**[[1]](#footnote-0)Effect of different fertilization on** **microorganism and** **chemical property of alfalfa’s rhizosphere in alkaline soil**

Xudong Gua, Fengju Zhangb, Xiaowei Xiea, Xing Xua,✱

a School Of Agriculture, NingXia University, Yinchuan, Ningxia 750021,China

b Institute of Environmental Engineering, Ningxia University, Yinchuan, Ningxia 750021,China

**Abstract**

Experiment of fertilization was carried out on two - year alfalfa of alkaline soil. Five treatments were set including no fertilizer (CK), single nitrogen (N), single phosphorus (P), single potassium (K), organic fertilizer(Y), to study effects of different fertilization on microorganism and chemical property of alfalfa’s rhizosphere. Providing theoretical and technical support of fertilization for alfalfa of high yield and high quality in alkaline soil of Yinbei, the results as follow:(1)Fertilization increased content of OC and TN of rhizosphere of alkaline soil, and decreased pH value. The N, P and K fertilizers increased content of nutrients in respective forms of N, P and K, changes of other chemical indexes showed certain differences under fertilization compared to CK treatment. (2) Fertilization changed microbial diversity, richness, abundance and structure of alfalfa’s rhizosphere in alkaline soil, and nitrogen and phosphorus increased microbial diversity and richness particularly. At the family level, fertilization had little effect on the abundance of dominant bacteria, but great effect on the abundance of dominant fungi. (3)Microorganisms of rhizosphere were greatly affected by environmental factors. Families of dominant bacteria were greatly affected by OC, AN, XN and AP, families of dominant fungi were greatly affected by AP, TP, TK, PH, XN and AN, among which AP was closely related to a variety of fungi families.Therefore, fertilization improved soil fertility and microbial diversity of alfalfa’s rhizosphere in alkaline soil, especially nitrogen and phosphate fertilizer, which was great significance to the yield and quality of alfalfa.

**Keywords：**alfalfa, nutrient, microorganism, alkaline soil, fertilization.

**Introduction**

Salinization cause a lot of problems such as shortage of land resources, deterioration of ecological environment and decline of soil fertility (Yupeng Jing et al. 2020). It has become an important factor in restricting the efficient use of regional ecological environment and agriculture.

problem that solve saline-alkali land is a worldwide challenge. Area of China's saline-alkali land was large and widely, with a total area around 99.13 million hm2, accounting for about 10% of the country's land area (Zhenfang Shang et al. 2019). Salinization is widespread across the country, especially more serious in arid and semi-arid region of the northwest, Hetao Plain, as the most important agriculture zone and the most fragile ecological zone in northwest China. Because of the arid climate, intense evaporation, high salt and unreasonable irrigation regime and other reasons(Run Chu et al. 2018), causing area of saline-alkali land and degree of salinization increased year by year, and soil quality and fertility were severely degraded, it has become the main obstacle of land-resource utilization in HeTao Plain. Alfalfa is a kind of perennial leguminous herbage of high yield, high quality, salt tolerance, cold resistance and drought resistance(Na Sun et al., 2020), not only increase content of N, P and SOC, and reduce pH in saline-alkali soil (Qiang Li et al., 2019), but also improve the ecological environment. It is of great significance to the biological improvement, utilization of land use and environmental protection of saline-alkali land.

How to improve soil fertility, yield and yield of alfalfa in saline-alkali land, which is very important to environmental protection and economic development.The technology of fertilization is one of the effective measures to improve the quality and yield of alfalfa in saline-alkali land at present, Compared with cash crops, there are few studies on fertilization of alfalfa compare to economic crops, especially on in saline-alkali land. Researches showed that N addition increase organic carbon components of soil, reducing the pH of soil (Wenkui Zheng etal., 2020), Wekha N et al found that P addition reduce the pH of soil , increasing TN of soil (Wekha N et al. 2016), Lu Cai et al found that addition of fertilizer could improve OC, TN, TP and so on by collating domestic literature. increasing yield of alfalfa, especially the effect of chemical fertilizer combined with organic fertilizer is the most prominent (Lu Cai et al. 2021). Dongtie Zhang et al found that N-P addition, N-K addition P-K addition could significantly improve the content of crude protein, crude fat and yield of alfalfa on saline-alkali land,but content of crude protein and crude fat decrease with the increase of fertilizing amount(Dongtie Zhang et al.2011). Sa 'owen et al. (Duowen SA et al.2019) found that organic fertilizer significantly increase the content of crude protein and crude fat of alfalfa, and reducing the content of ADF, NDF and ash compare to chemical fertilizer by field experiment. The results indicated that addition of fertilization is great significance to the improvement of nutrient quality of soil, and had great influence on the formation of yield and quality on alfalfa

Microorganism is an important component of the microenvironment of rhizosphere, microorganism involving in decomposition of organic matter , formation of humus and cycles of nutrient with the soil enzymes together (Peng Tian, et al. 2020),microorganism provide condition to balance of nutrient and growth of crop, and also is sources and storage of nutrient in rhizosphere (Wangsuo Liu, et al., 2020;Jingwei Liu et al.2021).Relevant studies have shown that changes in the species and proportion of microorganism can regulate and respond to the formation and supply of nutrient (Xiaobo Gu et al. 2019), microorganisms of rhizosphere are commonly used as one of the sensitive indicators to change of characterize soil quality. Hugo A et al found that activity of acid phosphatase has close relationship with decomposition and solubility of phosphorus in experiment of P addition(Hugo A et al. 2018) , which indicated that microorganisms have a strong regulation on concentration of AP in rhizosphere. Wang Qi et al found that N addition(N200) change survival strategy of bacteria by inducing bacteria on seedling of poplar in rhizosphere(Wang Qi et al., 2021), which reducing the alpha diversity of bacteria, and cause symbiosis-network simplification of bacterial.So it indicated that N addition had a certain effect on the abundance and structure of microorganism in rhizosphere.In addition, Tri Canda Setiawati et al found that microorganism of rhizosphere have a good role to compatibilization of K on sugarcane, and increasing the content of AP in the rhizosphere, which indicated that the microorganism had a certain relationship with the dissolution of K. Microorganisms of rhizosphere play a very important role in the growth and development of crop, so it is of great theoretical and practical significance to study microorganism of rhizosphere.

Therefore, carrying out a two-year experiment of single N, P, K and Y fertilizer addition on alfalfa of alkaline field in Yinbei, studying different fertilizer on effect of nutrients and microbial structure in rhizosphere of alfalfa, and relationship between microorganism and environmental factors, it will provide theoretical guidance and technical support for precise and scientific fertilization on alfalfa of alkaline field in Yinbei.

**1. Materials and method**

1.1 Site description

The experiment was carried out the experimental base of Qianyeqing Agricultural Company, Pingluo, Ningxia county, China in early July 2019 (38°50'N, 106°15'E; 1091~1110m above sea level ). Pingluo is zone of drought and desert in China. The amount of solar radiation is 4225.9kJ/m2 from April to October, and strongest amount of solar radiation is 716kJ/m2 in June. The hours of annual cumulative sunshine range from 2800 to 3200, with the most in June. Less rainfall and more evaporation, it’s annual precipitation is between 200~270 mm, precipitation account for 66.6% from July to September, the most amount of evaporation reachs up to 1500~1800mm.The average annual humidity is about 56%. Annual average temperature is 6.8, effective accumulated temperature of 10℃ or higher is 1638~2638℃, frost-free period about 150d. The soil type of the test field is alkaline soil, salt ions are mainly Na+, Cl- and SO4-2 .Physical and chemical characteristics of the tested soil are shown in table 1.

**Table 1 Physical and chemical characteristics of the tested soil**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  **Index****depth** | **SOC（g/kg）** | **OM(g/kg)** | **TN****(g/kg)** | **NH4+- N（mg/kg）** | **NO3-- N（mg/kg）** | **TP(g/kg)** | **AP（mg/kg）** | **TK(g/kg)** | **AK(mg/kg)** | **PH** |
| **20cm** | 16.04 | 27.65 | 1.18 | 11.30 | 193.96 | 5.34 | 112.91 | 53.21 | 126.97 | 8.57 |
| **40cm** | 10.52 | 18.14 | 0.97 | 7.41 | 256.21 | 3.92 | 42.06 | 50.52 | 130.99 | 8.50 |
| **60cm** | 8.12 | 14.00 | 0.86 | 9.12 | 286.87 | 3.14 | 121.57 | 48.94 | 143.05 | 8.40 |

**Note:** The SOC, OM, TN, NH4+- N, NO3--N, TP, AP, TK,AK refer to organic carbon, organic matter, total nitrogen, ammonium nitrogen, nitrate nitrogen, total phosphorus, available phosphorus, total potassium and available potassium respectively.

1.2 Experiment design and field management

The experiment adopted randomized design of block. Five treatments were set including no fertilization (CK), nitrogen (N), phosphorus (P), potassium (K) and organic fertilizer (Y). Fertilizer‘s amount of nitrogen (N), phosphorus (P), potassium (K) and organic fertilizer (Y) were 6, 9, 9 and 500kg/mu respectively. Management measures and basal fertilizer before all treatment of fertilization were the same in field. Repetition with 3 times, 15 plots, The plot adopted technology of artificial ridging. The interval of the plot was 1 meter, and guard row of the test field was 3 meters. The area of a single plot was 5m x 6m = 30m2, area of experiment was 450 m2, the actual area was 850m2.Alfalfa was mechanically sown with a sowing quantity of 1.5kg/mu, and a planting interval of 30cm in the middle of April 2017. Alfalfa was harvested artificially for 4 times on June 10, July 15, August 20 and October 10 respectively , and leave stubble of 5cm. The water was irrigated four times with depth of 5cm on May 15, June 15, July 15 and August 15 respectively. Fertilizer was artificially added, nitrogen was added twice a year, and half each time in April and July each year, phosphate, potassium and organic fertilizer was added once each year in April. Alfalfa's variety is salt-tolerant star (from Qianyeqing Agricultural of Ningxia). Fertilizers: Nitrogen fertilizer is Urea (N≥46.4%), phosphorus fertilizer is heavy calcium superphosphate (P2O5≥46%), and potash fertilizer is potassium sulfate for agricultural use (K2O≥50%), organic fertilizer is organic fertilizer（N+P2O5+K2O≥5%，organic matter≥45% ）.

The Five-point method was adopted to collect rhizosphere-soil samples with autoclaved sampling tools, 10-15 plants of well-growing alfalfa were selected from each collection point. Soil of 200g that attached to the alfalfa’s roots was collected after the alfalfa was uprooted and topsoil on the surface of root was shaken off. The rhizospheric  soil was divided into two parts, a part was treated routinely for the determination of the physical and chemical properties of the soil in the laboratory. Another part was stored in a sterilized sealed bag, was refrigerated in an icing box and quickly brought back to the laboratory, was stored at -80 ℃ for microbial determination.

**1.4 Measuring projection and method**

1.4.1 Method for physicochemical characteristics of soil: Organic carbon, total nitrogen, ammonium nitrogen, nitrate nitrogen, total phosphorus, available phosphorus, total potassium, available potassium and pH were measured in potassium dichromate volumetric method - external heating method, half minim kelvin method, phenol disulfonic acid method, 2mol/LKCI extraction and distillation, method of phenol disulfonic acid, HCIO4-H2SO4 method, method of 0.5mol/LNaHCO3, NaOH melting,-flame photometry and pH meter method respectively.

**1.5 Measuring method of rhizosphere microorganisms**

**1. 5.1 DNA extraction and PCR amplification**
 Microbial community genomic DNA was extracted from 30 samples using DNeasy 96 PowerSoil Pro QIAcube HT kit according to manufacturer’s instructions. The DNA extract was checked on 1% agarose gel, and DNA concentration and purity were determined with NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA). The hypervariable region V3-V4 of the bacterial 16S rRNA gene were amplified with primer pairs 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R(5'-GGACTACHVGGGTWTCTAAT-3') by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA)The fungi used ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS2R (GCTGCGTTCTTCATCGATGC) as primers, and  PCR amplification was the same as that of bacteria. The PCR amplification of 16S rRNA gene was performed as follows: initial denaturation at 95 ℃ for 3 min, followed by 27 cycles of denaturing at 95 ℃ for 30 s, annealing at 55 ℃ for 30 s and extension at 72 ℃for 45 s, and single extension at 72 ℃ for 10 min, and end at 4 ℃. The PCR mixtures contain 5 × *TransStart* FastPfu buffer 4 μL, 2.5 mM dNTPs 2 μL , forward primer (5 μM) 0.8 μL, reverse primer (5 μM) 0.8 μL, *TransStart* FastPfu DNA Polymerase 0.4 μL, template DNA 10 ng, and finally ddH2O up to 20 μL. PCR reactions were performed in triplicate. The PCR product was extracted from 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to manufacturer’s instructions and quantified using Quantus™ Fluorometer (Promega, USA).
 **1.5.2Illumina MiSeq sequencing**
 Purified amplicons were pooled in equimolar and paired-end sequenced (2 ×300) on an Illumina MiSeq platform (Illumina, San Diego,USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

**1.5.3 Processing of sequencing data**
 The raw 16S rRNA gene sequencing reads were demultiplexed, quality-filtered by Trimmomatic and merged by FLASH with the following criteria: (i) the 300 bp reads were truncated at any site receiving an average quality score of <20 over a 50 bp sliding window, and the truncated reads shorter than 50 bp were discarded, reads containing ambiguous characters were also discarded; (ii) only overlapping sequences longer than 10 bp were assembled according to their overlapped sequence. The maximum mismatch ratio of overlap region is 0.2. Reads that could not be assembled were discarded; (iii) Samples were distinguished according to the barcode and primers, and the sequence direction was adjusted, exact barcode matching, 2 nucleotide mismatch in primer matching.

Operational taxonomic units (OTUs) with 97% similarity cutoff (Liu et al. 2017) were clustered using UPARSE (version 7.1, http://drive5.com/uparse/), and chimeric sequences were identified and removed. The taxonomy of each OTU representative sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/) against the 16S rRNA database (bacterial database:SILVA 138, fungal database: unite8.0 ) using confidence threshold of 0.7. 3.

1.6 Data processing

Using Excel 20019 and data-analysis platform of majorbio for mapping, using DPSv7.05version analysis software for statistical analysis of data.

2.Analysis and results

2.1**Effects of fertilization on chemical property ofalfalfa’s rhizosphere in alkaline soil**

In general, different treatments of fertilization have different effects on physical and chemical properties of alfalfa’s rhizosphere in alkaline fields. Fertilization increase the contents of OC and TN , and decrease the pH value of rhizosphere compared to CK, the changes of other physical and chemical properties are not uniform among different treatments of fertilization(Table 1).In 2019, the OC、TN、XN、AN、TP、AP、TK and pH of CK are 16.2g/kg 1.16g/kg 209.9mg/kg 8.6mg/kg 0.40g/kg 70.2mg/kg 16.4g/kg and 8.44 in rhizosphere soil of alfalfa respectively. The N treatment increase contents of OC, TN, XN, AN and TK of rhizosphere soil, which increase by 22.60, 31.06, 14.86, 40.70 and 3.03% compare to CK treatment respectively, and OC, TN, XN and AN reach significant level (P<0.05), N treatment decrease the content of TP and AP, decrease pH of rhizosphere soil, which decrease by12.59, 21.51 and 2.96% compare to CK treatment respectively. The P treatment increase contents of OC, TN, TP, AP and TK of rhizosphere soil, which increase by 12.35，2.59，122.50,70.09 and 4.27% compare to CK treatment respectively, and OC, TP and AP reach significant level (P<0.05), P treatment decrease the content of XN and AN, decrease pH of rhizosphere soil, which decrease by 16.34, 10.47 and 2.25% compare to CK treatment respectively, and XN reach significant level (P<0.05). The K treatment increase contents of OC, TN, XN, AP and TK of rhizosphere soil, which increase by 6.79, 10.34, 5.24, 15.24 and 16.46% compare to CK treatment respectively, and AP and TK reach significant level (P<0.05), K treatment decrease the content of AN and TP, decrease and pH of rhizosphere soil, which decrease by 8.14, 2.50 and 0.60% compare to CK treatment respectively. The Y treatment increase contents of OC, TN, XN, AN, TP and AP of rhizosphere soil, which increase by 24.07, 15.52, 9.77, 12.79, 27.50 and 7.55% compare to CK treatment respectively, and OC reach significant level (P<0.05), Y treatment decrease the content of OC, decrease pH of rhizosphere soil, which decrease by 4.27 and 0.47% compare to CK treatment respectively.

These results indicated that different treatments of fertilization increased content of OC and TN of alfalfa‘s rhizosphere soil in alkaline field, and decreased pH.Other soil physical and chemical indexes showed certain differences among different treatments of fertilization. N, P and K treatments increased the nutrient content of N, P,and K forms respectively. Y treatment decreased the content of TK, and increased the content of other nutrient. In terms of comprehensive fertility of alfalfa’s rhizosphere, organic fertilizer has the best effect on formation of nutrients of alfalfa’s rhizosphere soil in alkaline field

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Year** | **Treatment** | **OC****(g/kg)** | **TN****(g/kg）** | **XN****（mg/kg）** | **AN****（mg/kg）** | **TP****(g/kg)** | **AP****（mg/kg）** | **TK****(g/kg)** | **pH** |
| **2019** | **CK** | 16.2士0.3a | 1.16士0.18a | 209.9士4.3a | 8.6士0.7a | 0.40士0.01a | 70.2士2.6a | 16.4士0.1a | 8.44士0.21a |
| **N** | 19.7士0.9b | 1.52士0.03b | 241.1士10.1b | 12.1士2.8b | 0.35士0.02a | 55.1士0.9a | 16.9士0.6a | 8.19士0.02a |
| **P** | 18.2士1.9b | 1.19士0.02a | 175.6士6.1c | 7.7士0.4a | 0.89士0.01b | 119.4士3.7b | 17.1士0.2a | 8.25士0.02a |
| **K** | 17.3士0.2a | 1.28士0.01a | 220.9士6.8ab | 7.9士0.3a | 0.39士0.05a | 80.9士3.4c | 19.1士0.6b | 8.39士0.13a |
| **Y** | 20.1士0.1b | 1.34士0.03ab | 230.4士22.3ab | 9.7士0.4a | 0.51士0.08a | 75.5士0.8ac | 15.7士0.6a | 8.40士0.01a |
|  |  |  |  |  |  |  |  |  |  |
| **2020** | **CK** | 16.5士0.2a | 1.17士0.16a | 213.1士5.3a | 8.6士0.8a | 0.38士0.02a | 51.9士0.8a | 16.6士0.3a | 8.44士0.03a |
| **N** | 19.9士0.7b | 1.54士0.04b | 244.7士9.1b | 12.3士2.4b | 0.41士0.02a | 105.7士3.9b | 17.7士0.8a | 8.18士0.12a |
| **P** | 18.5士1.8c | 1.27士0.03a | 174.3士6.9c | 7.6士0.3a | 0.91士0.04b | 121.2士4.8b | 18.5士0.6b | 8.24士0.21a |
| **K** | 17.4士0.2ac | 1.30士0.02a | 224.2士7.8a | 8.2士0.4a | 0.40士0.03a | 98.1士1.4b | 19.4士0.4b | 8.38士0.09a |
| **Y** | 20.5士0.1b | 1.36士0.04ab | 233.9士17.3ab | 9.9士0.3a | 0.52士0.05a | 76.5士2.8c | 15.6士0.5a | 8.41士0.11a |

 **Table 2. Effects of fertilization on physicochemical properties of alfalfa’s rhizosphere** **in alkaline soil**

**2.2** **Effects of fertilization on microbial diversity of alfalfa’s rhizosphere in alkaline soil**

**2.2.1 Diluted curves of rhizospheric microorganism**

Diluted curve of rhizosphere soil tends to be flat based on abundance of OTU (Figure 1), and

the sequencing coverage of bacteria and fungi are 0.98 and 1 respectively (Table 2). The results indicated that the sequencing data were reasonable, and could truly reflect basic information and species composition of microorganism in rhizosphere.



Fig1. Diluted curves of bacterial (a) and fungal (b) communities of rhizosphere based on OTU abundance

2.2.2 Effects of fertilization on microbial diversity of alfalfa’s rhizosphere **in alkaline soil**

As can be seen from Table.2, the N and P treatments increase bacterial diversity (shannon) of alfalfa’s rhizosphere compare to CK treatment in alkaline soil, and reach a significant level (P<0.05), the K and Y treatments had little effect on bacterial diversity (shannon). The N, P and K treatments increased the bacterial richness (ace and chao) of alfalfa’s rhizosphere compare to CK treatment in alkaline soil, N and P treatments reach a significant level (P<0.05) especially. Y treatment decreased the bacterial richness (ace and chao ) of alfalfa’s rhizosphere compare to CK treatment in alkaline soil, but don’t reach a significantly (P<0.05). The P and K treatment increase fungal diversity (shannon) of alfalfa’s rhizosphere compare to CK treatment in alkaline soil, and P treatment reach a significant level (P<0.05) especially, Y treatment decrease fungal diversity (shannon).The P treatment increase fungal richness (ace and chao ) of alfalfa’s rhizosphere compare to CK treatment in alkaline soil, the N, K and Y treatments decrease fungal richness, and P, K and Y treatments reach significant levels (P<0.05) especially. The results indicated that the addition of nitrogen and phosphorus increased microorganism’s richness and diversity, and enhanced the active level of microorganisms of alfalfa’s rhizosphere in alkaline soil.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Microorganism | **Treatment** | **Sobs** | **Shannon** | **Ace** | **Chao** | **Coverage** |
| **Bacteria** | **CK** | 2569.7士68.8a | 6.48士0.07a | 3409.2士154.1a | 3418.7士82.6a | 0.98  |
| **N** | 2748.3士93.1b | 6.91士0.20b | 3637.7士215.6b | 3632.9士143.5b | 0.98  |
| **P** | 2806.0士61.7c | 6.89士0.08b | 3732.5士76.2b | 3716.5士86.1b | 0.98  |
| **K** | 2676.7士90.8a | 6.44士0.24a | 3599.4士91.8ab | 3501.9士103.0a | 0.98  |
| **Y** | 2520.7士132.3a | 6.40士0.14a | 3346.3士178.6a | 3344.9士179.8a | 0.98  |
|  |  |  |  |   |  |  |
| fungi | **CK** | 312.3士17.1a | 3.27士0.37a | 362.3士5.4a | 367.6士6.6a | 1.00  |
| **N** | 296.3士26.3ab | 3.26士0.31a | 346.1士14.2ab | 349.9士21.6ab | 1.00  |
| **P** | 354.3士19.5bc | 3.66士0.03a | 388.1士16.2b | 393.5士22.9b | 1.00  |
| **K** | 289.7士22.2ac | 3.32士0.35a | 334.5士1.9b | 339.1士13.2bc | 1.00  |
| **Y** | 248.7士15.2d | 2.95士0.22a | 333.6士21.6b | 309.1士1.3c | 1.00  |

**Table 2. Effects of fertilization on bacterial and fungal diversity of alfalfa’s rhizosphere** **in alkaline soil**

**2.3 Analysis of fertilization on the relative abundance of microbial species of alfalfa’s rhizosphere in alkaline soil**

**2.3.1 Analysis of relative abundance of bacterial species in alfalfa’s rhizosphere**

The samples include 38 bacterial phylum at the phylum level, of which the phylum of dominant bacteria are proteobacteria, actinobacteriota, acidobota, chloroflexi, firmicutes, bacteroidota and gemmatimonadota(Figure.2). The phylum of dominant bacteria of CK treatment are proteobacteria(24.10%), actinobacteriota(19.09%), acidobacteriota(17.90%) chloroflexi(14.00%)and firmicutes(5.77%).

The phylum of dominant bacteria of N treatment are proteobacteria(26.86%), actinobacteriota(20.03%), acidobacteriota(15.95%), Chloroflexi(13.41%) and Firmicutes (5.41%) respectively. The abundance of proteobacteria and actinobacteriota increase by 2.76 and 0.94% compare to CK treatment respectively, and the abundance of Acidobacteriota, Chloroflexi and Firmicutes decrease by 2.05, 0.59 and 0.36% compare to CK treatment respectively. The phylum of dominant bacteria of P treatment are proteobacteria(24.12%), actinobacteriota(21.37%), acidobacteriota(16.34%), chloroflexi(13.68%) and firmicutes(5.64%) respectively. The abundance of actinobacteriota of P treatment increase by 2.28% compare to CK treatment, the abundance of acidobacteriota decrease by 1.56% compare to CK treatment, the abundance of chloroflexi proteobacteria and firmicutes almost unchange compare to CK treatment. The phylum of dominant bacteria of K treatment are proteobacteria(25.11%), actinobacteriota(24.40%), acidobacteriota(14,78%), chloroflexi(13.36%)and firmicutes(6.02%), respectively. The abundance of proteobacteria and actinobacteriota of K treatment increase by 1.01 and 5.31% compare to CK treatment, the abundance of acidobacteriota and chloroflexi decrease by 3.02 and 0.54% compare to CK treatment, and abundance of Firmicutes almost unchange compare to CK treatment. The phylum of dominant bacteria of Y treatment are proteobacteria(27.26%), actinobacteriota(23.56%), acidobacteriota(15.18%), chloroflexi(13.38%)and firmicutes(3.90%) respectively. The abundance of proteobacteria and actinobacteriota of K treatment increase by 3.16 and 4.47% compare to CK treatment, the abundance of Acidobacteriota Chloroflexi and Firmicutes decrease by 2.72, 0.62 and 1.87% compare to CK treatment. These results indicated that treatments of different fertilization changed the abundance of bacteria of alfalfa’s rhizospherea at phylum level which had different effects on the abundance of proteobacteria, acidobacteriota, actinobacteriota and firmicutes compared to CK treatmentin alkaline soil especially.



Fig2. Effects of fertilization on the relative abundance of rhizospheric bacteria of alfalfa at phylum level in alkaline soil

The richness and uniformity of bacteria are relatively good at the family level, including 487 families, among which the dominant families are norank\_o\_\_vicinamibacterales, vicinamibacteraceae, sphingomonadaceae, JG30-KF-CM45, micrococcaceae and orank\_o\_\_norank\_c\_\_KD4-96 in alfalfa rhizosphere of alkaline soil respectively from Fig.3. The families of dominant bacteria of CK treatment are norank\_O\_\_vicinamibacterales (7.18%), vicinamibacteraceae(5.80%), norank\_O\_\_norank\_c\_\_kd4-96(4.14%), JG30-KF-CM45(3.43%) and sphingomonadaceae(2.25%) in rhizosphere respectively.

The families of dominant bacteria of N treatment are norank\_o\_\_Vicinamibacterales（6.18%）,vicinamibacteraceae(4.80%), sphingomonadaceae（3.73）,JG30-KF-CM45(3.60%) and norank\_o\_\_norank\_c\_\_KD4-96(2.82%) in rhizosphere respectively. The abundance of rhizospheric norank\_o\_\_Vicinamibacterales, norank\_o\_\_norank\_c\_\_kd4-96 and vicinamibacteraceae of N treatment decrease by 1, 1.32 and 1% compare to CK treatment respectively, abundance of sphingomonadaceae increase by 1.48% compare to CK treatment, and abundance of JG30-KF-CM45 almost unchange compare to CK treatment. The families of dominant bacteria of P treatment are norank\_O\_\_vicinamibacterales (5.87%) vicinamibacteraceae(4.67%) micrococcaceae(4.40%) and norank\_O\_\_norank\_c\_\_KD4-96 (3.24%) in rhizosphere respectively. The abundance of rhizospheric norank\_o\_\_Vicinamibacterales, vicinamibacteraceae and norank\_o\_\_norank\_c\_\_KD4-96 of P treatment decrease by1.31, 1.13 and 0.90% compare to CK treatment respectively, and abundance of micrococcaceae increase by 2.09% compare to CK treatment. The families of dominant bacteria of K treatment are norank\_o\_\_vicinamibacterales (5.71%), vicinamibacteraceae(4.01%), ncardioidaceae (3.78%) and Micrococcaceae(3.76%) in rhizosphere respectively. The abundance of rhizospheric norank\_o\_\_Vicinamibacterales, vicinamibacteraceae and norank\_o\_\_norank\_c\_\_kd4 - 96 of K treatment decrease by 1.47、1.79% and 1.27% compare to CK treatment respectively, and abundance of noocardioidaceae and micrococcaceae increase by 1.41 and 1.74% compare to CK treatment respectively. The families of dominant bacteria of Y treatment are norank\_O\_\_vicinamibacterales (4.87%), vicinamibacteraceae(4.83%), ncardioidaceae (3.80%) and JG30-KF-CM45(3.76%) in rhizosphere respectively. The abundance of rhizospheric norank\_o\_\_Vicinamibacterales and vicinamibacteraceae of K treatment decrease by2.31 and 0.97% compare to CK treatment respectively, abundance of nocardioidaceae increase by 1.76% compare to CK treatment.and abundance of JG30-KF-CM45 almost unchange compare to CK treatment.

these results indicated that N treatment decreased the abundance of bacterial families of rhizospheric norank\_o\_\_vicinamibacterales, norank\_o\_\_norank\_c\_\_kd4-96 and vicinamibacter, and increased the abundance of Sphingomonadaceae. P treatment increased the abundance of rhizospheric nicrococcaceae, but decreased the abundance of rhizospheric norank\_o\_\_vicinamibacterales and vicinamibacteraceae. Although K treatment increased abundance of rhizospheric ncardioidaceae and micrococcaceae, but decreased the abundance of rhizospheric norank\_o\_\_vicinamibacterales vicinamibacteraceae and norank\_o\_\_norank\_c\_\_KD4-96. Y treatment increased the abundance of rhizospheric noocardioidaceae, but decreased the abundance of norank\_o\_\_vicinamibacterales. The results indicated that treatment of different fertilization improved the abundance of rhizospheric microorganism of alfalfa at family level in alkaline soil, which was beneficial to the transformation and utilization of rhizospheric nutrients combined with the growth situation of alfalfa and level of rhizospheric nutrients.



Fig.3. Effects of fertilization on the relative abundance of rhizospheric bacteria of alfalfa at family level in alkaline soil

**2.3.2 Analysis of relative abundance of fungal  species in alfalfa’s rhizosphere**

 The samples include 14 fungal phylum at the phylum level, and dominant fungi are ascomycota and mortierellomycota. The abundance of other fungi (unclassified\_k\_\_Fungi Chytridiomycota and Basidiomycota and so on) almost unchange. The phylum of dominant fungi of CK treatment are asoomycota(74.06%) and mortierellomycota(21.60%) respectively.

The phylum of dominant fungi of N treatment are asoomycota(83.30%) and nortierellomycota(14.18%) respectively. The abundance of ascomycota of N treatment increase by 7.24% compare to CK treatment, and the abundance of mortierellomycota decrease by 7.42%. The phylum of dominant fungi of P treatment are asoomycota(79.06%) and nortierellomycota(17.49%) respectively. The abundance of ascomycota of P treatment increase by 5.00% compare to CK treatment, and the abundance of mortierellomycota decrease by 4.11%. The phylum of dominant fungi of K treatment are asoomycota(77.37%) and nortierellomycota(18.27%) respectively. The abundance of ascomycota of P treatment increase by 3.31% compare to CK treatment, and the abundance of mortierellomycota decrease by3.33%. The phylum of dominant fungi of Y treatment are asoomycota(77.37%) and nortierellomycota(21.05%) respectively. The abundance of ascomycota of Y treatment increase by 2.46% compare to CK treatment, and the abundance of mortierellomycota decrease by 0.55%.

The results indicated that N, P, K and Y treatments mainly affected the abundance of Ascomycota and Mortierellomycota of alfalfa’s rhizosphere in alkaline soil. All treatments increased the abundance of ascomycota (N>P>K>Y), and decreased the abundance of mortierellomycota (N>P>K>Y), N treatment has the best effect especially. N treatment had the greatest change on the abundance of ascomycota and mortierellomyca ot among them.



Fig4. Effects of fertilization on the relative abundance of rhizospheric fungi of alfalfa at phylum level in alkaline soil

 The samples include 133 fungal families at the family level, which the family of dominant fungi are nectriaceae, mortierellaceae, bionectriaceae, plectosphaerellaceae, arthopyreniaceae, chaetomiaceae and unclassified\_o\_\_Hypocreales respectively. The family of dominant fungi of CK treatment are nectriaceae(32.78%), mortierellaceae(20.47%), plectosphaerellacea(10.80%), arthopyreniaceae(6.18%) and unclassified\_o\_\_hypocreales (5.15%) respectively.

The family of dominant fungi of N treatment are arenectriaceae(22.87%), mortierellaceae(13.65%), bionectriaceae(13.17%), plectosphaerellaceae(8.77%), arthopyreniaceae(13.85%) and unclassified\_o\_\_hypocreales (7.66%) respectively. The abundance of bionectriaceae, arthopyreniaceae and unclassified\_o\_\_hypocreales  increase by 12.06、7.67 and 2.51% compare to CK treatment respectively, and the abundance of  nectriaceae, mortierellaceae and plectosphaerellaceae decrease by 9.91、6.82 and 2.03% compare to CK treatment respectively.

The family of dominant fungi of P treatment are nectriaceae(16.42%), mortierellaceae(17.27%), bionectriaceae(9.70%), plectosphaerellaceae(8.66%), chaetomiaceae (12.42%) and unclassified\_o\_\_hypocreales (5.70%) respectively. The abundance of nectriaceae, mortierellaceae and plectosphaerellaceae decrease by 16.36、3.20 and5.85% compare to CK treatment respectively, and the abundance of   unclassified\_o\_\_hypocreales, bionectriaceae and chaetomiaceae increase by 2.55、8.59 and 9.27% compare to CK treatment respectively. The family of dominant fungi of K treatment are nectriaceae(27.37%), mortierellaceae(17.77%), bionectriaceae(19.63%), and plectosphaerellaceae(4.95%) respectively. The abundance of nectriaceae, mortierellaceae and plectosphaerellaceae decrease by 5.41, 2.70 and 1.55% compare to CK treatment respectively, and the abundance of   bionectriaceae increase by 18.52% compare to CK treatment. The family of dominant fungi of Y treatment are nectriaceae(20.60%), mortierellaceae(20.59%), plectosphaerellaceae(11.09), chaetomiaceae(8.46%), arthopyreniaceae(7.85%) and bionectriaceae(6.87）respectively. The abundance of nectriaceae decrease by 12.18% compare to CK treatment, the abundance of bionectriaceae和chaetomiaceae increase by 5.76和5.31% compare to CK treatment, and the mortierellaceae、plectosphaerellacea and arthopyreniaceae almost unchange compare to CK treatment.

The results indicated that all treatments of fertilization increased the abundance of fungal family bionectriaceae of alfalfa’s rhizosphere in alkaline soil, but decreased the abundance of nectriaceae. the abundance of fungal family of arthopyreniaceae, mortierellaceae, chaetomiaceae, plectosphaerellaceae and unclassified\_o\_\_hypocreales showed trend of increase or decrease under treatment of fertilization compared to CK treatment in different degrees.



Fig5. Effects of fertilization on the relative abundance of rhizospheric fungi of alfalfa at family level in alkaline soil

2.4 **Relationship between microbial community and chemical factor of environment of alfalfa’s rhizosphere in alkaline soil**

**2.4.1 Comparative analysis of samples based on PCoA**

Calculating distance of unweighted unifrac according to composition of microbial species (species) of each sample, to study the similarity and difference of composition of microbial community under treatments of different fertilization in rhizosphere based on PCoA. The contribution of PC1 and PC2 to the samples was 17.67 and 10.73% after dimension reduction, which accounted for difference of 28.40% on structure of bacterial community (Fig. 6a). There are difference in structure of bacterial community on treatment of fertilization compare to CK, and there are also difference in structure of bacterial community among treatment of fertilization. The contribution of PC1 and PC2 to the samples was 15.73 and 13.70% after dimension reduction, which accounted for difference of 29.43% on structure of fungal community (Fig. 6b). There are difference in structure of fungal community on treatment of fertilization compared to CK, and there are also difference in structure of fungal community among treatment of fertilization. The results showed that fertilization had effect on structure of microbial community in rhizosphere.



Fig6. PCoA analysis of rhizospheric bacteria (a) and fungi (b) based on distance of unweighted unifrac

**2.4.2 Relationship between microbial community and physicochemical factors of environment in rhizosphere**

Variance-inflation factor of VIF was used to screen environmental factors, spearman rank correlation coefficient analysis was used to conduct relationship between environmental factors and microbial species within top 30 of total abundances at the family level. The abundance of three families of bacteria have significantly positive relation with environmental factors (P<0.05), the abundance of two families of bacteria have significantly negative relation with environmental factors (P >0.05) from Fig. 7a. Blastocatellaceae has significantly  positive relation with AN, norank\_O\_\_Norank\_c\_\_KD4-96 has significantly positive relation with XN, sphingomonadaceae has significantly positive relation with OC. Bacillaceae have significantly negative relation with XN, and JG30-KF-CM45 have significantly negative relation with AP.

The abundance of 8 families of fungi have significantly positive relation with environmental factors (P<0.05), the abundance of 4 families of fungi have significantly negative relation with environmental factors (P >0.05) from Fig. 7b. Bionectriaceae has significantly  positive relation with TK, phaeosphaeriaceae has significantly positive relation with AP, pyronemataceae has significantly positive relation with TK, sordariales\_fam\_incertae\_sedis has significantly  positive relation with AP, trichocomaceae has significantly  positive relation with AP, tubeufiaceae has significantly  positive relation with TP, unclassified\_c\_\_dothideomycetes has significantly positive relation with AP, and unclassified\_p\_\_Chytridiomycota has significantly  positive relation with AP. Bionectriaceae have significantly negative relation with pH, pyronemataceae have significantly negative relation with pH, pyronemataceae have significantly negative relation with XN, sporormiaceae have significantly negative relation with AN, and unclassified\_c\_\_GS13have significantly negative relation with TP. The results indicated that the variation of rhizospheric microorganism of alfalfa was affected by physical and chemical properties of rhizosphere in alkaline field, which was related to the improvement of soil quality that was changed by addition of different fertilizer.

 

Fig6. Clustering-heat map of abundance of rhizospheric bacteria (a) , fungi (b) and environmental physicochemical factors at family level respectively

3.**Discussion**

The organic carbon and nitrogen of soil can not only provide nutrients for the growth and development of crops, but also play a crucial role in maintaining a good physical structure of soil (Renfei Li et al.2019). The pH value is an index to measure acidity and alkalinity of soil. High or low of pH value will affect the distribution and transformation of nutrients by changing physical, chemical and biological characteristics, thus leading to the availability of nutrient elements needed for growth and development of crop (Kevin E et al. 2012). pH value is limiting factor of efficient utilization of nutrients in alkaline soil particularly. Treatments of different fertilization increased the contents of OC and TN of alfalfa’s rhizosphere compared to CK treatment in alkaline soil , and decreased the pH value. This is consistent with conclusion of the N, P and K and Y fertilizer could increase OC and TN of crop’s rhizosphere (Wekha N et al.2016;Wenkui Zheng etal.2020) , this is consistent with conclusion of the N, P and K and Y fertilizer could decrease rhizospheric pH value(Hanxi Wang etal. 2019; Ruoyao Jing et al. 2019). The content of XN, AN, TP, AP, TK of alfalfa’s rhizosphere existed differences under treatments of different fertilization, the N, P and K treatments correspondingly increased content of the nutrient forms of N, P and K respectively. Y treatment increased nutrient contents of AN, XN, TP and AP of alfalfa's rhizosphere，but the decreased nutrient contents of TK. The results indicated that fertilization greatly improve the nutrient content of alfalfa’s rhizosphere in alkaline soil, and promote the of alfalfa’s absorption of nutrients. The Y fertilizer have the best comprehensive effect of improvement on nutrient of alfalfa’s rhizosphere in alkaline soil particularly, which could greatly play the comprehensive performance of fertilizer and alfalfa in alkaline soil, and improve the yield and quality of alfalfa.

Microorganism is main participants in the rhizosphere nutrient cycle as reserves and turnover warehouses of nutrients(Yuan Ma et al. 2020). Many factors affect species and diversity of rhizosphere microorganism, such as species of plants, type of soil , climatic type and species of fertilization and so on (Jingwei Liu et al.2021;Yi Ge et al. 2019). Fertilization can change the physical and chemical properties of soil, and then change the number and composition of microorganism’s community in rhizosphere. Microorganism’s physiological groups, metabolic functions and so on change under treatments of different fertilization in rhizosphere(Yiwei Li et al. 2020), which play an important role in fertility of soil and growth of crop. Studies have shown that treatments of different fertilization change the richness, uniformity and structure of rhizosphere microorganisms (Xingjia Xiang et al.2020). At present, there are few studies on the microorganism of alfalfa’s rhizosphere under application of single N, P, K and Y fertilizer in alkaline soil.Treatment of N and P improved the bacterial richness and diversity of alfalfa’s rhizosphere of alkaline soil in this experiment. The P treatment improved the fungal richness and diversity of alfalfa’s rhizosphere of alkaline soil in this experiment, and N, K and Y treatment decreased fungal richness and diversity of alfalfa’s rhizosphere of alkaline soil, Zhihong Jia et al found that N, P, K and organic fertilizers promoted the reproduction of rhizospheric microorganism, and improved the diversity and richness of rhizospheric microorganisms(Zhihong Jia et al. 2004). Different treatments of fertilization have different effects on microorganism of alfalfa’s rhizosphere in alkaline soil, the N and P treatments improve the microorganism’s diversity of alfalfa’s rhizosphere in alkaline soil particularly, which provide a variety of possibilities for absorption of nutrient in alfalfa’s rhizosphere.

Different treatments of fertilization changed the specie composition of -microorganisms of alfalfa’s rhizosphere at the phylum level. The N, K and Y treatments mainly increased abundance of proteobacteria, and decreased abundance of acidobacteriota, The P treatment mainly increased the abundance of actinobacteriota, and decreased the abundance of acidobacteriota. Studies have shown that proteobacteria is involve in the nitrogenous transformation of rhizosphere, and proteobacteria and acidobacteriota are related to pH value, which also explains the reason that fertilization decrease pH value, and increase content of nitrogen of alfalfa’s rhizosphere from the perspective of microorganism in alkaline soil. The Ascomycota and mortierellomycota belong to fungal phylum of dominance of alfalfa’s rhizosphere in alkaline soil. All treatments of fertilization increased fungal abundance of ascomycota(N > P > K > Y), decreased fungal abundance of mortierellomycota (N > P > K > Y). An increase in the abundance of ascomycota is likely to cause many diseases such as powdery mildew, black rot, and anthrax (Hagee Danielle et al. 2020;Pirrello Carlotta et al. 2019),  fertilizer of excessive nitrogen may lead to rot of alfalfa’s root in alkaline soi particularly, which is not conducive to growth of root and nutrient absorption. Therefore, we must pay attention to the dosage of nitrogenous fertilizer when applying nitrogenous fertilizer.

The effect of fertilization on the bacteria alfalfa’s rhizosphere was not very big at family leve in alkaline soil, little difference in the abundance of different bacteria under different fertilization treatments at family level. Families of dominant bacteria were norank\_o\_\_vicinamibacterales, Vicinamibacteraceae, sphingomonadaceae, JG30-KF-CM45, micrococcaceae and norank\_o\_\_norank\_c\_\_KD4-96. Fertilization had a great influence on fungi of alfalfa’s rhizosphere at family level in alkaline soil, all treatments of fertilization increased the abundance of bionectriaceae, and decreased the abundance of nectriaceae, it indicated that N, P, K and Y fertilizer promote the growth and reproduction of bionectriaceae of alfalfa’s rhizosphere in alkaline soil, and inhibite growth and reproduction of nectriaceae. The total abundance of bionectriaceae and nectriaceae were decreased, and the total abundance of other fungus were increased accordingly. Other families of dominant fungi were mortierellaceae, arthopyreniaceae, unclassified\_o\_\_Hypocreales, plectosphaerellaceae and chaetomiaceae, and these families of dominant fungi existed certain differences under treatment of fertilization compared to CK treatment. The results indicated that treatment of fertilization improve the fungal difference and diversity of alfalfa’s rhizosphere in alkaline soil, improving the coordination of fungal overall structure of alfalfa’s rhizosphere, increasing the ability of nutrient transformation of alfalfa’s rhizosphere, It is of great significance to carry out combination’s experiment of different fertilization from view of the microbial point in alkaline soil.

Rhizospheric microorganisms and physicochemical property exist relationship that are mutually promote and inhibite. Analysis of heat map showed that the families of dominant bacteria had significantly positive relation with OC, AN and XN (P <0.05) in alfalfa’s rhizosphere of alkaline soil, and significantly negative relation with XN and AP (P >0.05). The studies found that rhizosphere bacteria are more sensitive to nitrogen (TN, XN and AN) and organic carbon, which may be related to the fact that dominant actinobacteria and acidobacteria of alfalfa’s rhizosphere involve in nitrogen transformation(Zhang Lei et al. 2021; Jing Zhan et al. 2012). Licheng Zhang et al found that bacteria have significantly positive relation with total organic carbon in rhizosphere of rape - rape - rice rotation(Licheng Zhang et al. 2020）, which supports the results of this paper. Families of dominant fungi had significantly positive relation with AP, TP and TK (P <0.05) in alfalfa’s rhizosphere of alkaline soil, and significantly negative relation with pH, XN, AN and TP(P >0.05), Fungus families of Alfalfa’s rhizosphere are greatly affected by the environmental factors such as AP, TP, TK, PH, XN and AN. The AP had significantly positive relation with a variety of fungal families particularly, Which may promote the release rate of phosphorus and increase the content of AP in alfalfa’s rhizosphere of alkaline soil(Małgorzata Wyciszkiewicz et al. 2016;S. Banik et al. 1985). The results indicated that microorganism of alfalfa’s rhizosphere were affected by environmental factors under treatment of single N、P、K and Y fertilizer in alkaline soil.

**4.conclusion**

4.1 The N, P, K and Y treatments increased the content of OC, TN of alfalfa’s rhizosphere of alkaline soil, and decreased the pH value. The N, P and K fertilizers increased the content of nutrients in respective forms of N, P and K, and the changes of other chemical indexes showed certain differences among different treatments compared to CK treatment.

4.2 Fertilization changed the microbial diversity and richness of alfalfa’s rhizosphere in alkaline soil, and nitrogen and phosphorus fertilizer increased the microbial diversity and richness particularly

4.3 The N, P, K and Y treatments changed microorganism’s abundance and community structure of alfalfa’s rhizosphere in alkaline soil. At the phylum level, abundance of proteobacteria, acidobacteriota, actinobacteriota and firmicutes of dominant bacteria of alfalfa’s rhizosphere existed certain differences under different fertilization, fertilization increased abundance of ascomycota of fungi (N > P > K > Y), decreased abundance of Mortierellomycota of fungi (N > P > K > Y).At the family level, fertilization decreased the abundance of norank\_o\_\_vicinamibacterales, vicinamiaceae and norank\_o\_\_norank\_c\_\_kd4-96 of bacteria, fertilization increased the abundance of dominant fungi of bionectriaceae, but decreased the abundance of nectriaceae, other dominant bacteria and fungal existed certain differences under different fertilization.

4.4 The microorganisms of alfalfa’s rhizosphere were greatly affected by environmental factors in alkaline soil. the families of dominant bacteria were greatly affected by OC, AN, XN and AP, and the families of dominant fungi were greatly affected by AP, TP, TK, PH, XN and AN, among which AP was closely related to a variety of fungi families

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Wring author: Xudong Gu (1992 -), male, PhD candidate, E-mail:273323427@qq.com. ORCID iD : [0000-0002-1442-853X](https://orcid.org/0000-0002-1442-853X?lang=en" \t "https://mail.qq.com/cgi-bin/_blank)

✱Corresponding author: Xing Xu(1959-), male, professor, tutor of PhD candidate. [↑](#footnote-ref-0)