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ORIGINAL PAPER

Bacterial diversity within five unexplored freshwater lakes interconnected by surface channels in East Antarctic Dronning Maud Land (Schirmacher Oasis) using amplicon pyrosequencing

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Abstract The Schimacher Oasis, an ice-free plateau in East Antarctic Dronning Maud Land, consists of over 120 freshwater lakes. These lakes are connected largely through four major surface channels. The bacterial diversity in these lake ecosystems remains largely unexplored. In this study, we compared the bacterial diversity in five freshwater lakes (L42, L46, L47, L50, and L51) interconnected by two surface channels using bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) method. We further compared the resultant bacterial composition from these five lakes with another freshwater lake in the Schirmacher Oasis, Lake Tawani(P), which is not connected through the same surface channels. Using bTEFAP, we differentiated nine different phyla with the phyla Proteobacteria (especially the class Alphaproteobacteria) and Bacteroidetes (the class Sphingobacteria) dominating in lakes interconnected by surface channel 1, while the phyla Chloroflexi and Firmicutes were highly abundant in lakes interconnected by surface channel 2. The operational taxonomic unit (OTU) network and Principle Coordinate Analysis (PCoA) plot based on unweighted UNIFRAC determined that the bacterial assemblages found in these five lakes are different than the bacterial composition residing in Lake Tawani(P). The distribution and the diversity of the bacterial communities in Schirmacher Oasis freshwater lakes that are connected through surface channels may provide an insight into the role of the extreme physico-chemical parameters that help shape microbially driven functional ecosystems in other oases on this icy continent.

Keywords Antarctic freshwater lakes · Biodiversity · Qiime · 16S rRNA · Lake Tawani(P) · Heterotrophs

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Introduction

Antarctic oases are rocky landscapes that often consist of freshwater lakes and a ranging topography through various surface geomorphology and elevation. These oases were formed due to glaciation and tectonic lineation processes (Paech and Stackebrandt 1995; Richter and Bormann 1995). The Schirmacher Oasis (latitude 70°43′50″S–70°46′40″S and longitude 11°22′40″E–11°54′25″E) is approximately 17-km-long and 2–3-km-wide exposed bedrock spanning East to West of the Central Dronning Maud Land of East Antarctica and consists of over 120 freshwater lakes (Ravindra et al. 2002). This Oasis is surrounded by the Schirmacher Hills, a continental ice sheet in the south and an ice shelf to the north (Verlecar et al. 1996; Ravindra et al. 2002). Most of the lakes in the Schirmacher Oasis are landlocked freshwater category that were formed by natural processes of ice erosion and meltwater carried by surface channels from snow beds and ice slopes (Richter and Bormann 1995; Verlecar et al. 1996; Ravindra et al. 2002; Priscu and Foreman 2009). Previous ^{14}C isotopic analyses suggest that these lakes emerged approximately 10,000 years before present (BP) during the global warming event of the Holocene epoch (Bera 2004, 2006; Ellis-Evans 1996; Ingolfsson 2004; Laluraj

et al. 2010; Sinha et al. 2000 a, b). Previous studies of the bacterial diversity in the Schirmacher Oasis freshwater lakes and East Antarctic lakes reported an abundance of cyanobacteria and heterotrophic bacteria (Komarek and Ruzicka 1966; Matondkar and Gomes 1983; Sengupta and Qasim 1983; Shivaji et al. 1989, 2004, 2011; Laybourn-Parry and Marchant 1992; Ellis-Evans et al. 1998; Alam et al. 2003, 2006; Pandey et al. 2004; Arnaud et al. 2006; Singh et al. 2006; Mojib et al. 2008, 2009).

Unlike perennially ice-covered lakes in the Antarctic continent, the freshwater lakes in the Schirmacher Oasis are modulated primarily by annual weather cycles (wind, solar radiation during summer months, and seasonal/diurnal freeze–thaw cycles), resulting in the intermixing of lake waters through surface channels and the microbial communities inhabiting these lakes. These surface channels are known to carry ions, minerals, and nutrients that influence the physico-chemical parameters of the downstream lakes and the microbial communities inhabiting these lakes (Green and Canfield 1984; Stanish et al. 2012, 2013). Recently, Stanish et al. (2012) explored the heterotrophic and cyanobacterial assemblages residing within microbial mats in the McMurdo Dry Valleys (MCMDV) streams (Green, Onyx, Miers, and Commonwealth) of Antarctica but to our knowledge, no study has reported thus far the bacterial diversity in lakes that are connected by surface channels in Schirmacher Oasis. In contrast to the Wright Valley hydrology, 4 major surface channels connect most of the 120 distinct freshwater lakes in Schirmacher Oasis and the seasonal melting of ice and snow, katabatic winds and rising temperature in this region together contribute to form a microbial-dominated biologically active large freshwater lacustrine ecosystem.

We predict that during the Antarctic summer months, the snow and glacial meltwater intermix the bacterial population in lakes connected through surface channels. The objective of this study was to determine the relatedness of the bacterial communities in different seasonally ice-covered freshwater lakes that are interconnected through surface channels by comparing the 16S rRNA sequences, which were determined by bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) on metacommunity DNA. Additionally, we compared the bacterial diversity with a previously studied landlocked freshwater lake, Lake Tawani(P) (S70°45′13.6″S; E11°41′25.7″; Huang et al. 2013), that is also located within the Schirmacher Oasis but not connected by the same surface channels.

Materials and methods

Study sites and sample preparation

Five seasonal freshwater lakes interconnected by 2 distinct surface channels in the Schirmacher Oasis were selected in this study (Fig. 1). Surface channel 1 interconnects lakes L50 (S70°45.381′ E11°44.182′), L46 (S70°45.439′ E11°43.902′), and L42 (S70°45.998′ E11°42.460′); and channel 2 connects L50 (S70°45.381′ E11°44.182′), L51 (S70°45.525′ E11°44.145′) and L47 (S70°45.693′ E11°43.652′). Both surface channels begin with melted water flowing from the ice sheet located south of the Schirmacher Oasis. From here, surface channel 1 carries water into L42, then into L46 and finally into L50 while in surface channel 2, water drains from Lake Zub (Lake Zub = Russian nomenclature; Lake Priyadarshini = Indian nomenclature) (location of the Indian Antarctic Station, *Maitri*) into L51 followed by L50 (Fig. 1).

From each lake, a mixture of water and the surface sediment (50 ml) was collected in November 2008 at the rock–water interface (10–15 cm depth) from 3 adjacent locations (within a 10 ft radius). This interface is where freeze–thaw cycles occur as the freshwater systems transition from the polar winter to the summer months due to daily temperature variations (Fig. 1). After collection, samples were kept cold at 2–4 °C in an insulated bag with blue ice packs and then stored at −20 °C in *Maitri* Station; samples were then transported and stored at −20 °C in the laboratory at UAB until use. Metacommunity DNA from each of the 3 samples (50 ml each) collected from each lake was purified first by centrifugation (15,000*g* for 30 min at 4 °C) of the bacterial cells and then cell lysis and DNA recovery by using a MOBIO Power Soil DNA isolation kit (http://www.mobio.com/) (Huang et al. 2013). Delmont et al. (2012) compared different DNA purification kits, and based on their study, we selected MOBIO kit for the purification of metacommunity DNA in this and a previously reported study (Huang et al. 2013).

Purified DNA from each of the 3 samples was pooled, and the concentration (at 260 nm wavelength) and the purity (a ratio between 260 and 280 nm wavelengths) were determined using a Lambda II (Perkin Elmer) spectrophotometer. The DNA was then dried in a Savant Speedvac Evaporator SVC 100H, and stored at 4 °C until pyrosequencing (Huang et al. 2013).

Parallel bacterial tag-encoded FLX Amplicon pyrosequencing (bTEFAP) and downstream bioinformatics

Equal quantity (100 ng) of metacommunity DNA from each lake sample was subjected to bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) targeting the V3–V5 segment of the bacterial 16S rRNA gene (Dowd et al. 2008). The initial generation of the genomic library was performed by utilizing a one-step PCR with a total of 30 cycles of amplification (Dowd et al. 2008), using the oligonucleotide primers 341F (5′–CCT ACG GGA GGC

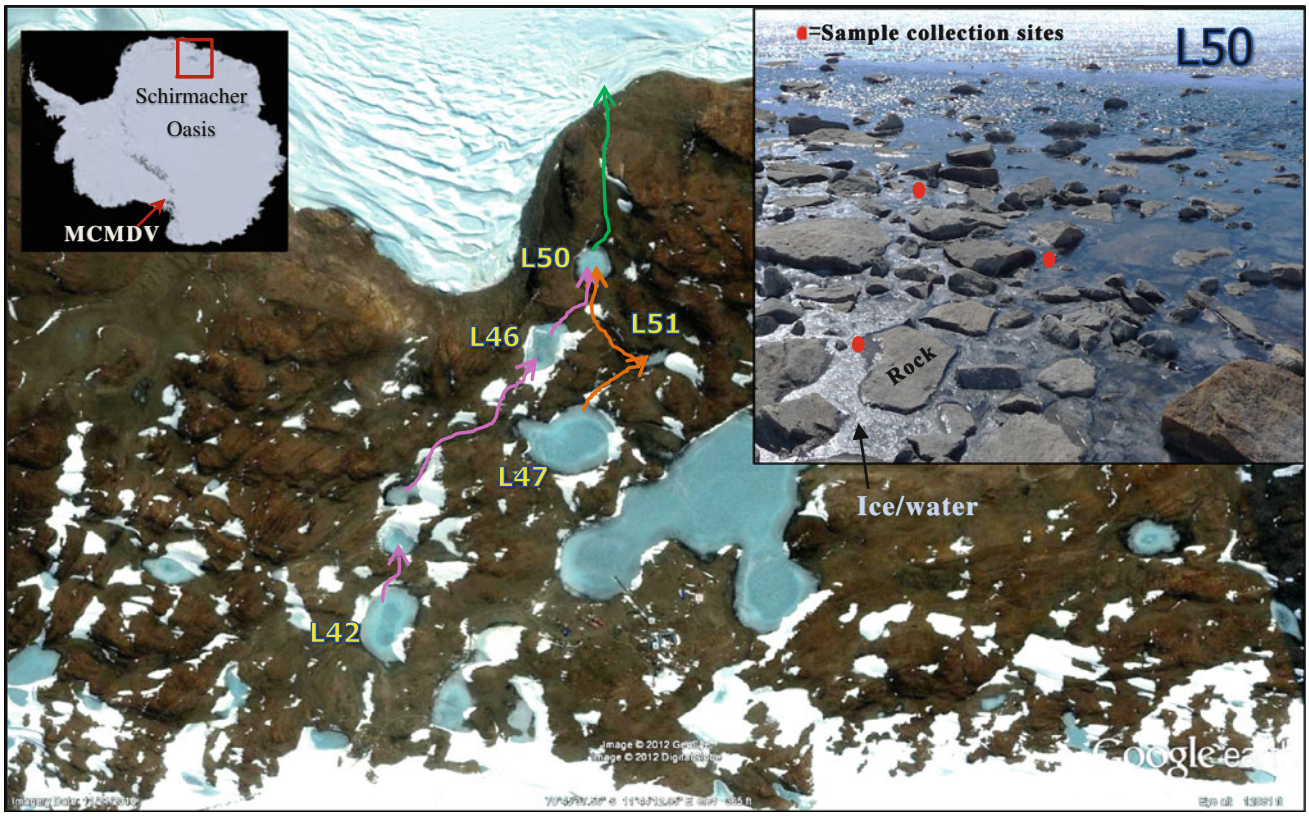

Fig. 1 Location of the five lakes (L50, L46, L42, L51, and L47) and the two surface channels that interconnects them in the Schirmacher Oasis, Antarctica. Image was obtained and then expanded from Google Earth (http://maps.google.com). Surface channel 1 interconnects lakes L50 (S70°45.381′ E11°44.182′), L46 (S70°45.439′ E11°43.902′), and L42 (S70°45.998′ E11°42.460′) while surface channel 2 connects L50 (S70°45.381′ E11°44.182′), L51 (S70°45.525′ E11°44.145′), and L47 (S70°45.693′ E11°43.652′). The arrows indicate the linement of the two surface channels in purple (surface channel 1) and orange (surface channel 2). These two surface channels join at L50, which is depicted in green and drain into the ice shelf to the north. *Left top inset* the location of the Schirmacher Oasis in relation to the McMurdo Dry Valleys (MCMDV). *Right top inset* a representative picture of Lake L50 showing the ice water–rock interface from where the sediment-mixed water samples were collected. *Red dots* indicate the locations (within an approximately 10 ft radius) of sample collection. Similar locations were used for all other lakes described in this study. (Color figure online)

AGC AG–3′ (Muyzer et al. 1993) and 907R (5′–CCG TCA ATT CMT TTG AGT TT–3′ (Lane et al. 1985) in a mixture of Hot Start and HotStar high-fidelity *Taq* DNA polymerase enzymes. After establishing the genomic library, pyrosequencing was conducted on a Roche 454 FLX with Titanium reagents (Roche, Indianapolis, IN) following the protocols described by the manufacturer (Dowd et al. 2008). All sequencing reactions were conducted by the Research and Testing Laboratories (RTL, Lubbock, TX) (www.researchandtesting.com).

The generated bTEFAP reads were analyzed and processed using QIIME 1.3.0 workflow (http://www.qiime.org/) as described by Caporaso et al. (2010). Briefly, QIIME processes 16S rRNA gene sequences (including chimera checking using integrated AmpliconNoise and ChimeraSlayer packages), clusters them into operational taxonomical units (OTUs) using uclust defined at 97 % sequence similarity (http://drive5.com/usearch/usearch3.0.html), classifies using the Ribosomal Database Project (RDP) classifier at >50 % confidence (http://rdp.cme.msu.edu/), aligns them using Pynast (http://pynast.sourceforge.net/), constructs phylogenetic trees using FastTree2 (http://www.microbesonline.org/fasttree/), and generates data summaries of the proportions of taxa.

Sequences obtained from Genebank for Lake Tawani(P) and Bioinformatics analyses

Sequences from Lake Tawani(P) were obtained through Genbank Short Read Archive under the accession SRP010437, added to sequences from the 5 sample sites, and an OTU table was generated through Qiime 1.3.0 as described above. A Principle Coordinate Analysis (PCoA) plot based on unweighted UniFrac distances (http://bmf.colorado.edu/unifrac/) was conducted to statistically compare the sequences obtained from the five sampling sites in the Schirmacher Oasis and Lake Tawani(P). An OTU table generated by Qiime was modified and exported into

Cytoscape (http://www.cytoscape.org/) to visualize as a neural network (Pope et al. 2010). Cytoscape allows OTU–OTU interactions to be mapped as an OTU network, which represent the microbial similarities and differences between locations.

Statistical analysis

The Shannon diversity index was defined as $H' = \sum_{i=1}^{s} (p_i \ln p_i)$ where s is the number of OTUs in the sample and p_i is the proportion of the organisms in the sample represented by the ith OTU (Shannon and Weaver 1964).

Results

The bTEFAP generated a total of 3,400, 2,928, 3,200, 2,984, and 2,400 preprocessed quality sequences from the lakes L42, L46, L47, L50, and L51 samples, respectively. The sequences were clustered into 355 distinct OTUs at 97 % sequence similarity. Rarefaction curves indicated the total quality sequences for each of the sampling sites either reached saturation or are approaching saturation when constructed at 3 % sequence variation (Fig. 2). Overall, the use of bTEFAP and downstream Bioinformatics identified a total of nine distinct phyla (Fig. 3; Table 1).

The comparison of bacterial distribution in the targeted lakes suggests that surface channels play a role in intermixing of bacteria between the freshwater lakes in Schirmacher Oasis. The bacterial diversity in L50, L46, and L42 interconnected through surface channel 1 exhibited an overall similar distribution in which the phylum Proteobacteria was found to be the dominant taxon (Fig. 3; Table 1). Within the phylum Proteobacteria, the class Alphaproteobacteria was the most abundant representing 56.93, 37.32, and 42.95 % of total bacterial reads in L50, L46, and L42, respectively (Table 2). Following phylum Proteobacteria, the phylum Bacteroidetes was the next most abundant taxon at 13.86 % (L50), 28.82 % (L46), and 20.14 % (L42) except phylum Chloroflexi, which highly represented in L50 (16.17 %) (Fig. 3; Table 1). Within phylum Bacteroidetes, the class Sphingobacteria represented 13.45, 24.32, and 20.11 % of total reads in L50, L46, and L42, respectively (Table 2). Minor phyla (Acidobacteria, Actinobacteria, Deinococcus-Thermus, Firmicutes, Gemmatimonadetes, and OP10) were not as well represented in all three lakes connected through surface channel 1, except the phyla Deinoccous-Thermus and Firmicutes were abundant in L42 (11.87 and 13.84 %, respectively), while the phyla Gemmatimonadetes and Actinobacteria were well represented in L46 (9.50 and 9.26 %, respectively) (Fig. 3; Table 1).

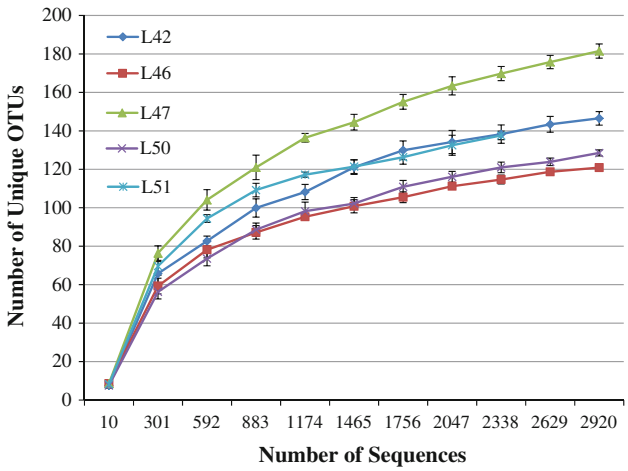

Fig. 2 Rarefaction *curve* of the sequences obtained from the bacterial 16S rRNA genes generated by bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) from Lakes L50, L46, L42, L51, and L47. Rarefaction *curves* derived through the accumulation of unique operation taxonomical units (OTUs) at 3 % sequence variations, and *standard deviation bars* were calculated by Qiime 1.3.0

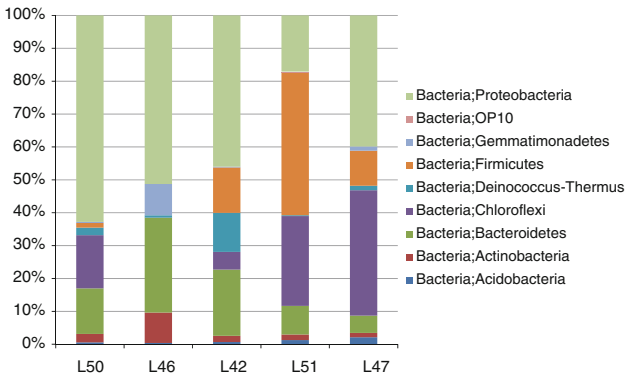

Fig. 3 Relative abundances of the bacterial phyla found in L50, L46, L42, L51, and L47 lakes based on the operational taxonomic units (OTUs) obtained by bTEFAP of amplified 16S rRNA genes. The RDP classifier used to determine the bacterial taxonomy was >50 % confidence through Qiime 1.3.0 workflow. Each bar represents pooled metacommunity DNA from 3 samples from the respective lake. (Color figure online)

Unlike the lakes interconnected through surface channel 1, the comparison of bacterial taxa in lakes L51 and L47 interconnected by surface channel 2 exhibited the phylum Firmicutes (43.39 %) to be the dominant taxon in L51 and the phylum Proteobacteria (39.85 %) in L47. Both lakes had a rich population of the phylum Chloroflexi (27.31 % for L51 and 38.14 % for L47) (Fig. 3; Table 1). In addition, an overall decrease in the number of representatives from the phylum Bacteroidetes (8.68 % in L51 and 5.27 % in L47) and the phylum Proteobacteria (17.07 % in L51) was noticed when compared with the lakes that are interconnected by the surface channel 1. Furthermore, there was a notable decrease in the abundance of class Alphaproteobacteria in L51

Table 1 The relative bacterial abundance and distribution of the different phyla identified in five seasonal freshwater lakes in Schirmacher Oasis

Taxon	L50 (%)	L46 (%)	L42 (%)	L51 (%)	L47 (%)
Bacteria; Acidobacteria	0.58	0.40	0.67	1.28	2.14
Bacteria; Actinobacteria	2.56	9.26	1.90	1.71	1.31
Bacteria; Bacteroidetes	13.86	28.82	20.14	8.68	5.27
Bacteria; Chloroflexi	16.17	0.04	5.37	27.31	38.14
Bacteria; Deinococcus-Thermus	2.31	0.72	11.87	0.28	1.35
Bacteria; Firmicutes	1.40	0.00	13.84	43.39	10.67
Bacteria; Gemmatimonadetes	0.33	9.50	0.23	0.00	1.27
Bacteria; OP10	0.00	0.00	0.00	0.28	0.00
Bacteria; Proteobacteria	62.79	51.25	45.98	17.07	39.85

Bacterial relative abundance and identification were conducted using Qiime 1.3.0 workflow

Table 2 The relative abundance of the different dominant taxa (class) found in each of the different lakes from this study

Taxon	L50 (%)	L46 (%)	L42 (%)	L51 (%)	L47 (%)
Phylum Proteobacteria					
Class Alphaproteobacteria	56.93	37.32	42.95	16.79	34.34
Class Betaproteobacteria	1.32	13.85	2.27	0.28	2.30
Class Gammaproteobacteria	0.00	0.00	0.03	0.00	0.28
Class Deltaproteobacteria	1.07	0.08	0.47	0.00	2.34
Phylum Bacteroidetes					
Class Sphingobacteria	13.45	24.32	20.11	8.11	5.19
Phylum Chloroflexi					
Class Anaerolineae	0.00	0.04	1.30	15.93	5.99
Class Caldilineae	11.72	0.00	0.40	1.56	7.93
Others	4.46	0.00	3.47	9.82	24.19
Phylum Deinococcus-Thermus					
Deinococcus	2.31	0.72	11.87	0.28	1.35
Phylum Firmicutes					
Others	1.40	0.00	13.84	43.39	10.67

Bacterial taxa were identified through the amplification of the partial 16S rRNA gene (V3–V5) through bTEFAP. Resulting sequences were then differentiated using RDP Classifier (>50 % confidence) within the Qiime 1.3.0 workflow

(16.79 %) and in class Sphingobacteria for both L51 (8.11 %) and L47 (5.19 %) (Table 2).

Despites these differences in bacterial abundance within taxa, overall the analyses of the PCoA plot and the OTU network (Fig. 4) indicate commonness of the bacterial communities in these seasonally ice-covered lakes. Similarly, the Shannon and Weaver diversity index predicts a similar diverse bacterial community between each of these Schirmacher Oasis freshwater lakes (4.768–5.719; Tables 2, 3).

In this study, the comparison of the 16S rRNA partial gene sequences from the Lake Tawani (P) with the lakes interconnected by surface channels exhibited similar bacterial distribution where phylum Proteobacteria dominated the bacterial composition followed by the phylum Bacteroidetes. Although Lake Tawani(P) also had a relative high abundance of members from the taxon Chloroflexi (11.33 %), which is consistent with the bacterial composition in the lakes within surface channel 2. However, Lake Tawani(P) consisted of relatively higher occurrence of bacteria belonging to the phyla Actinobacteria and Acidobacteria, which had a relatively low abundance in the current study. Moreover, the construction of OTU network and unweighted UNIFRAC-based PCoA plot displayed a different bacterial community composition in Lake Tawani(P) as compared to the lakes in this study (Fig. 4).

Discussion

Unlike other ice-free oases in Antarctic continent, the lacustrine systems in the Schirmacher Oasis uniquely harbor over 120 seasonally ice-covered freshwater lakes; most of them are interconnected through surface channels. The flow of meltwater through these surface channels causes an intermixing of nutrients and minerals that influence physical–chemical parameters and microbial composition of these lakes. In this study, we investigated the bacterial composition in lakes L50, L46, and L42 interconnected by surface channel 1. These lakes displayed similar bacterial phyla with the majority of taxa belonging to the phylum Proteobacteria, Bacteroidetes, and Chloroflexi. However, lakes L50, L51, and L47 interconnected by surface channel 2 had a slightly different microbial composition where the phyla Proteobacteria, Firmicutes, and Chloroflexi dominated the microbial fauna. Since L50 is interconnected by both surface channels, it had similar qualities in microbial composition from both channels.

Previous to this study, we explored the bacterial community in another nearby freshwater lake in the Schirmacher Oasis, Lake Tawani(P), which is not connected to any of the five lakes explored in this study (Huang et al. 2013). The bacterial composition in Lake Tawani(P) resembled the diversity found in the lakes connected by the surface channel 1. Similar to the bacterial communities in surface channel 1, the phylum Proteobacteria dominated the bacterial composition followed by the phylum Bacteroidetes in Lake Tawani(P). Even though Lake Tawani(P) and the lakes in this study are not directly interconnected by surface channels, the commonality between bacterial taxa was expected since katabatic winds also play a role in

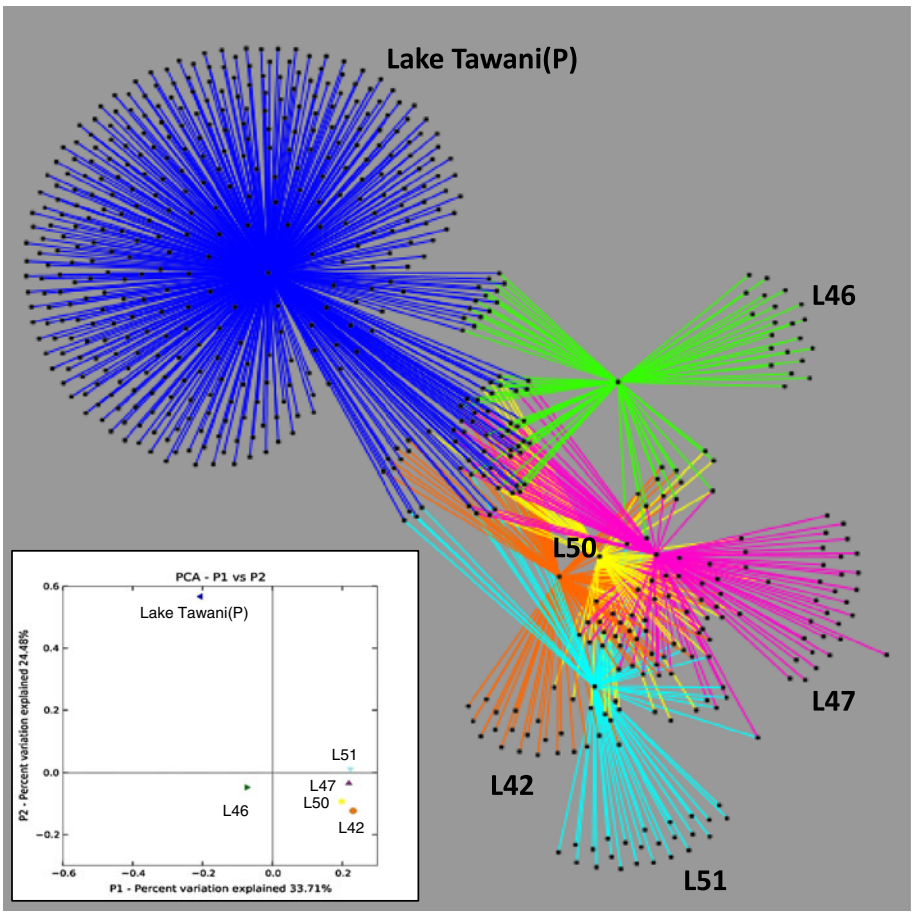

Fig. 4 Operational taxonomic unit (OTU) network map showing OTU interactions among all rarefied samples from the lakes identified in this study. Sequences for Lake Tawani(P) were obtained through the NCBI database (www.ncbi.nlm.nih.gov). OTU network was created using force-directed layout with weighted edges based on the number of sequences in an OTU. Edges radiating from the Lake Tawani (P) are represented in *blue*, L46 in *green*, L50 in *yellow*, L47 in *purple*, L51 in *turquoise*, and L42 in *brown*. The *inset* displays the PCoA plot that corresponds to the lakes from the aforementioned OTU network map. (Color figure online)

Table 3 Shannon Diversity and OTU richness (clustered at 97 %) calculated for the bacterial 16S rRNA gene analyses of L47, L51, L50, L46, and L42 using Qiime 1.3.0

Land-locked lakes	Shannon diversity index	OTU richness
L47	5.719	160
L51	5.347	85
L50	4.768	89
L46	5.320	110
L42	5.112	146

Bacterial diversity within five unexplored freshwater lakes interconnected by surface channels in East Antarctic Dronning Maud Land (Schirmacher Oasis) using amplicon pyrosequencing

intermixing bacterial populations within the Schirmacher Oasis lakes. However, the phyla Verrucomicrobia, Candidate Division TM7, and planctomycetes, which are generally unculturable, were only found in Lake Tawani(P). The difference could be attributed to differing phyisco-chemical parameters between Lake Tawani(P) and the five lakes in the current study. Overall, the PCoA (unweighted UNIFRAC) and OTU network indicated that Lake Tawani(P) had a dissimilar bacterial composition as compared to the lakes used in this study (Fig. 4), thus suggesting that surface channels may play important role in the establishment and sustenance of bacterial population in the Schirmacher Oasis freshwater lake ecosystems.

A recent study examined the microbial diversity found in streams, Green, Onyx, Miers, and Commonwealth, in the Antarctic MCMDV (Shaw and Healy 1980; Stanish et al. 2012; Stones et al. 2012). Stanish et al. (2012) primarily investigated the diatoms and their associated bacteria by targeting the V1–V2 region of the 16S rRNA in microbial mats within streams. Since we used the V3–V5 region of the 16S rRNA, direct comparisons using OTU and partial 16S rRNA sequence of the five lakes could not be made through Qiime 1.3.0. However, overall similar heterotrophic bacterial taxa were observed between Stanish et al. (2012) and our study. The bacterial taxa Bacteroidetes, Alphaproteobacteria, Betaproteobacteria, Chloroflexi, and Thermus-Deinococcus (Table 2) were dominant heterotrophic bacteria in both studies. Moreover, in our study, we identified a relative higher abundance of the class Alphaproteobacteria (16.79–56.93 % compared to 1.1–6.6 %; Table 2) than the study in the MCMDV streams. Nevertheless, these similarities suggest that the identified heterotrophic bacteria are highly adapted to the harsh conditions in the lakes and surface channels in Antarctica.

In conclusion, microorganisms dominate most Antarctic ecosystems including the seasonal and perennially ice-covered freshwater lakes and streams and form the basis of the ecosystem functioning and nutrient cycling (Laybourn-Parry 2009; Lauro et al. 2011; Wilkins et al. 2012). Unlike the West Antarctic McMurdo Dry Valley Lakes, the bacterial diversity in East Antarctic lacustrine ecosystems remains largely unexplored. The comparative analysis of the bacterial diversity in our study demonstrates that besides the Antarctic extreme conditions such as the subzero cold temperatures, high wind, solar UV radiation, desiccation, and seasonal freeze–thaw cycles (Laybourn-Parry 2002, 2009), the distribution and diversity of bacteria in Schirmacher Oasis seasonal freshwater lake ecosystems are modulated by the interconnecting surface channels. Thus, it is apparent that these surface channels mediate the flow of meltwater from snow, glacier, and ice shelf during the summer months causing intermixing of nutrients, ions, minerals, and bacteria in lakes connected by them. Therefore, the dynamic physicochemical conditions of the Schirmacher Oasis seasonal freshwater lakes and the surface channels that connect them could be interpreted as one single large freshwater lacustrine ecosystem. Future studies to determine the microbial putative gene function using metatranscriptomics and proteomics approaches in these seasonal freshwater lakes and the surface channels that connect them in Schirmacher Oasis and other oases will help delineate the structure–function relationships of the microbially driven lacustrine ecosystems on this icy continent.

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