**Resilience of planktonic bacterial community structure in response to short term weather deterioration during the growing season in an alpine lake**

Tianli Ma1,2, Yiming Jiang1,2, Ali Hassan Ali Elbehery1,2, Stephan Blank3, Rainer Kurmayer3,\*, Li Deng1,2\*

1 Institute of Virology, Helmholtz Zentrum München, German Research Center for Environmental Health, Ingolstädter Landstrasse 1, 85764 Neuherberg, Germany

2 Institute of Virology, Technical University of Munich, Trogerstrasse 30, 81675 München, Germany

3 Research Department for Limnology, Mondsee, University of Innsbruck, Mondseestrasse 9, 5310 Mondsee, Austria

Corresponding Authors: Rainer Kurmayer, Li Deng

E-mail: [rainer.kurmayer@uibk.ac.at](mailto:rainer.kurmayer@uibk.ac.at)

li.deng@helmholtz-muenchen.de

Telephone: 0043-512-507-50242

**Abstract**

The disturbing effect of a short term cooling period during the summer on planktonic bacterial community structure in the water column of an alpine lake was investigated using 16S rDNA pyrosequencing. Proteobacteria, Actinobacteria, and Bacteroidetes constituted the most abundant phyla. During the sampling period (July to August 2010), a sudden cooling period with high precipitation occurred, as indicated by a decrease in conductivity, calcium and dissolved organic carbon concentration resulting from increased runoff. The relative abundance of Actinobacteria, Betaproteobacteria, and Cyanobacteria decreased during this short term cooling period. Instead, a rapid shift from Betaproteobacteria to Gammaproteobacteria occurred which was mainly caused by an increase of *Acinetobacter rhizosphaerae*. Soon after the short term cooling period, warmer weather conditions got re-established and Betaproteobacteria recovered and became again dominant. NMDS analysis and Venn diagrams revealed a planktonic bacterial community composition with high similarity at the beginning and the end of the growing season. Air temperature and precipitation were significantly correlated with the observed variation in operational taxonomic unit (OTU) relative abundance. It is concluded that in response to the short term cooling period a distinct planktonic bacterial OTU community developed. It rapidly diminished, however, as summer conditions became re-established, implying recovery of the original bacterial community structure.

**Keywords**

High mountain lake, cooling period, runoff, resilience, community turnover, planktonic bacteria, soil bacteria

**Introduction**

Bacterial communities play crucial roles in decomposing organic matter and nutrient cycling in aquatic ecosystems (Salcher et al., 2010), and their activities are driven by multiple ecological factors such as temperature (Ren et al., 2013), pH (Stepanauskas et al., 2003) and nutrient concentration (Van Der Gucht et al., 2001; Yannarell & Triplett, 2004). In lake ecosystems, local and climatic factors can affect the bacterial community by abiotic and biotic drivers (Battarbee et al., 2002; Castro et al., 2010). While numerous studies have explored the effect of climatic changes on bacterial communities in lowland lakes (Allgaier & Grossart, 2006; Bertilsson et al., 2007; Comte et al., 2006), fewer studies have considered remote lakes located in the alpine vegetation zone. Compared with lowland lakes, these lakes are considered relatively pristine due to less intensive anthropogenic activities taking place on or around them (Weckström et al., 2016). In addition, alpine lakes are considered sensitive to disturbance, e.g. through nutrient introduction (Heiri and Lotter 2003), water temperature rises depending on altitude range where reduction in snow cover duration is most pronounced (Thompson et al. 2005) and predator effects by introduced fish (Magnea et al. 2013).

In recent years, studies have paid attention to the response of bacterial communities in alpine lakes to sudden changes in the weather conditions. For example, the bacterial community structure in an arctic lake showed a rapid change over a short time period following snow melting or rain (Crump et al., 2003), mainly because transient bacteria were washed into the lake and some rare bacteria profited from terrestrial organic matter entering the lake via terrestrial runoff (O’Brien et al., 1997; Whalen & Cornwell, 1985). In an experiment that simulated changes in soil characteristics induced by climate change, the availability of soil-derived phosphorous and carbon induced a change in bacterial community structure within three days (Rofner et al., 2017). In addition to these indirect factors, temperature is one of the most important direct factors affecting bacterial community structure (Lindström et al. 2005). Bacteria inhabiting in alpine lakes can adapt to local temperature by modulating membrane fluidity and gene expression (Yadav et al. 2016). Temperature can also indirectly mediate the release of nutrients from sediment and trophic transfer, and further affect bacterial growth and diversity (Rasconi et al., 2017). The resilience of bacterial communities to disturbance in general has been studied widely (e.g. Shade et al., 2012) and it has been generally argued that microbial composition is resilient and quickly return to its predisturbance state because of fast growth rate and physiological flexibility (e.g. Allison & Martiny, 2008). In lake ecosystems, studies have shown that planktonic bacterial community (as well as phytoplankton or zooplankton) indeed return to their predisturbance state after experiencing pesticide input (Downing et al. 2008), disturbance of the water column stability caused by typhoon (Jones et al. 2008) or human intervention (Shade et al. 2011). Less studies have been performed in rermote unproductive and relatively cold alpine lakes where the role of climatic disturbance might be more influential as compared with warmer and more productive lowland regions. In a recent study, the average water temperature after ice break in spring until sampling date has been observed as limiting factor during the earlier growing season in alpine lakes (Jiang et al. 2019).

It was the aim to investigate the changes in the bacterial community structure during the summer growing season occurring in a remote alpine lake, Unterer Giglachsee in the Niedere Tauern at 1922 m a SL, in response to a local cooling period disturbance linked to rain and snowfall. For this purpose (i) we monitored the local weather and environmental conditions, ii) described the bacterial community composition turnover at the phylum, class and genus level, (iii) analysed the effect size of meteorological conditions in comparison to chemical-physical parameters, (iv) compared the bacterial community composition at the beginning and the end of the observation period to describe the resilience after the disturbance by the short term cooling period.

**Materials and Methods**

**Site description and sampling**

Unterer Giglachsee, (47.28° N, 13.65° E) is an oligotrophic alpine lake, which is located in a catchment area dominated by carbonaceous bedrock (limestone and dolomite) at the tree and timberline. The altitude, length, width, depth, and area are 1922 m a. SL., 1 km, 40–280 m, 18 m, and 16.5 ha, respectively. Further detailed information is given by (Weckström et al., 2016). Meteorological parameters for this area (including air temperature, average wind speed, precipitation, sunshine hours and snow layer) were obtained from Zentralanstalt für Meteorologie und Geodynamik (ZAMG), who run three weather monitoring stations located more closely in the alpine region: Obertauern station (OBE, 1772 m), Rudolfshütte station (RUD, 1956 m) and Schmittenhöhe station (SCH, 1956 m). Water thermistors (MINILOG, Vemco Ltd) were installed at 2.5 m depth in the deepest parts of the lake and used to monitor water temperature every 4 h. Depth-integrated water samples (1.5–2 L) were collected at the deepest part of the lake on July 19, 21, 27, 31 and August 01, 04, 09, 18 of the year 2010. The water samples were first prefiltered in the field through glass fiber filters using a hand vacuum pump (GF/C) to remove most of the eukaryotic algae and particle-associated bacteria. Then the filtrate was filtered through nitrocellulose membranes (NC, pore diameter 0.2 µm). NC membranes were transferred into Eppendorf tubes and were stored at −20 °C until DNA extraction.

**DNA extraction, 16S rDNA amplification, and sequencing**

NC membranes were loaded into a bead beater tube to physically break the cells, DNA was subsequently isolated and purified using a NucleoSpin® Soil DNA extraction kit (Macherey-Nagel, Germany) and eluted in 40 µl elution buffer. The quality and quantity of extracted DNA were estimated using a NanoDrop® ND-1000 Spectrophotometer (Thermo Fisher Scientific). 100 ng of extracted bacterial genomic DNA were amplified in a 50 µl reaction mix, containing 10 µl 5 × buffer HF, 1 µl of 10 mM of each dNTP, one unit of a proofreading polymerase (Phusion Hot Start High-Fidelity DNA Polymerase, Thermo Fisher Scientific), and 2.5 µl of forward and reverse primer (10 pmol µl−1) binding to conserved regions (V3 and V6) within the 16S rDNA. The forward primer (338F: 5’-CGTATCGCCTCCCTCGCGCCA TCAG ACGAGTGCGT ACTCCTACGGGAGGCAGCAG-3’) and the reverse primer (1046R: 5’-CTATGCGCCTTGCCAGCCCGC TCAG ACGAGTGCGT CGACAGCCATGCANCACCT-3’) included the sequences representing the adaptor, key and barcode sequence (underlined and separated by a space), while the specific sequence binding to bacterial rDNA is indicated at the 3’ end (Huse et al., 2008). PCR products (709 bp) were obtained under the following cycling conditions: annealing temperature 67.8 °C, elongation 30 s, 20 cycles. After running the gel, PCR products were cut out from agarose gel and purified using the gel purification kit (Qiagen, Hilden, Germany). Four independent PCR amplicons were pooled. Purified amplicons were sequenced using a GS- FLX platform with 454 titanium chemistry. Four amplicons were sequenced in four regions separated by 4-region gaskets, loading approximately 3,100,000 amplicon-coated beads per run and recovering a total of 800,000 sequence tags.

**16S rDNA sequence processing**

The sequences were processed using QIIME 19.0 (Caporaso et al., 2010). The multiplex reads were assigned to the original samples based on their barcode sequences. The raw reads were filtered by quality (Phred quality score > 2) and the sequences were clustered into OTUs at 97% sequence similarity. The UCLUST (Edgar, 2010) consensus taxonomy classifier was used to identify bacterial phylotypes from assigned OTUs. Representative sequences from each phylotype were aligned using PYNAST (Caporaso et al., 2009) and the sequence which was found to be most abundant in each OTU cluster was selected as the representative sequence. The Greengenes database (DeSantis et al., 2006) was used to identify the taxonomy of each phylotype. The raw sequencing data have been deposited in the NCBI Raw Sequence Read Archive (SRA) under the following accession numbers: SAMN09906023, SAMN09906024, SAMN09906025, SAMN09906026, SAMN09906027, SAMN09906028, SAMN10743546, SAMN10743550.

**Statistical data analysis**

Non-metric multidimensional scaling (NMDS) based on ‘Bray-Curtis’similarities was used to compare bacterial community structure at OTU level between samples (including all OTUs > 0.1% in total reads). The analysis was performed using R (version 3.2, package vegan). Analysis of similarities (ANOSIM) was used to test whether there was a significant difference between groups of samples as identified by the NMDS. Venn diagram analysis was performed at <http://www.interactivenn.net/> and was used to illustrate the OTUs shared between samples. For clarity, we only chose the most abundant OTUs (> 1% in total reads) in each sample. All other graphs were generated with OriginLab 8.

In order to study the relationship between bacterial community composition at OTU level and meteorological variables, detrended correspondence analysis (DCA) was applied to test whether an unimodal or a linear relationship between OTUs and variables was appropriate. The length of gradient varied from 3 to 4 implying that both unimodal or linear models could be used. The linear redundancy analysis (RDA) method was chosen (Lepš & Šmilauer, 2003) including the time dependence of consecutive samples as a covariate. As deduced from variance inflation factors (VIFs), the variables air temperature (at noon) and sunshine hours showed a high correlation. Thus, the latter were excluded from further analysis and the meteorological variables (air temperature, precipitation, and average wind speed) were tested using the forward selection procedure (p < 0.05). A Monte Carlo permutation test (499 randomized data sets) was used to test whether the influence of axis 1 was statistically significant at p < 0.05. The ordination analysis was performed using CANOCO software for Windows (version 4.5).

**Results**

**Weather and environmental conditions during the study period**

In general, relatively warm (average air temperature at noon, 15.1°C ± 0.12 (SE)) and good weather during July 2010 was followed by a distinct cooling period at the end of July, as indicated by a decrease in air and water temperature (Fig. S1). The average air temperature during the cooling period was 6.7°C ± 0.31 (SE). On July 27, conductivity, calcium and dissolved organic carbon (DOC) values all declined, indicating significant runoff of rain and melting snow (Fig. 1). Since this change was observed for a short time only, it was concluded that runoff influence declined again in the second part of the study period, i.e. during August 2010. Nevertheless, the water temperature remained relatively low when compared to the summer period before the cooling event.

**Sequencing output**

A total of 104,612 raw sequences were obtained. Following the removal of linkers, barcodes, and primers, as well as low quality or ambiguous reads, in total 89,508 high-quality reads were identified. For individual sampling dates, read numbers ranged from 2,041 to 20,611 (Table 1). In total, 2,484 OTUs were selected by applying the 97% sequence similarity threshold. Low abundance reads (less than 0.1% in total reads) were removed for further analysis, resulting in 436 OTUs. The value of “Goods\_coverage” indicated that for all samples the sequencing depth was sufficient to represent the actual microbial diversity.

**Bacterial community composition at the phylum and class level**

Proteobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria, Chloroflexi, Firmicutes, Thermi (Deinococcus–Thermus), and Verrucomicrobia constituted the most abundant phyla in the bacterial community composition (Fig. 2a). On average, Proteobacteria, Actinobacteria, and Bacteroidetes accounted for > 90% of all OTU, with Proteobacteria constituting the most abundant phylum. Only on August 09, which was after the cooling period, the relative abundance of Actinobacteria reached 43.1% and exceeded that of Proteobacteria. Within the Proteobacteria, Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, and Gammaproteobacteria constituted the four major classes (Fig. 2b). In general, the relative abundance of Deltaproteobacteria was the lowest (< 1%). Alphaproteobacteria ranged in relative abundance from 5.0% to 16.8%. However, during the cooling period (on July 27 and July 31), the relative abundance of Gammaproteobacteria reached maxima of 55.9% and 46.8%, respectively. Furthermore, during the cooling period, the relative abundance of Betaproteobacteria was lower on July 27 (14.2%) and July 31 (14.6%), and lowest on August 09 (13.1%), compared with other sampling dates (30.2% – 47.8%). On average, Actinobacteria relative abundance was much lower during the cooling period (10.3%, 13.0% and 6.4% on July 27 and July 31, and August 01, respectively) when compared with the warmer weather conditions before and after. Similarly, Bacteroidetes relative abundance decreased on July 27 and July 31 (9.2% and 6.3%, respectively) when compared with the warmer weather conditions before and after. Cyanobacteria relative abundance ranged from 1.6% to 5.4% and showed a similar trend of decline during the cooling period, with results for July 27 and July 31 of 1.6% and 2.2%, respectively. On average, the other phyla (Nitrospirae, Gemmatimonadetes, Chlorobi, and Acidobacteria) contributed < 1% of all OTUs. Thus, while the Gammaproteobacteria class seemed to increase during the short term cooling event, other phyla and classes regained abundance once warmer weather conditions were re-established.

**Short term changes in bacterial community composition at the genus level**

In all samples, the genera that were taxonomically classified belonged to the phyla Proteobacteria, Actinobacteria, Firmicutes, Cyanobacteria, Bacteroidetes and Thermi (Fig. 3). The relative abundance of assigned genera was highest on July 27 and July 31, i.e. 64.5% and 62.8% and below 50% on all other sampling dates. Eight genera assigned to Proteobacteria were identified, with *Acinetobacter*, *Limnohabitans*, *Sphingomonas,* and *Polynucleobacter* found to be the most abundant (Fig. 4a). Several genera showed a significant increase in relative abundance in coinciding with the short term cooling period. During the study period, *Acinetobacter* (17 OTUs) increased in relative abundance and occurred in highest number during the short term cooling period, i.e. on July 27 and July 31 (54.7% and 45.5%, respectively). *Sphingomonas* was not detected on July 19 and August 18, while it occurred at high relative abundance on July 31, August 01 and August 04 (3.2%, 7.4% and 3.0%, respectively). However, the abundanceof *Limnohabitans* (13 OTUs) declined to a minimum during the cooling period (0.3% and 0.4% on July 27 and July 31, respectively), while its highest relative abundance was observed on July 21 (10.2%), followed by August 01 and August 09 (7.7% and 4.0%, respectively). Similarly, the relative abundance of *Polynucleobacter* was higher at the beginning (July 19, 9.1%) and at the end (August 18, 6.8%) of the study period, but declined during the short term cooling period.

Within Actinobacteria, three genera with low abundance were identified including *Propionibacterium* (1 OTU), *Rhodococcus* (2 OTUs) and *Mycobacterium* (3 OTUs) (Fig. 4b). Within Cyanobacteria, the relative abundance of *Synechococcus* (7 OTUs) ranged from 0.1% (August 09) to 5.1% (August 18) and decreased during the short term cooling period on July 27 (1.5%) and July 31 (2.1%) (Fig. 4c). Within Bacteroidetes, *Flavobacterium* (7 OTUs), *Fluviicola* (15 OTUs) and *Sediminibacterium* (14 OTUs) were identified (Fig. 4d). *Flavobacterium* relative abundance ranged from 0.04% on July 31 to 1.3% on July 19. *Fluviicola* decreased in relative abundance during the cooling period (0.4% and 0.6% on July 27 and July 31, respectively), and increased at the end of the study period (3.0%, 7.3% and 3.0% on August 04, August 09 and August 18, respectively). Similarly, *Sediminibacterium* relative abundance decreased on July 27 and July 31 (0.7% and 0.5%, respectively), while it occurred with higher relative abundance at the beginning and end of the observation period, i.e. on August 04 and August 09 (1.3% and 7.3%, respectively). In summary, these results show that a distinct change in the bacterial community composition coincided with the short term cooling period.

**The similarity of bacterial community structure at OTU level**

Accordingly, with changes in community composition, a lower richness and diversity was observed on July 27 and July 31, coinciding with the short term weather change (Table 1). Three groups of samples were identified using NMDS analysis (including OTUs > 0.1% in total reads). Group 1 included OTUs on July 27 and July 31 which was during the short term weather deterioration. Two technical replicates of sequencing (July 31a and July 31b) were found to be most similar, indicating that there was good reproducibility. Group 2 included OTUs in the beginning and the end of the study period, i.e. July 19, August 04 and August 18. The OTUs obtained on July 21, August 01 and August 09, were forming group 3 (Fig. S3). ANOSIM analysis revealed marginally significantdifferences between two groups (Group1 and Group2, Group2 and Group3, Group1 and Group3) (R=1, P=0.1). Indeed the he differences between the three groups were significantly greater than the differences within groups (R= 0.89, P <0.01) (Fig.S4). The bacterial community structure was compared using Bray-Curtis similarity indices among three dates representing the beginning (July 19), the cooling period (July 31) and the end of the study period (August 18), respectively. Notably, bacteria community structure on July 19 and August 18 (similarity index = 0.79) was found more similar when compared with July 19 and July 31(similarity index = 0.67) implying that bacterial community structure recovered under nice weather conditions.

In addition to NMDS analysis, the most abundant OTUs (> 1% in total reads, Table S1) were analyzed using Venn diagrams to illustrate which OTUs were shared between samples (15-22 OTUs per sample). Venn diagrams showed that three separate groups were formed (Fig. S2): Group 1 comprised OTUs from July 21, August 01, and August 09 only, sharing 5 OTUs belonging to the orders Acidimicrobiales, Cytophagales, Burkholderiales, and Pseudomonadales. In contrast group 2 comprised OTUs from the cooling period (July 27 and July 31) only, sharing 9 OTUs belonging to the orders Pseudomonadales, Burkholderiales, Sphingomonadales, Rhizobiales, Synechococcales, Cytophagales, and the families Mycobacteriaceae, C111. Group 3 comprised OTUs occurring at the beginning and the end of the study period (July 19, August 04 and August 18) sharing 8 OTUs belonging to the orders Burkholderiales, Sphingomonadales, Cytophagales, and the families Mycobacteriaceae, C111. In summary, the OTUs shared between the sampling dates at the beginning and the end of the study period supported the conclusion that the bacterial community became re-established after the short term cooling period.

**The relationship between bacterial community composition and meteorological variables**

Redundancy analysis (RDA) was used to explore the relationship between bacterial community composition at OTU level (> 1% in total reads) and meteorological variables. Using the most abundant taxa (75 OTUs), two variables were identified by forward selection (using time dependence as a covariate). Air temperature and precipitation significantly explained the observed variation in the bacterial community composition at OTU level (P = 0.012). The two factors were found to be relatively unrelated to each other since they were plotted roughly orthogonally (Fig. 5). The canonical axis 1 explained 33% of the total variability in the OTU data, implying a distinct gradient of air temperature, while the canonical axis 2 explained 15% of the variability related to precipitation. Variance partitioning revealed that the explanatory effect of air temperature only was still significant (p = 0.026), although the total variability explained decreased from 53% to 29%. The influence of precipitation was found marginally significant (p = 0.07), and the total variability explained declined to 34%. It is concluded that both variables represented nonredundant gradients determining bacterial OTU composition. Three groups of OTUs were identified: One group positively correlated with precipitation including the genera *Acinetobacter* and *Sediminibacterium* and unknown genera from families C111 and Cerasicoccaceae. The other two groups correlated negatively or positively with air temperature. One group related positively to air temperature including unknown genera of families Comamonadaceae, Cytophagaceae and the genera *Acinetobacter* and *Limnohabitans*. The other group related negatively to air temperature including unknown genera from the orders Sphingomonadales, Roseiflexales, the families C111, Cytophagaceae, Comamonadaceae, Methylobacteriaceae, and the genera *Rhodococcus*, *Mycobacterium, Synechococcus,* and *Polynucleobacter.* It is concluded that in response to the short term cooling period, a rather distinct aquatic bacterial OTU community developed, however, rapidly diminished when summer conditions got re-established.

Among the most abundant bacteria, *Acinetobacter* *rhizosphaerae* (OTU No3) was positively correlated with precipitation and negatively correlated with air temperature. Compared with other samples, OTU No3 had a pronounced increase in relative abundance (July 27, 53.05% and July 31, 40.47%) during the cooling period (Table S1). The sequence of OTU No3 was blasted against the NCBI database, and the metadata of the hits indicated that these bacteria originated from soil (Chanika et al. 2011; Kasana 2017) and plant root system (Jossi 2008; Marasco et al. 2013).

**Discussion**

**The influence of weather deterioration on lake planktonic bacterial community**

During the cooling period, there was a notable drop in conductivity and Ca2+ concentration, which indicated a terrestrial runoff influence. The Ca2+ concentration was reduced by almost one third within a few days implying that slow-growing species were washed out due to a lacking competitiveness (Hibbing et al., 2010). In addition, terrestrial runoff can increase organic matter transport and input to the lake (Hongve et al., 2004; Tranvik & Jansson, 2002; Worrall et al., 2018) leading to resource diversification, i.e. organic matter is carried into lakes from terrestrial plants and soils through catchment runoff (Crump et al., 2003). In this study, however, the DOC concentration declined during weather deterioration, implying that increased precipitation resulted in lake water dilution rather than enrichment with organic matter from terrestrial runoff. In summary, the cooling period led to a detectable disturbance of the alpine planktonic habitat which became reversed as soon as the source of disturbance disappeared. It has been argued that every ecosystem reacts to environmental changes in a relatively predictable manner depending on its biological capacity. In general, extreme environments such as arctic/alpine ecosystems are known to be more sensitive to various kinds of disturbance because of various growth limiting factors. Nevertheless, it has been suggested that disturbance and climate change might interact, e.g. early-successional ecosystems may be more sensitive to climate change influence compared with later successional states, thus resulting in state shifts only when disturbed (Kröel-Dulay et al. 2015). Aquatic ecosystems have long been studied for regime and state shifts induced by non-linear ecosystem behavior in relatively short periods (Scheffer and Carpenter 2003). Such regime shifts would have significant consequences on the ecosystem level, e.g. high algal biomass production during summer and oxygen consumption during the ice cover period resulting in a cascade of changes in the whole ecosystem. Modeling experiments have revealed that such regime shifts can be foreseen already through long-term monitoring by statistical anomalies, i.e. through the increased variance in residues from dependent variables in linear regression models (Seekell et al. 2011). In other words, weather induced disturbances might actually increase the variance in dependent variables to a potential extend that possibly increases the likelihood of state shifts. In a related study, run off events induced by precipitation were compared in five alpine lakes (Jiang et al. 2019). Notably, the evidence that richness or diversity may be influenced by run-off through rainfall or snow melting during two summer periods in 2010 and 2011 was found relatively low, implying that the pronounced cooling period observed in this study was rather the exception than the rule. In summary, a potential interaction between weather-induced disturbances and climate change effects should be considered, in particular for alpine systems known to be more sensitive to temperature rise effects than lowland aquatic ecosystems.

**Changes in bacterial community composition at the phylum level**

In general, five phyla have been frequently reported in lakes, including Proteobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria and Verrucomicrobia (Newton et al., 2011). In this study, the two main classes of Proteobacteria, namely the Beta- and Gammaproteobacteria alternated in relative abundance with dominance of Gammaproteobacteria during the cooling phase. Only a few studies showed that Gammaproteobacteria occurred in high abundance in mountain lakes (Power et al., 2005), while this class usually was found to be dominant under deep water conditions only (Bel'kova et al., 1996). Some authors also concluded that this group may be transiently washed into lakes with surface runoff (Lindström & Leskinen, 2002). In our study, the high relative abundance of Gammaproteobacteria during the cooling period was possibly caused by rapid growth of *A*. *rhizosphaerae*. In general, Betaproteobacteria are typically found to be dominant in the euphotic zone of a lake (Bel'kova et al., 1996) and are known to respond quickly to changes in nutrient availability (Hornák et al., 2006; Nelson, 2009; Posch et al., 2007): When nutrient concentrations are high, Betaproteobacteria tend to be fast-growing (Newton et al., 2011). In our study, after the cooling period, Betaproteobacteria rapidly regained dominance, probably because of their fast growth rate under more favorable DOC concentrations. In addition, the relative abundance of Cyanobacteria during the entire study period was relatively low. This low relative abundance might be a result of the overall oligotrophic conditions since higher abundance of Cyanobacteria typically occur in more eutrophic freshwater (Eiler & Bertilsson, 2004). In addition, Cyanobacteria are sometimes favored by increased temperature (Paerl & Huisman, 2008). Similarly to Betaproteobacteria, the growth of Bacteroidetes is also related to organic matter resulting from a phytoplankton bloom (Newton et al., 2011), and frequently occurs during periods with high external DOC loading and algae-derived DOC production (Eiler & Bertilsson, 2004; Kolmonen et al., 2004). Accordingly, in our study, the increased relative abundance of Bacteroidetes coincided with the period of higher DOC and higher Cyanobacteria relative abundance. Actinobacteria are ubiquitous in freshwater lakes (Haukka et al., 2006; Humbert et al., 2009), but favor less eutrophic conditions (Haukka et al., 2006). Previous studies confirmed that their abundance decreased with decreasing oxygen concentration (Taipale et al., 2009). Since Actinobacteria are typically small and slow-growing, they might have been disfavoured by the cooling period in our study. The Verrucomicrobia relative abundance were generally low in all samples, which can be explained by their overall dependence on more eutrophic conditions (Newton et al., 2011). Other phyla occurred in lowest relative abundance, which might be caused by specific local conditions including lake type, local weather conditions, nutrient availability, physical and chemical properties, catchment characteristics and others (Bertilsson et al., 2007; Corno, 2006; Corno et al., 2009; Crump et al., 2003).

**Short term succession of the bacterial community related to the cooling period**

It was obvious that *Acinetobacter*, affiliated to Proteobacteria, had a successional development along with temperature decrease. Previous studies have reported that many members of *Acinetobacter*, isolated from oligotrophic conditions, also had a higher tolerance to low temperatures and can grow well in cold environments (Huang et al., 2013; Yao et al., 2013). In our study, *A. rhizosphaerae* was frequently identified during the cooling period, and probably originated from rhizospheric soil (Kasana, 2017). The known higher growth rate of *A. rhizosphaerae* under low-temperature conditions (3.0-10.3°C) might have supported net bacterial growth even under higher flow-through conditions as indicated by the dilution of Ca2+ and DOC concentrations. However, another genus, *Limnohabitans*, which is also affiliated to Proteobacteria, is known to have a high growth and substrate uptake rate, as well as a high mortality rate (Kasalický et al., 2010). It was found to be positively related to air temperature. During the cooling period, the decrease in water temperature and DOC concentration might have actually reduced the adaptability and competitiveness of *Limnohabitans* compared with other bacteria. The relative rapid increase in their relative abundance may have been due to their rapid absorption of nutrients. *Synechococcus* was the only genus affiliated to Cyanobacteria. Typically it is found to be abundant in oligotrophic environments under well illuminated conditions in the euphotic zone (Waterbury, 1986). The relatively low growth rates might have been exceeded by higher flow through as observed during the cooling period. *Sediminibacterium*, affiliated to Bacteroidetes, has been isolated from sediment (Qu & Yuan, 2008), soil (Kim et al., 2013) and activated sludge (Ayarza et al., 2014). Nutrient supply and temperature were most closely related to *Sediminibacterium* net production and growth (Sander & Kalff, 1993). Compared to the cooling period, *Sediminibacterium* relative abundance was higher under warmer weather conditions and higher DOC concentrations. It is concluded that the two meteorological factors, air temperature and precipitation, were directly and indirectly related to the observed change in bacterial community composition.

**Conclusion**

In this study, the bacterial community composition changed substantially during a short term deterioration in weather conditions as revealed at the taxonomic level of phylum, class, and genus. This significant change seemed to be caused directly and indirectly by the decreased air temperature and increased precipitation resulting in the cooling of lake water by terrestrial runoff and increased flow-through. Notably, the planktonic bacterial community structure returned to the previous state implying a re-installation of the original environmental conditions. We conclude that in comparison with lowland lakes, changes in weather conditions during the summer growing season can have a more direct impact on planktonic bacterial community structure. Thus change in weather conditions can increase variability in planktonic bacterial community structure and can overrule other more constant factors such as nutrient availability. Moreover, the reestablishment of the bacterial planktonic community structure to a previous state at the start of the study period implies rather robust planktonic ecological conditions.

**Acknowledgments**

Several high school students, Anton Gimpl, Lisa Schindlegger, and Simon Urschitz from HLFS Ursprung/Elixhausen stayed at the lakeside overnight to perform sampling. Sabine Wanzenböck assisted in communication between high school students and teachers. We are most grateful to the local population for providing regular access to the Giglachsee Lake (‘Wegegemeinschaft Ursprungalm’) for sampling and to Mathias Keinprecht (Ignaz Mattis Hütte). Josef Franzoi and Roland Psenner (University of Innsbruck) performed the chemical analysis. Two anonymous reviewers and the editor commented on an earlier version of this manuscript. T.M. and Y.J. were supported by the Chinese Scholarship Council (CSC). The sampling and data acquisition was funded by the Nationalkomitee Alpenforschung of the Austrian Academy of Sciences, project: DETECTIVE (DEcadal deTECTion of biodIVErsity in alpine lakes) to R.K.

**References**

Allgaier, M. & H.-P. Grossart, 2006. Seasonal dynamics and phylogenetic diversity of free-living and particle-associated bacterial communities in four lakes in northeastern Germany. Aquatic Microbial Ecology 45: 115–128.

Allison, S. & J. Martiny, 2008. Resistance, Resilience, and Redundancy in Microbial Communities. In Avise JC, H. S., Ayala FJ, editors (ed) In the Light of Evolution: Volume II: Biodiversity and Extinction. National Academies Press (US), Washington (DC), 149-165.

Ayarza, J. M., E. L. Figuerola & L. Erijman, 2014. Draft genome sequences of type strain Sediminibacterium salmoneum NJ-44 and Sediminibacterium sp. strain C3, a novel strain isolated from activated sludge. Genome Announcements 2: e01073–e01013.

Battarbee, R. W., J.-A. Grytnes, R. Thompson, P. G. Appleby, J. Catalan, A. Korhola, H. Birks, E. Heegaard & A. Lami, 2002. Comparing palaeolimnological and instrumental evidence of climate change for remote mountain lakes over the last 200 years. Journal of Paleolimnology 28: 161–179.

Bel'kova, N., L. Denisova, E. Manakova, E. Zaĭchikov & M. Grachev, Species diversity of deep water microorganisms in Lake Baikal, detected by 16S rRNA sequences. In: Doklady Akademii nauk, 1996. vol 348. 692.

Bertilsson, S., A. Eiler, A. Nordqvist & N. O. G. Jørgensen, 2007. Links between bacterial production, amino-acid utilization and community composition in productive lakes. The ISME Journal 1: 532.

Caporaso, J. G., K. Bittinger, F. D. Bushman, T. Z. DeSantis, G. L. Andersen & R. Knight, 2009. PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics 26: 266–267.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Pena, J. K. Goodrich & J. I. Gordon, 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7: 335.

Castro, H. F., A. T. Classen, E. E. Austin, R. J. Norby & C. W. Schadt, 2010. Soil microbial community responses to multiple experimental climate change drivers. Applied and Environmental Microbiology 76: 999–1007.

Comte, J., S. Jacquet, S. Viboud, D. Fontvieille, A. Millery, G. Paolini & I. Domaizon, 2006. Microbial community structure and dynamics in the largest natural French lake (Lake Bourget). Microbial Ecology 52: 72–89.

Corno, G., 2006. Effects of nutrient availability and Ochromonas sp. predation on size and composition of a simplified aquatic bacterial community. FEMS Microbiology Ecology 58: 354–363.

Corno, G., B. E. Modenutti, C. Callieri, E. G. Balseiro, R. Bertoni & E. Caravatia, 2009. Bacterial diversity and morphology in deep ultraoligotrophic Andean lakes: the role of UVR on vertical distribution. Limnology and Oceanography 54: 1098–1112.

Crump, B. C., G. W. Kling, M. Bahr & J. E. Hobbie, 2003. Bacterioplankton community shifts in an arctic lake correlate with seasonal changes in organic matter source. Applied and Environmental Microbiology 69: 2253–2268.

DeSantis, T. Z., P. Hugenholtz, N. Larsen, M. Rojas, E. L. Brodie, K. Keller, T. Huber, D. Dalevi, P. Hu & G. L. Andersen, 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Applied and Environmental Microbiology 72: 5069–5072.

Downing, A. L., K. M. DeVanna, C. N. Rubeck-Schurtz, L. Tuhela & H. Grunkemeyer, 2008. Community and ecosystem responses to a pulsed pesticide disturbance in freshwater ecosystems. Ecotoxicology 17(6):539-548.

Edgar, R. C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26: 2460–2461.

Eiler, A. & S. Bertilsson, 2004. Composition of freshwater bacterial communities associated with cyanobacterial blooms in four Swedish lakes. Environmental Microbiology 6: 1228–1243.

Fierer, N. & R. B. Jackson, 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences 103: 626–631.

Haukka, K., E. Kolmonen, R. Hyder, J. Hietala, K. Vakkilainen, T. Kairesalo, H. Haario & K. Sivonen, 2006. Effect of nutrient loading on bacterioplankton community composition in lake mesocosms. Microbial Ecology 51: 137–146.

Heiri, O. & A. F. Lotter, 2003. 9000 years of chironomid assemblage dynamics in an Alpine lake: long-term trends, sensitivity to disturbance, and resilience of the fauna. Journal of Paleolimnology 30(3):273-289.

Hewitt, K. M., F. L. Mannino, A. Gonzalez, J. H. Chase, J. G. Caporaso, R. Knight & S. T. Kelley, 2013. Bacterial diversity in two neonatal intensive care units (NICUs). PloS One 8: e54703.

Hibbing, M. E., C. Fuqua, M. R. Parsek & S. B. Peterson, 2010. Bacterial competition: surviving and thriving in the microbial jungle. Nature Reviews Microbiology 8: 15.

Hongve, D., G. Riise & J. F. Kristiansen, 2004. Increased colour and organic acid concentrations in Norwegian forest lakes and drinking water–a result of increased precipitation? Aquatic Sciences 66: 231–238.

Hornák, K., J. Jezbera, J. Nedoma, J. M. Gasol & K. Simek, 2006. Effects of resource availability and bacterivory on leucine incorporation in different groups of freshwater bacterioplankton, assessed using microautoradiography. Aquatic Microbial Ecology 45: 277–289.

Huang, X., W. Li, D. Zhang & W. Qin, 2013. Ammonium removal by a novel oligotrophic Acinetobacter sp. Y16 capable of heterotrophic nitrification–aerobic denitrification at low temperature. Bioresource Technology 146: 44–50.

Humbert, J. F., U. Dorigo, P. Cecchi, B. Le Berre, D. Debroas & M. Bouvy, 2009. Comparison of the structure and composition of bacterial communities from temperate and tropical freshwater ecosystems. Environmental Microbiology 11: 2339–2350.

Huse, S. M., L. Dethlefsen, J. A. Huber, D. M. Welch, D. A. Relman & M. L. Sogin, 2008. Exploring microbial diversity and taxonomy using SSU rRNA hypervariable tag sequencing. PLoS Genetics 4: e1000255.

Jiang, Y., H. Huang, T. Ma, J. Ru, S. Blank, R. Kurmayer & L. Deng, 2019. Temperature response of planktonic microbiota in remote alpine lakes. Frontiers in microbiology 10.

Jones, S. E., C.-Y. Chiu, T. K. Kratz, J.-T. Wu, A. Shade & K. D. McMahon, 2008. Typhoons initiate predictable change in aquatic bacterial communities. Limnology and Oceanography 53(4):1319-1326

Kasalický, V., J. Jezbera, K. Šimek & M. W. Hahn, 2010. Limnohabitans planktonicus sp. nov. and Limnohabitans parvus sp. nov., planktonic betaproteobacteria isolated from a freshwater reservoir, and emended description of the genus Limnohabitans. International Journal of Systematic and Evolutionary Microbiology 60: 2710–2714.

Kasana, R. C., 2017. Bacterial Diversity in Cold Environments of Indian Himalayas Mining of Microbial Wealth and MetaGenomics. Springer, 83–99.

Kim, Y.-J., N.-L. Nguyen, H.-Y. Weon & D.-C. Yang, 2013. Sediminibacterium ginsengisoli sp. nov., isolated from soil of a ginseng field, and emended descriptions of the genus Sediminibacterium and of Sediminibacterium salmoneum. International Journal of Systematic and Evolutionary Microbiology 63: 905–912.

Knights, D., J. Kuczynski, E. S. Charlson, J. Zaneveld, M. C. Mozer, R. G. Collman, F. D. Bushman, R. Knight & S. T. Kelley, 2011. Bayesian community-wide culture-independent microbial source tracking. Nature Methods 8: 761.

Kolmonen, E., K. Sivonen, J. Rapala & K. Haukka, 2004. Diversity of cyanobacteria and heterotrophic bacteria in cyanobacterial blooms in Lake Joutikas, Finland. Aquatic Microbial Ecology 36: 201–211.

Kröel-Dulay, G., J. Ransijn, I. K. Schmidt, C. Beier, P. De Angelis, G. De Dato, J. S. Dukes, B. Emmett, M. Estiarte & J. Garadnai, 2015. Increased sensitivity to climate change in disturbed ecosystems. Nature communications 6:6682.

Lauber, C. L., M. Hamady, R. Knight & N. Fierer, 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and Environmental Microbiology 75: 5111–5120.

Lepš, J. & P. Šmilauer, 2003. Multivariate analysis of ecological data using CANOCO. Cambridge university press.

Lindström, E. & E. Leskinen, 2002. Do neighboring lakes share common taxa of bacterioplankton? Comparison of 16S rDNA fingerprints and sequences from three geographic regions. Microbial Ecology 44: 1–9.

Lindström, E. S., M. P. Kamst-Van Agterveld & G. Zwart, 2005. Distribution of typical freshwater bacterial groups is associated with pH, temperature, and lake water retention time. Appl Environ Microbiol 71(12):8201-8206

Magnea, U., R. Sciascia, F. Paparella, R. Tiberti & A. Provenzale, 2013. A model for high-altitude alpine lake ecosystems and the effect of introduced fish. Ecological modelling 251:211-220.

Nelson, C. E., 2009. Phenology of high-elevation pelagic bacteria: the roles of meteorologic variability, catchment inputs and thermal stratification in structuring communities. The ISME Journal 3: 13.

Newton, R. J., S. E. Jones, A. Eiler, K. D. McMahon & S. Bertilsson, 2011. A guide to the natural history of freshwater lake bacteria. Microbiology and molecular biology reviews 75: 14–49.

O’Brien, W. J., M. Bahr, A. E. Hershey, J. E. Hobbie, G. W. Kipphut, G. W. Kling, H. Kling, M. McDonald, M. C. Miller & P. Rublee, 1997. The limnology of Toolik lake Freshwaters of Alaska. Springer, 61–106.

Paerl, H. W. & J. Huisman, 2008. Blooms like it hot. Science 320: 57–58.

Posch, T., B. Mindl, K. Hornˇák, J. Jezbera, M. M. Salcher, B. Sattler, B. Sonntag, J. Vrba & K. Šimek, 2007. Biomass reallocation within freshwater bacterioplankton induced by manipulating phosphorus availability and grazing. Aquatic Microbial Ecology 49: 223–232.

Power, M. L., J. Littlefield‐Wyer, D. M. Gordon, D. A. Veal & M. B. Slade, 2005. Phenotypic and genotypic characterization of encapsulated Escherichia coli isolated from blooms in two Australian lakes. Environmental Microbiology 7: 631–640.

Qu, J.-H. & H.-L. Yuan, 2008. Sediminibacterium salmoneum gen. nov., sp. nov., a member of the phylum Bacteroidetes isolated from sediment of a eutrophic reservoir. International Journal of Systematic and Evolutionary Microbiology 58: 2191–2194.

Rasconi, S., K. Winter & M. J. Kainz, 2017. Temperature increase and fluctuation induce phytoplankton biodiversity loss–Evidence from a multi‐seasonal mesocosm experiment. Ecology and Evolution 7: 2936–2946.

Ren, L., D. He, J. Zeng & Q. L. Wu, 2013. Bacterioplankton communities turn unstable and become small under increased temperature and nutrient-enriched conditions. FEMS Microbiology Ecology 84: 614–624.

Rofner, C., H. Peter, N. Catalán, F. Drewes, R. Sommaruga & M. T. Pérez, 2017. Climate‐related changes of soil characteristics affect bacterial community composition and function of high altitude and latitude lakes. Global Change Biology 23: 2331–2344.

Salcher, M. M., J. Pernthaler & T. Posch, 2010. Spatiotemporal distribution and activity patterns of bacteria from three phylogenetic groups in an oligomesotrophic lake. Limnology and Oceanography 55: 846–856.

Sander, B. C. & J. Kalff, 1993. Factors controlling bacterial production in marine and freshwater sediments. Microbial Ecology 26: 79–99.

Shade, A., H. Peter, S. Allison, D. Baho, M. Berga, H. Buergmann, D. Huber, S. Langenheder, J. Lennon, J. Martiny, K. Matulich, T. Schmidt & J. Handelsman, 2012. Fundamentals of microbial community resistance and resilience 3(417) doi:10.3389/fmicb.2012.00417.

Shade, A., J. S. Read, D. G. Welkie, T. K. Kratz, C. H. Wu & K. D. McMahon, 2011. Resistance, resilience and recovery: aquatic bacterial dynamics after water column disturbance. Environmental microbiology 13(10):2752-2767.

Scheffer, M. & S. R. Carpenter, 2003. Catastrophic regime shifts in ecosystems: linking theory to observation. Trends in ecology & evolution 18(12):648-656.

Seekell, D. A., S. R. Carpenter & M. L. Pace, 2011. Conditional heteroscedasticity as a leading indicator of ecological regime shifts. The American Naturalist 178(4):442-451.

Stepanauskas, R., M. A. Moran, B. A. Bergamaschi & J. T. Hollibaugh, 2003. Covariance of bacterioplankton composition and environmental variables in a temperate delta system. Aquatic Microbial Ecology 31: 85–98.

Taipale, S., R. I. Jones & M. Tiirola, 2009. Vertical diversity of bacteria in an oxygen-stratified humic lake, evaluated using DNA and phospholipid analyses. Aquatic Microbial Ecology 55: 1–16.

Thompson, R., C. Kamenik & R. SCHMIDT, 2005. Ultra-sensitive Alpine lakes and climate change. Journal of Limnology 64(2):139-152.

Tranvik, L. & M. Jansson, 2002. Climate change (Communication arising): Terrestrial export of organic carbon. Nature 415: 861.

Van Der Gucht, K., K. Sabbe, L. De Meester, N. Vloemans, G. Zwart, M. Gillis & W. Vyverman, 2001. Contrasting bacterioplankton community composition and seasonal dynamics in two neighbouring hypertrophic freshwater lakes. Environmental Microbiology 3: 680–690.

Waterbury, J. B., 1986. Biological and ecological characterization of the marine unicellular cyanobacterium Synechococcus. Photosynthetic picoplankton 71–120.

Weckström, K., J. Weckström, K. Huber, C. Kamenik, R. Schmidt, W. Salvenmoser, M. Rieradevall, T. Weisse, R. Psenner & R. Kurmayer, 2016. Impacts of climate warming on Alpine lake biota over the past decade. Arctic, Antarctic, and Alpine Research 48: 361–376.

Whalen, S. & J. Cornwell, 1985. Nitrogen, phosphorus, and organic carbon cycling in an arctic lake. Canadian Journal of Fisheries and Aquatic Sciences 42: 797–808.

Worrall, F., N. J. Howden, T. P. Burt & R. Bartlett, 2018. Declines in the dissolved organic carbon (DOC) concentration and flux from the UK. Journal of Hydrology 556: 775–789.

Yadav, A. N., S. G. Sachan, P. Verma, R. Kaushik & A. K. Saxena, 2016. Cold active hydrolytic enzymes production by psychrotrophic Bacilli isolated from three sub‐glacial lakes of NW Indian Himalayas. Journal of basic microbiology 56(3):294-307.

Yannarell, A. C. & E. W. Triplett, 2004. Within-and between-lake variability in the composition of bacterioplankton communities: investigations using multiple spatial scales. Applied and Environmental Microbiology 70: 214–223.

Yao, S., J. Ni, T. Ma & C. Li, 2013. Heterotrophic nitrification and aerobic denitrification at low temperature by a newly isolated bacterium, Acinetobacter sp. HA2. Bioresource Technology 139: 80–86.

**Table 1** Sequencing output for samples from alpine lake Unterer Giglachsee analysed in the course of a cooling period in July/August 2010.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Sampling (date) | Total reads | Total OTUs | Goods\_coverage | Chao1 | fisher | Shannon | Simpson |
| July 19 | 5647 | 375 | 0.967 | 747 | 99.81 | 5.38 | 0.92 |
| July 21 | 10308 | 390 | 0.984 | 670.78 | 84.88 | 5.69 | 0.96 |
| July 27 | 11759 | 328 | 0.985 | 766.9 | 66.49 | 3.49 | 0.7 |
| July 31a | 20611 | 419 | 0.991 | 553.04\* | 50.56\* | 4.09\* | 0.82\* |
| July 31b | 7227 | 245 | 0.983 |
| August 01 | 2260 | 280 | 0.942 | 504.4 | 88.99 | 6.22 | 0.97 |
| August 04 | 19027 | 489 | 0.988 | 904.64 | 96.99 | 5.31 | 0.94 |
| August 09 | 10628 | 366 | 0.985 | 630.39 | 77.07 | 5.74 | 0.96 |
| August 18 | 2041 | 366 | 0.897 | 817.21 | 154.93 | 6.31 | 0.95 |

\*July31a and July31b were used as technical replicates and were combined subsequently

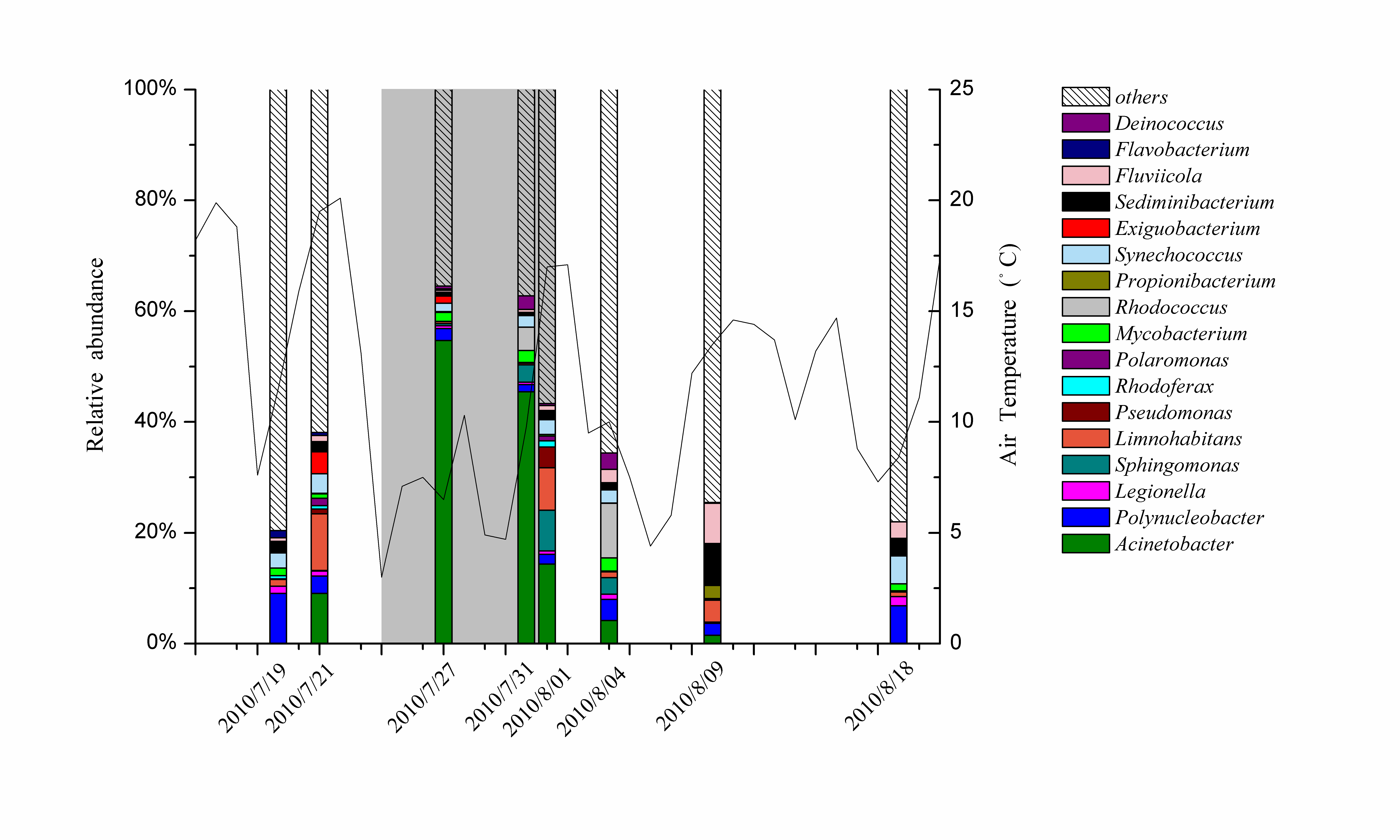
**Figures**

****

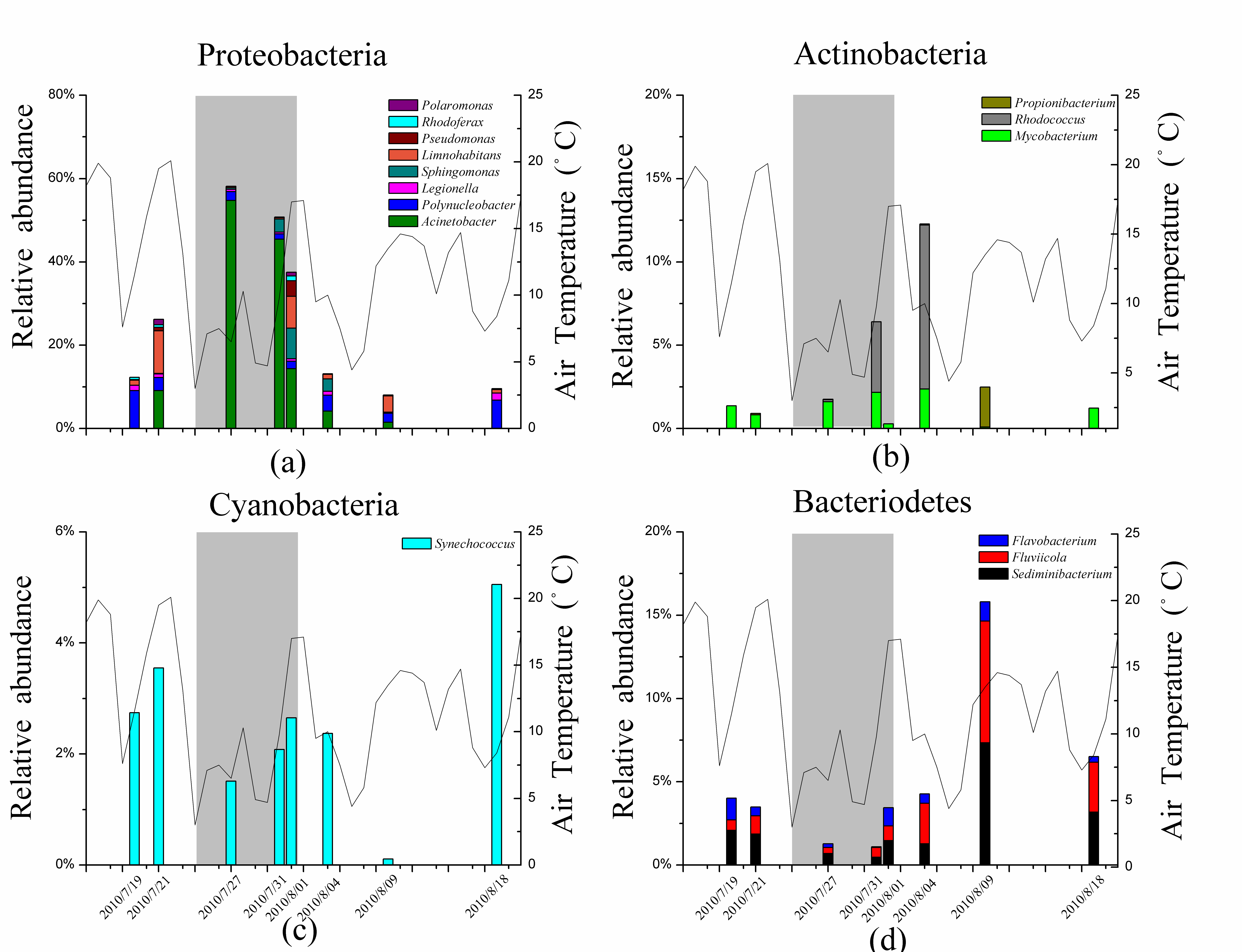
**Fig. 1** a) Parameters of water chemistry and b) air temperature and water temperature in the alpine lake Unterer Giglachsee indicating a short term winter period during July and August 2010. The short term winter period is indicated by the shaded area.



**Fig. 2** Relative abundance of **(a)** bacterial phyla and **(b)** classes of Proteobacteria in the alpine lake Unterer Giglachsee during July and August 2010. The air temperature at noon (12:00 midday) is indicated by the straight grey line. The deterioration in weather conditions is indicated by the shaded area.



**Fig. 3** Relative abundance of bacterial genera in the alpine lake Unterer Giglachsee during July and August 2010. The air temperature at noon (12:00 midday) is indicated by the straight grey line. The deterioration in weather conditions is indicated by the shaded area.



**Fig. 4** Relative abundance of abundant bacterial genera from different phyla (a) Proteobacteria, (b) Actinobacteria, (c) Cyanobacteria, (d) Bacteroidetes in the alpine lake Unterer Giglachsee during July and August 2010. The air temperature at noon (12:00 midday) is indicated by the straight grey line. The deterioration in weather conditions is indicated by the shaded area.



**Fig. 5** Redundancy analysis (RDA) of bacterial OTUs (> 1% in total reads) and environmental variables. The two canonical axes explained 47% of the microbial community differentiation, p value = 0.012. For clarity only the twenty most abundant OTUs are shown.