fig. 4). Additionally, the soil microbial community had an extremely significant correlation ($P < 0.001$) with soil temperature and SOC/TP (C/P) ratio (Fig. 4). Meanwhile, the SOC, TN, EC, and $KMnO_4$-C had significant ($P < 0.01$) roles in shaping the composition of the soil microbial community (Fig. 4). Moreover, the WSOC also had a significant ($P < 0.05$) effect on shaping the soil microbial community (Fig. 4). The F/B ratio had the most positive and negative connections with high pH and $KMnO_4$-C, respectively (Fig. 4). Meanwhile, the AMF/SF ratio had the most positive and negative connections with high temperature and C/P ratio, respectively; however, the Gm+/Gm− ratio had the most negative and positive connections with high temperature and C/P ratio, respectively (Fig. 4).

Discussion

Film mulching, N fertilization, and maize cultivation affect soil physicochemical properties

In this study, film mulching significantly increased the above ground biomass by 24.9% and 11.9% without and with N fertilization, respectively; meanwhile, N fertilization significantly increased the above ground biomass by 91.9% and 71.8% without and with film mulching, respectively (Online Resource 4). The

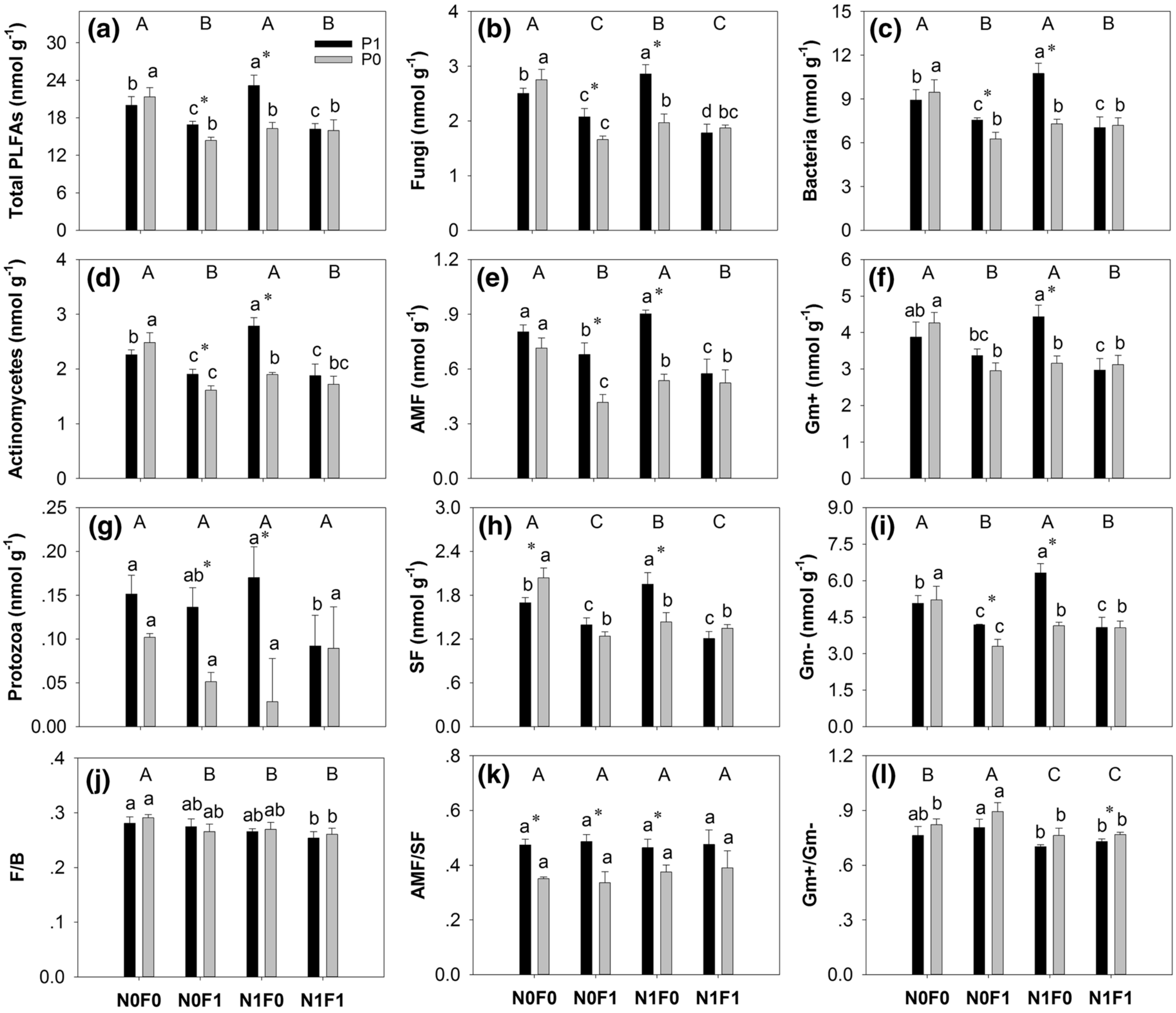

Fig. 3 Absolute abundance and structure of the soil microbial community under different film mulching, N fertilization, and maize cultivation treatments. Error bars are standard errors (n = 3). Different lowercase letters among the treatments within maize-planted (P1) or non-planted (P0) treatments indicate significant differences at $P < 0.05$. Different uppercase letters among the main treatments indicate significant differences at $P < 0.05$. N0, without N fertilization; N1, N fertilization with 225 kg N ha^{-1}; F0, no mulching; F1, film mulching. An asterisk (*) denotes significantly different means between the P1 and P0 treatments at $P < 0.05$. PLFA, phospholipid fatty acids; AMF, arbuscular mycorrhizal fungi; SF, saprotrophic fungi; Gm+, Gram-positive bacteria; Gm−, Gram-negative bacteria; F/B, fungi/bacteria. 'Bacteria' category includes Gm+ and Gm−, and 'Fungi' category includes AMF and SF

aboveground biomass changes resulted from the changes of soil physicochemical properties under film mulching and N fertilization, which in turn further affected soil physicochemical and microbial properties. In this study, N fertilization (N1F0 vs. N0F0) significantly decreased the soil moisture probably by the increases of plant utilization and transpiration resulted from increasing aboveground biomass and by other uncertain reasons, while soil moisture was higher under film mulching due to a reduction on water evaporation from the soil (Fig. 2a). Additionally, maize cultivation (P1 vs. P0) significantly decreased the soil mean temperature of the MS probably by increasing plant coverage to reduce solar radiation warming (Fig. 2b; $P < 0.001$, Online Resource 1). To a certain extent, film mulching mainly increased soil temperature, N fertilization mainly decreased soil moisture, and maize cultivation mainly

Table 1 The relative abundances of microbial community under film mulching, N fertilization, and maize cultivation effects in the 0–0.2 m depth

Treatments		AMF (%)	SF (%)	Fungi (%)	Gm+ (%)	Gm− (%)	Bacteria (%)	Actinomycetes (%)	Protozoa (%)
Maize-planted	N0F0	4.03 (0.20)a	8.51 (0.29)a	12.55 (0.43)a	19.35 (0.74)ab	25.40 (0.87)b	44.74 (0.67)ab	11.35 (0.42)a	0.76 (0.07)a
	N0F1	4.01 (0.26)a	8.25 (0.32)a	12.26 (0.52)a	19.92 (0.49)a	24.73 (0.80)b	44.65 (0.42)ab	11.26 (0.35)a	0.80 (0.11)a
	N1F0	3.91 (0.24)a	8.44 (0.14)a	12.35 (0.26)a	19.17 (0.09)ab	27.33 (0.41)a	46.50 (0.41)a	12.04 (0.23)a	0.74 (0.16)a
	N1F1	3.55 (0.38)a	7.46 (0.18)b	11.00 (0.42)b	18.33 (1.29)b	25.14 (0.98)b	43.47 (2.26)b	11.58 (0.67)a	0.56 (0.19)a
Unplanted	N0F0	3.35 (0.07)a	9.54 (0.06)a	12.89 (0.13)a	19.98 (0.15)a	24.35 (1.03)ab	44.32 (1.08)a	11.63 (0.21)a	0.48 (0.03)a
	N0F1	2.90 (0.19)a	8.65 (0.53)a	11.54 (0.41)b	20.52 (0.98)a	22.98 (1.10)b	43.50 (1.73)a	11.22 (0.59)a	0.36 (0.07)a
	N1F0	3.30 (0.18)a	8.78 (0.30)a	12.08 (0.35)ab	19.39 (0.26)a	25.47 (1.05)a	44.85 (0.88)a	11.68 (0.54)a	0.17 (0.29)a
	N1F1	3.29 (0.36)a	8.51 (0.90)a	11.79 (0.92)b	19.64 (0.55)a	25.58 (0.47)a	45.23 (0.80)a	10.82 (0.35)a	0.54 (0.23)a
N0F0		3.69 (0.07)A	9.03 (0.13)A	12.72 (0.16)A	19.66 (0.43)AB	24.87 (0.68)AB	44.53 (0.82)A	11.49 (0.11)AB	0.62 (0.02)A
N0F1		3.45 (0.22)A	8.45 (0.29)AB	11.90 (0.28)BC	20.22 (0.55)A	23.86 (0.16)B	44.08 (0.66)A	11.24 (0.44)B	0.58 (0.08)A
N1F0		3.60 (0.03)A	8.61 (0.22)A	12.21 (0.22)AB	19.28 (0.14)AB	26.40 (0.70)A	45.67 (0.57)A	11.86 (0.20)A	0.45 (0.20)A
N1F1		3.42 (0.29)A	7.98 (0.49)B	11.40 (0.65)C	18.99 (0.92)B	25.36 (0.56)AB	44.35 (1.47)A	11.20 (0.29)B	0.55 (0.08)A

Notes: Values in the parentheses indicate standard errors. Different letters among treatments indicate significant differences at $P < 0.05$. *PLFA* phospholipid fatty acids, *AMF* arbuscular mycorrhizal fungi, *SF* saprotrophic fungi, *Gm+* gram-positive bacteria, *Gm−* gram-negative bacteria. 'Bacteria' category includes Gm+ and Gm−, and 'Fungi' category includes AMF and SF

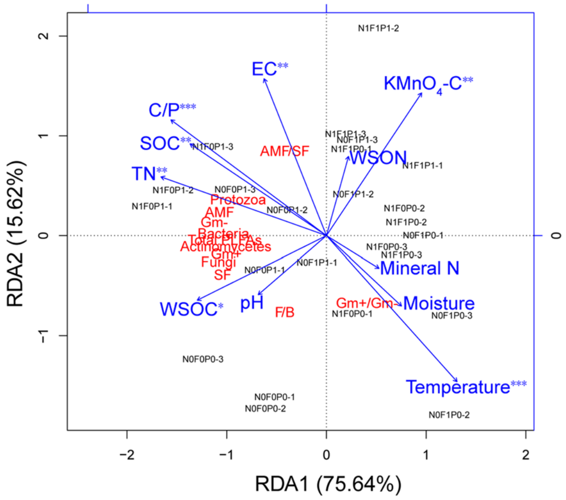

Fig. 4 Redundancy analysis (RDA) of the PLFA data explained by environmental variables and treatments. The explanatory variables followed by asterisks indicate significant influences on the PLFA data (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). AMF, arbuscular mycorrhizal fungi; SF, saprotrophic fungi; Gm+, Gram-positive bacteria; Gm−, Gram-negative bacteria; F/B, fungi/bacteria. SOC, soil organic C; TN, total N; C/P, soil organic C/total P; WSOC, water soluble organic C; WSON, water soluble organic N; KMnO$_4$-C, KMnO$_4$-oxidizable C; EC, electric conductivity. N0, without N fertilization; N1, N fertilization with 225 kg N ha^{-1}; F0, no mulching; F1, film mulching; P0, unplanted; P1, maize-planted. Numbers '1, 2, 3' represent three biological repeats

decreased soil temperature. Thus, film mulching, N fertilization and maize cultivation indeed changed the soil microclimate (temperature and moisture), which affected the SOM mineralization (i.e., decreases in SOC, WSOC, WSON, and KMnO$_4$-C) and nutrient cycle (i.e., changes of TN, mineral N, and WSON). Furthermore, we observed significantly deceased SOC and WSOC concentrations in the film mulching treatments (Fig. 2c, f). Previous studies have shown that short-term (1–3 years) plastic film mulching induced significant SOC losses (Li et al. 2004, 2007; Moreno and Moreno 2008; Zhang et al. 2015) probably due to enhanced mineralization rate with high temperature underside the film mulching. In this study, the highest soil temperatures under the plastic film during the MS even occasionally exceeded 50 °C; therefore, a solarization effect could be expected, which might lead to abundant organic C loss (even up to 85%) within 1 month (Simmons et al. 2013). In contrast, Liu et al. (2014c) found that 5-year plastic film mulching accelerated SOC mineralization but did not significantly affect the SOC concentration compared with no mulch. Moreover, our previous study indicated that the SOC stock decreased in the 0–0.2 m soil depth, whereas it increased in the 0.2–0.4 m soil depth after 4-year plastic film mulching (Luo et al. 2015b). A recent and important study by Wang et al. (2017) revealed that the 6-year plastic film mulching maintained the SOC concentration by balancing the enhanced SOC mineralization and improved root-derived C input. These findings supported "the decline in SOC is just temporary and would be compensated by root-derived C input after long-term (4–6 years) plastic film mulching", which is a hypothesis raised by Gan et al. (2013). Accordingly, short-term (1–3 years) plastic film mulching led to SOC losses in the top soil; however, SOC would be compensated for long-term (4–6 years) plastic film mulching compared with no mulch. Additionally, we observed significantly increased SOC and WSOC concentrations in the N fertilization treatments (Fig. 2c). In support of our findings, previous studies have shown that N fertilization decreased SOM decomposition (Zang et al. 2016; Li et al. 2017), especially at high nutrient addition (Liu et al. 2018). Considering that microbes intend to compete with plant roots for mineral N in soils (Dunn et al. 2006). When soil active N is depleted, microorganisms enhance N mineralization to release mineral N (Babujia et al. 2010). On the contrary, when the competition on N between plant roots and microbes turns to be weak, microorganisms reduce the demand of SOM mineralization to release mineral N. Meanwhile, root-derived C input is an important source of SOC and WSOC. Therefore, the increasing root biomass (resulted from N fertilization) induced the increases in SOC and WSOC concentrations.

We also observed a generally decreased TN concentration in the film mulching treatments of maize-planted treatments (Online Resource 2; $P < 0.05$, Online Resource 1). Regardless of cropping types or regional conditions, the higher productivity under plastic film mulching has often led to a lower N concentration compared with no mulch (Li et al. 2007; Domagała-Świątkiewicz and Siwek 2013). Furthermore, short-term plastic film mulching can enhance the risk of N mineralization, which is supported by Hai et al. (2015) and Zhang et al. (2012). However, these findings are different from Farmer et al. (2017), who found that film mulching had no significant effect on the TN concentration after 28 years of cultivation. Additionally, our previous study also showed that 5-year plastic film mulching had no significant effect on the TN concentration (Luo et al. 2015a). Accordingly, we may continue this investigation in the future. The present study also showed that N fertilization increased the mineral N concentration ($P < 0.001$, Online Resource 1), film mulching decreased the mineral N concentration ($P < 0.01$, Online Resource 1), and maize cultivation decreased the mineral N concentration ($P < 0.001$, Online Resource 1). These findings support earlier work. In addition, N fertilization significantly increased the WSON concentration, which is analogously reflected by a 28-year study in which N fertilization significantly increased the TN concentration (Farmer et al. 2017). Meanwhile, the more mineral N residual under N fertilization may retard the WSON mineralization and decomposition.

In the present study, the C/P ratios of all the treatments were less than 200 (Online Resource 2), which implies net mineralization (Paul 2006). Thus, film mulching significantly decreased the C/P ratio in the non-planted treatments, implying further net nutrient mineralization. The C/P ratio can be enhanced by N fertilization and maize cultivation, which are induced by root-derived C input. In this study, across the maize-planted and non-planted treatments, both film mulching and N fertilization significantly decreased the pH (Fig. 2d). N fertilization resulted in a significant drop of soil pH by 0.13 pH units in the present study, while the same effect has attracted extensive attention (Guo et al. 2010). We also need to pay attention to the pH decline induced by film mulching, which has been described by Wang et al. (2017). Moreover, film mulching generally increased the $KMnO_4$-C concentration (Online Resource 2). Similar results were reported by Zhou et al. (2012), Liu et al. (2013), and Luo et al. (2015b), who found that 2–4 years of plastic mulching increased the $KMnO_4$-C concentration up to twofold. Meanwhile, as an important source of $KMnO_4$-C, the increasing root biomass (resulted from film mulching and N fertilization) induced the $KMnO_4$-C concentration increase.

Film mulching, N fertilization, and maize cultivation affect soil microbial communities

Combined with shifts in soil physical properties and nutrient availability, plastic film mulching can cause alterations in the soil microbial community (Maul et al. 2014). Do microbial biomass changes spread equally across different microbial communities? In the present study, film mulching significantly decreased the total PLFAs and absolute abundance of most microbial communities (Fig. 3). Together with the higher soil temperatures induced by film mulching, the soil solarization effect occurs, which generally decrease fungal and bacterial richness by favoring anaerobic, detritivorous and thermophilic species and is non-selective to a certain extent (Bonanomi et al. 2008; Simmons et al. 2014). In support of our results, Wu et al. (2016) found that film mulching led to a significant decrease in the bacterial community compared with non-mulched paddy soil. Farmer et al. (2017) also found that film mulching significantly shaped the bacterial community structure regardless of fertilization after 28 years of cultivation. In contrast, Liu et al. (2012) and Chen et al. (2014) revealed that film mulching led to slight increases in AMF abundance and bacterial diversity compared to non-mulched soil. The altered soil environment (i.e., temperature, moisture, and nutrient status) may induce some microbial communities to become dominant while constraining other microorganisms, thereby causing shifts in the community composition and structure. Therefore, different microbial communities respond in different ways to climate characteristics, soil properties, and crop species.

In this study, N fertilization generally increased the most microbial communities in non-mulched soil but significantly decreased all the microbial communities in film-mulched soil under maize-planted treatments. Additionally, either N fertilization or film mulching generally decreased the most microbial communities with non-planted treatments (Fig. 3). Zhao et al. (2014) found that higher rates of N fertilization increased the soil fungal abundance and F/B ratio compared with lower N rates in non-mulched soil under maize-planted treatments. In addition, Farmer et al. (2017) found that long-term N application significantly decreased the soil bacterial diversity and richness compared to non-fertilized soil under maize-planted treatments. These results indicated that film mulching generally decreased the microbial communities in short-term cultivation, while the impact of N fertilization on soil microbial communities was quite complicated and requires further investigation.

Furthermore, film mulching and N fertilization change the soil microbial community structure, which may alter soil beneficial microorganisms, thereby affecting soil nutrient cycles. Generally, the F/B ratio decreased with the increase in nutrient availability (Rinnan et al. 2007), and a higher F/B ratio is an indicator of the dominant nutrient supplies for plant growth from organic matter decomposition and N mineralization (de Vries et al. 2007). In this study, film mulching and N fertilization significantly decreased the F/B ratio after the 2-year cultivation (Fig. 3j), which indicated that film mulching and N fertilization had the potential in retarding SOM decomposition and N mineralization. The general view is that Gm− bacteria prefer fresh plant material, whereas Gm+ bacteria prefer old organic matter (Fierer et al. 2003). Thus, film mulching significantly enhanced the Gm+/Gm− ratio without N fertilization in the non-planted treatments (Fig. 3l), which indicated that film mulching could reduce younger organic matter decomposition. In this study, film mulching caused the SOC decline after the 2-year cultivation and decreased the WSOC concentration, which is an important C source of microbial nutrients. The reduced WSOC would restrict the total microbial biomass, which favors the decline in SOM decomposition. The decreased microbial communities may be related to C metabolic processes. In summary, film mulching has the potential to enhance SOM preservation for a longer time. Accordingly, we may investigate film mulching further in the future.

Conclusions

Two-year film mulching and N fertilization altered the soil physicochemical properties, thereby changing the soil microbial community abundance and structure. Collectively, short-term film mulching increases SOM decomposition in the surface soil, accompanied by decreases in the total soil microbial biomass and most microbial communities. In turn, the changes in the soil microbial community induced by film mulching may affect the soil nutrient cycles. Soil microbial biomass and the F/B ratio declines would retard SOM decomposition and N mineralization, which require clear quantitative data as evidence in future studies. The decrease in soil microbial biomass was mainly linked to an increase in soil temperature, whereas the decrease in the F/B ratio was mostly associated to a decrease in soil pH induced by film mulching. Thus, combining our previous results, film mulching does not lead to a negative impact on soil quality in semiarid regions.

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