Bacterial diversity in the foreland of the Tianshan No. 1 glacier, China

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Abstract
There is compelling evidence that glaciers are retreating in many mountainous areas of the world due to global warming. With this glacier retreat, new habitats are being exposed that are colonized by microorganisms whose diversity and function are less well studied. Here, we characterized bacterial diversity along the chronosequences of the glacier No. 1 foreland that follows glacier retreat. An average of 10 000 sequences was obtained from each sample by 454 pyrosequencing. Using non-parametric and rarefaction estimated analysis, we found bacterial phylotype richness was high. The bacterial species turnover rate was especially high between sites exposed for 6 and 10 yr. Pyrosequencing showed tremendous bacterial diversity, among which the Acidobacteria, Actinobacteria, Bacteroidetes and Proteobacteria were found to be present at larger numbers at the study area. Meanwhile, the proportion of Bacteroidetes and Proteobacteria decreased and the proportion of Acidobacteria increased along the chronosequences. Some known functional bacterial genera were also detected and the sulfur- and sulfate-reducing bacteria were present in a lower proportion of sequences. These findings suggest that high-throughput pyrosequencing can comprehensively detect bacteria in the foreland, including rare groups, and give a deeper understanding of the bacterial community structure and variation along the chronosequences.

Keywords: bacterial community diversity, Tianshan No. 1 glacier, foreland, pyrosequencing, chronosequences

1. Introduction

Over the past 100 years, the average global temperature has increased by 0.74 °C (IPCC 2007). One consequence of this temperature increase is that glaciers are retreating in many mountainous areas of the world. As glaciers retreat, the newly exposed land is a new habitat for microorganisms (Schütte et al 2010). Microbial communities may be key determinants of glacier foreland ecosystem stability and function because of their important roles in soil development, biogeochemical cycles and heterotrophic activities (Schütte et al 2010). Most ecological studies of the glacier foreland are focused on plant communities, but few studies have investigated the succession of microbial communities on the glacier foreland (Schütte et al 2009). Most of those studies focus on Polar and European mountain areas but rarely on the high Asia area, so studies of bacteria community structure variation along the
chronosequences in the high Asia area including in Tianshan Mountains are still needed.

Some studies to assess bacterial diversity and succession in glacier forelands have been performed (Sigler et al 2002, Nicol et al 2005, Deiglmayr et al 2006, Nemergut et al 2007, Schmidt et al 2008, Sattin et al 2009, Schütte et al 2010). These studies provide conflicting information regarding the variation of microbial communities over time following glacier retreat. Of these studies, Schütte et al (2010) first used pyrosequencing to analyse bacterial diversity in a glacier foreland in the High Arctic and found that bacterial richness was high and increased significantly with glacier retreat. Nemergut et al (2007) analysed 16S rRNA gene sequences along a chronosequence of three unvegetated soil areas and found phylotype number, diversity and evenness increased over time at forelands of the Puca glacier. Meanwhile, Sigler et al (2002) found opposing results, suggesting that not only did the number of dominant organism types decrease with succession, so did community evenness. These findings suggest that the development of bacterial communities in soils following glacier retreat may be less predictable than the development of plant communities, where species richness consistently increases over time (Huston and Smith 1987, Matthews 1992, Hodkinson et al 2003, Walker and Moral 2003). Different glaciers have different thermal and hydrological conditions, potentially leading to different microorganism community variations at glacier forelands. Thus, the study of bacterial diversity in glacier forelands is needed.

Tianshan No. 1 glacier is located in the Eastern Tianshan Mountains of central Asia, which are surrounded by desert (Lee et al 2003). The climate in this area is a classical continental climate, and wind is an important climatic factor on the upper elevations of the Tianshan Mountain (Williams et al 1992). Tianshan No. 1 glacier has been studied intensively from the glaciological point of view since 1959 (Aizen et al 2006, Li et al 2006, Bolch 2007, Kutuzov and Shahgedanova 2009, Narama et al 2010), when the Tianshan Glaciological Station was built. With global warming, most mountain glaciers, including those in the Tianshan Mountains, have been in a state of rapid retreat over the past few decades and this retreat rate will increase in the future. Consequently, Tianshan No. 1 glacier separated into east and west branches in 1993. From 1959 to 1993 the glacier receded at an average rate of 4.5 m yr$^{-1}$ (a total of 139.72 m). From 1993 to 2004, the east branch and west branch of Tianshan No. 1 glacier retreated at an average rate of 3.5 m yr$^{-1}$ (a total of 38.7 m) and 5.8 m per year (a total of 64.1 m), respectively (Li et al 2008, Wang et al 2011, Xu et al 2011). Because of the availability of extensive glaciological data, this location is suitable for a study of microbial distribution and growth related to both climatic and other environmental records. Although the study of microorganisms in this area is very important, few studies have been performed. Bai et al (2006) examined the phylogenetic diversity of bacteria from permafrost in the Tianshan Mountains using a culture method and found four phyla: Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria. Yang et al (2008) studied the permafrost bacteria and archaea community structures and diversity by using denaturing gradient gel electrophoresis method and found seven phyla of bacteria, including the Acidobacteria, Gemmatimonadetes and Chloroflexi phyla not found by Bai et al. In addition, Sheng et al (2011) first described indigenous endophytic bacteria using a culture method and obtained 93 isolates from 20 different subnival plants. Studies on bacteria community structure and diversity variation along the chronosequences have not been carried out in the Tianshan Mountains. It is still unknown whether bacterial community structure and diversity change along the chronosequences, and which phylum is dominant in the glacier foreland of Tianshan No. 1 glacier. Based on the other related research (Jones et al 2009, Roesch et al 2007, Schütte et al 2010), we assume that bacterial community diversity may increase along the chronosequences, meanwhile Acidobacteria and Proteobacteria may be dominant phylum in the glacier foreland.

In this study, we used pyrosequencing to analyse the Tianshan No. 1 glacier foreland soil bacterial diversity along chronosequences. Investigations of bacterial diversity based on cultivation-independent methods are limited by the lack of high-throughput methods for sequencing 16S rRNA genes. The advent of high-throughput tag-encoded FLX amplicon pyrosequencing has eliminated this problem and provides the means necessary to conduct intensive and extensive analyses of bacterial diversity in different environments (Sogin et al 2006, Roesch et al 2007, Dethlefsen et al 2008, Huse et al 2008). Using this pyrosequencing method, we were able to show a remarkably high bacterial diversity as well as bacterial diversity change along the chronosequences. These results provide the database for the bacterial diversity information and bacterial succession rules on the Tianshan glacier foreland.

2. Materials and methods

2.1. Study site and sampling

The Tianshan Mountains run through China, Kyrgyzstan and Kazakhstan in Central Asia and have 15,953 glaciers and a
total area of 15416 km² (Wang et al. 2011). The sample sites were located at Tianshan No. 1 glacier (N 43°06’, E 86°48’), 120 km southwest of Urumchi, China (figure 1). The top elevation at this glacier was 4486 m. Samples were taken at the east branch of Tianshan No. 1 glacier foreland along the chronosequence in front of retreating glaciers, six soil samples were collected in August 2010. These soil samples representing six periods (table 1). The succession times of every sampling site were determined using Tianshan Glaciological Station (Chinese Academy of Sciences) annual glacier retreat observation data (from 1959 to 2010) and lichenometric chronology data (from 1958 to 1953) (Chen 1989). Each soil sample consisted of three subsample cores at 5 cm deep taken at random in an area approximately 2 m × 2 m and were mixed after the larger gravels had been removed. Pioneer plants appeared in the soil of deglaciation within 10–100 yr and the vegetation developed after 100 yr of deglaciation. Successional species arriving within 10–100 yr include Cancrinia tianschanica, Bryophyta spp., Poa tianschanica, Draba nemorosa, Saxifraga hirculus L., Melandrium apricum, Leontopodium lentopodioides, Saussurea gnaphalodes, Crepis flexuosa, Rhodiola coccinea, Oxyria digyna and Saussurea involucrata, while Senecio thianschanicus, Polygonum viviparum and Pedicularis spp. additionally appear outside the glacier foreland. The soil samples were placed in a sterile soil box and kept frozen on ice during transport to the laboratory and stored at −20°C until analysed. The samples were sieved (2 mm) before use. Soil pH was measured using soil/double-distilled water (1:1 w/v) with an acidity meter (Sartorius PT-10, Germany) (Zhang et al. 2007). Table 1 shows the sample location information along the glacier retreat.

2.2. DNA extraction, PCR and 454 Pyrosequencing

Genomic DNA was isolated from at least 1 g of three replicate mixed soils using the PowerSoil DNA Isolation Kit (MoBio) according to the manufacturer’s instructions. Extracted DNA was stored at −20°C.

To amplify a 16S rRNA gene fragment of the appropriate size and sequence variability for 454 pyrosequencing, primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′, Escherichia coli position 9–27) and 515R (5′-TTCACCCGCTGCTGGCAC-3′, E. coli position 533–515) were chosen (Watanabe et al. 2007), and the primers 27F and B515R contained the 454 Life Sciences(R) adaptors B and A respectively. Based on these, the V1–V3 region of bacterial 16S rRNA genes was amplified. Each PCR reaction contained 2 µl 10× buffer, 1.6 µl dNTP, 0.8 µl forward primer 454_27F, 0.8 µl reverse primer 454_533R, and 0.8 µl Pfu Taq DNA polymerase. Amplification of 16S rRNA genes was performed using an initial denaturation step at 95°C for 3 min, followed by 25 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s and an extension at 72°C for 30 s, with a final extension step of a 10 min step at 72°C.

Amplicons were purified using the AxyPrep DNA Gel Extraction Kit (Axygen) as directed by the manufacturer. The concentrations of amplicons were quantified by the TBS-380 system, and equal amounts of all amplicons were mixed in a single tube. Performed emulsion PCRs, which are described in Margulies et al (2005), and using a GS FLX Titanium System (454 Life Sciences, Roche Applied Science) obtained sequences.

2.3. Quality control, phylogenetic assignment, alignment and clustering of sequences

We followed previously described criteria to assess the quality of sequence reads (Sogin et al 2006, McKenna et al 2008). To pass, a sequence read had to (i) include a perfect match to the sequence tag (bar-code) and the 16S rRNA gene primer; (ii) be at least 200 bp in length and (iii) have no uncertain bases including chimeric sequences (Huse et al 2007). We obtained 75847 trimmed sequences. The 16S rRNA gene fragments were phylogenetically assigned according to their best matches in the SILVA database (Pruesse et al 2007). Various thresholds of sequence similarity among 16S rRNA gene sequences were commonly used as a proxy for different taxonomic levels in studies on microbial diversity with 95% and 97% similarity to differences between genera and species (Roesch et al 2007, Oakley et al 2008).

2.4. Statistical analysis

The rarefaction, Chao1 and ACE were used as richness estimators at 3%, 5% and 10% dissimilarity levels by using DOTUR (Roesch et al 2007). We used mothur software to calculate the changes in the bacterial community compositions described by Whittaker (1972), Diamond and May (1977) based on a 3% sequence dissimilarity. Statistical analyses were performed using the SPSS 13.0 (SPSS Inc., IL).

3. Results

Phyotype richness was estimated by using non-parametric (Chao, ACE) and rarefaction estimates (OTU) (table 2). All estimates showed that phyotype richness increased over time for all samples at three thresholds at all times since glacier retreat. For example, phyotypes were lowest in the youngest soils (at a threshold of 97% sequence similarity, OTU value approximately 4000) and peaked in the oldest soils (OTU value 5159).

The structures of bacterial communities were characterized by the phytype Shannon–Wiener index (H) and the Simpson index (D). As shown in table 3, the Shannon–Wiener index significance increased over time (F = 8.83, P = 0.04 < 0.05), from 6.65 to 7.82 and the Simpson index decreased.

| Table 1. Distance, retreated time, and pH value of the sample. |
|------------------|---------|---------|---------|---------|---------|
| Distance from the glacier front (m) | 23 | 40 | 73 | 235 | 290 | 370 |
| Times | 6a | 10a | 20a | 60a | 74a | 100a |
| pH | 8.91 | 8.23 | 7.48 | 7.75 | 7.38 | 7.48 |

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Table 2. Ability of three richness estimators and three thresholds to predict number of species in all samples.

| Time (years) | Number of sequence reads | 97% Similarity | 95% Similarity | 90% Similarity |
|--------------|--------------------------|----------------|----------------|----------------|
|              |                          | OTU Chao ACE   | OTU Chao ACE   | OTU Chao ACE   |
| 6            | 9 159                    | 1892 4 293 3 370| 1518 3 071 2 526| 1057 1 838 1 620|
| 10           | 14 126                   | 4107 13 669 8 767| 3483 10 169 6 736| 2486 5 521 4 117|
| 20           | 10 676                   | 3830 13 119 8 294| 3346 10 611 7 106| 2459 6 027 4 625|
| 60           | 12 791                   | 4366 12 236 8 585| 3648 9 222 6 691| 2544 5 227 4 160|
| 74           | 14 692                   | 5096 15 237 10 688| 4317 11 195 8 202| 3055 6 653 5 330|
| 100          | 14 403                   | 5159 14 906 10 366| 4327 10 952 7 863| 3034 6 615 5 069|

Table 3. Phylotype diversity changes along with chronosequences.

| Time (years) | Shannon Similarity | Simpson Similarity |
|--------------|--------------------|-------------------|
|              | 97% Similarity     | 95% Similarity     | 90% Similarity     |
| 6            | 6.65               | 0.0027             | 6.35               | 0.0037           | 5.87               | 0.0061            |
| 10           | 7.34               | 0.0021             | 7.09               | 0.0026           | 6.58               | 0.0048            |
| 20           | 7.37               | 0.0022             | 7.11               | 0.0031           | 6.61               | 0.0058            |
| 60           | 7.71               | 0.0009             | 7.42               | 0.0014           | 6.86               | 0.0026            |
| 74           | 7.74               | 0.0016             | 7.49               | 0.002            | 6.98               | 0.003             |
| 100          | 7.82               | 0.0013             | 7.55               | 0.0019           | 6.94               | 0.0037            |

Table 4. Turnover rate of bacterial communities along the chronosequence determined using the dissimilarity measure of Whittaker (1972).

| Years since glacier retreat | 6a | 10a | 20a | 60a | 74a | 100a |
|----------------------------|----|-----|-----|-----|-----|------|
| 6a                         | 0  | 0.76 | 0.85 | 0.89 | 0.90 | 0.91 |
| 10a                        | 0  | 0.85 | 0.91 | 0.91 | 0.92 | 0.93 |
| 20a                        | 0  | 0.86 | 0.89 | 0.89 | 0.90 | 0.91 |
| 60a                        | 0  | 0.79 | 0.72 | 0.72 | 0.73 | 0.74 |
| 74a                        | 0  | 0.72 | 0.70 | 0.70 | 0.71 | 0.72 |
| 100a                       | 0  | 0.72 | 0.70 | 0.70 | 0.71 | 0.72 |

Table 5. Change in bacterial community composition per year (turnover rate per year) along the chronosequence determined using the measure employed by Diamond and May (1977).

| Years since glacier retreat | 6a | 10a | 20a | 60a | 74a | 100a |
|----------------------------|----|-----|-----|-----|-----|------|
| 6a                         | 0  | 0.19 | 0.08 | 0.06 | 0.05 | 0.04 |
| 10a                        | 0  | 0.08 | 0.06 | 0.06 | 0.06 | 0.06 |
| 20a                        | 0  | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
| 60a                        | 0  | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| 74a                        | 0  | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| 100a                       | 0  | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |

over time ($F = 7.45, P = 0.053$), from 0.0027 to 0.0013 at a threshold of 97% sequence similarity.

We used mothur software to calculate the changes in the bacterial community compositions described by Whittaker (1972), Diamond and May (1977) based on a 3% sequence dissimilarity. These indices are measures of turnover and describe the dissimilarity of community compositions. The values range from zero to one and values near one correspond to large turnover. The measure by Whittaker (1972) showed high values in community composition for all pairwise comparisons of bacterial communities along the chronosequence (table 4). The measurements by Diamond and May (1977) showed relatively low values, because this index additionally accounts for time. It represents change in community per year. Using the method by Diamond and May (1977), we found that in the first 4 yr, the turnover rate was about 19% yr$^{-1}$ (0.190, table 5) and that this rate decreased to about 0.9% yr$^{-1}$ from 74 to 100 yr since glacier retreat (0.009, table 5). Overall, these results reflect the tremendous turnover of bacterial communities along the chronosequence and the change rate was particularly rapid in the early times and slower in the later times following glacier retreat.

The relative abundance of phylum in each soil sample was shown in figure 2. The Acidobacteria, Actinobacteria, Proteobacteria and Bacteroidetes were the predominant phyla in all samples; the proportion of Acidobacteria ranged from 5.15% to 17.77%, Actinobacteria 5.56%–12.71%, Proteobacteria 22.65%–39.75% and Bacteroidetes 7.04%–20.57%. The Chloroflexi, Cyanobacteria, Planctomycetes, Candidate division OD1, Candidate division OP10, Candidate division OP11, Candidate division TM7, Fibrobacteres, Fusobacteria, Gemmatimonadetes, Nitrospirae and Verrucomicrobia were all relatively low (<5%). Outside of these phyla, the Aquificae, Caldiserica, Candidate division BRC1, Candidate division OP3, Candidate division SR1, Candidate division TM6, Candidate division WS3, Candidate division WS6, Chlamydiae, Chlororbi, Deferribacteria, Deinococcus-Thermus, Elusimicrobia, Firmicutes and Lentisphaerae, occupied a very low proportion (<1%) of all sequences. Only a small part of the sequences was unclassified, ranging from 0.17% to 1.37%.
to 12.4% respectively. The Actinobacteria presented a more stable trend along the chronosequences with the proportion in most samples approximately 10%. Cyanobacteria first decreased and then increased from 5.25% to 0.38% and then to 2.87% (figure 2).

Some known functional bacterial genera (Uroz et al 2010) are shown in table 6. The samples tested contained a majority of nitrifying bacteria, such as genera *Nitrosococcus*, *Nitrosomonas*, *Nitrosospiria* and *Nitrospiria* and a large number of unclassified genera in *Nitrosomonadaceae*. Species from only three genera of nitrogen-fixing bacteria were detected in these samples: those from genera *Bradyrhizobium*, *Mesorhizobium* and *Rhizobium*. The methane-oxidizing bacteria had an abundant genera composition. The sulfur- and sulfate-reducing bacteria were present in only a small number of sequences; most genera were represented by less than 10 sequences. Some genera of bacteria were present at all ages from the glacier retreat, for example *Nitrospiria, Bradyrhizobium, Mesorhizobium, Methylobacterium, Methylocystaceae_uncultured* and *Methylophilaceae_uncultured*. Some existing in the early age following glacier retreat disappeared in the older ages, such as *Nitrosomonas* and *Nitrospiria*. Some did not exist in the early age and appeared in the later age, such as *Nitrosococcus, Rhizobium, Methylocapsa, Methylohalobius* and *Desulfurellaceae_uncultured*. The *Methylobacteriaceae_uncultured, Methylohalomonas, Methyloplana, Methylothenera, Desulfocella, Desulfonispora, Desulfosporosinus* genera did not exist in the earliest and oldest ages but only appear in middle samples.

Figure 2. Relative phyla abundance for each soil sample.
Table 6. Number of sequences classified to be within known functional bacterial genera.

| Time (years)       | 6a | 10a | 20a | 60a | 74a | 100a |
|--------------------|----|-----|-----|-----|-----|------|
| Nitrifying bacteria|    |     |     |     |     |      |
| Nitrosococcus      | 0  | 0   | 0   | 6   | 0   | 1    |
| Nitrosomonas       | 6  | 5   | 2   | 2   | 4   | 0    |
| Nitrospira         | 1  | 2   | 13  | 4   | 1   | 0    |
| Nitrospira         | 11 | 28  | 92  | 202 | 71  | 82   |
| Nitrosomonadaceae_uncultured | 292 | 324 | 401 | 903 | 356 | 477 |
| Nitrogen-fixing bacteria|    |     |     |     |     |      |
| Bradyrhizobium     | 9  | 4   | 13  | 26  | 25  | 55   |
| Mesorhizobium      | 25 | 33  | 80  | 37  | 14  | 38   |
| Rhizobium          | 0  | 1   | 3   | 17  | 6   | 9    |
| Methane-oxidizing bacteria|    |     |     |     |     |      |
| Methylhibium       | 5  | 14  | 0   | 13  | 0   | 3    |
| Methylbacteriaceae_uncultured | 0  | 0   | 1   | 10  | 0   | 0    |
| Methylbacterium    | 8  | 2   | 1   | 10  | 20  | 27   |
| Methylocapsa       | 0  | 15  | 15  | 4   | 7   | 4    |
| Methylcystaceae_uncultured | 22 | 49  | 82  | 23  | 14  | 39   |
| Methylhalobius     | 0  | 7   | 8   | 1   | 13  |      |
| Methylhalomonas    | 0  | 0   | 0   | 1   | 0   | 0    |
| Methylphilaceae_uncultured | 47 | 55  | 4   | 71  | 16  | 21   |
| Methylpila         | 0  | 0   | 1   | 1   | 0   | 0    |
| Methylothenera     | 0  | 4   | 1   | 0   | 0   | 0    |
| Sulfur- and sulfate-reducing bacteria|    |     |     |     |     |      |
| Desulfocella       | 0  | 0   | 0   | 1   | 0   | 0    |
| Desulfolina        | 1  | 0   | 3   | 0   | 0   | 3    |
| Desulfotogona      | 0  | 1   | 4   | 8   | 0   | 0    |
| Desulfofonsinus    | 0  | 1   | 0   | 0   | 0   | 0    |
| Desulfurellaceae_uncultured | 0  | 0   | 0   | 16  | 63  | 41   |

4. Discussion

This was the first intensively and extensively assessed high-throughput tag-encoded FLX amplicon pyrosequencing examination of the bacterial diversity in the foreland of a mountain glacier in China. Soil samples from six chronosequences were collected in the foreland of Tianshan No. 1 glacier that span a 100 yr period since deglaciation. By using high-throughput pyrosequencing methods, an average of 10,000 sequences was obtained for each of the six time points following deglaciation. Phytype richness varied using different indices, but both approaches indicated that phytype richness was high and increased over subsequent years. For example, at the 97% sequence similarity, the phytype richness (OTU) ranged from 1892 to 5159 per 1.0 g of soil, where the Chao estimates ranged from 4293 to 14,096 per gram of soil. There was significant increase of the Shannon–Wiener index in the early age that slowed in the intermediate age and peaked in the oldest age. The Shannon–Wiener index is derived from information theory. It measures the species diversity of communities, representing the complexity of the community. The larger the \( H \) value, the more species in the community. In our study, the change in Shannon–Wiener trend reflects that bacterial diversity became abundant, but that the rate of change slowed along the chronosequences. Values of another diversity index, the Simpson index, are greater than 0 and less than 1. Values near zero correspond to highly diverse or heterogeneous ecosystems, and values near one correspond to more homogeneous ecosystems. The Simpson index in our study showed a decreasing trend and reflects an increasing ecosystem heterogeneous along the chronosequences. Perhaps the most striking finding from this study was the extraordinarily high species turnover (beta diversity); the turnover rate is high at early times along the chronosequence (19% yr\(^{-1}\)), and then at later times the turnover rate decreased to 0.9% yr\(^{-1}\). This was consistent with the findings of Schütte et al (2010) that in the first 14 yr, turnover rate was about 7% yr\(^{-1}\)and that this rate decreased to about 0.7% yr\(^{-1}\) from 100 to 150 yr since glacier retreat in the High Arctic. Two reasons may account for species turnover change. First, there may be substantial changes in the soil environment following deglaciation, such as soil pH values dropping from 8.91 to 7.48 (table 1) and soil urease, protease, acid phosphatase, arylsulphatase, sucrase activity, microbial nitrogen mineralization and deamination increased along the chronosequences (Wang et al 2010). Second, the bacterial populations initially present may not be well suited to the newly exposed soil environment because environment conditions are harsh including high pH and low nutrient contents, such as organic matter, total phosphorus, available phosphorous, available potassium, and available
nitrogen (Wang et al. 2010). With the soil developing, immigrant populations may occupy ecological niches and weather the rocks and minerals. Once soil composition becomes stabilized, the bacteria present effectively prevent colonization by invading bacteria. Such varying trends accompanied the degree of soil development, which indicated that soil development was closely related to microorganisms and that these factors interacted with each other at the glacier foreland (Bruce 2005, Brankatschk et al. 2011).

In this study, all of the sequences fell into 31 phyla and a small proportion of unclassified sequences. Yang et al. (2008) using denaturing gradient gel electrophoresis, found seven phyla in the same location: Proteobacteria, Actinobacteria, Acidobacteria, Gemmatimonadetes, Bacteroidetes, Firmicutes and Chloroflexi. These phyla were all detected in our study. Besides these phyla, the Aquificae, Caldiserica, Chlamydiae, Chlorobi, Crenarchaeota, Cyanobacteria, Deferribacteres, Deinococcus-Thermus, Elusimicrobia, Fibrobacteres, Fusobacteria, Lentisphaerae, Nitrospirae, Planctomycetes, Verrucomicrobia, Candidate division BR1, Candidate division OD1, Candidate division OP10, Candidate division OP11, Candidate division OP3, Candidate division SR1, Candidate division TM6, Candidate division TM7, Candidate division WS3 and Candidate division WS6 groups were first detected in the study area. This indicates that the massive sequencing can more accurately reflect the bacterial community structure in the foreland of the glacier. Additionally, other studies (Schütte et al. 2010) using a high-throughput method found 20 phyla in the High Arctic, of which 19 phyla (Acidobacteria, Actinobacteria, Bacteroidetes, Chlamydiae, Chloroflexi, Cyanobacteria, Deinococcus-Thermus, Firmicutes, Gemmatimonadetes, Nitrospirae, Planctomycetes, Proteobacteria, Verrucomicrobia, Candidate division BR1, Candidate division OD1, Candidate division OP10, Candidate division OP11, Candidate division TM7 and Candidate division WS3) were included in our study; only Spirochaetes was additionally detected. Spirochaetes, Aquificae, Caldiserica, Chlorobi, Crenarchaeota, Deferribacteres, Elusimicrobia, Epsilonproteobacteria, Fibrobacteres, Fusobacteria, Lentisphaerae, Candidate division OP3, Candidate division SR1, Candidate division TM6 and Candidate division WS6 are novel communities at these two study sites. In our study, about 1.37% of all sequences could not be classified into known bacterial phyla, and about 42% of the sequences could not be classified into a known genus (Philippot et al. 2011). Thus, with each additional study we gain a better understanding of the full extent of prokaryotic diversity and its distribution on Earth. These data reflect that similar environments have similar bacterial communities and that different bacterial communities may be due to geographic specificity.

Four phyla, Acidobacteria, Actinobacteria, Bacteroidetes and Proteobacteria were found in larger numbers along the chronosequences. Acidobacteria are commonly found in soil (Neufeld and Mohn 2005, Roesch et al. 2007) and are known to grow under oligotrophic conditions and at low pH (Dion 2008, Jones et al. 2009). Thus, the abundance of Acidobacteria in samples from the entire chronosequence and their increase in number over time, similar to the study of Arctic foreland (Schütte et al. 2010), may reflect the low levels of nutrients present in foreland soils and the decrease in soil pH that occurs along the chronosequence (Hodkinson et al. 2003). Proteobacteria were abundant in all samples; this phylum has been cultured from similar glacial environments, such as glacial ice cores (Sheridan et al. 2003), permanent Antarctic lake ice (Gordon et al. 2000), sub-glacier ice above Lake Vostok, Antarctica (Priscu et al. 1999) and underneath the Bench glacier in Alaska and John Evans glacier in Nunavut, Canada (Skidmore et al. 2005). Some Actinobacteria are known to form mycelia, which is suggested to be advantageous in oligotrophic and desert environments because the hyphae allow the bacteria to reach water and nutrients in far off pores (Dion 2008). Bacteroidetes often are more abundant in water environments. Our study indicates that glacial melt water provides a suitable environment for Bacteroidetes. For any complex system, assessing the number and relative abundance of the parts is fundamental to a quantitative description (Gans et al. 2005). Based on this theory, our results provide a more detailed understanding of the bacterial community in the foreland environment.

Several N-fixing genera besides Cyanobacteria, such as Rhizobium, Mesorhizobium and Bradyrhizobium, were found in our study. Most studies of nitrogen fixation in terrestrial polar environments have focused on Cyanobacteria (Whitton and Potts 2000); however, our data suggest that additional genera should be considered for the contribution to N-fixation in glacier foreland soils. Some functional groups of bacteria always existed in all ages from the glacier retreat, some existed in the early age following glacier retreat and disappeared in the older age, some did not exist in the early age and appeared in the later age, and some did not exist in the earliest and oldest age but only appeared in the intermediate ages. For example, the Myelobacteriaceae_uncultured genera was not detected at the early age (6a, 10a) or older age (74a, 100a) but was detected at 20a and 60a. The reason for this change requires further study. This reflects that functional groups of bacteria undergo a succession process along the chronosequences.

5. Conclusion

This is the first time that bacterial community structure and variation along the chronosequences in Tianshan Mountains have been researched using high-throughput methods. Our data revealed that bacterial phylotype richness was high and increased along the chronosequences. The Shannon–Wiener index significantly increased, while the Simpson index decreased over time. The bacterial species turnover rate was especially high between sites exposed for 6 yr and 10 yr, up to 19% yr⁻¹. The Acidobacteria, Actinobacteria, Bacteroidetes and Proteobacteria were found to be dominant phyla at the study area and the sulfur- and sulfate-reducing bacteria were present in a lower proportion of sequences. In contrast, the methane-oxidizing bacteria were abundant genera and the nitrifying bacteria were more abundant than the nitrogen-fixing bacteria; the majorities were from unclassified
groups and the reason for this change requires further study. In conclusion, the bacterial phylotype richness increased along the chronosequence and these results are consistent with Schütte et al (2010).

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