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Artificial planting changes soil microbial community dynamics

ZiWei Tao[1], JinJuan Li[2], XiangTai Wang[1], and Guozhen Du[3]

[1]Lanzhou University
[2]Gansu Academy of Agricultural Sciences
[3]Key Laboratory of Arid and Grassland Ecology under the Ministry of Education

February 22, 2022

Abstract

The artificial planting of grassland serves as the most important means of grassland ecological restoration; however, the impact of artificial planting on soil microbial communities is not well understood. In this study, the evolution of the microbial community structure was studied using 16S and ITS gene sequencing techniques, and the microbial community differences between different forage grasses were analyzed, including different density cropping schemes, multi-year degraded grassland and natural grassland. It was found that the high-density planting scheme of multiple pastures exerts a great impact on soil nutrients as well as on the soil microbial community, effectively increasing the relative abundance of Actinobacteria and Basidiomycota, while the microbial community structure was found to be similar to that of natural grassland. However, in artificial planting treatment, the key node microflora group was noted to be bacteria, which was different from that in natural grassland, in which the key node microflora group was fungi. In comparison, fungi were found to be more sensitive than bacteria to different plantings.The rise in soil fungal diversity did not improve phosphate mineralization.Overall, this study may contribute to understanding the influence of artificial grassland on soil properties as well as the succession of microbial communities, How to accelerate the succession process of grassland ecosystem. which are of great significance in promoting artificial technology to restore the ecological environment.

Artificial planting changes soil microbial community dynamics

ZiWei Tao[1] JinJuan Li[2] XiangTai Wang[1] * GuoZhen Du[1] *

[1] Lanzhou Univ,Sch Life Sci, State Key Lab Grassland & Agroecosyst, Lanzhou, Gansu, Peoples R China

[2] Gansu Academy of Agricultural Sciences

* corresponding author:

XiangTai Wang: wangxt@lzu.edu.cn

GuoZhen Du: guozdu@lzu.edu.cn

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The artificial planting of grassland serves as the most important means of grassland ecological restoration; however, the impact of artificial planting on soil microbial communities is not well understood. In this study, the evolution of the microbial community structure was studied using 16S and ITS gene sequencing techniques, and the microbial community differences between different forage grasses were analyzed, including different density cropping schemes, multi-year degraded grassland and natural grassland. It was found that the high-density planting scheme of multiple pastures exerts a great impact on soil nutrients as well as on the soil microbial community, effectively increasing the relative abundance of Actinobacteria and Basidiomycota, while the microbial community structure was found to be similar to that of natural grassland. However, in

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artificial planting treatment, the key node microflora group was noted to be bacteria, which was different from that in natural grassland, in which the key node microflora group was fungi. In comparison, fungi were found to be more sensitive than bacteria to different plantings.The rise in soil fungal diversity did not improve phosphate mineralization.Overall, this study may contribute to understanding the influence of artificial grassland on soil properties as well as the succession of microbial communities, How to accelerate the succession process of grassland ecosystem. which are of great significance in promoting artificial technology to restore the ecological environment.

Compared with secondary bare land, artificial grassland treatment can increase soil water content by 6.3%, increase soil organic matter content by 52.25%, increase soil total potassium content by 10.67%, increase soil total phosphorus content by 22.58%, and increase soil total nitrogen content by 51.31%. The content of available potassium in the soil was 53.98%, the content of available phosphorus in the soil was increased by 29.48%, and the content of alkaline hydrolyzed nitrogen in the soil was increased by 72.66%. This experiment confirmed the changing trend of soil microbial community affected by grassland succession. The fungal community will show the community composition of multiple dominant species coexisting with the succession process, and the bacterial community will show the community structure of a single dominant population with the succession process. The composition of soil microbial community in the artificial grassland verification technology block is between the sparse grass and dense grass stages, which proves that the artificial grassland technology accelerates the succession of soil microbial communities.

Keywords:

Artificial planting; Ecological restoration; Microbial community; Community succession;Soil improvement

Introduction Soil microorganisms serve as an important component of soil. They are an important part of the nutrient cycle (Van Der Heijden et al, 2008) and help improve nutrient access and hormonal stimulation in order to promote plant growth (Berg 2009). Soil restoration usually refers to the improvement of soil nutrients and succession of the soil microbial community (Deng et al., 2014). Grassland, as a living and economic production area for herders, plays an irreplaceable role. However, due to the increasingly fragile ecological environment and long-term widespread use by humans, the global grasslands have experienced severe degradation (Babel et al., 2014; Hoppe et al., 2016;Kerven et al., 2012;Nesper et al., 2015; Pereira et al., 2018). The grassland ecological environment has deteriorated, and its ecological and production functions have been greatly affected. In addition, the regional ecological protection and socio-economic development functions of grasslands have been weakened (Dorre and Andrei 2012).However, much reliance has been given to the restoration of natural vegetation communities so as to improve the soil microbial community structure and restore land productivity (Chavarria et al, 2016; Guo et al, 2018; Liu et al, 2019). Therefore, how to efficiently carry out grassland restoration has become an important research topic in ecological restoration.

The concept of environmental friendliness and sustainable development has promoted research into soil microorganisms as biofertilizers (Kalayu 2019; Meena et al, 2017; Singh et al, 2020), though most studies have focused on directly adding microorganisms to improve the soil environment (Raymond et al, 2021; Lies et al, 2018; Itelima et al, 2018). The alpine meadow region in the eastern part of the Qinghai-Tibet Plateau is one of the main producing areas of animal husbandry in China. Accordingly, its extensive use has led to serious grassland degradation (Wen et al, 2018; Ma et al, 2020). The use of biofertilizer involves numerous costs, and herdsmen are usually not accustomed to investing in the pasture (Yan et al, 2011).

Artificial planting of grassland is the most effective method for grassland restoration (Wu et al, 2010); however, few studies exist that pertain to the response of soil microbial communities to different artificial planting schemes. In this study, the effects of different planting schemes on the soil microbial community were examined at the Alpine Meadow Wetland Ecosystem Positioning Research Station of Lanzhou University. Here, four main local pastures were used with a variety of unicast mixed sowing schemes for artificial planting. Moreover, a degraded alopecia areata that has been exposed for more than 7 years was added along with a piece of land. Natural succession occurred for over 10 years, and a comparison of top community blocks with stable community structure was conducted in order to understand the interaction of vegetation and

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microbial communities in the complete succession link. Accordingly, experiments were conducted at the Alpine Meadow Wetland Ecosystem Positioning Research Station of Lanzhou University. Four major local pastures were selected, and a variety of unicast and mixed sowing schemes were used for artificial planting. In addition, two comparative blocks were added: an alopecia areata plot that was degraded and exposed for more than 7 years, and a climax block that had natural succession for more than 10 years. Afterward, using high-throughput sequencing of 16S and ITS rRNA genes, the bacterial and fungal community structures were investigated, and their composition in different planting schemes were examined.

So, which planting method can effectively promote the process of grassland ecological succession and accelerate the restoration of grassland?

2. Materials and methods

2.1. Experimental materials

Four local wild fine pastures with complementary morphological characteristics were selected as test materials, namely Avena sativa, Poa pratensis, Elymus nutans and Festuca sinensis.

2.2. Experimental area

The experiment was set up in the Alpine Meadow Wetland Ecosystem Research Station of Lanzhou University (34°55'N, 102deg53'E). The site was located in the eastern part of the Qinghai-Tibet Plateau, in which the altitude was 2900 m, average annual temperature was 2.0 , and average annual precipitation was 557.8 mm.

2.3. Experimental program

2.3.1. Planting plan

The experiment is divided into two parts: unicast and mixed broadcast. 4 types of unicast: Avena sativa (As), Elymus nutans (En), Poa pratensis (Pp) and Festuca sinensis (Fs). Three kinds of mixed broadcasting: As+En+Pp (AEP), As+Fs+Pp (AFP) and As+En+Fs+Pp (AEFP). See Table 1 for seeding density.

For seeds in April 2019, when sowing, each treatment was broadcasted in three blocks with a block area of $4m^2$ (2mx2m), and 0.5m intervals were left between the plots. After the seeds were sowed, no mowing, grazing, fertilization, watering, and artificial intervention occurred during the growth period.

Table 1. Artificial planting seeding density

2.3.2 Sample Collection

Sampling was carried out in September 2019, which consisted of 22 plots that had previously been artificially constructed and planted and a barren areata plot that had been degraded and exposed for more than 7 years. In addition, a top community block that had a stable community structure after natural succession for more than 10 years was added for comparison.

After removing the plant residues on the soil surface of each plot, 5 samples were drilled along the S-shaped curve from the surface (0-30cm) using a soil drill (6cm in diameter). After getting the soil samples, they were mixed into one sample, which was then divided into two parts, for which one part was dried for 15 days and sieved using a 2 mm sieve to analyze the soil composition. After removing the sand and roots, the other part was divided into 3 parts and stored at -80degC in order to conduct an analysis of the microbial information.

2.3.3 Analysis of soil physical and chemical properties

Soil pH and moisture were determined using conventional methods (Liu et al, 2019), and soil organic matter (SOM) was quantified through potassium dichromate oxidation (Bao et al, 2011). Total nitrogen (TN) was determined via Kjeldahl nitrogen determination method (Purcell and King, 1996). Total potassium (TPo) was measured using alkaline fusion – flame photometry (Banerjee et al, 2020), while total phosphorus (TPh) was determined via alkaline fusion - molybdenum-antimony anticolorimetry (Qu et al., 2020). The content

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of alkali-hydrolyzed nitrogen (AN) was measured using alkali-hydrolyzed diffusion (Zhao et al, 2017). Determination of available potassium (APo) was carried out by ammonium acetate extraction-flame photometry (Sun, 2008). Finally, available phosphorus (AP) was extracted by sodium bicarbonate and then identified via anticolorimetry (Stevens et al., 2005).

2.3.4 Nucleic acid extraction

The genomic DNA was extracted using the PowerSoil DNA isolation kit (Mo Bio Laboratories, Solana Beach, CA, USA) according to the manufacturer's instructions. Three parts of each compost sample were extracted, after which the three extracts were mixed and detected using 1% agarose gel electrophoresis. The concentration and purity of the DNA were then determined via spectrophotometry with a micro-ultraviolet spectrophotometer (Nano Drop Technologies, USA). The absorbance ratios of all DNA samples were A260:A230>1.7 and A260:A280>1.8.

2.3.5 High-throughput sequencing

Quantitative PCR analysis of the 16S rRNA gene of the bacteria and ITS gene of the fungus was then carried out using the following primers: 338F: 5'-ACTCCTACGGGAGGCAGCAG-3'/806R:5'-GGACTACHVGGGTWTCTAAT-3' and ITS1F: 5'-CTTGGTCATTTAGAGGAAGTAA-3 '/ITS2R:5'-GCTGCGTTCTTCATCGATGC-3'. PCR was performed on the GeneAmp 9700 PCR system (Applied Biosystems, Foster City) (California, USA). The PCR reaction conditions occurred in the following order: 95degC (3 minutes), 95degC (30 s), 62degC (30 s), 72degC (45 s) for 30 cycles, and finally at 72degC for 10 minutes. Three copies of each sample were amplified, and the amplified products were mixed into one sample and detected by 2% agarose gel electrophoresis. The amplified products were then purified using the AxyPrep DNA gel extraction kit (Axygen Biosciences, Union City) (California, USA), which were then detected by 2% agarose gel electrophoresis. High-throughput sequencing was performed by Shanghai Meiji Biopharmaceutical Technology Co., Ltd. (Shanghai, China) on the Illumina MiSeq platform (San Diego, California, U.S.).

2.3.6 Sequencing data processing

In order to obtain higher quality and more accurate results in the biological information analysis, effective sequences were mixed, while optimized sequences were obtained for the data statistics. The sequence was less than 200 bp, the base was fuzzy or the average quality was less than 25. The chimeric sequence was deleted using mothur software (Schloss et al, 2010). Finally, sequence readings for each sample were clustered with 97% similarity as an operable classification unit (OTU) (Edgar et al., 2013). Based on the SILVA and Unite databases, the sequences represented by OTU were then sorted, which included bacterial and fungal ribosomal RNA sequences (version 119) (Pruesse et al. 2007) using the RDP classifier (Wang Q et al, 2007).

2.3.7 Statistical analysis

Based on OTU, R software was used to calculate the species composition of different samples at various taxonomic levels in order to understand the dominant species contained in each sample at the same taxonomic level as well as the relative abundance of each dominant species in the sample. In order to ascertain the similarities and differences of the flora of all groups under different treatment factors, PCoA analysis was performed on the samples. Circos software was then used to make the relationship diagram between the samples and species to obtain the composition proportion of dominant species in each sample and understand the distribution proportion of dominant species in different samples.

Lefse software was used to detect the characteristics of significant abundance difference according to non-parametric factorial Kruskal-Wallis (KW) Sum-rank test (non-parametric factorial Kruskal-Wallis rank sum test), for which the groups with significant abundance difference were found. Finally, linear discriminant analysis (LDA) was used to estimate the impact of each species' abundance on the effect of the difference. Spearman correlation of each OTU was paired with Gephi (R>0.8), after which the construction of a microbial symbiosis network and network module was performed.

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3.Results

3.1 Influence of different planting schemes on soil properties

Different planting schemes have different effects on soil properties (Table 2). Compared to other single-seeding treatments and low-density planting treatments for a variety of pastures, T7 treatments for high-density planting of four pastures in conjunction with the T22 treatments overgrown with weeds were found to significantly increase the organic matter (SOM) content in the soil. Compared to T22 treatment, T7 and T5 treatment of high-density planting of the three pastures were noted to greatly increase the content of alkali hydrolysable nitrogen (AN) in the soil. Moreover, T2 and T7 treatment of high-density planting of Elymus nutans (Elymus nutans) were observed to significantly raise the available phosphorus (AP) content in the soil. Compared to T23 treatment, which was barren for many years, and T24 treatment with top stable vegetation communities, T7 and T22 treatment had the highest levels of total soil nitrogen (TN) content. Furthermore, compared to T24 treatment, the soil moisture content of other treatments was found to have a large difference.

Table 2 Soil properties of different establishment blocks

3.2 Composition of soil microbial community

We selected five stages in the succession process from secondary bare land to natural grassland: secondary bare land stage (T23), primary and biennial weedy stage (T22), pioneer planting stage (T1), sparse bush Grass stage (T20), dense grass stage (T24) (Xiao et al., 1982; Sun, 1992), measure the bacterial and fungal gene sequences of soil samples, and understand the composition of soil microbial communities in different succession stages. The bacterial and fungal gene sequences of soil samples treated with T7 were measured to understand the similarity between the microbial community composition of this treatment and the five stages of grassland succession, and to verify whether artificial grassland technology can accelerate the succession process of grassland ecosystems.

Accordingly, 1,054,960 valid bacterial gene sequences were obtained from all soil samples, with an effective base number of 440,512,479 and an average sequence length of 417. Through clustering, 5498 OTUs were then obtained, which belonged to 33 Phylum, 92 Class, 239 Order, 405 Family, and 757 Genus. Similarly, 1,275,477 effective fungal gene sequences were obtained from the soil samples, in which the number of effective bases was 300,395,036 and the average sequence length was 235. Following clustering, 1965 OTUs were obtained, which belonged to 12 Phylum, 38 Class, 84 Order, 190 Family, and 361 Genus.

According to the influence of different treatments on soil characteristics, the 6 most representative treatment methods were then selected for analysis. As seen in Figure 1A, the main bacterial communities in the soil were found to be Proteobacteria, Actinomycetes, Acidobacteria and Chlorocurbillus, while other populations, including Bacteroidetes, Bacillomonas and Corynebacterium, were not observed to contribute much to the bacterial community in the soil. Compared to the bacterial community, the composition of the fungal community was observed to be relatively simple, in which the main fungal communities were Ascomycetes, Morphomyces and Basidiomycetes, while the abundance of Olpidiomycota, Chytrid fungi and Rozomycetes was noted to be relatively low (Figure 1B).

According to another perspective, the six treatments selected simulated the complete restoration and succession process of the grassland vegetation community from the barren land, invasion of weeds, and establishment of dominant species to the attainment of the top grassland vegetation community. From the barren T23 treatment to the T24 treatment with top community structure, no significant differences were present in microbial community composition at the Phylum level; however, there was a significant difference in the abundance of each Phylum ($p<0.05$) (Figure 1).

Figure 1. Relationship between soil microbial Circos samples and species. The figure reflects the composition proportion of dominant species in each sample as well as the distribution proportion of each dominant species in different samples.

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3.3. Relationships between soil properties and microbial taxa

The correlations among the soil properties and top 24 phyla of bacteria and fungi were then analyzed (Figure 2). Here, the Spearman's rank correlation coefficients showed that seven of the dominant phyla were positively correlated with most physicochemical factors, while six of the dominant phyla were negatively correlated with these factors. Specifically, SOM, AP, TN, and TPh were found to be positively correlated with Proteobacteria, Acidobacteria, Latescibacteria, and Ascomycota, which also had positive correlations with Gemmatimonadetes and Firmicutes. Firmicutes and Armatimonadetes were noted to be negatively correlated, while AN and APo were found to be positively correlated with Chloroflexi, Rokubacteria, and Basidiomycota and negatively correlated with Bacteroidetes and Mortierellomycota.

Figure 2. Correlations between environmental factors and dominant microbial taxa based on the Spearman correlation coefficient. The color represents the value of the Spearman correlation coefficient. "B_" represents the bacterial Phylum, and "F_" represents the fungal Phylum.

3.4 Differences in microbial communities among different treatments

The similarity of the bacterial community and fungal community under OTU water classification among different treatments were then compared (Figure 3). Comparing the composition of the bacterial community (Figure 3A) and composition of the fungal community (Figure 3B) of each treatment, T7 treatment with high-density establishment of the four pastures was found to possess the highest similarity with T24 treatment.

Figure 3. PCoA analysis of species diversity among microbial communities in different treatments

In order to refine and understand the differences among the microbial community samples of each treatment, a LEFSE analysis was conducted.

Comparing the differences in bacterial community abundance of each treatment, the difference in relative abundance between T23 and T24 treatment was found to be most significant (Figure 4A). The biomarkers having a LDA value greater than 2 was T23 dealt with 24, while the top three LDA values were O__chloroflexales, F__Roseiflexaceae and P_armatimonadetes. T24 treated 17, and the top three LDA values were C__Actinobacteria, P__Actinobacteria and F__Micrococcaceae.

Comparing the difference in fungal community abundance, T24 treatment was observed to have the most significant difference in relative abundance (Figure 4, B). There were 30 biomarkers with a LDA value greater than 2, while the top three with the largest LDA values were c__Agaricomycetes, p__Basidiomycota and o__Agaricales.

Figure 4. Discriminant analysis of LEFSE multi-energy level differences in soil microbial communities. A LEFSE linear discriminant analysis (LDA) was conducted on the samples according to taxonomic composition and different grouping conditions in order to ascertain the communities or species with significant differences in sample division. Nodes with different colors indicated microbial groups that were significantly enriched in the corresponding group and had a significant impact on differences between groups. Here, light yellow nodes indicated microbial groups with no significant differences in different groups or no significant impact on differences between the groups.

3.5 Interaction network between soil microbial communities

In order to further understand the correlation and difference between the dominant microbial groups (taxa) of each treatment, three treatments were selected: grasslands that have been degraded for many years, artificial planting treatments that have the greatest impact on soil properties, and natural top grassland communities. A network diagram was then constructed at the OTU classification level (Figure 5) in order to understand the interaction of microbial communities at the three key nodes of ecological restoration of degraded grasslands. Accordingly, the modulo quickening index of the three treatments was found to be greater than 0.6, serving as a typical module structure (Newman, 2006). Compared to the mean degree, the mean degree of T24 treatment was noted to be higher than that of T23 and T7, indicating that the microbial community of T24 treatment had a higher degree of interdependence.

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Figure 5. Interaction network and modular network of the soil microbial community. Each connection represents a strong positive correlation (Spearman's ϱ>0.8). The size of each node in A is proportional to the number of connections (degrees), and the connections between nodes are colored according to the different degrees of connection of the nodes. Picture B is colored according to modular classification. In the picture, the text "OTUB" represents the bacterial group, while "OTUF" represents the fungal group.

By comparing the three microbial groups with the highest degree of connection among different treatments (Fig. 5), T23 treatment, which was deserted for many years, was found to belong to two groups of Phylum: Bacteroidetes and Chloroflexi, which were not among the top three Phylum in the bacterial community in terms of abundance (Figure 1A, ChloroFlexi 13.94% and Bacteroidetes 5.75%). A possible reason for the low abundance but very active group is that the microbial community of T23 did not reach the top stable community level after many years of succession, while the inferior population with low abundance kept competing for a higher ecological niche. Compared to T24 treatment, the three species groups with the highest activity and the two species with the highest abundance were Phylum, in which the community structure was found to be more stable. T7 treatment was observed to be between the two.

As seen in Fig. 5B, the network structure of T24 treatment was noted to be clearer and more stable, while the network topology of T23 treatment was disorderly and T7 treatment was between T24 and T23 treatment. Another interesting phenomenon was that the three groups with the highest connectedness in T7 treatment were all bacterial groups, while those in T24 treatment were all fungal groups.

4. Discussion

Artificial planting has been demonstrated to be an important ecological restoration scheme according to previous studies (Yin et al, 2009; Li et al., 2017). Due to the interaction between plants and microorganisms (Trivedi et al, 2020), a top-level and stable grassland ecosystem should include top-level vegetation communities and top-level microbial communities. In this study, artificial planting was found to restore the aboveground vegetation community while influencing the soil's physical and chemical properties as well as the composition of soil microbial community, similar to previous studies (Menyailo et al, 2002; Han et al., 2007; Zhang et al., 2016). One possible reason is that litter and root biomass provide suitable habitat and sufficient energy for the soil microbial community to drive the succession of the microbial population (Yuan et al, 2015; Kyaschenko et al, 2017; Schirawski et al. al, 2018; Pathma et al, 2020). The increase in the abundance of microbial populations accelerates the cycle of carbon, nitrogen, and phosphorus (Xu et al, 2013; Karhu et al, 2014; Brabcová et al, 2018). Moreover, different planting schemes have different degrees of influence on soil characteristics, which was also confirmed in this study (Table 2).

In general, different cropping schemes have different effects on the microbial community due to the varied responses of different microbial groups to the environment (Fig. 1). The results of this study showed that the soil bacterial community was mainly comprised of Proteobacteria and Actinobacteria (Fig. 1), similar to previous studies (Spain et al., 2009; Barka et al, 2016). Proteobacteria, actinomycetes and acidobacteria were found to respond significantly to different treatments (Figure 1), while other studies have also proven that Proteobacteria, actinomycetes and acidobacteria are more sensitive to environmental changes (Verzeaux et al, 2016). In regard to the T7 treatment of high-density planting of the four pastures, the bacterial community structure was noted to be most similar to the natural top-level community T24 treatment (Figure 1).

Different planting schemes had varying degrees of influence on soil properties, which was also confirmed in this study (Table 2). Compared with the secondary bare land T23 treatment, the artificial grassland treatment can increase the soil water content by 6.3%, the soil organic matter content by 52.25%, the soil total potassium content by 10.67%, the soil total phosphorus content by 22.58%, and the soil total nitrogen content by 51.31%. The content of soil available potassium was increased by 53.98%, the content of soil available phosphorus was increased by 29.48%, and the content of soil alkali-hydrolyzed nitrogen was increased by 72.66%.

The experimental results illustrated that the soil fungal community was dominated by Ascomycota (Fig. 1), which was also confirmed in previous studies (Zhou et al., 2017), indicating that Ascomycota can be

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considered as the dominant population and indicator species, as confirmed through the interaction network (Fig. 5). The ascomycete community has also been shown to respond very positively to artificial planting (Li et al, 2017; Brundrett and Tedersoo 2018). However, the results of the present study demonstrated that by comparing the abundance of Mortierella species treated with T24 and T23 and other artificial establishment treatments, the abundance of Mortierella treated with T24 was lowest, which was different from the findings of previous studies. Specifically, "The relative abundance of Ascomycota is higher in disaffected soils, while Mortierellomycota is more abundant in healthy soils (Yuan et al, 2020)" was different compared to this study.

In comparison, fungi were found to be more sensitive than bacteria to different plantings. Compared to different artificial planting blocks, the difference in the fungal diversity coefficient was observed to be significantly higher than that of the bacterial diversity coefficient (Table 1). A possible reason may be due to fungi's important role in the degradation of plant residues (Bastida et al, 2016; Liang et al, 2017). Ascomycota and Basidiomycota are key microbial groups for the degradation of complex plant compounds (Bastida et al., 2016).

Soil microbial communities are also regulated by interspecific network relationships (Poole et al, 2018; Kuypers et al, 2018). In this experiment, differences were noted in the modularity, number of nodes and number of connections of soil microbial community network between artificial planting and natural grassland and degraded grassland (Figure 5). Compared to artificial grassland and degraded grassland, the microbial groups of natural grassland were found to be more closely related. Since the modularization index was greater than 0.4 and the average clustering coefficient was greater than 0.6, it can be inferred that these relationship networks are modular (Fig.4), which has been previously confirmed by past studies (Koskella et al., 2017; Belin et al., 2018). Compared to artificial grassland and degraded grassland, the modular classification of microbial groups in natural grassland was also clearer (Figure 5). However, in contrast to the experimental expectations, the key microorganism group in the artificially constructed T7 treatment was found to be the bacteria group, while that of the T24 treatment was fungi (Figure 5).

The rise in soil fungal diversity did not improve phosphate mineralization. Compared to T7 treatment, T1 had a higher index of soil fungal diversity (Table 1), while the available phosphorus content of T1 treatment was found to be lower than that of T7 treatment (Table 2). A possible reason is that the increased abundance of microbial populations may have led to increased competition, thereby reducing the utilization of organic matter (Maynard et al., 2017; Maynard &Crowther et al, 2017). Therefore, improving soil microbial diversity alone cannot improve soil restoration efficiency. This experiment confirmed the changing trend of soil microbial community affected by grassland succession. With the advancement of grassland succession, the highest abundance of Ascomycota in the fungal community accounted for 52.5% in the secondary bare land stage, and decreased to 44.95% in the dense grass stage, and the abundance was the second The third Basidiomycota accounted for 6.67% in the secondary bare field stage, and increased to 34% in the dense grass stage. The fungal community will present a community composition in which multiple dominant species coexist with the succession process. The abundance of Proteobacteria, which accounted for 25.28% of the bacterial community in the secondary bare land stage, further increased with the succession process, reaching 36.74% in the dense grass stage. Actinobacteria and The abundance of Acidobacteria decreased continuously, and the bacterial community showed a community structure of a single dominant population with the succession process. The comparison found that the composition of soil microbial community in the artificial grassland verification technology block was between the stage of sparse grass and the stage of dense grass, which proved that the artificial grassland technology accelerated the succession of soil microbial community.

In this work, we have gained some basic understanding of the relationship between cultivated grasslands, natural grasslands, degraded grasslands and soil microbes. But there are still some problems to be studied. Under the conditions of this experiment, the fungal community will show a community composition of multiple dominant species coexisting with the grassland succession process, and the bacterial community will show a community structure of a single dominant species with the succession process. In other environments and under different plant community conditions, more experiments are needed to confirm whether the microbial

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community still exhibits the same trend.

5. Conclusion

The results demonstrated that the high-density planting plan of multiple pastures is able to alter soil properties and soil microbial community structure more effectively than the planting plan of a single species of pasture schemes, and accelerate the succession process of the grassland ecosystem. As a result, this study confirmed that artificial grassland planting serves as an important means of grassland ecological restoration, which has great research benefit.

Acknowledgments Du Guozhen,XiangTai Wang is the project manager, Li Jinjuan helped organize the data, and Tao Ziwei wrote this article

Funding:the National Key Research and Development Program of China (Grant No. 2017YFC0504801)

Conflict of Interest: The authors declare that they have no conflict of interest.

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