Soil chemical properties drive the structure of bacterial communities in the cotton soil of arid Northwest China

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Abstract
Over the last few decades, much of the desert-oasis ecotone in northwestern China has been reclaimed for cotton cultivation. However, we still know little about the composition of the soil bacterial community relative to soil chemical properties in reclaimed soil after long-term monocultural cotton production in arid and extremely arid areas. Here, we collected soil samples from two farms where cotton has been continuously planted for more than 20 years. We found that the composition of the soil bacterial communities was significantly different between these two farms, variance in which could be explained by available K, N, and P, total N, and soil pH. The relative abundance of Verrucomicrobia was significantly higher at the Xinhu farm, while Bacteroidetes and Nitrospirae predominated at the Tenth farm. Across sites, the abundance of Bacteroidetes was positively correlated with available N and P, while the abundance of Verrucomicrobia was positively correlated with total N and available K. Moreover, bacterial community diversity, as quantified via phylogenetic diversity, Shannon diversity, and operational taxonomic unit richness, was higher at the Tenth farm. These soil bacterial diversities were all positively correlated with available N, but negatively correlated with available K in arid and extremely arid areas. Our results suggest that available K, N, and P, total N, and pH are all key factors in shaping soil bacterial communities. The influence of available N and K on bacterial community diversity should therefore be considered for cotton cultivation in reclaimed soil.

KEYWORDS
arid area, cotton, reclaimed soil, soil bacterial community, soil properties

1 | INTRODUCTION

Predicting how terrestrial organisms respond to environmental changes is crucial because their responses provide ecological feedback that can either enhance or reduce ecosystem functions (Zhao et al., 2014). Early studies have produced many insights regarding the impact of environmental changes on plant species, like altered plant diversity and biomass (Grabherr et al., 1994). Recent investigations have found that soil microbes,
which are critical to maintaining soil quality and ecological balance, also respond strongly and quickly to environmental changes (Hossain & Sugiyama, 2011; Pasternak et al., 2013), such as physical and chemical properties (Fierer et al., 2007) and vegetation types (Wu et al., 2008). Increasingly, research has demonstrated that soil microbial communities are strongly impacted by land-use type change (Ferreira et al., 2016; Tosi et al., 2016). For example, the global conversion of a desert to agricultural land can lead to the formation of large areas of desert-oasis ecotone. These ecotones are incredibly fragile as their soils are characterized by low nutrient and moisture levels ( Köberl et al., 2011). Therefore, a survey of bacterial communities in the desert-oasis ecotone is important for understanding maintaining soil health and sustainability.

The variation in bacterial community composition across ecosystems has been well studied, but different selection pressures in their environments could result in unpredictable changes in soil bacterial structure. For example, increasing nitrogen concentration after long-term fertilization regimes altered bacterial community composition in black soil (Yin et al., 2015). Another study showed that soil pH was a good predictor of bacterial community composition in croplands (Geisseler & Scow, 2014). We also know that soil texture affects soil bacterial structure. For example, silt and clay particles were more closely linked to bacterial diversity than sand particles in a long-term fertilizer field experiment (Sessitsch et al., 2001). Together, the physical and chemical properties of soil exert control over the composition and diversity of soil bacterial communities in agricultural and dry ecosystems (Zhang et al., 2019). Berdugo et al. (2020) found that increasing aridity led to abrupt changes in soil microbial communities. Soil relative humidity was another major factor determining bacterial community richness and diversity in the Atacama Desert (Neilson et al., 2017). In desert regions, land-use changes further influence the key driver of soil bacterial community composition. For example, Köberl et al. (2011) found that the changes in soil moisture and pH caused significant changes in bacterial communities after 30 years of agricultural practice on desert soil in Egypt. However, the effect of environmental factors on the composition and diversity of soil microbial communities may be different in reclaimed land from other desert land or cropland.

Due to the dry climate, only a few crops can grow in reclaimed land of Northwest China, with cotton being one of the most suitable cultivations (Feng et al., 2017). The introduction of a cotton system that combines drip irrigation with plastic film mulch has made it possible to reclaim some of the desert-oasis ecotones. Several previous studies have revealed the impact of reclamation on soil properties, enzyme activities, and nutrient use efficiency in this region (Tang et al., 2008; Yang et al., 2016), but the effect of soil properties on the composition and diversity of bacterial communities in cotton cultivation is little known. Soil microbial communities are particularly sensitive to environmental changes, which gradually alter the soil structure and function. Cheng et al. (2018) has found that in reclamation treatments, soil pH and EC play important roles in increasing bacterial community diversity in cultivated maize soil. Since much of the desert has already been reclaimed and planted highly profitable cotton, we need to understand the major factors driving the bacterial community change in order to evaluate the reclaimed outcomes and to develop land management strategies for cotton cultivation.

The Xinjiang Uighur Autonomous Region in Northwest China is one of the most substantial arid areas on the Eurasian continent. In the last 50 years, increasing population pressure has led to over-reclamation of the fragile desert-oasis ecotone for cotton cultivation (Gui et al., 2010). Xinjiang is ranked first among all the other cotton-producing provinces in the country, and the cotton production accounts for 60% of China’s output (Feike et al., 2015). In this study, we assessed the composition and diversity of soil bacterial communities in continuous cotton cropping soil. Our goal was to explore the possible soil factors driving the changes in soil bacterial communities in the cotton reclaimed soil, and whether the major drivers of bacterial communities were similar in arid and extremely arid areas. Soil samples were collected from two sites, with each site containing three different soil textures. We conducted 16S rRNA gene sequencing using Illumina MiSeq in order to analyze soil bacterial community composition. We also determined soil properties, including salinity, total organic carbon (TOC), total nitrogen (total N), available nitrogen (available N), available phosphorus (available P), available potassium (available K), and pH.

# 2 MATERIALS AND METHODS

## 2.1 Site description and soil sampling

Soil samples were collected at two sites in the Xinjiang region of northwestern China (40°61′–44°96′N, 81°19′–86°63′) (Figure 1). These sites have been used as productive cotton cropland for more than 20 years. Cotton is one of the most important economic crops in Xinjiang. The Xinhu farm (approximately 200,000 km²) is situated in the northern region of Xinjiang and represents a typical arid ecosystem. This site is approximately 471 m above sea level with a mean annual temperature of
10.7°C and mean annual precipitation of 200 mm. The Tenth farm (approximately 150,000 km²) is situated in the southern region of Xinjiang and represents a typical extremely arid ecosystem. This site is approximately 1,104 m above sea level with a mean annual temperature of 11.9°C and mean annual precipitation of 50 mm.

As each site contained three different types of soils (sandy loam, clay loam, and loam soil), we collected multiple samples of each soil type at each site (18 samples from the Xinhu farm and 15 from the Tenth farm). In total, 33 soil samples were collected from April to May 2018 (Table S1). The soil samples were immediately refrigerated before being transported to the laboratory. After removing fine roots and visible organic debris, approximately 20 g of topsoil was frozen at −80°C for later molecular analysis. The remaining sample material was air-dried and sieved (<2 mm) for analysis of soil chemical properties (listed in Table S2).

### 2.2 Soil chemical properties

Soil pH was determined at a soil-to-water ratio of 1:2.5 using a pH meter (F-20, Shanghai Mettler-Toledo International Trading Co., Ltd., Shanghai, China). We quantified TOC and total nitrogen (TN) using dichromate oxidation and titration with ferrous ammonium sulfate (Walkley & Black, 1934). Ammonium nitrogen (AN) was extracted using 2 M KCl solution and subjected to flow injection analysis. We measured available potassium (AK) and available phosphorus (AP) colorimetrically after extraction with 3% ammonium carbonate.

### 2.3 DNA extraction, purification, and sequencing

For each sample, we used 0.5 g of frozen soil to extract total DNA using a Fast DNA SPIN Kit for Soil (MP Biomedicals, Heidelberg, Germany). DNA quality and concentration were determined with a Nano Drop 2000 spectrophotometer (Thermo Scientific, USA). DNA was then stored at −80°C.

We conducted PCR amplification using forward primer F515 (5'-GTGCCAGCMGCAGCGGTAA-3') and reverse primer R806 (5'-GGACTACHVGGGTWTCTAAATT-3') (Caporaso et al., 2011). These primers were designed to target the V4 region of 16S rRNA gene. To identify samples, the forward primer was ligated to adapters using a six-base barcode-specific sequence. A QIA quick Gel Extraction Kit (QIAGEN, Germany) was used to purify the PCR products and then pyrosequenced using the MiSeq PE250 platform (Illumina, San Diego, CA, USA) at Majorbio (Shanghai, China).

The raw sequence data were processed and analyzed using QIIME (V 1.9.1) (Caporaso et al., 2010). To improve sequence quality, we removed sequencing reads that had an average quality value ≤20, ambiguous nucleotides in their barcodes, and/or read lengths smaller than 200. Paired ends were merged using the fast length adjustment of short reads (FLASH). Chimeric sequences were detected and eliminated using the Uchime algorithm (Edgar et al., 2011). Finally, we clustered all sequences into operational taxonomic units (OTUs) based on 97% similarity. Singletons were removed using QIIME before analysis. We selected the most abundant sequence of each OTU as the representative sequence, which was classified taxonomically using the Greengenes database and aligned against the template Greengenes alignment using PyNAST.

### 2.4 Statistical analysis

The diversity indices (phylogenetic diversity, Shannon index, OTU richness) were calculated with the OTU table by using “vegan” package in R (version 3.5.2) (Kembel et al., 2010). We used non-metric multidimensional scaling (NMDS) ordination plots to identify differences in bacterial community structure. Permutational multivariate analysis of variance (PERMANOVA) was used to test the significant effects of two sites, soil texture and their interaction on soil bacterial community structure using function “adonis” (package “vegan”) (Anderson, 2001). We used a Mantel test to evaluate correlations between community structure and soil chemical variables (package “vegan”) (Borcard et al., 1992). The relative abundance of phylum was calculated as the proportions of reads corresponding to different phyla in each sample.
taxa in a sample. We performed Student’s t-tests to assess the effects of two sites on the soil properties, community diversity (phylogenetic diversity, Shannon index, OTU richness), and relative abundance of abundant phyla. We calculated Pearson correlation coefficients to measure the relationship between bacterial community diversity, relative abundance, and soil chemical properties. All data were tested for normality and homogeneity of variance using the Shapiro–Wilk and Levene’s tests, respectively. Any data that were not normally distributed were square-root transformed before statistical analyses. These statistical analyses were performed using SPSS 21.0 software.

3 | RESULTS

3.1 | Diversity and composition of bacterial communities

After performing quality control and removing chimeric sequences, we amassed a total of 1,943,721 useable sequences from the collected soil samples. The mean number of sequences per sample (n = 33) was 59,540. Based on the genetic distance at 0.03, we identified 3,000–4,547 and 3,880–5,046 OTUs at the Xinhu farm and the Tenth farm, respectively. The calculated metrics of bacterial community diversity for each sample are shown in Table S3.

For community comparisons, we performed an analysis of distribution of only the 10 most abundant phyla (i.e., those accounting for more than 0.3% of all sequences; Figure 2). The most represented bacterial phyla were Proteobacteria (average relative abundance 38.38%), Actinobacteria (13.94%), Acidobacteria (13.08%), and Gemmatimonadetes (10.52%). We observed no difference in community composition at phylum level among different soil textures. However, there was a pronounced difference in the relative community abundance of three phyla across the two sites. The relative abundance of Verrucomicrobia was higher at the Xinhu farm than that at the Tenth farm (p = 0.012) (Figure 4d), while the relative abundances of both Bacteroidetes (p = 0.024) (Figure 4a) and Nitrospirae (p = 0.031) were higher at the Tenth farm than those at the Xinhu farm.

3.2 | Effect of soil properties on diversity and composition of bacterial communities

Soil chemical properties varied significantly between two sites (Table 1). The salt content, available N, and

![Relative abundance of the major bacterial groups (10 most abundant phyla) in different soil texture (sandy, clay and loam) at the two study sites (Xinhu farm and the Tenth farm)]

| Study site | Sandy | Clay | Loam | Sandy | Clay | Loam |
|------------|-------|------|------|-------|------|------|
| Xinhu farm |       |      |      |       |      |      |
| Tenth farm |       |      |      |       |      |      |

![TABLE 1 Physical and chemical properties of the soil samples collected at the Xinhu farm and the Tenth farm in arid Northwest China](Note: Data (mean ± SE) followed by different lowercases in each row indicated significant differences (p ≤ 0.05) between the two sites.)
available P were significantly higher at the Tenth farm than those at the Xinhu farm. On the other hand, total N and available K were present in greater quantities at the Xinhu farm than those at the Tenth farm.

The composition of bacterial communities differed between the two sites (Figure 3), but this difference was not driven by the effects of soil textures or site × texture interaction (Table 2). However, we detected a substantial effect of soil properties (including available K, total N, available N, available P, and pH) on the composition of bacterial communities (Figure 3; Table 3). The Tenth farm possessed elevated available N and available P, which correlated with an increase in the abundance of Bacteroidetes (Figure 4b,c). Higher concentrations of total N and available K at the Xinhu farm also corresponded to increase the abundance of Verrucomicrobia (Figure 4e,f). Across sites, increasing pH was negatively correlated with the relative abundance of Actinobacteria ($r = -0.429$, $p < 0.05$), but positively correlated with the abundance of Acidobacteria ($r = 0.415$, $p < 0.05$) (Table S4).

The phylogenetic diversity, Shannon index, and OTU richness were higher at the Tenth farm (Figure 5a,d,g). These biodiversity indices were all positively correlated with available N (Figure 5b,e,h), but negatively correlated with available K (Figure 5c,f,i). The Shannon index also increased with increasing available P

![Non-metric multidimensional scaling (NMDS) ordination plot of soil bacterial communities on different sites and soil texture. Blue symbols: Xinhu farm; red symbols: the Tenth farm. Circle, squares, and triangles refer to sandy, clay, and loam respectively. Permutational multivariate analysis of variance (PERMANOVA) was adopted to compare community composition on different sites and the results are shown on the plot. Ellipses represent 95% confidence intervals around the centroid of all samples. AK, available K; AN, available N; AP, available P; TN, total N [Color figure can be viewed at wileyonlinelibrary.com]](image)

| Table 2 | The relative importance of site, soil texture, and their interaction for soil bacterial communities of willow as revealed by PERMANOVA |
|-----------------|-----------------|-----------------|-----------------|
| Parameter | Degrees of freedom | $F$-value | $R^2$ | $p$-Value |
| Site | 1 | 5.219 | 0.143 | 0.001*** |
| Soil texture | 1 | 1.283 | 0.035 | 0.196 |
| Site × texture | 1 | 0.949 | 0.026 | 0.457 |
| Residuals | 29 | | | |
| Total | 32 | | 0.796 | 1.00000 |

Note: Bold indicates significance:

***$0.001 > p$.
and pH \( (r = 0.366, \ p < 0.05) \) (Table S5).

**4 | DISCUSSION**

Many desert-oasis ecotones have been reclaimed for planting cotton in Xinjiang, China. We evaluated the soil bacterial communities at the two farms in arid and extremely arid environments that had continuously grown cotton for 20 years. We discovered that the response of the soil bacterial community diversity to soil properties was similar across sites. Available soil N and available soil K were found to be the primary drivers of soil bacterial community diversity and composition in the cotton soil of these two farms.

We found that the soil chemical properties were more closely correlated to the composition of bacterial communities than soil texture. Overall, our data identified the available K, N, and P, total N, and pH as the important determinants of bacterial community composition in cotton soils. Similarly, soil chemical properties have been found to have a significant influence on soil bacterial community composition after 5 years of cotton cropping elsewhere in China (Li et al., 2021) as well as in different cropping systems across Europe (Plassart et al., 2019). Other studies have also detected that pH was a key indicator of soil bacterial community composition in the agricultural system (Shen et al., 2010), and that N, P, and K levels in soil constitute important components for bacterial community structure across land-use types (Chemidlin et al., 2014; Lauber et al., 2008).

Proteobacteria was the most dominant phylum in our study areas, followed by Actinobacteria and Acidobacteria. Despite investigating a previously unexplored region, we did not find any new bacterial species. Proteobacteria has been observed at relative abundances of 27.06% after 5 years of reclamation (Cheng et al., 2019) and 17.87% after 60 years of reclamation (Li et al., 2021). We found it to occur at relative abundance of 38.38% after 20 years of cotton cultivation. These are the most common phyla across ecosystems and are specifically adapted to arid environment (Zhang et al., 2017). The high relative abundance of Actinobacteria found at our study sites (13.9%) suggests that the continuous use of agricultural pesticides may increase the prevalence of these bacteria (Li et al., 2021). Actinobacteria was negatively correlated with pH, which is consistent with previous research in reclaimed forest soils.
(Dimitriu & Grayston, 2010) and wheat soil (Stroobants et al., 2014). The Acidobacteria phylum, which contains oligotrophs, was also abundant in our study sites and positively correlated with pH, which is consistent with a previous study (Wang et al., 2019), but is contrary to another one (Shen et al., 2013). The relative abundance of these three phyla differed across these two farms. At the Tenth farm, we found fewer Verrucomicrobia phyla (positively correlated with total N and available K) and higher concentrations of Bacteroidetes and Nitrospirae phyla (positively correlated with available N and P), suggesting that Bacteroidetes and Verrucomicrobia may serve as good predictors of available N, P, and K in soil. Collectively, these results demonstrate that changes in soil nutrient concentrations ultimately lead to changes in soil bacterial composition. Soil bacterial diversity is critical to the functioning and long-term sustainability of soil ecosystems (Liu et al., 2014). We found that, across arid and extremely arid environments, available N and K content strongly influence the diversity of soil bacterial communities in fields. A possible explanation is that nitrogen is an important resource for bacterial growth, and thus high nitrogen content may promote soil bacterial biomass and diversity. Our results are consistent with the former study that the soil fertilizer is likely to directly influence soil bacterial diversity (Sugiyama et al., 2008). On the other hand, high soil potassium results in low diversity of soil bacterial communities. The recent study also found that the amount of available potassium increased over time and reached the highest at 25 years of cotton cropping in this region, which suggested that cotton cultivation exerts a significant influence on soil potassium content, which in turn affected bacterial richness and diversity over a long time (Li et al., 2020). Xinjiang is a desert region and microbial biomass production is generally low. Reclaimed soils in the area are irrigated and fertilized, which is helpful to increase soil microbial biomass and

**FIGURE 5** The phylogenetic diversity, Shannon index and OTU richness of soil bacterial communities at different sites (a, d, and g). Bars represent mean value ± SE. Pearson correlations analysis between these diversity index and soil chemical variables (b, c, e, f, h, and i) (n = 33)
diversity, and lead to a more stable ecosystem (Cheng et al., 2019). Our results suggest that the input of more available N and less available K in fertilizer may have substantially increased the diversity of soil bacterial communities in cotton cultivation farms, although the climate conditions were different between the two areas. Our result highlighting that environmental selection was mainly based on soil chemical characteristics but not on climatic conditions (Chemidlin et al., 2014). This is in accordance with previous studies that the weak influence of climate on soil bacterial diversity (Auguet et al., 2010; Dequiedt et al., 2009; Dequiedt et al., 2011). Nevertheless, recent studies also demonstrated strong differences between bacterial communities across different temperate and precipitation conditions, suggesting that climate may play a role at very large spatial scales (Fierer et al., 2012). Therefore, it is of critical importance to control the amount of available N and K, thereby escalating the diversity of soil bacterial communities in the cotton cultivation in these study areas.

5 | CONCLUSION

Our study helps us to better understand the role of soil properties in altering the composition and diversity of soil bacterial communities in cotton soil in Northwest China. The soil available N and available K contents are major drivers of bacterial community diversity and composition across ecotones, although the relative abundances of Verrucomicrobia, Bacteroidetes, and Nitrospirae differed between sampling locations. We suggest that the higher available N and lower available K fertilizer application is necessary for cotton cultivation to promote soil bacterial diversity and sustainability in reclaimed soil.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Haiyan Liu, Zhiyong Huang, Rong Yang, and Xin Lv designed the experiments. Haiyan Liu, Jingjing Wang, Wei Zhao, Yun Chen, Ning Lv, and Zhengbing Yi carried out the sampling and biochemical analyses. Haiyan Liu, Zhiyong Huang, Rong Yang, and Xin Lv wrote the manuscript. All authors read the final manuscript.

DATA AVAILABILITY STATEMENT

All data generated and analyzed in this study are provided in the article. The raw sequencing data are deposited to the NCBI sequence read archive (SRA) under the accession number SRP188329 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA526808).

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**SUPPORTING INFORMATION**
Additional supporting information may be found online in the Supporting Information section at the end of this article.

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