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The effects of functional microbial agents on the soil microbial communities of high-frigid grassland under desertification in Northwest Sichuan

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ABSTRACT:
Grassland desertification seriously destroys the structure of the soil microbial communities and further accelerates the deterioration of grassland. In this study, functional microbial agents were sprayed on degraded grasslands and studied by means of real-time fluorescence quantitative PCR and high-throughput sequencing methods. The aim was to investigate the effects of microbial agents on the soil microbial community structure and soil remediation functions in different degraded grasslands (LDG: light desertification grassland, MDG: medium desertification grassland, and HDG: heavy desertification grassland). The results showed that after treatment with the microbial agents, bacterial abundance increased by 96.24% (LDG), 95.19% (MDG), and 93.47% (HDG), respectively, and fungal abundance increased by 85.77% (LDG), 95.85% (MDG) and 22.49% (HDG), respectively. Further, with the colonisation and acclimatisation of foreign functional microorganisms, the microbial agents greatly influenced the structure of the soil microbial community, increased the microbial diversity index, and significantly changed the microbial community composition. The application of the microbial agents did not only improve the forage yield and quality, but also guided the soil restoration, improved the soil water content of sandy grassland, adjusted the soil pH, significantly increased the content of soil organic matter, total nitrogen, and total phosphorus, and also inhibited the growth of soil-borne pathogens. Our findings provide new ideas and guidance for the management of degraded grassland.

Keywords:
Desertification of grassland, soil microbial community, functional microbial agents, remediation, the management of degraded grassland

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INTRODUCTION

China is one of the countries in the world most seriously affected by land desertification (Wang *et al*. 2015a, b). Soil desertification is one of the major and most serious ecological and environmental problems in the world, which leads to soil sanding, the formation of australopithecine landscapes, and significantly reduces the land production potential (Gad *et al*. 2000; Gomes *et al*. 2003; Jin *et al*. 2018).

Located on the eastern edge of the Tibetan Plateau, the alpine grassland of northwest Sichuan is one of China's five major pastoral areas. The area is an important livestock production base in the Sichuan Province and an important water source protection area for the Yangtze and Yellow rivers in China, with important ecological status, ecological value, and strategic significance (Jacobs *et al*. 2003; Wu *et al*. 2018). Under the combined influence of various natural and human factors, the human-land conflict in alpine grassland ecosystems has become more prominent (Wu *et al*. 2018). Sanding caused by degraded grasslands has severely restricted the sustainable socio-economic development of the region and weakened the role of the ecological barrier of the region.

Table 1. An overview of the sample sites with different degrees of desertification

Desertification degree	Longitude and latitude	Elevation (*h*/a.s.l.)	Species richness	Average vegetation coverage (%)	Desertification condition
Light desertification	N 33°11' E 102°35'	3640	18–22	60–75	Desertification was not obvious; the soil layer was thick, and some soil was relatively dry.
Medium desertification	N 33°39 E 102°56'	3469	14–17	40–50	It is characterised by obvious sanding, more sand grains, dryness, severe rodent infestation, and grazing.
Heavy desertification	N 33°12' E 102°36'	3462	9–13	20–35	It was characterised by serious desertification, a large amount of exposed sand, and dryness.

Table 2. The dry weight of the underground biomass and forage yield of the sample plots with different desertification degree

Sample plots	The dry weight of underground biomass (g/m^2)	The dry weight of forage yield (g/m^2)
LDG	6.69±0.01	8.69±0.27
C-LDG	6.54±0.02	8.22±0.02
MDG	7.02±0.01	7.64±0.16
C-MDG	6.79±0.03	5.20±0.08
HDG	7.07±0.01	5.97±0.34
C-HDG	6.84±0.05	4.99±0.05

LDG: Light desertification grassland with microbial agent treatments, C-LDG: Light desertification grassland without microbial agent treatments, MDG: medium desertification grassland with microbial agent treatments, C-MDG: medium desertification grassland without microbial agent treatments, HDG: heavy desertification grassland with microbial agent treatments, C-HDG: heavy desertification grassland without microbial agent treatments (the same below).

In recent years, although the expansion of land desertification has been restrained due to the gradual development of desertification control and ecological protection projects (Liao *et al*. 2011; Duan *et al*. 2019), the desertification of grassland is still on the increase. More and more attention has been paid to studying and controlling the desertification process of the grassland in this area (Xiong *et al*. 2011). With the deepening of research, many scientists have realized that the underground part has become the most uncertain factor in the study of the structure, function, and process of the grassland degradation ecosystem (Karaca *et al*. 2010; Jiang *et al*. 2017). Microorganisms are an important part of the soil ecosystem, participating in the cycle of soil fertility and nutrients, and are important indicators for characterising soil quality and evaluating soil restoration performance; soil microbial communities and diversity are closely related to the health of grassland soil quality and ecosystems (Sun *et al*. 2016). Microbial agents, with different functional microorganisms, can not only promote plant growth, improve soil structure and soil fertility, and increase crop yield and quality, but also make up for the shortcomings of indigenous microorganisms (Udeani *et al*. 2010; Vimal *et al*. 2017). The application of microbial agents in many crops has also achieved remarkable results (Demain & Sanchez 2009; Choi & Cho 2016; Wang *et al*. 2020). In this study, we (1) examine the abundance and community structure of soil microbial communities in grassland with different degradation degrees, and (2) investigate the impact of microbial agents on the microbial community structure of grassland desertification. The results could serve to provide a reference for the scientific control of alpine grassland desertification.

MATERIALS AND METHODS

Study site and soil sampling. The study site, Hongyuan County, the central part of Aba, is located on the eastern margin of the Tibetan Plateau (N 33°39', E 102°56'; about 3469 m a.s.l.), China. The terrain is undulating and flat, which is typical of the transition from mountain to hilly plateau and belongs to the continental plateau cold temperate monsoon climate. It is affected by the tropical and maritime monsoon climate and is regularly exposed to subfreezing temperatures with a mean annual temperature of 1.1°C, with maximum and minimum temperatures of 11.2 and -9.3°C, respectively. Mean annual precipitation is about 753 mm, and the rainy season is from June to September. The main vegetation types are subalpine coniferous forest, alpine shrub grassland, and alpine meadow and the main soil types are swamp soil, subalpine meadow soil, and alpine meadow soil (Tang *et al*. 2013; Dao *et al*. 2017).

The typical sandy grassland was selected as the field location and observation sampling point and divided into light desertification grassland (LDG), medium desertification grassland (MDG), and heavy desertification grassland (HDG) (Table 1). The grasslands are rich in *Elymus sibiricus*, and *Deschampsia caespitosa* (Linn.) Beauv, *Elymus nuts*, *Pedicularis* Linn., *Leymus scalenus* (Georgi) Tzvel, *Carex praeclara*, and so on (Wang *et al*. 2009).

Two 25 × 25 m plots were established in each study sampling point, respectively, and three 5 × 5 m sub-plots were established in each plot of 0.5 km. The microbial

Table 3. The physical and chemical properties of the soil in the sample plots with different desertification degrees

Sample plots	pH	Soil water content (%)	Soil organic matter content (g/kg)	Soil total nitrogen (%)	Soil total phosphorus (%)
LDG	6.54±0.02	8.69±0.27	17.01±0.38	5.35±0.12	5.94±0.47
C-LDG	6.69±0.01	8.22±0.02	15.32±0.33	4.65±0.11	4.45±0.51
MDG	6.79±0.03	7.64±0.16	8.43±1.14	3.94±0.37	4.52±0.40
C-MDG	7.02±0.01	5.20±0.08	5.85±0.31	2.84±0.19	2.26±0.35
HDG	6.84±0.05	5.97±0.34	5.65±0.75	1.98±0.10	3.67±0.67
C-HDG	7.07±0.01	4.99±0.05	4.26±0.73	1.92±0.15	2.09±0.29

agents were sprayed into one of the plots at each sampling point in May, June, July, and August 2019, respectively (187 ml/sample, diluted 100 times before spraying, the main components of the agent were *Bacillus subtilis* MZS1 50%, *Rhodococcus* sp. PG 42 30%, *Alcaligenes* sp. CJ1 5%, and *Delftia* sp. CDN1 15%.). For the corresponding control group at each sampling site, the same volume of water was used instead of the microbicides, and other routine treatments were the same as for the experimental group. Forage yield and underground biomass were detected in August when the grass was ripe (Table 2). At the same time, soil samples (10–20 cm) from each plot were collected and stored in freezer boxes and transported to the laboratory within 24 h. Each soil sample was divided into two. One was frozen (-80°C) and subsequently freeze-dried using a vacuum freeze dryer at -80°C (Wizard2.0, VirTis USA) before the extraction and purification of the community DNA. The other was oven-dried at 65°C for 72 hours and then analysed to determine the quality (Lv 1999; Bao 2000) (Table 3).

Quantification of rDNA genes. The genomic DNA of the freeze-dried soil samples was extracted by a Soil DNA kit (Tiangen, China) and the purity was determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific). The primer pairs ITS1/5.8S and 338F/518R (White *et al*. 1990; Gardes & Bruns 1993) were used to amplify the internal transcribed spacer (ITS) region of fungi rDNA and bacteria 16S ribosomal DNA (rDNA), respectively. The universal primer pair for determining the 16S rRNA gene copy number of the soil bacteria was 338F/518R: 338F (5-ACTCCTACGGGAGGCAGCAG-3') and 518R (5'-ATTACCGCGGCTGCTGG-3'). The common primer pair to determine the copy number of the soil fungal rRNA genes was ITS1/5.8S: ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and 5.8S (5'-CGCTGCGTTCTTCATCG-3') (Pang *et al*. 2017). PCR was performed using the C1000^{TM} Thermal Cycler (Bio-Rad, Hercules, USA) in 25 µL reactions containing 12.5 µL Premix Ex Taq (TaKaRa, Dalian, China), 0.4 mg·mL^{-1} bovine serum albumin, 200 nmol·L^{-1} of each primer, and 1 µL of purified DNA (1–10 ng) as a template. The PCR protocol was 95°C for 3 min followed by 40 cycles of 95°C for 30 s, 55°C for bacteria (or 56°C for fungi) for 30 s, and 72°C extensions for 12 s. The plates were then read at 72°C after each cycle. The product specificity was confirmed using melting curve analysis (65–95°C, 0.5°C per reading with a hold time of 5 s).

The PCR fragments obtained from amplification were ligated with pUC19 vector at 16° for 30 min; the ligated products were transferred into pre-thawed DH5α receptor cells in an ice bath for 30 min, activated at 42° for 45 s, and placed in ice for 1 min. 890 µl of sterile SOC liquid medium was added and incubated in a shaker for 1 h at 37° with shaking; white colonies were selected on LB agar plate medium containing Ampicillin, IPTG, and X-Gal on LB agar plate medium at 37° for 24 h. White colonies were selected, and positive clones were detected using PCR and sent for sequencing.

The verified positive clones were selected for plasmid DNA extraction using an E.Z.N.A Plasmid Extraction Kit (Omega Bio-Tek. Norcross, USA), and these plasmids were used as the rDNA gene standard. A Biophotometer UV-vis photometer (Eppendorf, Hamburg, Germany) was used to determine the concentration of both plasmid DNA, and the number of both gene copies was directly calculated from the concentration of plasmid DNA (Okano *et al*. 2004; Zhang *et al*. 2017a). Tenfold serial dilutions of the known number of copies of the plasmid DNA containing sequenced bacterial 16S rDNA or fungi ITS region fragments were subjected to q-PCR in triplicate to generate standard curves. The PCR efficiency and correlation coefficients for the standard curves were 107.5% and $r^2 = 0.998$ for bacteria, and 98.3% and $r^2 = 0.997$ for fungi, respectively.

The same primers for the real-time PCR quantification of bacterial 16S rDNA and the fungi ITS region fragment were used as previously mentioned. Real-time PCR was performed using the MiniOpticon Real-Time system (Bio-Rad, USA) in each 20 µL reaction containing 9 µL Real Master Mix (SYBR Green FP202; Tiangen, China), 1 µM of each primer, and 1 µL of purified DNA (1–10 ng) as a template. The abundance of fungi and bacteria was then expressed as the number of ITS or 16S rRNA gene copies in g-1 soil.

High throughput sequencing. The V4-V5 region of the bacterial 16S rRNA gene (the amplification primers,

Table 4. Pearson's correlation analyses between gene abundances and environmental variables in each study site

Site		pH	Soil water content	Soil total nitrogen	Soil organic matter	Soil total phosphorus	The dry weight of underground biomass	The dry weight of forage yield
LDG	Soil water content	-0.798						
	Soil total nitrogen	-0.977**	0.714					
	Soil organic matter	-0.972**	0.769	0.912*				
	Soil total phosphorus	-0.822*	0.737	0.802	0.73			
	The dry weight of underground biomass	-0.778	0.385	0.807	0.788	0.394		
	The dry weight of forage yield	-0.934**	0.632	0.940**	0.927**	0.592	0.940**	
	Bacterial abundance	-0.916*	0.803	0.914*	0.825*	0.936**	0.504	0.738
	Fungal abundance	-0.997**	0.768	0.980**	0.967**	0.830*	0.801	0.938**
MDG	Soil water content	-0.986**						
	Soil total nitrogen	-0.870*	0.904*					
	Soil organic matter	-0.828*	0.865*	0.992**				
	Soil total phosphorus	-0.936**	0.979**	0.915*	0.875*			
	The dry weight of underground biomass	-0.884*	0.945**	0.860*	0.834*	0.958**		
	The dry weight of forage yield	-0.888*	0.943**	0.975**	0.958**	0.969**	0.946**	
	Bacterial abundance	-0.79	0.852*	0.951**	0.969**	0.881*	0.875*	0.956**
	Fungal abundance	-0.959**	0.982**	0.958**	0.939**	0.969**	0.937**	0.974**
HDG	Soil water content	-0.816*						
	Soil total nitrogen	-0.168	0.359					
	Soil organic matter	-0.661	0.868*	0.111				
	Soil total phosphorus	-0.844*	0.792	0.282	0.547			
	The dry weight of underground biomass	-0.606	0.769	0.361	0.915*	0.415		
	The dry weight of forage yield	-0.717	0.343	0.232	-0.014	0.743	-0.022	
	Bacterial abundance	-0.888*	0.890*	0.42	0.66	0.970**	0.586	0.68
	Fungal abundance	-0.685	0.382	0.158	0.364	0.22	0.538	0.427

* Correlation is significant at the 0.05 level (one-tailed).
** Correlation is significant at the 0.01 level (two-tailed)

Table 5. Diversity indexes of the bacterial and fungal community

Sample plots	Shannon-Wiener diversity indexes		Simpson diversity indexes		Coverage	
	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi
LDG	10.3670	6.2315	0.9952	0.9085	0.9970	0.9997
C-LDG	9.9986	5.3730	0.9981	0.9438	0.9957	0.9999
MDG	10.0196	5.5998	0.9961	0.9136	0.9969	0.9998
C-MDG	9.9361	4.9526	0.9966	0.9366	0.9964	0.9999
HDG	10.1335	5.2940	0.9964	0.9185	0.9964	0.9998
C-HDG	9.9297	4.0743	0.9975	0.9649	0.9973	0.9999

515F/926R) and the ITS1 region of the fungal ITS1 gene (the amplification primers, ITS1F/ ITS1R) were sequenced and analysed, respectively, on the Illumina HiSeq platform. Chengdu Mailesai Biotechnology Co., Ltd. was entrusted with the construction and sequencing work, FLASH (v1.2.7, http://ccb.Jhu.Edu/software/FLASH/) for sequence splicing, UCHIME (v4.2, http://drive5.com/uchime) for identifying and removing chimeric sequences, and the QIIME (v1.8.0, http://qiime.org/) platform for OTU division based on a 97% sequence similarity level (Caporaso *et al.* 2010; Edgar *et al.* 2010). The OTUs were annotated based on the Silva (https://www.arb-silva.de/)

and UNITE (https://unite.ut.ee/) taxonomic databases, and Mothur software (Version1.35.1, https://www.mothur.org/) was used to calculate the diversity index of the bacterial and fungal communities.

Statistical Analysis. Statistical analysis was conducted using IBM SPSS Statistics (version 19). An independent samples t-test was used to analyse the statistical differences in the soil physical, chemical, and diversity indexes at the level of $p = 0.05$ in different desertification degrees and the corresponding control. The correlations between the microbial abundance and the environmental factors were analysed using Pearson's correlation coefficient in SPSS Statistics (version 20.0, IBM, USA). Redundancy analysis (RDA) between the microbial community structures and environmental variables was performed using Canoco for Windows version 4.5.

RESULTS

The abundance of the microbial rDNA gene. The bacterial 16S rDNA and fungus 18S rDNA genes were detected in the different desertification soils (Figs. 1 & 2). Both bacterial and fungal abundance was significantly higher in the LDG soil than that in the MDG and HDG soils ($p < 0.05$), and was lowest in the HDG soil. In each degraded grassland, both bacterial and fungus abundance was significantly higher in the soil treated with microbial agents than that in the corresponding control soil ($p < 0.05$). After four-month treatment with microbial agents, bacterial abundance increased by 96.24% (LDG), 95.19% (MDG), and 93.47% (HDG), respectively, while fungus abundance increased by 85.77% (LDG), 95.85% (MDG) and 22.49% (HDG).

Pearson's correlation analyses showed that the pH, soil water content, soil total nitrogen, soil organic matter content and soil total phosphorus had a profound impact on soil microbial abundance (Table 4). This was especially obvious in LDG and MDG. In LDG, bacterial abundance was negatively correlated with pH ($r = -0.916$, $p < 0.05$) and positively correlated with soil organic matter ($r = 0.825$, $p < 0.05$) and soil total nitrogen ($r = 0.914$, $p < 0.05$), respectively, and significantly positively correlated with soil total phosphorus ($r = 0.936$, $p < 0.01$). Fungal abundance was significantly negatively correlated with pH ($r = -0.997$, $p < 0.01$), significantly positively correlated with soil organic matter ($r = 0.967$, $p < 0.01$) and soil total nitrogen ($r = 0.980$ $p < 0.01$), respectively, and positively correlated with soil total phosphorus ($r = 0.830$, $p < 0.05$). In MDG, bacterial abundance was positively correlated with soil water content ($r = 0.852$, $p < 0.05$) and soil total phosphorus ($r = 0.881$, $p < 0.05$), respectively, and significantly positively correlated with soil organic matter ($r = 0.969$, $P < 0.01$) and soil total nitrogen ($r = 0.951$, $p < 0.01$), respectively. While in HDG, bacterial abundance was negatively correlated with pH ($r = -0.888$, $p < 0.05$), positively correlated with soil water content ($r = 0.890$, $p < 0.05$) and significantly positively correlated with soil total phosphorus ($r = 0.970$, $p < 0.01$), there was no significant correlation between fungal abundance and the soil physical and chemical indexes.

Community structure analysis. According to the Illumina HiSeq platform, the coverage of each soil DNA was more than 99% (Table 5), the V4-V5 region of the 16SrRNA fragment of each soil microbial community was double-end sequenced (paired-end), and the length distribution of the high-quality sequences contained in all the samples was counted using R language scripts. Among the species localisation based on 97% similarity, a total of 1496750 high-quality sequences were obtained from six soil samples, and 537,973 valid bacterial sequences were obtained, of which 107,962 were LDG. C-LDG 928,19, MDG 87,101, C-MDG 85,612, HDG 86,837, C-HDG 77,642, heavily desertified grassland, without microbial preparation treatment. Meanwhile, the fungus obtained 404,159 high-quality sequences with 61,610 LDG, 59,700 C-LDG, 73,545 MDG, 52,530 C-MDG, 81,490 HDG, and 71,734 C-HDG.

The dominant groups of bacterial and fungal communities were analysed, respectively, at the genus level, as shown in Figs. 3 & 4. The differences in the microbial relative abundance indicated the differences in the microbial community composition and structure at different sites.

The top20 bacterial groups based on relative abundance are shown in Fig. 3. Overall, the dominant bacteria changed a great deal in different degrees of soil desertification after treatment with the microbial agents. In LDG, compared with C-LDG, *Bacillus*, *Pyrinomonadaceae*, *Delftia*, *Aeromicrobium*, and *Marmoricola* increased, while *Acidibacter*, *Massilia*, *Devosia*, *Pseudomonas*, *Acinetobacter*, *Sphingomonas,* and *Candidatus Udaeobacter* were significantly reduced. In MDG, compared with C-MDG, with the exception of the dramatic decrease of *Delftia* and *Bryobacter*, there were no other obvious changes. In HDG, also compared with C-HDG, there was a significant increase in *Bacillus*, *Rhodopseudomonas palustris, Pseudonocardia,* and *Bryobacter*, while an obvious decrease in *Delftia*, *Candidatus Udaeobacter,* and *Pyrinomonadaceae.*

The top 20 fungal groups based on relative abundance are shown in Fig. 4. Compared with the changes in the bacterial groups, the fungal community structure changed more dramatically after treatment with the microbial agents. Both in LDG and MDG, *Fusarium* initially represented a small percentage of the fungal community. However, when treated with the microbial agents, it rapidly became predominant, accounting for more than 60% of the community. In contrast, *Hygrocybe* declined rapidly from the dominant position in the community. In HDG, in addition to the rapid spread and

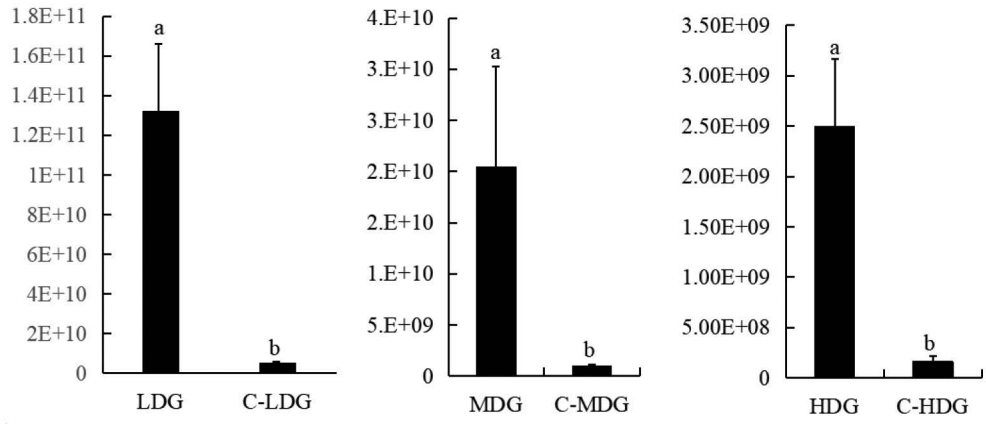

Fig. 1. The abundance of bacterial rDNA gene at each site**.** Different lowercase letters indicate significant differences ($p < 0.05$) between the treatment group and the control group at the same site.

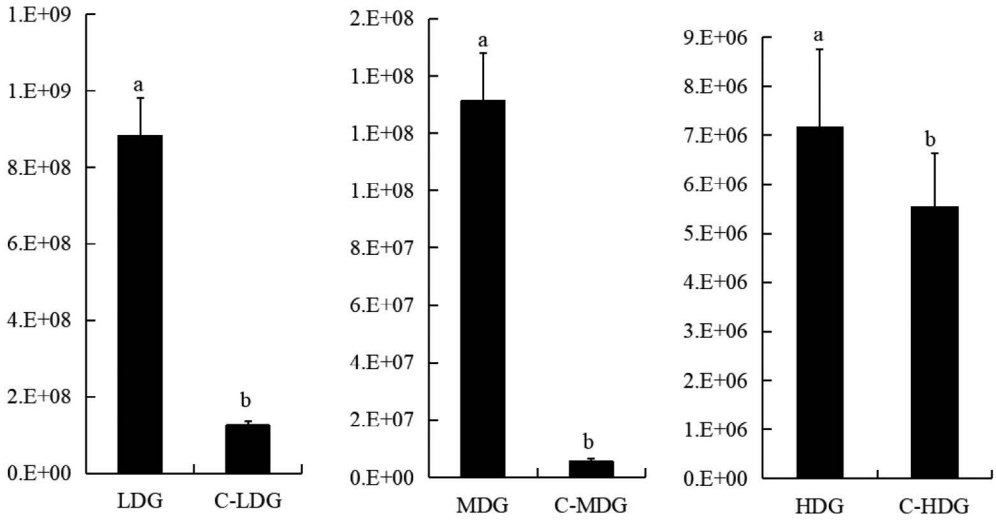

Fig. 2. The abundance of fungal rDNA genes at each site. Different lowercase letters indicate significant differences ($p < 0.05$) between the treatment group and the control group at the same site.

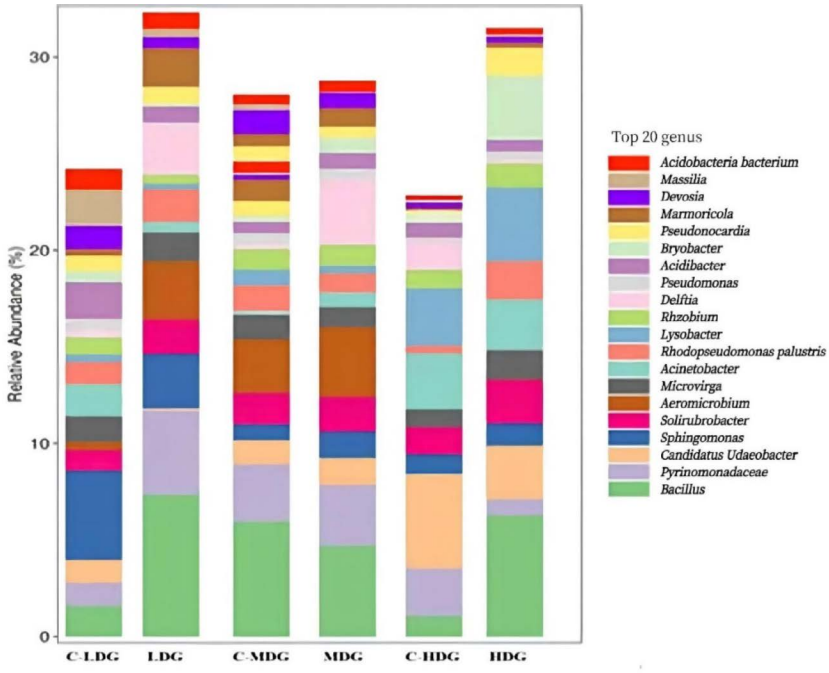

Fig. 3. The relative abundance of the dominant groups of bacteria at the genus level in grasslands with different sanding gradients. C-LDG: Light desertification grassland without microbial agent treatments, LDG: Light desertification grassland with microbial agent treatments, C-MDG: medium desertification grassland without microbial agent treatments, MDG: medium desertification grassland with microbial agent treatments, C-HDG: heavy desertification grassland without microbial agent treatments, HDG: heavy desertification grassland with microbial agent treatments, (the same below).

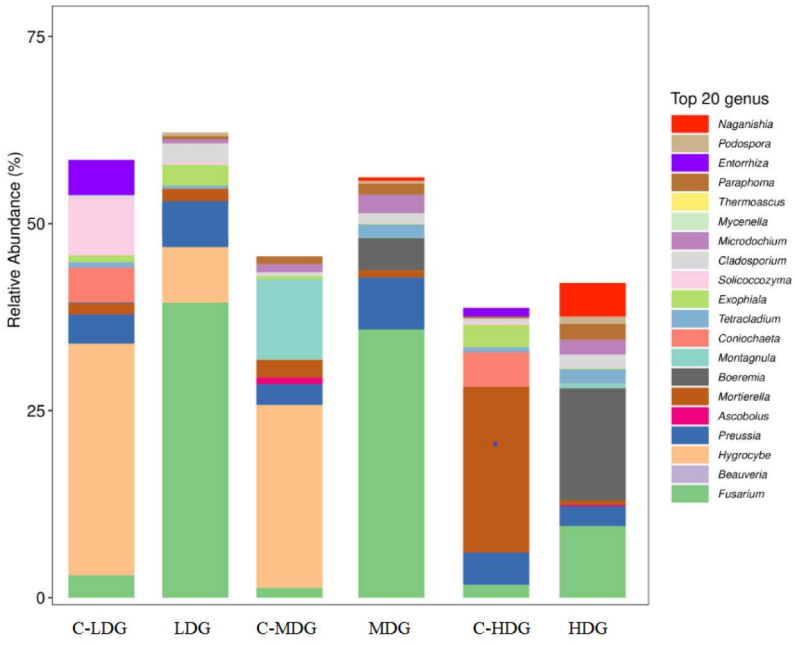

Fig. 4. The relative abundance of the dominant fungal groups at the genus level in grasses with different sanding gradients

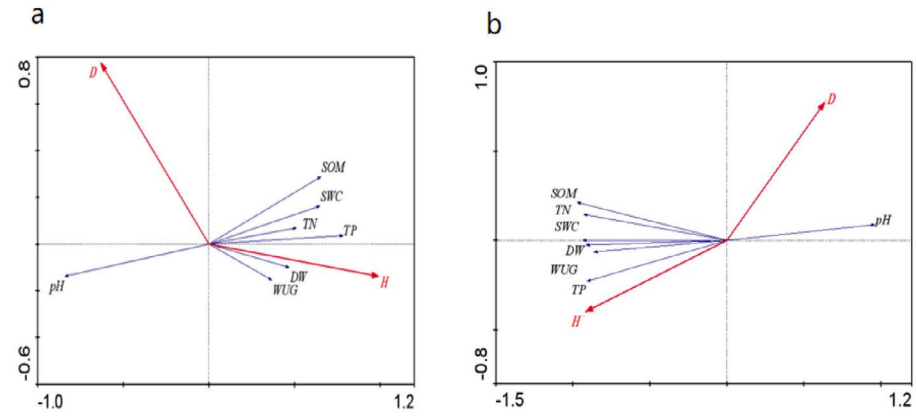

Fig. 5. Redundancy analysis of the soil microbial community structure in relation to soil environmental factors. **(a)** Graph describing the bacterial community structure and soil environmental factors, **(b)** Graph describing the fungal community structure and soil environmental factors. *H*: the Shannon-Wiener diversity index of bacterial communities, *D*: the Simpson diversity index of bacterial communities, SOM: Soil organic matter, SWC: Soil water content, TN: Soil total nitrogen, TP: Soil total phosphorus, DW: Dry weight of forage yield, WUG: Dry weight of underground biomass.

predominance of *Fusarium* and *Boeremia*, the relative abundance of other species was also highly variable. The absolute dominant *Mortierella* decreased sharply to only approximately 1.74% of the community, for example. In addition, there was a significant decrease in *Coniochaeta*, *Exophiaia,* and *Entorrhiza*, while *Naganishia*, *Paraphoma*, *Microdochium*, *Cladosporium,* and *Tetracladium* showed a significant increase.

The Shannon-Weiner index and Simpson's diversity index are diversity indices used to measure biodiversity, i.e. to evaluate species richness and species evenness. The Shannon-Weiner index is used to describe disorder and uncertainty in the occurrence of individuals of a species, with larger values indicating richer species diversity. Simpson's diversity index values range from 0 to 1, with values closer to 1 indicating greater diversity and closer to 0 indicating less diversity. The Shannon-Wiener diversity index and Simpson diversity index of microorganisms at each site are shown in Table 5. After treatment with the microbial agents, the Shannon-Wiener diversity index significantly improved, and the Simpson diversity index decreased slightly for all the different degrees of grassland degradation. Redundancy analysis (RDA) showed that the pH value had a considerable influence on the diversity of both the bacterial and fungal communities (Fig. 5), the Shannon-Wiener diversity index of bacterial and fungal communities was negatively correlated with pH, while positively correlated with the soil water content, soil organic matter content, soil total nitrogen and soil total phosphorus, respectively. On the other hand, Simpson's diversity index of bacterial and fungal communities suggested the opposite.

DISCUSSION

The grassland ecosystem in Northwest Sichuan is a region sensitive to climate change (Jacobs *et al*. 2003; Tu *et al*. 2013; Wu *et al*. 2019). The desertification of the grassland and the subsequent reduced litter yield of vegetation have had a major negative impact on the physical and chemical properties of the soil. Furthermore, the absorption, utilisation, and transformation of nutrients by microorganisms have also been weakened, and the structure, quantity, and proportion of the soil microbial population seriously unbalanced. Soil-borne diseases have been aggravated, and some pathogens have increased dramatically, such as grey mould caused by grey staphylinid (*Botrytis cinerea*), early blight caused by *Streptomyces* sp. (*Alternaria* sp.) fungi, and root-knot nematode disease caused by nematodes (*Meloidogyne* spp.), etc. (Arita *et al*. 2020; Hernández *et al*. 2020; Zhao *et al*. 2020).

The ecological restoration of grassland desertification has mainly been based on "fence and replant" (Fu *et al*. 2010; Zhu *et al*. 2016; Li *et al*. 2020), which to some extent has prevented and controlled the process of land desertification. However, the vegetation community structure tends to be single due to long-term enclosure, when dominant species have gradually replaced other species, and above-ground biomass and diversity have also decreased. What is more, due to the lack of living biological substances, the transformation of material and energy is low, and fertilization time long, which results in no essential improvement in the physical and chemical properties and biological properties of the soil (Xia *et al*. 2016; Chen *et al*. 2019). Microbial agents containing active microorganisms with specific fertilizer effects are generally considered to be environment-friendly products with high efficiency to improve crop yield and soil (Vimal *et al*. 2017; Wang *et al*. 2020). In this study, the soil moisture content, soil organic matter, soil total nitrogen, and soil total phosphorus increased as a result of treatment with the microbial agents (Table 3). This provided abundant carbon and energy for forage growth, resulting in a significant increase in both the dry weight of the underground biomass and the dry weight of the forage yield (Table 2). In the meantime, the microbial agents promoted the reproduction of microorganisms in the whole environment, followed by the further release of organic nutrients and an increase in available nutrients and soil fertility.

The soil microbiome is an important part of the ecosystem, which has a significant impact on soil quality maintenance, remediation, and the materials cycle (Zhang *et al*. 2017b; Liu *et al*. 2020a). Previous studies demonstrated that the abundance and diversity of soil microorganisms were significantly improved when treated with a microbial agent (Zhao *et al*. 2019; Liu *et al*. 2020b). The same result was obtained in other research, where environmental factors were shown to have a profound impact on this process (Li *et al*. 2004). Firstly, because of the different degrees of soil desertification, the influence on the microbial community from soil water content proved to be different at each site. In this study, there was a significant positive correlation between the soil water content and microbial abundance, whether bacteria or fungi, in both HDG and MDG. However, in LDG, there was no significant correlation between the soil water content and microbial abundance (Table 4). Secondly, the change in environmental pH has always been one of the important factors affecting microbial communities (Mudhoo & Garg 2011; Liu *et al*. 2020c). The serious desertification of grassland resulted in an increase in soil pH and a decrease in soil water content, soil carbon, nitrogen and the soil C/N ratio, etc. (Li *et al*. 2004; Jin *et al*. 2018; An *et al*. 2020). Treatment with the microbial agents meant that active microorganisms entered the soil and propagated rapidly, which stimulated the metabolism of soil microorganisms, brought the concentration of the environmental ions closer to the adaptive range of microbial enzymes, enhanced soil biological activity, and accelerated organic matter decomposition and transformation, thus resulting in a significant increase in soil organic matter, total nitrogen and total phosphorus. In turn, this further promoted the growth of microorganisms and the improvement of crop yield and quality. Remarkably, the colonisation of foreign functional microorganisms showed a significant inhibitory effect on some soil-borne pathogens, especially in LDG and MDG. For example, while functional microorganisms were able to secrete some antibacterial peptides to increase the cell permeability of *Fusarium* spp., *Fusarium* spp. rapidly died and reduced because of cell content exosmosis (Liu *et al*. 2019).

In summary, the application of the microbial agents greatly improved the soil water content of the sandy grassland, adjusted the soil pH, significantly increased the content of soil organic matter, total nitrogen, and total phosphorus, increased the abundance and diversity of the bacterial and fungal community and then inhibited the growth of soil-borne pathogens. The application of microbial agents can not only improve the yield and quality of forage, but also guide soil restoration. Although further research is needed, our findings provide a microbial consortium with the potential for the management of degraded grassland.

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REZIME

Botanica SERBICA

Efekti funkcionalnog mikrobnog agensa na mikrobne zajednice tla visoko hladnih dezertifikacionih travnih staništa u severozapadnom Sečuanu

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Dezertifikacija travnjaka bi ozbiljno uništila strukturu mikrobne zajednice zemljišta i dodatno ubrzala propadanje travnjaka. U ovoj studiji, funkcionalni mikrobni agensi su korišćeni za prskanje degradiranih travnjaka, sa fluorescentnim kvantitativnim PCR-om u realnom vremenu i metodama sekvenciranja visoke propusnosti. Cilj je bio proučavanje efekata mikrobnih agenasa na strukturu mikrobne zajednice u tlu i funkciju remedijacije zemljišta u različitim degradiranim travnjacima(LDG: slabo dezertifikovana travna staništa, MDG: srednje dezertifikovana travna staništa i HDG: teško dezertifikovana travna staništa). Rezultati su pokazali da se nakon tretmana mikrobiološkim agensima broj bakterija povećao za 96.24% (LDG), 95.19% (MDG) i 93.47% (HDG), respektivno, a broj gljivica je povećan za 85.77% (LDG), 95.85% (MDG).) i 22.49% (HDG), respektivno. Dalje, kolonizacijom i aklimatizacijom stranih funkcionalnih mikroorganizama, mikrobni agensi su imali veliki uticaj na strukturu mikrobne zajednice zemljišta, povećanje indeksa mikrobne raznovrsnosti i značajnu promenu sastava mikrobne zajednice. U međuvremenu, primena mikrobnog agensa ne samo da poboljšava prinos i kvalitet stočne hrane, već i vodi obnovi zemljišta, poboljšava sadržaj vode u zemljištu na peščanim travnjacima, podešava pH zemljišta, značajno povećava sadržaj organske materije u zemljištu, ukupnog azota i ukupnog fosfora, a zatim i inhibira rast patogena u zemljištu. Naši rezultati su dali nove ideje i smernice za upravljanje degradiranim travnim staništima.

Ključne reči: dezertifikacija travnih staništa, mikrobna zajednica zemljišta, funkcionalni mikrobni agensi, remedijacija, upravljanje degradiranim travnim staništima