Long-term ground cover affects soil bacterial community and carbon metabolism in the Loess Plateau, China

wenting Zhang[1], Yi Wang[2], Chunyue Li[1], Shun Chang[1], Yinglong Xue[1], Tinhui Dang[3], and Xiaomin Zeng[1]

[1]School of Geography and Tourism, Shaanxi Normal University, Xi'an, 710119, China
[2]Bio-Agriculture Institute of Shaanxi, State Key Laboratory of Loess and Quaternary Geology, Institute of Earth Environment, CAS, Xi'an, 710061, China
[3]Institute of Soil and Water Conservation, Northwest A&F University, Yangling, 712100, China

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Abstract

Farmland mulching can maintain soil temperature and moisture, increase crop yields, which plays a positive role in agricultural planting in the arid area. However, the composition of the soil microbial community and the carbon cycle involved is not well understood under the ground cover scenario. Based on the Changwu Agro-Ecological Experiment Station, this study set up a long-term positioning Experiment to explore the impact of long-term land cover pattern on soil bacterial community structure and carbon metabolism capacity. In this study, we include five treatments: control uncovered (CK), plastic film mulching (F), high amount of straw mulching is carried out in July, August and September every year (St90), low amount of straw mulch throughout the growth period (S45), high amount of straw cover throughout the growth period (S90). We combined Illumina MiSeq sequencing and Biolog-ECO technology to analyze the functional characterization of soil bacterial community composition in terms of carbon source metabolism. The results indicated that the soil bacteria showed high degree of epistatic clustering after mulching treatments, and the operational taxonomic units (OTU) numbers of soil bacteria under different treatments decrease as St90, S45, F, CK, S90. Redundancy analysis (RDA) showed that AP, NH4+-N, MC were the main environmental factors affecting bacterial community composition. Proteobacteria, Acidobacteria and Actinobacteria are the main dominant flora in this area. Long-term surface cover resulted in significant differences in soil bacterial carbon metabolism, which was highest in the F treatment. Structural equation modeling (SEM) shows that surface cover has direct and indirect effects on bacterial carbon metabolism capacity and diversity through soil physicochemical properties. Reasonable mulching patterns can greatly change the community structure and carbon metabolism of soil bacteria, and play a positive role in the sustainable development of agro-ecosystems in arid area.

SUPPLEMENTARY MATERIALS

TABLE 1 Loading values of 31 carbon sources on PC1 and PC2 in different mulching treatments ($|r| > 0.6$).

Carbon source chemical category	Substrate	PC1	PC2
Miscellaneous	Pyruvic acid methyl ester	0.896	0.348
	lucose-1-phosphate	0.162	0.617
	D, L-α-Glycerol phosphate	0.969	0.039
Polymers	Tween 80	0.958	0.232
	Tween 40	0.974	0.137

Carbon source chemical category	Substrate	PC1	PC2
	α-Cyclodextrin	0.723	0.572
	Glycogen	-0.743	0.486
Carbohydrates	D-Cellobiose	0.839	-0.529
	α-D-Lactose	0.676	0.482
	Methyl-D-glucoside	0.811	-0.458
	D-Xylose	0.665	-0.474
	i-Erythritol	0.816	0.334
	D-Mannitol	0.650	-0.569
	N-Acetyl-D-glucosamine	0.736	-0.518
Carboxylic acids	D-Glucosaminic acid	0.725	-0.619
	4-Hydroxy benzoic acid	0.868	0.396
	D-Galactonic acid latone	0.976	-0.153
	D-Galacturonic acid	0.646	-0.673
	2-Hydroxy benzoic acid	-0.396	-0.697
	γ-Hydroxy butyric acid	0.721	-0.388
	Itaconic acid	0.294	-0.368
	α-Keto butyric acid	-0.934	-0.178
	D-Malic acid	0.521	0.842
Amino acids	L-Arginine	0.839	-0.436
	L-Asparagine	0.990	-0.096
	L-Phenylalanine	0.805	-0.536
	L-Serine	0.991	-0.035
	L-Threonine	0.529	0.769
	Glycyl-L-glutamic acid	0.611	0.540
Amines/amides	Phenylethylamine	0.445	0.869
	Putrescine	0.972	0.130

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[1] School of Geography and Tourism, Shaanxi Normal University, Xi'an, 710119, China

[2] Bio-Agriculture Institute of Shaanxi, Xi'an, 710043, China

[3] State Key Laboratory of Loess and Quaternary Geology, Institute of Earth Environment, CAS, Xi'an, 710061, China

[4] Institute of Soil and Water Conservation, Northwest A&F University, Yangling, 712100, China

Correspondence: Chunyue Li, School of Geography and Tourism, Shaanxi Normal University, Xi'an 710119, Shaanxi, China

Email: chunyue_li@snnu.edu.cn

Abstract: Farmland mulching can maintain soil temperature and moisture, increase crop yields, which plays a positive role in agricultural planting in the arid area. However, the composition of the soil microbial community and the carbon cycle involved is not well understood under the ground cover scenario. Based on the Changwu Agro-Ecological Experiment Station, this study set up a long-term positioning Experiment to explore the impact of long-term land cover pattern on soil bacterial community structure and carbon metabolism capacity. In this study, we include five treatments: control uncovered (CK), plastic film mulching (F), high amount of straw mulching is carried out in July, August and September every year (St_{90}), low amount of straw mulch throughout the growth period (S_{45}), high amount of straw cover throughout the growth period (S_{90}). We combined Illumina MiSeq sequencing and Biolog-ECO technology to analyze the functional characterization of soil bacterial community composition in terms of carbon source metabolism. The results indicated that the soil bacteria showed high degree of epistatic clustering after mulching treatments, and the operational taxonomic units (OTU) numbers of soil bacteria under different treatments decrease as St_{90}, S_{45}, F, CK, S_{90}. Redundancy analysis (RDA) showed that AP, NH_4^+-N, MC were the main environmental factors affecting bacterial community composition. *Proteobacteria*, *Acidobacteria* and *Actinobacteria* are the main dominant flora in this area. Long-term surface cover resulted in significant differences in soil bacterial carbon metabolism, which was highest in the F treatment. Structural equation modeling (SEM) shows that surface cover has direct and indirect effects on bacterial carbon metabolism capacity and diversity through soil physicochemical properties. Reasonable mulching patterns can greatly change the community structure and carbon metabolism of soil bacteria, and play a positive role in the sustainable development of agro-ecosystems in arid area.

Abbreviations:

AP, soil available phosphorus; AWCD, average well color development; CK, control uncovered; F, plastic film mulch; MC, soil moisture content; MFC, soil maximum water holding capacity in the field; NH_4^+-N, Soil ammonium nitrogen; NO_3^--N, nitrate nitrogen; OTU, operational taxonomic units; RDA, redundancy analysis; St_{90}, high amount of straw mulching is carried out in July, August and September every year; S_{45}, low amount of straw mulch throughout the growth period; S_{90}, high amount of straw cover throughout the growth period; SOC, soil organic carbon; SEM, structural equation modeling; TN, total nitrogen; TP, total phosphorus; TC, soil total carbon.

Core Ideas

Straw mulching significantly increased soil organic carbon content.

The AP, NH_4^+-N, MC were the main environmental factors affecting bacterial community.

The carbon source metabolism of bacteria was the strongest under plastic film mulch.

1 INTRODUCTION

Soil microorganisms are an important component of terrestrial ecosystems and are the most active part of the soil, bacterial community diversity can reflect soil quality to a certain extent (Niemi et al. 2001). Bacteria are the most abundant and widely distributed major taxa in the soil, accounting for 70 ~ 80% of the total soil microorganisms (Bardgett et al. 2008). It was showed that bacteria participate in almost all biochemical cycles in terrestrial ecosystems (Han et al. 2014), and play an important role in regulating soil nutrient and water cycling and influencing ecosystem stability (Chen et al. 2015).

The Loess Plateau is a typical rain-fed agricultural area, and character for water scarcity, poor soil fertility, severe soil erosion and desertification (Wang & Shao 2013). Appropriate farmland conservation management not only improve crop yields, but also enhance soil quality conditions and soil fertility, which are important for sustainable development of agriculture (Hou et al., 2012; Sun et al., 2020; Wang et al., 2012). Ground mulching, include straw, film and gravel mulching is one of the important farming protection managements, can improve soil nutrient content and soil water utilization (Yin et al. 2018, Zheng et al. 2019, Ma et al. 2021).

Many researches on the change of soil microbial community under different ground mulching patterns have been done in recent years. Straw mulching could significantly improve the soil bacterial community structure and optimize soil quality in Weibei rainfed apple orchards Chen et al. (2015). The diversity indices of soil bacteria under gravel mulch in the dry farming area were all higher than those in the unmulched treatment, and the bacterial community structure was similar to the functional diversity of carbon source metabolism (Hao et al.,

2017). Mulching with manure application can significantly increase the diversity of soil dominant flora and play an important role in shaping the bacterial community structure (Farmer et al., 2016). But, continuous use of biodegradable plastic film had no effect on soil bacterial community structure in a Japanese farmland soil (Masui et al., 2011). Although many studies have shown that mulching have positive effects on improving soil microbial community structure characteristics, there are still different results for different soil types, different fertilizer application methods, and different crop types. Therefore, it is necessary to investigate the soil microbial community structure in different areas and different tillage patterns to obtain more comprehensive research results.

The functional characteristics of carbon source metabolism and the rate of carbon mineralization are closely related to the diversity of soil microorganisms (Garland J L & Millsal, 1991; Juarez et al., 2013). Long-term straw mulching significantly increases the content of organic carbon and microbial residual carbon in topsoil (Liang et al., 2017; Sokol et al., 2019). Ground mulching intensifies plant-soil interactions, accelerates the mineralization of organic matter in the soil, and increases soil microbial activity (Li et al., 2012). Straw decomposition inputs large amounts of exogenous carbon to the soil and changes the activity of bacterial taxa, resulting in an increase in their ability to utilize carbon sources (Yu et al., 2020). Proper farming practices can enhance the carbon sequestration capacity of soils and increase the abundance of soil microorganisms (Six et al., 2006).

The research on the diversity of soil microbial community structure and the characteristics of carbon metabolism function under different mulching methods is still in its infancy, especially the comparison of plastic ground mulching with different straw mulching amount and mulching time. Therefore, this study intends to use the Illumina Miseq platform high-throughput sequencing technology and the Biolog microplate method, using the Changwu Agro-ecological Experimental Station in Shaanxi province of China as the research platform, and analyzing the effects of 10-year-long different surface coverage methods on soil microbial diversity, community structure and carbon metabolism. We hypothesis that (i) straw mulching facilitate soil organic carbon accumulation and enhance carbon metabolism capacity; (ii) soil bacterial community structure and carbon metabolism capacity of dry farmland under long-term ground cover were mainly affected by soil physicochemical properties. The results of this research are expected to provide a theoretical basis for choosing a suitable mulching method for the farmland soil in the Loess Plateau.

2 MATERIALS AND METHODS

2.1 Experimental site

The experiments were conducted in an agricultural area in Changwu (35°14 N, 107°41 E) which is a national field scientific observation and research station in farmland system (Figure 1). It is a typical semi-arid area located to the west of the Loess Plateau in China at an altitude of approximately 1200 m.

The average annual precipitation is 580 mm, the frost-free period is 171 days, and the average annual temperature is 9.1℃. The tested soil was Heilu soil (Cumulic Haplustoll, USDA classification) with appropriate permeability. The experimental plots were managed according to the field high yield model. The background values of the soil before the test are as follows: Organic matter 10.50 g kg^{-1}, total nitrogen (TN) 0.80 g kg^{-1}, total phosphorus (TP) 1.26 g kg^{-1}, pH=8.10.

2.2 Experimental design

This long-term location coverage experiment started in 2008. The experiment had five treatments: uncovered control (CK), plastic film mulching (F), high amount of straw mulching is carried out in July, August and September every year (St_{90}), low amount of straw mulch throughout the growth period (S_{45}), high amount of straw cover throughout the growth period (S_{90}). And Changwu's reproductive period is: sowing stage (early September to middle September), seedling stage (7 ~ 8 days after sowing), overwintering stage (mid-November), turning green stage (early March to middle March), tillering stage (early April), jointing stage (early April to middle April), heading stage (early May), filling stage (late May), harvesting stage (mid-June). Each plot was 4 m long and 6 m wide. The test treatments are the same for each plot in each year, and the planting mode is continuous wheat cropping and conventional farming. Urea (N) and calcium superphosphate (P_2O_5) were applied as wheat base fertilizers, fertilization rates were 90 kg $ha^{-1}$and 135 kg ha^{-1}, respectively. Cover the whole plot with 1.2 m white transparent plastic mulch. The straw mulch is 100% covered by crushed wheat straw. The experimental field with high straw mulching treatment is 90 kg hm^{-2}, and the low straw mulching treatment is 45 kg hm^{-2}.

2.3 Soil sampling

The samples were collected in late March 2019. Samples were collected by the five-point sampling method, taking the cultivated layer of soil (0-20 cm). Fresh soil samples of about 1 kg were collected, gravel and impurities larger than 1 cm were removed and passed through a 2 mm sieve. A portion of each soil sample was collected in 100 ml centrifuge tubes, transported from the field to a laboratory refrigerator, and then stored at -80°C for soil DNA extraction. The remaining soil samples were air dried and used for the determination of soil physical and chemical properties.

2.4 Soil physicochemical measurements

Soil total carbon (TC) was determined by carbon analyzer (Vario TOC, Elementar, Hanau, Germany); soil total nitrogen (TN) was determined by automatic Kjeldahl nitrogen analyzer (Wang et al. 2021); soil total phosphorus (TP) was determined by concentrated sulfuric acid-perchloric acid digestion with molybdenum antimony anti-colorimetric method. Soil ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N): 0.5 M K_2SO_4 extraction followed by continuous flow analyzer (Autoanalyzer 3, Bran Luebbe, Germany). The soil organic carbon (SOC) content was assayed using the potassium dichromate oxidation

method. Soil available phosphorus (AP) was determined by Olsen method (Bao 2005). Soil moisture content (MC): determined by the drying method at 105°C; soil maximum water holding capacity in the field (MFC): determined by the ring knife method. Soil pH: measured with a water-soil ratio of 2.5:1.

2.5 DNA extraction, PCR amplification and high-throughput sequencing

The total DNA was extracted from each replicate sample using a Soil DNA Kit (Omega, USA) according to the manufacturer's instructions. The concentration was assessed using a NanoDrop ND-1000 Spectrophotometer. After the concentration was completed, the total DNA was stored in a refrigerator at -20℃.

For all of the DNA samples, we amplified the V3-V4 regions of the bacterial 16S rDNA gene with primer pair 338F (5'-ACT CCT ACG GGA GG CAG CA-3') and 806R (5'-GGA CTA CHV GGG TWT CTA AT-3') (Wang & Qian 2009). Thermal cycling comprised initial denaturation at 98°C for 1 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 30 s, with a final extension at 72°C for 5 min. The purified products were sequenced on Illumina Miseq platform (Shanghai Personal Biotechnology Co. Ltd., Shanghai, China).

The Illumina Miseq sequencer was used for 2×300 bp paired-end sequencing. First, use the Flash software (v1.2.7) to splice the sequences that have passed the preliminary quality screening, and discard the sequences that cannot be connected. According to the test requirements, first remove the bases with an average quality lower than Q20 in the window, and cut the sequence from the first window with an average quality lower than Q20. The length of the truncated sequence is not less than 150 bp, and it is required that there should be no ambiguous bases (Ambiguous base). Secondly, the paired-end sequences that have passed the preliminary quality screening are paired and connected according to overlapping bases: the overlapping base length of the two sequences of Read 1 and Read 2 is required to be 10 bp, and base mismatches are not allowed. Finally, according to the Index information corresponding to each sample (i.e. the Barcode sequence, which is a short base sequence used to identify the sample at the beginning of the sequence), the connected sequence identification is assigned to the corresponding sample (the index sequence is required to match exactly), so as to obtain the effective sequence of each sample. The Quantitative Insights Into Microbial Ecology pipeline was used to define operational taxonomic units (OTUs) by combining reads of the clustered OTUs with 97% similarity (Edgar 2013). Subsequently, according to the number of sequences contained in each OTU in each sample, a matrix file (OTU table) of the abundance of OTU in each sample is constructed.

2.6 Soil microbial catabolic diversity

Functional diversity of the bacterial community was determined by the Biolog-ECO method (Garland J L and Millsal, 1991). For the Biolog-ECO assay, the enzymes generated by the metabolism of soil microorganisms and 31 kinds of

carbon sources react with tetrazole purple dye to reflect the utilization capacity of carbon sources by the depth of color. The carbon sources were classified according to the nature of their chemical groups as amino acids (6 kinds), sugars (7 kinds), carboxylic acids (9 kinds), amphiphiles (3 kinds), polymers (4 kinds) and amines/amino compounds (2 kinds) (Choi and Dobbs 1998). In this study, we chose to weigh 10 g of fresh soil sample in 100 ml of 0.85% sterilized saline, shake at 160 r min^{-1}, 28°C for 30 min, leave for 15 min, and take the supernatant to dilute to 10^{-3} g ml^{-1} of soil bacterial suspension. The prepared bacterial suspension was poured into a sterile V-shaped spiking tank, 150 ml of sterile saline was added to the three control wells, and the suspension was inoculated into the other 93 microwells of the ecological plate with an 8-channel spiker, 150 ml was added to each well, covered and wrapped with tinfoil and placed in a constant temperature incubator at 28℃, and measured every 24 h using Microplate Reader. The absorbance values at 590 nm were measured continuously for 10 d to ensure that all samples reached the saturation stage of carbon utilization.

The metabolic activity of microbial carbon sources was expressed as Average well color development (AWCD) per well of the microplate to judge the ability of the microbial community in the soil to utilize carbon sources. The AWCD was expressed as the absorbance value at 590 nm. Simpson index (D), Shannon index (H) and McIntosh index (U) were used to characterize the functional diversity of soil microorganisms. The specific calculation is as follows (Dang et al. 2015):

$$\mathrm{AWCD} = \frac{\sum(Ci-R)}{31} \mathrm{AWCD} = \frac{\sum(Ci-R)}{31} \quad (1)$$

C_i is the absorbance at 590 nm of the sample bacterial suspension in well i, R is the absorbance of the control well, and n is the number of carbon substrates included in the measurement, which is 31 in this study. If $Ci\text{-}R<0$, the hole is counted as zero in the calculation.

Shannon-Wiener index: $\mathrm{H} = -\sum P_i \cdot \mathrm{In}\ (P_i)\ \mathrm{H} = -\sum P_i \cdot \mathrm{In}\ (P_i)$ (2)

Simpson index: $\mathrm{D} = 1 - \sum(P_i)^2 \mathrm{D} = 1 - \sum(P_i)^2$ (3)

McIntosh index: $\mathrm{U} = \mathrm{SQRT}\ (\sum N_i^2)\ \mathrm{U} = \mathrm{SQRT}\ (\sum N_i^2)$ (4)

P_i is calculated by subtracting the control value from the absorbance of each substrate, and then dividing this value by the total color change recorded by all 31 substrates, which is P_i=(Ci-R)/Σ(Ci-R); N_i is the relative OD in each carbon source.

2.7 Statistical Analyses

Significant differences in soil properties, bacterial diversity among samples were determined with a one-way ANOVA and least significance difference (LSD) using SPSS (version 19.1) statistical software (SPSS, Chicago, IL, United States). Redundancy analysis (RDA) was carried out using CANOCO 5.0 to determine correlations between environmental variables and bacteria community composition with the Monte Carlo permutation test. Structural equation modeling (SEM) was applied to gain a mechanistic understanding of how soil properties in bacterial carbon source utilization diversity under different cover system. SEM analysis was carried out under the standard of hypothesis relationship conceptual model, which assumed that long-term land cover would change the physical and chemical properties of soil, thus affecting the carbon source utilization capacity of soil bacterial community.

3 RESULTS

3.1 Soil basic physicochemical properties

The physical and chemical properties of the soil were affected by different long-term mulching treatments (Table 1). The TN, NH_4^+-N, and NO_3^--N were reduced under long-term mulching treatments compared with CK. Compared with the CK, soil organic carbon content increased from 7.08 g kg^{-1} to 7.78-8.46 g kg^{-1} under the straw mulching conditions, while decreased in the F treatment. The TP was significantly higher in the S_{90} treatment than in the other treatments. Soil AP increased from 47.00 mg kg^{-1} to 51.90 - 52.80 mg kg^{-1} under the straw mulching treatments, while significantly reduced in F treatment ($P < 0.05$). Plastic film and straw mulching treatments significantly increased soil pH ($P < 0.05$), and there was no significant difference among the mulching treatments ($P \quad 0.05$). The MC and MFC differed significantly among different mulching treatments ($P < 0.05$). The F treatment has the highest soil water content and the lowest maximum field water holding capacity.

3.2 Analysis of soil bacterial groups

3.2.1 Alpha diversity and OTU cluster analysis

High quality effective sequences under different coverage treatments were screened based on 97% sequence similarity, and the Alpha diversity index was calculated (Table 2). Chao1 index and ACE index represent species richness, and the higher the value, the higher the richness. Simpson index and Shannon index represent species diversity, and the greater the value, the higher the diversity. The results showed that S_{45} treatment had the highest bacterial community richness, while St_{90} treatment had the highest bacterial community diversity. The OTU Venn diagram of soil bacteria (Figure 2) can reflect the overlapping relationship of bacterial groups. Under the five treatments, the bacteria produced a total of 1072 OTUs, accounting for 16.72% of the total 6410 OTUs. The unique OTUs in the CK, F, St_{90}, S_{45}, and S_{90} treatments were 603, 602, 545, 576, and 450, which accounted for 9.41%, 9.39%, 8.50%, 8.99%, and 7.02% of the total number of OTUs. Compared with CK, the number of OTUs processed by F, St_{90}, and S_{45} shows an increasing trend,

while the number of OTUs processed by S_{90} shows a decreasing trend. The results showed that the OTU number of soil bacteria under different mulching treatments was decrease as St_{90}, S_{45}, F, CK, S_{90}.

3.2.2 Diversity analysis of bacterial community structure

A total of 20 phylum and unidentified taxa were obtained after high-throughput sequencing of the five treatments (Figure 3A). Each treatment had similar phylum, but the relative abundance varied. The top eight phylum in relative abundance were *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Gemmatimonadetes*, *Planctomycetes*, *Bacteroidetes*, and *Rokubacteria,* which accounting for 33.4%, 20.8%, 18.4%, 7.2%, 6.2%, 5.0%, 4.2% and 1.4%, respectively. The dominant soil bacterial phyla under each treatment were mainly *Amoebacteria*, *Acidobacteria* and *Actinobacteria*, the sum of their relative abundance reached more than 70%. The relative abundance of *Proteobacteria* was the highest in all treatments, 37.8% in F, 36.4% in CK, 32.2% in S_{45}, 31.6% in S_{90}, and 29.0% in St_{90}. The results showed that the clustering tree can be divided into two categories ((Figure 3C). The composition of soil bacteria treated by CK and F tends to one type, and the clustering relationship is strong. The S_{90}, S_{45}, and St_{90} treatments tend to be the other class. It shows that under the three straw mulching treatments, the bacterial community composition is relatively closely related, and the bacterial community composition under the uncovered and plastic mulching treatment conditions is relatively close, that is, the structural changes of the soil bacterial community under the long-term mulching treatment will tend to be uncovered.

At the class level (Figure 3B), there are 14 dominant classes in the five treatments, and the top ten relative abundances are: *-Proteobacteria* (23.5%), *Subgroup_6* (13.7%), *Actinobacteria* (8.6%), *Thermooleophilia* (7.3%), *AlphaProteobacteria* (6.1%), *Gemmatimonadetes* (5.0%), *Bacteroidia* (4.1%), *DeltaProteobacteria* (3.8%), *Phycisphaerae* (3.0%), *Chloroflexia* (2.9%). The proportion of dominant classes in each treatment is relatively small. The relative abundance of *-Proteobacteria* under the F treatment was the largest, accounting for 29.0%. For *Subgroup_6*, the relative abundance under the St_{90} treatment was the largest (15.8%).

3.2.3 Relationship between bacterial community structure and soil properties

The horizontal abundance of soil bacteria under the film and straw mulching conditions was used as the response variable, and the physical and chemical indexes of the soil were used as the explanatory variable, the redundancy analysis (RDA) was carried out (Figure 4). The first axis explained 81.59% of the variation, and the second axis explained 15.35%. Soil AP has the most obvious effect, explaining 65.80% of the bacterial community change ($P < 0.05$), followed by NH_4^+-N, which explains 19.00% ($P < 0.05$). According to the analysis of the distribution of soil environmental factors and the connection length, soil AP NH_4^+-N MC NO_3^--N MFC and pH are the main factors affecting the

changes of soil bacterial communities under different mulching.

3.3 Soil Bacterial Carbon Source Metabolism

3.3.1 AWCD

The AWCD value can reflect the ability of microorganisms to utilize the same carbon source (Figure 5). The AWCD shows an increasing trend with the extension of the incubation time under each treatment. The mulch management enhanced soil microbial activity and the F treatment was the highest and significantly higher than other treatments ($P < 0.05$). It shows that the microorganisms in the soil under F treatment have the strongest carbon source utilization ability. The AWCD values under the three treatments have the largest slope between 24-120 h. After 120 h, the growth rate of AWCD under each treatment has slowed down and tended to be stable.

3.3.2 Functional diversity index

According to the utilization of carbon sources under different coverage treatments and comprehensive consideration of its changing trends, the AWCD value of 120 h was selected for the analysis of the microbial diversity index (Table 3). The results showed that the Shannon indexes (H) and Simpson indexes (D) in each treatment were significantly higher than the control ($P < 0.05$), while St_{90} was significantly lower than F ($P < 0.05$). There was no significant difference between F, S_{45}, and S_{90}, and they were significantly higher than St_{90} ($P < 0.05$). The McIntosh index (H) of CK significantly lower than the other mulch treatments ($P < 0.05$) and F was the highest treatment.

3.3.3 Main component analysis of carbon source

Principal component analysis was performed on the AWCD values of each carbon source of soil samples cultured for 120 h under different mulching treatments. The cumulative contribution rates of the first and second principal components were 30.2% and 13.0% respectively (Figure 6A). The first component is the main source of variation, and CK and St_{90} treatments are distributed on the positive semi-axis, while F, S_{45}, S_{90} are distributed on the negative semi-axis. The dispersion between samples under F treatment was the smallest. The scores of F and S_{90} treatments were close, and both were in the first quadrant.

The utilization intensity of other carbon sources has been significantly improved by coverage treatments (Figure 7). And the increase in carboxylic acids, amino acids, and sugars is greater. Among them, the metabolic intensity of soil microorganisms to carboxylic acid showed F>S_{90}>S_{45}>St_{90}>CK, and the metabolic intensity of soil microorganisms to carboxylic acid under F treatment was significantly higher than that of CK and other mulching treatments. The utilization intensity of polymers and saccharides by soil microorganisms under different treatments was similar, which is expressed as F>S_{45}>S_{90}>St_{90}>CK. For amino acids, the order of soil microorganisms' utilization intensity is: F>S_{90}>S_{45}>St_{90}>CK. Combined with the loading values of 31 carbon sources on the two principal components (Supplementary Table1), it was considered

that carboxylic acids were the main carbon sources utilized by microorganisms, followed by carbohydrates, amino acids and amines.

3.3.4 Relationship between soil bacterial carbon metabolism and soil properties

Soil microbial carbon source metabolism under film and straw mulching was used as response variables, and soil environmental factors were used as explanatory variables for redundancy analysis (Figure 6B). The first ordination axis explained 97.18% of the variation in soil bacterial carbon source metabolism, and the second ordination axis explained 0.61%. Soil MC had the most obvious effect, explaining 83.50% of the variation of soil microbial carbon source metabolism ($P < 0.05$), followed by TP and pH. According to the distribution of soil environmental factors and line length analysis, AWCD, Shannon index, Simpson index and McIntosh index were positively correlated with MC and pH. Shannon index was negatively correlated with NO_3^--N. MC and TP were the main factors affecting the variation of soil microbial carbon source metabolism under different mulching treatments.

Combined with the structural equation model analysis (SEM) of the mechanism between soil physicochemical properties, bacterial community structural diversity, and carbon source metabolic capacity under long-term mulching (Figure 8). The results show that the interpretation rates of AWCD, Shannon index and Mclntosh index reach 67%, 84% and 99% respectively. The bacterial diversity index under long-term surface cover was directly affected by soil pH and bacterial carbon metabolism, and indirectly by other soil physicochemical properties. Under long-term surface cover, there was a significant positive correlation between bacterial carbon source metabolism and Shannon index and Mclntosh index.

4 DISCUSSIONS

4.1 Effects of ground cover on the soil properties

Long-term ground cover measures changed the physical and chemical properties of the soil (Table 1). The results showed that wheat growing rapidly at the green stage had a higher demand for nitrogen in soil, which might be one of the reasons for the decrease of total nitrogen content in soil (Li et al. 2019). Straw decomposition will directly supplement nitrogen in the soil, leading to the increase of nitrogen content in the soil. However, the release process of straw nitrogen in the soil is very complicated and affected by a variety of environmental factors, which may also be the reason why the nitrogen content in the soil does not increase but decreases under mulching treatment (Xu et al. 2017).

In addition, plastic film mulching will increase the soil temperature of the cultivated layer, promote the metabolic process of soil microorganisms, accelerate the mineralization of soil nitrogen, and reduce the total nitrogen of the soil (Li et al. 2004). In this study, the soil organic carbon content under the straw mulching treatment was significantly higher than that of the CK and F, which

is consistent with the results of Ussiri & Lal (2008). The straw can supplement the soil organic carbon content through humification under suitable conditions. Meanwhile, the temperature stabilization, water storage and water retention effects under the straw mulching condition also favorite to the accumulation of soil organic carbon (Perucci et al. 1997). Long-term straw mulching can significantly increase the content of soil available phosphorus and increase the utilization rate of soil phosphorus (Akhtar et al. 2019), this is one reason why AP content in soil increased significantly under straw mulching. In addition, straw mulching increases soil pH and soil negative charged particle, which increasing the solubility of the soil and increasing the solubility of the soil, thereby reducing to adsorb and fix phosphorus, all these improve the effectiveness of the plant's absorption of soil phosphorus (Xu et al. 2014, Zhou et al. 2021). Long-term ground cover changed soil temperature, hydrothermal condition and other soil environment, which may be the reason for the significant increase of soil water content in the arid area (Li, et al. 2004).

4.2 Effects of ground cover on soil microbial community composition

Microorganisms, as an important component of the soil, can characterize the soil quality status by change their community structure (Spedding et al. 2004). Reasonable ground mulching measures can effectively improve soil structure and regulate soil temperature and humidity, which have ecologically positive effects (Jumpponen & Brown 2014). Therefore, different ground mulching management affect the abundance, growth and reproduction of soil microbe and their community structure and diversity. Govaerts et al. (2008) and others showed that no-till combined with straw residue mulching can increase the number of soil bacteria and fungi, which is important for sustainable agricultural development. In this research, the dominant bacterial communities at the phylum level with a sum of relative abundance of more than 90% (Figure 3A), which was similar to the results of Chen, et al. (2015). The relative abundance of *Proteobacteria* was the highest under different mulching treatments, and did not differ significantly among treatments, which may be due to the fact that *Proteobacteria* is the largest phylum of bacteria and is not greatly influenced by the external environment (Roesch et al. 2007). Furthermore, *Proteobacteria* can fix nitrogen in soil nitrogen cycle and adapt to various complex environments, the change in environmental conditions have little effect on their distribution and relative abundance (Liu et al. 2014). In this study, the relative abundance of *Acidobacteria* phylum was increased under all mulching treatments. *Acidobacteria* can effectively degrade plant residues while participating in the metabolism of single carbon compounds (Naether et al. 2012). Under St_{90} treatment, the relative abundance of *Acidobacteria* phylum was the highest, because the high temperature in summer was in the early stage of sowing, and straw decay provided a large number of external carbons, which further promoted the growth and reproduction of *Acidobacteria* (Jiang et al. 2019). From the perspective of class level, there are 14 dominant classes shared by different treatments, mainly including: *-Proteobacteria*, *Subgroup_6*, *Actinobacteria*, *Thermoleophilia*, *-Proteobacteria*, etc (Figure 3B). Most of them are different types of *Proteobacteria*, which was

similar to the mulching treatment on the diversity of soil bacterial community structure in Lei bamboo forest (Zhai et al. 2017). The same conclusion was obtained in dryland apple orchards under different mulching treatments (Shen et al. 2019).

4.3 Effects of ground cover on soil bacterial community diversity

Soil microbial community diversity indices can reveal differences in soil microbial species and functions (Nsabimana et al. 2004). In this study, compared with CK, long-term land cover increased the alpha diversity of soil bacteria (Table 2), which was consistent with the research results of Huang et al. (2019) on loess dryland. The metabolic functional diversity index of bacteria under long-term mulching was significantly higher than that without mulching (Table 3), which is consistent with the findings that different mulching treatments increased the soil microbial community diversity index (Gu et al. 2016). Straw mulching changes the diversity of soil bacteria, probably due to reducing water evaporation, producing saccharides, amino acids and organic acids during its decomposition soil, which provides nutrient elements for microorganisms (Ndzelu et al. 2021), then increasing the variety of microorganisms and making bacterial diversity increase (Guo et al. 2016). The moisture retention effect of ground mulching improves the soil microenvironment and changes the transformation of soil nutrients, which is an important influencing factor in regulating the structure of soil microbial communities (Hou et al. 2007). The difference of diversity indexes may be related to the amount of straw mulching and the mulching time, which resulting in large differences in the physical and chemical properties of the soil, and this have a significant impact on the soil bacterial community structure and species diversity (Huang et al. 2019). Such differences, indeed, are thought to be the result of a combination of multiple external environmental factors such as crop species, soil type, soil moisture and soil temperature (Zhang et al. 2018).

4.4 Differences in the utilization of soil bacterial carbon sources under ground cover

The variation of AWCD values with incubation time can characterize the average microbial activity, and the higher AWCD value, the higher microbial metabolic activity and the utilization of carbon sources (Konopka et al. 1998). Studies have shown that plant residue, soil physicochemical property, and carbon source type affect the diversity of carbon source utilization by soil microorganism (Xu et al. 2015). Straw return significantly increased soil microbial carbon metabolic activity and diversity (Tang et al. 2018). In this study, the carbon metabolism capacity of soil microorganism at all incubation stages were significantly higher in the mulching treatment than in the CK, indicating that the mulching treatment could effectively increase the activity of soil microorganisms and promote carbon metabolism of carbon sources (Yang et al. 2019). Crop residues and root secretions under straw mulch provide substrate energy for soil microbial activities, which increases microbial metabolic activity and population (Han et al., 2014). The F treatment with plastic film mulch has the strongest carbon

metabolism ability in this research. This may be due to the long-term plastic film mulching improving the hydrothermal conditions of the soil, providing material and energy for the growth and reproduction of microorganism, then resulting in an increase in the ability of carbon source metabolism (Cao et al. 2016).

The CK and St_{90} treatments were mainly concentrated in their positive direction of the PC1, indicating that the carbon source utilization was similar. This may during the increased competition between microorganisms and crops for soil moisture due to the decomposing process with high temperature in summer, which lead to the reduction of soil microbial carbon source metabolic activities, and not changed significant (Yang, et al. 2019). F, S_{45}, and S_{90} are mainly separated in the negative direction of PC1, indicating that different mulching treatments lead to changes in the use of carbon. After the straw was decomposed and mixed with soil sources by soil microorganisms. First of all, after the straw decomposes and enters the soil, it provides carbon and nutrients for the growth and reproduction of soil microorganism, so the organic matter content is an important factor to the changes in the characteristics of soil microbial carbon source metabolism (Ayres et al. 2009). Secondly, plastic film mulching can increase soil temperature, inhibit water evaporation, provide a suitable environment for the soil microorganism, and change the number and structure of the microorganism. This may be one of the main reasons for the differences in soil microbial carbon source metabolism characteristics under different mulching treatments (Ramakrishna et al. 2006). Similar with this study, other research also found that soil microorganisms can utilize a variety of carbon sources such as saccharides, alcohols and carboxylic acids (Liu et al. 2011). The main carbon source utilization types in this study area were carboxylic acids, saccharides and amino acids, which can be used as the main basis for distinguishing the carbon source utilization types of soil microorganisms under different mulching treatments.

5 CONCLUSIONS

In conclusion, long-term ground mulching can improve soil bacterial community diversity and carbon metabolism, which is of positive significance for the sustainable development of agro-ecosystems in arid regions of the Loess Plateau. Long-term surface cover changes soil physicochemical properties, which is one of the main reasons for changes in bacterial community diversity. *Proteobacteria*, *Acidobacteria* and *Actinobacteria* were the main dominant flora. The AP, $NH_4{}^+$-N, MC were the main environmental factors affecting the composition of bacterial community. The carbon metabolism ability of bacteria was the strongest under F treatment, and the SOC content was the higher under straw mulching. Amino acids, carboxylic acids and sugars are the main carbon sources used by soil microorganisms. This study provides a theoretical basis for the effects of different mulching patterns on soil bacterial communities. According to different climatic conditions and crop growing seasons, selecting reasonable materials and methods is conducive to maximizing the benefits of covering and

realizing the sustainable development of agriculture.

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AUTHOR CONTRIBUTIONS

Wenting Zhang: Conceptualization; Data curation; Writing- Original draft preparation; Data sorting and analysis, Experiment implementation. Yi Wang: Data curation, Method and model design, Data sorting and analysis. Chunyue Li: Conceptualization, Guidance, Method and model design, Visualization. Shun Chang: Sample collection. Yinglong Xue: Experiment implementation. Tinghui Dang, Xiaomin Zeng: Visualization.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Figure captions and tables

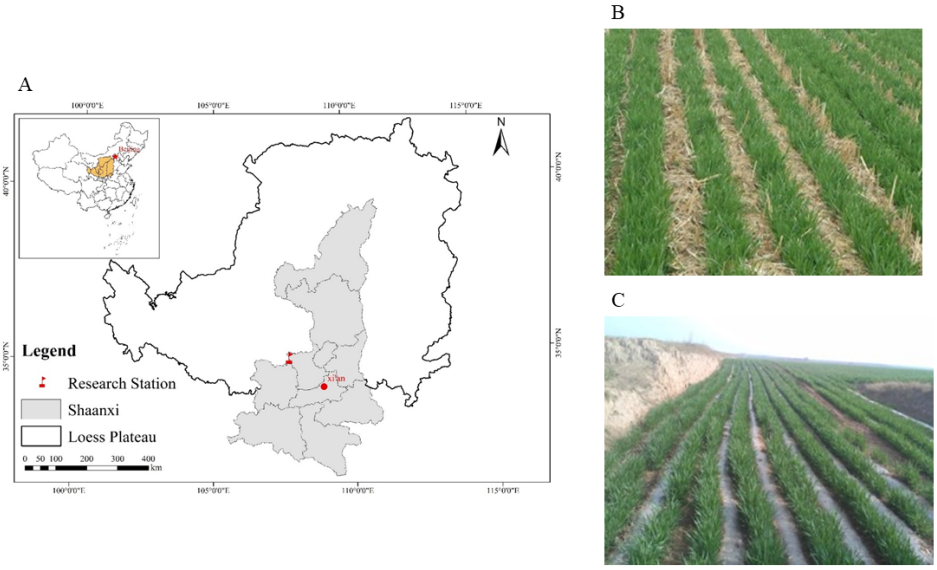

FIGURE 1 Location of the research station (A), straw mulching (B), plastic film mulching (C).

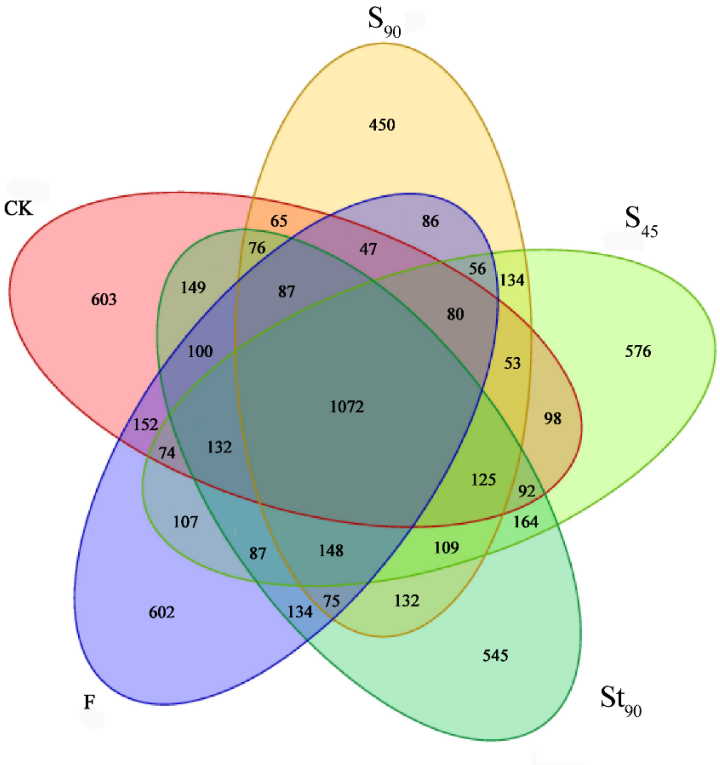

FIGURE 2 OTUs Venn diagram of soil bacteria under different mulching treatments.

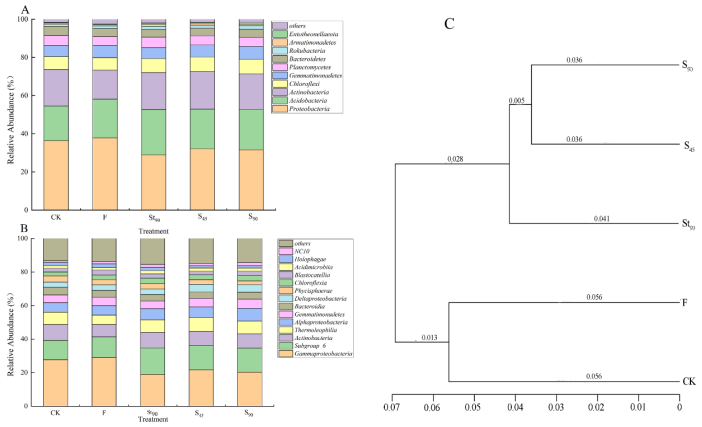

FIGURE 3 Soil bacterial community structure under different mulching treatments. Soil bacterial phylum horizontal community composition (A); soil bacterial class horizontal community composition (B); similarity clustering tree of soil bacterial community (C).

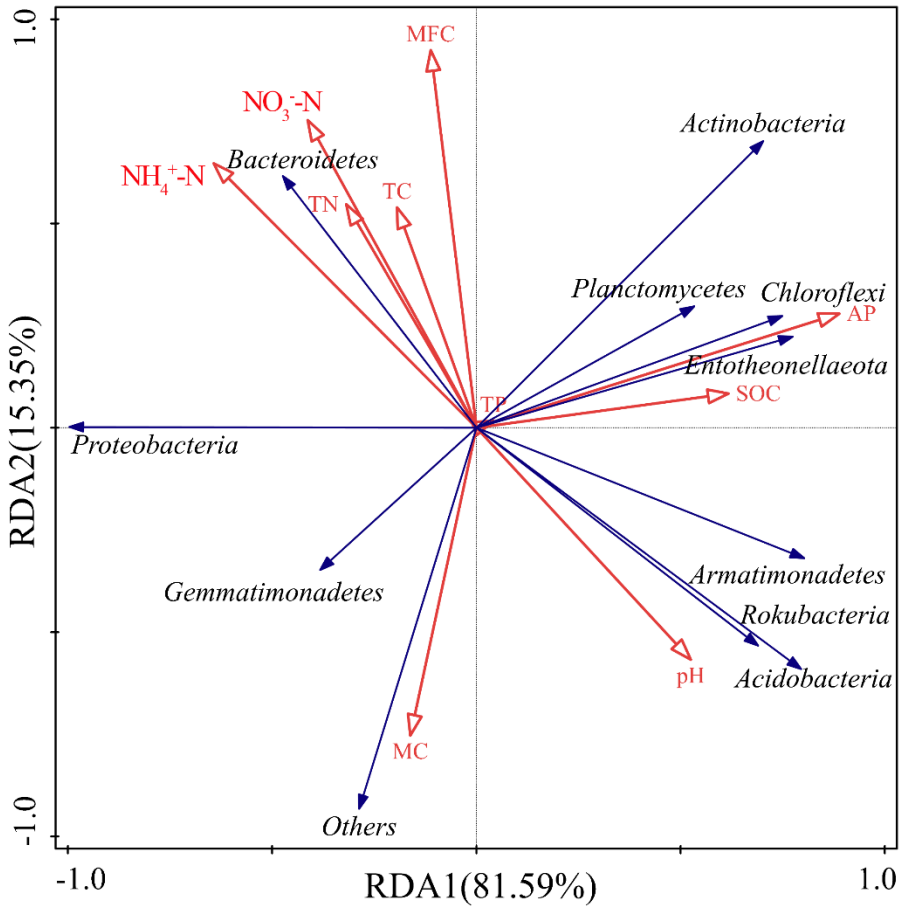

FIGURE 4 Redundancy analysis of soil bacterial community structure and soil environmental factors under different mulch treatments.

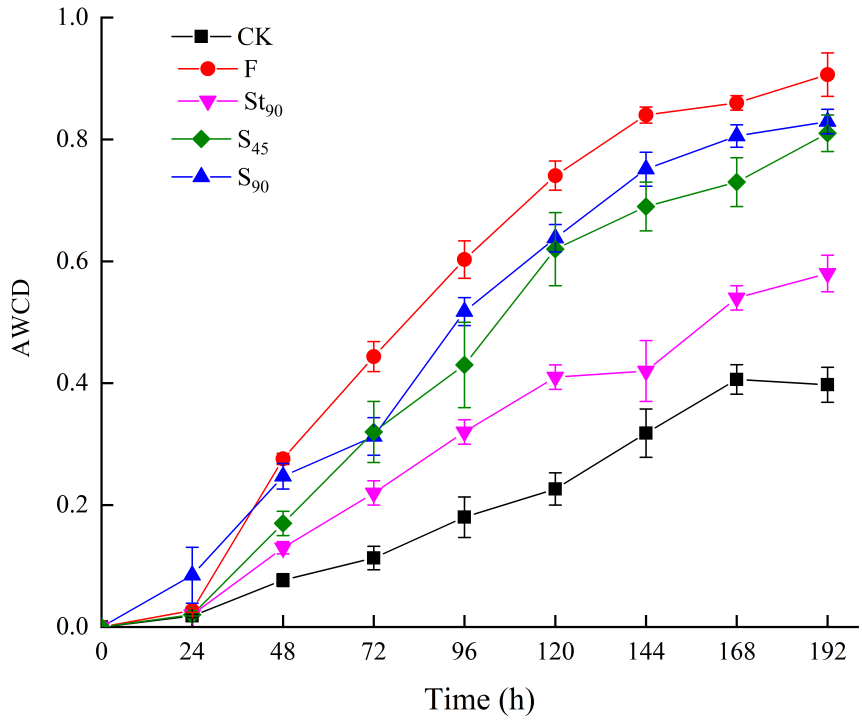

FIGURE 5 The average well color development (AWCD) dynamics changes over incubation time in five soil samples.

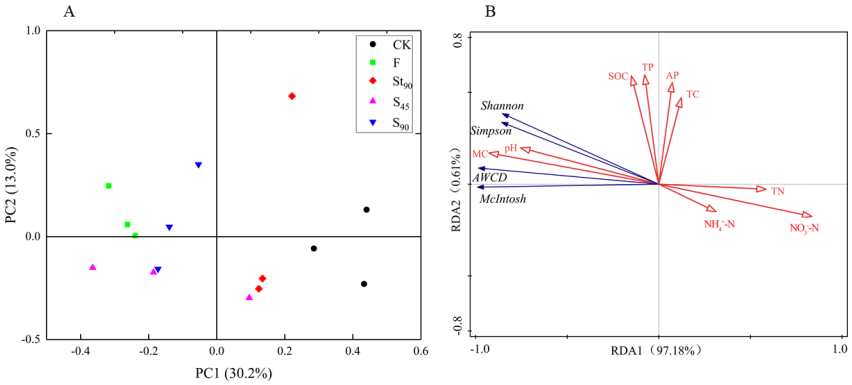

FIGURE 6 Principal component analysis (A) and redundancy analysis (B) of soil microbial carbon source metabolism under different mulching treatments.

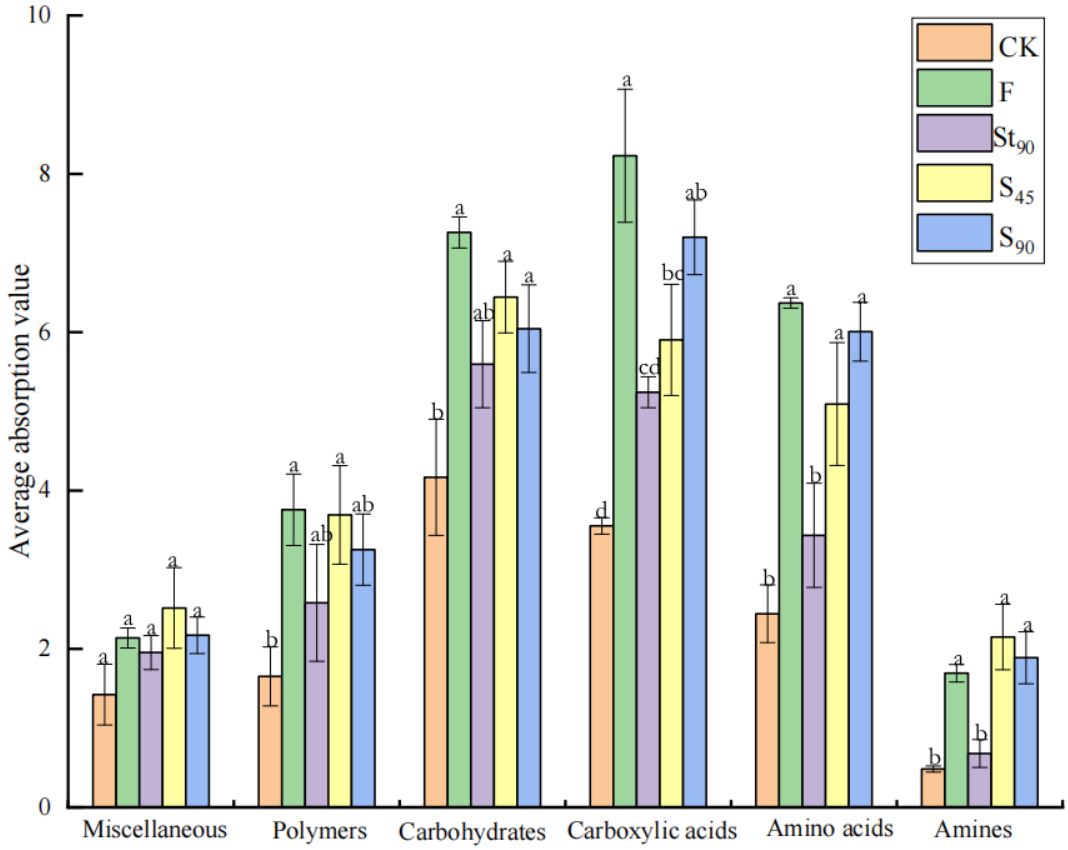

FIGURE 7 The utilization intensity of soil microorganisms to various carbon sources under different mulching treatments. Notes: Miscellaneous (Pyruvic acid methyl ester, lucose-1-phosphate, D, L- -Glycerol phosphate); Polymers (Tween 80, Tween 40, -Cyclodextrin, Glycogen); Carbohydrates (D-Cellobiose, -D-Lactose, Methyl-D-glucoside, D-Xylose, i-Erythritol, D-Mannitol, cetyl-D-glucosamine); Carboxylic acids (D-Glucosaminic acid, 4-Hydroxy benzoic acid, D-Galactonic acid latone, D-Galacturonic acid, 2-Hydroxy benzoic acid, -Hydroxy butyric acid, Itaconic acid, -Keto butyric acid, D-Malic acid); Amino acids (L-Arginine, L-Asparagine, L-Phenylalanine, L-Serine, L-Threonine, Glycyl-L-glutamic acid); Amines/amides (Phenylethylamine, Putrescine).

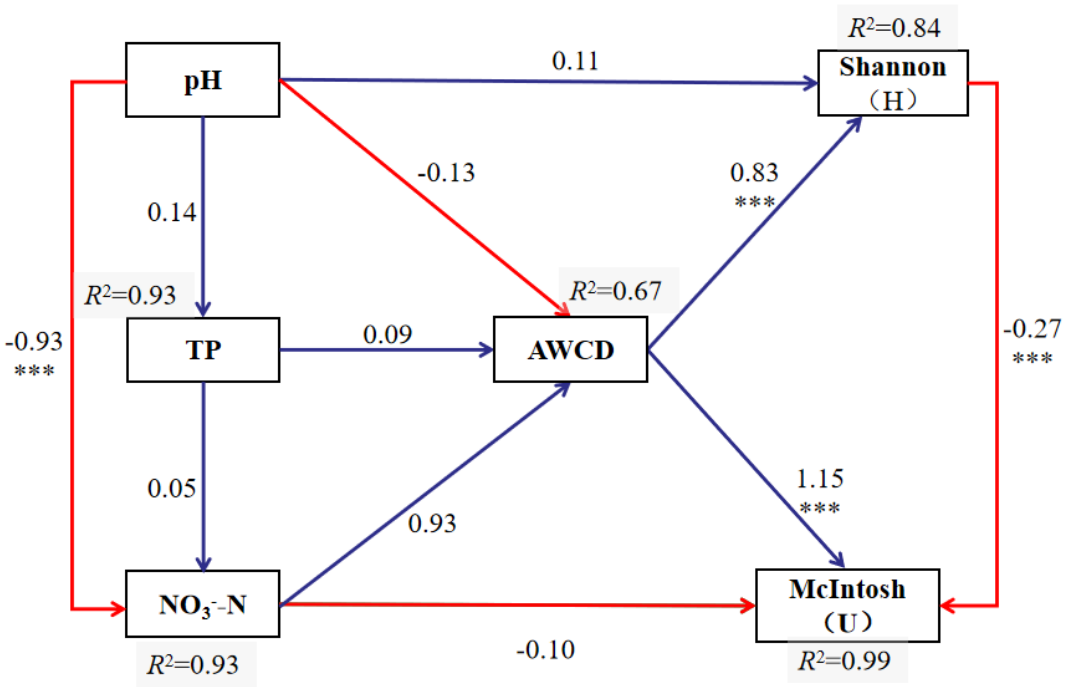

FIGURE 8 Structural equation modeling (SEM) path diagram of carbon source metabolism of soil bacteria for each factor. Notes: the model resulted in a good fit to the data, with a mode $^2 = 5.101$, $df = 4$, $P = 0.277$. The numbers beside the path are standardized path coefficients, where red is negative effect and bule is positive effect. R^2 represents the explanatory rate of this variable in the model. *** indicates $P < 0.001$.

TABLE 1 Basic physicochemical properties of soil under different mulching treatments.

Treatment	CK	F	St_{90}	S_{45}	S_{90}
TN (g kg^{-1})	1.14±0.06a	1.01±0.02b	1.01±0.04b	1.04±0.03ab	1.00±0.02b
NH_4^+-N mg kg^{-1})	0.24±0.01a	0.17±0.01bc	0.11±0.01d	0.19±0.02b	0.15±0.01c
NO_3^--N (mg kg^{-1})	74.97±1.60a	15.68±0.74c	19.12±0.21b	18.25±0.51bc	15.71±0.11c
TC (g kg^{-1})	17.91±0.10ab	17.43±0.06bc	17.03±0.11c	18.45±0.37a	17.53±0.19bc
SOC (g kg^{-1})	7.08±0.05bc	6.99±0.38c	7.78±0.24abc	7.97±0.40ab	8.46±0.18a
TP (g kg^{-1})	1.19±0.00b	1.19±0.01b	1.18±0.01b	1.17±0.00b	1.24±0.01a
AP (mg kg^{-1})	47.00±0.53b	43.50±0.75c	51.90±0.29a	51.90±0.29a	52.80±0.61a
pH	7.81±0.01b	8.06±0.06a	8.10±0.02a	8.12±0.02a	8.14±0.01a
MC (%)	8.66±0.04e	13.42±0.09a	10.29±0.11d	11.15±0.04c	12.57±0.15b
MFC (%)	22.47±0.09a	19.04±0.04e	19.54±0.11d	22.07±0.05b	19.99±0.08c

Different letters in the same column indicate significant differences at ($P < 0.05$) according to Duncan's test. The same below. TN, total nitrogen; NH_4^+-N, Soil ammonium nitrogen; NO_3^--N, nitrate nitrogen TC, soil total carbon. SOC, soil organic carbon; TP, total phosphorus AP, soil available phosphorus; MC, soil moisture content; MFC, soil maximum water holding capacity in the field.

TABLE 2 Total effective sequence number and diversity index of soil bacterial (at 97% sequence similarity).

Treatment	Sequence number	Simpson index	Shannon index	Chao1 index	ACE index
CK					
F					
St_{90}					
S_{45}					
S_{90}					

TABLE 3 Metabolic functional diversity index of soil microorganisms under different mulching treatments.

Treatment	Shannon index	Simpson index	McIntosh index
CK	2.51±0.11c	0.89±0.01c	2.30±0.17d
F	3.00±0.04a	0.95±0.00a	5.34±0.14a
St_{90}	2.72±0.06b	0.92±0.00b	3.59±0.15c
S_{45}	2.97±0.02a	0.94±0.00a	4.56±0.42b
S_{90}	3.04±0.01a	0.95±0.00a	4.52±0.14b

Different lowercase letters indicate significant differences between different treatments ($P < 0.05$).