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Article

Forage mixed planting can effectively improve soil enzyme activity and microbial community structure and diversity in agro-pastoral interlacing arid zone

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Abstract

Aiming at the problems of serious soil desertification and continuous reduction of effective soil nutrients in the agricultural and animal husbandry interlaced arid area in Inner Mongolia, this study used Aohan alfalfa, old awn wheat, and fodder oats at the Siziwang Banner, Ulanchabu City, Inner Mongolia Autonomous Region. There were a total of five treatments, namely, single seeding alfalfa, single seeding old awn wheat, single seeding fodder oats, alfalfa and old awn wheat mixed sowing, and control. The results showed that the urease activity and microbial biomass nitrogen content of mixed planting were higher than other treatments. With the advancement of the growth period, the bacteria α-diversity index showed an upward trend; β-diversity analysis showed that planting method was the main factor affecting bacteria diversity and sampling time was the main factor affecting fungi diversity. In summary, mixed planting treatment was more helpful to improve soil enzyme activity, microbial biomass, and enrich soil microbial diversity, which was of great significance to maintain the balance of soil ecosystem. It is hoped that this study will contribute to the theoretical basis and practical experiences for efficient utilization of microbial resources in the field of soil improvement.

Key words: herbage, planting pattern, microbial diversity, enzyme activity

Résumé

Dans l'espoir de résoudre de sérieux problèmes de désertification et de perte continue des oligoéléments dans le sol d'un région aride de la Mongolie intérieure consacrée à la fois à l'agriculture et à l'élevage, les auteurs ont semé de la luzerne Aohan, une antique variété de blé barbu et de l'avoine fourragère dans la région autonome de Siziwang Banner, à Ulanchabu. Les traitements étaient au nombre de cinq : semis simple de luzerne, semis simple de blé barbu, semis simple d'avoine fourragère, semis de luzerne et de blé barbu, et témoin. Les résultats indiquent que la plantation mixte favorise une plus grande activité de l'uréase et une hausse de la concentration d'azote venant de la biomasse microbienne, comparativement aux autres traitements. À mesure qu'on progresse dans la période végétative, l'indice α de diversité des bactéries affiche une tendance à la hausse. L'analyse de l'indice de diversité β révèle que la technique de plantation est le paramètre principal qui affecte la diversité des bactéries, alors que le moment de l'échantillonnage est celui qui influe le plus sur la diversité des cryptogames. Bref, les semis mixtes accroissent davantage l'activité enzymatique dans le sol, la biomasse microbienne et la diversité des unicellulaires, ce qui revêt une grande importance pour équilibrer l'écosystème tellurique. Les auteurs espèrent que leur étude ajoutera aux fondements théoriques et à l'expérience pratique existants en vue d'un usage efficace des ressources microbiennes pour l'amélioration du sol. [Traduit par la Rédaction]

Mots-clés : herbage, mode de plantation, diversité microbienne, activité enzymatique

1. Introduction

In recent years, due to human factors such as overgrazing, reclamation, and irrational use of farmland, soil desertification in the ecotone has become increasingly serious, and soil degradation and agricultural area have reduced crop productivity (Tuo et al. 2018). The decline of land quality caused by desertification has a significant impact on the ecological environment and the sustainable development of human society (Lyu et al. 2020; Zeng et al. 2021). Effective control of the rate of soil degradation urgently needs to be put on the agenda. Research shows that planting patterns not only affect crop growth but also significantly affect soil biological properties (Wang et al. 2021). Reasonable planting patterns can improve land quality and soil biological properties (Chen et al. 2019).

Therefore, different planting patterns can be used as one of the important ways to improve soil desertification.

Soil enzymes are considered to be key soil components that catalyze important transformations related to decomposition and nutrient turnover (Cwab et al. 2021; Nannipieri et al. 2018). At present, the research on the factors affecting the soil enzyme activity mainly focuses on the physical and chemical properties of soil, soil types, plant communities, fertilization and farming, other agricultural measures, and external factors, and there are few studies on the effects of different planting methods (Wang et al. 2021). In addition, under farmland conditions, changes in crops and planting methods may affect soil microbial community composition, microbial diversity and activity (Bolo et al. 2021; Zuber and Villamil 2016; Vimal et al. 2017). In recent years, research on soil microorganisms has received extensive attention. Many studies have reported on the diversity and distribution characteristics of bacteria communities in desert soils in different regions of China. It has been found that the diversity, structure, and composition of bacterial communities in different regional types of desert soils were different in the dominant flora (Zhang et al. 2018; Zuo et al. 2019; Kang et al. 2021).

To understand the effects of different planting methods of forage on some biological properties of soil in the agro-pastoral ecotone arid area of Inner Mongolia, five treatments were set up under the two planting methods of unicast and mixed sowing. The changes in soil enzyme activity, microbial biomass, and microbial diversity in different periods have been compared and analyzed by a series of methods such as fumigation culture and high-throughput sequencing. The purpose of this study was to (I) study the dynamic changes in soil enzymes and microbial biomass in different treatments in different periods; (II) study the composition and abundance of bacteria and fungi in soil under different treatments; (III) explore the relationship between soil enzymes, microbial biomass, and microbial diversity.

2. Materials and methods

2.1. Experimental site

The experimental site is located in Siziwang Banner, Wulanchabu City, central Inner Mongolia Autonomous Region. It is located in the mid-temperate continental monsoon climate zone. The annual average temperature is 1–6 °C. The average frost-free period is 108 days. The annual average precipitation is 110–350 mm. The soil is mostly chestnut soil. The content of soil organic matter in this area is 22.96–28.74 g/kg. The total nitrogen content is 1.39–1.86 g/kg, the alkali hydrolyzable nitrogen content is 79.44–100.89 mg/kg, and the available potassium content is 189.09–208.67 mg/kg.

2.2. Experimental materials

Forage materials include taproot-type high-quality legume forage Aohan alfalfa (*Medicago sativa* cv. 'Aohan'), rhizome-type perennial gramineous forage (*Elymus sibiricus* L.), and annual forage oat grass (*Avena sativa*). The above materials were provided by the Inner Mongolia Grass Industry Research Center of the Chinese Academy of Sciences. Fertilizer urea 75 kg/hm^2 and diammonium phosphate 150 kg/hm^2 were applied during sowing. Five treatments were set up in the experimental field, which were unicast alfalfa (DM), unicast rye (DL), unicast forage oat (SYYM), mixed sowing of alfalfa and rye (HB), and control treatment (CK). After deep ploughing (25 cm) and harrowing, the seeds shall be sown by manual trenching and drilling, and the row spacing shall be 20 cm. Each treatment was repeated three times for a total of 15 plots with an area of 50 m^2.

2.3. Experimental design

Soil samples were collected to determine enzyme activity, microbial biomass, and microbial diversity. In each sampling area, soil samples were collected with a soil drill at the depths of 0–10, 10–20, and 20–40 cm, respectively, and the soil samples were brought back to the laboratory. Some samples were naturally air-dried and screened for 1 mm for soil enzyme determination; the other part was directly stored at −20 °C after being screened for 2 mm, and then used for the determination of microbial biomass. In the determination of microbial diversity, CK, DM, DL, and HB were selected. 0–20 cm soil layer was taken for each treatment, and a total of 24 samples were obtained. Removed 0–5 cm of floating soil before sampling. After collecting the soil, removed stones and plant residues from the surface. Each treated soil sample was screened through a 2 mm sieve, and 5–10 g of soil sample was put into a centrifuge tube for rapid liquid nitrogen freezing, and then put into an ultralow temperature refrigerator (DW-HL398, Anhui, China) at −80 °C for DNA extraction and high-throughput sequencing. All samples in this test were collected by random sampling method.

2.4. Measurement index and method

Soil catalase activity was determined by potassium permanganate titration, soil urease activity by indophenol blue colorimetry, soil invertase activity by 3,5-dinitrosalicylic acid colorimetry, and soil alkaline phosphatase activity by phenylenedisodium phosphate colorimetry (Guan 1986; Zhou 1989; Franke et al. 2015). The contents of soil microbial biomass carbon and microbial biomass nitrogen were determined by the fumigation culture method (Vance et al. 1987).

For DNA extraction, the soil sample was taken from the refrigerator and centrifuge for 2 minutes to remove the supernatant and sediment. Then DNA was extracted according to the steps recommended by Omega (Omega Bio-Tek, USA). The successfully extracted samples were labeled and stored in a 1.5 mL centrifuge tube and stored in a refrigerator at −20 °C until 16S rRNA and ITS rRNA sequencing. Subsequently, 16S rRNA, ITS rRNA, and bioinformatics analysis were carried out in Shanghai Meiji biological company. According to the standard operating procedures of Illumina Mi SEQ platform (Illumina, San Diego, USA), the miseq pe300 platform library of Illumina company was used for construction and sequencing.

2.5. Data analysis

Excel was used for data processing of soil enzyme activity and microbial biomass, and SPSS 17.0 and graphpad prism 8 were used for mapping and data analysis. The composition

and diversity of soil microbial community were analyzed by i-sanger Shengxin cloud platform.

Using trimmatic (version 0.35) software, for the original data sequence generated by Illumina miseq sequencing, first scan the sequence that used the sliding window method. When the quality was lower than 20, the sliding window with the average base quality lower than the threshold was cut off, and the sequence with the length less than 50 bp was removed. Use flash (version 1.2.11) software to splice the qualified double ended raw data in the previous step. The maximum overlap during sequence splicing was 200 bp to obtain the complete paired end sequence. Use split in qiime_Libraries (version 1.8.0); the software removed the sequence containing N base in the paired end sequence, removed the sequence with single base repeat greater than 8, and removed the sequence with length less than 200 bp to obtain the clean tag sequence. Use uchime (version 2.4.2); the software removed the chimera in the clean tags and finally obtained the valid tags for the subsequent OTU division.

Qime2 (2019.4) software was used to calculate the microbial alpha diversity index, and the R language ggplot2 package was used to draw the visual map of the composition distribution of samples at the phylum classification levels, and Wilcox rank sum test in Agricola software package was used to analyze the difference between groups of alpha diversity index. The R software draws principal coordinate analysis (PCoA) diagram analysis.

3. Results

3.1. Effects of different forage planting patterns on soil enzyme activities

In the whole growth period of forage, the activities of catalase, urease, sucrase, and alkaline phosphatase in 0–40 cm soil layer under each planting mode were higher than control. Except catalase, the activities of other enzymes peaked in July. From May to August, catalase activity in all soil layers showed an upward trend. Except for the CK, the catalase activity of each treatment from July to August was significantly higher than that from May to June (Figs. 1*a*–1*c*). In 0–40 cm soil layer, the urease activity of mixed sowing treatment was higher than that of other treatments at each stage (Figs. 1*d*–1*f*). With the extension of sampling date, the sucrase activity of each treatment decreased first and then increased, and the enzyme activity in August was higher than that in May (Figs. 1*g*–1*i*). In 0–10 and 10–20 cm soil layers, the alkaline phosphatase activity of mixed sowing treatment was higher than that of other treatments. With the advance of sampling date, the enzyme activity first decreased, then increased, and then decreased (Figs. 1*j*–1*l*).

3.2. The influence of different planting ways of forage on soil MBC, MBN

The MBC content of each treatment in August was higher than that in May. There was no significant difference in MBC content of each soil layer in May, and the MBC content of mixed sowing treatment in June and July was higher than other treatments. In 0–10 cm soil layer in June and July, and 20–40 cm soil layer in July, the MBC content of mixed sowing treatment was higher than other treatments. In August, the MBC content of all soil layers treated with CK was the highest (Figs. 2*a*–2*d*).

Except in May, the MBN content of mixed sowing treatment was higher than that of other treatments. In addition, the MBN content in each soil layer in June, 10–20 cm soil layer in July, and each soil layer in August was higher than that in other soil layers. Except for the mixed sowing treatment, the MBN content of 10–20 cm soil layer in August was lower than that in May (Figs. 2*e*–2*h*).

3.3. Effects of different forage planting patterns on soil microbial species composition

With the advance of growth period, the number of common fungi in different planting methods decreased with time, the number of specific fungi in DM and CK treatment increased, the number of specific fungi in DL treatment decreased, and the changes of DM and HB were not obvious. The number of fungi in HB treatment was the least in August (Figs. 3*a* and 3*b*). From July to August, the number of common bacteria in different planting methods decreased. Except DM, the number of unique bacteria in other treatments increased, and the HB treatment increased the most. In August, the number of bacteria treated by HB was the largest (Figs. 3*c* and 3*d*).

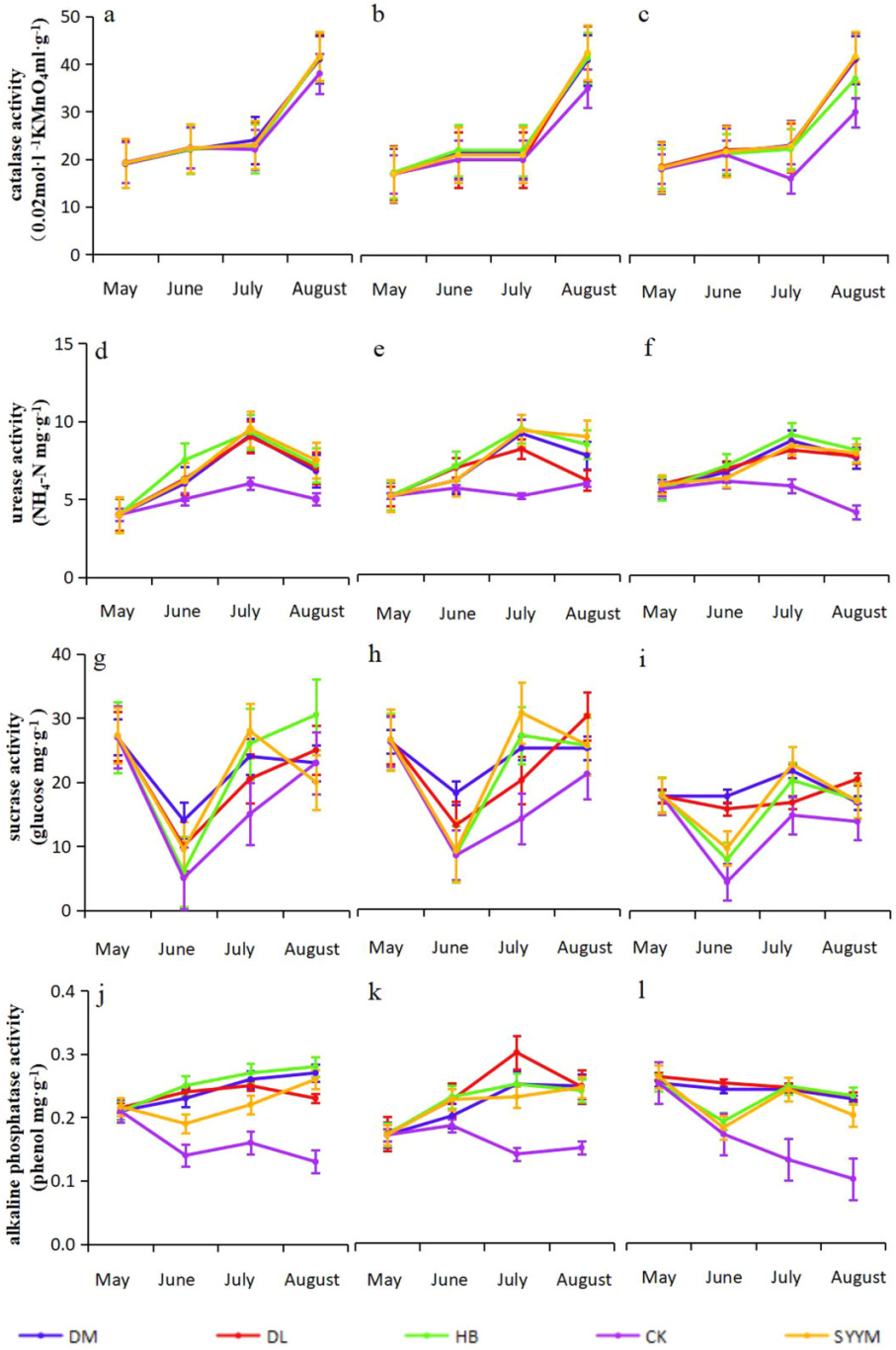
The results showed that the dominant phylum of fungi was Ascomycota. The relative content of Ascomycota in HB treatment was low in July. In August, the relative content of Ascomycota in HB treatment increased significantly (Figs. 4*a* and 4*b*). Under different planting methods, Proteobacteria and Actinobacteria were always the dominant bacteria. The bacteria with relative abundance >5% at the phylum level also included *Chloroflex*, *Acidobacteria*, and *Firmicum*. The relative proportion of Proteobacteria in August was significantly higher than that in July. In July, the relative abundance of *Chloroflex*, *Acidobacteria*, and *Firmicum* in the mixed sowing treatment was higher than that in the CK (Figs. 4*c* and 4*d*).

3.4. Effects of different forage planting patterns on soil microbial diversity

Except ace index, there was no significant difference in fungi diversity index among treatments. The fungi diversity index in August was generally lower than that in July, and the fungi diversity index in August was lower than that in other treatments. The bacteria α-diversity index of DM and HB treatment in July was lower than that in August, but CK and DL treatments were opposite. In July, the bacteria α-diversity index of each planting mode was higher than CK, and in August, the bacteria α-diversity index of DM and DL treatments was higher than CK and HB (Table 1).

PCoA analysis of fungi and bacteria communities under different planting patterns showed that sampling time was an important factor affecting fungi community ($R^2 = 0.02993$), and planting pattern was an important factor affecting bacteria community ($R^2 = 0.1414$). The difference of fungi community in July was greater than that in August (see

Fig. 1. The effect of different planting patterns of forage grass on soil catalase activity (0.02 mol/L $KMnO_4$ mL/g) urease activity (NH_4–N mg/g) sucrase activity (glucose mg/g soil) alkaline phosphatase activity (phenol mg/g). (*a*–*c*) Catalase activities in 0–10, 10–20, and 20–40 cm soil layers respectively; (*d*–*f*) urease activities; (*g*–*i*) sucrase activities; (*j*–*l*) alkaline phosphatase activities. The blue icon represents DM processing, the red icon represents DL processing, the green icon represents HB processing, the purple icon represents CK processing, and the yellow icon represents SYYM processing. [Color online]



Appendix A for relevant analysis results). Compared with each treatment, DM treatment was discrete in July and August. There was no significant difference in fungi community between DL treatment and CK treatment, and the difference between HB and CK decreased in August and July (Figs. 5*a*, 5*b* and 5*e*). The difference of bacteria community in August was greater than that in July (see Appendix A for relevant analysis results). There was no significant difference between DM and HB in July, and CK and DL were relatively discrete. In August, except HB, DL, DM, and CK were relatively concentrated. It can be seen that with the extension of growth period, the difference of bacteria community between HB treatment and other planting methods is more and more obvious (Figs. 5*c*, 5*d* and 5*f*).

Fig. 2. The effect of different planting methods on MBC and MBN at different stages (mg/kg). (*a*–*d*) Represent the content of MBC under each treatment in May, June, July, and August. (*e*–*h*) Represent the content of MBN under each treatment in May, June, July, and August. [Color online]

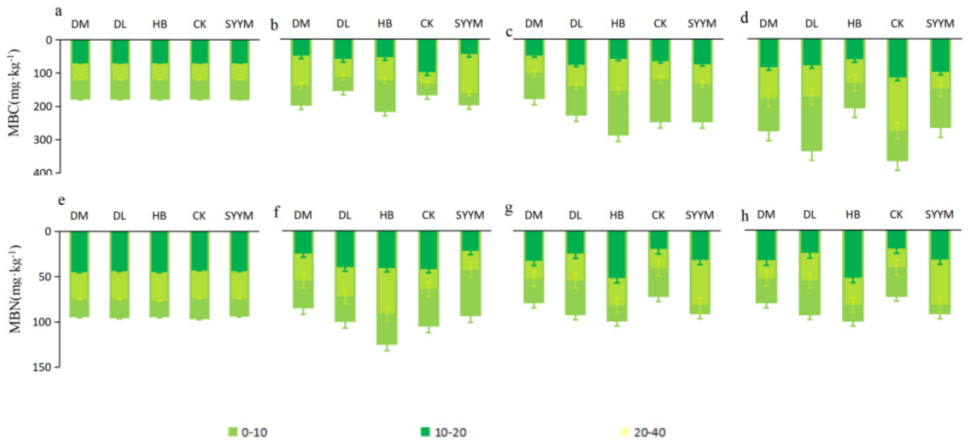
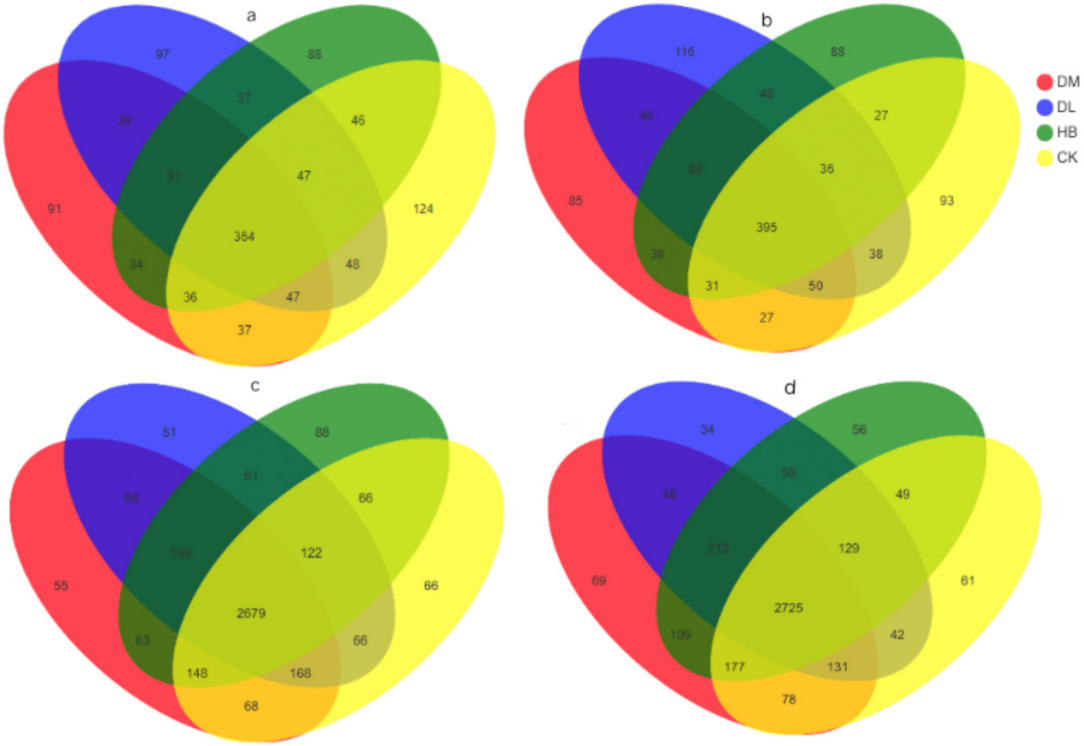


Fig. 3. The Venn map of fungi and bacteria in July and August. (*a*, *b*) The Venn map of fungi in July and August under different treatments. (*c*, *d*) The Venn map of bacteria in July and August under different treatments. [Color online]



3.5. Correlation analysis of soil enzyme activity and microbial biomass with soil microbial diversity.

In the same period, the four enzymes were positively correlated with each other, and urease, sucrase, and alkaline phosphatase were extremely positively correlated with each other, while MBN and MBC were negatively correlated. There was no significant correlation between soil enzyme activity and microbial biomass. Catalase was positively correlated with MBC and negatively correlated with MBN, while urease, sucrase, and alkaline phosphatase were on the contrary (Table 2).

In the fungi part, the four enzymes were negatively correlated with the three indexes, and the Shannon index was extremely negatively correlated with urease and sucrase. MBC was positively correlated with Shannon index and negatively correlated with sobs index and Chao index. MBN was positively correlated with the three indexes (Table 2).

In the bacteria part, catalase was negatively correlated with Sobs and Chao index, sucrase was negatively correlated with Chao index, and other enzymes were positively correlated with the three indexes, among which Shannon was extremely positively correlated with sucrase and alkaline phosphatase.

Fig. 4. Community composition of fungi and bacteria in July and August. (*a*, *b*) The community composition diagram of fungi in July and August under different treatments. (*c*, *d*) The community composition diagram of bacteria in July and August under different treatments. [Color online]

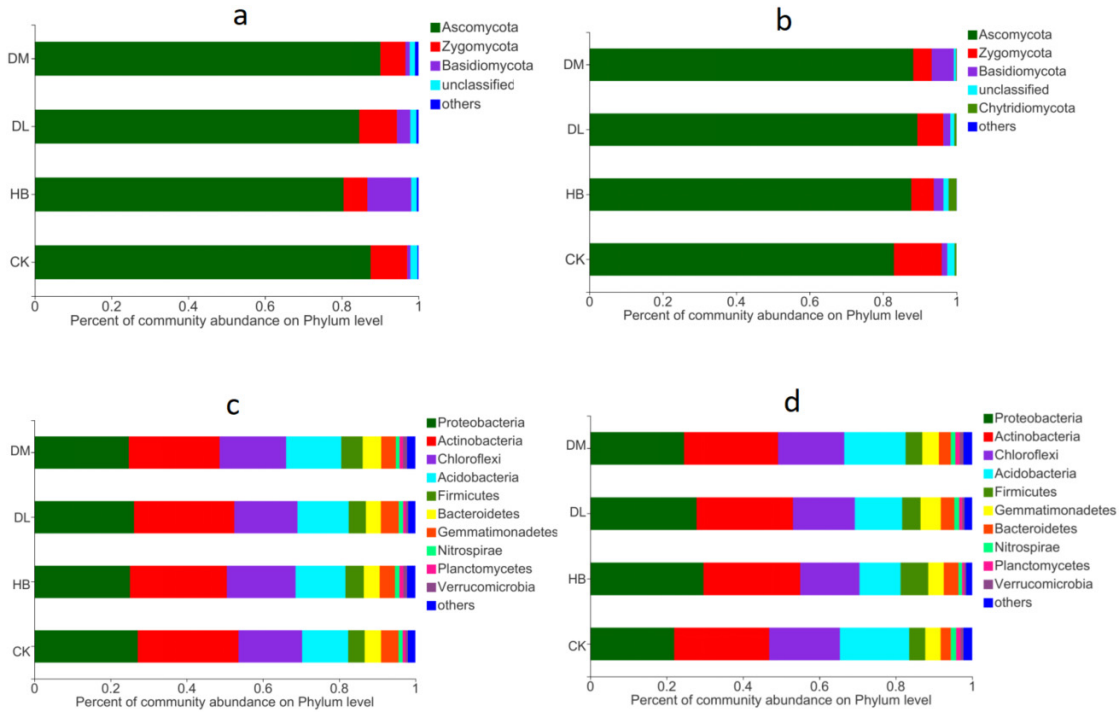


Table 1. Alpha diversity index table.

		Samples	Sobs	Shannon	Ace	Chao	Coverage
Fungi	July	CK	418 ± 37.24a	3.64 ± 0.42a	491 ± 5.78a	497 ± 5.28a	99.48%
		DM	463 ± 35.69a	2.53 ± 0.29a	580 ± 53.26a	595 ± 80.43a	99.77%
		DL	516 ± 6.13a	3.56 ± 0.16a	605 ± 12.39a	597 ± 3.57a	99.80%
		HB	447 ± 83.69a	2.93 ± 0.65a	541 ± 65.11ab	537 ± 70.02a	99.81%
	August	CK	452 ± 49.95a	3.30 ± 0.13a	514 ± 68.04a	518 ± 63.18a	99.84%
		DM	373 ± 185.87a	2.50 ± 0.84a	454 ± 195.44a	456 ± 190.56a	99.83%
		DL	434 ± 45.98a	2.95 ± 0.54a	520 ± 37.7a	513 ± 40.64a	99.82%
		HB	429 ± 69.58a	2.88 ± 1.03a	537 ± 58.35a	533 ± 73.26a	99.79%
Bacteria	July	CK	256 ± 75.14b	6.56 ± 0.04a	3197 ± 47.62b	3166 ± 15.36b	97.51%
		DM	2705 ± 65.7a	6.67 ± 0.05a	3371 ± 98.32a	3365 ± 116.28a	97.35%
		DL	2614 ± 47.39ab	6.62 ± 0.05a	3245 ± 59.53a	3249 ± 77.97ab	97.46%
		HB	2709 ± 30.87a	6.66 ± 0.07a	3352 ± 31.24a	3335 ± 52.16ab	97.40%
	August	CK	2589 ± 104.91a	6.62 ± 0.02a	3220 ± 124.04a	3207 ± 87.86a	97.49%
		DM	2648 ± 46.09a	6.66 ± 0.04a	3269 ± 82.76a	3243 ± 74.78a	97.48%
		DL	2619 ± 36.44a	6.68 ± 0.03a	3264 ± 80.22a	3295 ± 46.35a	97.44%
		HB	2584 ± 47.23a	6.65 ± 0.12a	3202 ± 26.63a	3215 ± 26.30a	97.52%

Note: Different lowercase letters in the same column indicate that in different periods, the planting patterns are significant at the level of $P < 0.05$.

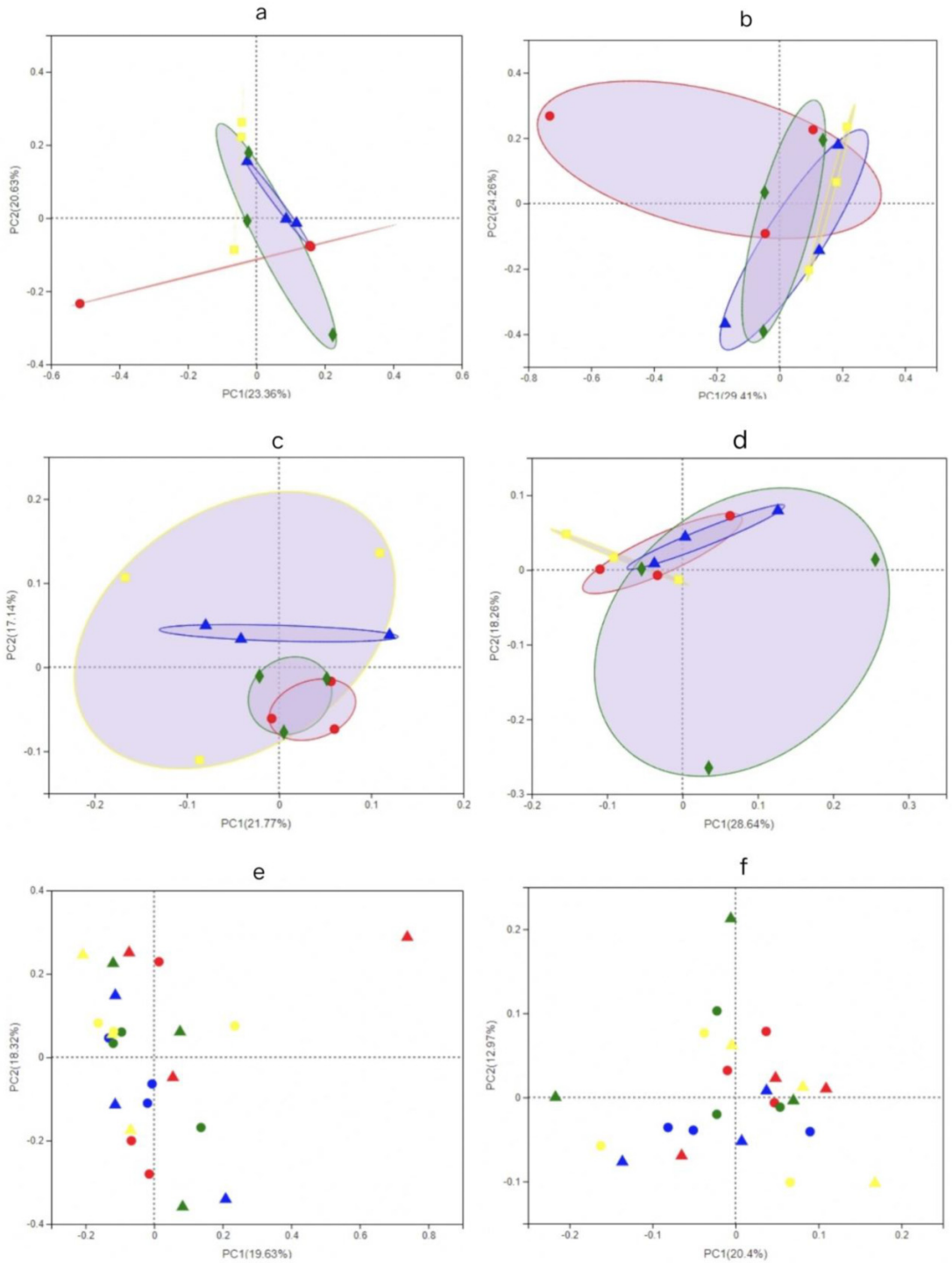
MBC was negatively correlated with each index, while MBN was positively correlated with each index (Table 2).

4. Discussion

4.1. The effect of different planting methods on soil enzyme activity

With the changes in crop planting methods, soil enzyme activities have also changed to varying degrees (Li et al. 2021). The results of this study indicated that the activity of catalase gradually increases over time. This may be because planting forage absorbs soil nutrients and reduces soil pH, which indirectly affects enzyme activity. In addition, alfalfa is a high-quality forage that can maintain or even improve soil fertility through biological nitrogen fixation (Li et al. 2016). The study found that, similar to catalase, the urease activity of each treatment gradually increased with time, and the soil enzyme activity of the mixed seeding treatment was higher than that of the other treatments. During the entire forage

Fig. 5. PCoA of fungi and bacteria under different treatments in July and August. (*a*, *b*) The PCoA of fungi in July and August under different treatments. (*c*, *d*) The PCoA of bacteria in July and August under different treatments. (*e*, *f*) The PCoA of fungi and bacteria under different treatments, respectively. The blue icon represents DL processing, the red icon represents DM processing, the green icon represents HB processing, and the yellow icon represents CK processing. [Color online]



growth period, the urease activity of each planting method was significantly higher than that of the fallow method. The sucrase activity of SYYM treatment and CK treatment did not change much in different periods, and the changing trend of sucrase under the other three treatments first decreased and then increased. In addition, the increase in sucrase activity in August was not significant. The continuous drought from May to June in the test area may be one of the reasons for the decrease of soil sucrase activity. Therefore, when the rainy season comes, the soil sucrase activity increases again,

Table 2. Correlation analysis between soil enzyme activity and microbial biomass and soil microbial diversity.

								Fungi			Bacteria	
	Catalase	Urease	Sucrase	Alkaline phosphatase	MBC	MBN	Sobs	Shannon	Chao	Sobs	Shannon	Chao
Catalase	1	0.037	0.354	0.047	0.298	−0.034	−0.263	−0.222	−0.287	−0.241	0.22	−0.195
Urease		1	0.66**	0.86**	−0.372	0.126	−0.224	−0.524**	−0.074	0.187	0.328	0.277
Sucrase			1	0.589**	−0.109	0.15	−0.357	−0.447**	−0.315	0.088	0.501*	−0.03
Alkaline phosphatase				1	−0.276	0.218	−0.147	−0.387	−0.02	0.325	0.493*	0.313
MBC					1	−0.344	−0.083	0.257	−0.221	−0.249	−0.148	−0.143
MBN						1	0.357	0.288	0.38	0.329	0.196	0.016
Sobs							1	0.744**	0.946**	1	0.558**	0.774**
Shannon								1	0.576**		1	0.379
Chao									1			1

Note: Different asterisks (*) in the same column indicate that in different periods, the planting patterns are significant at the level of $P < 0.05$; ** stands for more significant.

which is consistent with the research conclusion of Yu et al. (2017). Planting methods have little effect on soil alkaline phosphatase. Except for CK treatment, the changing trend of alkaline phosphatase activity in all treatments showed that the enzyme activity increased with time, but the increase was not great. The alkaline phosphatase activity mainly increases with the increase of soil organic matter content, which is the same as the reason for the change of catalase.

4.2. The impact of different planting methods on soil microbial biomass

Microbial biomass carbon has been proposed as an important indicator of soil quality changes under different land uses (Sheng et al. 2015). This study showed that different planting methods have significant effects on soil microbial biomass carbon, and the microbial biomass carbon of each treatment was higher than that of the CK treatment. The HB treatment has the least microbial carbon content because the competition between seedings in the first year is greater than the reciprocity between seedings (Bi et al. 2019). With the passage of forage growth period, the soil microbial biomass carbon of each treatment showed a gradual upward trend. Research on the effects of different pasture planting methods on soil microbial biomass nitrogen showed that the soil microbial biomass nitrogen level of mixed planting treatments was higher than other treatments at each stage. Studies have shown that in the mixed sowing system, legume forages transfer nitrogen to gramineous forages, so as to reduce the absorption of soil nitrogen by crops (Fernandez et al. 2016; Stern 1993). Mixed sowing was conducive to improving the nitrogen fixation of soil microorganisms. This is consistent with the fact that the microbial biomass nitrogen level of mixed sowing treatment was higher than that of other treatments.

4.3. The impact of different planting methods on soil microbial diversity

With the advancement of the forage growth period, the bacteria α-diversity index of different planting methods showed an upward trend, and the fungi showed a downward trend. This may be due to the rainy season in July, and the microbial species showed less species diversity in the rainy season. The comparison of this study found that the α-diversity indexes of mixed seeding and single seeding alfalfa treatments were always higher than those of the control. The comparison of β-diversity showed that there were certain differences in the microbial communities of fungi and bacteria in mixed seeding and single seeding alfalfa compared with single seeding sage wheat and CK treatments, and the planting method was the main factor affecting the bacteria. Planting mode was the main factor affecting bacteria community, and the sampling period has a greater impact on fungi community (Zhao et al. 2019). This study showed that as the growth period of crops was prolonged, the relative abundance of Proteobacteria in the mixed seeding treatment increases, while the control treatment decreases. Another study showed that the Proteobacteria have a positive effect on soil circulation and enriched in an environment with sufficient nutrients (Chang et al. 2021), so it can be seen that mixed seeding is more conducive to the enrichment of soil nutrients.

4.4. Relationship among soil enzyme activity, microbial biomass, and microbial diversity under different planting methods

Enzyme activity was negatively correlated with fungi and positively correlated with bacteria, which positively indicates that the increase of enzyme activity improves the metabolic capacity of soil, promotes the biochemical process in soil, metabolizes the bacteria that have a negative impact on life in soil, and promotes the survival of beneficial bacteria. In turn, increased microbial activity also produced more soil enzymes (Duchene et al. 2017; Veres et al. 2015). Microbial biomass carbon represents the carbon in living and dead organisms in soil; microbial biomass nitrogen promotes soil nitrogen cycle and provides nitrogen and nutrition for microorganisms, both of which were beneficial to maintaining microbial survival rate and species richness. The size of soil microbial biomass directly reflected the size of soil activity. The larger the microbial biomass, the higher the soil quality (Shao et al. 2002). The Shannon index represents the number of liv-

ing microorganisms, so continuous change was reasonable. In short, there were few studies on the correlation between enzyme activity, microbial biomass, and microbial diversity, which still needs further research and improvement.

5. Conclusion

Analyzing the effects of different pasture planting methods, different growth periods, and soil depths on the changes of four soil enzymes, soil microbial biomass C, N, and microbial diversity, it was concluded that different planting methods increase the activities of the four enzymes. With the advancement of the growth period of forage grass, the α-diversity index of bacteria showed an upward trend, while fungi showed a downward trend. The comparison of β-diversity found that the planting method was the main factor that caused the composition of the bacterial community, and the sampling time was the main factor that affected the fungi. From the community composition, it is found that the dominant phylum of bacteria are Proteobacteria and Actinobacteria, and the dominant phylum of fungi is Ascomycota.

In general, mixed planting achieves a complementary and mutually beneficial state in enhancing the absorption of light energy and soil fertility absorption, and has shown strong advantages in practice. The study of microbial diversity in the agro-pastoral interlace can provide a theoretical basis for farmland ecological restoration. However, its effects on local soil improvement and agricultural evaluation need to be further studied.

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Data availability

All data included in this study are available upon request by contacting with the author Dejian Zhang.

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Author notes

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All authors certify that they have participated sufficiently in the work of the paper. The authors have reviewed the final version of the manuscript and approved it for publication.

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Appendix A

	Factor	July	August	ALL	Period	Treatment
	R^2	0.28518	0.25946	0.29166	0.02993	0.12464
Fungi	P	0.371	0.552	0.665	0.857	0.563
	F	1.0639	0.93432	0.94113	0.67868	0.94921
	R^2	0.26226	0.28065	0.31434	0.05732	0.14143
Bacteria	P	0.592	0.385	0.335	0.117	0.268
	F	0.94798	1.01332	1.04788	1.33773	1.09822

Note: The data for this table comes from Table 1 and Table 2.