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Variations of bacterial and fungal communities along a primary successional chronosequence in the Hailuogou glacier retreat area (Gongga Mountain, SW China)

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Abstract: New terrestrial habitats have emerged and a primary succession has developed in the retreat area (29°34'N, 102°00'E, 2951–2886 m) after the retreat of the Hailuogou glacier. To investigate soil microbial changes along the primary successional chronosequence, mixed soil samples were collected at six sites at different ages (2 young sites, 2 mid-aged sites, and 2 old sites). The RNA was extracted and amplified. Bacterial 16S rRNA and fungal 18S rRNA were analyzed using high-throughput 454 pyrosequencing analysis. Overall, pyrosequencing showed that Proteobacteria, Acidobacteria, Bacteroidetes and Actinobacteria were the main bacterial phyla, and the fungal communities were strongly dominated by the phyla Ascomycota and Basidiomycota in the retreat area. The Shannon diversity index ($H_{shannon}$) of bacteria was 6.5 – 7.9, and that of fungi was 2.2 – 4.1 in these sites. For the bacterial communities, diversity and evenness values were highest on the mid-age sites and were relatively low on the young and old sites. A similar trend was observed for the fungal communities. In contrast, soil properties showed significant linear distributional trends (increase or decrease) with the age of the site. Combining the linear change patterns of soil properties, the highest values of bacterial and fungal evenness and diversity in the mid-aged sites indicated that there was less environmental stress and more niches for microbial communities in the middle successional stage compare with other stages. In addition, our analysis showed that microbial communities were the main drivers that build a soil organic matter pool to expedite pedogenesis for ecosystem succession. This primary succession in the Hailuogou glacier retreat area is developing rapidly compared with that in other glacier retreats.

Keywords: Primary successional chronosequence; Microbial community; Soil properties; 454 sequencing; Rapid succession

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Introduction

With global climate change, glacier retreats have occurred widely around the world and could become more rapid (Oerlemans 2005; Zemp et al. 2006). New bare lands have emerged because of glacial retreats and a successional process towards a zonal ecosystem has begun. Glacier forefield chronosequences can reveal an ecosystem developmental process and offer an excellent opportunity to study the successional development of a terrestrial process by substituting space for time. Most studies of environmental succession based on this system have focused on plant and animal communities and soil development (He and Tang 2008; Hodkinson et al. 2003; Kaufmann 2001). In contrast, we know very little about microbial patterns across a glacier forefield chronosequence.

In light of the key roles of microorganisms in pedogenesis, biogeochemical cycling and plant colonization (Brunner et al. 2011; Schutte et al. 2010), studies of microbial succession are becoming more important (Schmidt et al. 2014; Chen et al. 2015). Genetic cloning and sequence analysis (Jumpponen 2003; Zumsteg et al. 2013) and analyses of phospholipid fatty acids and enzymes (Tscherko et al. 2005) have often been used in studies of microbial succession on glacier forefields. Though some progress has been made concerning microbial diversity and succession along the glacier forefield chronosequence, researchers have often reported different observations and reached inconsistent conclusions. On the basis of an Arctic glacier foreland, Schutte et al. (2010) indicated that bacterial richness increased significantly with site age. However, Jangid et al. (2013) tested bacterial community patterns along a long chronosequence of a retreating glacier and concluded that bacterial richness and diversity declined significantly with site age. Meanwhile, Wu et al. (2012) found opposite results in a foreland of an Asia glacier: not only did bacterial richness increase with successional time, so did bacterial diversity.

In addition, Sigler and Zeyer (2002) analyzed genetic fingerprinting along the forefields of two receding glaciers and revealed that bacterial diversity and succession differed significantly between the two chronosequences, even when the two glaciers are located in the same region. As for fungal succession on a glacier forefield, only a few studies have been performed, which did not draw general conclusions. Brown and Jumpponen (2014) showed that fungal richness and diversity were static across the Lyman Glacier chronosequence. Their data highlight different drivers for fungal and bacterial succession trajectories. In contrast, Blaalid (2012) found that in Norway, fungal richness increased significantly along the glacier forefield chronosequence. Their results suggested that fungal richness patterns were similar to those of bacteria. These contradictory results indicate that fundamental knowledge about microbial succession in glacier forefields is still lacking, and more studies are needed to understand the underlying mechanisms.

The Hailuogou glacier is located on Gongga Mountain in southwestern China. It is a typical temperate glacier and is more sensitive to climate changes than polar or continental glaciers (Liu et al. 2010). To our knowledge, very few studies have provided information about microbial changes in such glaciers. The Gongga Mountain area is one of the few remaining refuges for the Tertiary Geoflora (Yin 1987), and the soils can be expected to harbor relatively unique microbial communities. In this study, we analyzed microbial community changes along a primary successional chronosequence in front of the Hailuogou glacier using 454 sequencing. Our objectives were to examine the following information: (1) what are the dominating microorganisms in the temperate glacier forefield? and (2) how do microbial communities change with increasing terrain age? This study is expected to provide additional information about microbial succession on the glacier forefield and to produce important clues for understanding the driving factors of successional dynamics in terrestrial ecosystems.

1 Materials and Methods

1.1 Study site

We chose the Hailuogou glacier retreat area (29°34'N, 102°00'E, 2951–2886 m) located on Gongga Mountain (summit: 7556 m) (Figure 1). Gongga Mountain is located in the transition zone

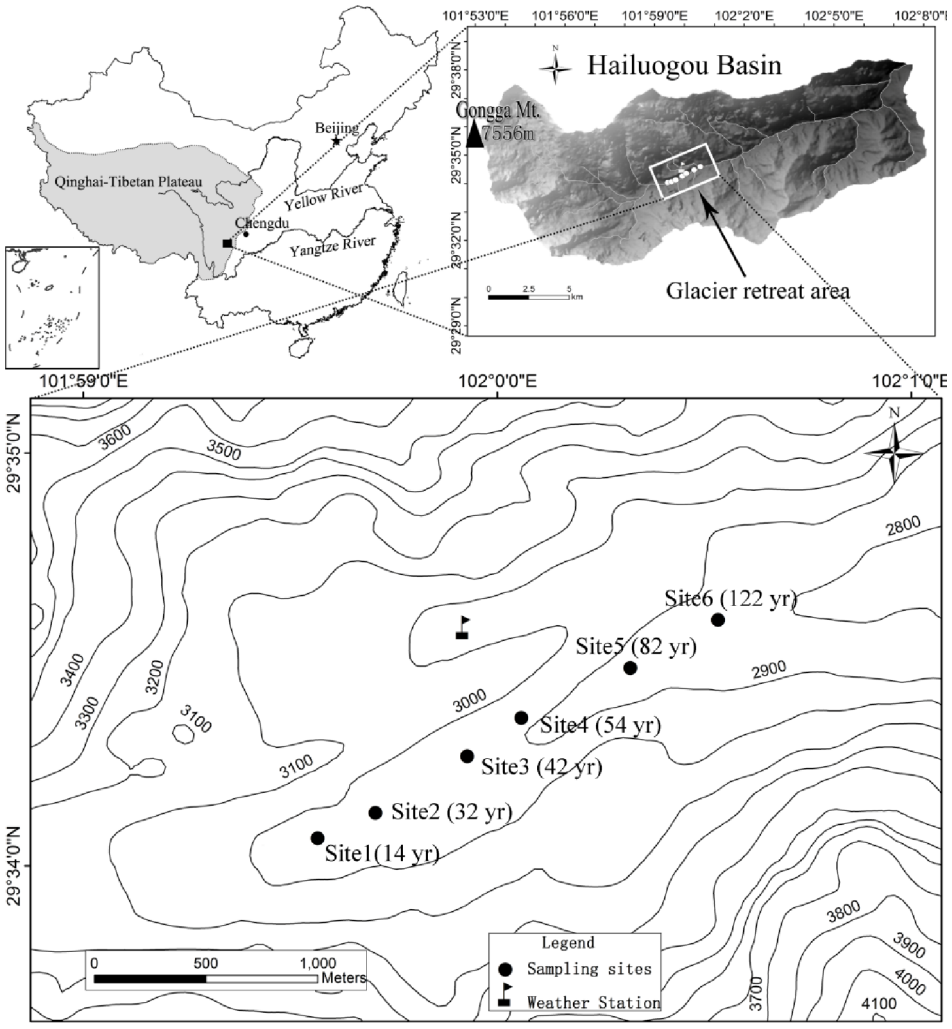

Figure 1 Location of the Hailuogou chronosequence and sampling sites.

between the Tibetan Plateau and Sichuan Basin. On the east slope of Gongga Mountain, Hailuogou glacier is the largest glacier, with an area of 25 km². The regional climate is dominated by the warm-humid subtropical monsoon. The mean annual precipitation is 1949 mm, the mean annual temperature is 4°C, and the mean annual relative humidity is 90%.

The Hailuogou glacier retreat area has extended approximately 2000 m since the 1820s and has developed a chronosequence ranging from deglaciated moraines to forest soil (Zhou et al. 2013). On this chronosequence, quartzite, granodiorite, biotite schist, chlorite schist, slate and phyllite constitute the parent soil materials (Xu 1989). With increasing soil age, different successional stages of vegetation have gradually appeared. The first stage community is mainly composed of *Astragalusadsurgens* Pall., *Anaphalis*sp., *Epilobiumhirsutum* Linn., and other leguminous plants (site 1, young site). The second stage is dominated by *Hippophaerhamnoides* L., *Salix rehderiana* Schneid, *Populuspurdomii* Rehder (site 2, young site). Subsequently, *Populuspurdomii* Rehder becomes the dominant species through inter-species competition; together with mixed *Rhododendron — H. rhamnoides— Salix dolia* Schneid. It forms the community of the third stage (site 3, mid-aged site). In the next stage, *Betulautilis*, *Piceabrachytula,* and *Abiesfabri* begin to appear in large numbers (site 4, mid-aged site); The subdominant conifers grow up into the canopy layer and *Populuspurdomii* gradually disappears (site 5, old site); Finally, the ecosystem developed into a community mainly composed of *Piceabrachytula* and *Abiesfabri* (site 6, old site). Some properties of the sites are shown in Table 1.

Table 1 Site characteristics

Site	Young sites		Mid-aged sites		Old sites		rs	*p*
	1	2	3	4	5	6		
Stand age (year)	14	32	42	54	82	122		
Elevation (m)	2944	2934	2924	2911	2883	2856		
Mean ST (°C)	6.2	6.1	5.8	5.4	4.7	4.5	-0.899	*
Mean SM (%)	28.4	35	31.3	35.4	36.7	39.8	0.943	**
pH	6.7	5.8	5.4	5.2	4.7	4.5	-1	***
BD (g cm^{-3})	1.84	1.31	1.28	1.06	0.72	0.60	-1	***
SOM (g kg^{-1})	7.7	66.5	227.1	324.6	419.9	458.2	1	***
Total N (g kg^{-1})	0.04	0.30	1.12	2.00	3.40	3.15	0.943	**
Total P (mg kg^{-1})	1244.8	1275.6	1169.8	1068.7	776.2	893.8	-0.886	*
Total K (mg kg^{-1})	21.1	18.2	13.9	12.9	5.3	6.3	-0.943	**
Total Na (mg kg^{-1})	18.7	14.2	10.1	9.3	3.4	3.4	-1	***
Total Ca (mg kg^{-1})	43.5	37.9	30.8	27.8	17.4	15.2	-1	***
Total Mg (mg kg^{-1})	19.9	18.6	14.5	11.0	4.0	4.3	-0.943	**
Total Fe (mg kg^{-1})	40.3	38.0	31.6	22.6	10.6	11.6	-.943	**
Total Al (mg kg^{-1})	68.1	58.4	46.0	39.1	16.6	18.8	-0.943	**

Notes: ST= Soil Temperature; SM = Soil Moisture; BD = Bulk Density; SOM = Soil Organic Matter. rs denoted Spearman rank correlation coefficient between stand age and sites characteristics. *denoted significance at the $p < 0.02$ probability level; **denoted significance at the $p< 0.005$ probability level; ***denoted significance at the $p< 0.0001$ probability level.

1.2 Site selection and soil sampling

Soil samples were collected in September 2012 at the six sites along the chronosequence. For each site, three 10 m × 10 m plots were set up in the core zone of each successional stage. Five sampling points were randomly selected (with> 2 m intervals between the sampling points) along one diagonal line of each plot. Soil samples (0 – 10 cm below the litter layer) were collected using a sterile blade at each sampling point. Soil samples from all sampling points at each site were combined as the representative soil sample of each site. These representative soil samples were analyzed for soil physicochemical characteristics and genetic material.

1.3 PCR amplification and pyrosequencing

Soil genetic material was extracted from 0.5 g of fresh soil using the Soil DNA/RNA Kit (OMEGA, Bio-Tek, USA) following the manufacturer's instructions. The extracted genetic material was dissolved in sterilized double-distilled water for sequencing (Yang et al. 2007). The genetic material concentration was determined using a spectrophotometer (NanoDrop® ND-100, USA).

Two primer sets were selected to amplify the bacterial and fungal gene fragments for 454 pyrosequencing. The fungal 18sRNA genes were amplified using the primers V4_euk_R2 (5′-ACGGTATCT(AG)ATC(AG)TCTTCG-3′) (Brate et al. 2010) and 3DNF (5′- xxxxGGCAA GTCTGGTGCCAG-3′) (Cavalier-Smith et al. 2009). The bacterial primer pair 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 533R (5′-xxxxTTACCGCGGCTGCTGGCAC-3′) (Lane 1991; Weisburg et al. 1991) were used to amplify the 16sRNA genes. In primers 3DNF and 533R, xxxx was used as a barcode that allowed sample identification during pyrosequencing.

All PCR reactions were performed in 20 μl mixtures containing 1μl of each primer (8 pmol μl^{-1}), 0.5 μl of template DNA (45 ng μl^{-1}), 100 mM KCl, 15 mM Tri-HCl, 3 mM $MgCl_2$ and 400 μM dNTP, and 0.5 μl of Taq DNA polymerase (2.5 U ul^{-1}). The PCR reactions were carried out using the following sequences: 95°C for 2 min, 25 cycles at 95°C for 30 s denaturation, 55°C for 30 s annealing, 72°C for 30 s extension, followed by 72°C for 5 min. The presence of PCR products was examined by DGGE (denaturing gradient gel electrophoresis, 2μl PCR products, 2% agarose gel). The PCR products of all samples were purified using a PCR purification kit (Axygen Bio, USA). The sequencing tasks were completed by Majorbio Biotech Co., Ltd (Shanghai, China) using high-throughput 454 pyrosequencing.

MOTHUR software was used to analyze the sequences (Schloss et al. 2009). Quality sequence

reads were selected by removing the sequences with lengths < 200 bp, homopolymers > 8 nt, > 1 difference with the barcode, or > 2 differences with the primer region. The commands "screen.seqs," "filter.seqs" and "unique.seqs" were used to further trim the sequences and eliminate the redundant reads. Chimeras were removed using the "chimera.slayer" command. The "dist.seqs" command was performed to cluster unique sequences into operational taxonomic units (OTUs) at a similarity of 97%, and rarefaction curves were produced at this level. The MOTHUR "unique.seqs" command was used for simplifying the sequence reads to generate a unique set of sequence reads. The "align.seqs" command was then run to align the unique sequences and compare them with the SILVA comprehensive rRNA database (http://www.arb-silva.de/). Moreover, the representative sequence from each OTU was analyzed by SILVA Incremental Aligner (SINA) (http://www.arb-silva.de/aligner/) against SILVA comprehensive rRNA database (http://www.arb-silva.de/), and taxonomy was assigned to OTU with the minimum sequence similarity (99% to species, 95% to genus, 90% to class/order/family, and 80% to domain/phylum) (Pruesse et al. 2007; Wei et al. 2015).

1.4 Data analysis

All OTUs were obtained using cluster analyses with a 97% sequence similarity. The Shannon diversity index ($H_{shannon}$) was calculated as follows: $H_{shannon} = -\sum_{i=1}^{S} P_i lnP_i$, and evenness was calculated using evenness = $H_{shannon}$/ln(S), where, S is the total number of observed OTUs, $P_i = n_i/N$ (n_i is the number of sequences in OTU i and N is the total number of sequences in the community).

2 Results

2.1 Sequence data

The number of sequence reads varied among the sites. The number of fungal sequences showed greater variation than the bacterial sequences across the entire chronosequence (the coefficients of variation were 0.11 for bacteria, 0.38 for fungi). After sequence clean-up, the average length was 495 base pairs (bp) for the bacteria and 450 bp for the fungi. The unclassified sequences at the phylum level were a relatively small proportion (5.7% of the bacterial reads, 2.3% of the fungal reads). After clustering at 97% sequence similarity, a total of 16,710 bacterial and 564 fungal OTUs were recovered.

2.2 Overall characteristics of microbial communities

OTUs were assigned to twenty-four bacterial phyla by pyrosequencing analysis. Most of OTUs were assigned to the Proteobacteria and Acidobacteria; 28,347 sequences (43%) and 5764 OTUs (34%) were assigned to Proteobacteria, and 10,933 sequences (16%) and 1919 OTUs were assigned to Acidobacteria (Figure 2). Moreover, some OTUs with abundant sequences were aligned with several taxa: 1832 OTUs (11%) with 6138 sequences (9.2%) were assigned to Bacteroidetes, 1249 OTUs (7.5%) with 5105 sequences (7.7%) to Actinobacteria, and 1659 OTUs (9.9%) with 4025 sequences (6.0%) to Planctomycetes. Sequences assigned to Verrucomicrobia and Chloroflexi were also abundant (Figure 2). We detected fewer sequences associated with the other bacterial phyla.

For example, the sequences related to

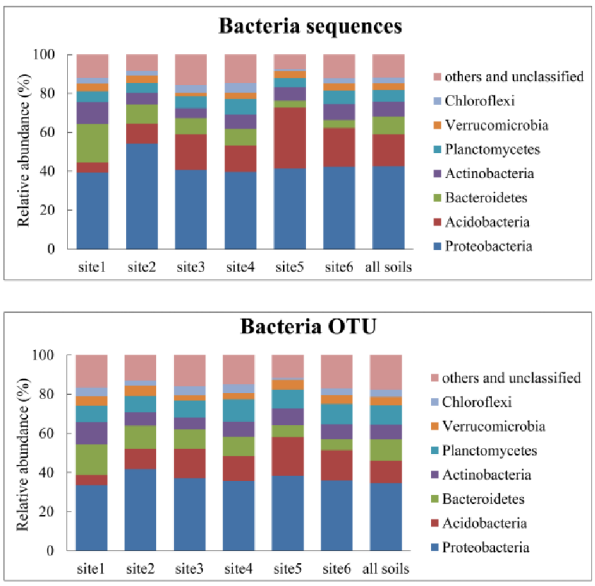

Figure 2 Taxonomic proportions of bacteria in the Hailuogou glacier retreat area.

Gemmatimonadetes, Nitrospirae and Cyanobacteria all accounted for less than 2% of the bacterial sequences. It is noteworthy that we found a large number of OTUs related to photosynthetic bacteria. For example, 639 OTUs were assigned to the photosynthetic Chloroflexi with 2015 sequences (3%), 145 OTUs to the Cyanobacteria with 517 sequences (0.8%) and 67 OTUs to the Chlorobi with 219 sequences (0.3%). Of the 35 classified classes, sequences related to the Acidobacteria (10154 sequences, 15%) were most abundant, and OTUs with 27572 sequences (41%) were assigned to 4 classes: Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria and Deltaproteobacteria. Sequences related to Sphingobacteria (3919 sequences, 5.9%), Planctomycetacia (2940 sequences, 4.4%) and Actinobacteria (1936 sequences, 2.9%) were also abundant. OTUs were assigned to 62 bacterial orders. Those assigned to Burkholderiales (6063 sequences, 9.1%), Sphingobacteriales (3919 sequences, 5.9%), Rhizobiales (3554 sequences, 5.3%) and Planctomycetales (2940 sequences, 4.4 %) comprised the major bacterial groups. Of 6995 OTUs assigned to 126 families, those related to Comamonadaceae (3221 sequences, 4.8%), Planctomycetaceae (2940 sequences, 4.4%), Acidobacteriaceae (2433 sequences, 3.7%) and Sinobacteraceae (2160 sequences, 3.2%) were most abundant.

Many of the detected sequences may come from novel taxa, as 33,922 sequences could not be classified to family. More sequences assigned to some bacteria were found on the older terrain, but fewer (e.g., Isosphaera) or no (e.g., Labrysmiyagiensis) sequences were recorded on the younger terrain. Conversely, sequences associated with other bacteria (e.g., Arenimonas oryziterrae, Pseudoxanthomonas ginsengisoli and Pedobacter cryoconitis) were abundant on the younger sites, but scarce on the older sites.

Most of the OTUs in the study sites were assigned to 10 fungal phyla or subphyla. The fungal OTUs were strongly dominated by Ascomycota (387 OTUs and 69%, 7672 sequences and 85%), Basidiomycota (68 OTUs and 12%, 723 sequences and 8%) (Figure 3). OTUs assigned to other phyla were present but rare: 27 OTUs in the Chytridiomycota, 6 in the Neocallimastigomycota and 13 in the Glomeromycota; their sequence reads were 163 (1.8%), 54 (0.6%) and 47 (0.5%), respectively. We also encountered some fungal subphyla, and 19 OTUs and 177 sequences (2%) were matched to the Mucoromycotina. At each taxonomic level, we encountered a large number of sequences that remained unclassified. Fifteen fungal classes were matched, of which the following were most abundant: 712 sequences (7.9%) matched with Agaricomycetes, 679 (7.5%) with Pezizomycetes, 567 (6.3%) with Sordariomycetesand 507 (5.6%) with Leotiomycetes. In addition, we detected OTUs related to 42 fungal orders, of which 679 sequences (7.5 %) matching with Pezizales, 431 sequences (4.8%) with Hypocreales and 287 sequences (3.2%) with Helotiales were the major sequences. Of OTUs related to the 57 fungal families, those related to Pyronemataceae (234 sequences, 2.6%), Sarcosomataceae (199 sequences, 2.2%), Tuberaceae (158 sequences, 1.7%) and Cantharellaceae (119 sequences, 1.3%) were most abundant.

Many sequences could not be assigned to higher taxonomic levels (e.g., 318 OTUs were not assigned to the class level) and may have come from unknown fungi. Across the chronosequence, large numbers of sequences associated with *Craterellus tubaeformis* (family Cantharellaceae) were found on the older sites (site 5 and 6), but fewer were found on the younger sites (site 1 and 2). Moreover, no sequences associated with the

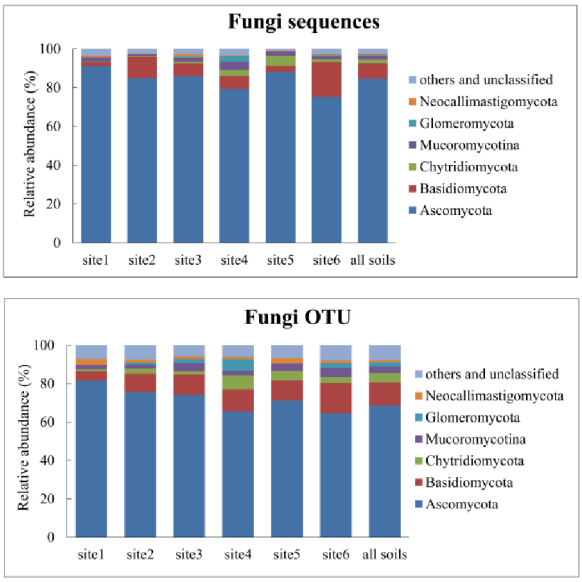

Figure 3 Taxonomic proportions of fungi in the Hailuogou glacier retreat area.

Cantharellaceae were detected from the young sites. Similar patterns were found for *Chytriomyces*, *Mortierella_verticillata* and *Leotia_lubrica*. *Mortierella_alpina* and *Bionectria_ochroleuca* were widespread on the young terrain but were scarce on the older sites.

2.3 Microbial communities along the primary successional chronosequence

At each individual site, the bacterial communities were dominated by sequence reads related to Proteobacteria, Acidobacteria, Bacteroidetes, Actinobacteria, Planctomycetes, Verrucomicrobia and Chloroflexi, and the percentages of these phyla varied between 84% and 92% of the sequence reads (Figure 2). All sites showed little difference in the percentage of Proteobacteria (from 39%-42%) except for site 2. The percentage of Acidobacteria was smaller in the young sites than in the mid-aged and old sites. In contrast, the percentage of Bacteroidetes was greater in the young sites than in the mid-aged and old sites. The percentages of Chloroflexi and Planctomycetes were greatest and that of Verrucomicrobia was smallest in the mid-aged sites. Along the chronosequence, the percentage of OTUs related to Proteobacteria ranged from 34% to 42% in the individual sites (Figure 2). The percentage of OTUs related to Acidobacteria and Planctomycetes showed a tendency to increase with soil age. However, a decreasing tendency was obvious with soil age for the percentage of OTUs related to Bacteroidetes. In addition, the percentage of OTUs related to Verrucomicrobia was on the low side in mid-aged sites in contrast to the young and mid-aged sites.

At each site, Ascomycota accounted for a very large proportion of the fungal sequence reads (from 75% to 91%), and the percentage decreased slightly with soil age (Figure 3). In the fungal sequences, the percentage of sequences assigned to Basidiomycota tended to be low in mid-aged sites compared with that in the young and mid-aged sites. For the Chytridiomycota, the percentage of sequence reads was 0.1% to 5% and increased markedly from site 1 to site 5 (Figure 3). In addition, the Mucoromycotina at all sites were more than 1.5% (the highest was 4%) except for site 2 (0.8%).

Although the OTUs assigned to Ascomycota also accounted for a very large proportion along the chronosequence, they showed a decreasing tendency (from 81% to 64%) (Figure 3). In contrast, the OTUs related to Basidiomycota increased markedly along the chronosequence (from 4% to 16%). Overall, the OTUs related to Mucoromycotina increased along the chronosequence (from 2% to 5%).

The rarefaction curves (Figure 4) showed that the great majority of the bacterial and fungal OTUs were captured by pyrosequencing, but the bacterial curves did not reach complete saturation, suggesting that a greater pyrosequencing effort was needed. Diversity estimators of the bacterial communities reached their highest values on the mid-age sites and to show relatively low value on the young and old sites (Figure 5). A similar pattern was also observed for the fungal communities. In addition, the pattern was more pronounced in the fungal communities compared with the bacterial communities.

Along the chronosequence, the richness of fungal OTUs tended to decrease with site age (rs =

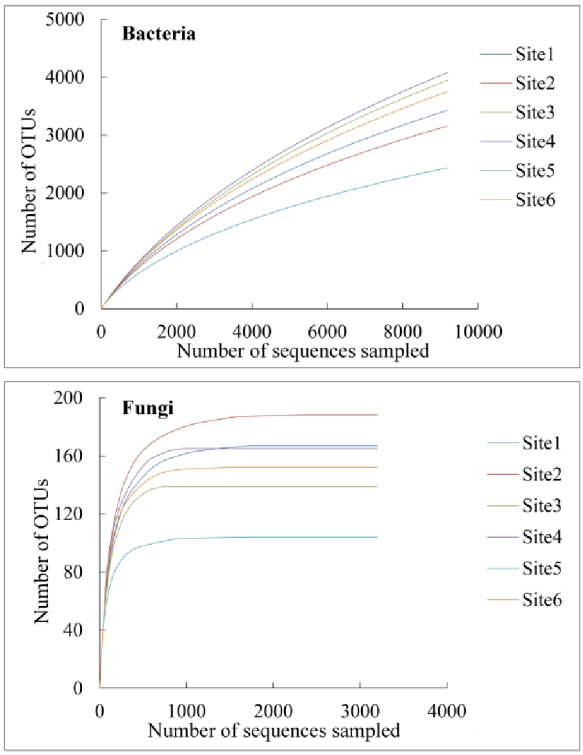

Figure 4 Rarefaction analysis of bacterial and fungal pyrosequencing.

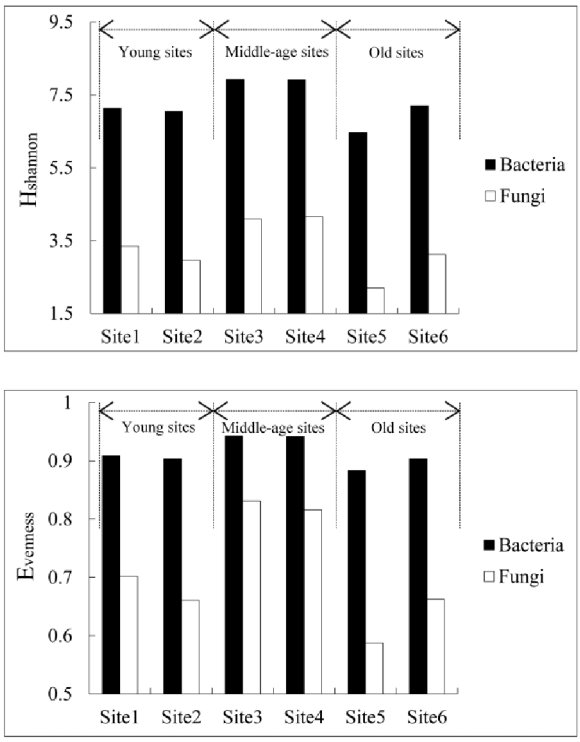

Figure 5 Diversity and evenness of bacterial and fungal communities in different sites.

-0.67, p = 0.078). In contrast, bacterial OTU richness did not respond linearly to site age. A tendency to increase or decrease with site age was found for 0.8% of the bacterial OTUs at a very significant level (p < 0.01), for 6.1% of the bacterial OTUs at a significant level (p < 0.05) and for 40% of the bacterial OTUs at a marginally significant level (p < 0.1). For individual fungal OTUs, an obvious variation tendency was seen with site age for 1.4% of fungal OTUs at a very significant level (p < 0.01), for 7.4% of the fungal OTUs at a significant level (p < 0.05) and for 37% of the fungal OTUs at a marginally significant level (p< 0.1).

3 Discussion

Along a deglaciated chronosequence, we employed pyrosequencing to reveal the bacterial and fungal communities from an early primary successional system that has continued to deglaciate for a century. Previous studies have reported that the relative abundance of Proteobacteria sequences is 30%-50% of the soil bacterial community (Zumsteg et al. 2012; Knelman et al. 2012; Miyashita et al. 2013; Shen et al. 2013; Brown and Jumpponen 2014). This is quite consistent with our results. It may suggest that the relative abundance of Proteobacteria has changed little between the soils during primary succession and the zonal soils. In contrast, the relative abundance of Bacteroidetes appears to change readily. In some soils during primary succession, the relative abundance of Bacteroidetes was 6% to 13% (Zumsteg et al. 2012; Knelman et al. 2012), very similar to our observations. However, in the zonal soils of the temperate forest, the relative abundance of Bacteroidetes is very low, and its average value is approximately 2.4%-3.5% (Shen et al. 2013; Miyashita et al. 2013). This likely implies that Bacteroidetes are a special type of bacteria for the early stage of soil development.

For the fungal community, this and other studies have shown that Ascomycota are the major fungi in soils during primary succession (Cutler et al. 2014; Brown and Jumpponen 2014). In these studies, the relative abundance of Ascomycota sequences was very high (65%-85%) compared with less than 50% in other soils (Blaalid et al. 2014; Shen et al. 2014; Liu et al. 2015). Cutler (2014) found that the relative abundance of Mucoromycotina were higher in soils on a volcanic primary succession gradient (≈ 2.6%) compared with other soils (≈ 0.1%) on the Arctic archipelago of Svalbard (Blaalid et al. 2014). In other studies of fungal sequencing in zonal soils, the relative abundance of Mucoromycotina may have been too small to report (Rincon et al. 2015; Shen et al. 2014; Liu et al. 2015). Therefore, Mucoromycotina may be important early colonizing taxa.

The successional system showed positive successional species (Yang et al. 2014; Zhou et al. 2013), that could be considered as part of the process of building a zonal and mature ecosystem. The accumulation of autochthonic organics and the arrival of organisms (or debris) from allochthonic sources are probably the drivers establishing the early ecosystem establishment (Brown and Jumpponen 2014). In this process, soil microorganisms revealed their importance in ecological functions establishing an organic pool in the soil. First, certain photosynthetic bacteria (e.g., Chlorobi and Cyanobacteria) could produce organic matter using air, water, sunlight and

minerals for soil, and these photosynthetic bacteria were distributed frequently in young sites with sparse vegetation.

Second, some microorganisms are pathogens that kill soil animals. For example, it was found that most of bacterial OTUs (43% of all bacterial sequences) were assigned to the phylum Proteobacteria in the studied area. The phylum Proteobacteria may include a wide variety of pathogens (Madigan and Martinko 2005). It likely implies that the phenomena of pathogenic bacteria killing soil animals may exist. The bodies of animals killed by pathogenic microorganisms could become soil organic matter.

Third, many mycorrhizal fungi (e.g., the families Tuberaceae and Russulaceae, the genus *Geopora* and *Craterellustubaeformis*) were found in the early primary successional system. Mycorrhizal fungi are responsible for most nutrient uptake by the majority of land plants and are increasingly recognized as important drivers of terrestrial ecosystem processes (Mohan et al. 2014). They should be important microbial species promoting ecosystem succession. In addition, many taxonomic groups (family Sarcosomataceae, genus *Saccharomyces* and *Coniochaetavelutina*) that include saprotrophic communities are widely distributed in the glacier retreat area. These saprophytic microorganisms are responsible for litter decomposition and play an important role in the cycling of nutrients (Koukol et al. 2006). It is suggested that building a complete system of decomposed biological residues for a mature ecosystem may be a necessary task during early primary succession. Most importantly, a large amount of microbial necromass is added to the soil organic matter with rapid turnover. According to a recent study, carbon in the microbial necromass could account for 80% of soil organic carbon (Liang and Balser 2011). The ecological functions described above show microbial communities are one of the main drivers that build soil organic matter pool and expedite pedogenesis for ecosystem succession.

Vegetation can impact the physical and chemical properties of soil through litter, root growth and root exudates (van der Heijden et al. 2008). Our data support the important impact of plants on soil properties. Particularly along the primary successional chronosequence with different plant types, soil temperature, pH, moisture and a variety of nutrients (SOM, TN, TP, K, Ca, Na, Mg, Fe, Al) exhibited statistically significant distributional trends (increases or decreases) (Table 1). These changes in soil properties could influence the microbial communities. As shown by Read (1994), close links exist between plant and microbial communities that influence the soil environment.

In contrast to the linear changes in soil properties and the positive succession of plant communities during primary succession, the microbial communities exhibited different developmental patterns in this study area. The highest values of bacterial and fungal evenness and diversity indicated that there was a relatively good soil environment for microbial communities in the mid-aged site successional stage. Indeed, a previous study in this area also demonstrated that the dominant species of plants were abundant and many trees were young and energetic in the mid-aged sites (Cheng and Luo 2002). In addition, the degree of soil weathering was low in this successional stage compared to the old sites (He and Tang 2008), and low soil weathering could easily provide the available mineral nutrition. We suggest this is a successional stage with less environmental stress and more niches for microbial communities in a complete primary succession (from bare land to a zonal ecosystem).

In contrast to our findings, Brown and Jumpponen (2014) observed a static microbial diversity (bacteria and fungi) and a declining bacterial evenness across the Lyman Glacier chronosequece. Moreover, they noted that their findings may not be universal. The possible explanations for this incongruence were the differences in successional speeds and ecosystem types between the two study areas. According to survey and previous data, the growing season is much longer in the Hailuogou glacier retreat area (approximately 6 months of the year) than that in Lyman Glacier area (approximately 3 months of the year) (Cazares et al. 2005).

The long growing season led to the rapid succession of vegetation in our study area. For example, our site 1 reached the vegetation phase which their site reached after 20– 30 years of glacier retreat. After 82 years of glacier retreat, our site had reached the lush forest stage of *Abiesfabri*

and *Piceabrachytyla* while their site was still in the stages of the shrub and meadow parkland communities containing individuals of *Abieslasiocarpa*, *Larixlyallii* and *Tsugamertensiana* among patches dominated by several members of Ericaceae (Jumpponen et al. 1998). In addition, the accumulation rates of organic C and N in our study area were significantly higher than in other chronosequences (He and Tang 2008). For example, the accumulation rates of C and N were 3-4 times and 7-11 times as high as those of other chronosequences, respectively (Egli et al. 2001; Lichter 1998).

The above discussion thus indicates that primary succession sequence in the Hailuogou glacier retreat area is developing rapidly compared to some chronosequences.

4 Conclusions

This study revealed the characteristics of microbial community changes on a primary succession in the Hailuogou glacier retreat area. The highest values of bacterial and fungal evenness and diversity indicated that there were less environmental stress and more niches for microbial communities in the middle successional stage compare with other stages. In addition, the microbial taxa were identified and their ecological functions showed that microbial communities are among the main drivers which build the soil organic matter pool and expedite pedogenesis for ecosystem succession. The primary succession in the Hailuogou glacier retreat area is developing rapidly.

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