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Response of lotic microbial communities to altered water source and nutritional state in a glaciated alpine floodplain

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Abstract

Factors driving bacterial community composition (BCC) and linkages to ecosystem function (EF) are a fundamental interest in microbial ecology. Climate warming is expected to cause a shift from glacial- to groundwater-dominated water sources in alpine catchments due to receding glaciers, which is likely accompanied by a shift in BCC and EF. In this context, we performed a reciprocal transplant experiment of hyporheic sediments within a Swiss alpine floodplain. We assessed the influence of water source (groundwater = krenal, glacial water = kryal) and nutritional state (C, N, and P) on BCC and EF. Experimental response was tested using automated ribosomal intergenic spacer amplification and potential activities of eight different enzymes. BCC from both kryal and krenal systems was highly resistant to changes in water source, yet exhibited pronounced EF flexibility. Major factors determining BCC and EF response were sediment origin followed by seasonal variation in BCC. The gradient in seasonal change in BCC showed different strengths in the two water systems. Krenal BCC was more seasonally stable compared with kryal BCC, although functional plasticity showed the same extent in both. This difference in connectivity between BCC and EF suggests that krenal BCC was dominated by generalists, whereas kryal BCC was dominated by specialists. The weak effect of altered nutritional state on BCC and EF indicates a complex but hierarchically structured relationship among these factors. We conclude that microbial communities in alpine catchments are able to rapidly buffer the effect of shifts in water source on ecosystem functioning.

Most alpine glaciers have been retreating over the past decades due to climate change (Intergovernmental Panel on Climate Change 2007), thereby strongly influencing alpine floodplain ecosystems. Water source is a major environmental factor in alpine landscapes, which shapes floodplain structure and function. Future predictions based on climate models suggest that glacial (kryal) water will become temporarily more important in conjunction with strong glacial mass loss. Over longer time periods, it is likely that contributions by groundwater (krenal water) and precipitation run-off will predominate (Uehlinger et al. 2010) as habitat shaping agents of alpine floodplains.

Along with climate change, other anthropogenic pressures are altering the nutritional state and hydrology of alpine waters (Dickson et al. 2012). For instance, atmospheric deposition is a primary contributor of nitrogen (N) into these systems (Hiltbrunner et al. 2005). As deposition levels increase and N release is strongly coupled to snow storage, an elevated but less seasonally pronounced N input can be expected (Burns 2003; Hiltbrunner et al. 2005). In addition, phosphorous (P) plays an important role in alpine ecosystems and may represent the limiting nutrient for heterotrophic growth, primary production, and decomposition (Robinson et al. 2002). Glacial run-off and the hydrologic linkage of stream sediments with surrounding soils also influence the availability and forms of P (Tockner et al. 1997; Brady and Weil 2008). Lastly, alpine vegetation is sensitive to increasing temperatures, CO_2, and altered precipitation patterns and is continuously changing within alpine regions (Theurillat and Guisan 2001), thereby influencing organic matter (OM) input (quality and quantity) into floodplain waters (Zah and Uehlinger 2001).

Microbes, such as heterotrophic bacteria, within hyporheic sediments play a major ecological role in alpine waters due to their ability to carry out a wide range of functional processes. The hyporheic zone represents the interface between surface waters and groundwaters, where nutrient exchange and cycling occurs and where water and nutrient flows create linkages between riparian and alluvial zones (Findlay 1995). In this respect, kryal and krenal systems show different dynamics in their physical and biogeochemical characteristics, which creates diverse habitat patches inhabited by heterotrophic bacteria, and with krenal systems being more temporally stable.

These changes in environmental conditions and water sources restructure habitat patches, potentially causing microbial assemblages to differ in ecosystem function (EF). The underlying mechanisms dictating these shifts in EF are poorly understood because they are a result of differential changes within the bacterial community (i.e., changes in single-cell metabolism, changes in total cell numbers, and changes in bacterial community composition [BCC; Comte and del Giorgio 2011]). How bacterial communities cope with new environmental conditions may be strongly dependent on present BCC characteristics and the gradient of change in environmental conditions. Ecosystem functions can have different levels of redundancy and plasticity within a community, ultimately determining potential EF resistance

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to altered conditions (Allison and Martiny 2008). Generalist-dominated bacterial communities may be able to withstand environmental changes as they adapt their metabolism at the single-cell level, whereas specialist-dominated communities lack plasticity and thus are forced to shift community composition to those specialists able to perform a specific EF (Comte and del Giorgio 2011).

This study assessed the effect and interaction strength of differing water source and nutritional state on BCC and EF within hyporheic sediments of a proglacial floodplain. We conducted a reciprocal transplant experiment of hyporheic sediments between krenal and kryal systems in combination with nutrient amendments (C, N + P, and C + N + P). The experiment was repeated during three different seasons to cover temporal changes in landscape properties on microbial assemblages and functioning in both water systems. We hypothesized that each water system harbors a distinct bacterial community pool (i.e., generalists vs. specialists) and thus will react differently to changing environmental conditions created by a change in water source. We further hypothesized that seasonal fluctuations in chemistry would drive BCC and EF and thus influence the outcome of the transplant experiment.

Methods

Study site—Two streams (kryal, krenal) were chosen within the Val Roseg catchment in the Swiss Alps for the sediment transplant experiment. The catchment is part of the austroalpine Bernina nappe of crystalline bedrock. Around 30% of the catchment area is glaciated by the Roseg and Tschierva glaciers (http://www.hydrodaten.admin.ch/en/2256.html#stationsinfos). The Roseg River is an 11.3 km second-order tributary of the Inn River and represents the main channel within the floodplain. Its flow is mainly via water from the two glaciers; ~ 30% of the annual water volume is glacial meltwater. The glacial-water contribution is highest during glacial ablation from spring until autumn and lowest during winter. The Roseg gains lateral inputs from groundwater-fed tributaries with distinct seasonal inputs from snow melt. The relative importance of this water source increases when glacial ablation is low in winter (Tockner et al. 1997). The glacial stream (kryal) was situated within the floodplain and the nearby groundwater-fed stream (krenal) was situated next to the valley side.

Study design and sampling—A full-factorial reciprocal transplant experiment was performed using sediment in flow-through mesocosms, taking into account two sediment origins (SO; krenal or kryal), two incubation sites (IS; krenal or kryal), and four treatments (T; zero control [Z], C, NP, CNP; Fig. 1). The experiment was replicated across three seasons (summer, Aug [A]; winter, Oct [O]; spring, May [M]). At each site (krenal, kryal), hyporheic sediment was collected after removing the top 10 cm, and a < 8 mm (by sieving) size fraction was placed into an open-ended double-chambered mesocosm tube (6.5 cm diameter, 30 cm long) with both ends capped with 1 mm mesh nylon screen.

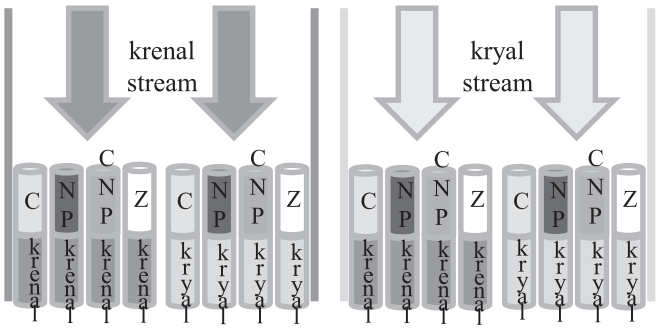

Fig. 1. Experimental set-up. Arrows depict the incubation-site water source, whereas cylinders illustrate the mesocosms. Treatments and sediment origin are annotated within the cylinders.

The second chamber was separated by a 1 mm mesh and kept empty (zero treatment) or used for three different nutrient amendments: carbon (C), nitrogen (N), and phosphorous (P), or C, N, and P combined. Osmocote Exact slow-release fertilizer tablets (Hauert HBG Dünger AG, Grossaffoltern) were used to add N as nitrate (NO_3-N) and ammonium (NH_4-N; 7.5% weight[wt] wt^{-1}, each), and P expressed as water-soluble phosphorus pentoxide (P_2O_5; 10% wt wt^{-1}). Two tablets were used per treatment (~ 10 g). Potassium acetate (Sigma-Aldrich, P1147) was used for C amendment; 36 g potassium acetate (8.82 g C) was dissolved with stream water in a 50 mL Falcon tube and placed within the respective mesocosm. An osmotic membrane was used to seal the Falcon tube (Spectra Por® Type F, Spectrum Laboratories, 250 kDa) to ensure a constant release during the experiment.

In the field, mesocosms were inserted into each channel with the nutrient supply chamber facing upstream. Three mesocosms per treatment were either embedded within the sediment source stream or transplanted into the nonnative water-source stream. Preliminary tests confirmed the slow-release properties of the nutrient sources. Osmocote tablets showed an average weight loss of 0.15 ± 0.02 g d^{-1}. Potassium acetate had a linear release rate when tested in the lab (−0.0189 g C h^{-1}, R^2 = 0.93) and showed an average release of 0.24 ± 0.09 g C d^{-1} in the field experiments as determined by the total C left in the Falcon tubes after the experiment. Taking into account flow velocity within the boundary layer of the streams (~ 0.1 m s^{-1}) and ~ 20-fold decrease in water velocity through the mesocosm sediment, one can expect an increase of C (compared with dissolved organic carbon [DOC] concentrations) of around 40% and 35% compared with averaged background concentrations of krenal and kryal waters, respectively. NH_4-N would show an increased concentration of 400% and 200%, whereas NO_3-N concentration would increase about 10% and 5% in krenal and kryal streams, respectively. P_2O_5-P would increase P concentrations around 200% and 150% for krenal and kryal streams, respectively, compared with background phosphate (PO_4-P) concentrations. These numbers are estimates because we do not know the exact flow rate of water within each mesocosm.

Incubations were performed in August and October 2009, and May 2010 for 21 d each. These months were chosen due to their specific hydrologic conditions driven by glacial ablation and snowmelt dynamics (i.e., high glacial water input into the kryal system in Aug, reduced glacial water input into the kryal system in Oct, and snowmelt water input into krenal and kryal systems during May with some additional glacial water input into the kryal system). At the end of incubation, sediments were collected and either directly processed for microscopic examination or frozen at −20°C for automated ribosomal intergenic spacer amplification (ARISA) and assessment of potential enzymatic activities. Freezing is thought to have only minor effects on enzyme activities or bacterial community profiling (Wallenius et al. 2010) within a uniform type of sample, although DeForest (2009) shows larger but inconsistent effects across widely varying soil types. In this study, equal effects on a specific enzyme can be expected due to the similarity in nature of the sediment types and environmental conditions. To counteract a potential bias in the interpretation of our results, we used statistical approaches that rely on patterns of dissimilarities of transformed and double-standardized potential enzyme activities rather than the absolute values or ratios between different enzymes (*see* below).

Specific conductivity (μS cm^{-1} at 20°C) and temperature were measured at each site with a conductivity meter (LF323; WTW, Weilheim, Germany). Surface-water samples (1 liter) 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 DOC, particulate organic carbon, total inorganic carbon, ammonium (NH_4-N), nitrite (NO_2-N), nitrate (NO_3-N), dissolved organic nitrogen, particulate nitrogen, phosphate (PO_4-P), dissolved phosphorus, and particulate phosphorus following standard protocols (Tockner et al. 1997). These data are summarized in Table 1 and Fig. 2, showing the expected differences between kryal and krenal streams, and are not discussed further.

Enzyme assays—Eight enzymes were tested for their potential activity using Methylumbelliferone (MUF)-labeled substrate analogues (Sigma-Aldrich Co.). Enzymes degrading polysaccharides were tested using 4-MUF-α-D-glucoside for Alpha glucosidase (*Alph*), which hydrolyses α-1,4- and 1,6-glucosidic linkages of polysaccharides; 4-MUF-β-D-glucoside for Beta glucosidase (*Bet*), which hydrolyzes beta-1,4-glucans; and 4-MUF-β-D-xylopyranoide for β-xylosidase (*Xyl*), which hydrolyses xylose residues. 4-MUF-N-acetyl-β-D-glucosaminidase was used to assess hydrolysis of 1,4-β-linkages of glucosamines by N-acetyl-glucosaminidase (*Nac*; Sinsabaugh et al. 2008). Esterase (*Est*) activity was measured using 4-MUF-acetate (Arpigny and Jaeger 1999). Leucine aminopeptidase (*Leu*) and endopeptidase (*Epep*) activity were measured using L-leucine-7-amido-4-methylcoumarin and 4-MUF-4-guanadinobenzoate, respectively (Vihinen and Mäntsälä 1989; Makoi and Ndakidemi 2008). 4-MUF-phosphate was used to assess phosphatase (*Phos*) activity.

Table 1. Physico–chemical characteristics of the kryal and krenal system during the different hydrological periods. DOC, dissolved organic carbon; POC, particulate organic carbon; TIC, total inorganic carbon; NH_4-N, ammonium; OM, organic matter; NO_2-N, nitrite; NO_3-N, nitrate; DN, dissolved organic nitrogen; PN, particulate nitrogen; PO_4-P, phosphate; DP, dissolved phosphorus; and PP, particulate phosphorus.

Incubation month	Stream type	Conductivity [μS cm^{-1}]	DOC [mg C L^{-1}]	POC [mg C L^{-1}]	TIC [mg C L^{-1}]	NH_4-N [μg L^{-1}]	OM [g 100 g^{-1}dw]	NO_2-N [μg N L^{-1}]	NO_3-N [mg N L^{-1}]	DN [mg N L^{-1}]	PN [mg N L^{-1}]	PO_4-P [μg P L^{-1}]	DP [μg P L^{-1}]	PP [μg P L^{-1}]
A	kryal	69.00	0.60	0.30	3.20	8.20	0.26±0.11	2.80	0.10	0.03	0.30	2.50	2.50	169.70
	krenal	30.00	0.30	0.12	6.60	2.50	0.25±0.09	0.50	0.10	0.01	0.30	2.50	2.50	6.10
O	kryal	43.00	0.30	0.28	3.80	2.50	0.35±0.10	0.50	0.10	0.02	0.30	2.50	6.80	8.20
	krenal	92.00	0.30	0.72	9.10	2.50	0.35±0.29	0.50	0.10	0.02	0.30	2.50	2.50	1.90
M	kryal	59.00	0.80	0.16	6.80	1.30	0.36±0.18	0.50	0.40	0.03	0.03	3.30	5.20	6.10
	krenal	85.00	0.30	0.08	9.50	2.50	0.57±0.74	0.50	0.10	0.01	0.03	2.30	2.80	6.00

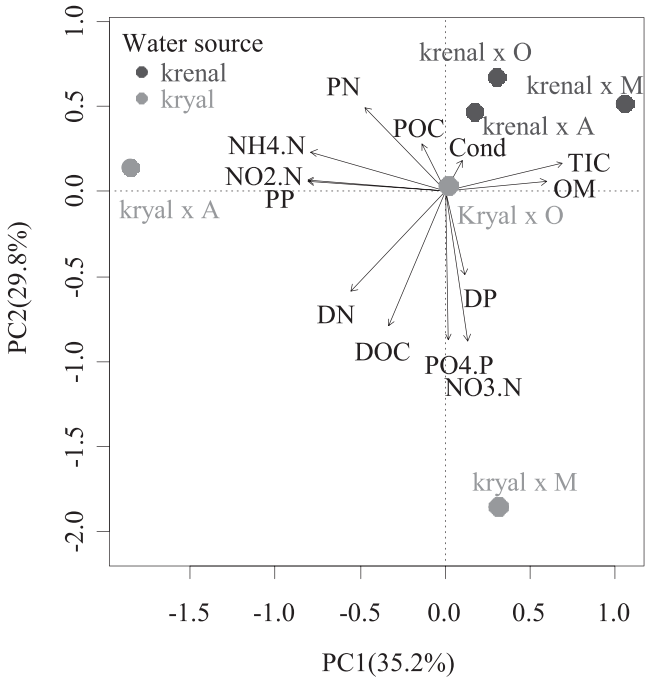

Fig. 2. Principal component analysis (PCA) biplot of physico–chemical characteristics. Dark and light grey dots depict the distinct water systems (kryal, krenal) at different hydrological periods (summer, Aug [A]; winter, Oct [O]; spring, May [M]). Given is the explained variance of PC1 and PC2.

Approximately 10 g of the frozen sediment sample was thawed overnight at 4°C and subsequently resuspended in 10 mL Milli-Q ultrapure water (MQ) and vortexed for 1 min. Supernatant was transferred into a 96 well microplate and substrate stock solution was added to a final concentration of 400 μmol L^{-1} (Findlay et al. 2001). The remaining sediment and MQ were dried at 60°C for 48 h to measure the dry weight of measured sediments. Fluorimetric enzyme assays were performed after adding the substrate over 24 h using a microplate reader (Tecan Infinite® 200). The excitation wavelength was set at 365 nm and fluorescence emission was measured at 445 nm. Plates were stored on a plate shaker at 15°C between measurements. Fluorescence values were corrected for quenching by adding a known quantity of free MUF to the samples and pure MQ or MQ and bicarbonate buffer mix, respectively. Reaction rates were calculated using the linear part of the fluorescence reaction curve. Subsamples of the collected sediments were air-dried at 50°C and total sediment OM was determined as ash-free dry mass by combusting the samples at 450°C for 4 h. Potential enzyme activities were standardized to nmol substrate g^{-1} OM h^{-1}.

Bacterial 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 (PBS) and 5 min centrifugation at 10,000 × *g* between washing steps. Samples were then stored at −20°C in a 1 : 1 mix of PBS and ethanol until further processing (Pernthaler et al. 2001). Biofilm-associated bacteria were detached with sonication (Branson Digital Sonifier 250, 5 mm tapered microtip, actual output of 20 W) using 1 s sonication pulses for 30 s. Samples were vortexed for 7 s followed by a short spin centrifugation for 5 s to settle coarse sediment particles interfering with subsequent processing of samples. The supernatant was transferred into a new Eppendorf tube and used for total cell counting of 4′,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich) stained cells. Between 10 μL and 60 μL of template solution was pipetted into 5 mL MQ ultrapure water and stained with DAPI for 7 min (1 μg mL^{-1} final concentration) and then filtered onto a black polycarbonate filter by applying a gentle vacuum (0.2 μm pore size, 25 mm diameter; Millipore, Molsheim, GTBP02500; Porter and Feig 1980). Filters were embedded into citifluor AF1 after air drying (Linaris Biologische Produkte). At least 16 photographs of each filter were taken with an epifluorescence microscope (Leica Microsystem, DMI6000b) and at least 800 cells were counted per filter using the CellC software (Selinummi et al. 2005). Cell numbers were then standardized to the dry mass of initially suspended sediments.

Bacterial community fingerprinting—ARISA was used to determine bacterial community structure (Fisher and Triplett 1999). Sediment samples were extracted using the PowerSoil DNA isolation Kit (MoBio, Carlsbad) following manufacturer's instructions. Fluorescein- (6-FAM) labeled universal forward primer 1406f-6FAM (16S ribosomal ribonucleic acid (rRNA) gene, 5′-FAM-TGYACA CACCGCCCGT-3′, Y = T,C), and the bacteria-specific reverse primer 23Sr (5′-GGGTTBCCCCATTCRG-3′, B = G,T,C, R = G,A) were used to amplify bacterial ribosomal intergenic spacers (Yannarell et al. 2003). Polymerase chain reaction (PCR) was performed using an TProfessional thermocycler (Biometra GmbH, Göttingen). A final reaction volume of 25 μL contained a mix of 1× GoTaq®Flexi buffer, 3 mmol L^{-1} $MgCl_2$, 0.25 mmol L^{-1} of each dNTP, 0.05 U μL^{-1} of GoTaq®Flexi DNA Polymerase (Promega), 0.25 mg mL^{-1} bovine serum albumin (Sigma-Aldrich), 0.4 μmol L^{-1} of each primer (Microsynth), and 1 μL of template DNA (~ 1 ng) was amplified.

Initial denaturation for 2 min at 94°C was followed by primer annealing at 55°C for 35 s and extension of 2 min at 72°C. Subsequently, 29 cycles with denaturation for 35 s and annealing and extension as above were performed, followed by a final extension of 2 min at 72°C. PCR products were subjected to electrophoresis on a 2% agarose gel with a 100 base pair (bp) ladder (Promega) and stained with ethidium bromide to verify successful amplification. ARISA fragments were processed as previously described (Bürgmann et al. 2011). A 1 μL aliquot of PCR product was mixed with 9 μL highly deionized (HiDi) formamide and 0.5 μL Liz1200 size standard (Applied Biosystems) followed by denaturation on a PCR thermocycler for 3 min at 95°C. Subsequently, samples were placed on ice. Denaturing capillary electrophoresis of each fragment was performed on a 3130XL Capillary Genetic Analyzer (Applied Biosystems) equipped with a 50 cm capillary using POP-7 polymer. ARISA fragments between 200 bp and 1400 bp were analyzed with the Southern size-calling

method and a background cut-off level of 50 fluorescence units. Binning of peaks was done with the automatic and interactive binning R scripts (Ramette 2009). Relative fluorescence intensity of binned peaks data was exported for further analysis.

Data analysis—Effects of treatment, incubation site, and sediment origin on single potential enzyme activities, total cell abundances, OM, and number of operational taxonomic units (OTUs) were tested by ANOVA separately for each season. Tukey's test was performed if there was a significant interaction of all three fixed factors present. An additional complete ANOVA model including season as a factor was performed to assess the effect of different hydrological conditions on the experimental outcome. Normality of residuals was assessed using a Shapiro-Wilk's test and examining the quantile–quantile-plot of the residuals. Levene's test was used to check homogeneity of variance. If one of the assumptions was violated, data were transformed by ln(x + 1). Percentage values were arcsine (sqrt)-transformed prior to analysis. Lastly, a principal component analysis was performed with physico–chemical data to assess qualitatively the temporal changes within and between the two water systems.

Results from community fingerprinting and enzymatic activities were analyzed using nonmetric multidimensional scaling (NMDS) constrained to two dimensions. Dispersion ellipses using standard error of the weighted score averages were drawn for season × sediment origin. The weighted correlation defines the direction of the principal axis of the ellipse (Oksanen et al. 2011). Enzymatic activities were used for vector fitting and tested by permutation tests. Additionally, NMDS including the season results was produced to illustrate shifts in EF or BCC. Generalized additive models (GAM) were fitted for enzyme activities in the NMDS plots to visually check whether there was a linearity of enzymatic factor fittings in the ARISA NMDS plots and to assess the relationship of the enzyme centroids to their surrounding within the EF NMDS (Bennion et al. 2011; Oksanen et al. 2011; graphics and data not shown).

We performed multivariate homogeneity of groups dispersion (MHGD), an analogue of Levene's test for homogeneity of variance, to test whether fingerprinting profiles and enzymatic activities were distinguished in their variability. Bray–Curtis dissimilarities of transformed and standardized data sets were reduced to principal coordinates and distances to defined group centroids were then calculated. Significance between group dispersions was assessed using a permutation test (999 permutations; Anderson et al. 2006).

Permutational multivariate analysis of variance (PERMANOVA) was used to assess the influence of treatment, incubation site, sediment origin, and season on community and potential enzyme activity structures (Anderson 2001). A complete model with all factors was used to assess changes in community structure and function in relation to season, sediment origin, incubation site, and treatment. Hierarchical structured models, testing the effect of incubation site within one sediment origin, the effect of incubation site within one sediment origin and within a season, and the effect of treatment additionally within a distinct incubation site, were used to illustrate changes of community structure and function that were likely covered by strong effects of sediment origin and season. A procrustes analysis of the corresponding NMDS ordinations of ARISA profile and potential enzyme activities was used to assess potential linkage between structure and function (Gower 1975). This leads to a m^2 statistic, which is a measure of congruence of the two ordinations. The procrustes correlation r was calculated as $r = \sqrt{1 - m^2}$. Configurations of NMDS were scaled to equal dispersion. The two configurations were tested for nonrandomness by the means of permutations with the protest function (Peres-Neto and Jackson 2001). NMDS, MHGD, and PERMANOVAs were based on Bray–Curtis dissimilarity matrices calculated from the Wisconsin double-standardized relative fluorescence intensity of ARISA profiles and the Wisconsin standardized square-root–transformed potential enzyme activities (Bray and Curtis 1957). All analyses were done using the vegan package in R (Oksanen et al. 2011, R Development Core Team 2011).

Results

Enzymatic activity—Enzymatic activities are shown in Figs. 3, 4 and detailed results of ANOVAs separated by season are reported in Table 2. In addition, ANOVAs incorporating season as a fixed factor revealed that the total potential enzyme activity (sum of the eight potential enzyme activities) had an interaction effect with season and sediment origin and with season and incubation site (ANOVA: $F_{2,96} = 8.48$, $p < 0.001$ and $F_{2,96} = 14.12$, $p < 0.001$, respectively). Krenal sediments had equally low total enzymatic activity in summer as kryal sediments in all seasons and highest activities in spring compared with kryal sediments in summer and spring (Tukey's honestly significant difference test [HSD]: $p < 0.05$). Sediments incubated in the kryal system showed generally lower enzymatic activities except for spring where activities were equally high as in sediments incubated in the krenal system (Tukey's HSD: $p < 0.05$).

Five enzymes showed a highly significant effect of season (*Bet, Leu, Est, Epep,* and *Phos,* ANOVAs: $p < 0.001$). The interaction term for season and sediment origin was likewise significant for six potential enzyme activities (*Alph, Bet, Leu, Nac, Epep,* and *Phos*), and the interaction term for season and incubation site was significant for five enzymes (*Xyl, Leu, Est, Epep,* and *Nac*). Significant treatment effects were observed for five enzymes (*Bet, Nac, Est, Epep,* and *Leu,* ANOVAs: $p < 0.05$). Treatment effects were mainly interconnected with sediment origin (*Nac*), with sediment origin and season (*Epep*), or with sediment origin, incubation site, and season (*Leu* and *Bet*).

Bacterial abundance and biomass—Bacterial cell abundance was affected by season, with lowest cell densities during spring compared with winter in kryal sediments (ANOVA: $F_{2,96} = 34.22$, $p < 0.001$, Tukey's HSD: $p < 0.05$; *see* Fig. 4 bottom). Krenal sediments had lowest cell densities in summer, and were higher than kryal cell

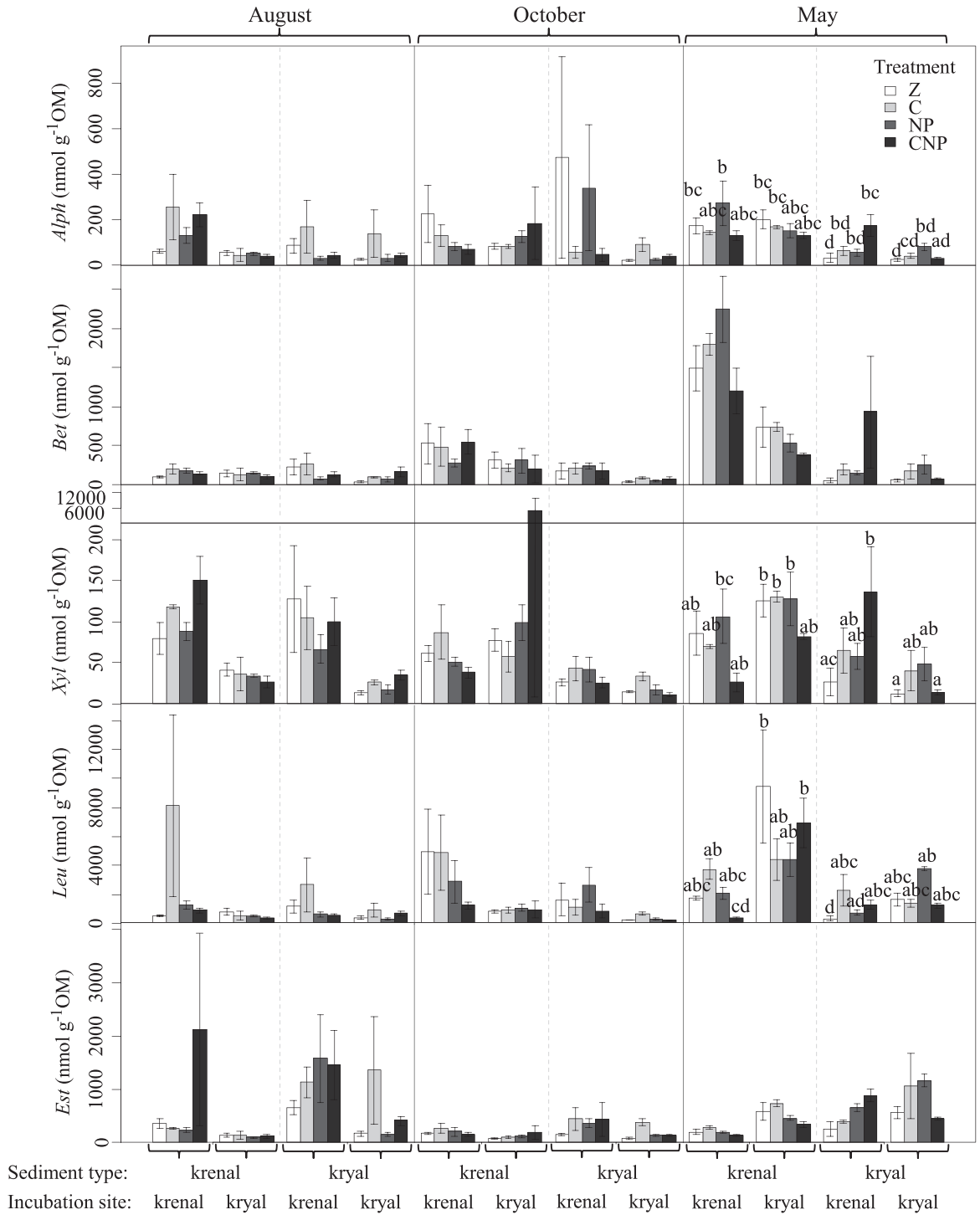

Fig. 3. Bar plots of measured enzymatic activities. The graph is divided into the different hydrologic periods by the solid line, and within the season the graph is split by the dashed line into the different sediment types. The incubation site of the specific sediment is annotated at the bottom. Bars that are significantly different (Tukey test after significant three-way interaction ANOVA at $p < 0.05$) are annotated by different letters. Note the axis break in the *Xyl* activity bar plot.

densities in any season (Tukey's HSD: $p < 0.05$). Krenal sediments had generally higher cell densities than kryal sediments (mean ± SE: krenal = $9.78 \times 10^7 \pm 8.00 \times 10^6$ cells g^{-1} dry weight [dry wt], kryal = $19.94 \times 10^6 \pm 2.85 \times 10^6$ cells g^{-1} dry wt; ANOVA: $F_{1,96} = 788.05$, $p < 0.001$).

The dependence of bacterial cell density on treatment varied with season and sediment type (ANOVA: $F_{6,96} = 10.30$, $p < 0.001$). Incubation site influenced the effect of treatment on bacterial densities depending on season (ANOVA: $F_{6,96} = 7.25$, $p < 0.001$). Native krenal

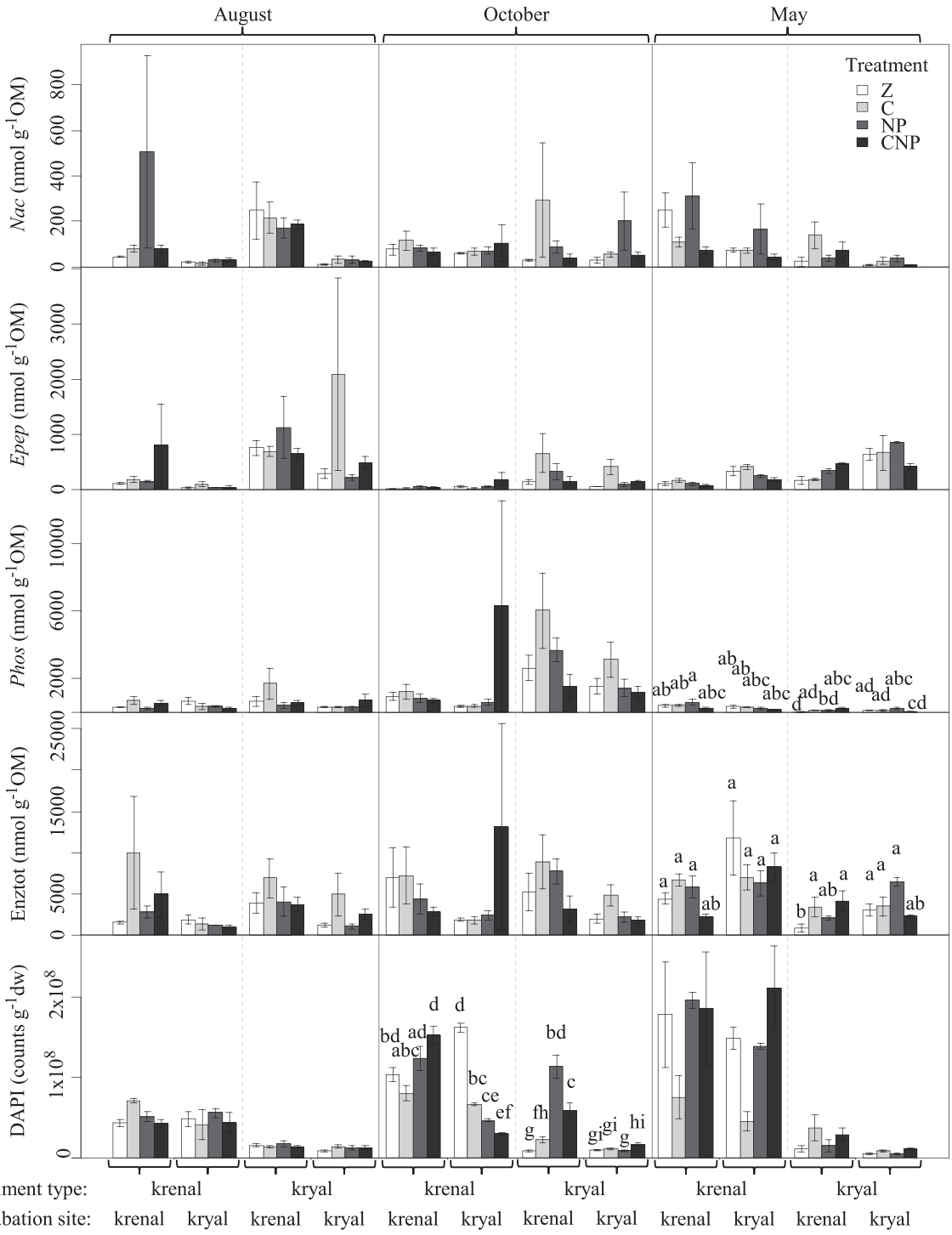

Fig. 4. Bar plots of measured enzymatic activities, total enzymatic activities (Enztot), and total cell abundances. The graph is divided into the different hydrologic periods by the solid line, and within the season the graph is split by the dashed line into the different sediment types. The incubation site of the sediment is annotated at the bottom. Bars that are significantly different (Tukey test after significant three-way interaction ANOVA at $p < 0.05$) are annotated by different letters.

sediments had decreased bacterial density when exposed to any nutrient treatment in addition to a nonnative water-source effect in winter. In contrast, kryal sediments had increased cell densities due to any nutrient treatment after transplantation into the nonnative water source in winter (Tukey's HSD: $p < 0.05$; Fig. 4).

Mean krenal sediment OM was 0.40% ± 0.13% of dry wt, whereas kryal sediments had mean OM of 0.27% ±

Table 2. ANOVA results (*F*-values) for bacterial abundance, operational taxonomic units (OTUs), and enzymatic activities. DAPI: 4′,6-diamidino-2-phenylindole.

F-statistic parameter	Incubation month	Treatment (T)	Incubation site (IS)	Sediment origin (SO)	T × IS	T × SO	IS × SO	T × IS × SO
df		3	1	1	3	3	1	3
DAPI	Aug	0.78	4.28*	180.35***	0.44	0.24	0.24	2.49
	Oct	14.66***	237.49***	754.16***	74.45***	67.31***	22.2633*	13.27***
	May	3.63*	14.9***	267.16***	0.71	9.25***	7.8**	0.11
Number of OTUs	Aug	3.33*	0.28	3.08	2.1	1.27	0.01	6.68**
	Oct	1.50	0.02	0.12	1.21	0.78	2.51	0.35
	May	1.19	0.04	18.97***	0.46	1.16	0.24	0.51
Total enzymes	Aug	1.77	16.89***	2.61	0.43	0.63	0.23	2.17
	Oct	1.25	8.27**	0.31	0.53	0.65	0.01	0.34
	May	1.98	12.5**	24.88***	2.73	3.67*	0.03	4.12*
α-Glucosidase (*Alph*)	Aug	1.22	10.00**	5.25*	0.16	1.48	2.68	1.76
	Oct	0.78	1.58	4.07	1.2	0.11	0.8	1.35
	May	2.62	1.78	65.19***	1.98	4.53**	1.07	3.24*
β-Glucosidase (*Bet*)	Aug	0.53	5.17*	2.24	0.69	1.79	1.04	2.34
	Oct	0.53	14.69***	18.55***	0.25	0.77	0.22	2.16
	May	3.14*	11.15**	104.73***	2.01	4.13*	3.39	2.02
Xylosidase (*Xyl*)	Aug	0.81	19.54***	2.83	0.27	0.59	0.04	0.31
	Oct	0.27	0.16	9.48**	0.05	0.26	1.92	0.44
	May	3.01*	0.21	25.22***	0.42	4.45*	14.89***	3.31*
Leucine-aminopeptidase (*Leu*)	Aug	1.67	10.79**	0.73	1.21	0.72	0.63	2.04
	Oct	2.72	25.75***	18.44***	0.51	0.42	0.07	1.15
	May	2.56	36.78***	24.22***	4.84**	4.49**	0.86	5.89**
Esterase (*Est*)	Aug	1.56	25.78***	15.86***	0.55	1.32	0.04	1.11
	Oct	2.75	8.48**	3.78	0.32	1.16	0.01	0.77
	May	3.18*	25.19***	16.54***	2.23	4.74**	2.93	1.88
N-acetyl-glucosaminidase (*Nac*)	Aug	1.62	45.48***	0.88	0.31	0.76	1.95	0.57
	Oct	2.35	0.08	1.62	0.68	1.23	0.44	0.27
	May	2.99*	12.93**	36.53***	0.50	3.59*	0.12	2.03
Endopeptidase (*Epep*)	Aug	1.12	13.17***	55.93***	0.83	0.07	1.53	0.29
	Oct	1.85	0.56	50.37***	2.01	4.07*	8.46**	0.66
	May	1.34	47.25***	33.84***	2.89	4.36*	0.06	1.39
Phosphatase (*Phos*)	Aug	1.14	2.92	0.87	1.55	0.29	0.74	1.07
	Oct	1.22	4.78*	15.58***	0.06	0.34	0.27	0.35
	May	1.88	0.17	54.44***	3.16*	3.07*	3.40	4.83**

*** $p<0.001$; ** $p<0.01$; * $p<0.05$.

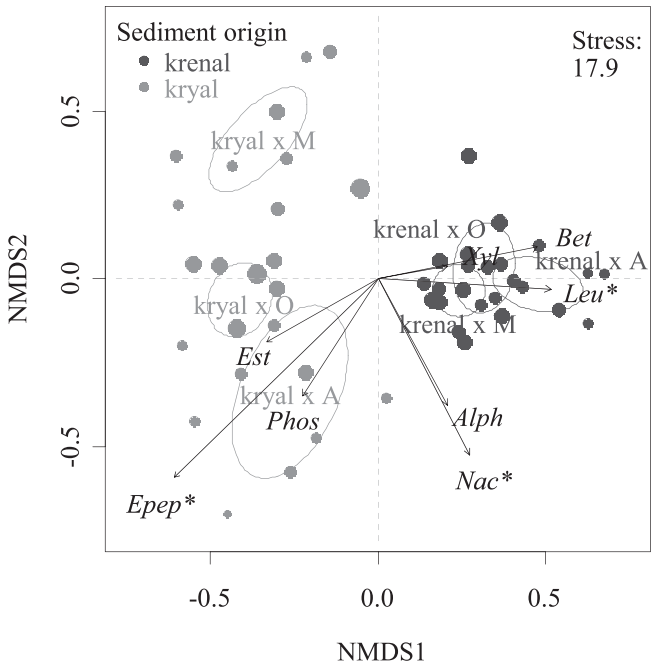

Fig. 5. NMDS of ARISA profiles. Dots depict ordination of bacterial community structure. Light and dark grey dots correspond to the distinct sediment origins and the size is relative to the numbers of OTUs. Strength and direction of fitted enzyme activity gradients are depicted as arrows. Enzymes fitted with permutational power of $p < 0.05$ are denoted by an asterisk (*see* Methods for abbreviations). Dispersion of standard error of the weighted scores of factors sediment origin × season (summer, Aug [A]; winter, Oct [O]; spring, May [M]) are depicted as ellipses in the respective grey tone (Confidence limit (CL) = CL = 0.95).

0.19%. OM showed an interaction effect of incubation site, season, and treatment (ANOVA: $F_{6,96} = 3.17$, $p < 0.01$). Generally, OM was highest in krenal sediments and lowest in summer in both sediment types (ANOVA: $F_{1,96} = 11.13$, $p < 0.001$, Tukey's HSD: $p < 0.001$ and $F_{2,96} = 17.62$, $p < 0.001$, Tukey's HSD: $p < 0.05$, respectively).

Bacterial community composition—In summer, transplanting sediment of either origin into a nonnative water source induced a shift in community structure. This response also was true for the experiment performed in winter, but only for krenal sediments (Table 3). In spring, no significant shift in community structure was observed in transplanted sediments (Table 3). A highly significant effect of sediment origin and interaction of sediment origin and season on BCC was apparent in the total model ($F_{1,48} = 27.04$, $r^2 = 0.17$, $p < 0.001$ and $F_{1,48} = 6.74$, $r^2 = 0.08$, $p < 0.001$, respectively). Indeed, the NMDS ordination and single-term PERMANOVA revealed that bacterial communities differed mainly between the two sediment origins and was influenced by a seasonal shift that was more distinct within kryal sediments (Fig. 5; Table 3).

The complete PERMANOVA model showed an interaction of treatment, incubation site, season, and sediment origin, indicating a high dependence of a treatment effect on the other factors ($F_{6,48} = 1.35$, $r^2 = 0.05$, $p < 0.05$). Accordingly, a treatment effect was mainly found in kryal

Table 3. PERMANOVAs of ARISA profiles ($n = 2$). *F*-values of structured pairwise comparison for sediment origin (18.95***; df = 1, 95), season (df = 2, 47), incubation site (df = 1, 22), and treatment (df = 1, 7). Italic numbers indicate the kryal incubation site, and nonitalic numbers indicate the krenal incubation site. For treatments, see Fig. 1.

		Kryal						Krenal					
	Season	Incubation site	Treatment	Z	C	NP	CNP	Season	Incubation site	Treatment	Z	C	NP	CNP
Summer	1.66*		Z	—	*2.24***	*1.65**	*0.92*			Z	—	*0.76*	*1.98**	*1.47*
			C	0.90	—	*1.64**	*1.20*			C	1.23	—	*1.45*	*0.87*
			NP	1.23	0.92	—	*1.06*		1.96*	NP	1.43	0.96	—	*0.88*
			CNP	2.23**	0.99	1.26	—			CNP	0.99	0.84	1.29	—
Winter	5.21***	1.52	Z	—	*1.17*	*1.29*	*1.25*	6.73***	2.29*	Z	—	*1.31*	*0.63*	*0.51*
			C	0.98	—	*1.33*	*2.15***			C	1.1	—	*1.98*	*0.66*
			NP	1.13	1.59	—	*1.44*			NP	0.31	0.71	—	*1.07*
			CNP	1.50	2.04*	1.69	—			CNP	2.12*	1.89	1.51	—
Spring	1.04		Z	—	*1.54*	*0.98*	*1.44*		1.11	Z	—	*0.82*	*1.4*	*2.49**
			C	1.78*	—	*1.92**	*1.18*			C	0.89	—	*1.12*	*2.02*
			NP	0.49	1.45	—	*0.96*			NP	1.55	1.25	—	*0.39*
			CNP	1.14	0.85	1.50	—			CNP	0.98	0.85	1.29	—

*** $p<0.001$; ** $p<0.01$; * $p<0.05$.

Table 4. PERMANOVAs of enzymatic activity profiles ($n = 3$). *F*-values of structured pairwise comparison for sediment origin (53.05***; df = 1, 143), season (df = 2, 71), incubation site (df = 1, 23), and treatment (df = 1, 11). Italic numbers indicate the kryal incubation site, and nonitalic numbers indicate the krenal incubation site. For treatments, see Fig. 1.

		Kryal					Krenal					
							Incubation					
	Season	Incubation site	Treatment	Z	C	NP	CNP	Season	site	Treatment	Z	C	NP	CNP
Summer			Z	—	*1.36*	*0.33*	*0.40*			Z	—	*2.04*	*1.28*	*3.24***
			C	0.62	—	*1.59*	*1.50*			C	1.11	—	*1.82*	*1.31*
		3.48**	NP	1.32	0.88	—	*0.27*		9.46***	NP	1.24	1.52	—	*0.88*
			CNP	1.04	0.61	0.18	—			CNP	0.42	1.3	2.03	—
Winter			Z	—	*1.45*	*2.40*	*1.04*			Z	—	*0.28*	*0.19*	*4.00***
			C	1.63	—	*3.81**	*1.05*			C	0.75	—	*0.11*	*3.66***
	21.61***	2.89*	NP	0.02	1.36	—	*2.48*	9.98***	6.33***	NP	1.02	0.06	—	*3.06**
			CNP	0.97	1.38	0.55	—			CNP	3.54**	2.61*	2.64*	—
Spring			Z	—	*2.48*	*1.57*	*0.70*			Z	—	*1.51*	*0.94*	*0.33*
			C	3.31*	—	*0.77*	*0.72*			C	2.12	—	*1.42*	*3.41**
		15.94***	NP	0.09	2.20	—	*0.38*		47.65***	NP	0.24	2.88*	—	*2.1*
			CNP	0.89	1.85	0.86	—			CNP	3.63**	4.21**	2.92*	—

*** $p<0.001$; ** $p<0.01$; * $p<0.05$.

sediments in summer, where the C and NP treatment in the nontransplanted and the CNP treatment in the transplanted sediments significantly influenced community structure (Table 3). The C treatment also was different from the CNP treatment in kryal sediments in winter whether they were transplanted or not, and a C treatment effect was observed in spring in the transplanted kryal sediments (Table 3).

Multivariate dispersion showed that krenal sediment bacterial communities showed, in general, less variation and thus a more seasonally stable community composition (MHGD: $F_{1,94} = 87.27$, $p > 0.001$). This pattern also was found when single seasons were analyzed for dispersion differences between sediment origins (MHGD: summer: $F_{1,30} = 39.23$, $p > 0.001$, winter: $F_{1,30} = 6.04$, $p > 0.05$, spring: $F_{1,30} = 97.80$, $p > 0.001$).

The number of detected OTUs differed in spring between the two sediment types, with more OTUs detected within krenal sediments (ANOVA: $F_{2,96} = 9.98$, $p < 0.001$). Kryal sediments had reduced numbers in OTUs in the C treatment in the native water source and after transplantation and CNP treatment in summer compared with the CNP treated and nontransplanted kryal sediments (Table 2; Tukey's HSD: $p < 0.05$).

Enzymatic activity structure and dynamics—There was an incubation site effect on enzymes in all seasons, being most pronounced in spring (Table 4). A nutrient treatment effect on kryal sediment enzymatic activity structure was mainly apparent in the C-treated nontransplanted sediments in winter and in the transplanted kryal sediments in spring. Krenal sediments were more often affected by nutrient treatments. There was a CNP effect in summer and winter in the transplanted sediment on potential enzyme activity structure compared with the nontreated sediment. The nontransplanted krenal sediments were influenced by CNP treatment in winter and spring (Table 4). The complete PERMANOVA model revealed highly significant differences in enzymatic activity structure between the two sediment origins ($F_{1,143} = 113.57$, $r^2 = 0.27$, $p < 0.001$) and a seasonal shift in sediment functioning ($F_{2,143} = 40.35$, $r^2 = 0.19$, $p < 0.001$; Fig. 6).

Epep and *Est* were the primary enzymes characterizing kryal sediments (Fig. 6), and this pattern remained for *Epep* within the different seasons (Fig. 5). *Alph, Bet,* and *Leu* showed mainly an activity gradient directed toward krenal sediments (Figs. 5, 6; GAM fittings not shown). Seasonal differentiation between the two sediment types was mainly apparent in spring where six enzymes dominated krenal sediments, whereas *Epep and Est* still characterized kryal sediments (Fig. 6; GAM fittings not shown). Potential enzyme-activity–based NMDS ordination revealed a seasonal shift in EF, where *Bet* and *Leu* showed more pronounced activity in krenal sediments in spring. *Alph* dominated krenal sediments in summer, whereas *Est* and *Epep* activity was most pronounced in kryal sediments during this period. *Phos* dominated kryal sediments in winter (Fig. 6; GAM fittings not shown).

Although enzymatic activity structure was less variable in krenal sediments when all samples were analyzed

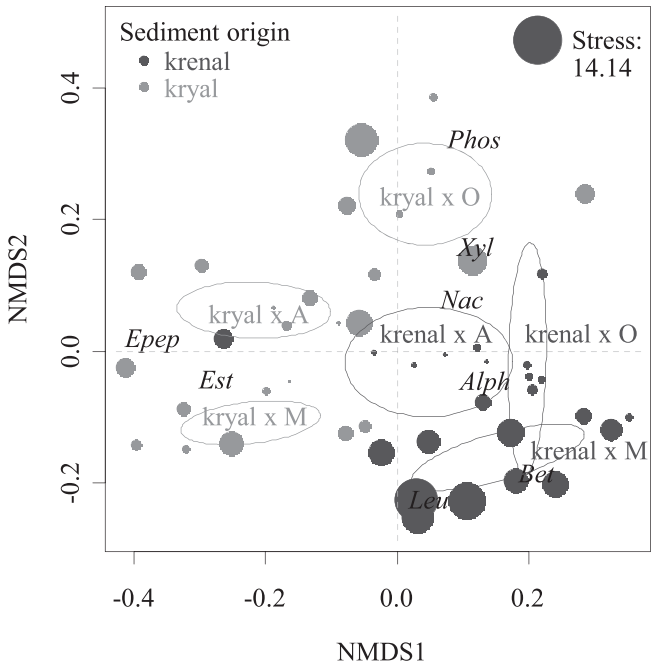

Fig. 6. NMDS of enzymatic activities. Dots depict samples in ordination based on enzymatic activity structure. Distinct enzyme scores are annotated. Light and dark grey dots correspond to the distinct sediment origin and the size is relative to the log of the sum of the measured enzymes activities. Dispersion of standard error of the weighted scores of factors sediment origin × season (summer, Aug [A]; winter, Oct [O]; spring, May [M]) are depicted as ellipses in the respective grey tone (CL = 0.95).

(MHGD: $F_{1,142} = 11.83$, $p > 0.001$), no difference in variability was detected within each season between kryal and krenal sediments (MHGD $_{(summer,\ winter,\ spring)}$: $F_{1,46} = 2.11, 1.14, 0.88$; $p = 0.154, 0.291$, and 0.352, respectively).

Linkage of bacterial structure and function—Although a BCC shift was less pronounced in krenal sediments (i.e., size of dispersion ellipses in Fig. 5; Table 3), there was a clear change in EF in both sediment types when transplanted, indicating a response to a change in water source. Transplanted sediments became functionally adapted to the new environment, as apparent in the relative movement of the dispersion ellipses (depicting specific sediment origin, incubation site, and season) toward the native sediment EF activity pattern (figures not shown). We observed a linkage between community structure and function when NMDS ordinations based on ARISA and potential enzyme activity, respectively, were compared by a procrustes test ($r = 0.593$, $p < 0.001$; *see* Figs. 5, 6). However, the correlation was significant only in kryal sediments compared with krenal sediments ($r = 0.588$, $p < 0.001$ and $r = 0.227$, $p = 0.570$, respectively) when the different sediment origins were analyzed separately.

Discussion

Our results suggest that hierarchically structured drivers influence the patterns of bacterial community structure and functioning within hyporheic sediments in alpine running waters. For instance, sediment origin was the main factor driving BCC and EF patterns, and BCC changed seasonally based on the characteristics of the specific sediment type. Lastly, water source and partly nutrient state modified BCC and EF at the finer scale.

Sediment origin: A strong delineator of BCC and EF—The NMDS ordinations and PERMANOVA model results showed a clear separation of BCC between the two sediment origins independent of any other factor. This differentiation between kryal and krenal channels has been described before, although for another alpine catchment and at a shorter temporal scale (Logue et al. 2004). Higher cell densities in krenal sediments supported this separation of BCC by sediment origin. This finding suggests that microbial communities in the two sediment types are adapted to the sediment-specific physico–chemical habitat template. Spatial variability across habitats has been shown to be a major separator of BCC (Fortunato et al. 2012), and this variation is driven by different mechanisms or environmental factors such as pH (Fierer et al. 2007), temperature (Adams et al. 2010), and hydrogeochemical conditions.

Bacterial function showed the same trends as those in community structure (i.e., differentiation between the two sediment origins as also reported from different systems; Romaní 2000). Distinct potential enzyme activities could be assigned to each sediment type, although dependent somewhat on season and modulation by water source. High *Epep* and *Est* activity typically characterized kryal sediments, whereas *Bet* and *Leu* were more active in krenal sediments. This result indicates that the relative importance of gathering N and C from distinct resources can change. For example, C may be a nonlimiting resource due to the relatively higher input of cellulose into krenal waters, and thus *Bet* plays a more important role in krenal sediments (Zah and Uehlinger 2001). In kryal systems, it is probably more favorable to gather C from small ester-containing molecules such as lipids and, for example, N from peptides, and thus investment in *Est* and *Epep* is favored in kryal systems (Grzesiak et al. 2009).

Different seasons: Different communities and associated functions—Seasonal change is a common observation within riverine bacterioplankton and hyporheic bacterial communities (Lowell et al. 2009). In this study, seasonal shifts in BCC and EF also were apparent for both sediment types, and the physico–chemical habitat template changed with season. Seasonality in aquatic bacterial biofilm community structure and activity has been connected to the shifting physico–chemical habitat template (Sekar et al. 2002), and thus may also be a main driver in streams in alpine catchments.

Our results further showed that krenal sediments have a less pronounced seasonal BCC dynamic compared with kryal sediments, and therefore likely represent a more temporally persistent bacterial community. Such patterns in separation of BCC between the sediment types and their strength of compositional seasonality was observed within

the same floodplain for a longitudinal and lateral sampling program and seems to represent a general trend in alpine floodplains (R. Freimann unpubl.). The lack of strong seasonal variation in krenal BCC in their native stream habitat may be due to relatively small temporal shifts in the physico–chemical habitat template that allow for a stable bacterial community, or the potential of krenal bacterial communities to better cope with environmental change. The latter point includes physiological plasticity of krenal bacteria at the genetic level resolved in this study. Although krenal waters are more stable in physico–chemical characteristics, the latter statement also is supported by the fact that transplanted krenal BCC shifted little but still showed strong changes in EF.

Environmental milieu and life strategies: How bacteria handle altered water source and nutritional state—Distinctions between BCC of the two sediment origins remained clear following transplantation in all seasons. This relatively high resistance in bacterial community structure to a changing environment (here, water source) has been observed in several other studies. For instance, Zumsteg et al. (2011) transplanted soils from glacier forefields to soils of different temperature regimes and found no assimilation of nonnative communities to the new environment after 8 weeks of incubation, and only slight adaptation after 16 weeks of incubation. Waldrop and Firestone (2006) showed a soil origin effect on BCC mediated by altered environmental conditions over a 2 yr time scale. They observed a compositional and functional community shift of transplanted oak ecosystem soil into grassland but not vice versa. The fact that we could still see the signature of sediment origin BCC when transplanted indicates a relatively autonomous native bacterial assemblage that undergoes changes due to adaptation of resident taxa instead of colonization by stream bacteria from outside the system. We would therefore expect that seasonal changes are part of a recurring and, in itself, stable successional pattern typical for each sediment origin, while the time needed for species replacement of transplanted communities toward a local water source community composition clearly extends beyond the 21 d duration of our experiment.

In contrast to BCC, EF in transplanted sediments showed a response to a new water source, which was most pronounced in spring. During spring, bacteria that show strong functional plasticity could be favored due to diurnal fluctuations in physico–chemical habitat characteristics. A previous study showed a relative homogeneous and thus spatially weak structured BCC within the water-logged riparian zone of the krenal incubation site (R. Freimann unpubl.). Nevertheless, a distinct shift in EF along the hydrological flow path was present, indicating a high functional plasticity during this period. Although the kryal BCC did not show a significant shift after transplantation to the krenal channel in spring, there was a larger variability between transplanted and nontransplanted communities as seen by a higher dispersion in the NMDS (figure not shown).

We observed varying strengths in the coupling of structure and function in the procrustes analysis. There was a weak and nonsignificant linkage in krenal communities between function and community structure, whereas shifts in kryal BCC was more congruent with shifts in EF. Krenal microbial communities responded to seasonal changes in the physico–chemical habitat by changing their functional output with relatively small changes in BCC compared with the larger shift observed in kryal bacterial communities. This finding suggests that different sediment origins have a different level of functional plasticity (Allison and Martiny 2008). Environmentally driven functional adjustments may occur at the single cell level, thus changing the single cell metabolic activity distribution within the community and finally EF.

Kryal bacterial communities faced a stronger seasonal shift in environmental factors. The plasticity of dominant phylotypes appears to be limited in relation to environmental shifts, rendering kryal communities less resistant. Selection toward phylotypes present in the metacommunity that functionally and metabolically fit the new habitat template changes EF by rearranging single cell characteristics within the community. Comte and del Giorgio (2011) referred to this as an adjustment and replacement scenario. They analyzed bacterio–plankton community composition and metabolic response to a resource gradient along aquatic ecotones and found that composition influenced the pathway but not the outcome in metabolic response to shifts in available resources.

The linkage of species composition to ecosystem function is not written in stone, but rather seems to be a continuum from strong functional linkages to functional redundancy and plasticity depending on the complexity of the system and the scope in function (Boucher and Debroas 2009; Comte and del Giorgio 2010). Frossard et al. (2012) investigated EF and BCC in a nascent stream corridor and found a pronounced uncoupling between community composition and ecosystem functioning, with EF being mainly driven by seasonality, whereas BCC showed no seasonal pattern. They concluded that bacterial communities have a high functional redundancy within these early successional landscapes. In this context, krenal sediments appeared to be habitat for bacterial generalists that do not reorganize their community structure to environmental change, at least within the magnitude of change and temporal scope covered in this study. In contrast, kryal sediment bacteria showed a simultaneous transition in community structure and EF, which suggests that kryal bacterial communities act more as functional specialists.

The relative lack of response in bacterial abundance to nutrient additions in this study could be due to various mechanisms. For instance, alpine streams, in general, have low amounts of DOC and lower temperatures compared with streams in other studies. Olapade and Leff (2005, 2006) found a significant positive DOM effect on total bacterial abundance and shift in BCC in a low-elevation stream, mainly induced by labile DOM. The BCC in alpine streams may lack the functional ability to incorporate additional readily available C into biomass, at least within the 3 week experimental period in our study. Another issue may be differences in the general experimental set-ups, the resources used, and their applied concentrations in different studies. For instance, Olapade and Leff (2005, 2006) used clay pots filled with relatively high concentrations of a

glucose solution (~ 1 mol L^{-1}) as artificial substratum, thus instilling DOC directly into the biofilm.

Kryal bacterial communities showed highest diversity within each season that was driven by BCC shifts due to transplantation and nutrient treatment effects. Although the PERMANOVA results do not show shift in community composition due to nutrient addition, kryal sediment BCC was more influenced by the amended nutrient state (*see* Table 3). The relatively weak response pattern of BCC to nutrient amendment could be due to the relatively low increase in nutrient concentrations. For example, Bowen et al. (2009) found that even a severe increase (15× above background) in N and P in salt-marsh sediments did not necessary lead to a shift in BCC. Furthermore, Lage et al. (2010) found within the same habitat a shift in ammonia-oxidizing bacteria composition when N and P were applied separately, but this effect was not present when N and P were applied together.

Distinct strategies of bacterial taxa contribute to the assembly and functional maintenance of bacterial communities. Global change–induced changes in water source will be reflected in shifts in EF. The high functional flexibility in both sediment types is likely to sustain EF in track with newly introduced environmental gradients. Although microbial functionality adapts to a new environment, such a shift still will have implications on C and N cycles at the ecosystem scale (Shen and He 2011). For instance, decomposition rates were shown to be influenced by historical imprints on BCC (Strickland et al. 2009). Bacterial diversity may also change at the ecosystem scale in response to the strength in environmental change, the new temporal dynamics occurring in alpine systems, and the initial community composition present in the respective system.

Although kryal systems may become less temporally dynamic, there could be an increased frequency of climate change–induced extreme events disturbing alpine waters. The strength in such disturbances could influence which life strategy is favored and thus community composition. According to our analysis, functional resistance was linked to the functionality of single OTUs. This fact suggests that distinct strategies of different bacteria contribute to the assembly and functional maintenance of bacterial communities. Also evolutionary priority effects can increase the resistance of native bacterial communities (Urban and De Meester 2009) and dampen a shift in BCC induced by dispersal-related colonization mechanisms. Thus far, it is unclear whether and how long kryal communities can resist changes in water source. Predictions concerning future shifts in EF and their effect on biogeochemical cycling should consider apparent BCC and functional flexibility, as they guide possible future shifts in alpine ecosystems facing novel environmental conditions.

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