Effects of reclamation years on composition and diversity of soil bacterial communities in Northwest China

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Effects of reclamation years on composition and diversity of soil bacterial communities in Northwest China

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Abstract: The objective of this study was to evaluate bacterial community structure and diversity in soil aggregate fractions when salinized farmland was reclaimed after > 27 yr of abandonment and then farmed again for 1, 5, 10, and 15 yr. Illumina MiSeq high-throughput sequencing was performed to characterize the soil bacterial communities in five aggregate size classes in each treatment. The results indicated that reclamation significantly increased macro-aggregation (> 0.25 mm), as well as soil organic C, available N, and available P. The 10-yr field had the largest proportion (93.9%) of soil in the macro-aggregate size classes (i.e., >0.25 mm) and the highest soil electrical conductivity. The five most dominant phyla in the soil samples were Proteobacteria, Actinobacteria, Gemmatimonadetes, Acidobacteria, and Bacteroidetes. The phylogenetic diversity, Chao1, and Shannon indices increased after the abandoned land was reclaimed for farming, reaching maximums in the 15-yr field. Among aggregate size classes, the 1–0.25 mm aggregates generally had the highest phylogenetic diversity, Chao1, and Shannon indices. Soil organic C and soil electrical conductivity were the main environmental factors affecting the soil bacterial communities. The composition and structure of the bacterial communities also varied significantly depending on soil aggregate size and time since reclamation.

Key words: Abandoned farmland, reclamation time, soil aggregates, bacterial community, Illumina sequencing.

Introduction

Soils are highly complex, heterogeneous environments which play an important role in selecting the resident microbial community (O'Donnell et al. 2007; Torsvik & Øvreås 2002; Soman et al. 2016). Bacteria are the most abundant and diverse group of soil microorganisms (Gans et al. 2005). As primary drivers of C, N, and P cycling, bacteria greatly influence the health and sustainability of agroecosystems (Matson et al. 1997; Steenwerth et al. 2002). Many studies have reported that various factors affect soil microbial community membership. These factors include soil properties (Shen et al. 2013; Liu et al. 2015; Nacke et al. 2011), fertilization practices (He et al. 2007; Tian et al. 2017), cropping patterns (Dorrede et al. 2012; Li et al. 2010), and seasonal variation (Schutter et al. 2001; Kennedy et al. 2006). Soil pH is often a main factor in determining the soil bacterial community (Fierer and Jackson 2006; Rousk et al. 2010).

Soil aggregates are important for maintaining soil porosity and for providing stability against

erosion (Mikha and Rice 2004; Lupwayi 2001). Soil structure is mainly evaluated by the degree of aggregate stability (Six et al. 2004). Soil bacteria are one of the main agents in aggregate formation and stabilization (Rashid et al. 2016). Knowledge of microbial communities and their activities within different aggregate fractions is limited, but essential for increasing crop production and maintaining agricultural sustainability (Trivedi et al. 2015). Recently developed molecular techniques using rDNA (e.g., Illumina sequencing) are excellent tools for assessing microbial diversity and microbial community composition under different agriculture practices (Nakatsu 2007).

The Manasi River Basin in the Xinjiang Region of northwest China is the country's fourth largest agricultural area. The amount of cultivated land in the basin increased from 156 km² in 1949 to 6,221 km² in 2010. In the past, unreasonable irrigation practices destroyed the water-salt balance in many parts of the Manasi River Basin. Intense evaporation caused secondary salinization. Serious ecological problems arose when large areas of farmland were abandoned due to declining productivity (Eynard et al. 2005; Fan et al. 2008). However, large areas of this abandoned farmland have been reclaimed since the introduction of drip irrigation in 2000.

This work aimed to determine whether soil bacteria communities changed dramatically when salinized farmland was reclaimed after many years of abandonment and how these changes occurred over time. The specific objectives of this study were (i) to identify changes in soil properties and aggregation after abandoned, salinized farmland was reclaimed, (ii) to study the difference in the composition, diversity, and species richness of bacterial communities in different aggregate fractions, and (iii) to identify the environmental factors that significantly influence the bacterial community in soil at different stages in the reclamation process.

Materials and methods

Site description and design

The study was conducted at the middle reaches of the Manasi River Basin of Xinjiang, China (44°37'N, 86°08'E, 400 m above sea level) (Fig. 1). The area has an arid continental climate. The average annual frost-free period is 169 days. The average annual precipitation and evaporation are 153 mm and 2,005 mm, respectively. Excess flood irrigation in the 1970s and 1980s caused the water table to rise near the soil surface in the area. The salt content of the shallow groundwater was high; thus evaporation led to salt accumulation at the soil surface. Over time, a large area of salinized land

developed in the basin. More than a quarter of the farmland was eventually abandoned because of salinization.

The experimental site was a 105 hm² area in Shihutan Township. The site had previously been farmed, but then it was abandoned for more than 27 yrs due to soil salinization. The land has been reclaimed for cotton production with drip irrigation and plastic film mulch at different times during the past ten years. The experiment involved five field sites (i.e., treatments): (1) original abandoned farmland (CK, 5 hm² area); (2) 1-yr of cotton production (20 hm² area); (3) 5-yr of cotton production (30 hm² area); (4) 10-yr of cotton production (20 hm² area); and (5) 15-yr of cotton production (30 hm² area).

The management practices were the same in all of the cotton fields. The cotton was planted at the end of April each year. The plant density was 2.4×10^4 plants hm⁻². The fields were drip-irrigated 8 to 10 times each growing season. Nitrogen (300 kg hm⁻²) and phosphorus (200 kg hm⁻²) fertilizers were applied via drip irrigation. The total irrigation amount was 4,500 m³ hm⁻² yr⁻¹. Calcium superphosphate (450 kg hm⁻²) was applied before planting. Cotton gin screenings (2 250 kg hm⁻²) and cotton residue (6 000 – 7 500 kg hm⁻²) were returned to the soil each autumn.

The soil at the site is classified as grey desert soil according to the local classification (Gong et al. 1988) and as Yermosol by the FAO-UNESCO system. A soil survey done by the local Soil and Fertilizer Station in 2000 showed no significant spatial variation in the distribution of soil salinity and soil organic matter in the reclamation area. Vegetation on the abandoned farmland is sparse, mainly consisting of *Suaeda glauca* Bunge, *Kalidium foliatum*, and *Salsola collina* Pall. The vegetation was uniformly distributed across the landscape. Grazing was prohibited in the area. Due to the nature of this study, there were no true replications of the different reclamation times. However, considering the uniformity of the soil, vegetation, and climate conditions, we believe that there were no inherent differences among the five field sites at the beginning of the study.

Soil sampling

Soil sampling was carried out in July 2014 during the bloom period of cotton. Three undisturbed soil samples were collected by randomly digging three pits (50 cm width $\times$ 100 cm length) at each site. Undisturbed soil samples were removed from the 0–20 cm depths, and the samples were put in sterilized, hard plastic boxes to maintain the primary structure of the soil. Samples were immediately taken to the lab where three undisturbed soils were combined to form one composite sample per site

for aggregates fractionation.

The soil samples were sieved according to methods described by Schutter and Dick (2002) and Wei et al. (2014). First, large soil clods were gently broken up by hand, and then the soil samples were spread on brown, sterilized paper to dry slowly for several days until the gravimetric water content reached approximately 80 g kg⁻¹ soil. The dry sieving method could be effectively implemented at this moisture level. This process was conducted at 4°C to minimize the effect of air drying on the microbial communities and activities.

The dry-sieving method was chosen because it disrupted the physical habitat of microbial communities less than wet-sieving (Schutter and Dick 2002). Fractionation was achieved by placing 100 g of cold air-dried, sieved soil on nested sieves mounted on a Sieve Shaker (JK-VWS, Shanghai, China). The sieves were mechanically shaken (10 min, amplitude 1.5 mm) to separate the soil into the five aggregate fractions (> 5, 5–2, 2–1, 0.25–1, and < 0.25 mm). Preliminary experiments showed that shaking for 10 min at 1.5 mm amplitude was adequate for separating the soil aggregates without destroying the large aggregates. The aggregate distribution was determined by weighing the soil in each aggregate size class. The fractionated samples were later combined to make composite samples for each aggregate size class. The bulk soil and all the soil aggregate samples were stored in sterilized polyethylene bags at 4°C until they were analyzed for biological properties and DNA extraction.

Physicochemical analysis

The following physicochemical properties were measured: soil water content, pH, electrical conductivity (EC), soil organic content (SOC), available N, Olsen P, and soil bulk density (BD). Soil pH and EC were measured in soil-water suspensions (1:5 and 1:2.5 soil-water ratio, respectively). The SOC was determined using the Walkley-Black dichromate oxidation method (Diaz-Zorita 1999). Available soil N was determined with the alkaline hydrolysis diffusion method (Mulvaney and Khan 2001). Available P was determined using the 0.5 M NaHCO₃ method (Page et al. 1982). The BD of the 0–20 cm depth was also determined using a soil core (100 cm³).

DNA extraction, PCR amplification, Illumina MiSeq high-throughput sequencing, and sequencing data processing

The soil microbial DNA was extracted from 0.5 g soil samples using a FastDNA SPIN Kit for soil (Mo Bio PowerSoil DNA Isolation Kit) according to the manufacturer's instructions. The V3-V4 regions of the bacterial 16S rRNA gene were amplified using PCR (30 cycles of predenaturation at

95°C for 5 min; 25 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 30 s; with a final extension at 72°C for 10 min). The primer sets were as follows: 336F: 5'-GTACTCCTACGGGAGGCAGCA-3'; 806R: 5'-GTGGACTACHVGGGT WTCTAAT-3'. The PCR reactions were performed in a 50 µL mixture containing 1 µL of template DNA (30 ng), 2 µL of each primer (10 µM), 4 µL of 2.5 mM dNTPs, 5 µL of 10 × Pyrobest buffer, 0.3 µL of Pyrobest DNA polymerase (2.5 U/µL, TaKaRa), and 35.7 µL of ddH₂O.

After the PCR process, the DNA amplicons were extracted from 2 % agarose gels and purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions and then quantified using QuantiFluorTM-ST (Promega, Madison, WI, USA). Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 250) on an Illumina MiSeq platform (MajorBio Shanghai Technologies, Shanghai, China) according to standard protocols.

Raw FASTQ files were de-multiplexed and quality-filtered using QIIME (version 1.17) with the following criteria: (i) 250 bp reads were truncated at any site receiving an average nucleotide length < 20 over a 10 bp sliding window and (ii) truncated reads shorter than 50 bp were discarded. Following exact barcode matching, we removed sequences with two nucleotide mismatches in primer matching as well as reads containing ambiguous characters. Only sequences that overlapped in regions longer than 10 bp were assembled according to their overlapping sequence. Finally, the reads that could not be assembled were discarded. To characterize microbial diversity, operational taxonomic units (OTUs) were clustered with a 97 % similarity cutoff using UPARSE. Chimerical sequences were identified and removed using UCHIME (Caporaso et al. 2010; Kunin et al. 2010). The Chao1 index (S_{Chao1}) (the estimated number of OTUs), which indicates species richness, was calculated using the following equation:

$$S_{Chao1} = S_{obs} + \frac{n_1(n_1 - 1)}{2(n_2 + 1)}$$

where S_{obs} is the observed number of OTUs, n_1 is the number of OTUs with only one sequence, and n_2 is the number of OTUs with only two sequences (Liu et al. 2014).

The Shannon index ($H_{Shannon}$) (Schloss et al. 2009), which indicates community diversity within the sample (Jurasinski et al. 2009), was determined as follows:

$$H_{Shannon} = - \sum_{i=1}^{S_{obs}} \frac{n_i}{N} \ln \frac{n_i}{N}$$

where S_{obs} is the number of observed OTUs, n_i is the number of individuals in the OTU, and N is the total number of individuals in the community.

Statistical analyses

Software R (R Development Core Team, 2008) was used to calculate the percentage of classifiable reads and generate the figures. Analyses of variance (ANOVA) and the least significant range test (Tukey's method) were performed with SPSS 17.0 to test the significance ($p < 0.05$) of the differences among the treatments. The principal component analysis (PCA) and canonical corresponding analysis (CCA) were generated by Canoco 4.5 software.

Results

Soil physicochemical properties

Soil pH and SOC in the 0–20 cm depth were greatest in the 15- and 5-yr fields (Table 1). Reclamation had no significant effect on either soil BD or soil water content ($P > 0.05$). Soil EC was greatest in the abandoned and 1-yr fields. Available N (69.89 mg kg^{-1}) and available P (52.06 mg kg^{-1}) were greatest in the 5-yr field.

The abandoned and 1-yr fields were characterized by a high degree of micro-aggregation (< 0.25 mm fraction), accounting for 69.69 and 36.41 % of the soil mass in those two fields, respectively. The micro-aggregate content decreased as reclamation time increased, reaching 6.14 % of the soil mass in the 10-yr field. The 10- and 5-yr fields had a significantly greater proportion of soil in the large macro-aggregate (> 5 and $5\text{--}2$ mm) fractions than any of the other fields ($P < 0.05$). The two size classes combined contained 37.47 % of the soil mass in the 10-yr field and 31.55 % of the soil mass in the 5-yr field ($P < 0.05$). There was no significant difference in the proportion of soil in the small macro-aggregate ($2\text{--}1$ and $1\text{--}0.25$ mm) fractions among the 1-, 5-, 10-, and 15-yr fields.

Bacterial community composition

A 16S rRNA gene sequence analysis was performed on 25 soil samples (5 samples per field). A total of 1 187 438 high-quality sequences were obtained from the samples after all of the raw sequences had been subjected to quality control processing (including trimming and filtering) and the low quality sequences were removed. The effective number of sequences was 1 122 101 (Table 2). The overall number of OTUs detected by analysis was 51 007, based on 97 % nucleotide sequence

identity between reads. To assess whether sampling provided sufficient OTU coverage to describe the bacterial composition of each site accurately, individually-based rarefaction curves were generated for each site (Fig. S1). All of the rarefaction curves tended to approach the saturation plateau. This indicated that the data volume of sequenced reads was reasonable and that the discovery of a high number of reads made a small contribution to the total number of OTUs. The rarefaction curves indicated large variation in the total number of OTUs among the different samples. The OTU numbers of the 25 soil samples ranged from 1 361 to 2 522, with the 15-yr field having the richest diversity (2 364 OTUs), followed by the 10-yr (2 242 OTUs) and 5-yr (2 210 OTUs) fields. The 1-yr field showed the lowest diversity with only 1 464 OTUs.

At the phylum level, the top 11 phyla were selected, and the remaining sequences were assigned to a cluster named “Others” (Fig. 2). The results revealed that Proteobacteria was the most abundant phylum across all 25 soil samples. The proportions of Proteobacteria ranged from 26.00% in the 10-yr field to 50.70% in the 15-yr field. The other dominant phyla were Actinobacteria (3.20%–31.50%, averaging 13.87%), Gemmatimonadetes (8.20%–21.10%, averaging 16.30%), Acidobacteria (1.20%–33.50%, averaging 12.42%), Bacteroidetes (2.80%–24.40%, averaging 8.16%), Chloroflexi (0.80%–12.60%, averaging 5.11%), and Planctomycetes (0.20%–4.20%, averaging 2.10%). These seven phyla dominated (93.27 %) the bacterial communities in all soil samples. The following phyla were present at less than 2%: Nitrospirae (averaging 1.87%), and Verrucomicrobia (averaging 1.24%).

We performed LefSe analyses to examine how taxa differed most in relative abundance among the five fields. The cladogram in Supplementary Fig. S2 shows that many taxa were common among the five fields; however, there were some specific differences. For example, the abandoned field had the highest relative abundances of Chloroflexi and Planctomycetes. The 1-yr field had the highest relative abundances of Actinobacteria, Bacteroidetes, and Firmicutes. The 5-yr field had the greatest relative abundance of Proteobacteria. Finally, the 10-yr field had the highest relative abundance of Acidobacteria, Proteobacteria, and Nitrospirae.

The composition of the bacterial community varied not only among reclamation times, but also among aggregate size classes (Fig. 2). In abandoned farmland, the abundance of the Proteobacteria community was greatest in the > 5 mm fraction and least in the 1–0.25 mm fraction. In the 10-yr field, Proteobacteria abundance in the 10-yr field increased as aggregate size decreased; however, in the 1-, 5-, and 15-yr fields the micro-aggregate fraction (< 0.25 mm) had the lowest Proteobacteria

abundance among the aggregate size classes.

Bacterial community richness and diversity

The PD, Chao1, and Shannon indices confirmed that reclamation increased soil bacterial diversity, except for the 1-yr field (Table 2). The ANOVA analysis of the PD, Chao1, and Shannon indices also shown significant differences ($P = 0.0001$), and the post-hoc pairwise tests showed that the 15-yr field was significantly different from the other fields. The average Shannon diversity indices were 9.05 in the 15-yr field, 8.79 in the 10-yr field, and 8.61 in the 5-yr field. These results indicated that the Shannon diversity increased with time since reclamation.

The PD, Chao1, and Shannon values tended to be greatest in the 1-0.25 mm fraction (Table 2). The following exceptions should be noted: (i) in the 1-yr soil the PD and Shannon values were greatest in the 2-1 mm fraction, (ii) in the 5-yr soil the Shannon index was greatest in the < 0.25 mm fraction, and (iii) in the 10-yr soil the 5-2 mm fraction had the highest PD value and the 2-1 mm fraction had the highest Chao1 value.

The similarity of the soil samples was evaluated according to principal coordinate analysis (PCoA) and cluster analysis (CA). The PCoA was conducted to estimate β -diversity, which represents the community diversity between samples (Lozupone and Knight 2005) in the Illumina-MiSeq sequencing data based on the UniFrac distance matrix (Peiffer et al. 2013) created by QIIME (Caporaso et al. 2010). The PCoA indicated that β -diversity changed with time since reclamation (Fig. 3). As shown by the weighted UniFrac analysis, the contributions of the first principal component, the second principal component, and the third principal component were 56.69, 15.78, and 9.05%, respectively. It is noteworthy that separation existed among the abandoned, 1-yr, 5-yr, 10-yr, and 15-yr fields. However, some overlap was observed among samples from the 5-, 10-, and 15-yr fields. This meant that the 5-, 10-, and 15-yr fields had some of the same OTUs but at different levels.

Cluster analysis was conducted to analyze the similarity of the soil samples (Fig. 4). The results showed that the soil samples were clustered into six groups. In the abandoned field, the five aggregate fractions were clustered in two groups, with > 5 and 5–2 mm aggregates in the same group, and 2–1, 1–0.25 and < 0.25 mm aggregates in the other group. This result was similar to that obtained by PCoA. In the 1-yr field, the > 5 mm fraction was slightly separated from the other size fractions. In the 5-yr field, the <0.25 mm aggregates formed a cluster that was separate from the other four size fractions. The 10- and 15-yr fields showed similar patterns, with the five

aggregate fractions grouped together in one cluster. Overall, the results of cluster analysis were consistent with those of PCoA (Fig. 4). Hierarchical cluster analysis of the similarity of the bacterial communities further confirmed that time since reclamation is the major determinant of bacterial community structure.

The PCA results showed differences in the diversity of the bacterial community among reclamation times (Fig. 5). The PC1 and PC2 accounted for 82.9% of total variance. All of the bacterial communities at the phylum level were distributed in the four quadrants of the PCA results. For Actinobacteria and Acidobacteria, the only two sources were observed in the second and fourth quadrants, and they had loading scores that were higher on PC1 than those of Bacteroidetes, Firmicutes, TM7, Chloroflexi, Verrucomicrobia, Planctomycetes, Nitrospirae, Gemmatimonadetes and Proteobacteria. Actinobacteria scored highest in PC1, whereas TM7 and Gemmatimonadetes scored highest in PC2. This showed that Actinobacteria and Acidobacteria were the most important bacterial communities in the five fields. The abandoned, 1-, 10-, and 15-yr fields were separated in PC1. The blue circles representing different aggregate classes in the 5-yr field were mainly distributed in the positive direction of PC2 and separate from the other fields. Moreover, the bacterial community structures of aggregate fractions in the 10- and 15-yr fields were clustered in the second principal component axis, whereas the aggregate classes in the 5-yr field were separated from each other. This indicated that 10- and 15-yr fields have more similar bacterial assemblages than those of the other fields.

Environmental factors and bacterial community diversity

Pearson correlations were calculated to investigate correlations between diversity parameters and environmental factors (Table 3). The results showed that SOC was significantly positively correlated with bacterial richness (OTUs) and with the diversity parameters (Chao1 and PD) ($P < 0.05$). The other environmental factors, including pH, EC, BD, EC, available N, and available P, were not significantly correlated with bacterial richness or with any of the diversity parameters ($P > 0.05$).

Redundancy analysis (RDA) was performed to investigate the relationship between the bacterial communities and the environmental factors (Fig. 6). The results showed that that 71.40% of the variation was explained by RDA axis 1 and 22.48% was explained by RDA axis 2. The bacterial communities within the five fields were distributed in four quadrants. The RDA analysis revealed that EC, SOC, and available N were the main environmental factors influencing soil bacterial community

composition. Additionally, Pearson correlations were calculated between the most abundant bacterial phyla and environmental factors. The relative abundance of Actinobacteria and Bacteroidetes was significantly positively correlated with EC and negatively correlated with SOC and available N ($P < 0.05$). The relative abundance of Gemmatimonadetes was positively correlated with SOC and soil available P. In addition, the relative abundance of Acidobacteria and Nitrospirae was negatively correlated with soil EC. Soil pH, soil moisture content, and BD showed no correlation with any bacterial phyla.

Two-way ANOVA was performed to better understand the effect of soil aggregate size and reclamation time on the bacterial community (Table S1). The ANOVA showed that time since reclamation ($P = 0.001$) and soil aggregate fractions ($P = 0.004$) were both statistically significant determinants of community composition ($P < 0.05$, Table S1).

Discussion

Soil properties

Soil salinization has become a serious environmental problem worldwide, causing major reductions in arable land, crop productivity, and crop quality (Shahbaz and Ashraf, 2013). China has less than 9% of the world's arable land with which to feed over 22% of the global population (Chen 2007). Under the pressure of rapid population growth, more and more abandoned salinized farmland will be reclaimed for use as arable land. Therefore, knowledge about how soil physicochemical properties change after reclamation could be helpful for understanding soil productivity.

Significantly lower soil EC was observed in the 5-, 10-, and 15-yr fields than in the abandoned field. This is consistent with our previous study which showed that soil EC declined as time since reclamation increased (Zhang et al. 2017). The SOC concentrations in the 5-, 10-, and 15-yr fields were significantly greater than those in the abandoned field ($P < 0.05$). Xinjiang is a desert region and biomass production is generally low. Agricultural soils are irrigated using drip irrigation, thus increasing biomass production. Cotton residue was mixed into the soil after harvest in the autumn. Cotton roots are also an important source of organic C. This additional organic matter would change the structural, biological, and chemical properties of the soil. This may explain why SOC increased after the reclamation of the abandoned farmland. Soil available N and P content significantly increased after reclamation, probably because of fertilization application (Zu et al. 2014). The

proportion of macro-aggregates (>0.25 mm) increased as the number of reclamation years increased. After 10-yr of cotton production, the >0.25 mm aggregates accounted for 93.9% of total soil mass. This was 67.7% greater than that in abandoned farmland. The increases in the >0.25 mm fraction indicate that the soil structure became more stable after reclamation.

Bacterial community composition and diversity

Illumina sequencing of bacterial 16S rRNA indicated notable shifts in bacterial community structure after the reclamation of abandoned salinized land. The number of OTUs in the 25 samples ranged between 1 361 to 2 522 OTUs, with an average of 1 161 OTUs. The rarefaction curves also tended to plateau, indicating that the library was large enough to reflect the bacterial diversity among the different fields.

The most dominant phylum in all soil samples was related to Proteobacteria, which was consistent with the findings of previous studies (Zhang et al. 2012; Sun et al. 2008; Shi et al. 2014). The other dominant phyla were Actinobacteria (13.87%), Gemmatimonadetes (16.30%), Acidobacteria (12.42%), Bacteroidetes (8.16%), Chloroflexi (5.11%), and Planctomycetes (2.10%). These results suggest that the soil bacterial community in the Manasi River Basin is not unique, despite the rather severe environment. Significant differences in soil bacterial composition were observed among the five reclamation treatments. In particular, the relative abundance of Proteobacteria was high in the 5-yr farmland, whereas Acidobacteria and Gemmatimonadetes had the highest relative abundance in the 10- and 15-yr fields. Our results were consistent with the finding that the relative proportion of Proteobacteria is closely associated with soil available nutrient content (Fierer et al. 2007; Buée et al. 2009). Among the treatments in this study, the 5-yr field had the highest SOC, available N, and available P. This may explain why the 5-year field also had the highest relative abundance of Proteobacteria.

Soil microbial diversity is critical to the integrity, function, and long-term sustainability of soil ecosystems (Kennedy and Smith 1995). Species diversity indices are important measures of microbial community and are determined by a combination of both historical and contemporary effects (Martiny et al. 2006; Sun et al. 2014). Increasingly, research suggests that greater species diversity is associated with greater ecosystem stability (McNaughton 1994); therefore, the maintenance and restoration of microbial biodiversity is becoming a key issue in sustainable agricultural systems (Zhao et al. 2014). In our study, we found that the PD, Chao1, and Shannon indices were lowest in

abandoned farmland and increased gradually after 1-yr of cotton production. Overall, the PD, Chao1, and Shannon values were highest in the 10- and 15-yr fields ($P < 0.05$). This is because relatively little residue was incorporated in the 1-yr field and because SOC, available N, and available P were significantly less in the 1-yr field than in the other treatments. Hence the increase of soil bacterial abundance and diversity might have contributed to the reclamation of abandoned farmland.

Soil chemical properties, reclamation time, and soil aggregation

Significant positive correlations were found between SOC and bacterial richness ($r = 0.91$, $P < 0.05$), Chao1 ($r = 0.98$, $P < 0.01$), and PD ($r = 0.89$, $P < 0.05$). A previous study in Argentina indicated that bacterial richness and diversity were linearly related with SOC (Gomez et al., 2006), which was consistent with our study. The SOC is closely related to nutrient supplying power, and frequently identified as a key indicator of soil quality (Reeves, 1997). Therefore, we can hypothesize that SOC can drive the soil bacterial community. In addition, Spearman correlation and RDA suggested that EC was the second largest explanatory factor (Table 4). This result is consistent with Gao et al. (2015), who used CCA to analyze the relationships between bacterial communities and environmental factors in the 0-20 cm soil depth in the Yellow River Delta region.

Soil pH was reported as a strong indicator of soil bacterial community composition (Rousk et al. 2010; Liu et al. 2015). However, no correlations were found between soil pH and the most abundant phyla in this study. Our results were inconsistent with previous studies which showed that soil pH is a better predictor of soil bacterial community structure in alkaline sediment than in soils with other pH levels (Xiong et al., 2012). This phenomenon may have been caused by variations in soil pH in this study (ranging from 7.85 to 8.58). In addition, neither soil moisture content nor BD were correlated with abundant phyla (Fig. 7). One possible explanation is moisture content and BD did not change significantly after the reclamation of abandoned farmland (Table 1).

Soil available N was negatively correlated with Actinobacteria and Bacteroidetes, whereas Gemmatimonadetes was positively correlated with soil available P. Ramirez et al. (2010) observed that the relative abundance of Actinobacteria was positively correlated with soil N. Na et al. (2016) found that Gemmatimonadetes was positively correlated with soil available P. Thus, we hypothesize that soil properties such as soil available P and available N can drive bacterial communities in some specific situations. The exact effects of these nutrients depend on land use, cropping system, fertilization, and elevation (Lauber et al. 2008; Shen et al. 2013; Pan et al. 2014). The bacterial

communities were well grouped according to time since reclamation (Fig. 3, 4, 5). The relationship between soil aggregate fractions and time since reclamation demonstrated that soil bacterial species was associated with those two factors (Table S1, $P < 0.05$). These results provide a small but important piece of information necessary for better understanding soil microbial diversity in northwest China. The information is important for the reclamation of abandoned salinized farmland.

Conclusions

The results indicate that reclamation of abandoned farmland for cotton production can increase SOC, available N, available P, and macro-aggregation (>0.25 mm). High throughput Illumina MiSeq sequencing was used to investigate bacterial community structure. Over 1 122 101 sequences were analyzed. The result indicated that the most abundant phyla across all 25 soil samples were Proteobacteria, Actinobacteria, Gemmatimonadetes, Acidobacteria, Bacteroidetes, and Chloroflexi. The PD, Chao1, and Shannon indices increased as time since reclamation increased. The PD, Chao1, and Shannon values were generally, but not always, greatest in the 1–0.25 mm fraction. Soil SOC and EC were the main environmental factors affecting changes in the bacterial communities. Soil aggregate size and time since reclamation significantly affected bacterial species composition and diversity; however, the exact nature of this relationship is uncertain. Longer term, field-based experiments will be needed to evaluate which factors have the most important effect on microbial diversity.

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Figure legends

Fig. 1. Sketch map of the Manasi River Basin and the study region.

Fig. 2. Relative abundances of bacterial phyla (> 1%) in the soil samples. Phyla accounting for less than 1 % of all sequences were grouped in the category “other”.

Fig. 3. Principal coordinate analysis (PCoA) scores plot of beta diversity of unweighted UniFrac pairwise distances. The percentages of variation explained by PC1, PC2 and PC3 are indicated in the axis. A, B, C, D and E stand for abandoned, 1-, 5-, 10-, and 15-yr fields, respectively. 1, 2, 3, 4, and 5 represent different aggregates fractions (i.e. > 5, 5-2, 2-1, 1-0.25, and < 0.25 mm).

Fig. 4. Cluster analysis that contain the essential 16S rDNA of soil bacteria under different years of reclamation farmlands. A, B, C, D and E stand for abandoned, 1-, 5-, 10-, and 15-yr fields, respectively. 1, 2, 3, 4, and 5 represent different aggregates fractions (i.e. > 5, 5-2, 2-1, 1-0.25, and < 0.25 mm).

Fig. 5. Principle component analyses (PCA) with similarity instrumental variable were carried out with 25 soil samples from the study areas. A, B, C, D and E stand for abandoned, 1-, 5-, 10-, and 15-yr fields, respectively. 1, 2, 3, 4, and 5 represent different aggregates fractions (i.e. > 5, 5-2, 2-1, 1-0.25, and < 0.25 mm).

Fig. 6. Cross analysis of bacterial community and soil physicochemical characteristics using redundancy analysis (Canoco 4.5).

Fig. S1. Rarefaction curves of each soil sample at the 3 % cutoff level of. A, B, C, D and E stand for abandoned, 1-, 5-, 10-, and 15-yr fields, respectively. 1, 2, 3, 4, and 5 represent different aggregates fractions (i.e. > 5, 5-2, 2-1, 1-0.25, and < 0.25 mm).

Fig. S2. Cladogram indicating the phylogenetic distribution of bacterial lineages as affected by time since reclamation; the threshold on the logarithmic LDA score for discriminative features was 4.0. Differences are represented in the color of the most abundant taxa (red indicating taxa in the abandoned field, green in the 1-yr field; dark blue in the 5-yr field; purple in the 10-yr field; light blue in the 15-yr field; and yellow is non-significant). Each circle's diameter is proportional to the given taxon's relative abundance. Circles represent phylogenetic levels from domain to genus from the inside outwards.

Table 1. Selected physical and chemical properties of the soil after abandoned salinized farmland was reclaimed for cotton production.

Treatments	pH	Moisture content (%)	Bulk density ($\text{g}\cdot\text{cm}^{-3}$)	Electric conductivity ($\text{ms}\cdot\text{cm}^{-1}$)	SOC (g kg^{-1})	Available N ($\text{mg}\cdot\text{kg}^{-1}$)	Olsen P ($\text{mg}\cdot\text{kg}^{-1}$)	Aggregate size distribution (%)				
								>5 mm	5-2 mm	2-1 mm	1-0.25 mm	<0.25 mm
Abandoned	7.96 bc*	0.16 a	1.77 a	2.77 a	10.08 bc	44.26 d	12.15 d	6.49 d	4.00 c	8.26 b	11.57 a	69.69 a
1 year	8.02 b	0.14 a	1.72 a	2.12 a	8.63 c	37.11 e	15.12 d	13.61 cd	12.26 d	21.08 a	16.63 a	36.41 b
5 years	8.20 ab	0.13 a	1.63 a	0.39 b	17.20 a	69.89 a	52.06 a	21.05 bc	31.55 a	23.01 a	15.69 a	8.70 c
10 years	7.85 c	0.12 a	1.71 a	0.34 b	15.59 ab	61.58 b	23.09 c	37.47 a	23.47 b	20.50 a	12.40 a	6.14 c
15 years	8.58 a	0.15 a	1.82 a	0.34 b	20.59 a	52.88 c	46.65 b	32.12 ab	21.42 b	23.36 a	12.29 a	10.82 c

* Different lowercase letters within a column indicate significant differences ($P < 0.05$).

Table 2. MiSeq sequencing results and diversity estimates for each sampling site.

Treatments	Aggregate size (mm)	Sequencing results			Diversity estimates		
		Raw tags	Clean tags	Total OTUs	PD	Chao1	Shannon
Abandoned	> 5	33 626	31288	1 595	99.88 ± 1.70	1 973.71 ± 25.76	8.44 ± 0.01
	5-2	52 335	44 013	1 842	107.39 ± 1.40	2 221.96 ± 43.19	8.43 ± 0.00
	2-1	45 834	42 680	2 055	122.21 ± 1.41	2 503.26 ± 28.36	8.80 ± 0.00
	1-0.25	56 445	53 098	2 207	128.67 ± 1.38	2 661.70 ± 11.04	8.88 ± 0.01
	< 0.25	38 108	37 062	1 908	108.27 ± 2.29	2 346.91 ± 39.55	8.53 ± 0.01
	Mean value	45 270	41 628	1 921	113.28	2 341.51	8.62
1 year	> 5	49 442	48 265	1 495	89.45 ± 0.86	1 878.03 ± 12.89	7.84 ± 0.00
	5-2	33 876	32 524	1 361	82.19 ± 1.24	1 637.56 ± 25.36	8.02 ± 0.00
	2-1	36 784	35 272	1 524	91.41 ± 1.39	1 771.70 ± 1.73	8.18 ± 0.00
	1-0.25	51 589	49 843	1 511	86.66 ± 0.91	1 818.72 ± 3.69	7.84 ± 0.00
	< 0.25	63 298	60 885	1 429	81.27 ± 0.94	1 772.74 ± 2.23	7.54 ± 0.00
	Mean value	46 998	45 358	1 464	86.19	1 775.75	7.89
5 years	> 5	59 124	56 614	2 094	117.79 ± 1.46	2 700.45 ± 21.64	7.95 ± 0.00
	5-2	59 065	46 698	1 946	105.79 ± 1.57	2 479.99 ± 38.75	7.96 ± 0.00
	2-1	44 622	43 283	2 170	118.15 ± 2.13	2 759.13 ± 49.66	8.32 ± 0.00
	1-0.25	65 371	63 287	2 448	133.01 ± 0.96	3 046.03 ± 37.27	8.50 ± 0.00
	< 0.25	51 958	49 980	2 391	130.76 ± 2.08	2 946.72 ± 40.69	8.61 ± 0.00
	Mean value	56 028	51 972	2 210	121.10	2 786.46	8.27
10 years	> 5	47 375	46 119	2 011	116.59 ± 1.73	2 518.36 ± 25.06	8.34 ± 0.00
	5-2	49 204	48 002	2 351	131.63 ± 1.75	2 796.63 ± 19.89	8.64 ± 0.00
	2-1	39 768	38 843	2 293	127.78 ± 2.86	2 847.93 ± 48.66	8.74 ± 0.01
	1-0.25	35 192	33 904	2 273	125.44 ± 2.18	2 745.51 ± 28.82	8.97 ± 0.01
	< 0.25	47 784	40 580	2 283	125.66 ± 1.93	2 805.71 ± 26.27	8.79 ± 0.01
	Mean value	43 865	41 490	2 242	125.42	2 742.83	8.70
15 years	> 5	54 031	52 064	2 274	134.88 ± 1.55	2 785.42 ± 33.48	8.72 ± 0.00
	5-2	41 253	40 286	2 298	131.88 ± 2.25	2 866.18 ± 31.71	8.86 ± 0.01
	2-1	46 179	44 854	2 399	137.46 ± 1.86	2 945.63 ± 35.39	8.97 ± 0.00
	1-0.25	46 914	45 379	2 522	143.41 ± 1.98	3 137.16 ± 42.47	9.11 ± 0.00
	< 0.25	38 261	37 278	2 327	131.26 ± 2.15	2 869.14 ± 54.72	9.05 ± 0.00
	Mean value	45 328	43 972	2 364	135.78	2 920.71	8.94

Table 3. Correlations between diversity parameters and environmental factors.

Factor	OTUs	Chao1	Shannon	PD
pH	0.45	0.47	0.37	0.49
MC	-0.21	-0.23	0.20	-0.08
BD	0.09	0.04	0.60	0.24
EC	-0.78	-0.79	-0.39	-0.70
SOC	0.91*	0.92**	0.65	0.89*
AN	0.78	0.81	0.30	0.67
AP	0.70	0.74	0.26	0.63

Note: OTUs, operational taxonomic units; PD, Faith's phylogenetic diversity; Shannon, nonparametric Shannon diversity index; MC, soil moisture content; BD, soil bulk density; EC, electrical conductivity; SOC, soil organic carbon; AN, available nitrogen; AP, available phosphorus. Pearson correlation coefficients were calculated. *, $P < 0.05$; **, $P < 0.01$.

Table 4. Spearman’s rank correlations (r) between the 11 most abundant phyla (Relative abundance > 1% in all samples combined) and selected soil properties.

	pH	MC	BD	EC	SOC	AN	AP
<i>Proteobacteria</i>	-0.04	0.20	-0.67	0.19	-0.18	0.27	0.27
<i>Gemmatimonadetes</i>	0.63	-0.44	-0.18	-0.96[†]	0.95	0.77	0.92
<i>Actinobacteria</i>	-0.36	0.38	0.00	0.84	-0.90	-0.83	-0.68
<i>Acidobacteria</i>	0.14	-0.67	0.02	-0.85	0.75	0.67	0.43
<i>Bacteroidetes</i>	-0.34	0.37	0.02	0.82	-0.88	-0.84	-0.67
<i>Chloroflexi</i>	0.13	0.50	0.70	0.17	0.19	-0.06	-0.13
<i>Planctomycetes</i>	0.24	0.57	0.76	0.16	0.23	-0.10	-0.08
<i>Firmicutes</i>	-0.23	0.06	-0.09	0.50	-0.69	-0.71	-0.49
<i>Nitrospirae</i>	0.07	-0.75	-0.01	-0.83	0.68	0.60	0.35
<i>Verrucomicrobia</i>	-0.13	0.52	0.64	0.40	-0.09	-0.21	-0.39
<i>TM7</i>	-0.52	0.19	0.49	0.45	-0.33	-0.29	-0.69

Note: MC, soil moisture content; BD, soil bulk density; EC, electrical conductivity, SOC, soil organic carbon; AN, available nitrogen; AP, available phosphorus.

[†]Values in bold are significant ($P < 0.05$).

Fig. 1. Sketch map of the Manasi River Basin and the study region.

Relative abundances of bacterial phyla (> 1%) in the soil samples. Phyla accounting for less than 1 % of all sequences were grouped in the category “other”.

Fig. 3. Principal coordinate analysis (PCoA) scores plot of beta diversity of unweighted UniFrac pairwise distances. The percentages of variation explained by PC1, PC2 and PC3 are indicated in the axis. A, B, C, D and E stand for abandoned, 1-, 5-, 10-, and 15-yr fields, respectively. 1, 2, 3, 4, and 5 represent different aggregates fractions (i.e. > 5, 5-2, 2-1, 1-0.25, and < 0.25 mm).

Fig. 4. Cluster analysis that contain the essential 16S rDNA of soil bacteria under different years of reclamation farmlands. A, B, C, D and E stand for abandoned, 1-, 5-, 10-, and 15-yr fields, respectively. 1, 2, 3, 4, and 5 represent different aggregates fractions (i.e. > 5, 5-2, 2-1, 1-0.25, and < 0.25 mm).

Fig. 5. Principle component analyses (PCA) with similarity instrumental variable were carried out with 25 soil samples from the study areas. A, B, C, D and E stand for abandoned, 1-, 5-, 10-, and 15-yr fields, respectively. 1, 2, 3, 4, and 5 represent different aggregates fractions (i.e. > 5, 5-2, 2-1, 1-0.25, and < 0.25 mm).

Fig. 6. Cross analysis of bacterial community and soil physicochemical characteristics using redundancy analysis (Canoco 4.5).

Table S1. Two-way analysis of variance (ANOVA) of reclamation years (Y) and aggregate size (A) on soil bacterial communities (B).

Source of variation	Sum of squares	df	Mean square	F value	P value
Reclamation Years (Y)	1.69	4	0.42	0.060	0.9933
Aggregates (A)	0.06	4	0.02	0.002	0.9999
Bacteria (B)	27326.08	10	2732.61	386.185	0.0001
Y×A	0.24	16	0.015	0.002	0.9999
Y×B	5233.20	40	130.83	18.490	0.0001
A×B	614.65	40	15.37	2.172	0.0004

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- Abandoned
■ 1-yr
■ 5-yr
■ 10-yr
■ 15-yr

a: mb2424	k: Euzebyales	t: Flavobacteriia	a2: Gemmatimonadetes	b1: Sphingomonadaceae
b: iii1_15	l: Nitriliruptoraceae	u: Anaerolineae	a3: 0319_6A21	b2: Sphingomonadales
c: Acidobacteria_6	m: Nitriliruptorales	v: Gitt_GS_136	a4: Nitrospirales	b3: Alphaproteobacteria
d: RB41	n: Nitriliruptoria	w: Thermomicrobia	a5: Nitrospira	b4: MND1
e: _Chloracidobacteria_	o: Flammeovirgaceae	x: Bacillales	a6: Phycisphaerae	b5: Betaproteobacteria
f: DS_18	p: Cytophagales	y: Bacilli	a7: Rhizobiales	b6: Deltaproteobacteria
g: iii1_8	q: Cytophagia	z: Gemm_1	a8: Rhodobacteraceae	b7: Sinobacteraceae
h: Actinomycetales	r: Flavobacteriaceae	a0: Gemm_5	a9: Rhodospirillaceae	b8: Xanthomonadaceae
i: Actinobacteria	s: Flavobacteriales	a1: Gemm_5	b0: Rhodospirillales	b9: Xanthomonadales
j: Euzebyaceae				