Ecological networks reveal contrasting patterns of bacterial and fungal communities in glacier-fed streams in Central Asia

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ABSTRACT

Bacterial and fungal communities in biofilms are important components in driving biogeochemical processes in stream ecosystems. Previous studies have well documented the patterns of bacterial alpha diversity in stream biofilms in glacier-fed streams, where, however, beta diversity of the microbial communities has received much less attention especially considering both bacterial and fungal communities. A focus on beta diversity can provide insights into the mechanisms driving community changes associated to large environmental fluctuations and disturbances, such as in glacier-fed streams. Moreover, modularity of co-occurrence networks can reveal more ecological and evolutionary properties of microbial communities beyond taxonomic groups. Here, integrating beta diversity and co-occurrence approach, we explored the network topology and modularity of the bacterial and fungal communities with consideration of environmental variation in glacier-fed streams in Central Asia. Combining results from hydrological modeling and normalized difference of vegetation index, this study highlighted that hydrological variables and vegetation status are major variables determining the environmental heterogeneity of glacier-fed streams. Bacterial communities formed a more complex and connected network, while the fungal communities formed a more clustered network. Moreover, the strong interrelations among the taxonomic dissimilarities of bacterial community (BC) and modules suggest they had common processes in driving diversity and taxonomic compositions across the heterogeneous environment. In contrast, fungal community (FC) and modules generally showed distinct driving processes to each other. Moreover, bacterial and fungal communities also had different driving processes. Furthermore, the variation of BC and modules were strongly correlated with hydrological properties and vegetation status but not with nutrients, while FC and modules (except one module) were not associated with environmental variation. Our results suggest that bacterial and fungal communities had distinct mechanisms in structuring microbial networks, and environmental variation had strong influences on bacterial communities but not on fungal communities. The fungal communities have unique assembly mechanisms and physiological properties which might lead to their insensitive responses to environmental variations compared to bacterial communities. Overall, beyond alpha diversity in previous studies, these

results add our knowledge that bacterial and fungal communities have contrasting assembly mechanisms and respond differently to environmental variation in glacier-fed streams.

INTRODUCTION

Glaciers cover approximately 10% of the land surface on the Earth (*Milner et al., 2017*) and are important components of the hydrological cycle providing vital water resources (*Barnett, Adam & Lettenmaier, 2005*; *Gardner et al., 2013*; *Zemp et al., 2015*). However, glaciers are shrinking rapidly across the world due to accelerating global warming (*Immerzeel, Van Beek & Bierkens, 2010*; *Sorg et al., 2012*; *Marzeion et al., 2014*), and most of them are expected to disappear by 2050 (*Zemp et al., 2006*; *IPCC, 2014*). As a prominent component of the glacier forefront, glacier-fed streams have a highly heterogeneous environment due to longitudinal alterations of landcover, river hydrology and morphology, sediment transport, and biogeochemical processes (*Hood & Scott, 2008*; *Laghari, 2013*; *Hotaling, Hood & Hamilton, 2017*; *Milner et al., 2017*). For example, from glacier terminus to downstream, terrestrial vegetation increases (*Zhang et al., 2013*; *Raynolds et al., 2015*), stream channel lengthens (*Milner, Brown & Hannah, 2009*; *Robinson, Thompson & Freestone, 2014*), and water source compositions changes (*Brown, Hannah & Milner, 2003*).

Biofilms are hot spots of microbial diversity and activity in stream ecosystems (*Geesey et al., 1978*; *Battin et al., 2016*). Within stream biofilms, bacteria, fungi, and algae are the major components driving the bulk of metabolism and biogeochemical processes (*Brittain & Milner, 2001*; *Battin et al., 2003*; *Von Schiller et al., 2007*). The changing environment presents significant challenges for glacier-fed stream ecosystems. Previous studies have revealed that factors associated with glacier shrinkage have significant influences on the composition, diversity, and functional potential of bacterial communities in stream biofilms (*Wilhelm et al., 2013*, *2014*; *Ren, Gao & Elser, 2017*; *Ren et al., 2017*). However, fungal communities in glacial systems are rarely studied (*Edwards, 2015*; *Anesio et al., 2017*). With the decrease in elevation, glacier coverage, and glacier source contribution to streamflow, as well as increase in distance to glacier terminus, bacterial communities showed increased alpha diversity as well as distinct taxonomic and functional compositions (*Wilhelm et al., 2013*, *2014*; *Ren, Gao & Elser, 2017*; *Ren et al., 2017*). Biodiversity is important for generating and stabilizing ecosystem structure and functions (*Loreau et al., 2001*; *Tilman, Isbell & Cowles, 2014*). The positive effects of local species richness (alpha diversity) on ecosystem functioning have been widely confirmed by a growing number of studies (*Chapin et al., 2000*; *Cardinale et al., 2012*; *Duffy, Godwin & Cardinale, 2017*). However, comparing to alpha diversity, beta diversity is an underexplored facet of biodiversity (*Mori, Isbell & Seidl, 2018*), which accumulates from compositional variations among local assemblages and provides insights into the

mechanisms underlining biodiversity changes and their ecological consequences (*Anderson et al., 2011*; *Socolar et al., 2016*). For ecological communities suffering intensive environmental fluctuations and disturbances, focusing on beta diversity is especially important (*Mori, Isbell & Seidl, 2018*). In addition, microorganisms in many environments often coexist in a complex network with positive and negative interactions among members, playing pivotal roles in community assembly (*Fuhrman, 2009*; *Barberán et al., 2012*; *Shi et al., 2016*). These interactions may imply biologically or biochemically meaningful relationships between microorganisms (*Weiss et al., 2016*). Microbial co-occurrence networks can reveal how taxa potentially interact with each other, how diverse taxa structure networks, and how networks are compartmentalized into modules of closely associated taxa, as well as how microbial communities responded to environmental variations (*Newman, 2006*; *Fuhrman, 2009*; *De Menezes et al., 2015*; *Banerjee et al., 2016*). In addition, modularity (the tendency of a network to contain sub-clusters of nodes) is an important ecological feature in many biological systems, providing opportunities to identify highly connected taxa and integrate high dimension data into predicted ecological modules (*De Menezes et al., 2015*; *Shi et al., 2016*). A module is defined as a group of densely connected operational taxonomic units (OTUs), which have less links with OTUs belonging to other modules (*Shi et al., 2016*), forming a clustered network topology (*Barberán et al., 2012*). Modules can help to reveal more ecological and evolutionary properties (*Thompson, 2005*; *Olesen et al., 2007*), which are easily overlooked when communities are studied as a whole or in taxonomic groups (*Porter, Onnela & Mucha, 2009*; *Bissett et al., 2013*; *De Menezes et al., 2015*). The relationships between microbial modules and environmental variables can improve our understanding of the influences of environmental variation on microbial community assembly (*Lindström & Langenheder, 2012*; *De Menezes et al., 2015*; *Toju et al., 2016*). However, previous studies in glacier-fed streams have only focused on the whole communities or certain taxonomic groups of bacteria and fungi (*Robinson & Jolidon, 2005*; *Milner, Brown & Hannah, 2009*; *Wilhelm et al., 2013*; *Ren, Gao & Elser, 2017*). The network and modularity features of bacterial and fungal communities in glacier-fed streams are remaining one of our knowledge gaps. Integrating beta diversity and network modularity can provide novel insights into assembly mechanisms of microbial communities in glacier-fed streams.

Glacier-fed streams in Tian Shan Mountains in Central Asia are particularly vulnerable to climate change, where glaciers contribute significantly to stream runoff (*Aizen et al., 1997*; *Hagg et al., 2007*; *Sorg et al., 2012*). Glacier shrinkage has been observed in the past decades (*Farinotti et al., 2015*) and will accelerate in the coming decades as temperature increases (*Kraaijenbrink et al., 2017*; *Gao et al., 2018*). Here, we investigated bacterial and fungal communities in two glacier-fed streams using high-throughput sequencing combined with hydrological modeling. We aimed to examine the microbial co-occurrence networks (considering both fungal and bacterial communities) and to assess their response patterns to environmental variation in these glacier-fed streams. In glacier foreland soil, bacterial and fungal communities had contrasting community structures and response patterns to environmental variables (*Blaalid et al., 2012*; *Bradley, Singarayer & Anesio, 2014*; *Brown & Jumpponen, 2014*). Glacier-fed streams have intimate connections with terrestrial ecosystems
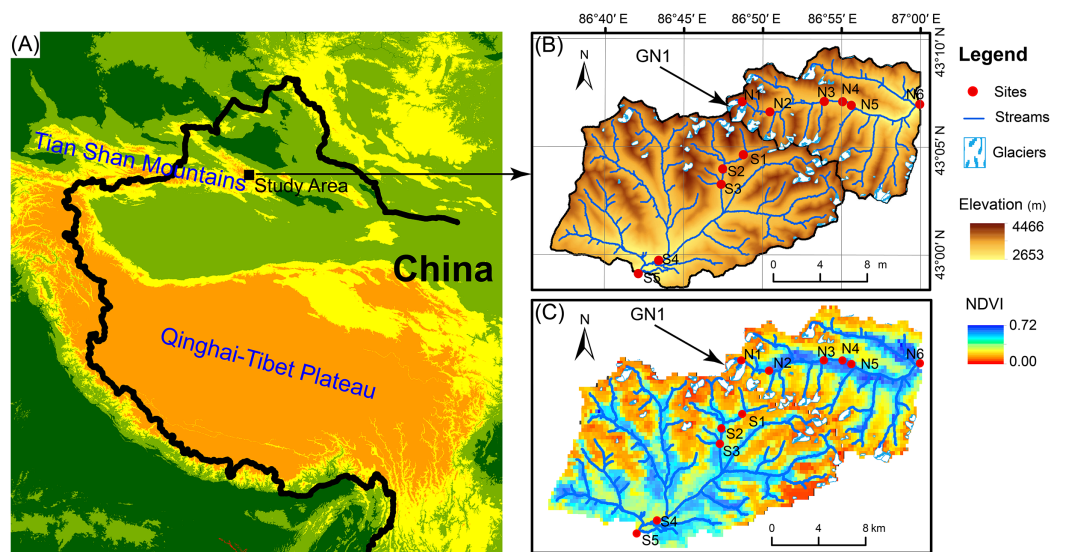
Figure 1 Map of the study area. (A) Location of the study area. (B) Elevation distribution of the study area. GN1 represents Urumqi Glacier No. 1. (C) The Normalized difference of vegetation index (NDVI) of the study area. Full-size 🖼 DOI: 10.7717/peerj.7715/fig-1

in the glacier foreland through multiple ways (*Crump, Amaral-Zettler & Kling, 2012*; *Gutiérrez et al., 2015*; *Ruiz-González, Niño-García & Del Giorgio, 2015*). Thus, we hypothesize that bacterial and fungal communities in glacier-fed streams have contrasting assembly mechanisms and respond differently to environmental variation.

MATERIALS AND METHODS

Study area

The Tian Shan mountains, known as the "water tower of Central Asia," span a large area of Central Asia from northwestern China to southeastern Kazakhstan and from Kyrgyzstan to Uzbekistan (Fig. 1). In Tian Shan area, glaciers contribute considerably to water resources and play an important role in streamflow regimes (*Sorg et al., 2012*; *Unger-Shayesteh et al., 2013*). In China's portion of the Tian Shan Mountains, there are 7,934 glaciers with a total area of 7,179 km^2 and a total volume of 707 km^3 (*Guo et al., 2014*). However, glaciers in the Tian Shan Mountains are extremely sensitive to global warming (*Kraaijenbrink et al., 2017*) and have been shrinking rapidly due to climate warming since the 1970s (*Narama et al., 2010*; *Farinotti et al., 2015*). For example, Urumqi Glacier No. 1 (GN1, 43°06′N, 86°49′E) is located in eastern Tian Shan Mountain at the headwater of the Urumqi River (Fig. 1) and retreated from an area of 1.95 km^2 in 1962 to 1.65 km^2 in 2010 (*Zhang et al., 2014*). In 2050, GN1 will likely lose up to 54% of the glacier area and 79% of the ice volume relative to 1980 (*Gao et al., 2018*).

Field sampling

In June 2016, we investigated two glacier-fed streams in the Tian Shan Mountain. Water samples and benthic biofilm samples were collected from 11 sample sites in total spanning from the elevation of 3,828 to 2,646 m (Fig. 1). The sample sites were chosen along the

streams in order to have heterogeneous environments, including different land vegetation and hydrological properties such as glacier contributions to stream flow. However, due to the constraint of accessibility, the sites were not spaced at equal intervals. At each sample site, six to nine submerged rocks were randomly sampled from the stream cross section below 10 cm. A sterilized nylon brush was used to remove the benthic biofilm from each stone in an area of 4.5 cm diameter on the upper surface. The slurry was rinsed with 500 mL sterile water. Approximately 10 mL of the mixed slurry was filtered through a 0.2-μm polycarbonate membrane filter (Whatman, Maidstone, UK) which was immediately frozen in liquid nitrogen in the field. After transported to the lab, the benthic biofilm samples were stored under −80 °C until DNA extraction. In addition, 500 mL water samples were collected for chemical analyses with three replications and stored under 4 °C.

Environmental factors

At each sample site, pH, conductivity (Cond), and elevation were measured in situ using a handheld pH meter (PHI 400 Series; Beckman Coulter, Brea, CA, USA), YSI meter (model 80, Yellow Springs, OH, USA), and GPS unit (Triton 500; Magellan, Santa Clara, CA, USA), respectively. Unfiltered water samples were directly used to measure total nitrogen (TN) and total phosphorus (TP). TN was analyzed by ion chromatography with prior persulfate oxidation (EPA 300.0). TP was analyzed using the ammonium molybdate method with prior oxidation (EPA 365.3). Filtered water samples (filtered with pre-combusted GF/F filters) were used to test nitrate (NO_3^-), ammonium (NH_4^+), soluble reactive phosphorus (SRP), and dissolved organic carbon (DOC). NO_3^- was analyzed using ion chromatography (EPA 300.0). NH_4^+ was analyzed using the indophenol colorimetric method (EPA 350.1). SRP was measured according to the ammonium molybdate method (EPA 365.3). DOC was measured using a total organic carbon (TOC) analyzer (TOC-VCPH; Shimadzu Scientific Instruments, Columbia, MD, USA). Water chemistry data was reported in our previous research (Ren, Gao & Elser, 2017).

In glacier-fed streams, both biotic and abiotic environments are tightly linked to the relative contributions of glacier melt and runoff to the stream flow (Milner et al., 2001; Hannah et al., 2007; Kuhn et al., 2011). According to the landscape-based hydrological model proposed by Gao et al. (2016, 2017), we classified the landscape into glaciated and non-glaciated. For each sample site, the proportion of glaciated area (GA) in its sub-catchment was calculated and the proportion of glacier source water (GS) in the total runoff was derived from the model (Gao et al., 2016, 2017). The hydrological distance to glacier terminus (GD) was measured according to the river channel network (Fig. 1). These hydrological parameters were also reported in our previous research (Ren, Gao & Elser, 2017).

In the study area, the vegetation (grassland) status was measured by the normalized difference vegetation index (NDVI) using the Terra moderate resolution imaging spectroradiometer Vegetation Indices (MOD13Q1) Version 6 data downloaded from USGS (https://earthexplorer.usgs.gov/) (Fig. 1). The MOD13Q1 product was generated on June 26, 2017 and has a resolution of 250 m. NDVI is calculated based on the absorption of red light and the reflection of infrared radiation by vegetation (Rouse et al., 1974).

The equation is represented as NDVI = (NIR − RED)/(NIR + RED), where NIR is near infrared reflectance and RED is visible red reflectance. It has been demonstrated that NDVI exhibits close relationships with above-ground vegetation biomass and coverage (*Carlson & Ripley, 1997*; *Eastwood et al., 1997*; *Ren et al., 2019*). For each stream site, the average NDVI of its sub-catchment (the upstream area of the stream site) was calculated as the mean NDVI of each pixel in the sub-catchment.

DNA extraction, PCR, and sequencing

Bacterial 16S rRNA gene sequences and fungal 18S rRNA gene sequences were analyzed to determine the bacterial community (BC) and fungal community (FC), respectively. To determine the fungal community, the internal transcribed spacer regions and 18S rRNA genes are commonly used and provide similar results and congruent conclusions (*Brown, Rigdon-Huss & Jumpponen, 2014*). We used 18S rRNA gene sequencing to detect the fungal community in this study. DNA was extracted using the PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA, USA) following the manufacturer's protocol. The V3–V4 regions of the 16S rRNA genes were amplified using the bacterium-specific forward and reverse primers 338F-ACTCCTACGGGAGGCAGCA and 806R-GGACTACHVGGGTWTCTAAT (Invitrogen, Vienna, Austria) (*Huws et al., 2007*; *Masoud et al., 2011*; *Caporaso et al., 2012*). The V4–V5 regions of the 18S rRNA genes were amplified using the fungus-specific forward and reverse primers 817F-TTAGCATGGAATAATRRAATAGGA and 1196R-TCTGGACCTGGTGAGTTTCC-3′ (Invitrogen, Vienna, Austria) (*Borneman & Hartin, 2000*). The forward primers were barcoded, and the barcodes were designed considering the balanced guanine–cytosine content, minimal homopolymer runs, and no self-complementarity of more than two bases to reduce internal hairpin propensity (*Hawkins et al., 2018*). PCR reaction systems were prepared using a Premix Taq Kit (Code No. RR902A; Takara, Kusatsu,, Japan) according to the manufacturer's instructions. The total volume of each PCR reaction was 20 μL, containing 10 μL of 2×EX Premix Taq^{TM} Polymerase, one μL of forward primer, one μL of reverse primer, one μL of NDA extraction, and seven μL of Nuclease-free water. The PCR reactions were conducted with a thermal cycler (ABI 2700, SeqGen, Torrance, CA, USA) with a temperature profile of 1-min hot start at 80 °C, followed by pre-denaturation at 94 °C for 5 min, 30 cycles of amplification (denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C for 90 s), and a final extension at 72 °C for 10 min. The PCR amplicons were verified in 1.0% agarose with 1× TAE buffer using electrophoresis, purified using the Gel Extraction Kit (Qiagen, Hilden, Germany), and quantified by Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). One of the fungi sample (N2) was not successfully amplified. The purified and quantified DNA libraries were then pooled together according to their concentrations. The pooled library was sequenced on an Illumina MiSeq (PE300) sequencing platform.

Analyses

Raw sequence data of bacterial 16S rRNA (available at NCBI, PRJNA398147, SRP115356) and fungal 18S rRNA (available at NCBI, PRJNA542974, SRP198430) were processed

using QIIME 1.9.0 (*Caporaso et al., 2010*). The forward and reverse reads were merged. The merged sequences were then assigned to samples based on the barcode. The barcode and primer sequence were cut off to truncate the sequences. The sequences with length >200 bp and mean quality score <20 were discarded. Using UCHIME algorithm (*Edgar et al., 2011*), the chimeric sequences were detected and removed. Finally, the effective sequences of 16S and 18S rRNA were grouped into OTUs against the Silva 132 database at 99% threshold.

All the analyzed environmental variables, including GD, GA, GS, NDVI, elevation, pH, Cond, TN, NO_3^-, NH_4^+, TP, SRP, and DOC, were standardized using the "normalize" method in *decostand* function in vegan 2.5-3 package (*Oksanen et al., 2007*). Spearman correlation analyses (*cor* function in stats v3.6.0 package) were used to assess the pairwise relationships between environmental variables and visualized using *corrplot* function in corrplot 0.84 package. Mantel tests (*mantel* function in vegan 2.5-3 package) were used to assess the relationships among spatial dissimilarity (represented by geographic distance), overall environmental distance, hydrological dissimilarity, nutrient dissimilarity, and vegetation dissimilarity. Partial Mantel tests (*mantel.partial* function in vegan 2.5-3 package) were used to assess those relationships by controlling nutrient dissimilarity. The geographic distance was calculated based on the GPS coordinates of sample sites using *distGeo* function in geosphere 1.5-10 package. The environmental distance was represented by Euclidean distance based on all analyzed environmental variables. Hydrological dissimilarity was represented by Euclidean distance based on GD, GA, and GS. Nutrient dissimilarity was represented by Euclidean distance based on TN, NO_3^-, NH_4^+, TP, and SRP. Vegetation dissimilarity was represented by Euclidean distance based on NDVI. Euclidean distances were calculated using *vegdist* function in vegan 2.5-3 package (*Oksanen et al., 2007*). All the above statistical analyses were conducted in R 3.5.1 (*R Development Core Team, 2017*).

The co-occurrence networks of bacterial and fungal communities were assessed and visualized using Cytoscape (version 3.7.1) (*Shannon et al., 2003*). In network analyses, the pairwise correlations between OTUs (OTUs with a relative abundance >0.01%) were calculated using Spearman's correlation based on the relative abundance of OTUs (data was transformed by Hellinger transformation using *decostand* function in vegan 2.5-3 package). Only strong ($R > 0.7$ OR $R < -0.7$) and significant ($P < 0.01$) correlations were considered in network analysis. ClusterMaker app (*Morris et al., 2011*) was used to analyze the modular structures of the co-occurrence networks. Modularity values greater than 0.4 suggest that the network has a modular structure (*Newman, 2006*). The group attributes layout algorithm was used to construct the networks based on modules. The basic topological metrics of networks were calculated, including number of nodes, number of edges, clustering coefficient, characteristic path length, network density, network heterogeneity, and modularity. The taxonomic dissimilarities (beta diversity) of the BC and FC were calculated based on Bray–Curtis distance in terms of the relative abundance of OTUs using R package vegan 2.5-3 (*Oksanen et al., 2007*). Moreover, the taxonomic dissimilarities of the major modules (modules have more than 15 nodes) were also calculated based on Bray–Curtis distance in terms of the relative abundance of OTUs in
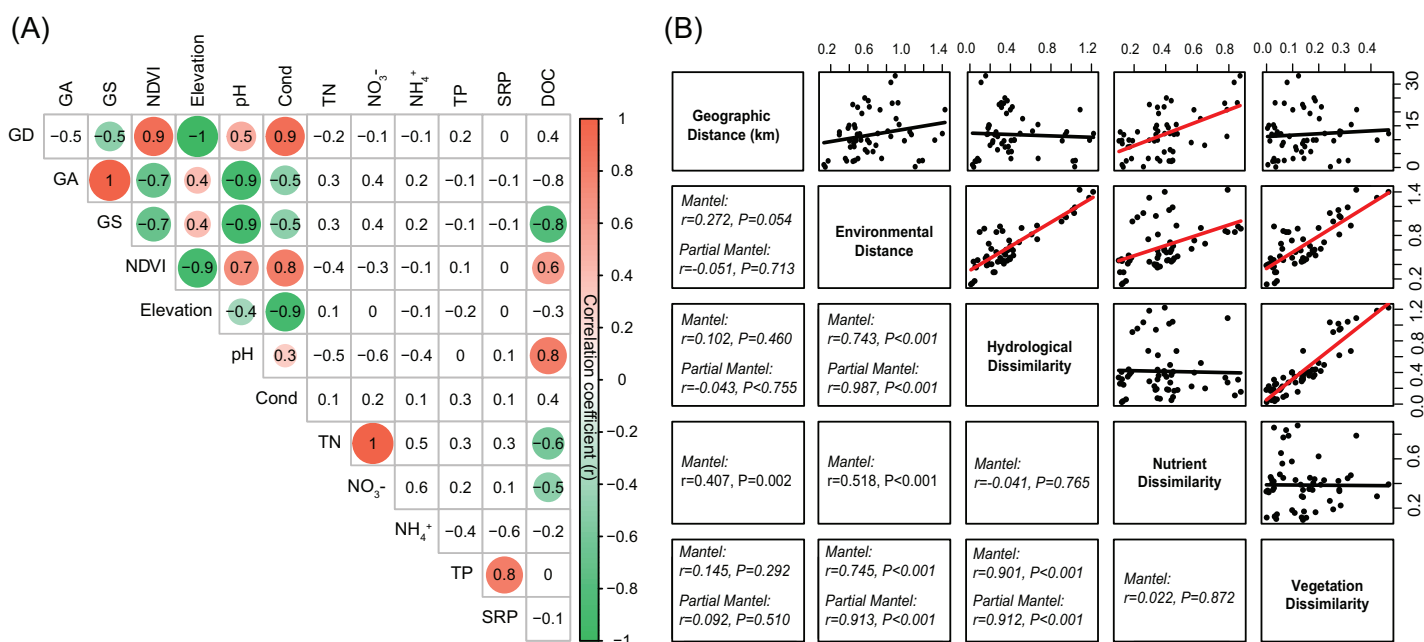
(A)

(B)

Figure 2 (A) Pairwise-Spearman correlations between environmental variables. The magnitude of correlation coefficient is only shown graphically with color scale when correlation is significant ($P < 0.05$). The color intensity and the size of the circle are proportional to the correlation coefficients. (B) Correlations between the different components of environmental dissimilarities. Spearman correlations (r) and associated P-values were calculated by Mantel test. Nutrient dissimilarity was controlled in Partial Mantel test. Full-size 🔳 DOI: 10.7717/peerj.7715/fig-2

the module. BM1, BM2, BM3, and BM4 represent four major modules in the bacterial network. FM1, FM2, FM3, FM4, FM5, and FM6 represent six major modules in the fungal network. Mantel tests were used to assess the correlations between spatial and environmental dissimilarities and taxonomic dissimilarities of bacterial and fungal communities and modules. The relationships among different modules and communities for bacteria and fungi were also assessed using Mantel tests.

RESULTS

Environmental variations and community taxonomic dissimilarities

The environmental variables of the streams varied across the sampling sites and showed differing interrelationships (Fig. 2). GD, GA, GS, NDVI, elevation, pH, and conductivity were closely correlated with each other (Fig. 2A). DOC was negatively correlated with GS, TN, and NO_3^-, while positively correlated with NDVI and pH (Fig. 2A). Across the sampling sites, hydrological and vegetation dissimilarities had strong linear relationships with overall environmental dissimilarity, while nutrient dissimilarity only had a weak relationship (Fig. 2B). Moreover, hydrological and vegetation dissimilarities had a strong interrelationship with each other but without significant relationships to nutrient dissimilarity (Fig. 2B). Geographic distance only had a significant relationship with nutrient dissimilarity (Fig. 2B). In addition, Mantel tests showed that environmental, hydrological, and vegetation dissimilarities had significant positive relationships to taxonomic dissimilarities of BC but not to FC (Fig. 3). However, geographic distance and
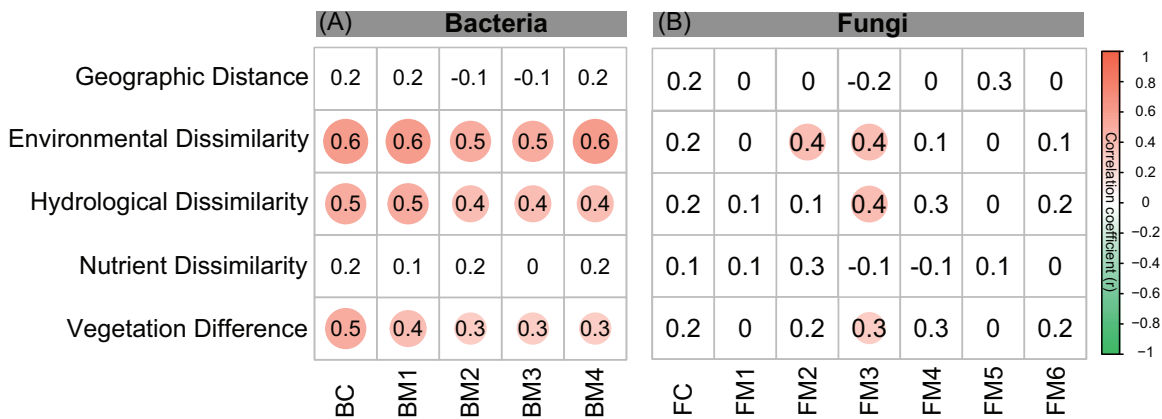
Figure 3 Correlations between different components of environmental distance (environmental dissimilarity, hydrological dissimilarity, nutrient dissimilarity, and vegetation dissimilarity) and taxonomic dissimilarities of (A) bacterial and (B) fungal communities and modules. Spearman correlations were calculated by Mantel test. The magnitude of correlation coefficient is only shown graphically with color scale when correlation is significant ($P < 0.05$). The color intensity and the size of the circle are proportional to the correlation coefficients.

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nutrient dissimilarity did not have significant relationships with taxonomic dissimilarities of both bacterial and fungal communities (Fig. 3).

Bacteria and fungi co-occurrence patterns and modular structures

In the studied glacier-fed streams, bacteria and fungi formed complex co-occurrence networks (Fig. 4). The bacterial and fungal networks consisted of 904 and 238 nodes and 21,463 and 1,348 edges, respectively (Table 1). The bacterial network had a higher number of nodes and edges as well as a higher network density (Fig. 4; Table 1), indicating the bacterial network was more complex and connected than the fungal network. However, the fungal network exhibited a higher clustering coefficient, characteristic path length, network heterogeneity, and modularity, indicating that the fungal network had a more clustered topology than the bacterial network (Fig. 4; Table 1).

Co-occurrence networks can be compartmentalized into modules within which nodes are closely associated and are expected to share environmental preferences. We found that the OTUs in bacterial and fungal networks were grouped into four and six major modules (modules with more than 15 nodes), respectively (Figs. 4A and 4B). All the modules were formed by various microbial taxa (Figs. 4C and 4D), which shown differently across sample sites (Figs. S1 and S2). Modules had significantly different taxonomic compositions to each other (Tables S1 and S2). Mantel tests showed that the taxonomic dissimilarities of the BC and modules had strong interrelationships (Fig. 5). The taxonomic dissimilarities of FC and modules only had weak interrelationships. Between bacterial and fungal networks, only FM3 had strong relationships with BC and BMs. Mantel tests also revealed that all bacterial modules (BM1, BM2, BM3, and BM4) and one fungal module (FM3) were positively correlated with environmental, hydrological, and vegetation dissimilarities, but not with geographic distance and nutrient dissimilarity (Fig. 3).
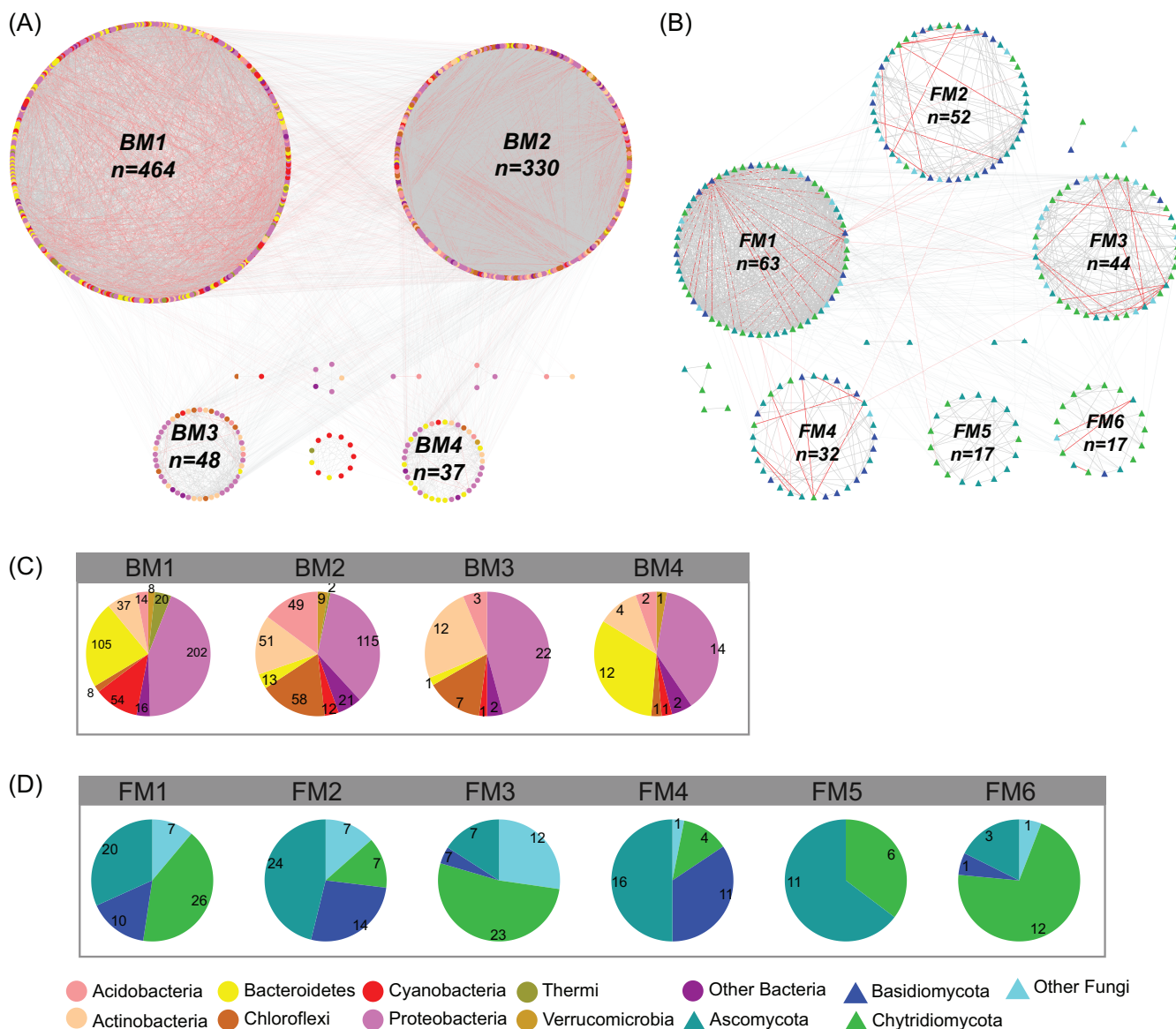
Figure 4 Co-occurrence network of (A) bacterial and (B) fungal communities. Dot and triangle represent bacterial and fungal OTUs (OTUs with a relative abundance >0.01%), respectively. OTUs were colored according to major phylum (phylum with relative abundance >1%). Edges represent Spearman correlation relationships ($P < 0.01$). Gray lines indicate positive associations and pink lines indicate negative associations. The circles represent modules of the networks. The pie graphs in the below two panels show the composition of the modules in (C) bacterial and (D) fungal networks with the number representing the number of OTUs. Full-size 🖼 DOI: 10.7717/peerj.7715/fig-4

DISCUSSION

Bacterial and fungal communities in stream biofilms are major components of glacier-fed stream ecosystems. The strong correlations between bacterial communities and environmental, hydrological, and vegetation characteristics suggest substantial influences of spatial heterogeneity and potential influences of glacier shrinkage on bacterial communities (Fig. 3). However, the variation of bacterial communities was not associated with stream nutrient variations and geographic distance. In our studied glacier-fed streams, the proportion of GA, the proportion of glacier source water (GS), the

Table 1 Topological parameters of the network of bacterial and fungal communities.

| Topological parameters	Description	Bacterial	Fungal
Number of nodes	Number of OTUs in the network	904	238
Number of edges (in total)	Strong and significant correlations	21,463	1,348
Number of edges (positive)	Positive correlations	18,419	1,253
Number of edges (negative)	Negative correlations	3,044	95
Clustering coefficient	The fraction of observed vs. possible clusters for each node	0.440	0.451
Characteristic path length	The median of the means of the shortest path lengths connecting each vertex to all other vertices	2.887	3.829
Network density	The ratio of the number of edges and the number of possible edges	0.053	0.048
Network heterogeneity	Density distribution of connections between nodes	0.929	1.107
Modularity	Tendency of a network to contain sub-clusters of nodes	0.52	0.599
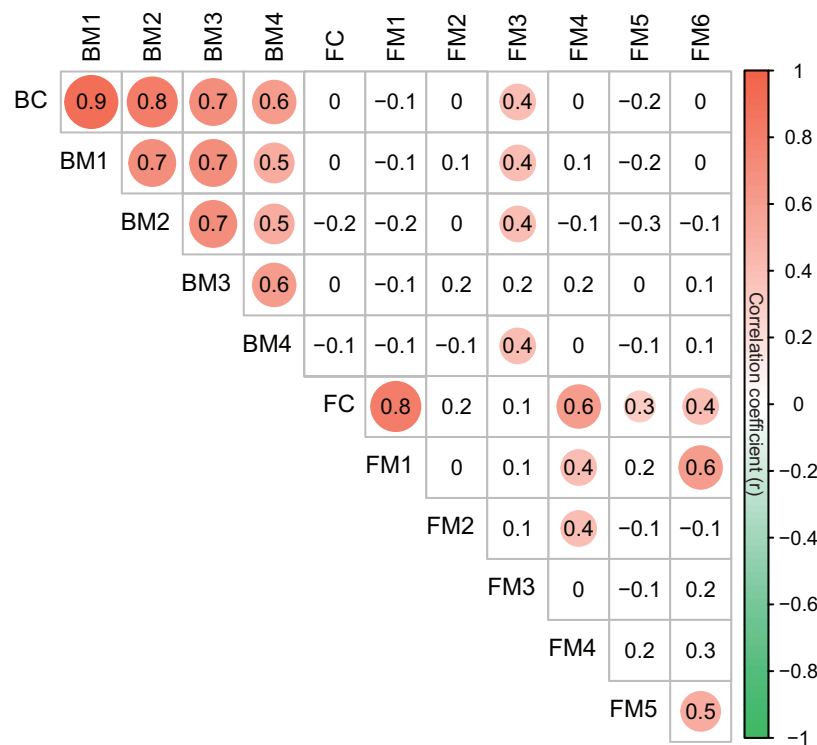
Figure 5 Correlation matrix of the taxonomic dissimilarities for bacterial and fungal communities and modules. The magnitude of correlation coefficient is only shown graphically with color scale when correlation is significant ($P < 0.05$). The color intensity and the size of the circle are proportional to Spearman correlation coefficients. Full-size ☑ DOI: 10.7717/peerj.7715/fig-5

hydrological distance to glacier terminus (GD)and the NDVI were the environmental variables associated with longitudinal patterns of glacier-fed streams. Glacier-fed streams are fed by various sources, including ice-melt, snowmelt, and groundwater (*Brown, Hannah & Milner, 2003*). The contribution of different water sources varies longitudinally from the glacier terminus to downstream reaches and temporally with glacier shrinkage (*Brittain & Milner, 2001*; *Milner, Brown & Hannah, 2009*; *Gao et al., 2016*), resulting in distinct hydrology and physicochemical features which control the ecological structures

and processes in glacier-fed streams (*Brown, Hannah & Milner, 2003*, *2007*; *Sertic Peric et al., 2015*). Synchronizing with the hydrological changes in glacier-fed streams, vegetation growth and aboveground biomass in the catchment also has a clear elevation gradient (*Carlyle, Fraser & Turkington, 2014*) which will likely be amplified due to climate warming (*Zhang et al., 2013*; *Raynolds et al., 2015*). The changed landcover modifies terrestrial and aquatic biogeochemistry (*Sadro, Nelson & Melack, 2012*), and affects stream biofilms (*Figueiredo et al., 2010*; *Nielsen et al., 2012*). Thus, hydrological variables (GD, GA, and GS) and vegetation variable (NDVI) determine the environmental heterogeneity of glacier-fed streams and can potentially indicate glacier shrinkage. It has been widely demonstrated that glacier shrinkage alters watershed landcover and instream environments (*Hood & Scott, 2008*; *Jacobsen & Dangles, 2012*; *Laghari, 2013*; *Raynolds et al., 2015*; *Milner et al., 2017*), imposing impacts on bacterial communities (*Nelson, Sadro & Melack, 2009*; *Hotaling, Hood & Hamilton, 2017*). For example, the alpha diversity of biofilm bacteria decreased with the increases of elevation, the proportion of glacier area in the watershed, and the relative contribution of glacier sources to stream runoff (*Wilhelm et al., 2013*; *Ren, Gao & Elser, 2017*). Potential functions of bacterial communities are also significantly associated with hydrological factors (*Ren et al., 2017*). Our results further suggest strong influences of hydrological and vegetation characteristics on bacterial communities, leading to more different (biotic heterogenization) bacterial communities in glacier-fed streams.

In contrast to bacterial communities, environmental, hydrological, and vegetation dissimilarities were not significantly associated with the dissimilarity of fungal communities. Moreover, the variation fungal communities were also not associated to nutrient variations. The results suggest that FC variations were not affected by the environmental variation and might be insensitive to glacier shrinkage. We proposed that the unique responses of fungi may relate to the low temperature which can suppress the response of fungal communities to environmental variation. Fungi can survive and grow in harsh conditions with low temperatures such as glacier and snow by evolving various adaptive features (*Hassan et al., 2016*). These fungi are known as psychrophiles and psychrotrophs. Although they exist widely in cold environments, the optimum temperature for the growth of psychrophilic fungi is around 15 °C and for psychrotrophic fungi is 20 °C (*Gounot, 1986*; *Robinson, 2001*; *Cavicchioli et al., 2002*; *Turchetti et al., 2008*). In glacier-fed streams, water temperature is usually below 10 °C or even close to 0 °C in summer (*Milner & Petts, 1994*). More interestingly, the contrasting response patterns of bacterial and fungal communities were also found in glacier foreland soil ecosystems, where bacterial communities are strongly influenced by the presence of vegetation and environmental heterogeneity and show convergence (*Brown & Jumpponen, 2014*). In contrast to the bacterial communities in glacier foreland soil, fungal richness and diversity were more static and the community structure and distribution show a large extent of stochastic processes across the glacier foreland (*Blaalid et al., 2012*; *Bradley, Singarayer & Anesio, 2014*; *Brown & Jumpponen, 2014*). It has been revealed that bacteria and fungi in headwater streams are similar to communities in adjacent soil due to intimate associations between headwater streams and terrestrial ecosystems

(*Crump, Amaral-Zettler & Kling, 2012*; *Gutiérrez et al., 2015*; *Ruiz-González, Niño-García & Del Giorgio, 2015*). The immigration and advection of allochthonous bacteria and fungi from terrestrial environments can influence bacterial and fungal communities in glacier-fed streams (*Crump, Amaral-Zettler & Kling, 2012*; *Gutiérrez et al., 2015*). The unique response of fungal communities in glacier-fed streams is congruent with the observations in the periglacial soils, suggesting differing trajectories of fungal and BC variations in glacier-fed streams.

The different patterns of bacterial and fungal communities in glacier-fed streams were further supported by network analysis, which showed that the bacterial communities formed a more complex and connected network, while the fungal communities formed a more clustered network (Fig. 4; Table 1). Microbial communities are complex assemblages comprised by highly interactive taxa (*Fuhrman, 2009*; *Jones, Hambright & Caron, 2018*). This study is the first to explore the organization of the bacterial and fungal communities in glacier-fed streams using a co-occurrence approach with consideration of the driving forces. In general, communities with tight co-occurrence interactions and high complexity have a lower stability and are more susceptible to disturbance (*Montoya, Pimm & Sole, 2006*; *Saavedra et al., 2011*). The highly connected and complex bacterial network suggests that bacterial communities in the glacier-fed streams were more sensitive to environmental variations, especially to instream hydrological properties and land vegetation. Moreover, in a complex network, the highly interconnected species are grouped into a module (*Barabási & Oltvai, 2004*; *Newman, 2006*). The strong interrelations among the taxonomic dissimilarities of BC and modules suggest that they had common processes in driving diversity and composition across various environments (*Delgado-Baquerizo et al., 2018*). In contrast, FC and modules generally had distinct driving processes to each other. Moreover, the fungal and bacterial communities also had different driving processes (except FM3 and bacterial communities and modules). Consistent with these findings, the variation of all bacterial modules and FM3 were strongly associated with environmental variation except nutrient dissimilarity, while fungal modules (except FM3) did not respond to environmental variation. Thus, our results suggest that, in our studied area, environmental variation had strong influences on bacterial communities and their assembly mechanisms but not on fungal communities (except one module) in biofilms of glacier-fed streams. The fungal communities may have unique assembly mechanisms which lead to their insensitive responses to environmental variations.

CONCLUSION

Glacier shrinkage imposes significant influences on glacier-fed streams. Integrating beta diversity, our study provides the first co-occurrence network analyses of bacterial and fungal communities in glacier-fed streams. Firstly, this study highlighted that hydrological variables and vegetation status are important components in determining environment heterogeneity of glacier-fed streams and are indicator variables of glacier shrinkage. Then we identified co-occurrence properties of the microbial communities and their responses to environmental variations. Bacterial communities formed a more complex and

connected network, while the fungal communities formed a more clustered network. Nutrients were insignificant to the assemblies of both bacterial and fungal communities in these glacier-fed streams. However, hydrological properties and vegetation status impose significant influences on assemblies of the BC but not on the FC. The results suggest the influences of glacier shrinkage on bacterial communities. However, fungi communities might be insensitive to glacier shrinkage. The results would add our knowledge of microbial community assembly mechanisms and the responses of microbial communities to environmental variations caused by glacier shrinkage.

ADDITIONAL INFORMATION AND DECLARATIONS

Competing Interests
The authors declare that they have no competing interests.

Author Contributions
- Ze Ren conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.

- Hongkai Gao conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.

Data Availability

The following information was supplied regarding data availability:

Data is available at NCBI: SRP115356 and SRP198430.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.7715#supplemental-information.

REFERENCES

Aizen VB, Aizen EM, Melack JM, Dozier J. 1997. Climatic and hydrologic changes in the Tien Shan, Central Asia. *Journal of Climate* **10**:1393–1404 DOI 10.1175/1520-0442(1997)010<1393:CAHCIT>2.0.CO;2.

Anderson MJ, Crist TO, Chase JM, Vellend M, Inouye BD, Freestone AL, Sanders NJ, Cornell HV, Comita LS, Davies KF, Harrison SP, Kraft NJB, Stegen JC, Swenson NG. 2011. Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. *Ecology Letters* **14(1)**:19–28 DOI 10.1111/j.1461-0248.2010.01552.x.

Anesio AM, Lutz S, Chrismas NAM, Benning LG. 2017. The microbiome of glaciers and ice sheets. *NPJ Biofilms Microbiomes* **3(1)**:1–10 DOI 10.1038/s41522-017-0019-0.

Banerjee S, Baah-Acheamfour M, Carlyle CN, Bissett A, Richardson AE, Siddique T, Bork EW, Chang SX. 2016. Determinants of bacterial communities in Canadian agroforestry systems. *Environmental Microbiology* **18(6)**:1805–1816 DOI 10.1111/1462-2920.12986.

Barabási AL, Oltvai ZN. 2004. Network biology: understanding the cell's functional organization. *Nature Reviews Genetics* **5(2)**:101–113 DOI 10.1038/nrg1272.

Barberán A, Bates ST, Casamayor EO, Fierer N. 2012. Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME Journal* **6(2)**:343–351 DOI 10.1038/ismej.2011.119.

Barnett TP, Adam JC, Lettenmaier DP. 2005. Potential impacts of a warming climate on water availability in snow-dominated regions. *Nature* **438(7066)**:303–309 DOI 10.1038/nature04141.

Battin TJ, Besemer K, Bengtsson MM, Romani AM, Packmann AI. 2016. The ecology and biogeochemistry of stream biofilms. *Nature Reviews Microbiology* **14(4)**:251–263 DOI 10.1038/nrmicro.2016.15.

Battin TJ, Kaplan LA, Denis Newbold J, Hansen CME. 2003. Contributions of microbial biofilms to ecosystem processes in stream mesocosms. *Nature* **426(6965)**:439–442 DOI 10.1038/nature02152.

Bissett A, Brown MV, Siciliano SD, Thrall PH. 2013. Microbial community responses to anthropogenically induced environmental change: towards a systems approach. *Ecology Letters* **161**:128–139 DOI 10.1111/ele.12109.

Blaalid R, Carlsen T, Kumar S, Halvorsen R, Ugland KI, Fontana G, Kauserud H. 2012. Changes in the root-associated fungal communities along a primary succession gradient analysed by 454 pyrosequencing. *Molecular Ecology* **21(8)**:1897–1908 DOI 10.1111/j.1365-294X.2011.05214.x.

Borneman J, Hartin RJ. 2000. PCR primers that amplify fungal rRNA genes from environmental samples. *Applied and Environmental Microbiology* **66(10)**:4356–4360 DOI 10.1128/AEM.66.10.4356-4360.2000.

Bradley JA, Singarayer JS, Anesio AM. 2014. Microbial community dynamics in the forefield of glaciers. *Proceedings of the Royal Society B: Biological Sciences* **281(1795)**:e20140882 DOI 10.1098/rspb.2014.0882.

Brittain JE, Milner AM. 2001. Ecology of glacier-fed rivers: current status and concepts. *Freshwater Biology* **46(12)**:1571–1578 DOI 10.1046/j.1365-2427.2001.00845.x.

Brown LE, Hannah DM, Milner AM. 2003. Alpine stream habitat classification: An alternative approach incorporating the role of dynamic water source contributions. *Arctic, Antarctic, and Alpine Research* **35(3)**:313–322 DOI 10.1657/1523-0430(2003)035[0313:ASHCAA]2.0.CO;2.

Brown LE, Hannah DM, Milner AM. 2007. Vulnerability of alpine stream biodiversity to shrinking glaciers and snowpacks. *Global Change Biology* **13(5)**:958–966 DOI 10.1111/j.1365-2486.2007.01341.x.

Brown SP, Jumpponen A. 2014. Contrasting primary successional trajectories of fungi and bacteria in retreating glacier soils. *Molecular Ecology* **23(2)**:481–497 DOI 10.1111/mec.12487.

Brown SP, Rigdon-Huss AR, Jumpponen A. 2014. Analyses of ITS and LSU gene regions provide congruent results on fungal community responses. *Fungal Ecology* **9**:65–68 DOI 10.1016/j.funeco.2014.02.002.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, Mcdonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Tumbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7(5)**:335–336 DOI 10.1038/nmeth.f.303.

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G, Knight R. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME Journal* **6(8)**:1621–1624 DOI 10.1038/ismej.2012.8.

Cardinale BJ, Duffy JE, Gonzalez A, Hooper DU, Perrings C, Venail P, Narwani A, Mace GM, Tilman D, Wardle DA, Kinzig AP, Daily GC, Loreau M, Grace JB, Larigauderie A, Srivastava DS, Naeem S. 2012. Biodiversity loss and its impact on humanity. *Nature* **486(7401)**:59–67 DOI 10.1038/nature11148.

Carlson TN, Ripley DA. 1997. On the relation between NDVI, fractional vegetation cover, and leaf area index. *Remote Sensing of Environment* **62(3)**:241–252 DOI 10.1016/S0034-4257(97)00104-1.

Carlyle CN, Fraser LH, Turkington R. 2014. Response of grassland biomass production to simulated climate change and clipping along an elevation gradient. *Oecologia* **174(3)**:1065–1073 DOI 10.1007/s00442-013-2833-2.

Cavicchioli R, Siddiqui KS, Andrews D, Sowers KR. 2002. Low-temperature extremophiles and their applications. *Current Opinion in Biotechnology* **13(3)**:253–261 DOI 10.1016/S0958-1669(02)00317-8.

Chapin FS III, Zavaleta ES, Eviner VT, Naylor RL, Vitousek PM, Reynolds HL, Hooper DU, Lavorel S, Sala OE, Hobbie SE, Mack MC, Díaz S. 2000. Consequences of changing biodiversity. *Nature* **405(6783)**:234–242 DOI 10.1038/35012241.

Crump BC, Amaral-Zettler LA, Kling GW. 2012. Microbial diversity in arctic freshwaters is structured by inoculation of microbes from soils. *ISME Journal* **6(9)**:1629–1639 DOI 10.1038/ismej.2012.9.

De Menezes AB, Prendergast-Miller MT, Richardson AE, Toscas P, Farrell M, Macdonald LM, Baker G, Wark T, Thrall PH. 2015. Network analysis reveals that bacteria and fungi form

modules that correlate independently with soil parameters. *Environmental Microbiology* **17(8)**:2677–2689 DOI 10.1111/1462-2920.12559.

Delgado-Baquerizo M, Reith F, Dennis PG, Hamonts K, Powell JR, Young A, Singh BK, Bissett A. 2018. Ecological drivers of soil microbial diversity and soil biological networks in the Southern Hemisphere. *Ecology* **99(3)**:583–596 DOI 10.1002/ecy.2137.

Duffy JE, Godwin CM, Cardinale BJ. 2017. Biodiversity effects in the wild are common and as strong as key drivers of productivity. *Nature* **549(7671)**:261–264 DOI 10.1038/nature23886.

Eastwood JA, Yates MG, Thomson AG, Fuller RM. 1997. The reliability of vegetation indices for monitoring saltmarsh vegetation cover. *International Journal of Remote Sensing* **18(18)**:3901–3907 DOI 10.1080/014311697216739.

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27(16)**:2194–2200 DOI 10.1093/bioinformatics/btr381.

Edwards A. 2015. Coming in from the cold: potential microbial threats from the terrestrial cryosphere. *Frontiers in Earth Science* **3**:e12 DOI 10.3389/feart.2015.00012.

Farinotti D, Longuevergne L, Moholdt G, Duethmann D, Mölg T, Bolch T, Vorogushyn S, Güntner A. 2015. Substantial glacier mass loss in the Tien Shan over the past 50 years. *Nature Geoscience* **8(9)**:716–722 DOI 10.1038/ngeo2513.

Figueiredo RO, Markewitz D, Davidson EA, Schuler AE, Watrin ODS, De Souza Silva P. 2010. Land-use effects on the chemical attributes of low-order streams in the eastern Amazon. *Journal of Geophysical Research* **115**:1–14 DOI 10.1029/2009JG001200.

Fuhrman JA. 2009. Microbial community structure and its functional implications. *Nature* **459(7244)**:193–199 DOI 10.1038/nature08058.

Gao H, Ding Y, Zhao Q, Hrachowitz M, Savenije HHG. 2017. The importance of aspect for modelling the hydrological response in a glacier catchment in Central Asia. *Hydrological Processes* **31(16)**:2842–2859 DOI 10.1002/hyp.11224.

Gao H, Han T, Liu Y, Zhao Q. 2016. Use of auxiliary data of topography, snow and ice to improve model performance in a glacier-dominated catchment in Central Asia. *Hydrology Research* **48(5)**:1418–1437 DOI 10.2166/nh.2016.242.

Gao H, Li H, Duan Z, Ren Z, Meng X, Pan X. 2018. Modelling glacier variation and its impact on water resource in the Urumqi Glacier No. 1 in Central Asia. *Science of the Total Environment* **644**:1160–1170 DOI 10.1016/j.scitotenv.2018.07.004.

Gardner AS, Moholdt G, Cogley JG, Wouters B, Arendt AA, Wahr J, Berthier E, Hock R, Pfeffer WT, Kaser G, Ligtenberg SRM, Bolch T, Sharp MJ, Hagen JO, Van Den Broeke MR, Paul F. 2013. A reconciled estimate of glacier contributions to sea level rise: 2003 to 2009. *Science* **340(6134)**:852–857 DOI 10.1126/science.1234532.

Geesey GG, Mutch R, Costerton JW, Green RB. 1978. Sessile bacteria: an important component of the microbial population in small mountain streams. *Limnology and Oceanography* **23(6)**:1214–1223 DOI 10.4319/lo.1978.23.6.1214.

Gounot AM. 1986. Psychrophilic and psychrotrophic microorganisms. *Experientia* **42(11–12)**:1192–1197 DOI 10.1007/BF01946390.

Guo WQ, Liu SY, Yao XJ, Xu JL, Shangguan DH, Wu LZ, Zhao JD, Liu Q, Jiang ZL, Wei JF, Bao WJ, Yu PC, Ding LF, Li G, Li P, Ge CM, Wang Y. 2014. The Second Glacier Inventory Dataset of China (Version 1.0). Cold and Arid Regions Science Data Center at Lanzhou. *Available at http://card.westgis.ac.cn/data/f92a4346-a33f-497d-9470-2b357ccb4246.*

Gutiérrez MH, Galand PE, Moffat C, Pantoja S. 2015. Melting glacier impacts community structure of Bacteria, Archaea and Fungi in a Chilean Patagonia fjord. *Environmental Microbiology* **17(10)**:3882–3897 DOI 10.1111/1462-2920.12872.

Hagg W, Braun LN, Kuhn M, Nesgaard TI. 2007. Modelling of hydrological response to climate change in glacierized Central Asian catchments. *Journal of Hydrology* **332(1–2)**:40–53 DOI 10.1016/j.jhydrol.2006.06.021.

Hannah DM, Brown LE, Milner AM, Gurnell AM, McGregor GR, Petts GE, Smith BPG, Snook DL. 2007. Integrating climate–hydrology–ecology for alpine river systems. *Aquatic Conservation: Marine and Freshwater Ecosystems* **17(6)**:636–656 DOI 10.1002/aqc.800.

Hassan N, Rafiq M, Hayat M, Shah AA, Hasan F. 2016. Psychrophilic and psychrotrophic fungi: a comprehensive review. *Reviews in Environmental Science and Bio/Technology* **15(2)**:147–172 DOI 10.1007/s11157-016-9395-9.

Hawkins JA, Jones SJ, Finkelstein IJ, Press WH. 2018. Indel-correcting DNA barcodes for high-throughput sequencing. *Proceedings of the National Academy of Sciences of the United States of America* **115(27)**:E6217–E6226 DOI 10.1073/pnas.1802640115.

Hood E, Scott D. 2008. Riverine organic matter and nutrients in southeast Alaska affected by glacial coverage. *Nature Geoscience* **1(9)**:583–587 DOI 10.1038/ngeo280.

Hotaling S, Hood E, Hamilton TL. 2017. Microbial ecology of mountain glacier ecosystems: biodiversity, ecological connections, and implications of a warming climate. *Environmental Microbiology* **19(8)**:2935–2948 DOI 10.1111/1462-2920.13766.

Huws SA, Edwards JE, Kim EJ, Scollan ND. 2007. Specificity and sensitivity of eubacterial primers utilized for molecular profiling of bacteria within complex microbial ecosystems. *Journal of Microbiological Methods* **70(3)**:565–569 DOI 10.1016/j.mimet.2007.06.013.

Immerzeel WW, Van Beek LPH, Bierkens MFP. 2010. Climate change will affect the Asian water towers. *Science* **328(5984)**:1382–1385 DOI 10.1126/science.1183188.

IPCC. 2014. *Climate change 2014: synthesis report*. Geneva: Intergovernmental Panel on Climate Change.

Jacobsen D, Dangles O. 2012. Environmental harshness and global richness patterns in glacier-fed streams. *Global Ecology and Biogeography* **21(6)**:647–656 DOI 10.1111/j.1466-8238.2011.00699.x.

Jones AC, Hambright KD, Caron DA. 2018. Ecological patterns among bacteria and microbial Eukaryotes derived from network analyses in a low-salinity lake. *Microbial Ecology* **75(4)**:917–929 DOI 10.1007/s00248-017-1087-7.

Kraaijenbrink PDA, Bierkens MFP, Lutz AF, Immerzeel WW. 2017. Impact of a global temperature rise of 1.5 degrees Celsius on Asia's glaciers. *Nature* **549(7671)**:257–260 DOI 10.1038/nature23878.

Kuhn J, Andino P, Calvez R, Espinosa R, Hamerlik L, Vie S, Dangles O, Jacobsen D. 2011. Spatial variability in macroinvertebrate assemblages along and among neighbouring equatorial glacier-fed streams. *Freshwater Biology* **56(11)**:2226–2244 DOI 10.1111/j.1365-2427.2011.02648.x.

Laghari JR. 2013. Melting glaciers bring energy uncertainty. *Nature* **502(7473)**:617–618 DOI 10.1038/502617a.

Lindström ES, Langenheder S. 2012. Local and regional factors influencing bacterial community assembly. *Environmental Microbiology Reports* **4(1)**:1–9 DOI 10.1111/j.1758-2229.2011.00257.x.

Loreau M, Naeem S, Inchausti P, Bengtsson J, Grime JP, Hector A, Hooper DU, Huston MA, Raffaelli D, Schmid B, Tilman D, Wardle DA. 2001. Biodiversity and ecosystem functioning:

Current knowledge and future challenges. *Science* **294(5543)**:804–808
DOI 10.1126/science.1064088.

Marzeion B, Cogley JG, Richter K, Parkes D. 2014. Attribution of global glacier mass loss to anthropogenic and natural causes. *Science* **345(6199)**:919–921 DOI 10.1126/science.1254702.

Masoud W, Takamiya M, Vogensen FK, Lillevang S, Al-Soud WA, Sørensen SJ, Jakobsen M. 2011. Characterization of bacterial populations in Danish raw milk cheeses made with different starter cultures by denaturing gradient gel electrophoresis and pyrosequencing. *International Dairy Journal* **21(3)**:142–148 DOI 10.1016/j.idairyj.2010.10.007.

Milner AM, Brittain JE, Castella E, Petts GE. 2001. Trends of macroinvertebrate community structure in glacier-fed rivers in relation to environmental conditions: a synthesis. *Freshwater Biology* **46(12)**:1833–1847 DOI 10.1046/j.1365-2427.2001.00861.x.

Milner AM, Brown LE, Hannah DM. 2009. Hydroecological response of river systems to shrinking glaciers. *Hydrological Processes* **23(1)**:62–77 DOI 10.1002/hyp.7197.

Milner AM, Khamis K, Battin TJ, Brittain JE, Barrand NE, Fuereder L, Cauvy-Fraunie S, Gislason GM, Jacobsen D, Hannah DM, Hodson AJ, Hood E, Lencioni V, Olafsson JS, Robinson CT, Tranter M, Brown LE. 2017. Glacier shrinkage driving global changes in downstream systems. *Proceedings of the National Academy of Sciences of the United States of America* **114(37)**:9770–9778 DOI 10.1073/pnas.1619807114.

Milner AM, Petts GE. 1994. Glacial rivers: physical habitat and ecology. *Freshwater Biology* **32(2)**:295–307 DOI 10.1111/j.1365-2427.1994.tb01127.x.

Montoya JM, Pimm SL, Sole RV. 2006. Ecological networks and their fragility. *Nature* **442(7100)**:259–264 DOI 10.1038/nature04927.

Mori AS, Isbell F, Seidl R. 2018. β-Diversity, Community assembly, and Ecosystem functioning. *Trends in Ecology & Evolution* **33(7)**:549–564 DOI 10.1016/j.tree.2018.04.012.

Morris JH, Apeltsin L, Newman AM, Baumbach J, Wittkop T, Su G, Bader GD, Ferrin TE. 2011. clusterMaker: a multi-algorithm clustering plugin for Cytoscape. *BMC Bioinformatics* **12(1)**:e436 DOI 10.1186/1471-2105-12-436.

Narama C, Kääb A, Duishonakunov M, Abdrakhmatov K. 2010. Spatial variability of recent glacier area changes in the Tien Shan Mountains, Central Asia, using Corona (~1970), Landsat (~2000), and ALOS (~2007) satellite data. *Global and Planetary Change* **71(1–2)**:42–54 DOI 10.1016/j.gloplacha.2009.08.002.

Nelson CE, Sadro S, Melack JM. 2009. Contrasting the influences of stream inputs and landscape position on bacterioplankton community structure and dissolved organic matter composition in high-elevation lake chains. *Limnology and Oceanography* **54(4)**:1292–1305 DOI 10.4319/lo.2009.54.4.1292.

Newman MEJ. 2006. Modularity and community structure in networks. *Proceedings of the National Academy of Sciences of the United States of America* **103(23)**:8577–8582 DOI 10.1073/pnas.0601602103.

Nielsen A, Trolle D, Søndergaard M, Lauridsen TL, Bjerring R, Olesen JE, Jeppesen E. 2012. Watershed land use effects on lake water quality in Denmark. *Ecological Applications* **22(4)**:1187–1200 DOI 10.1890/11-1831.1.

Oksanen J, Kindt R, Legendre PO, Hara B, Stevens MHH, Oksanen MJ, Suggests MASS. 2007. The vegan package. *Community Ecology Package* **10**:631–637.

Olesen JM, Bascompte J, Dupont YL, Jordano P. 2007. The modularity of pollination networks. *Proceedings of the National Academy of Sciences of the United States of America* **104(50)**:19891–19896 DOI 10.1073/pnas.0706375104.

Porter MA, Onnela JP, Mucha PJ. 2009. Communities in networks. *Notices of the AMS* **56**:1082–1097.

R Development Core Team. 2017. *R: a language and environment for statistical computing.* Vienna: R Foundation for Statistical Computing. *Available at https://www.R-project.org*.

Raynolds M, Magnusson B, Metusalemsson S, Magnusson SH. 2015. Warming, sheep and volcanoes: land cover changes in Iceland evident in satellite NDVI trends. *Remote Sensing* **7(8)**:9492–9506 DOI 10.3390/rs70809492.

Ren Z, Gao H, Elser JJ. 2017. Longitudinal variation of microbial communities in benthic biofilms and association with hydrological and physicochemical conditions in glacier-fed streams. *Freshwater Science* **36(3)**:479–490 DOI 10.1086/693133.

Ren Z, Gao H, Elser JJ, Zhao Q. 2017. Microbial functional genes elucidate environmental drivers of biofilm metabolism in glacier-fed streams. *Scientific Reports* **7(1)**:e12668 DOI 10.1038/s41598-017-13086-9.

Ren Z, Niu D, Ma P, Wang Y, Fu H, Elser JJ. 2019. Cascading influences of grassland degradation on nutrient limitation in a high mountain lake and its inflow streams. *Ecology* **100**:e02755 DOI 10.1002/ecy.2755.

Robinson CH. 2001. Cold adaptation in Arctic and Antarctic fungi. *New Phytologist* **151(2)**:341–353 DOI 10.1046/j.1469-8137.2001.00177.x.

Robinson CT, Jolidon C. 2005. Leaf breakdown and the ecosystem functioning of alpine streams. *Journal of the North American Benthological Society* **24(3)**:495–507 DOI 10.1899/04-100.1.

Robinson CT, Thompson C, Freestone M. 2014. Ecosystem development of streams lengthened by rapid glacial recession. *Fundamental and Applied Limnology* **185(3)**:235–246 DOI 10.1127/fal/2014/0667.

Rouse JW, Haas RH, Schell JA, Deering DW. 1974. Monitoring vegetation systems in the Great Plains with ERTS. In: *Third Earth Resources Technology Satelite-1 Symposium, Washington, D.C., USA,* 309–317.

Ruiz-González C, Niño-García JP, Del Giorgio PA. 2015. Terrestrial origin of bacterial communities in complex boreal freshwater networks. *Ecology Letters* **18(11)**:1198–1206 DOI 10.1111/ele.12499.

Saavedra S, Stouffer DB, Uzzi B, Bascompte J. 2011. Strong contributors to network persistence are the most vulnerable to extinction. *Nature* **478(7368)**:233–235 DOI 10.1038/nature10433.

Sadro S, Nelson CE, Melack JM. 2012. The influence of landscape position and catchment characteristics on aquatic biogeochemistry in high-elevation lake-chains. *Ecosystems* **15(3)**:363–386 DOI 10.1007/s10021-011-9515-x.

Sertic Peric M, Jolidon C, Uehlinger U, Robinson CT. 2015. Long-term ecological patterns of alpine streams: an imprint of glacial legacies. *Limnology and Oceanography* **60(3)**:992–1007 DOI 10.1002/lno.10069.

Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Research* **13(11)**:2498–2504 DOI 10.1101/gr.1239303.

Shi S, Nuccio EE, Shi ZJ, He Z, Zhou J, Firestone MK. 2016. The interconnected rhizosphere: high network complexity dominates rhizosphere assemblages. *Ecology Letters* **19(8)**:926–936 DOI 10.1111/ele.12630.

Socolar JB, Gilroy JJ, Kunin WE, Edwards DP. 2016. How should beta-diversity inform biodiversity conservation? *Trends in Ecology & Evolution* **31(1)**:67–80 DOI 10.1016/j.tree.2015.11.005.

Sorg A, Bolch T, Stoffel M, Solomina O, Beniston M. 2012. Climate change impacts on glaciers and runoff in Tien Shan (Central Asia). *Nature Climate Change* **2(10)**:725–731 DOI 10.1038/nclimate1592.

Thompson JN. 2005. *The geographic mosaic of coevolution*. Chicago: University of Chicago Press.

Tilman D, Isbell F, Cowles JM. 2014. Biodiversity and ecosystem functioning. *Annual Review of Ecology, Evolution, and Systematics* **45(1)**:471–493 DOI 10.1146/annurev-ecolsys-120213-091917.

Toju H, Kishida O, Katayama N, Takagi K. 2016. Networks depicting the fine-scale co-occurrences of fungi in soil horizons. *PLOS ONE* **11(11)**:e0165987 DOI 10.1371/journal.pone.0165987.

Turchetti B, Buzzini P, Goretti M, Branda E, Diolaiuti G, D'Agata C, Smiraglia C, Vaughan-Martini A. 2008. Psychrophilic yeasts in glacial environments of Alpine glaciers. *FEMS Microbiology Ecology* **63(1)**:73–83 DOI 10.1111/j.1574-6941.2007.00409.x.

Unger-Shayesteh K, Vorogushyn S, Farinotti D, Gafurov A, Duethmann D, Mandychev A, Merz B. 2013. What do we know about past changes in the water cycle of Central Asian headwaters? A review. *Global and Planetary Change* **110**:4–25 DOI 10.1016/j.gloplacha.2013.02.004.

Von Schiller D, Marti E, Riera JL, Sabater F. 2007. Effects of nutrients and light on periphyton biomass and nitrogen uptake in Mediterranean streams with contrasting land uses. *Freshwater Biology* **52(5)**:891–906 DOI 10.1111/j.1365-2427.2007.01742.x.

Weiss S, Van Treuren W, Lozupone C, Faust K, Friedman J, Deng Y, Xia LC, Xu ZZ, Ursell L, Alm EJ, Birmingham A, Cram JA, Fuhrman JA, Raes J, Sun F, Zhou J, Knight R. 2016. Correlation detection strategies in microbial data sets vary widely in sensitivity and precision. *ISME Journal* **10(7)**:1669–1681 DOI 10.1038/ismej.2015.235.

Wilhelm L, Besemer K, Fasching C, Urich T, Singer GA, Quince C, Battin TJ. 2014. Rare but active taxa contribute to community dynamics of benthic biofilms in glacier-fed streams. *Environmental Microbiology* **16(8)**:2514–2524 DOI 10.1111/1462-2920.12392.

Wilhelm L, Singer GA, Fasching C, Battin TJ, Besemer K. 2013. Microbial biodiversity in glacier-fed streams. *ISME Journal* **7(8)**:1651–1660 DOI 10.1038/ismej.2013.44.

Zemp M, Frey H, Gärtner-Roer I, Nussbaumer SU, Hoelzle M, Paul F, Haeberli W, Denzinger F, Ahlstrøm AP, Anderson B, Bajracharya S, Baroni C, Braun LN, Cáceres BE, Casassa G, Cobos G, Dávila LR, Delgado Granados H, Demuth MN, Espizua L, Fischer A, Fujita K, Gadek B, Ghazanfar A, Ove Hagen J, Holmlund P, Karimi N, Li Z, Pelto M, Pitte P, Popovnin VV, Portocarrero CA, Prinz R, Sangewar CV, Severskiy I, Sigurðsson O, Soruco A, Usubaliev R, Vincent C. 2015. Historically unprecedented global glacier decline in the early 21st century. *Journal of Glaciology* **61(228)**:745–762 DOI 10.3189/2015JoG15J017.

Zemp M, Haeberli W, Hoelzle M, Paul F. 2006. Alpine glaciers to disappear within decades? *Geophysical Research Letters* **33(13)**:L1350413 DOI 10.1029/2006GL026319.

Zhang Y, Gao J, Liu L, Wang Z, Ding M, Yang X. 2013. NDVI-based vegetation changes and their responses to climate change from 1982 to 2011: a case study in the Koshi River Basin in the middle Himalayas. *Global and Planetary Change* **108**:139–148 DOI 10.1016/j.gloplacha.2013.06.012.

Zhang G, Li Z, Wang W, Wang W. 2014. Rapid decrease of observed mass balance in the Urumqi Glacier No. 1, Tianshan Mountains, central Asia. *Quaternary International* **349**:135–141 DOI 10.1016/j.quaint.2013.08.035.