RESEARCH ARTICLE

Diversity and succession of autotrophic microbial community in high-elevation soils along deglaciation chronosequence

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One sentence summary: Autotrophic microorganisms rapidly colonized young deglaciated soils and their abundance positively correlated with total organic carbon and total nitrogen, suggesting that soil TOC and TN originated from autotrophs.

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ABSTRACT

Global warming has resulted in substantial glacier retreats in high-elevation areas, exposing deglaciated soils to harsh environmental conditions. Autotrophic microbes are pioneering colonizers in the deglaciated soils and provide nutrients to the extreme ecosystem devoid of vegetation. However, autotrophic communities remain less studied in deglaciated soils. We explored the diversity and succession of the cbbL gene encoding the large subunit of form I RubisCO, a key CO₂-fixing enzyme, using molecular methods in deglaciated soils along a 10-year deglaciation chronosequence on the Tibetan Plateau. Our results demonstrated that the abundance of all types of form I cbbL (IA/B, IC and ID) rapidly increased in young soils (0–2.5 years old) and kept stable in old soils. Soil total organic carbon (TOC) and total nitrogen (TN) gradually increased along the chronosequence and both demonstrated positive correlations with the abundance of bacteria and autotrophs, indicating that soil TOC and TN originated from autotrophs. Form IA/B autotrophs, affiliated with cyanobacteria, exhibited a substantially higher abundance than IC and ID. Cyanobacterial diversity and evenness increased in young soils (<6 years old) and then remained stable. Our findings suggest that cyanobacteria play an important role in accumulating TOC and TN in the deglaciated soils.

Keywords: autotrophic microbial community; deglaciated soil; Tibetan Plateau; cyanobacteria
INTRODUCTION

Global warming has significantly accelerated glacier retreats in polar and mountainous regions over the past 100 years. Tibetan Plateau (TP), with an average elevation of over 4000 m above sea level (a.s.l.) and an area of ~2.5 × 10^6 km², is the highest and the most extensive highland in the world and is usually called the ‘Third Pole’ (Qiu 2008). TP is one of the largest ice masses on Earth with more than 100 000 km² of glaciers and accounts for more than 80% of the total glacial mass in China (Kang et al. 2010). The TP glaciers have been exhibiting rapid retreat due to climate warming since 1980s (Su et al. 1999; Yao, Pu and Liu 2006; Yao et al. 2012).

Glacier retreat exposes new terrains to high levels of UV radiation and large-scale daily fluctuations in moisture and temperature. Deglaciated soils on the new terrains are typically dominated by sands with low nutrients and are particularly devoid of organic carbon (Strauss, Ruhland and Day 2009; Eckmeier et al. 2013). Deglaciated soils are rapidly colonized by functionally diverse microbial communities (Tscherko et al. 2003; Philippot et al. 2011), and the soil biological, physical and chemical characteristics are highly dependent on deglaciation chronosequence (Bernasconi et al. 2011). The exposed terrains provide a natural laboratory to study microbial community succession, function and soil development (Schuette et al. 2010; Wu et al. 2013). Microbial succession in deglaciated soils is dynamic (Sigler, Crivili and Zeyer 2002), and is highly correlated or controlled by organic carbon and nitrogen contents (Yoshitake et al. 2006, 2007; Zumsteg et al. 2012; Bajeraki and Wagner 2013).

Autotrophic microorganisms, including photoautotrophs and chemosynthetic autotrophs, are pioneering colonizers in deglaciated soils (Kašťovská et al. 2005). Establishment of pioneering microbial communities is key determinant of deglaciated soil development and its ecosystem function and stability (Schmidt et al. 2008; Kabala and Zapart 2012), and facilitates to colonization of pioneering plants (Bradley, Singarayer and Anesio 2014). Photosynthetic and nitrogen-fixing bacteria play important roles in the acquisition of nutrients and ecological succession in high-elevation deglaciated soils (Schmidt et al. 2008, 2011). Despite the importance of autotrophic microbial communities in deglaciated soils, their diversity, structure and succession remain largely unexplored along deglaciation chronosequence.

Autotrophic microorganisms fix atmospheric CO₂ by several pathways. The Calvin–Benson–Bassham cycle (CBB) is the most widespread CO₂-fixation pathway on Earth. The key enzyme of the CBB cycle is Rubisco, 5-bisphosphate carboxylase/oxygenase (RubisCO), which catalyzes the first step in CO₂ fixation into biomass. The chlb gene, encoding the large subunit of the Rubisco enzyme, is categorized into four forms (form I–IV), of which form I is the most prevalent (Tabita et al. 2008). Form I Rubisco could be further subdivided into IA, IB, IC and ID. chlb has been widely used as a phylogenetic biomarker to characterize the diversity and structure of autotrophic microbial communities in diverse habitats (Elsaied, Kimura and Naganuma 2007; John et al. 2007; Kong et al. 2012a,b; Yuan et al. 2012).

Microbial community diversity and structure shift along deglaciated soil chronosequences (Bradley, Singarayer and Anesio 2014; Brown and Jumpponen 2014). We hypothesized that (i) autotrophic microbial communities could quickly colonize newly deglaciated soils and their abundance and diversity shift along deglaciated soil chronosequence; (ii) the accumulation of organic carbon originated from autotrophic microorganisms. To test the hypothesis, we used primer sets specifically designed to target forms IA/B, IC and ID chlb gene to explore the abundance, diversity and succession of autotrophic microbial communities in deglaciated soils along a 10-year chronosequence on the plateau. A quantitative real-time PCR (qPCR) approach was applied to estimate the abundance of both total bacteria (16S rRNA gene) and autotrophic microorganisms. Clone library and sequencing of chlb gene fragments were performed to determine the diversity and structure of autotrophic communities across the deglaciated soil chronosequence.

MATERIALS AND METHODS

Study site and sample collection

The study site was in Zhadang (ZD) glacier termini (30°28.540’ N, 90°38.362’ E) with an elevation of 5200 m a.s.l. on the southern TP (Zhou et al. 2010). Daily mean temperature markedly fluctuated with seasons with a range of −22.1°C–5.8°C in the region (Zhang et al. 2013). ZD deglaciation and its mass balance have been observed on a yearly basis since 2006 and the deglaciated soil chronosequence was 10 years old (Kang et al. 2007).

A total of 21 soil samples were collected along the deglaciation chronosequence in September 2012. The soils were sampled every 5 m starting from the ZD glacier terminus representing the newly deglaciated soil to the far side representing 10-year-old soils. The first sample (IT1) under the ZD glacier terminus was roughly estimated at zero year old and the far side (100 m to the glacier terminus, IT21) was estimated to be 10 years old, based on the glacier retreat rate of 10 m yr⁻¹ (Kang et al. 2007). Surface soils were collected in sterile sampling bags (Labplas, Canada). Three surface soils were collected and mixed at each distance because the local Tibetan religion rule allows minimum soil for sampling. Soil samples were transported to laboratory in coolers with ice bags and passed through a 2.0-mm sieve. Subsamples for DNA extraction were stored at −80°C and the remaining samples were air-dried for physicochemical analysis.

Soil physicochemical analysis

Soil electrical conductivity (EC) and pH were measured by electrode method with a soil to water ratio of 1:5 (Yan and Marschner 2013). Soil total organic carbon (TOC) was measured by total organic carbon analyzer (TOC-L, Shimadzu, Kyoto, Japan) and total nitrogen (TN) was determined by elemental analyzer (vario MAX, Elementar, Hanau, Germany).

Soil DNA extraction and gene abundance quantification

Soil genomic DNA was extracted from 0.5 g of frozen soil using a FastDNA spin kit for soil (MP Biomedicals, Solon, OH, USA) following the manufacturer’s protocol. The quantity of the DNA was determined using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Gene abundance of bacterial 16S rRNA and chlb (form IA/B, IC and ID) was determined by qPCR using primer sets from previous publications (Paul, Alfreider and Wawrik 2000; Corredor et al. 2004; Alfreider et al. 2009; Philippot et al. 2011) (Table 1). qPCR was conducted on a LightCycler® 480 system (Roche Diagnostics, Indianapolis, IN, USA) with the thermal program of 40 cycles of 30 s at 94°C, 30 s at 54°C for form IA/B chlb gene, 30 s at 60°C for form IC and ID chlb gene.
at 52°C for forms IC and ID cbbL genes and 53°C for 16S rRNA gene and 30°C at 72°C (Kong et al. 2012a, b). The total volume reaction was 20 µL, containing 10 µL SYBR Premix ExTaq (Takara, Dalian, China), 20–50 ng of genomic DNA and 0.6 µM of each primer. Standard curves were generated using a 10-fold dilution series from plasmids as previously described (Kong and Nakatsu 2010). Briefly, plasmids were extracted from clones containing each target gene fragment using Plasmid Miniprep Kit (Kangwei, Beijing, China) and quantified by spectrometry (NanoDrop-1000, Thermo Scientific, Wilmington, DE, USA). The gene copy number was calculated from the concentration of the extracted plasmid DNA assuming the nucleotide molecular weight of 1.096 × 10⁻¹² g bp⁻¹.

**Clone library construction, sequencing and phylogenetic analysis of cbbL gene fragments**

Clone libraries of cbbL gene fragments (form IA/B, IC and ID) were constructed for five soil samples: IT2 (0.5 year old), IT6 (2.5 years old), IT13 (6 years old), IT18 (8.5 years old) and IT21 (10 years old). A total of 15 DNA-based clone libraries were thus generated. These samples were chosen because they represented a cross section of soil ages and exhibited differential levels of cbbL gene abundance along the deglaciated soil chronosequence. The specific PCR amplicons (623 bp for IA/B, 552 bp for IC and 557 bp for ID) were amplified with triplicates for each sample using Takara Master Mix (Takara), the same primer sets and 25 cycles of the thermal program as described above. Gel-purified PCR products were ligated into pGEM-T Easy vector (Promega, WI, USA), and the latter was chemically transferred into Escherichia coli DH-5α cells. Randomly selected clones (40–50 per sample) were screened for positive inserts by PCR using the primer set of M13F and M13R. The PCR products containing the correct inserts were then sequenced using an ABI model 3730xl DNA analyzer (Applied Biosystems, CA, USA).

Multiple sequence alignments were conducted using CLUSTALW in MEGA6.0 (Tamura et al. 2013). Sequences with the nucleotide sequence similarity of >97% were defined as an operational taxonomic unit (OTU) using the Mothur program v.1.33.3 (Schloss et al. 2009). BLASTn (www.ncbi.nlm.nih.gov/BLAST/) was employed to search GenBank for the nearest related sequences to each OTU. Phylogenetic trees were generated using the neighbor-joining method with the maximum composite likelihood model in MEGA6.0 (Tamura et al. 2013). The reliability of phylogenetic trees was tested using bootstrap with 1000 iterations. Sequences generated in this study have been deposited in the National Center for Biotechnology Information GenBank database under the accession numbers KJ700657–KJ700707 for form IA/B, KJ700708–KJ700766 for form IC cbbL and KJ700767–KJ700838 for form ID cbbL, respectively.

**Data analysis**

All figures and fitting curves were generated using SigmaPlot 10 software (Systat Software, San Jose, CA, USA). For the fit curve, polynomial linear model was used for soil pH and EC, and logarithm model was used for gene abundance, TOC and TN. Shannon’s diversity and evenness were calculated using the software PC-ORD 5.0. Pearson correlations were performed by SPSS 18 (SPSS Inc., Chicago, IL, USA).

**RESULTS**

**Soil physicochemical characteristics along deglaciation chronosequence**

Soil EC substantially decreased from 35 to 15 µS cm⁻¹ within the first year of deglaciation and then gradually decreased along the 10-year deglaciation chronosequence (Fig. 1A). The deglaciated soils were alkaline and their pH rapidly decreased from 9 to 6.7 within the 10-year deglaciation (Fig. 1B). Soil TOC and TN were generally low, ranging from 0.03% to 0.11% and 0.05% to 0.016%, respectively (Fig. S1, Supporting Information). Soil TOC gradually increased in the first 6 years of deglaciation, and then exhibited large fluctuations in old soils. The fitting curve showed that soil TOC gradually increased with the soil age (P = 0.03). In contrast, TN increased in the first 2 years of deglaciation, remained relatively stable to 6 years and then exhibited large fluctuations in older soils.

**Gene abundance of 16S rRNA and cbbL**

The abundances of bacterial 16S rRNA and three forms of cbbL (form IA/B, IC and ID) were quantified using qPCR across the deglaciated soil chronosequence. Abundance of 16S rRNA gene increased rapidly during the first half year of deglaciation, and then gradually increased across the 10-year deglaciation chronosequence, ranging from 10⁴ to 10⁸ gene copies g⁻¹ soil (Fig. 2). The cbbL abundance exhibited similar patterns as the 16S rRNA gene along the chronosequence, and ranged from 10⁴ to 10⁶ gene copies g⁻¹ soil. Form IA/B exhibited the highest abundance among the cbbL genes. The ratio of form IA/B cbbL gene to 16S rRNA gene ranged from 1.6% to 12.4%, and the highest occurred in the new exposed soil (Fig. S2, Supporting Information). Curve fitting revealed that bacteria (indicated by 16S rRNA gene) and autotrophic microbes (forms IA/B, IC and ID cbbL) exponentially colonized deglaciated soils within a year of the
Correlations between the abundance of all genes and soil TOC or TN were calculated by Pearson correlation analysis. Gene abundances of 16S rRNA and cbbL forms were positively correlated with both TOC and TN (Table 2). The abundances of 16S rRNA gene and all forms of cbbL gene were positively correlated with each other, and the abundances of bacterial and cbbL genes were negatively correlated with EC and pH.

Succession and diversity of autotrophic microbial community

Clone libraries of all cbbL gene fragments (IA/B, IC and ID) were constructed for five deglaciated soil samples (0.5 year, 2.5 years, 6 years, 8.5 years and 10 years old). cbbL gene sequences retrieved from the five soils revealed that autotrophic microbial community diversity and succession shifted along the 10-year deglaciation chronosequence.

Form IA/B autotrophs, which dominated the autotrophic microbial communities, were only affiliated with cyanobacteria (Fig. 3), many of which shared high similarity (sequence similarity ranging from 91% to 97%) with those observed in Antarctic lakes (Kong et al. 2012b). The dominance of cyanobacteria was further confirmed by a clone library of 0.5-year-old soil based on 16S rRNA gene, which showed that the relative frequency of cyanobacteria was 59.4% (Fig. S3, Supporting Information).

The form IA/B autotrophs were assigned to three orders, Nostocales, Oscillatoriales and Chroococcales, and the relative frequency of the three orders in all soils was 9.86%, 54.23% and 35.92%, respectively. The relative frequency was herein defined as the number of clones assigned to a specific taxa in relation to all picked clones. Nostocales-like OTUs were not detected in relatively young deglaciated soils (0.5 and 2.5 years old), but abundantly occurred in older soils with a peak in 6-year-old soil sample (28.0% in relative frequency). Oscillatoriales-like OTUs exhibited the highest relative frequency in 0.5-year-old soil (100%) and the least in 2.5-year-old soil (3.6%). The Chroococcales relative frequency exhibited a substantially temporal variation with a peak in 2.5-year-old soil (96.4%) (Fig. S4A, Supporting Information). Pearson correlation analysis demonstrated that the Chroococcales relative frequency negatively correlated with that of Oscillatoriales (R = −0.954, P < 0.05).

Form IC cbbL sequences were affiliated with Proteobacteria, consisting of Actinomycetales of Actinobacteria and Proteobacteria containing Burkholderiales, Rhodospirillales and Chromatiales (Fig. 4). The average relative frequency of the four orders across all the soils was 5.9%, 57.6%, 20.3% and 16.1%, respectively. All the Burkholderiales sequences were closely affiliated (similarity > 93%) with those retrieved from a supraglacial cryoconite (Cameron, Hodson and Osborn 2012). Burkholderiales dominated all samples with the relative frequency ranging from 50% to 80% across the five soil ages (Fig. S4B, Supporting Information). Rhodospirillales relative frequency gradually increased and Chromatiales kept stable with soil age. The relative frequency of Rhodospirillales was negatively correlated with that of Burkholderiales (R = −0.913, P < 0.05).

Form ID cbbL sequences were affiliated with Chroococcales, Nostocales, Oscillatoriales, Coscinodiscophyceae and Xanthophyceae (Fig. 5). The first three orders belonged to cyanobacteria, and the last two were assigned to Stramenopiles. Oscillatoriales rarely occurred in young soils (0.5 and 2.5 years old), but dominated older soils (6–10 years old) (>60% in relative frequency). In contrast, Xanthophyceae dominated the young soils (0.5 and 2.5 years old), and substantially decreased in older soils (6–10 years old) (Fig. S4C, Supporting Information). A negative correlation between Oscillatoriales and Xanthophyceae in relative frequency was observed (R = −0.944, P < 0.05).

Autotrophic microbial diversity and evenness showed a clear temporal pattern along the deglaciated soil chronosequence. Shannon’s diversity of form IA/B autotrophic microbial
Table 2. Pearson correlations between gene abundance and soil physicochemical factors in deglaciated soils.

<table>
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<tr>
<th></th>
<th>EC</th>
<th>pH</th>
<th>TOC</th>
<th>TN</th>
<th>16S rRNA gene</th>
<th>IA/B cbbL gene</th>
<th>IC cbbL gene</th>
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<tr>
<td>16S rRNA gene</td>
<td>−0.770&lt;sup&gt;a&lt;/sup&gt;</td>
<td>−0.747&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.834&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.709&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>IA/B cbbL gene</td>
<td>−0.606&lt;sup&gt;a&lt;/sup&gt;</td>
<td>−0.655&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.778&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.670&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.937&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC cbbL gene</td>
<td>−0.700&lt;sup&gt;a&lt;/sup&gt;</td>
<td>−0.649&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.895&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.757&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.944&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.910&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>ID cbbL gene</td>
<td>−0.678&lt;sup&gt;a&lt;/sup&gt;</td>
<td>−0.643&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.621&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.461</td>
<td>0.928&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.905&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.856&lt;sup&gt;a&lt;/sup&gt;</td>
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EC: electrical conductivity; TOC: total organic carbon; TN: total nitrogen.
<sup>a</sup>Significant level at 0.01 levels.

Figure 3. Neighbor-joining phylogenetic tree of IA/B cbbL sequences (623 bp) retrieved from environmental DNA in deglaciated soils of ZD glacier. Bar, 0.02 substitutions per nucleotide position. The GenBank accession number was listed in brackets after each sequence name.
Figure 4. Neighbor-joining phylogenetic tree of form IC cbbL gene sequences (552 bp) retrieved from environmental DNA in deglaciated soils of ZD glacier. Bar, 0.02 substitutions per nucleotide position. The GenBank accession number was listed in brackets after each sequence name.
Figure 5. Neighbor-joining phylogenetic tree of form ID cbbL gene sequences (557 bp) retrieved from environmental DNA in deglaciated soils of ZD glacier. Bar, 0.05 substitutions per nucleotide position. The GenBank accession number was listed in brackets after each sequence name.
community gradually increased with increasing soil age and then showed a slight decrease in old soils (>6 years old) (Fig. 6A). The diversity of form IC and ID followed a similar trend as IA/B along the deglaciated soil chronosequence. The maximum Shannon’s diversity of all forms reached a similar level in older soils. Curve fitting revealed that the diversity of forms IA/B ($R^2 = 0.99, P < 0.01$) and IC ($R^2 = 0.97, P < 0.05$) exponentially increased along the 10-year soil age. Similar to the Shannon’s diversity, the evenness of form IA/B autotrophic communities substantially increased and then kept stable in old soils (>6 years old) (Fig. 6B), while the evenness of IC and ID communities kept relatively constant along the 10-year soil age.

**DISCUSSION**

Bacteria and cyanobacteria abundantly colonized the newly deglaciated soils, increased substantially in 0.5–2.5-year-old soils and then remained a relatively stable trend in older soils (Fig. 2). Our finding is consistent with that 16S rRNA gene abundance increased with soil development after deglaciation (Kandeler et al. 2006). A similar trend was observed in Peru deglaciated soils, where phylotype abundance was lowest in the youngest soils, increased in the intermediate aged soils and plateaued in the oldest soils (20 years old) (Nemergut et al. 2007). Our data showed that cyanobacteria dominated the autotrophic microbial communities along the whole 10-year soil chronosequence. This is in the line with the increasing documents that cyanobacteria widely habitat harsh environments. They could soon colonize deglaciated soils after deglaciation in a high-elevation area (5000 m a.s.l.) (Schmidt et al. 2008). Recent studies have shown that cyanobacteria dominated soil biocrusts across all elevations in TP regions (Janatkova et al. 2013), and were frequently detected in glacier retreat barren soils in Svalbard and southeastern Peru (Kaštovská et al. 2005; Nemergut et al. 2007). Our data also demonstrated that Oscillatoriales dominantly occurred in the deglaciated soils, and Nostocales was less in young soils. This is consistent with that Oscillatoriales dominated barren deglaciated soils and Nostocales dominated sparsely vegetated soils (Segawa and Takeuchi 2010). These studies indicate that cyanobacteria are dominant primary colonizers in deglaciated soils (Reháková et al. 2010), and Oscillatoriales are frequently observed in barren soils. We have to mention that the lack of sampling replicates for the local Tibetan religion reason might lightly reduce the more general implications of our findings, although the data and findings were robust in the study.

The diversity and evenness of form IA/B and IC autotrophic communities dramatically increased in the first 5 years and then kept relatively stable in the older soils (6–10 years old) (Fig. 6). This temporal trend is similar to the findings in a 20-year deglaciation chronosequence (Nemergut et al. 2007), which showed that bacterial diversity was lowest in youngest soils, increased in the intermediate aged soils and plateaued in the oldest soils. Our results suggested that autotrophic diversity and evenness were mainly structured by deglaciated soil age.

The increase of both TOC and TN along the deglaciation chronosequence indicates that the nutrients have been accumulating by microbial autotrophs since the deglaciation. The significant correlations of TOC and TN with abundances of bacteria and autotrophs suggest that soil TOC and nutrients mainly originated from soil autotrophs and bacteria, minimally from ancient or external carbon sources. Studies have observed that TOC positively correlated with soil age (Bradley, Singarayer and Anesio 2014), while our findings further extend that the TOC accumulation results from autotroph abundance increase with soil age, and highlight the autotrophic microbial role in accumulating nutrients and soil formation in young deglaciated soils. This is supportive of previous studies that turnover of the microbial community is the major source of dissolved nitrogen nutrients for plant uptake during the plant growing season (Schmidt et al. 2007). Among the three forms of autotrophs, cyanobacteria carrying form IA/B cbbL gene exhibited the highest abundance, suggesting that photoautotrophs play a key role in accumulating TOC and TN in the high-elevation deglaciated soils. This is in accordance with that photosynthetic bacteria play important roles in acquiring nutrients and facilitate ecological succession in soils near some of the highest elevation receding glaciers (Schmidt et al. 2008, 2011). Our findings extend that photoautotrophic microorganisms could play a key role in accumulating organic carbon and nutrients in young deglaciated soils (Stibal et al. 2008). It has to be mentioned that large fluctuations of TOC and TN occurred in older soils. This might be caused by unevenly scattered cyanobacterial mats. Findings showed that bacterial communities in Antarctic soils exhibited large variations at different sampling sites, which was caused by large variations in soil physicochemical compositions (Kim et al. 2015). In our study, soil pH exhibited a large fluctuation in older soils, which is in accordance with the TOC and TN, suggesting that pH variations at sampling sites might lead to the large fluctuations of TOC and TN. Therefore, enough sampling replicates may minimize the variations in the future studies. Another shortage is that carbon-fixing capacity and activity were not experimentally determined in the study, although there was a robust positive relationship.
between TOC and cbbL gene abundance. Stable isotope probing method and mRNA of cbbL gene could further elucidate the roles of autotrophs in TOC accumulating in the future research.

The dominance of cyanobacteria and their quick colonization in deglaciated soils indicate that cyanobacteria are likely capable of enduring extreme environmental conditions, including high UV, daily freeze-thaw cycles and low water availability and alkalinity. Cyanobacteria have been globally detected, particularly in extreme environments, including polar regions (Cameron, Hodson and Osborn 2012; Makhalanyane et al. 2015) and dry mountainous soils of Himalaya (Rehkáková, Chlumská and Doležal 2011; Janatkova et al. 2013). The cyanobacterial cbbL gene sequences retrieved in the current study are similar to those observed in Swiss Alps deglaciated soils, Qilian glaciers (Segawa and Takeuchi 2010; Frey et al. 2013) and Antarctic quartz rocks (Khan et al. 2011). In addition, the cyanobacterial sequences exhibited high similarity with those retrieved from Antarctic lake waters (Kong et al. 2012a,b) and Antarctic pillar mosses (Nakai et al. 2012). These findings collectively indicate that cyanobacteria are well adapted to extreme environments in high elevations and high latitudes. Cyanobacteria can survive high UV exposure (Castenholz and Garcia-Pichel 2000; Sinha and Häder 2002), for they have developed photo protective machinery to overcome solar UVR damage in extreme environments (Rastogi et al. 2014). Temperature and water availability are the most important environmental factors driving microbial community in extreme environments (Cary et al. 2010). Cyanobacteria have evolved strategies to survive freezing and desiccation, including low metabolic activity and producing specific proteins and molecules (e.g., anti-freezing proteins) (Makhalanyane et al. 2015). Cyanobacteria were frequently dominant in alkaline environments (Uetake et al. 2010; Kovaleva et al. 2011; Rehkáková, Chlumská and Doležal 2011) for they could adjust their metabolism to maintain pH homeostasis under alkaline conditions (Summerfeld and Sherman 2008).

Betaproteobacteria were dominant phylotypes of form IC cbbL in ZD deglaciated soils and exhibited high phylogenetic similarity with those in supraglacial cryoconites (Cameron, Hodson and Osborn 2012). These autotrophic bacteria have also been observed in cryoconite holes in Svalbard (Edwards et al. 2011), deglaciated soils of Tianshan No. 1 glacier (Wu et al. 2012) and on the glacier surface (Philippot et al. 2011). The occurrence indicates that Betaproteobacteria could tolerate various environmental stressors, e.g., low temperature and low water availability. Our results demonstrated that Betaproteobacteria were dominated by Burkholderiales in the high-elevation deglaciated soils (Fig. 4). Burkholderiales have been observed to dominate high-elevation airborne microbial communities and potentially act as atmospheric ice nuclei (Bowers et al. 2009). They could inhabit debris-rich basal ice (Montross et al. 2014) and are the most efficient mineral-weathering bacteria (Ma et al. 2012) with high pH tolerance (Stopnisek et al. 2014). Our results were in line with these findings and expand that abundant Burkholderiales in high-elevation deglaciated soils.

Soil physicochemical factors exhibited a clear temporal pattern along the deglaciation chronosequence. Soil EC and pH substantially decreased along the deglaciation chronosequence. Similar findings were observed in Antarctica glacier forefield soils, where pH decreased from 8.3 to 6.7 along deglaciation chronosequence (Bajerski and Wagner 2013). The higher levels of EC and pH in the glacier terminus soils may result from snow and ice melting, for snow samples in this area were generally alkaline with high concentrations of Ca\(^{2+}\) and HCO\(_3^-\) (83%), and their pH values ranged from 7.24 to 8.39 with a mean value of 7.82 (Huang et al. 2012). It has been shown that snow and ice EC positively correlated with alkalinity and land dust source Ca\(^{2+}\) ions (Xiao et al. 2001). It is reasonable to assume that snow and glacier melting waters in summer seasons induce higher values of pH and EC in new deglaciated soils adjacent to the glacier terminus, and they substantially decrease with increasing distance to the glacier terminus. Additionally, biological weathering and yearly round strong winds could decrease the EC and pH of deglaciated soils with the soil-age increase.

**CONCLUSION**

EC and pH significantly decreased along deglaciation chronosequence in deglaciated soils of ZD glacier on the TP. Soil TOC and TN increased in the first few years, and they positively correlated with bacterial and autotrophic microbial abundance, indicating that barren soil nutrients were originated from autotrophic microbes. Bacteria and autotrophic microbes rapidly colonized deglaciated soils in a year of deglaciation. Cyanobacteria dominated the autotrophic microbial communities with a substantially higher abundance than their partners (IC and ID), and Oscillatoriales were the dominant Cyanobacterial order. Most of the cyanobacteria had high similarity with those in Antarctic lake waters and supraglacial cryoconites. Shannon diversity and evenness of autotrophic microbial communities gradually increased in young soils (<5 years after glacial recession), and then kept stable. The autotrophic microbial abundance and their diversity were structured by deglaciated soil age, but not by ancient nutrients.

**SUPPLEMENTARY DATA**

Supplementary data are available at FEMSEC online.

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