




Division - Soil Processes and Properties | Commission - Soil Biology

Winter cover crops effects on soil microbial characteristics in sandy areas of Northern Shaanxi, China

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ABSTRACT: In sandy areas of Northern Shaanxi, after potato harvest, there are large areas of soil exposed to wind erosion from winter to spring, leading to degradation of soil quality. Planting cover crops in fallow fields is considered effective to improve soil biological properties; however, there is scarce study on the effect of winter cover crops on fallow soils in this region. In the study lasting from 2017 to 2019, four winter cover crops, i.e., alfalfa (AC), sweetclover (SC), winter wheat (WC), and ryegrass (RC), as well as bare land (CK), were used to study the effect of winter cover crops on soil microbial characteristics. The experiment showed that the soils in AC treatment had the highest values of microbial biomass N (MBN) content, dehydrogenase and urease activities, bacteria colonies, and Shannon and Richness indices. The soils in SC treatment had the maximum values of microbial biomass C (MBC) content, protease activity, fungi colonies, and Simpson index. Under AC and SC treatments, microbial communities in soil showed the highest percentage of carbon source utilization for amino acids, carbohydrates, and amines. Alfalfa and sweetclover as cover crops were helpful to improve the activity and diversity of soil microorganisms, exerting a positive effect on soil quality. This finding is of great significance for improving the methods of mitigating soil degradation in winter fallow fields of Northern Shaanxi, China.

Keywords: soil microorganism, winter fallow soil, soil quality, enzyme activity.

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Received: December 12, 2019

Approved: April 29, 2020

How to cite: Wang W, Han L, Zhang X. Winter cover crops effects on soil microbial characteristics in sandy areas of Northern Shaanxi, China. Rev Bras Cienc Solo. 2020;44:e0190173.
<https://doi.org/10.36783/18069657rbcs20190173>

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INTRODUCTION

The sandy area of Northern Shaanxi is located in the southeast of Maowusu desert, China. The potato is the main food crop in that area. In autumn, after potato harvest, the soil is usually turned over, resulting in six to seven months (November to May) of bare soil exposure. Sandy loam soils are more vulnerable to erosion by wind, and some farmlands even cannot be cultivated due to severe soil desertification (Zobeck et al., 2013; Sirjani et al., 2019). Studies reported that the wind erosion to topsoil in the arid and semi-arid regions causes the loss of fine-grained soil that is rich in nutrients such as C and N (Wang et al., 2015; Zhang et al., 2015), leading to the dwindling of soil organic carbon (SOC) and total nitrogen (TN) as well as the degradation of soil (Li et al., 2017; García-González et al., 2018; Chappell et al., 2019).

Winter cover crops are often sowed after the harvest of cash crops (Daryanto et al., 2018). The aboveground litter (Veiga et al., 2017), underground root exudates, and dead roots (Austin et al., 2017) of winter cover crops are accumulated in the soil, changing nutrient cycling in the topsoil. Winter cover crops can create a favorable environment for soil microorganisms (Balota et al., 2014; de Cima et al., 2016; Nevins et al., 2018) by inputting decomposable organic substrates (Poeplau and Don, 2015; Plaza-Bonilla et al., 2016; Alvarez et al., 2017) to improve soil structure and corrosion resistance (Wang et al., 2015; Zhang et al., 2015; Daryanto et al., 2018). So, they are beneficial to increasing microbial biomass (Frazão et al., 2010; Nair and Ngouajio, 2012; Brennan and Acosta-Martinez, 2017), enhancing soil enzyme activities (such as urease, dehydrogenase, and phosphatase activities) (Hamido and Kpomblekou, 2009; Calderon et al., 2016; Zheng et al., 2018), shifting carbon source utilization patterns of soil microorganisms (Nivelle et al., 2016), stimulating microbial diversity (Simpson index and Shannon index, etc.) (Nair and Ngouajio, 2012) and maintaining microbial richness (Verzeaux et al., 2016; Daryanto et al., 2018).

There have been some reports evaluated the influence of winter cover crops in the humid and sub-humid soils (Alvarez et al., 2017), most of the researches were related to legume crops (Pantoja et al., 2016; Snapp and Surapur, 2018) and cereal crops (Roldán et al., 2003; Plaza-Bonilla et al., 2016; Ordóñez-Fernández et al., 2018). Manici et al. (2018) found that soil covered by hairy vetch and by barley showed different effects on soil bacteria and fungi communities. However, there are very few reports about the changes of soil microbial characteristics under different cover crops in winter fallow soils of semi-arid area, and it is not clear about the microbial mechanism in the soil.

In the sandy area of Northern Shaanxi, many large-scale farms can be properly irrigated through existing irrigation system for planting winter cover crops. In this study, the cold-tolerant legume cover crops (alfalfa and sweetclover) and cereal cover crops (ryegrass and winter wheat) were cultivated between 2017 and 2019 after potato being harvested. The hypothesis of this study is that legume cover crops and cereal cover crops have different effects on soil microbial characteristics. This study aimed to (1) determine the changes of soil microbial biomass, microbial colonies, enzyme activities, microbial diversities, and carbon source utilization patterns under the four cover crops; (2) reveal the relationships between microbial parameters and chemical properties in soils; and (3) clarify the effect of the four cover crops on the soil quality in winter fallow soils.

MATERIALS AND METHODS

Site description

The experiment site is in the agricultural technology demonstration park, which locates 10 km north of Yulin, Shaanxi Province, China (38° 23' N, 109° 43' E). The area has a typical semi-arid climate with average annual precipitation of 407 mm, average

annual evaporation of 1900 mm, annual sunshine of 1900 h, annual total radiation of $606.7 \times 10^7 \text{ J m}^{-2}$, annual average temperature of $8.6 \text{ }^\circ\text{C}$, and $\geq 10 \text{ }^\circ\text{C}$ accumulated temperature of 3000-3300 $^\circ\text{C}$. Due to the widespread irrigated agriculture, the area is convenient for irrigation with flat terrain and high groundwater level ($>10 \text{ m}$). The soil is Calcaric Arenosol (489 g kg^{-1} sand, 305 g kg^{-1} of silt, and 206 g kg^{-1} of clay), loose, and rich in potassium, with $\text{pH}(\text{CaCl}_2)$ value of 7.60, SOC content of 12.50 g kg^{-1} , TN content of 1.41 g kg^{-1} , and available N content of 38.76 mg kg^{-1} . The monthly average air temperature and total precipitation during the experiment are shown in figure 1.

Experimental design

The experiment was set up with five treatments, including covered with alfalfa (AC, *Medicago sativa* L.), sweetclover (SC, *Melilotus alba* Desr.), winter wheat (WC, *Triticum aestivum* L.), ryegrass (RC, *Lolium perenne* L.), and bare land (CK, conventional practice). The area per treatment was 0.1 ha^{-1} (hereinafter referred to as the 'sample plot'). Before the experiment was conducted, the potatoes in the field had been harvested by a potato harvester with 0.25-0.30 m digging depth on September 1, 2017, and August 29, 2018, respectively. Then, both potato tubers and residues were transferred out of the experiment field. After harvesting, except for the CK, the other treatments were plowed with a rotary tiller to a depth of 0.15 m on September 4, 2017, and September 1, 2018. On the same days, experimental seeds were sowed at 375 kg ha^{-1} of winter wheat and ryegrass and 30 kg ha^{-1} of sweetclover and alfalfa. No organic and chemical fertilizers, pesticide, and herbicide were applied to the plots during the whole experimental period. A fixed center pivot irrigation machine (Reinke Manufacturing Co., Inc., Texaco, Nebraska, USA) was used to irrigate once every 15 days with the irrigation amount of $600 \text{ m}^3 \text{ ha}^{-1}$. Irrigation was not done in the case of daily precipitation higher than 3 mm and the daily average temperature lower than $0 \text{ }^\circ\text{C}$.

Soil sampling

Soil samples were collected on May 2, 2018, and April 30, 2019, three steel wire quadrats with a size of 2 m^2 were randomly placed in each sample plot. For each quadrat, five soil samples from the layer 0.00-0.20 m were taken with a soil auger (5 cm diameter) and mixed up to produce a pooled sample. There were three soil samples for each plot

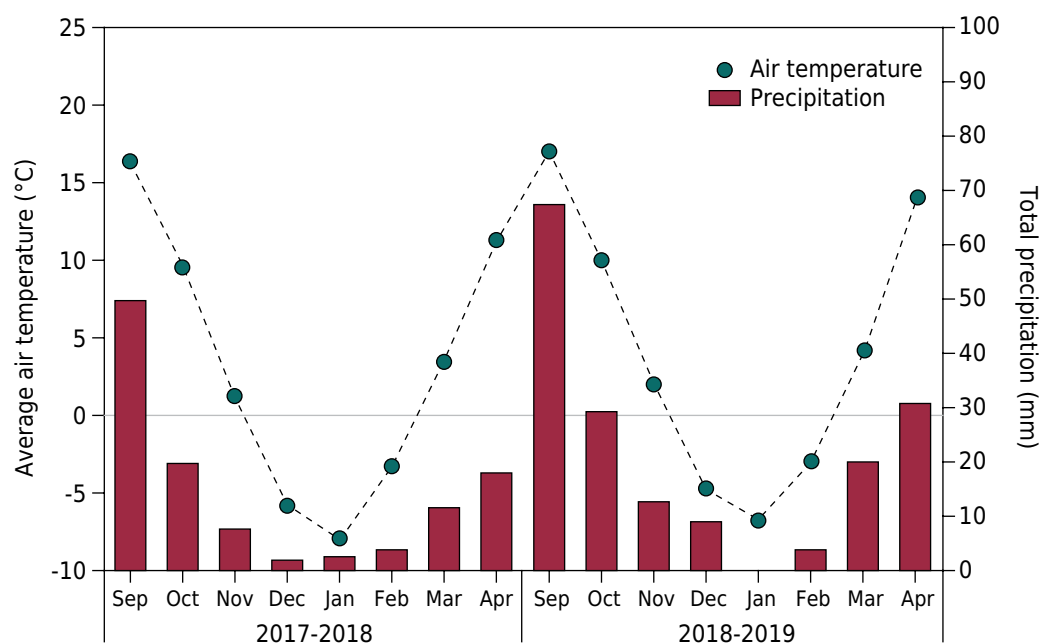


Figure 1. Changes of monthly average temperature and total precipitation during the 2017-2019 experiment in winter fallow fields in sandy areas of Northern Shaanxi of China.

in each year. Fresh samples were sieved through a 2 mm mesh and placed in plastic sample bags (TP001, 0.30 × 0.40 m), then brought back to the laboratory at the Yulin University and kept in a refrigerator at 4 °C (not more than 48 h) for analysis.

Chemical properties of soil

Total nitrogen content was determined with a Kjeldahl analyzer using $K_2Cr_2O_7$ - H_2SO_4 digestion method as described by Bao (2000). The SOC content was measured by $K_2Cr_2O_7$ oxidation-spectrophotometry method (Bao, 2000) with a total organic carbon analyzer (TOC-VCPH, Shimadzu, Kyoto, Japan). The pH value was determined using 10 g of soil sample and 10 mL of $CaCl_2$ 0.01 mol L^{-1} as extractant. The C/N ratio was calculated by dividing the SOC and TC concentration, respectively.

Microbial characteristics of soil

Soil enzyme activities: urease (EC 3.5.1.5) activity was measured with sodium carbonate colorimetric method based on Guan (1986) using a UV spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan); sucrase (EC 3.2.1.26) activity was measured with 3,5-dinitrosalicylic acid method and colorimetrically at 500 nm (Guan 1986). Dehydrogenase activity was determined using the reduction method of 2,3,5-triphenyltetrazolium chloride (TTC) to triphenyl formazan (TPF) as described by Casida et al. (1964). Protease activity (EC 3.4.21-24) was measured with folin-phenol reagent method as described by Ladd and Butler (1972).

Soil microbial biomass: microbial biomass C (MBC) and N (MBN) was analyzed by chloroform fumigation- K_2SO_4 0.5 mol L^{-1} extraction method (water to soil ratio of 4:1). After treated by fumigation, the soluble organic C in the extracting solution was assayed by the total organic carbon analyzer, and the MBC content was calculated based on Vance et al. (1987) with a conversion coefficient (K_c) of 0.38. The total nitrogen in the extracting solution was measured by H_2SO_4 digestion method using Kjeldahl analyzer, and the MBN content was calculated based on Brookes et al. (1985) with a conversion coefficient (K_c) of 0.54.

Microbial colonies in soil were measured with the dilution-plate method (Blum and Shafer, 1988). Specifically, the bacteria colonies were grown on beef extract peptone agar medium (BPA) at 28 °C for 3 days; the fungi colonies were grown on Martin's medium (MA) at 25 °C for 5 days, and the actinomyces strain were grown on Gause's synthetic agar medium (GA) at 28 °C for 7 days. Colony forming units (CFU) were represented as the number of colonies per gram of dry soil.

The structure of the soil microbial community was analyzed through the sole-carbon-source utilization profiles using Biolog EcoPlates™ (BiologInc., Hayward, CA). In the microplate, there were 95 wells, including 31 different carbon sources plus one control well with no carbon (water), and the wells were in three replications. Optical density (OD) values were recorded with Biolog Emax™ (BiologInc., Hayward, CA) automatically at 590 nm per 24 h until reaching 240 h, and the data recorded at the 96th hour (the end of the exponential phase) were selected for statistical analysis. The average well color development (AWCD) of each EcoPlate was measured once every 24 h and expressed as the mean of the OD values for all 95 wells minus the control well values (Garland and Mills, 1991). The Richness index (S) was calculated as the total number of metabolic micropores of carbon source; if OD of a micropore ≥ 0.2 , the carbon in that pore is considered as being utilized, and the pore is defined as a metabolic micropore. The diversity indices, including Shannon index (H), Simpson index (D), and Pielou index (E) were calculated according to equations 1 to 4 (Keylock, 2005).

$$AWCD = \sum(C_i - R_0)/n \quad \text{Eq. 1}$$

$$H = \sum[(C_i - R_0)/\sum(C_i - R_0)] \times \ln[(C_i - R_0)/\sum(C_i - R_0)] \quad \text{Eq. 2}$$

$$D = 1 - \sum[(C_i - R_0)/\sum(C_i - R_0)]^2 \quad \text{Eq. 3}$$

$$E = H/\ln S \quad \text{Eq. 4}$$

In which C_i is the OD value of each micropore in Biolog EcoPlates, R_0 is the OD value of the control micropore, and n is the types of carbon sources ($n = 31$).

Statistical analysis

All sample data were conducted to normality test and the homogeneity of variance test using Bartlett's method. After all assumptions were met to one-way analysis of variance (ANOVA), using SPSS software (version 20.0), the significant differences among different treatments within the same year were calculated by the Duncan's new multiple range test ($p < 0.05$), and the significant differences among years of 2018 and 2019 within the same treatment were calculated by the t-test ($p < 0.05$). Redundancy analysis (RDA) was used to explore the simultaneous relationships between soil microbial variables and soil environmental variables with CANOCO software (version 4.5). Multivariate regression analysis was carried out for each microbial variable and all environmental variables (SOC, TN, C/N ratio, pH) to obtain the fitting matrix. Principal component analysis was carried out for the fitting matrix to obtain the eigenvectors matrix which was performed sorting. The results were visualized by RDA biplots, where the position, angle, and length of arrows indicate the direction, degree, and scope of the response of the microbial variables to environmental variables (Gonzalez et al., 2003).

RESULTS

Soil enzyme activity

In topsoil (0.00-0.20 m), the urease (Figure 2a) and dehydrogenase activities (Figure 2b) under AC treatment were the 10.29-62.75 and 1.06-31.28 % higher than that under other treatments, and significantly higher than CK ($p < 0.05$). The protease activity (Figure 2c) under SC treatment was significantly higher (32.64-56.83 %) than that under WC and CK treatments ($p < 0.05$). The sucrase activity (Figure 2d) in RC treatment was the largest (13.52 and 15.23 mg g⁻¹). In general, the soil enzyme activities under all treatments in 2019 treatments were higher than that in 2018; however, the differences were not significant.

Soil microbial biomass

The contents of MBC under SC treatment was 9.18-71.03 % higher than that under other treatments and significantly higher than WC and CK treatments ($p < 0.05$) (Figure 3a); and the contents of MBN under AC treatment was 8.95-81.11 % higher than other treatments and significantly higher than RC, WC, and CK treatments ($p < 0.05$) (Figure 3b). The ratio of MBC to SOC and the ratio of MBN to TN denote the contribution rates of MBC and MBN to soil organic carbon and total nitrogen. The SC treatment displayed the largest MBC/SOC ratio (0.022 and 0.027) (Figure 3c), and AC treatment showed the maximum MBN/TN ratio (0.038 and 0.050) (Figure 3d). In general, the soil microbial biomass in 2019 was higher than that in 2018, and the MBN content under AC treatment in 2019 was 14.39 % significantly higher than that in 2018.

Soil microbial colonies

The log number of colonies for the three major groups showed the order of bacteria > actinomycetes > fungi (Table 1). The number of bacteria colonies under AC treatment and fungi colonies under SC treatment was 0.02-0.49 and 0.02-0.24 log₁₀ CFU g⁻¹ higher

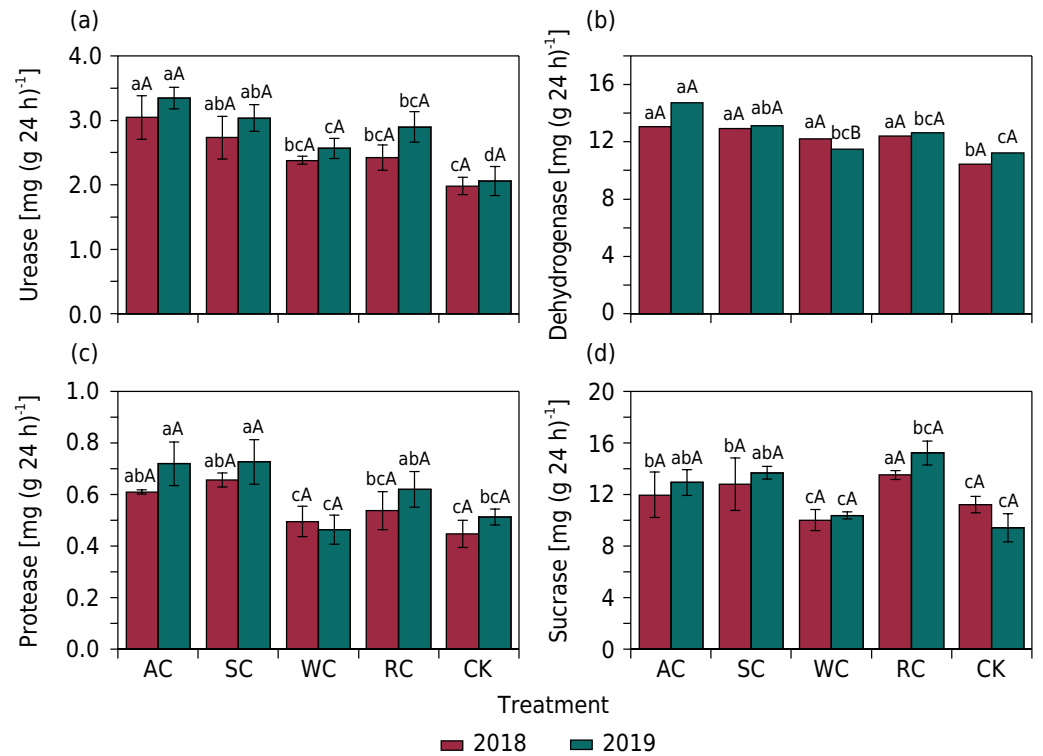


Figure 2. Changes of soil urease activity (a), dehydrogenase activity (b), protease activity (c), and sucrase activity (d) under different treatments. AC: alfalfa cover; SC: sweetclover cover; WC: winter wheat cover; RC: ryegrass cover; CK: bare land. Different lowercase letters denote significant differences among the treatments within the same year (Duncan's new multiple range test; $p < 0.05$), different uppercase letters indicate significant differences among the years within the same treatment (t-test; $p < 0.05$). Bars represent the standard error.

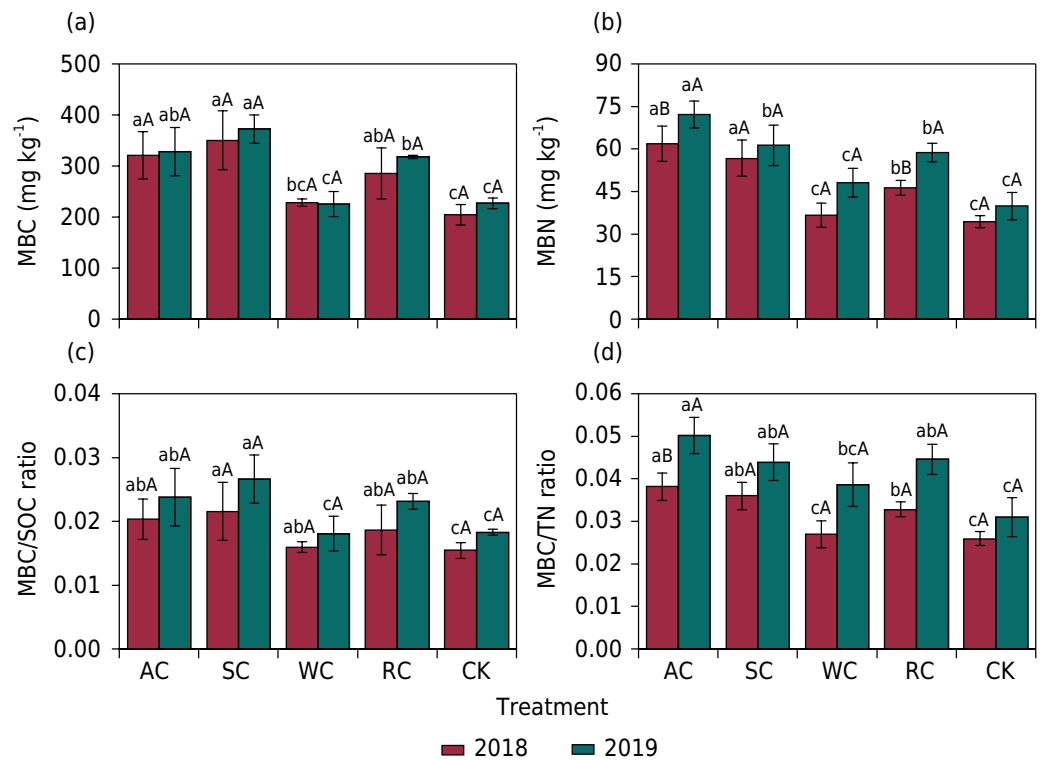


Figure 3. Changes of MBC (a), MBN (b), MBC/SOC ratio (c), and MBN/TN ratio (d) under different treatments. MBC: Microbial Biomass Carbon; MBN: Microbial Biomass Nitrogen; MBC/SOC ratio: ratio of microbial biomass carbon to soil organic carbon; MBN/TN ratio: ratio of microbial biomass nitrogen to total nitrogen. AC: alfalfa cover; SC: sweetclover cover; WC: winter wheat cover; RC: ryegrass cover; CK: bare land. Different lowercase letters indicate significant differences among the treatments within the same year (Duncan's new multiple range test; $p < 0.05$), and different uppercase letters indicate significant differences among the years within the same treatment (t-test; $p < 0.05$). Bars represent the standard error.

than that under other treatments, respectively; and WC treatment showed the maximum number of actinomycetes colonies (5.72 and $5.82 \log_{10} \text{CFU g}^{-1}$). The bacteria and actinomycetes colonies in 2019 were higher than that in 2018; however, the fungi colonies in 2019 were lower than that in 2018.

Soil microbial diversity

Shannon index and Richness index under different treatments showed the order of $\text{AC} > \text{SC} > \text{RC} > \text{WC} > \text{CK}$ (Figures 4a and 4b). The two indices under AC treatment were

Table 1. Changes in the number of microbial colonies in soil under different treatments

Treatment	Bacteria number		Fungi number		Actinomycetes number	
	2018	2019	2018	2019	2018	2019
	$\log_{10} \text{CFU g}^{-1}$					
AC	6.86 ± 0.01 aB	6.95 ± 0.02 aA	4.91 ± 0.04 abA	4.86 ± 0.01 aA	5.67 ± 0.05 abA	5.70 ± 0.06 bcA
SC	6.81 ± 0.05 aA	6.93 ± 0.03 aA	4.94 ± 0.05 aA	4.88 ± 0.04 aA	5.65 ± 0.05 abA	5.77 ± 0.02 abA
WC	6.68 ± 0.07 bA	6.66 ± 0.04 cA	4.83 ± 0.03 bA	4.70 ± 0.01 bA	5.59 ± 0.04 bB	5.68 ± 0.05 cA
RC	6.72 ± 0.03 bA	6.79 ± 0.07 bA	4.88 ± 0.07 abA	4.83 ± 0.04 aA	5.72 ± 0.05 aB	5.82 ± 0.03 aA
CK	6.37 ± 0.06 cA	6.56 ± 0.07 dA	4.70 ± 0.06 cA	4.67 ± 0.03 bA	5.63 ± 0.03 bA	5.57 ± 0.02 dA

AC: alfalfa cover; SC: sweetclover cover; WC: winter wheat cover; RC: ryegrass cover; CK: bare land. Values are means of three replications \pm standard error. Means in each column followed by a different lowercase letter denote significant differences among the treatments within the same year (Duncan's new multiple range test; $p < 0.05$); Means in each row followed by a different uppercase letter denote significant difference among the years within the same treatment (t-test; $p < 0.05$).

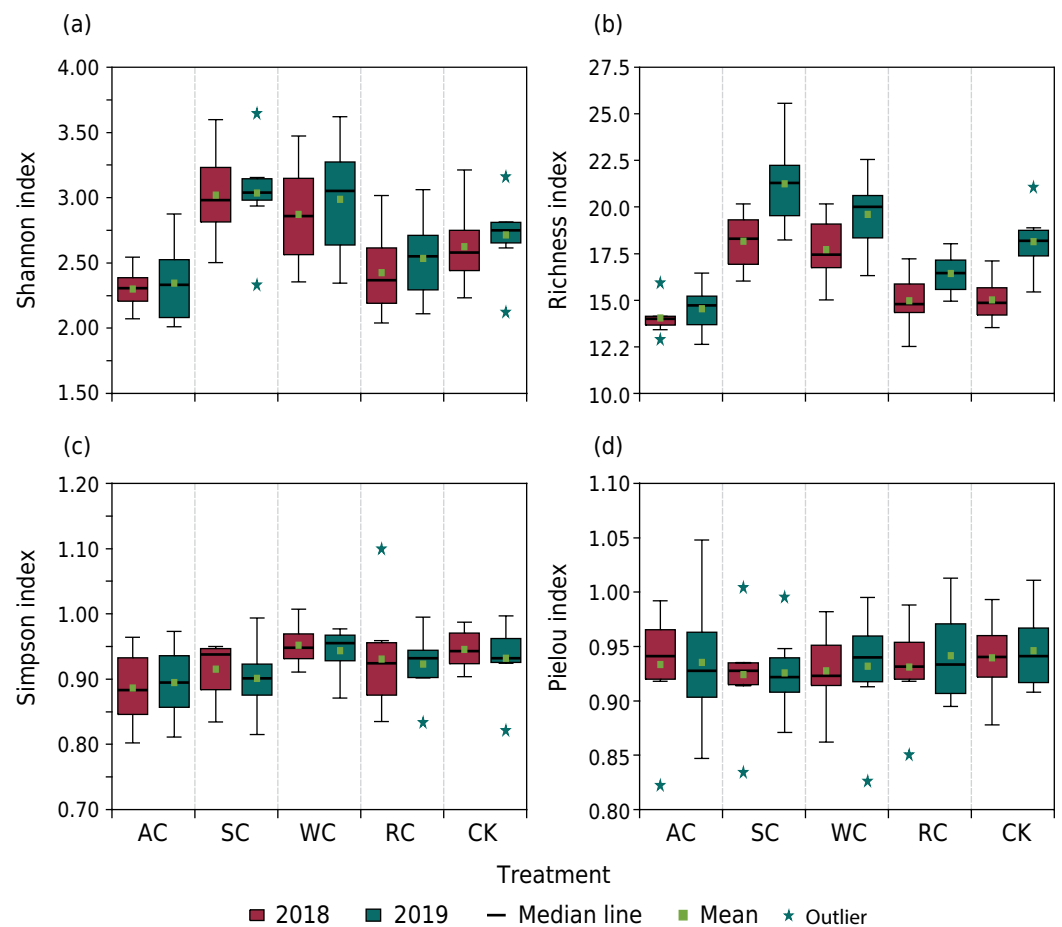


Figure 4. Changes of Shannon index (a), Richness index (b), Simpson index (c), and Pielou index (d) under different treatments. The box-whisker plot shows the quartiles with the median line and means (box), 1.5 times of the inter-quartile range (whiskers), and outliers. AC: alfalfa cover; SC: sweetclover cover; WC: winter wheat cover; RC: ryegrass cover; CK: bare land.

significantly higher than those under RC, WC, and CK treatments ($p < 0.05$). The Simpson index (Figure 4c) was the largest in SC treatment, and the Pielou index was highest in RC treatment (Figure 4d). The diversity indices under all treatments in 2019 were higher than those in 2018.

Carbon source utilization by soil microbial communities

The Average Well Color Development (AWCD) is an important indicator to reflect the capability of microbial communities to utilize carbon sources. Under different treatments, there were differences in utilization patterns to six types of carbon sources which had been incubated for 96 h on Biolog EcoPlates™ (Figure 5). Under AC and SC treatments, the main carbon sources utilized by microbial communities were amino acids (percentages of utilization ranged from 27 to 32 %), carbohydrates (ranged from 25 to 32 %), and amines (ranged from 24 to 33 %) (Figures 5c and 5d), and the AWCD values of carbon source were significantly higher than those under other treatments ($p < 0.05$) (Figures 5a and 5b). Under RC treatment, microbial communities had the highest AWCD values (Figures 5a and 5b) and the percentage utilization of phenolic compounds (ranged from 27 to 29 %) and carboxylic acids (28 %) (Figures 5c and 5d).

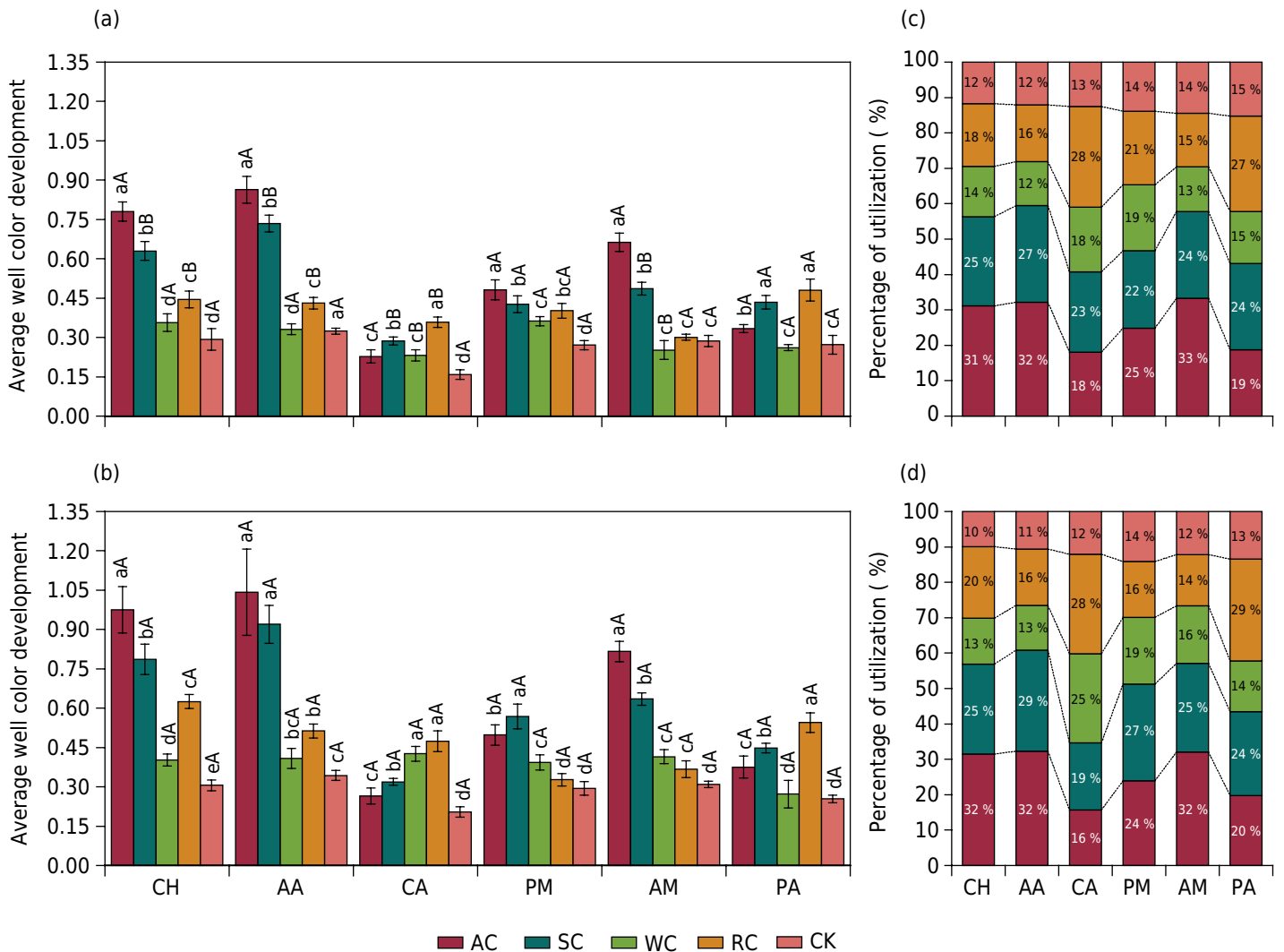


Figure 5. Changes of Average Well Color Development (AWCD) of carbon source in 2018 (a), Average Well Color Development (AWCD) of carbon source in 2019 (b), percentage of sole carbon source utilization in 2018 (c), and percentage of soil carbon source utilization in 2019 (d). AC: alfalfa cover; SC: sweetclover cover; WC: winter wheat cover; RC: ryegrass cover; CK: bare land; CH: carbohydrates; AA: amino acids; CA: carboxylic acids; PM: polymers; AM: amines; PA: phenolic acid compounds. Different lowercase letters represent significant differences among the treatments within the same year (Duncan's new multiple range test; $p < 0.05$), and different uppercase letters represent significant differences among the years within the same treatment (t-test; $p < 0.05$).

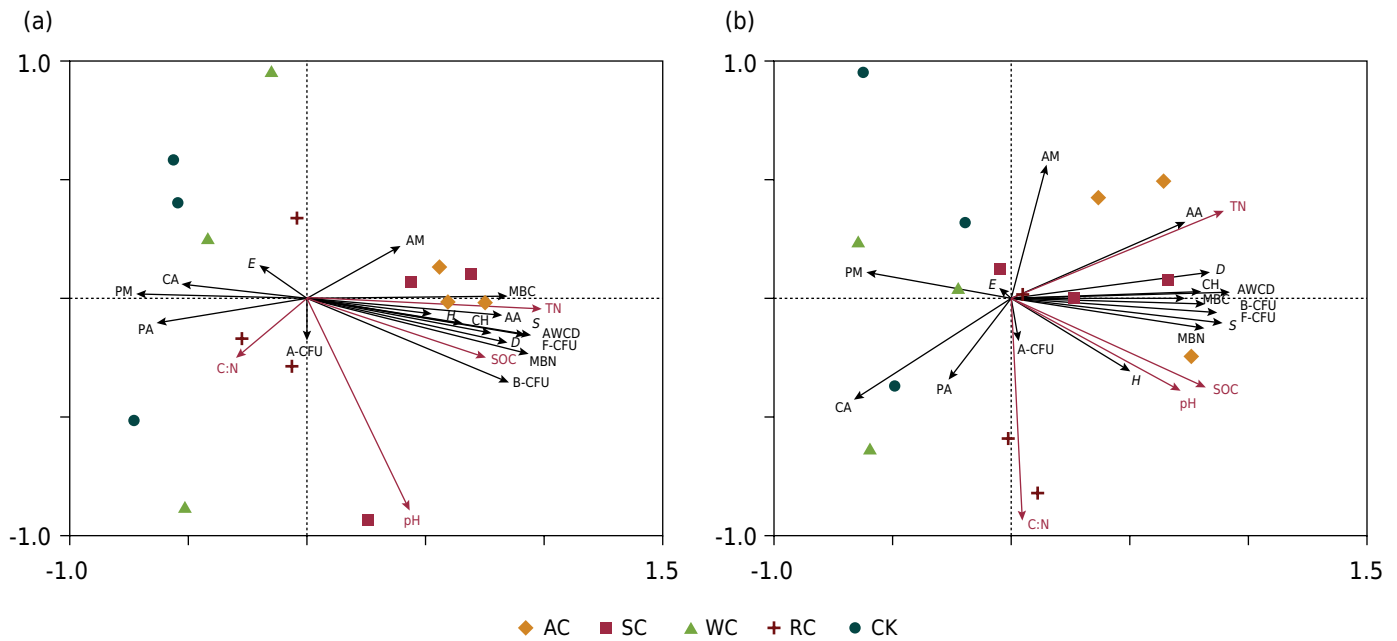


Figure 6. Redundancy analysis (RDA) of soil microbial variables vs. soil environmental variables in 2018 (a) and microbial variables vs. environmental variables in 2019 (b). Plots in the plane of the first two axes showed the relations between the microbial variables (blue arrows) and environmental variables (red arrows). Microbial variables include AWCD (average well color development); the percentage of carbon source utilization of CH (carbohydrates), AA (amino acids), CA (carboxylic acids), PM (polymers), AM (amines), PA (phenolic acid compounds); *H* (Shannon index), *D* (Simpson index), *S* (Richness index), *E* (Pielou index); MBC (microbial biomass carbon); MBN (microbial biomass nitrogen); B-CFU (bacteria colonies), F-CFU (fungi colonies), and A-CFU (actinomycetes colonies). Environmental variables include SOC (soil organic carbon), TN (total nitrogen), C/N (ratio of SOC to TN), and pH (soil pH value).

The AWCD values of six types of carbon sources in 2019 (Figure 5b) were higher than that in 2018 (Figure 5a).

Relationships between soil microorganisms and soil properties

The RDA biplots showed that the SOC, TN, and pH were positively correlated to the contents of MBC and MBN, fungi and bacteria colonies, AWCD values, the indices of Simpson, Shannon and Richness, and the percentages of carbon source utilization (carbohydrates, amino acids, and amines) (Figure 6). However, the C/N ratio was negatively correlated to these microbial parameters ($r = -0.3302$ and -0.1048 , $p = 0.032$) in 2018. The actinomycetes colonies were less affected by soil chemical properties. The relationship between soil microbial and environmental variables in 2019 (Figure 6b) was similar to that in 2018 (Figure 6a). The AC and SC treatments were mainly distributed in the first and fourth quadrants.

DISCUSSION

Effects of winter cover crops on soil microbial characteristics

To reveal the effects of four winter cover crops on the microbial characteristics of the surface soil (0.00-0.20 m) during the winter fallow period, we conducted a field experiment from 2017 to 2019 in the semi-arid soils of northern China. The results showed that under cover of legume crops (AC and SC), the activities of urease (Figure 2a), dehydrogenase (Figure 2b) and protease (Figure 2c), the contents of MBC (Figure 3a) and MBN (Figure 3b), the log number of bacteria and fungi colonies (Table 1), the microbial diversity indices of Shannon (Figure 4a), Richness (Figure 4b), and Simpson (Figure 4c), and the AWCD values of microbial communities to utilize carbon source of amino acids, carbohydrates and amines (Figure 5) were higher than those under

cover of cereal crops (RC and WC). The findings suggested that the soil microbial characteristics were different for the four cover crops, and there were higher activity and diversity in soil microorganisms under the legume cover crops in this region, and thus further verified our initial hypothesis.

Soil enzyme activities are important indicators for characterizing the activity of microbial metabolisms in soil (Hamido and Kpombekou, 2009; Calderon et al., 2016). Soils covered by alfalfa and sweetclover had the largest values of dehydrogenase, urease, and protease activities (Figure 2), suggesting that the microorganisms under the legume cover crops had good redox capability, high metabolic activity, and strong mineralization of nitrogen; so, there was a high capability of converting organic N to available N in soil under the two treatments, indicating that the soils had a large inventory of active nitrogen. It is worth noting that the activities of four enzymes in 2019 were higher than that in 2018 (Figure 2), which may be due to the monthly average temperature and total precipitation in spring 2019 (from March to April) were higher than that in the same period in 2018 (Figure 1). The higher temperature and precipitation were conducive for accelerating the decomposition of crop residues in surface soil, providing more substrates for microorganisms (Khan et al., 2018), and thus increasing the metabolic activity of microorganisms.

As the most active part of soil organic matter, MBC, and MBN contents are closely related to nutrient cycling, so they are important indicators to reflect soil environmental quality (Austin et al., 2017; Coombs et al., 2017). Studies in different climatic regions showed that cover crops significantly increased soil MBC and MBN levels compared to bare soil (Frazão et al., 2010; Nair and Ngouajio, 2012). This study found that the highest MBC and MBN values existed in AC and SC treatments (Figures 3a and 3b). The C/N ratio of the residues (litter and dead fine roots) of legume cover crops was low (Roldán et al., 2003), so the residues in the soil reduced the C/N ratio of the soil, provided a large amount of readily decomposable organic substrates for soil microorganisms (Ordóñez-Fernández et al., 2018), promoted the growth and reproduction of microorganisms (Hontoria et al., 2019), and increased the microbial biomass (Liang et al., 2014). The AC and SC treatments also showed the largest ratios of MBC/SOC and MBN/TN (Figures 3c and 3d), indicating that microorganisms in soil covered by legume crops could fix more nutrients and increase the availability of C and N nutrients in the soil (Manici et al., 2018).

The log number of microbial colonies in the soil is an important indicator of soil fertility and health (Franchini et al., 2007; Balota et al., 2014; Mangalassery et al., 2015; Nivellet al., 2016). We found the maximum numbers of bacteria and fungi colonies recorded in AC and SC treatments (Table 1). During the growth of legumes, a large number of fine roots and nodules died, became a part of the soil, and contributed to increasing the number of rhizobium bacteria (Dubach and Russelle, 1994) and arbuscular mycorrhizal fungi (Hontoria et al., 2019) in the soil. While, the number of actinomycetes colonies in AC and SC treatments was lower than that in WC treatment; and according to RDA analysis, SOC and TN content had little influence on actinomycetes colonies (Figure 6), suggesting that soil actinomycetes in the sandy area of Northern Shaanxi might have stronger tolerance to the adverse environment (Bhatti et al., 2017). The bacteria colonies in 2019 were higher than those in 2018, while the fungi colonies in 2019 were lower than that in 2018 (Table 1), and that might be due to the antagonism between fungi and bacteria (Bahram et al., 2018).

Microbial communities had higher diversity and richness in soils covered by alfalfa and sweetclover which have the largest indices of Shannon and Richness (Figures 4a and 4b); however, there was the smallest Pielou index existed in the two legume cover crops (Figure 4d), implying the existence of dominant species of bacteria and fungi. The RDA analysis (Figure 6) showed that soil chemical properties were positively correlated with the percentage of utilization by microbial communities to amino acids, carbohydrates, and amines, indicating that good soil conditions promoted more

participation of microorganisms in C and N cycling. However, the above-mentioned chemical properties were inversely correlated to the percentage of utilization by microbial communities to phenolic compounds, polymers and carboxylic acids, indicating that adverse environmental conditions had little influence on microbial decomposition of the three types of carbon sources.

Effects of winter cover crops on soil quality

Soil microbial characteristics are important parameters for evaluating the quality of soil environment (Hontoria et al., 2019), and RDA analysis (Figure 6) showed that the two legume cover crops had a positive effect on soil environmental quality. Due to AC treatment with the highest soil MBN/TN ratio (Figure 3d), the microbial metabolism was vigorous, decomposition and mineralization ability were strong, and increased nutrient availability in topsoil (Lagomarsino et al., 2009; Balota et al., 2014; Liang et al., 2014; Plaza-Bonilla et al., 2016). The WC treatments had negative effects on the soil environment (Figure 6). The MBC and MBN contents, enzyme activities, bacteria, and fungi colonies, microbial diversity indices (except for Pielou index) were significantly lower than that under other winter cover crops ($p < 0.05$), indicating the inventory C and N in soil was too low to meet the demand of microorganisms for growth and reproduction, so the overall quality of soil lowered. Some studies have found that ryegrass cover is helpful to improve the environmental quality of soil (Pantoja et al., 2016; Alvarez et al., 2017; Snapp and Surapur, 2018), whereas in this study the microbial parameters in soil (except for sucrase activity and Pielou index) covered by ryegrass were lower than that covered by legume cover crops. Therefore, ryegrass was less effective than legume cover crops in improving soil quality in sandy areas of Northern China. In the 2017-2019 experiment, the effects of four winter cover crops on soil microbial parameters showed the same trend. Remarkably, as the temperature and precipitation in spring 2019 were higher than those in 2018 (Figure 1), the soil microorganisms had higher activity and diversity, which may accelerate the cycling of C and N nutrients in the soil. Therefore, the cover crop strategy was affected by climate change in the fallow period, but the irrigation conditions in this region may weaken this part of the impact.

Varieties with strong resistance against cold and drought should be selected as winter cover crops for winter fallow fields in sandy areas of Northern Shaanxi. Many studies showed that in the humid and semi-humid areas (Alvarez et al., 2017), crops with strong cold tolerance were often used as green manure (Ćupina et al., 2017), which could be turned into the soil after decomposing to provide nutrients for crops sowed in summer. In the sandy area of Northern Shaanxi, many crops need to be planted in spring (April or May). If the winter cover crops are buried in the soil, there may be no enough time and temperature to decompose fully, and that may not be conducive to the growth of the later crops (e.g., pest and disease); so, the cover crops should be considered to be included in the rotation system.

CONCLUSIONS

Planting legume cover crops (alfalfa and sweetclove) in winter fallow soils in the sandy area of Northern Shannxi is able to improve soil enzyme activity, facilitate the growth and reproduction of soil microorganisms, promote microbial metabolic activity, diversity and richness, enhance the carbon source utilization capability of microbial communities, and increase the availability of C and N in the soil. Therefore, legume cover crops could exert a more positive effect on soil quality than cereal cover crops could do. This finding may be helpful for further understanding of the microbial mechanism of winter cover crops in mitigating soil degradation in the fallow soils.



APPENDIX A. SUPPLEMENTARY DATA



Supplementary data to this article can be found online at https://www.rbcjournal.org/wp-content/uploads/articles_xml/1806-9657-rbcs-44-e0190173/1806-9657-rbcs-44-e0190173-suppl01.pdf

ACKNOWLEDGMENTS

This research was financially supported by the National Natural Science Foundation of China (Grant No. 41763011), the Technology Department of Shaanxi Province (Grant No. 2015NY017), the Education Department of Shaanxi Province (Grant No. 16JK1893), and the of Yulin University (Grant No. 14GK41).

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