Highlight

High-throughput sequencing reveals soil bacterial community structure and their interactions with environmental factors of the grassland fairy ring

Jun-xia Wang,† Shan-shan Liu,† Shou-yan Han and Ai-ying Wang O†
College of Life Sciences, Shihezi University, Shihezi, 832000, China.

Summary
Fairy rings (FRs) are common ecological grassland landscapes that have been studied for a long time. However, little is known about their interactions with soil physicochemical properties and bacterial communities. This study performed high-throughput sequencing of the 16S rRNA V3–V4 variable regions of soil bacteria in the three concentric zones of chosen FR, namely, the ON zone, on the ring; IN zone, inside the ring; and OUT zone, outside the ring. Also, the change in physicochemical properties and enzyme activities of the soil were determined. This study found that the nutrients and enzyme activities on the ring were higher than inside and outside of the ring. The activities of microorganisms were frequent and the plant grew splendidly. The bacterial species diversity was the lowest on the ring with the main genera Pseudonocardia, Streptosporangium, Kribella and Promicromonospora. The imbalance of the microbial community structure at different ring zones may be the driving factor for the continuous outward expansion of FRs. Soil available phosphorus, electrical conductivity, total nitrogen and organic matter positively correlated with the distribution of FR soil bacteria.

Introduction
Soil microbial communities play important regulatory roles affecting nutrient cycling, plant growth and the balance of the ecosystem (Porazinska et al., 2003; Mendes et al., 2015; Soong et al., 2018; Schleuss et al., 2019). Grassland soil microorganisms participate in the decomposition and transformation of various soil organic matter (OM) promoting energy flow through material circulation, maintaining the normal operation of the ecosystem (Bardgett and van der Putten, 2014; Thakur and Geisen, 2019). Notably, bacteria are considered to be the most abundant and diverse soil microorganisms accounting for about 70%–90% of total soil microorganisms (Lamar and Dietrich, 1990; Acosta-Martinez et al., 2008). Soil bacteria–environment interactions influence soil secreted substances, including inorganic substances, enzymes and other active substances improving soil environment (Dong et al., 2017; Chen et al., 2020; Yin and Yan, 2020; Wu et al., 2021; Liu et al., 2021b). Meanwhile, the distribution of other soil microorganisms, plants and additional surrounding environment factors also have a certain impact on the distribution of soil bacteria (Jing and Ming-Hua, 2010).

Fairy rings (FRs) are common grassland ecological landscape caused by basidiomycetes in the soil (Edwards, 1984; Yang et al., 2018a). It is a complete system composed of soil-microbes-plants that plays an important role in the replacement and evolution of grassland vegetation (Cowles, 1918). The spread and radial growth of fungal mycelium form an FR, which affects surrounding soil microorganisms and plants through metabolic activities influencing their distribution in a ring-like manner (Caesar-TonThat et al., 2013). The current research on the FRs mainly focuses on the composition and characteristics of FR soil microorganisms (Yang et al., 2018b; Mari et al., 2020; Duan and Bau, 2021) studying their influence on plant biomass and soil physicochemical properties (Ji et al., 2003; Yang et al., 2018b; Yang et al., 2019; Liu et al., 2021a). A recent study suggests that the grassland FRs represent the centre of a rich soil microbial community (Duan and Bau, 2021). FRs are conducive to the growth and reproduction of soil microorganisms affecting the growth of plants by modulating carbon/nitrogen metabolism (Liu et al., 2021a). Studies also suggest that the community structure of FR
soil bacteria is largely affected by FR fungi (Yang et al., 2018b).

In recent years, there have been many studies on FR soil fungi communities and plants, but there are few studies on bacteria. This study selected the Tricholoma mongolicum S.Lmai FR of the Bayanbulak Grassland in Xinjiang to examine the community structure and diversity of soil bacteria on, inside and outside the rings during the peak of the FR using high-throughput sequencing (Zhang et al., 2021) of the bacterial 16S rRNA V3–V4 variable regions. Also, the soil physicochemical properties and enzyme activities were determined to examine their effect on the bacterial structure and plant growth to understand the outward expansion of the FR. By analysing the biological and non-biological constituents of the FR, we hope to reveal the relationship at the soil-FR-microbes-plant axis to systematically and comprehensively understand the formation and growth of FRs. At the same time, the change in soil bacterial diversity and community structure due to altered soil physicochemical properties in the FR of the Bayanbulak Grassland can provide a theoretical basis for the study of grassland soil microorganisms in this region.

Results

Soil physicochemical properties in the FR

Except for pH, the other soil physicochemical properties showed significant differences in the three zones of the FR (Table 1). The soil electrical conductivity (EC), soil moisture content (SM) and soil organic matter content (OM) were significantly higher in the ON zones compared with the IN and OUT zones (p < 0.05). The content of total potassium (TK), total phosphorus (TP) and total nitrogen (TN) were significantly higher in the ON zone than in the OUT and IN zones (p < 0.05). In general, the TK content was higher than TP and TN; TP content was relatively lower in this area. The soil contents of alkali-hydrolyzable nitrogen (AN) and available potassium (AK) were significantly higher than that of available phosphorus (AP); and the contents of AN, AP and AK were significantly higher in the ON zone than in the OUT and IN zones (p < 0.05).

Soil enzyme activity in the FR

The soil enzyme activity of different samples is shown in Table 2. The activities of urease, sucrase and catalase were significantly higher in the ON than in the OUT and IN zones (p < 0.05); sucrase activity was the highest among these enzymes. Soil cellulase activity showed a trend of ON > IN > OUT and was five times higher in the ON zone than in the OUT zone. The activity of protease was significantly lower in the ON zone than in the OUT and IN zones (p < 0.05).

Composition of soil bacterial community in the FR

From the sequencing analysis, after filtering out low-quality and short-sequence reads, a total of 1 447 122 high-quality sequences were obtained from 18 samples, with an average of 80 396 reads. After clustering based on >97% similarity criterion, a total of 49 970 operational taxonomic units (OTUs) were obtained, with an average of 2776 OTUs per sample (Supplementary Table 1). After annotations, these were divided into 44 phyla, 102 classes, 141 orders, 266 families and 424 genera. Among the 102 classes, the top 10 classes with highest relative abundance were unclassified_Actinobacteria (19%), Alphaproteobacteria (18.3%), Sphingobacteria (10%), Blastocatellia (8.8%), Acidimicrobia (6%), Spartobacteria (4.4%), Thermoleophilia (4.3%), unclassified_Gemmamonadetes (3%), Gammaproteobacteria (2.5%) and Cytophagia (1.4%) (Fig. 1A and C). Among the 424 genera, the top 10 genera with highest relative abundance were RB41 (4.9%), Kribbella (4.47%), Sphingomonas (2.56%), Pseudonocardia, (1.15%), Nocardioides (1.89%), Mycobacterium (1.43%), Rhodoplanes (1.58%), Halalangium (1.55%), Bradyrhizobium (1.34%) and Terrimonas (1.25%) (Fig. 1B and D).

The heat map showing the relative abundance of the 35 most abundant genera in the soil samples of the FR and the corresponding statistical data are shown in Fig. 2 and Supplementary Fig. 1. The top four genera with the highest relative abundance in the ON zone of the FR were Pseudonocardia (10.98%), Streptosporangium (9.81%), Promicromonospora (8.5%) and Kribbella (8.37%). Among these, the relative abundance of Streptosporangium was >25 times higher in the ON zone than in the OUT zone and seven times higher than in the IN zone. Although the relative abundance of Paenibacillus in the FR was not high, its relative abundance was significantly higher in the ON zone than in the IN and OUT zones (p < 0.05) (Fig. 3). Sphingomonas (3.92%), RB41 (3.64%), Nocardioides (2.99%) and Promicromonospora (2.94%) were the genera with higher relative abundance in the IN zone. Kribbella (5.11%), RB41 (5.39%), Sphingomonas (3.02%) and Nocardioides (1.73%) were the genera with relatively high abundance in the OUT zone.

Classification and differential analysis of bacterial OTUs in the soil samples of the FR

A Venn diagram (Fig. 4B and C) can intuitively reflect the difference and similarity between the OTU composition of the soil bacterial community in the FR. The soil samples of plot 1 XB1.W, XB1.N and XB1.S had 3103, 5256 and
2466 OTUs respectively (Fig. 4B). These three shared 1633 OTUs and had 657 unique OTUs in XB1.W (21.17%), 2844 unique OTUs in XB1.N (54.11%) and 261 unique OTUs in XB1.S (10.58%). The soil samples of Plot 2 XB2.W, XB2.N and XB2.S had 3071, 2989 and 2838 OTUs respectively (Fig. 4C). These three shared 2125 OTUs and had 360 unique OTUs in XB2.W (11.72%), 308 unique OTUs in XB2.N (10.30%) and 317 unique OTUs in XB2.S (11.17%).

To better visualize the changes in bacterial communities, linear discriminant analysis (LDA) is displayed as cladograms for the ON regions of the FR in the two plots (Fig. 4D). The bacterial groups that played important roles in the ON zone of plot 1 were Streptosporangiales, Pseudonocardiales, Micromonosporales, Micrococcales and Bacillales; the bacterial groups that played important roles in the ON zone of plot 2 were Acidimicrobiales and unidentified Blastocakiia. These species showed extremely significant differences (LDA scores >4) in the ON zones of the two plots (Fig. 4A).

α-Diversity analysis of the soil bacterial community in the FR

α-diversity, the average species diversity, reflects the richness and diversity of the microbial community. As shown in Table 3, the number of bacterial species in the FR ranged from 1791 to 2689; the number of bacterial species was lowest in the ON zone, which was significantly lower than the IN zone (p < 0.05). The Chao1 index ranged from 2225 to 3838 and the ACE index ranged from 2275 to 4125. The Chao1 and ACE indices were the lowest in the ON zone, followed by the OUT zone, which was significantly lower than the IN zone (p < 0.05). The Shannon index, which characterizes the diversity of microbial communities, ranged from 7.86 to 9.13. It was lowest for the ON zone, which was significantly lower than the OUT and IN zones (p < 0.05).

β-Diversity analysis of the soil bacterial community in the FR

The distance matrix calculated from the species composition was analysed by principal coordinates analysis (PCoA) (Fig. 5A), and then the samples were clustered by unweighted group average method (UPGMA) based on the species abundance information (Fig. 5B). Based on the β-diversity analysis of the soil bacterial community and the weighted unifrac PCoA, the abscissa and ordinate indicate the variation in fungal community structure at the OTU level 51.24% and 17.55% respectively. Combined with the Adonis test ($R^2 = 0.305, p = 0.001$), it was seen that the distribution of bacteria in soil samples showed relatively less difference in the OUT zones of the

| Sample | EC (ms µm⁻¹) | pH | SM (%) | AN (mg kg⁻¹) | AK (mg kg⁻¹) | AP (mg kg⁻¹) | AK (mg kg⁻¹) | TK (g kg⁻¹) | TN (g kg⁻¹) | TP (g kg⁻¹) | OM (g kg⁻¹) |
|--------|--------------|----|--------|--------------|--------------|--------------|--------------|-------------|-------------|-------------|--------------|
| ON     | 320.5 ± 22.87a | 7.37 ± 0.28a | 12.09 ± 1.75a | 29.81 ± 1.03a | 61.63 ± 0.77a | 1.97 ± 0.25a | 1.03 ± 0.05a | 0.19 ± 0.28a | 0.05 ± 0.23a | 0.03 ± 0.21a | 23.54 ± 1.75a |
| OUT    | 151.7 ± 13.02b | 7.21 ± 0.15a | 8.29 ± 0.15a | 8.44 ± 0.15a | 5.21 ± 0.15a | 4.91 ± 0.15a | 1.58 ± 0.15a | 1.97 ± 0.15a | 0.77 ± 0.15a | 0.05 ± 0.15a | 61.63 ± 0.15a |
| IN     | 134.5 ± 13.02c | 7.37 ± 0.28c | 12.09 ± 1.75c | 29.81 ± 1.03c | 61.63 ± 0.77c | 1.97 ± 0.25c | 1.03 ± 0.05c | 0.19 ± 0.28c | 0.05 ± 0.23c | 0.03 ± 0.21c | 23.54 ± 1.75c |

Different lowercase letters indicate significant differences (p < 0.05). OUT, IN and ON represent the outside the ring, the ring and the on the ring of the fairy ring respectively. EC, electrical conductivity; PH: pH; SM: soil moisture content; AN: alkaline hydrolysis nitrogen; AP: available phosphorus; AK: available potassium; TK: total potassium; TN: total nitrogen; TP: total phosphorus; OM: organic matter.
And the distribution of soil bacteria in the IN zone of plot 2 is close to OUT zones in both plots. On the contrary, the soil samples from the ON zones from the two plots showed a significant difference in the bacterial community structure. Meanwhile, within the individual plots, the distribution characteristics of soil bacteria in different zones were quite different in Plot 1 but were indistinct in Plot 2.

Based on the weighted unifrac distance matrix analysis (Fig. 5B), the FR bacterial community can mainly be divided into three groups at the phylum level. The bacteria in the IN and ON zones in plot 1 formed individual branches indicating a significant difference in the bacterial composition of different zones in plot 1. XB1.W and XB2. W got clustered into one group indicating that the bacterial community structure outside the fairy ring was the same in both places. Also, the bacterial community structure inside and on the fairy ring was relatively close.

Soil bacteria diversity at the species level in the FR

UPGMA cluster analysis revealed that the bacterial community composition was significantly different between the IN and ON regions of the FR in two plots. To explore the micro-ecological characteristics and possible mechanism of FR formation, the bacterial species in the IN and ON zones were analysed at taxonomic levels. We observed a big difference at the phylum level. To investigate significant
differences ($p < 0.05$) between groups at the species level, Fisher’s exact test was performed for species abundances (mean percentages) in the IN and ON zones of the respective FR plots (Fig. 6).

In Plot 1, *Paenarthrobacter nitroguajacolicus*, *Micromonospora chokorienis*, *Paenibacillus sepulcri*, *Bacillus nealsonii*, *Amycolatopsis saalfeldensis*, *Saccharopolyspora gregorii*, *Rhodococcus erythropolis* and *Luteibacter rhizovicinus* were significantly more abundant in the ON zone ($p < 0.05$), while *Luteimonas mephitis*, *uncultivated soil bacterium clone C112*, *Mesorhizobium ciceri*, *Actinomadura alba* and *Brevundimonas alba* were significantly higher ($p < 0.05$) in the IN zone. In plot 2, the abundance of *Mesorhizobium tianshanense*,

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uncultivated_soil_bacterium_clone_C112 and Mesorhizobium_ciceri was significantly higher in the IN zone \((p < 0.05)\), while Luteibacter_rhizovicinus was significantly higher in the ON zone \((p < 0.05)\).

**Correlation between soil physicochemical factors and bacterial composition**

RDA analysis, based on a linear model, can analyse the correlation between the flora and environmental factors. The relationship between the soil bacteria in the three zones of the FR and different sampling points was analysed at the class level (Fig. 7). The distribution of the samples in the ON, OUT and IN zones of the FR showed a significant difference, especially in plot 1. As shown in Fig. 7, the soil samples of the OUT, IN and ON zones from plot 1 are distributed in different quadrants. The soil samples on the ring are mainly distributed at the junction of the first and fourth quadrants, the soil samples inside the ring are distributed in the fourth quadrant, and the soil samples outside the ring are distributed in the third quadrant. Notably, the composition of soil bacteria on and inside the ring was relatively similar in Plot 1. In plot 2, the difference between the soil samples was small, which mainly concentrated in the second quadrant. Also, the difference between the soil samples inside and on the ring was small. Overall, the difference between the IN and ON zones was small, while the difference between the OUT and ON zones was prominent. This phenomenon can be attributed to the continuous outward growth of the FR each year.

According to the RDA analysis, the P1 and P2 axes explained 68.41% and 14.91% of the soil physicochemical factors respectively, indicating that the P1 axis is the main factor for the differences in soil physicochemical properties. Soil AP, EC, TN, OM and soil water content (SM) showed greater correlations with different plots of the FR. Soil AP, EC, OM and TN positively correlated with the distribution of soil bacteria in the ON zone of the FR. TN positively correlated with the distribution of soil bacteria in the ON zone. There was little correlation between the distribution of soil bacteria in the OUT zone and soil physicochemical factors. It shows that the distribution of bacteria outside the ring was less affected by soil physicochemical factors. Overall, the distribution of bacteria on and inside the ring showed a greater correlation with soil physicochemical factors.

**Discussion**

**ON zone has higher levels of soil nutrients than the OUT and IN zones**

Soil physicochemical properties reflect the status of soil fertility that affects the structure and function of soil microbial communities (Yuan et al., 2013; Liu et al., 2018; Pan et al., 2020). FR fungi can mineralize soil nitrogen (N) and phosphorus (P), thereby stimulating plant growth in the surrounding soil (Fisher, 1977; Edwards, 1988; Bonanomi et al., 2012). The analysis of the soil physicochemical properties of the Bayanbulak Grassland FR in Xinjiang revealed that the soil nutrient contents varied among the ON, OUT and IN zones. Except for total soil potassium content, the change in other soil nutrients showed a trend of \(\text{ON} > \text{OUT} \geq \text{IN}\). Moreover, the ON zone showed splendid vegetation with higher plant height than the sides, which reflects the high soil nutrient status of the ON zone. This could be attributed to vigorous microbial metabolism involving higher decomposition of soil nutrients increasing the nutrient content of the ON zone. Also, a large number of dominant fungal communities in the ON zone promoted the accumulation of soil nutrients. Research shows that soil properties are related to plant interactions (Harrison and Bardgett, 2010; Xia et al., 2016). The soil of the FR ON zone is nutrient-rich which promotes the growth of plants. At the same time, the growth of microorganisms and large fungi facilitate water and nutrient retention in the region.

The vigorous growth of microorganisms and plants in the ON zone promotes decomposition and utilization of soil nutrients. In addition, the annual or seasonal cover of vegetation changes the local water and heat balance. The soil lacking complete water leaching causes salt accumulation on the soil surface. This explains the higher
Fig. 4. A. LDA effect size analysis showed that there were significant differences between the bacterial communities of plot 1 and plot 2; LDA score (log10) > 4. B and C. Venn diagrams of OTUs in different soil samples of the fairy ring. D. Cladogram using the LEfSe method shows the differential bacteria between the XB1.ON and XB2.ON samples. OUT, IN, and ON denote outside, inside and on the ring regions of the fairy ring respectively; XB1 and XB2 represent two different plots.
soil conductivity in the ON zone than in the OUT and IN zones. Decomposition of the remains of animals, plants and microorganisms is the major source of soil OM. A higher OM in the ON zone indicates higher soil microbial activity. The TK content in the ON zone was significantly higher than that of TN and TP; also, the content of available potassium was higher than that of alkali-hydrolyzable nitrogen and AP. This may be related to the activities of special microbial populations in the soil community of the ON zone. Overall, FRs have poor soil fertility and low nutrient levels. Soil fungi and plants need a continuous supply of nutrients forcing outward growth of the FR.

Fairy rings affect soil enzyme activity

Soil enzymes are active substances secreted into the soil by the metabolic activities of animals, plants and microorganisms. These are important indicators reflecting the status of soil fertility and nutrient transformation in the ecosystem (Lide et al., 2016; Luo et al., 2020). Soil enzymes play an important role in the conversion of nutrients, especially the conversion of soil organic and inorganic matters affecting the process of material transformation (Caldwell, 2005; Gianfreda, 2015). This study found that in the ON zone, in addition to protease, the activities of sucrase, cellulase, catalase and urease were significantly higher than in the OUT and IN zones. In general, the life metabolism activity of the rhizosphere microbial community is relatively vigorous in FRs. The analysis of the soil enzyme activity revealed that overall soil enzyme activity was relatively low in the FR, which may be due to the relative lack of soil nutrients in the Bayanbulak Grassland.

The change in the trend of cellulase and catalase activities showed the same effect ON > IN > OUT. Cellulose is the main source of soil OM, and its activity significantly affects the process of material circulation. The degradation of soil cellulose is mainly driven by microorganisms, specially, involving fungi with a relatively complete cellulase-producing enzyme system. Jingwei et al. pointed out a significant correlation between the number of decomposing bacteria in the soil, cellulase activity and soil nutrient content. The growth and reproduction of cellulolytic bacteria in the forest are conducive to the decomposition and transformation of soil OM balancing the forest ecosystem (Jingwei et al., 2000). Catalases enhance the antioxidant capacity and resistance of plants by removing excessive active oxygen. Catalase level can be used to indicate the degree of soil oxidation and is closely related to the conversion of soil OM (Jun et al., 2005; Liu et al., 2019). This study found that the catalase activity in the ON zone was significantly higher than in the IN zone, followed by the OUT zone. This indicated vigorous OM conversion activity in the ON zone. Urease and protease are important catalysts of soil nitrogen promoting the nitrogen cycle and utilization of soil nitrogen (Caldwell, 2005). Urease hydrolyzes amide peptide bonds releasing ammonia, an easily absorbable N source for plants (Gong et al., 2015). Protease participates in the conversion of amino acids, proteins and other nitrogen-containing organic compounds. Its level indicates the status of soil nitrogen mineralization. In presence of sufficient organic nutrients, a higher soil enzyme activity ensures higher mineralization and metabolic rate, which is conducive to the balance of the soil ecosystem (Yu-fan et al., 2020). The soil enzyme activity directly affects the circulation of soil nutrients and in turn can change the composition of soil microorganisms (Burns et al., 2013). Also, it plays a key role in plant interactions (Wanting et al., 2021). This study found that urease activity was the highest in the ON zone with a relatively high utilization rate of soil nitrogen. The vigorous growth of plants in the ON zone can be related to the higher urease activity. The protease activity in the ON zone was significantly lower than in the OUT and IN zones, indicating lower soil organic nitrogen content in the ON zone. Overall, this suggests that the available organic nitrogen for microorganisms was low, but the utilization rate of soil nitrogen was relatively high in this FR. Activity of sucrase, also called soil invertase, is closely related to the level of soil OM and microorganisms. Sucrese promotes fertility in carbon-rich soils (Yue-hong et al., 2021). This study found that the invertase activity in the ON zone was significantly higher than in the OUT and IN zones. Also, the invertase activity was higher than other enzyme activities. It shows higher soil microbial metabolism in the ON zone that facilitated plant vegetation.

Table 3. α-Diversity index.

| Sample | Observed_species | Shannon | Chao1 | ACE | Good’s coverage | PD |
|--------|------------------|---------|-------|-----|----------------|----|
| OUT    | 2234.33<sup>ab</sup> | 9.13<sup>a</sup> | 2578.71<sup>ab</sup> | 2596.22<sup>ab</sup> | 0.982 | 216.34 |
| IN     | 2689.17<sup>a</sup> | 9.06<sup>a</sup> | 3838.74<sup>a</sup> | 4125.44<sup>a</sup> | 0.973 | 264.93 |
| ON     | 1791.00<sup>b</sup> | 7.88<sup>b</sup> | 2225.87<sup>b</sup> | 2275.20<sup>b</sup> | 0.981 | 180.83 |

Different lowercase letters indicate significant differences (p < 0.05). Community richness index (Chao1 and ACE), Community coverage index (the Good’s coverage), Community diversity index (PD: Phylogenetic Diversity and Shannon).
Diversity of soil bacterial community in the FR

Bacterial community composition and species diversity are important indicators of soil ecological functions (Wagg et al., 2019; Delgado-Baquerizo et al., 2020). These showed certain differences in the ON, OUT and IN zones of Bayanbulak Grassland FR. The $\alpha$-diversity index analysis revealed poor bacterial community diversity and low richness in the ON zone, while the same was highest in the IN zone, showing significant

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It can be inferred that certain factors affected or inhibited the distribution of bacterial community and growth of certain species in the ON zone. Oh et al. studied the soil bacterial diversity of the fairy and non-FR areas produced by *Tricholoma matsutake* and pointed out low bacterial diversity in the ring area. The ring had *Tricholoma matsutake* dominated bacterial groups on the ring, while other microorganisms were inhibited (Oh and Lim, 2018).

OTU analysis of the FR bacterial groups suggests that the ON zone is very likely related to the formation of FR. Cladogram analysis showed obvious differences in the abundance of bacterial groups that played important roles in the two plots and might be the reason for differences in bacterial diversity. These microorganisms are very likely to affect the microbial abundance and the growth of fungi and plants in the ON zone. This study found big differences in the composition of soil bacteria in the FR. The relative abundance in the IN, OUT and ON zones was ranked with the top four genera; the OUT and IN zones had the most common genera. This also may be the key reason for the difference in the composition of the FR bacteria. There were many saprophytic bacteria in the ON zone, which can decompose the soil OM for...
the growth of mushroom fruit bodies. Meanwhile, other bacteria in the ON zone, such as *Paenibacillus* and *Streptosporangium*, can produce antibiotic substances to inhibit the growth of bacteria, especially pathogenic bacteria. This creates a nutrient-rich healthy environment for the growth of FR. A low soil bacterial diversity in the ON zone may also be related to it. *Paenibacillus* is a biocontrol bacterium that can be used to control plant pathogens and promote plant growth (Wang and Liu, 2008; Lal and Tabacchioni, 2009; Grady et al., 2016; Wen-qing et al., 2020). Importantly, we found that most of the highly abundant bacteria in the ON zone were nitrogen-fixing or phosphorus-solubilizing bacteria, such as *Paenarthrobacter*, *Bacillus* and *Rhodococcus*. This further shows that the rich nutrients and vigorous vegetation on the FR are closely related to their soil bacteria community composition. Overall, dominating bacteria affect the micro-ecological environment of the FRs promoting their growth.

Soil bacteria in the FR are correlated with environmental factors

The composition of soil microbial community depends on soil physicochemical properties (Delmont et al., 2014) affecting the inter-species and intra-species interactions (Luo et al., 2018; Yang et al., 2018b). This study too showed that the relative abundance and diversity index of the FR soil bacteria were affected by environmental factors. Soil TP, EC, TN and OM positively correlated with differences in soil bacterial community composition and relative abundance at different zones. AK, AP, EC and TK play a major role in the composition of soil bacteria. The FR bacteria change the physicochemical properties of soil, activate soil nutrients and improve soil nutrient status. *Tricholoma mongolicum* S.Imai mainly produces saprophytic life increasing the content of soil OM. Accordingly, a previous study showed relative high abundance of saprophytic bacteria in the ON zone (Duan and Bau, 2021). The soil content of TN and TP positively relates to the growth of plants increasing vegetation at the FR.

Experimental procedures

Study site

The research site is located in the Bayanbulak Grassland in the middle of the Tianshan Mountains, Xinjiang, China. The region is dominated by a cold and arid climate, with an average elevation of >2400 m, an average annual
temperature of -4.5°C and average annual precipitation of 276.9 mm. The region is a vast area with various types of grasslands. It has the largest alpine swamp meadow and alpine grassland in Xinjiang. The main soils in the region are fluvo-aquic soil and meadow soil. The region is a very important pasture in Xinjiang. FR plots selected in this study have an altitude of 2646.41 m and a geographical location of 86.34°E, 42.56°N.

Soil sample collection

In this study, two *Tricholoma mongolicum* S.Imai FR plots, named XB.1 and XB.2, were selected in the Bayanbulak Grassland. Three FRs were selected in each plot to retrieve samples using the five-point sampling method for the three concentric zones: on the ring, ON zone; inside the ring, IN zone; outside the ring, OUT zone (Fig. 8). After removing the surface humus soil layer, sample profile soil (0-20 cm) was collected using a 5 cm wide auger. The soil collected from the five sampling points in the same concentric zone of each FR was mixed to form an individual sample; in total, 18 such samples were prepared. All fresh soil samples were divided into two parts: one part was stored at 4°C for physicochemical analysis and the other part was stored at -80°C for DNA extraction.

Soil physicochemical properties

The soil physicochemical properties were determined as per the methods described in the book ‘*Soil Agrochemical Analysis*’ (Shidan, 2000). Soil conductivity and pH values (soil-water ratio 1:5) were measured using the conductivity and acidity meters respectively. SM was measured using the specific gravity method and drying at 105°C. Soil alkali-hydrolyzable nitrogen content was determined using the alkaline hydrolysis diffusion method. Available and TP contents were determined by the NaHCO₃ extraction-molybdenum antimony colorimetric method. Available and TK contents were determined by NaOH fusion-flame photometry. TN was determined by the Kjeldahl method. The soil OM was determined by the potassium dichromate volumetric method. All measurements were repeated three times to obtain an average value.

**Determination of soil enzyme activity**

The soil enzyme activity was determined based on methods in Songyin *et al.* (Songyin, 1986; Likai, 1987). Urease activity was determined by sodium phenate-sodium hypochlorite colorimetry (Songyin, 1986). Protease activity was determined by ninhydrin colorimetry. Cellulase and sucrase activities were determined by 3,5-dinitrosalicylic acid colorimetry. Catalase activity was determined by the potassium permanganate titration method (Likai, 1987).

**DNA extraction and PCR amplification**

Total soil DNA was extracted using the Dehumus kit (Power Soil® DNA Isolation kit, Mo Bio Laboratories, Solana Beach, CA, USA) as per the kit instructions. The quality of the extracted DNA was analysed by 1% agarose gel electrophoresis and spectrophotometry (optical density at 260 nm/280 nm ratio). The extracted DNA was stored at -20°C for further analysis. Genomic DNA was used as a template to amplify the V3–V4 hypervariable region of bacterial 16S tRNA using the universal primers: forward 341F (5′-CCTAYGGGRBGCASCAG-3′) and reverse 806R (5′-GGACTACNNGGGTATCTAAT-3′) (Sundberg *et al.*, 2013; Machuca *et al.*, 2016; Yang *et al.*, 2020). These primers contain a set of eight nucleotide barcode sequences unique to each sample. PCR reactions were performed in triplicates in 30 μl mixtures containing 15 μl of 2× Phusion Master Mix (New England Biolabs), 3 μl of each primer (2 μmol L⁻¹), 2 μl of ddH₂O and 7 μl of genomic DNA (1 ng μl⁻¹). Samples were subjected to the following PCR program: 94°C for 1 min, 30 cycles of 94°C for 20 s, 56°C for 30 s, 72°C for 30 s and a final extension at 72°C for 5 min.

**Illumina HiSeq sequencing**

The amplified PCR product was analysed by 2% agarose gel electrophoresis, and the target band was recovered
from agarose gel. TruSeq®DNA PCR-Free Sample Preparation Kit (Illumina Company, USA) was used to construct the library from the qualified recovered products. The established library was qualified by Qubit and q-PCR quantitative tests before sequencing at Illumina HiSeq (2 × 250) platform at Novogene (Beijing, China, https://www.novogene.cn/).

### Statistical analysis

The sequencing data were analysed by bioinformatics. QIIME 1.7.0 was used to filter the quality raw sequences, and UCLUST was used to perform OTU clustering and species classification analysis of valid sequences. Sequences with ≥97% similarity were labelled from the same OTUs. Alpha diversity was used to analyse the species diversity through six indices, including observed species, Chao1, Simpson, Shannon, ACE and good_coverage. All indices were calculated and analysed using QIIME 1.7.0. Beta diversity analysis was performed to assess changes in species composition between samples. PCoA based on the weighted and unweighted Unifrac distance algorithm was performed to compare the similarity analysis between different samples. The UPGMA was used for cluster analysis. Statistical analysis was performed using Excel, R programming language and SPSS 20.0 software.

### Conclusions

This study of the soil micro-ecological environment by high-throughput sequencing showed the variation in soil physicochemical properties, soil enzyme activity and soil microbial community structure at different spatial locations in the FR. The production and growth of FR showed close relation with bacterial community structure. Bacteria played a greater role in the transformation of soil nutrients and plant growth affecting the micro-ecological environment of the FR through metabolic activities. Also, the micro-ecological environment of the FR affected the distribution and types of soil bacteria. The main reason for the expansion of FRs may be the imbalance of microbial community structure in different regions of FR.

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Supporting Information
Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

Appendix S1. Supporting Information.