The ISME Journal (2013), 1–13

www.nature.com/ismej

ORIGINAL ARTICLE

Bacterial structures and ecosystem functions in glaciated floodplains: contemporary states and potential future shifts

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Glaciated alpine floodplains are responding quickly to climate change through shrinking ice masses. Given the expected future changes in their physicochemical environment, we anticipated variable shifts in structure and ecosystem functioning of hyporheic microbial communities in proglacial alpine streams, depending on present community characteristics and landscape structures. We examined microbial structure and functioning during different hydrologic periods in glacial (kryal) streams and, as contrasting systems, groundwater-fed (krenal) streams. Three catchments were chosen to cover an array of landscape features, including interconnected lakes, differences in local geology and degree of deglaciation. Community structure was assessed by automated ribosomal intergenic spacer analysis and microbial function by potential enzyme activities. We found each catchment to contain a distinct bacterial community structure and different degrees of separation in structure and functioning that were linked to the physicochemical properties of the waters within each catchment. Bacterial communities showed high functional plasticity, although achieved by different strategies in each system. Typical kryal communities showed a strong linkage of structure and function that indicated a major prevalence of specialists, whereas krenal sediments were dominated by generalists. With the rapid retreat of glaciers and therefore altered ecohydrological characteristics, lotic microbial structure and functioning are likely to change substantially in proglacial floodplains in the future. The trajectory of these changes will vary depending on contemporary bacterial community characteristics and landscape structures that ultimately determine the sustainability of ecosystem functioning.
The ISME Journal advance online publication, 11 July 2013; doi:10.1038/ismej.2013.114
Subject Category: Microbial ecology and functional diversity of natural habitats
Keywords: glacier; biofilm; hyporheic sediment; stream; bacterial communities

Introduction

Heterotrophic bacteria are crucial in the functional ecology of aquatic ecosystems, being the driving force behind metabolic processes like respiration and productivity, nutrient cycling and fluxes, trophic links with secondary consumers and numerous biogeochemical processes (Edwards *et al.*, 1990; Kirchman, 1994; Hall and Meyer, 1998; Acuna *et al.*, 2008). The hyporheic zone and its heterotrophic components have an important role by integrating many of these ecosystem functions (EFs) at the interface between surface waters, groundwaters and the riparian zone (Hendricks, 1993; Stanford and Ward, 1993; Findlay, 1995; Battin, 1999). Alpine aquatic systems are undergoing rapid change in response to glacier recession, thereby providing the opportunity to examine structural and functional responses of bacterial communities (see Milner *et al.*, 2009) to potential changes in environmental conditions, especially in high elevation lotic systems.

Globally, alpine catchments are major sources of freshwater because of relatively high levels of precipitation, often stored as snow and ice in glaciers. This stored water is then released during warm periods as snow and glacial meltwaters. Groundwater-fed streams also are common in alpine catchments. Hence, the majority of running waters in glaciated alpine floodplains can be characterized as either glacier-meltwater-fed (kryal) or groundwater-fed (krenal) channels, or streams dominated by snowmelt (rhithral) during spring (Brown *et al.*,

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Received 18 February 2013; revised 16 May 2013; accepted 7 June 2013

2003). These different types of streams have distinct annual and diel discharge patterns (flow regimens), hydrological linkages and physicochemical characteristics (Ward, 1994; Tockner *et al.*, 1997; Brown and Fuge, 1998; Smith *et al.*, 2001). Krenal systems, for example, are less influenced by discharge fluctuations, whereas kryal systems show high discharge during summer ablation and an increasing influence of groundwater towards winter (Brown and Fuge, 1998). Owing to these different dynamics in biogeochemical and physical characteristics, diverse habitat patches are created.

Regional climate models predict an increase in mean temperature in European Alpine regions and more rapid glacial melting (Horton *et al.*, 2006; Zemp *et al.*, 2006; IPCC, 2007). Krenal systems will likely become more common as glaciers retreat and precipitation patterns change, for example, projections suggest that precipitation periods will shift from reduced precipitation in summer towards increased precipitation in late winter (Swiss Climate Change Scenarios CH2011, 2011). Landscape heterogeneity, as influenced by glaciers, will be reduced and a consequent shift in flow source and regimen towards more krenal-regulated systems is expected. This shift in water source will have a large effect on the physicochemical and ecological state of alpine lotic systems (Hall and Fagre, 2003). For instance, the quality, quantity and timing of resources, such as organic matter (OM) and nutrient inputs, are highly affected by shifts in environmental and hydrological conditions, and will likely influence heterotrophic bacteria assemblages and their ecological services or functioning (Boyer *et al.*, 1997; Findlay and Sinsabaugh, 1999; Horton *et al.*, 2006).

Although the above-mentioned changes in environmental conditions will potentially affect EFs mediated by bacterial assemblages, the underlying mechanisms and future trajectories of EF are poorly understood. This is mainly because altered EF can be either linked to changes in bacterial community composition (BCC), single-cell metabolism or changes in total cell numbers (Comte and Del Giorgio, 2011). Which mechanism has the important role in potential future shifts in EF may depend on present bacterial community characteristics (i.e. apparent functional redundancy and plasticity, domination of generalists vs specialists) and the strength of changes in environmental variables (Allison and Martiny, 2008). In this study, our main objective was to characterize BCC and EF (assessed as potential enzymatic activity) within hyporheic sediments in glaciated alpine floodplains in relation to potential environmental drivers and their spatio-temporal dynamics. We examined streams at different spatial scales (within and between catchments) covering a diverse set of landscape features (such as glaciers and lakes) and different degrees of spatial connectivity. In particular, we chose kryal and krenal streams within three different alpine catchments, focusing on spatial and temporal differences in physicochemical and microbial characteristics of the streams. Because of the more pronounced heterogeneity (spatial and temporal) within kryal systems, we hypothesized that microbial communities will either show strong resistance and steady-state characteristics because of high functional plasticity or a high degree of community turnover because of competing species that are adapted to a specific temporary condition. We interpret the results in the context of current landscape structures (degree of deglaciation, geological background and stream network structure) and discuss the findings in the light of potential future trajectories of BCC and linked EF because of environmental change and contemporary BCC characteristics. This study provides new information on the potential shifts in bacterial communities and their ecosystem functioning within alpine catchments in relation to expected scenarios of changing alpine water regimens and landscape structures resulting from global change.

Materials and methods

Study floodplains

Geographical location, geological characterization and hydrological conditions in the three study sites, Val Roseg (VR), Loetschental (L) and Macun (M), are

Table 1 Characteristics of the three alpine catchments

Catchment	*Val Roseg*	*Loetschental*	*Macun*
Coordinates	9°53′53″E, 46°29′24″N	07°49′03″E, 46°25′08″N	10°07′31″E, 46°43′51″N
Altitude (m a.s.l.)	1766–4049	1375–3200	2616–3046
Catchment area (km^2), (% glaciated)	66.5 (30.1)	77.8 (36.5)	3.6 (18.8)[a]
Annual precipitation (m)	1.6	1.1	0.9
Mean discharge ($m^3\ s^{-1}$)	28.5	37.2	ND
Mean water temperature of main channel (°C) (range)	3.6 (1–12)	4 (0.1–10.9)	2.9 (0.1–19.2)
Geology, dominating rock types	Crystalline bedrock, diorite, granite	Crystalline bedrock, amphibolite, gneiss	Crystalline bedrock, orthogneiss

Abbreviation: ND, no data.
[a]Rock glaciers.

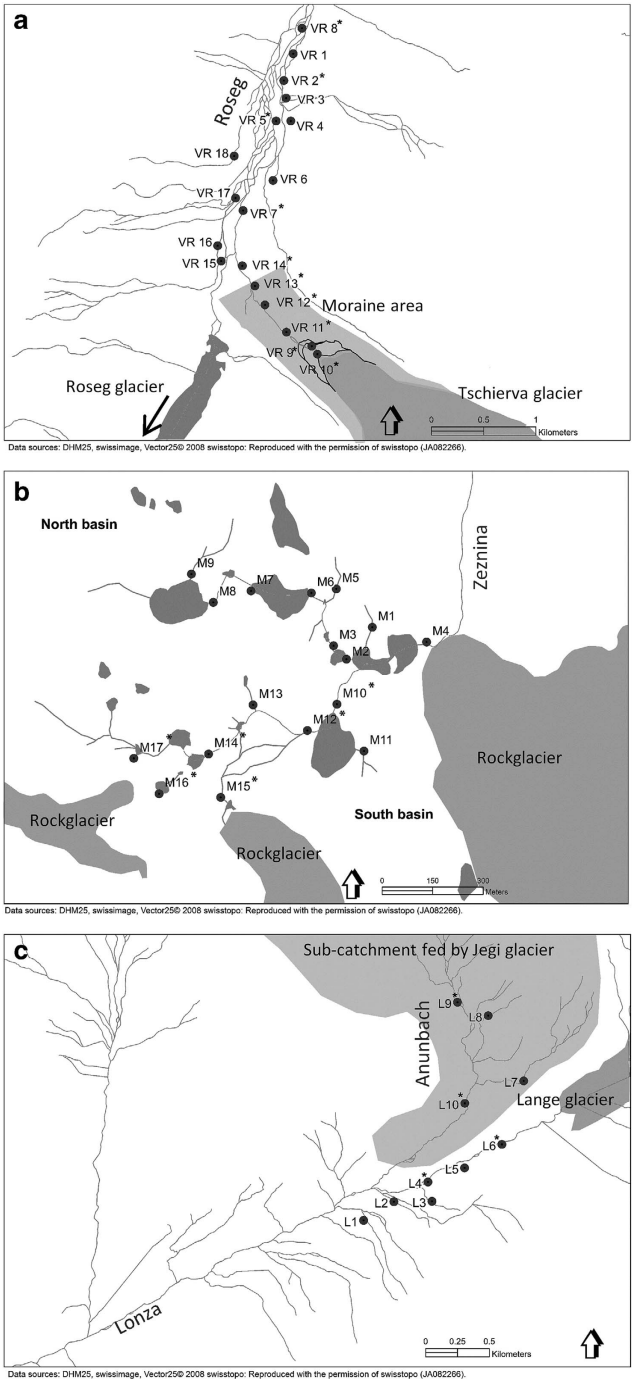
given in Table 1 (sources: Labhart, 1998; Malard *et al.*, 1999; Robinson and Kawecka, 2005; Schmidt *et al.*, 2009; BAFU, 2010).

The waters of the Roseg and Loetschental catchments are partly fed by valley glaciers, which have retreated continuously over the past century (Maisch, 1988; Tockner *et al.*, 1997, 2002; Malard *et al.*, 2000). Permanently flowing first-order tributaries contribute groundwater and snowmelt to kryal main channels, which have peak flows during spring and summer (Malard *et al.*, 2000). Loetschental study sites are divided between two subcatchments (Figure 1). The Macun Lakes region is a high alpine cirque. The catchment is divided into a southern and northern basin that differs in their water source (Robinson and Matthaei, 2007). The northern basin is mainly groundwater and snowmelt fed, whereas the southern basin is fed mostly by rock glaciers. All catchments experience contraction of surface channels in winter (Robinson and Matthaei, 2007).

Sediment sampling

Hyporheic sediment samples for analysis of bacteria were collected from selected sites in all three catchments during July/August (A) and October (O) 2008. The Val Roseg and Loetschental catchments were also sampled in June (J) 2009. A total of 45 sites were sampled: 10 kryal sites and 8 krenal sites in the Val Roseg (VR1–VR18), 4 kryal sites and 6 krenal sites in Loetschental (L1–L10) and 6 kryal sites and 11 krenal sites in the Macun catchment (M1–M17) (Figure 1). Water systems were distinguished based on previous studies (Tockner *et al.*, 1997; Robinson *et al.*, 2007) or determined by geographical position relative to the glaciers. Thirteen out of 118 potential samples were either not sampled or included in the data analysis: 5 sites that were dry in Val Roseg and Loetschental in October (VR3, VR17, L2, L3 and L8), Roseg site VR11 in June because of potential contamination and Macun samples M1, M5, M11, M13, M15, M16, M17 that were snow-covered in October and thus inaccessible. Sample sites were chosen owing to their relative position along the flow path (longitudinal and lateral) and owing to their position in context of landscape structures (lakes and glaciers).

For each sediment sample, the upper ~10 cm of streambed sediment was removed to avoid sampling bacteria associated with benthic biofilms. Sediment samples were then taken to a depth of ca. 20 cm, being sieved through an 8-mm mesh sieve (Retsch GmbH, Haan, Germany). All samples were transported in a cooling box to the laboratory, where samples for DNA extraction and enzyme assays were frozen at −20 °C. Fixation for microscopic analysis was performed within 12 h of sampling (see below).



Data sources: DHM25, swissimage, Vector25© 2008 swisstopo: Reproduced with the permission of swisstopo (JA082266).

Figure 1 Map of the study catchments and location of sampling sites in (**a**) Val Roseg, (**b**) Macun, (**c**) Loetschental. Kryal sites have an asterisk. Streams are delineated in gray. Lakes and glaciers are depicted as dark gray and gray areas, respectively. Light gray indicates the subcatchment in Loetschental and the moraine area in Val Roseg.

Physicochemical water variables

Specific conductivity (μS cm^{-1} at 20 °C) and temperature were measured in the field with a conductivity meter (LF323; WTW, Weilheim, Germany). Surface water samples (1 L) were collected and transported in a cooling box to the laboratory. The

water was then filtered through preashed glass fiber filters (GF/F, Whatmann) and the filtrate analyzed for dissolved OM, particulate organic carbon (POC), total inorganic carbon (TIC), ammonium (NH_4-N), nitrite (NO_2-N), nitrate (NO_3-N), dissolved organic nitrogen, particulate organic nitrogen, phosphate (PO_4-P), dissolved phosphorus and particulate phosphorus (PP) according to standard protocols detailed in Tockner *et al.* (1997). We chose to sample surface water instead of pore water as most sites showed relatively coarse substrate (see sediment sorting coefficients below and in the Supplementary Material); thus, a strong similarity of surface and subsurface waters within the upper sediment layers was likely. Results of the most important physico-chemical parameters are presented in Figure 2 and further discussed in the Supplementary Information.

Sediment characteristics

Subsamples of the collected sediments were air-dried at 50 °C and then used to measure pH as described in Schofield and Taylor (1955). Total sediment OM was determined as ash-free dry mass by combusting the samples at 450 °C for 4 h. The remaining material was then used to determine

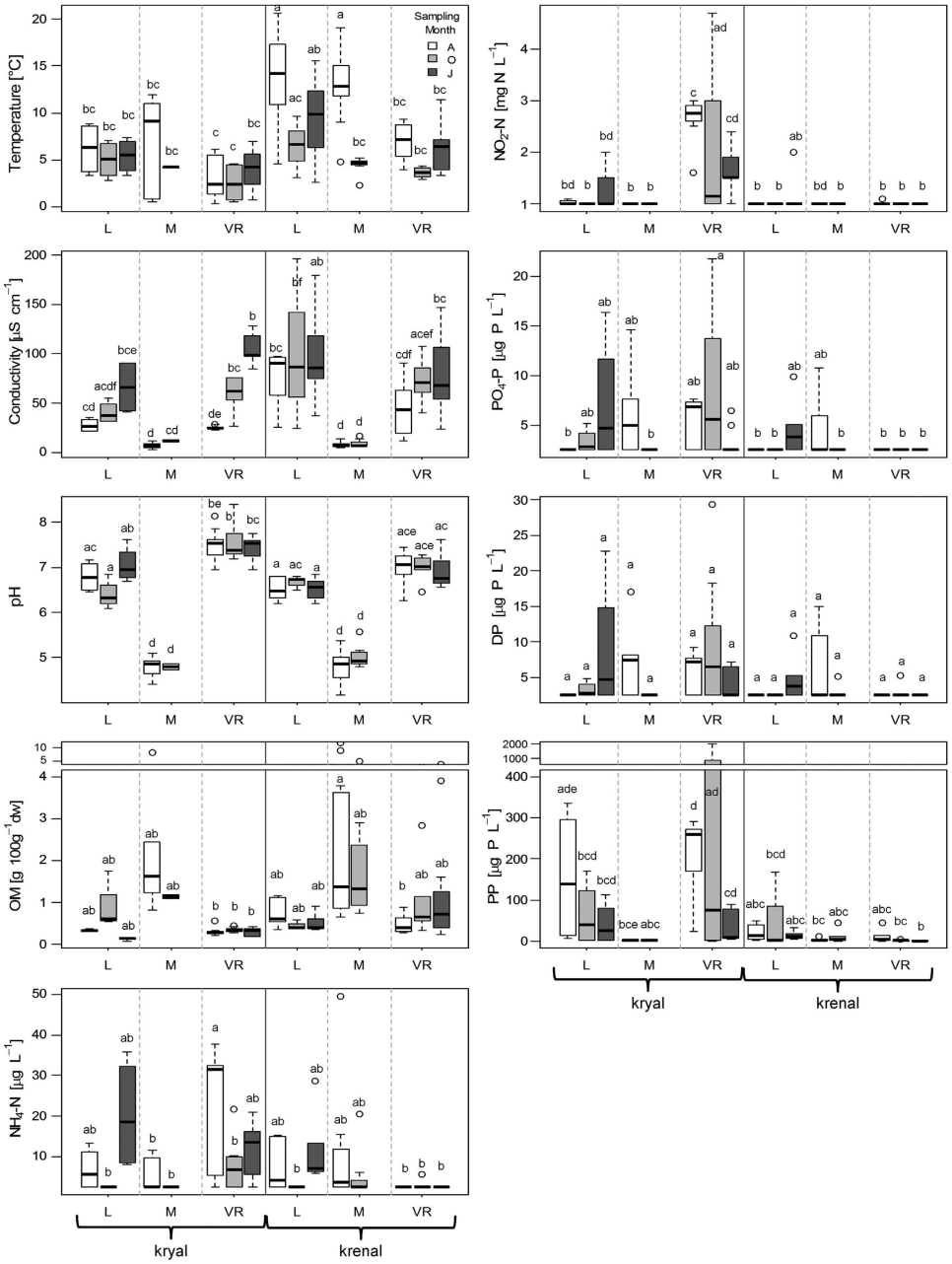


Figure 2 Boxplots of environmental variables. Whiskers indicate 1.5 × interquartile range. Groups are split by water source (brackets), catchments and sampling dates. Letters show significant differences based on Tukey's honestly significant difference ($P<0.05$).

grain size distribution using a sieving machine (Retsch GmbH, Haan, Germany) at mesh sizes of 6.3, 2.0, 1.0, 0.5, 0.25, 0.125 and 0.063 mm. The size distribution was analyzed using the GRADISTAT software (Simon and Blott, 2001) to give the D90/D10 sorting coefficient as a measure of sediment interpacking.

Bacterial total cell numbers
A 0.5-ml aliquot of collected sediment was suspended in 1.11-ml paraformaldehyde (2%, final concentration) in an Eppendorf tube and fixed for 24 h at 4 °C followed by three washing steps with 1 × phosphate-buffered saline and 5 min centrifugation at 10 000 *g* between washing steps. Samples were then stored at −20 °C in a 1:1 mix of phosphate-buffered saline/ethanol until further processing (Pernthaler *et al.*, 2001). Cell detachment was carried out by sonication (Branson Digital Sonifier 250, Danbury, CT, USA; 5-mm tapered microtip, actual output of 20 W, 30 s). The homogenate served as a template for filtration-based counting of 4′,6-diamidino-2-phenylindole- (Sigma-Aldrich Co., St Louis, MO, USA) stained cells (Porter and Feig, 1980). Photographs taken with an epifluorescence microscope (Leica Microsystem, Heerbrugg, Switzerland; DMI6000b) were analyzed with the CellC software (CellC Cell Counting, Tampere, Finland) (Selinummi *et al.,* 2005) or counted manually in case of high background fluorescence. Detailed information for this and the following sections of Materials and methods, statistical analysis and an in-depth discussion on specific results are available in the Supplementary Material.

Enzyme assays
Eight different enzymes were tested for their activity using methylumbelliferone-labeled substrate analogs. They were chosen based on their potential role in bacterial metabolism. (Table 2, Vihinen and Mäntsälä, 1989; Sinsabaugh *et al.*, 1991; Arpigny and Jaeger, 1999; Makoi and Ndakidemi, 2008; Sinsabaugh *et al.*, 2008). Fluorometric enzyme assays were performed under standardized conditions as described before (Findlay *et al.*, 2001). All values were corrected for quenching and potential autofluorescence, that is, because of the presence of small mineral particles, and subsequently standardized to OM. The activity of single enzymes is presented in Figure 3.

Bacterial community fingerprinting
BCC was assessed by automated ribosomal intergenic spacer analysis (ARISA). Samples (~2 g) were extracted using the PowerSoil DNA isolation Kit (MoBio, Carlsbad, CA, USA) following the manufacturer's instructions. DNA was amplified using the fluorescein (6-FAM)-labeled universal forward primer 1406f-6FAM and the bacteria-specific reverse primer 23Sr (Yannarell *et al.*, 2003). ARISA fragment analysis was performed as described in Bürgmann *et al.* (2011).

Data analysis
All statistical analyses were carried out using the vegan, relaimpo and mgcv packages in R (Grömping, 2006; Wood, 2006; Oksanen *et al.*, 2011; R Development Core Team, 2012).

Community fingerprinting results and enzymatic activities were analyzed with redundancy analysis (RDA) based on forward selected environmental explanatory variables. RDA-based variation partitioning on ARISA and enzyme activity data were performed to evaluate the influence of chemical and physical (temperature and D90D10) variables on BCC and EF. Unique fractions of RDA were tested by analysis of variance (ANOVA)-like permutation tests (Peres-Neto *et al.*, 2006; Blanchet *et al.*, 2008). A Mantel test was performed to assess the linkage between BCC and EF (Mantel, 1967).

Permutational multivariate ANOVA (PERMANOVA) was used to assess the influence of water source,

Table 2 Enzymes analyzed in this study, substrate used for assays and their biogeochemical functions

Enzyme (abbreviation)	*Substrate analog*	*Acquiring element*	*Target*	*Function in ecosystem*
α-Glucosidase (Alph)	4-MUF-α-D-glucoside	Carbon	α-1,4- and 1,6-Glucosidic linkages	Starch degradation
β-Glucosidase (Bet)	4-MUF-β-D-glucoside	Carbon	β-1,4-Glucans	Cellulose degradation
β-Xylosidase (Xyl)	4-MUF-β-D-xylopyranoide	Carbon	Xylose residues	Hemicellulose degradation
Esterase (Est)	4-MUF-acetate	Carbon	Small ester containing molecules	Glyceride hydrolization
N-acetyl-glucosaminidase (Nac)	4-MUF-*N*-acetyl-β-D-glucosaminide	Nitrogen	1,4-β-Linkages of glucosamines	Chitin degradation
Leucine aminopeptidase (Leu)	L-leucine-7-amido-4-methylcoumarin	Nitrogen	Hydrophobic amino acids from N terminus	Peptide degradation
Endopeptidase (End)	4-MUF-4-guanadinoenzoate	Nitrogen	Peptide bonds	Peptide degradation
Phosphatase (Phos)	4-MUF-phosphate	Phosphorous	Phosphomono- and diester	Protein, nucleotide degradation

Abbreviation: MUF, methylumbelliferone.

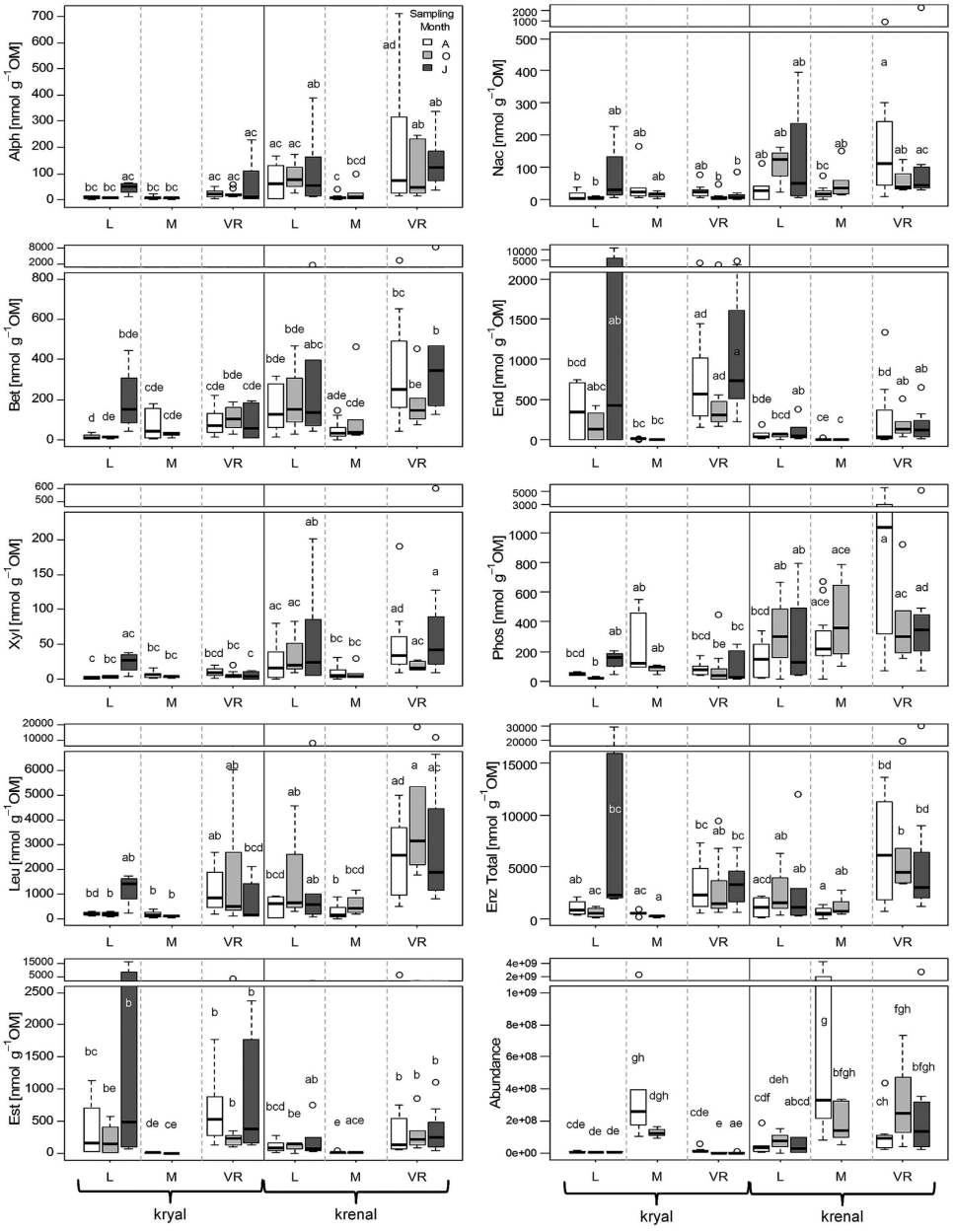


Figure 3 Boxplots of single and total enzymatic activities and cell abundance. Whiskers indicate 1.5 × interquartile range. Groups are split by water source, catchments and sampling dates. Letters show significant differences based on Tukey's honestly significant difference ($P<0.05$).

catchment and sampling date on physicochemical, community and enzyme activity structure with a full factorial model (Anderson, 2001).

Correlations of single enzymes to physicochemical variables were tested by multiple linear regression. OM was omitted as an independent variable before analysis to prevent autocorrelation. The models were selected with the Akaike information criterion. Significance of predictors were tested by permutational ANOVA and their relative importance in the linear model was assessed using the lmg metric (Chevan and Sutherland, 1991; Grömping, 2006).

For descriptions of data transformations before the analysis consult the Supplementary Information. To verify the main patterns seen with the above-mentioned analysis tools, we used additional methods that are described and presented in the Supplementary Information.

Results

Physicochemical characteristics

The catchments differed significantly in many physicochemical variables, including temperature, sediment pH, conductivity, particulate organic nitrogen and OM (Figure 2). For instance, sediment pH was different between all catchments, being

lowest in Macun and highest in Val Roseg, mirroring the geological differences. Significant differences were also found between krenal and kryal systems, for example, there were generally higher temperatures in krenal than kryal systems. Other variables such as conductivity differed only for specific sites or depended on sampling date or catchment, and showed the influence of glacial meltwater during the year and the importance of site positioning along the longitudinal flowpath relative to respective landscape structures. Macun, for instance, showed fewer differences in physicochemical characteristics between water systems because of a decrease in glacial water inputs in the southern subcatchment. Val Roseg, on the other hand, showed the strongest distinction between water systems, that is, PP, NH_4 and NO_2 showed higher concentrations in kryal sites because of the overall large glacial water input.

The PERMANOVA revealed an interaction of physicochemical variables with catchments and dates, and catchments and water source, indicating different strengths in temporal fluctuations of physicochemical variables and the separation of the two water systems within each catchment ($P<0.001$). Again, these findings mirror the actual state of the three catchments concerning their deglaciation and present landscape structures, and thus the present temporal and spatial variability in stream physicochemistry.

Cell abundance

Bacteria cell abundance in sediments differed between the three catchments ($P<0.001$) and showed high variability within all catchments. Loetschental had the lowest mean cell densities (range: 2.65×10^6–1.90×10^8 cells g^{-1} dry weight, $n=27$) followed by Val Roseg (range 1.67×10^6–2.75×10^9 cells g^{-1} dry weight, $n=51$), and then Macun (range: 5.25×10^7–4.44×10^9 cells g^{-1} dry weight, $n=27$). Krenal systems had generally higher cell abundances than kryal systems ($P<0.001$). There was a significant interaction between water source and catchment, with lowest cell abundances in kryal sediments in Loetschental and Val Roseg ($P<0.001$; Figure 3).

Enzymatic activities

Patterns of enzyme activities varied considerably among sites. ANOVA revealed various significant differences between catchments and water source (Figure 3), whereas temporal effects were generally less pronounced. Highest total potential enzyme activities were found in krenal systems. Comparisons between catchments revealed highest mean values in Roseg, intermediate values in Loetschental and lowest values in Macun ($P<0.05$).

There was a separation between catchments, water systems and their interactions, with clear separation of the three catchments, and of kryal from krenal sites in Val Roseg and Loetschental (PERMANOVA: $P<0.05$; Figure 4). Macun showed no separation in enzyme patterns between water sources.

Results of multiple linear regression show relationships of single enzymes with water chemistry, sediment characteristics and some nutrient variables (PP and pH most strongly). Results are summarized in Table 3. The fitted biplot vectors showed the relative physicochemical variables potentially driving the functional separation between water systems (Figure 4). In particular, this approach identified pH, TIC, POC, PP, dissolved phosphorus and PO_4-P as potential drivers of EF. According to the RDA analysis, 55.8% of the variation in EF can be explained by the physicochemical variables. Variation partitioning of separated water sources revealed an equally high contribution of chemical variables for both systems (kryal: 48.0%; krenal: 47.9%), whereas pure physical variables showed no significant influence on EF or were intercorrelated with chemical variables.

Bacterial community structure and linked functions

A total of 191 operational taxonomic units (OTUs) were detected across all sites. Ten OTUs were unique to a single site each, whereas 95 OTUs occurred in more than 50% of all sampled sites. Diversity (Shannon index) was highest in krenal systems ($P<0.001$).

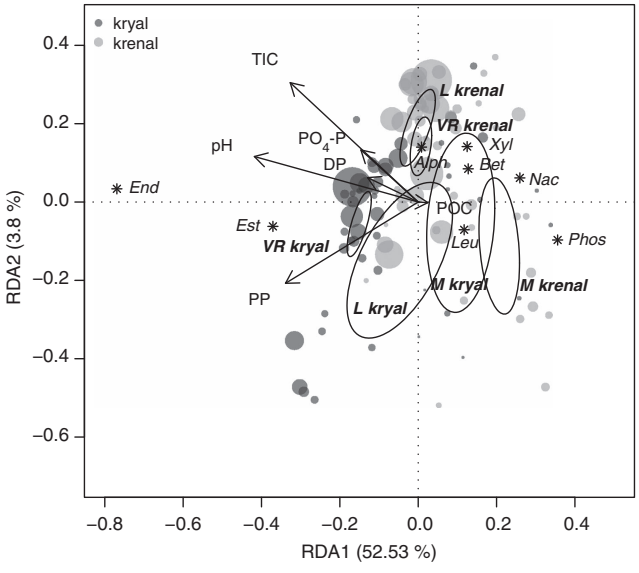


Figure 4 RDA correlation biplot of enzymatic activities. Dots indicate individual sites. The size of dots is relative to the sum of logarithms of all measured enzymes standardized to OM. Dark gray dots correspond to kryal sites and light gray dots to krenal sites. Dispersion ellipses depict the standard error of weighted average scores of catchment groupings (Macun = M; Loetschental = L; Val Roseg = VR; confidence limits = 0.95). Environmental variables are fitted as arrows and response variables centroids (enzymes) are depicted with an asterisk. The explained variance for RDA axes 1 and 2 are given.

Table 3 Relative importance of multiple linear regression of enzymatic activities standardized to OM and physicochemical parameters

	Physicochemical parameters													*Variance explained by model*
Enzyme	*Temp.*	*Cond*	*pH*	*D90D10*	*DOC*	*POC*	*TIC*	*NO_2-N*	*NO_3-N*	*PN*	*PO_4-P*	*DP*	*PP*	
Alph	0.025		0.577***	0.021				0.112*		0.039**	0.058**	0.038	0.129**	48.49%
Bet			0.398***	0.054*				0.096		0.086***	0.089***	0.039	0.238***	48.10%
Xyl	0.099*		0.308***	0.098**				0.148*		0.063**	0.111***		0.173***	41.33%
Nac			0.091**	0.098*						0.084*	0.076*	0.073	0.578***	26.29%
Est			0.496***	0.021*	0.007	0.02	0.255***		0.009				0.191***	77.31%
Leu		0.182	0.525***	0.059*				0.061*		0.055**	0.024*		0.095***	45.08%
End			0.353*		0.009	0.032***	0.524***	0.128*	0.014	0.017*			0.275***	76.69%
Phos	0.106	0.077*	0.058*	0.128**		0.053*	0.082	0.198					0.297**	40.58%

Abbreviations: AIC, Akaike information criterion; Alph, α-glucosidase; Bet, β-glucosidase; End, endopeptidase; Est, esterase; DOC, dissolved organic matter; DP, dissolved phosphorus; Leu, leucine aminopeptidase; Nac, *N*-acetyl-glucosaminidase; NO_2-N, nitrite; NO_3-N, nitrate; OM, organic matter; Phos, phosphatase; PN, particulate organic nitrogen; POC, particulate organic carbon; PO_4-P, phosphate; PP, particulate phosphorus; TIC, total inorganic carbon; Xyl, β-xylosidase.
*$P<0.05$.
**$P<0.01$.
***$P<0.001$.
Underlined values are contributing negatively to the distinct enzyme activity. Values are scaled to 100% of the explained variance, and models were selected with AIC.

RDA differentiated BCC between catchments and water sources (Figure 5). BCC were similar in Loetschental and Val Roseg in the krenal sediments. Macun was more separated from the other two catchments and showed a less pronounced separation of kryal and krenal sites compared with Val Roseg (Figure 5). Krenal systems in Val Roseg showed no temporal pattern compared with the temporal shift in BCC in kryal sites in Val Roseg ($P=0.96$ and $P<0.001$, respectively). Temporal BCC changes were not significant in Loetschental krenal and kryal sites ($P>0.05$). Macun BCC showed no temporal pattern in kryal sites but did in krenal sites ($P=0.79$ and $P<0.05$, respectively).

Biplots showed which chemical variables correlated with trends in the BCC distinguishing kryal and krenal systems. High values in nutrient and carbon variables were generally associated with kryal systems. In particular, gradients in sediment pH seemed to be of high importance for driving community composition (Figure 5). The importance of glacial ablation in August is mirrored by high PP and NO_2-N. High temperature and OM appeared correlated with krenal community composition. RDA revealed that 19.9% of the total variation was explained by forward-selected environmental variables. Variation partitioning of physical and chemical variables applied only to kryal systems showed that 23.2% of the variation in community structure could be accounted for by water chemistry. Krenal systems, in contrast, had just 10.9% of the variation explained by water chemistry, reflecting their more stable environmental conditions.

Relating enzyme activity patterns to BCC by means of Mantel tests showed differences in the strength of association, with a maximum correlation for Val Roseg (0.561, $P<0.01$), intermediate correlation for Loetschental (0.389, $P<0.01$) and nonsignificant correlation for Macun.

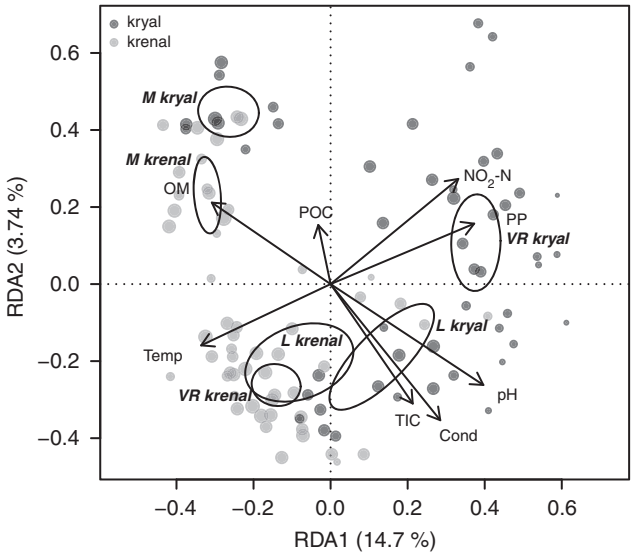


Figure 5 RDA correlation biplot of ARISA profiles. Dots indicate individual sites. The size of dots is relative to the numbers of OTUs at a site. Dark gray dots correspond to kryal sites and light gray dots to krenal sites. Dispersion ellipses depict the standard error of weighted average scores of catchment groupings (Macun = M; Loetschental = L; Val Roseg = VR; confidence limits = 0.95). Environmental variables are fitted as arrows. The explained variance for RDA axes 1 and 2 are given.

Discussion

Our results showed a strong influence of water source on BCC, EF and spatiotemporal dynamics of bacterial assemblages within hyporheic sediments of streams in glaciated alpine floodplains. Although

the study catchments showed distinct patterns in BCC, EF and temporal dynamics, water source influenced heterotrophic bacteria occurrence, functioning or both in all catchments. Finding larger and more tightly linked temporal shifts in BCC and EF in kryal systems in Val Roseg mirrored their higher temporal heterogeneity in physical and chemical characteristics and supported our hypothesis that typical kryal BCC are dominated by generalists. Although temporal dynamics in BCC were not as apparent within krenal sites, there still was a remarkable difference in BCC and EF between catchments.

The patterns we observed appeared to follow a hierarchical template: geological differences such as sediment pH or conductivity had a strong influence and acted as a principal separator of bacterial community structure and enzymatic expression patterns between catchments. Fierer and Jackson (2006) compared soil samples across North and South America and found that soil pH was the best predictor of bacterial community composition and richness. Landscape features such as glaciers have the potential to create strong landscape heterogeneity by dictating coarse-scale physicochemical characteristics of habitats over time and space. Hydrogeochemical conditions, disturbance as well as spatial connectivity between aquatic systems can be altered by glaciers, and it has been shown that these mechanisms can influence BCC (Sekar *et al.*, 2002; Frey *et al.*, 2009; Langenheder *et al.*, 2011). In contrast, lake outlet streams or groundwater-fed streams provide more spatiotemporal stability and a more homogeneous landscape (Tockner *et al.*, 1997; Brown *et al.*, 2003), and the smaller fluctuations in physicochemical characteristics in such habitats cause reduced variability in bacterial communities.

Glaciers and lakes: landscape features that drive hydrology and bacterial communities

Seasonal glacial melt-water dynamics have a major influence on a suite of floodplain characteristics and therefore have a strong role in the ecology of alpine streams. Summer ablation leads to distinct physicochemical water characteristics, increased sediment load, decreased channel stability and a greater hydrological linkage between aquatic and terrestrial compartments (Milner and Petts, 1994; Ward, 1994; Tockner *et al.*, 1997; Brown *et al.*, 2003; Battin *et al.*, 2004). PP was an important driver for a whole set of enzymes and equalized EF over a large spatial scale. Altered activities owing to phosphorous amendments have been observed before in other systems such as Mediterranean rivers or several types of soils (Romaní *et al.*, 2004; DeForest *et al.*, 2012). Phosphorus availability also can be decreased with sediment pH and is strongly dependent on the parent material, weathering and glacial-mediated scouring of the bed rock (Robarge, 2008). Similarly PO_4-P, dissolved phosphorus, POC, NH_4-N and temperature influenced EF and BCC, typically distinguishing krenal from kryal systems during summer ablation and being strong delineators for BCC and EF. The degree of deglaciation thus influences, in concert with geological factors, EF and BCC characteristics within a landscape because of its impact on the physicochemical properties of a habitat patch (i.e. within a subcatchment or reach along the flow path). Indeed, the Loetschental subcatchment fed by the Jegi glacier has a physicochemical characteristic resembling krenal systems, indicating a reduced glacial water input and consequently an EF and BCC characteristic that is more congruent with that of a krenal system.

The fact that Macun EF did not differ between water sources can be related to mitigated glacial water input by lakes, essentially homogenizing the two water systems. Kryal streams in Macun had no affiliation in either BCC or EF with kryal streams in the other two catchments. Patterns in benthic biofilms having lower bacterial abundance in kryal channels, as occurring in Val Roseg and Loetschental, also were not present within this catchment (e.g. Battin *et al.*, 2004). As a mitigating agent of physical and chemical disturbance, lakes can act as sinks of glacial-induced high sediment loads, consequently reducing PP concentrations at the lake outlet. As PP was shown to be an important driver for EF and BCC, the presence of lakes may thus partially offset the differences between stream types in the Macun catchment. Phosphorous availability can additionally be reduced by the low sediment pH further separating EF and BCC from the other two catchments.

Different habitat, different strategy

The linkage of species composition and EF appears to be a continuum extending from functional plasticity and redundancy to a complete coupling, depending on the investigated functions, the complexity of the system and the players involved (Langenheder *et al.*, 2006; Boucher and Debroas, 2009; Comte and Del Giorgio, 2010). The results of this study showed that the coupling between bacteria structure and function can be stronger or weaker depending on environmental constraints that determine local community structure. Temporal shifts in BCC and EF in kryal channels were linked to shifting physicochemical templates as shown by the large fraction of variance explained by physicochemical variables and the broader fluctuations in the physicochemical template within kryal systems. Variation partitioning only explained a small part of BCC dynamics in krenal systems via the influence of chemical and physical variables or their interactions.

Enzymatic functions, however, were influenced to the same extent by physicochemical variables in kryal as in krenal systems, indicating a large functional plasticity in both types of systems. The underlying mechanisms leading to this systemic EF

plasticity differ between the typical kryal and krenal water systems. Krenal bacterial communities probably withstand environmental changes by adapting their single-cell metabolism, whereas kryal communities lacking this plasticity shift towards a community composition dominated by specialists able to perform a specific EF under the given conditions. The lower Shannon diversity index in kryal systems may also be interpreted as a result of a dominance of a few specialists adapted to the current set of environmental conditions.

Implications of global change for alpine stream microbiomes

Glaciers continue to recede and the future loss of snow and ice will alter alpine ecosystems in fundamental ways. It is expected that there will be a strong decrease in glacial meltwater input to alpine floodplains, altering spatial and temporal runoff dynamics. In general, a shift towards a more groundwater-dominated landscape is likely to occur in the near future in most alpine areas. This change in water source also entails a shift in the physicochemical and structural habitat templates. General differences between kryal and krenal sediment types have been shown before (Logue *et al.*, 2004), but the findings of this study show that microbial communities are adapted to habitat-specific physicochemical conditions defined by their community metabolic capabilities. Global change induced shifts in water sources are therefore expected to result in a shift in both microbial communities and mediated ecosystem services.

Temperature in kryal channels will increase in the future, whereas PP will decrease (Milner *et al.*, 2009). As water temperature and PP were correlated with BCC, a loss of diversity because of a narrower bandwidth and less fluctuation could be expected. This change is even more likely when resident kryal specialists must compete with invading krenal generalists in an environment becoming more suitable for the latter. Although we did not see a lower overall OTU richness in kryal systems, a future shift towards a more even community structure and loss of specialists could be caused by decreased glacial runoff in kryal systems. This shift in water source would lead to a reduced temporal and spatial turnover of microbial species, and decreased beta diversity at the landscape scale. As environmental complexity is linked to biodiversity and ecosystem functioning, an impact on water properties can be expected when landscape heterogeneity and biodiversity are reduced (Langenheder *et al.*, 2010).

Changes in vegetation in alpine landscapes that occur in concert with glacial retreat can alter carbon inputs substantially (Theurillat and Guisan, 2001). As OM, POC and TIC influenced BCC, there will be an impact on krenal microbial communities. OM originates from benthic algal inputs that grow well within krenal systems. In-stream primary production could become more important in the future for kryal systems, thus fueling hyporheic sediments with OM and thus promoting a shift towards a krenal BCC characteristic (Logue *et al.*, 2004; Uehlinger *et al.*, 2010).

Total cell abundance is also expected to change in alpine stream sediments. The quantity and quality of OM has been shown to correlate with shifts in BCC and bacterial abundance (Crump *et al.*, 2003; Olapade and Leff, 2005, 2006). Physical changes in the habitat template such as increasing channel stability because of reduced discharge may additionally favor generalists and reduce stochastic proliferations as apparent in kryal channels (Milner *et al.*, 2009). For instance, Füreder (2007) proposed an increasing importance of macroinvertebrate generalists within kryal systems because of reduced glacial runoff.

An altered physicochemical habitat template is likely to influence EF in both water systems. As BCC are more stable within krenal systems and they maintain a high functional plasticity, we would expect to see a less pronounced shift in community composition and a longer time horizon until changes appear. In contrast, kryal system BCC could change more rapidly and be more pronounced. They seem to be more constrained by physicochemical variables because of a reduced functional plasticity, and thus are forced to restructure their community with the changing habitat template. A reciprocal transplant experiment of krenal and kryal sediment types previously conducted in the Val Roseg showed that after 21 days of incubation in the non-native water source, there still was the signature of the native communities apparent with a generally smaller shift in BCC in krenal sediments (Freimann *et al.*, 2013). EF, on the other hand, adapted relatively fast to the new environment in both sediment types. The apparent situation in Macun is likely to represent such a state, where communities adapt their EF to the reduced glacial water inputs, whereas BCC still show a historical imprint. Regardless of shifts in BCC, EF could experience a significant change with reduced or lost kryal water inputs.

Distinct enzymes correlated well with physicochemical characteristics and BCC of each system. Increased ester and endopeptidase activity characterized kryal sediments, whereas α-glucosidase, β-glucosidase, β-xylosidase, *N*-acetyl-glucosaminidase, leucine aminopeptidase and phosphatase were more expressed in krenal systems. The importance of these typical krenal enzymes has been described for lowland rivers in both the water column and sediments (Wilczek *et al.*, 2005) and mirrors the importance of gathering carbon, nitrogen and phosphorous from different sources. For instance, krenal systems have high carbon input via cellulose, thus expressing Bet becomes important within krenal sediments to gather carbon from this non-limiting resource (Zah and Uehlinger, 2001). In kryal

systems, ester and endopeptidase seem to be favored to gather carbon and nitrogen, that is, from ester-containing molecules such as lipids and from peptides. Phosphorus seems to be a limiting resource in krenal systems, thus investment in phosphatase is favored compared with kryal systems that experience high PP loads that may be partly bioavailable (Hodson *et al.*, 2004). Decreased concentrations of PP could rapidly promote a functional shift towards krenal characteristics or lead to generally higher enzymatic activity. Specific enzymes that correlate well with physicochemical characteristics of kryal water could become less expressed, whereas typical krenal enzymes would dominate EF.

Our study shows how lotic microbial structure and function in glaciated alpine floodplains will potentially change as a consequence of altered hydrological conditions in the context of apparent landscape structures. Functional flexibility was high in both water systems, but it is unclear to what extent a shift of kryal BCC would be buffered if their provided EF could completely adapt to a changed physicochemical environment. Even if a functional response lag and high redundancy prevents microbial functionality from collapse, there still will be consequences for carbon and nitrogen cycles at the larger scale, that is, historical legacies altering EF (Strickland *et al.*, 2009; Shen and He, 2011). As microbes have an important role linking geochemical organic matter, nutrient cycling and higher trophic levels, a significant shift in alpine floodplain foodwebs and EF can also be expected. Long-term studies in these environments would give an opportunity to examine how persistent (kryal) bacterial communities are when substantial changes in habitat templates occur and how ecosystem functionality adapts or collapses. Predicting future EF and their impact on biogeochemical cycling should consider functional flexibility of apparent BCC as they can react differently to altered alpine ecosystems.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

We thank the Swiss National Park for access rights to the Macun catchment, Simone Blaser and Christa Jolidon for field and laboratory assistance and the anonymous reviewers for their valuable comments and suggestions to improve the quality of the paper. This study was funded by the Swiss National Foundation (No. 31003A-119735).

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