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# Regional hydrology controls stream microbial biofilms: evidence from a glacial catchment

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## Abstract

Glaciers are highly responsive to global warming and important agents of landscape heterogeneity. While it is well established that glacial ablation and snowmelt regulate stream discharge, linkage among streams and streamwater hydrogeochemistry, the controls of these factors on stream microbial biofilms remain insufficiently understood. We investigated glacial (metakryal, hypokryal), groundwater-fed (krenal) and snow-fed (rhithral) streams – all of them representative for alpine stream networks – and present evidence that these hydrologic and hydrogeochemical factors differentially affect sediment microbial biofilms. Average microbial biomass and bacterial carbon production were low in the glacial streams, whereas bacterial cell size, biomass, and carbon production were higher in the tributaries, most notably in the krenal stream. Whole-cell in situ fluorescence hybridization revealed reduced detection rates of the *Eubacteria* and higher abundance of  *$\alpha$ -Proteobacteria* in the glacial stream, a pattern that most probably reflects the trophic status of this ecosystem. Our data suggest low flow during the onset of snowmelt and autumn as a short period (hot moment) of favorable environmental conditions with pulsed inputs of allochthonous nitrate and dissolved organic carbon, and with disproportional high microbial growth. Krenal and rhithral streams with more constant and favorable environments serve as possible sources of microbes and organic matter to the main glacial channel during periods (e.g. snowmelt) of elevated hydrologic linkage among streams. Ice and snow dynamics have a crucial impact on microbial biofilms, and we thus need better understanding of the microbial ecology and enhanced consideration of critical hydrological episodes in future models predicting alpine stream communities.

## 1. Introduction

Alpine glaciers are prominent agents of environmental heterogeneity. At large temporal scales, glacial retreat shapes landscapes by generating chronosequences of revegeta-

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tion and humus build-up which in turn alters nitrogen fixation and carbonate leaching – factors that can affect solute fluxes at the catchment level and even lake phytoplankton communities (Engstrom et al., 2000). At smaller temporal scales, the seasonal dynamics of glaciers can trigger different hydrologic reservoirs, change flowpaths of water and solutes through the catchment, and drastically shape the fluvial geomorphology of lotic networks (e.g. Malard et al., 1999; Smith et al., 2001). For instance, during summer, supraglacial and subglacial ice-melt feeds glacial streams and replenishes groundwater stores; groundwater becomes increasingly important in autumn and together with subglacial waters constitutes the main source to winter flow (e.g. Smith et al., 2001). Each of these hydrological sources generates distinct chemical signatures, which, depending on the level of hydrologic connectivity among streams, can induce remarkable physical and geochemical heterogeneity at the landscape level (e.g. Tockner et al., 1997; Smith et al., 2001). Along with this shifting dominance of hydrologic flowpaths, seasonal variation in discharge also leads to cycles of expansion and contraction of the lotic network (Malard et al., 1999). Meltwaters from glacial ablation and snowmelt can dramatically increase discharge, expand channel networks and increase hydrologic connectivity whereas receding discharge in autumn and winter contracts networks.

This heterogeneity results in a complex network of kryal, rhithral and krenal stream types (Ward, 1994; McGregor et al., 1995). Kryal reaches are located within the main channel directly affected by the glacial flow pulse during summer ablation and characterized by low temperatures, high sediment loads, low channel stability and large diel fluctuations. Rhithral streams are largely fed by snowmelt and krenal streams by groundwater and are generally more stable. This environmental heterogeneity constitutes a major organizing agent of the habitat template for algal and macroinvertebrate communities in alpine streams (Hieber et al., 2001; Peterson et al., 2001; Füreder et al., 2001; Burgherr et al., 2002). Brittain and Milner (2001) proposed a conceptual model that relates longitudinal gradients in macroinvertebrate community structure to channel stability and maximum water temperature – both being a function of the distance from the glacier and time since deglaciation. Habitat heterogeneity was also proposed to

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mitigate glacial-induced disturbances, and to increase macroinvertebrate biodiversity and community stability at the floodplain level (Burgherr et al., 2002). Streams that are less prone to the glacial meltwaters and snowmelt pulses may serve as refugia for macroinvertebrates and post-disturbance sources of recolonization for the main glacial channel (Burgherr et al., 2002).

Seasonal variation is also pronounced in glacial streams with snow cover and glacial flow pulses as major agents of environmental harshness, and low flow conditions in early spring and autumn can act as relatively short periods of more favorable conditions. Such windows of opportunity (Brittain and Milner, 2001) or hot moments (sensu McClain et al., 2003) are characterized by disproportional high reaction and activity rates, and are thought to greatly contribute to the functioning of glacial stream ecosystems. Despite the awareness that alpine aquatic ecosystems are particularly prone to climate change (Chapin and Körner, 1994; McGregor et al., 1995), the response of this predictable seasonal variation to global warming and its consequences for community and ecosystem ecology remain poorly understood.

While considerable effort has addressed the influence of glacial dynamics on the more traditional algal and macroinvertebrate communities, virtually nothing is known on the microbial ecology of alpine streams – despite the pivotal role of microbes for stream ecosystem functions (Battin et al., 2003a). The central objective of the present paper is to study the variability of stream microbial biofilms within a glacial floodplain and to assess the influence of glacial ablation and snowmelt as major hydrologic factors determining the physical template for stream biofilms. We selected kryal, rhithral and krenal streams in a small glacial catchment in the Austrian Alps and expected different impacts of glacier and snow dynamics on the hydrology and biogeochemistry of these streams depending on their distance from the glacier and their position in the catchment. We hypothesized that this combination of linkage and location dictates bulk microbial biomass and activity, cell size structure, and microbial community composition.

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## 2. Study sites

Study sites were located within the glacial catchment (Rotmoos, 11°03' N 46°50' E, 2280 to 2450 m above sea level) in the Austrian Alps (Fig. 1). Approximately 40% of the catchment area (10 km<sup>2</sup>) are glaciated. Annual mean temperature is -1.3°C (1997/1998) and annual precipitation averaged 820 mm from 1970 to 1996 (Kaufmann, 2001). Depending on elevation, snow cover usually lasts from mid October to late May. Feldspar, micaschists, and a local metacarbonate outcrop in a southern subcatchment near the glacier terminus characterize the catchment geology. The Rotmoos catchment contains a well-preserved chronosequence with a neoglacial (1858) terminal moraine marking the limit of the glacier foreland. Within the past 160 years, the Rotmoos glacier has retreated approximately 2 km at an average rate of 14 m yr<sup>-1</sup> developing at least three prominent terminal moraines (see Fig. 1). The area below the 1858 terminal moraine has been ice-free for about 9500 years and is characterized by a large peat deposit. Parent soils largely consist of neoglacial moraine rubble (mica slate and granite with grains of carbonate) and fluvio-glacial sands. The entire study area lies above tree line. Vegetation is characterized by pioneering plants such as *Saxifraga aizoides*, *Saxifraga oppositifolia* or *Cerastium uniflorum*, and acidic alpine grasslands containing *Carex sempervirens* (Kaufmann 2001).

We selected one upstream (metakryal) and one downstream (hypokryal) study site within the main channel (Fig. 1, Table 1). The rhithral site is largely fed by meltwater from the metacarbonate subcatchment and located between the 1920 and 1994 terminal moraine. The groundwater-fed krenal stream is located in within the peat deposit. Bulk sediment density ranged from 1.50 to 1.68 g ml<sup>-1</sup>, and sediment organic matter was higher in the tributaries than in the glacial stream (Table 1). Major algae associated with sediments were *Hydrurus foetidus* in the glacial stream and representatives of the diatom genera *Fragilaria*, *Synedra*, *Cymbella*, *Diatoma*, *Navicula* and *Nitzschia* dominated the tributary samples.

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### 3. Sample collection

We collected random grab-samples of benthic sediments (at a depth of 0 to 4 cm) on 9 to 12 occasions from September 1998 to December 1999 at all four sites. Avalanches and snowcover up to 3 m thick made access to the sample sites impossible during winter. Sediment samples were gently passed through a 1000  $\mu\text{m}$  sieve in the field and the fraction  $>200 \mu\text{m}$  was retained to achieve sandy samples with median grain size from 355 to 528  $\mu\text{m}$ . Samples for bacterial secondary production were incubated in the field with radiochemicals to ensure in situ temperature, which varied among streams and with time. Aliquot samples were immediately transferred into sterile polypropylene tubes containing 2.5% (v/v) formaldehyde and a 1:1 mixture of ethanol and paraformaldehyde (4%, w/v) for the determination of bacterial biomass and whole-cell in situ hybridization, respectively. The remaining sediment was frozen ( $-20^\circ\text{C}$ ) for carbohydrate and chlorophyll *a* analyses, which were performed within 2 months. One-liter samples of stream water were collected in acid rinsed bottles for chemical analyses.

### 4. Methods

#### 4.1. Flow gauging

Highly unstable channels and logistic constraints prevented continuous flow monitoring in all four study sites. However, discrete flow measurements from the hypokryal site could be related ( $r^2=0.987$ , slope=1.02,  $n=27$ ,  $p<0.001$ ) to flow records from a downstream gauging station (TIWAG, Obergurgl). Based on this regression model, we reconstructed the annual hydrograph for the hypokryal site, which we then used to define hydrological periods. An unusually large storm event ( $49\,200 \text{ L s}^{-1}$  at the TIWAG gauging station) that caused massive channel alterations in September 1999 was excluded from the hydrograph reconstruction.

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## 4.2. Stream water chemistry

Conductance was measured in the field with a conductivity meter (WTW LF196, Germany). Anions ( $\text{SO}_4^-$ ,  $\text{NO}_3^-$ ,  $\text{Cl}^-$ ) were analyzed with ion chromatography (DIONEX DX-120) and cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ) with an atomic absorption spectrometer (Perkin Elmer 3110). Filtered (Whatman, GF/F) samples were analyzed with a Shimadzu TOC-5000 for DOC. Fluorescence measurements were made on aliquot acidified (pH 2 with HCl) samples using a Shimadzu fluorescence spectrophotometer with a xenon lamp. The fluorescence index (FI) was calculated according to McKnight et al. (2001) as the ratio of the fluorescence intensities measured at 450 nm and 500 nm using an excitation of 370 nm. The FI is used as a simple indicator to distinguish fulvic acid precursors and to differentiate between DOC of allochthonous and autochthonous sources (McKnight et al., 2001). We used simple two end-member mixing analysis to assess the relative source contribution to the DOC pool as described by Hood et al. (2003). We used glacial ice and peat water samples as end-members.

We computed the Renkonen index (Krebs 1989) based on ion concentrations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^-$ ) to describe the geochemical similarity between pairs of streams (hypokryal and metakryal, rhithral and krenal). We used the relationship of this similarity index with the main channel discharge to evaluate the geochemical linkage among streams and its seasonal variation.

## 4.3. Bacterial biomass and chlorophyll *a*

Bacterial abundance was estimated by epifluorescence microscopy after staining with 4', 6'-diamidino-2-phenylindole (DAPI, Sigma, St. Louis USA) according to Porter and Feig (1980). Sediment preserved in formaldehyde (2.5%) was treated with  $0.1 \text{ mol L}^{-1}$  tetrasodium pyrophosphate and sonicated (180 s, 40 W output) to detach bacterial cells (Velji and Albright, 1985). To reduce the high background fluorescence caused by minerals, 2 ml of the thoroughly mixed supernatant were transferred into Eppendorf tubes, sonicated (microtip, 30 s, 30 W output) and centrifuged (Eppendorf 5415C) to

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pellet mineral particles. This procedure significantly reduced background fluorescence and recovered on average >93% of the bacterial cells, as revealed by microscopic analyses of the residual particles. Bacterial cells were enumerated on a black 0.2  $\mu\text{m}$  GTBP Millipore filter in 10–30 randomly selected fields to account for 300 to 500 cells.

Two filters were counted per site and date. Cell size and shape were determined on 400 to 600 bacterial cells per filter using image analysis as described by Posch et al. (1997). Cell volume was derived from  $V = (w^2 \times p/4) \times (l - w) + (p \times w^3/6)$ , where  $V$  is the cell volume ( $\mu\text{m}^3$ ),  $w$  and  $l$  are cell width and length ( $\mu\text{m}$ ), respectively. We used the allometric relationship  $C = 120 \times V^{0.72}$  between cell volume ( $V$ ,  $\mu\text{m}^3$ ) and cell carbon content ( $C$ , fg) to calculate bacterial biomass (Norland, 1993).

Sediment chlorophyll *a* was extracted with methanol (99%, v/v) from approximately 2–3 g wet sediment during 12 h in the dark (4°C). After centrifugation, the supernatant was assayed fluorometrically (EX435/EM675) and chlorophyll *a* from spinach (Sigma, St. Louis, USA) was used as standard.

#### 4.3.1. Bacterial secondary production

The incorporation of [ $^3\text{H}$ ]leucine was used to estimate sediment bacterial production (Marxsen 1996). Approximately 2–3 g (wet weight) sediment were incubated with [ $^3\text{H}$ ]leucine (specific activity 60 Ci  $\text{mmol}^{-1}$ , American Radiochemicals) at saturating concentrations of 200  $\text{nmol L}^{-1}$  (made up with cold leucine) for 2 to 4 h in the field at in situ temperatures in the dark. Time series confirmed linear [ $^3\text{H}$ ]leucine uptake during this incubation time. Triplicate [ $^3\text{H}$ ]leucine assays and duplicate formaldehyde-killed controls (2.5% final concentration, 30 min prior to [ $^3\text{H}$ ]leucine addition) were employed per run. The [ $^3\text{H}$ ]leucine incorporation was terminated in the field with 5 ml formaldehyde and samples were frozen ( $-20^\circ\text{C}$ ) within 2 to 3 h. Upon return to the laboratory, samples were repeatedly washed with 5% formaldehyde, and proteins were extracted, precipitated and radioassayed as described elsewhere (Battin et al., 2001).

#### 4.4. Whole-cell in situ hybridization

Whole-cell fluorescence in situ hybridization (FISH) was performed to describe the community composition of biofilm samples from 12 August, 6 September, and 3 October 1999. Within 3 days after sampling, bacterial cells were detached from the sediment as described above. Aliquot samples (3 to 5 ml) of the supernatant were filtered onto 0.2  $\mu\text{m}$  polycarbonate membrane filters (Millipore GTTP, 47 mm diameter) and subsequently washed with phosphate buffer saline (PBS, pH 7.2). The following oligonucleotide probes (Interactiva, Ulm, Germany) were used as in Battin et al. (2001): ARCH915 for members of the domain *Archaea* (16S rRNA, positions 915 to 934), EUB338 for members of the domain *Bacteria* (16S rRNA, positions 338 to 355), BET42a for the beta subclass of *Proteobacteria* (23S rRNA, positions 1027 to 1043), ALF968 for the alpha subclass of *Proteobacteria* (16S rRNA, positions 968 to 986) and CF319a for the *Cytophaga-Flavobacterium* cluster (16S rRNA, positions 319 to 336). Probes were labeled with the indocarbocyanine fluorescent dye CY3 (Biological detection Systems, Pittsburgh, PA USA). The unlabeled probe GAM42a served as competitor for BET42a. Hybridization and epifluorescence microscopical (Axioplan Carl Zeiss Inc.) counts of hybridized and DAPI stained cells were performed as previously described (Alfreider et al., 1996; Battin et al., 2001). As the fluorochrome CY3 sorbed to minerogenic particles in some samples, thereby increasing the background fluorescence, each particle emitting a CY3 signal was checked against DAPI staining. We also checked each filter section for autofluorescence signals of phototrophic and cyanobacterial cells using the filter sets 510–560 (FT580, LP590) and 450–490 (FT510, LP520).

#### 4.5. Microbial exopolysaccharides

Exopolysaccharides (EPS) were harvested from lyophilized sediment (ca. 10 g dry mass) by extraction with 50 mmol L<sup>-1</sup> ethylenediaminetetraacetic acid (EDTA) on a rotary shaker (1 h). The extract was filtered (0.2  $\mu\text{m}$  membrane filter) to remove particles

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and bacterial cells, and EPS was precipitated in the filtrate with ice-cold 99% (v/v) ethanol and left at  $-20^{\circ}\text{C}$  for 48 h. The polymeric fraction was pelleted by centrifugation (2500 rpm, 30 min) and redissolved in MilliQ water. EPS was analyzed for bulk exopolysaccharides with the sulfuric acid – phenol method (Dubois et al., 1956).

#### 5 4.6. Statistical analyses

Repeated-measures analysis of variance (rmANOVA) was used to test the influence of the hydrological period (winter flow, snow melt, ablation, recession) as the repeated variable and stream type (metakryal, hypokryal, rhithral, krenal) on an array of dependent variables related to streamwater chemistry and biofilm structure and function. Hydrological periods (see Fig. 2) were identified using the multivariate Wilk's  $\lambda$  instead of the univariate statistics where data did not meet sphericity (Max and Onghena, 1999). Tukey's multiple comparison post-hoc test followed rmANOVA to assess how levels of stream type differ. Variation in selected microbial parameters was explored with step-wise forward multiple regression on data pooled from all stream types to account for catchment-scale variation and to achieve degrees of freedom high enough to ensure robust results. Entrance criterion was  $P=0.15$  and minimum tolerance for entry into the model was 0.01. We computed principal component analyses (PCA) with the median of cell length, width, volume and circularity to explore bacterial cell size structure. Community composition was also analyzed using PCA on the relative abundance of the various FISH probes. We used correlation matrices for the PCA and 2 factors were retained for analysis. Data were natural-log or arcsin-square-root transformed prior to analysis. All data are given as mean  $\pm$  standard error (S.E.) and statistical tests were considered significant at the level  $\alpha=0.05$ . All analyses were performed with SYSTAT (Evanston, SYSTAT, Inc.).

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## 5. Results

### 5.1. Hydrogeochemistry

Four distinct periods characterized the reconstructed hydrograph in the hypokryal stream (Fig. 2). Flow was low and constant during winter snow cover, dramatically increased during snowmelt in spring, was high and variable during ablation in summer, and receded in autumn.

Most geochemical descriptors differed significantly among streams and hydrologic periods, and with significant interaction terms (i.e. time  $\times$  site interaction,  $P < 0.05$ , Table 2). Streamwater conductance (rmANOVA, Wilk's  $\lambda$ ,  $P = 0.03$ ) and calcium concentration (rmANOVA, Wilk's  $\lambda$ ,  $P = 0.003$ ) – as representative geochemical descriptors – were significantly elevated during snowmelt, decreased during ablation to increase again during the recession period (Table 2, Fig. 3). The metacarbonate outcrop in the southern subcatchment imparted distinct signatures with higher concentration of calcium (ANOVA, Tukey  $P < 0.001$ ), magnesium (ANOVA, Tukey  $P < 0.01$ ) and potassium (ANOVA, Tukey  $P < 0.001$ ) to the rhithral stream. This effect was attenuated in both the metakryal and the hypokryal and was virtually absent from the krenal site (Table 2).

$\text{NO}_3\text{-N}$  concentration showed significant (rmANOVA, Wilk's  $\lambda$ ,  $P = 0.02$ ) temporal patterns in all sites with high concentrations at the beginning of the snowmelt that immediately decreased to reach minimal values during glacial ablation (Table 2, Fig. 3). Average DOC concentrations exhibited no significant seasonal trend (rmANOVA, Wilk's  $\lambda$ ,  $P = 0.125$ ), except a concentration peak in the hypokryal site at the onset of the snowmelt (Table 2, Fig. 3). Average DOC concentrations were lower in the sites close to the glacier forefield (metakryal:  $0.32 \text{ mg C L}^{-1}$ , rhithral:  $0.39 \text{ mg C L}^{-1}$ ) than in the downstream sites adjacent to the peat deposit (hypokryal:  $0.60 \text{ mg C L}^{-1}$ , krenal:  $0.55 \text{ mg C L}^{-1}$ ).

Because of missing FI data from the metakryal site during ablation, we did not compute a rmANOVA. However, average FI values (seasons pooled) from the metakryal and rhithral were significantly (one-way ANOVA,  $P = 0.042$ ) higher than from the hy-

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pokryal and krenal. FI values peaked during snowmelt and the beginning of the ablation in the hypokryal and metakryal, respectively. FI values of shallow glacial ice averaged  $1.92 \pm 0.16$  with maximum values as high as 2.20 during a bloom of *Chlamydomonas nivalis*, whereas the FI values of peat draining waters averaged  $1.29 \pm 0.13$ .

Mixing model analysis using these FI values as end-members suggests that  $62 \pm 7\%$  of the fulvic acids in the metakryal originate from glacial ice, a relative contribution that decreased to  $43 \pm 8\%$  in the hypokryal. The krenal stream had lowest contributions ( $33 \pm 6\%$ ) of glacier-derived fulvic acids.

Stream discharge correlated significantly with most chemical parameters in the hypokryal with high ionic concentrations during low flow (Table 3). While we found no such correlation with DOC concentration, FI and the percentage contribution of glacial-derived fulvic acids were inversely related to discharge.

Clear hysteresis patterns result from plots of the Renkonen similarity index from pairwise comparison of streamwater geochemistry and discharge (Fig. 4). The geochemical fingerprint became increasingly similar between the hypokryal and metakryal as winter flow progressed, decreased with the onset of snowmelt and reached minimal similarity during recession. Pairwise comparisons between the rhithral and krenal give similar results.

## 5.2. Spatial and temporal patterns of microbial biomass and activity

Average microbial biomass and activity ranged 37 and 14-fold, respectively, among stream types and 3 and 2-fold, respectively, among hydrologic periods, and the influence of stream type remained consistent across hydrologic periods (i.e. time  $\times$  site interaction not significant,  $P > 0.05$ ) (Table 4, Fig. 5). Chlorophyll *a* was highest in the krenal stream (ANOVA, Tukey  $P < 0.05$ ), and significantly (rmANOVA, Wilk's  $\lambda$ ,  $P = 0.016$ ) changed with hydrological periods with low values during both winter flow and glacial ablation, and high values during recession (Fig. 5). Bacterial biomass was significantly higher (rmANOVA, followed by Tukey  $P < 0.001$ ) in the rhithral and krenal than in the metakryal and hypokryal sites (Table 4). It showed a 5-fold peak at the onset of

snowmelt and crashed during ablation in the hypokryal. This temporal variation was less clear in the tributaries (Fig. 5) and did not differ significantly among hydrological periods (rmANOVA, Wilk's  $\lambda$ ,  $P=0.164$ ).

Bacterial carbon production significantly differed among stream types with highest values in the tributaries (rmANOVA followed by Tukey,  $P<0.05$ ) (Table 5). Although our estimates of bacterial carbon production are few during winter, there is evidence for a peak at the onset of the snowmelt in the hypokryal site (Fig. 5). Yet rmANOVA did not reveal any significant effect of hydrological period on bacterial carbon production (Table 4). Chlorophyll *a*, bacterial biomass and DOC FI values accounted for 77% ( $F=27.54$ ,  $P<0.001$ ) of the temporal and spatial variation in bacterial carbon production.

Average concentrations of exopolysaccharides were highest in the krenal tributary (ANOVA, Tukey  $P<0.001$ ), whereas average exopolysaccharide to cell ratios were highest in the metakryal (ANOVA, Tukey  $P<0.001$ ) (Table 4). Exopolysaccharide to cell ratios differed significantly (rmANOVA, Wilk's  $\lambda$ ,  $P=0.014$ ) among hydrologic periods with lowest values during snowmelt and ablation and elevated values during low flow in autumn and winter (Table 4, Fig. 5). Multiple regression analysis revealed that calcium concentration and bacterial carbon production explained 36% ( $F=10.59$ ,  $P<0.001$ ) of the spatial and temporal variation in exopolysaccharide to cell ratios.

Principal component analysis clearly grouped hydrological periods based on morphological parameters of bacterial cells (Fig. 6a). Median cell length and volume loaded strongly on PC1 (component loading: 0.93) illustrating the differences between winter flow and snowmelt versus ablation and recession. Whereas median cell circularity loading on PC2 (component loading: 0.80) indicated a shift from more rod-shaped cells during recession and winter flow to small and coccoid cells during snowmelt and glacial ablation. Representative plots of cell length frequency during winter flow and ablation illustrate this shift in cell morphology in each of the stream types (Fig. 6b). Repeated-measures ANOVA (Wilk's  $\lambda$ ,  $P<0.05$ ) confirmed the statistical significance of the temporal course of these morphological cell parameters (Table 4).

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### 5.3. Community composition

Principal component analysis of the FISH data revealed clear temporal and spatial patterns of the community composition between the glacial stream and tributary sites (Fig. 7, Table 5). Rhithral and krenal communities were characterized by elevated eubacterial abundance (PC2 component loading: 0.61), whereas the *α-Proteobacteria* (PC2 component loadings: -0.85) characterized the metakryal and hypokryal sites. These differences were statistically significant for the *Eubacteria* (ANOVA,  $P=0.009$ ) and the *α-Proteobacteria* (ANOVA,  $P=0.015$ ). Probes for the *β-Proteobacteria*, *Cytophaga-Flavobacterium* cluster and the *Archaea* did not reveal any site-specific pattern. The temporal shift in community composition was shaped by the September storm that increased the numbers of *Cytophaga-Flavobacterium* and *Archaea* probes in all streams but did not affect the partitioning effect of *α-Proteobacteria*.

### 6. Discussion

Our results reveal snowmelt and glacial meltwaters as important hydrogeochemical agents of the template that shapes microbial biofilm structure and function in alpine streams. Effect and strength of this template depend on the landscape positioning of the streams relative to the glacier and the snowpack, and relative to catchment geology.

Landscape positioning and linkage among streams (i.e. induced by hydrology) were clearly reflected by the hydrogeochemistry – as suggested by the significant interaction terms of the repeated measures ANOVA (Table 2). For instance, the metacarbonate outcrops increased ion concentrations in the rhithral, to some extent in the metakryal and with an even lower signature in the downstream hypokryal. This was particularly pronounced during low winter flow and became less apparent during ablation. Conversely the krenal stream had lower ion concentrations throughout the entire year with apparently less temporal variation as it is typical for groundwater-fed streams. Furthermore, increasing geochemical similarity between the hypokryal and metakryal during

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low winter flow indicates reduced inputs from additional hydrologic flowpaths along the glacial stream. For instance, discharge from small nival tributaries draining elevated sub-catchments are insignificant during this hydrological period when streamwater largely originates from subglacial and groundwater sources (cf. Füreder et al., 2001; Smith et al., 2001). This contrasts with snowmelt and ablation as periods of higher hydrological linkage that reduce the geochemical similarity between the hypokryal and metakryal sites. Depending on landscape positioning various hydrological reservoirs are progressively activated during these periods, produce water parcels with different geochemical fingerprints that enter the glacial streams along multiple flowpaths. These patterns agree with other glacial catchments, where shifts from groundwater to surface water dominated ecosystems result in considerable chemical heterogeneity at the landscape level (e.g. Malard et al., 2000).

Furthermore, spatial patterns of DOC fluorescence properties strongly suggest accompanying terrestrial vegetation as a significant carbon source to the stream network. While the elevated FI values in the metakryal are evidence for algal/microbial derived precursor material in the upper reaches of the glacial stream, lower FI values in the downstream hypokryal and krenal sites indicate increasing inputs of terrestrially-derived fulvic acids. The relationship between discharge and mixing-model results suggest that fulvic acids of terrestrial origin are highest when the terrestrial/aquatic linkage is elevated, and that the glacier constitutes a major DOC source to the stream during winter when the terrestrial/aquatic linkage is reduced. Yet care should be taken in interpreting yields from end-member mixing analyses because they are based on the assumption that the fluorescence index can be used quantitatively (Hood et al., 2003). Nevertheless, the spatial pattern of our estimates agrees with the chronosequence of revegetation and soil build-up from the glacier forefield with almost barren recent glacial sediments to the downstream peat deposit, and with the soil organic carbon contents along this gradient (cf. Tschérko et al., 2003). These findings also support other reports on soils as DOC sources and longitudinal shifts in DOC chemistry in alpine catchments (e.g. Boyer et al., 1997; Hood et al., 2003).

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Our data suggest that snowmelt is a major factor for microbial biofilms for several reasons. Snowmelt increases the hydrologic linkage with the terrestrial milieu and among streams, reactivates and transports resources (DOC, nutrients) and most likely also microbes to the streams. Moreover, the onset of snowmelt is generally characterized by low turbidity and flow-induced disturbance. Therefore, the onset of snowmelt can be considered as a hot moment (sensu McClain et al., 2003) that is crucial for rapid biofilm recovery from winter transient state, followed by a period of flow-induced stress caused by snowmelt and glacial ablation.

We found microbial biomass peaks in the metakryal and tributaries shortly after the onset of snowmelt and subsequent decreases, which suggests – among other factors – inputs of microbial cells from the snowpack. Snow cover was in fact reported to harbor an active and diverse microbial community (e.g. Alfreider et al., 1996; Sattler et al., 2001), yet flushing of soils during snowmelt should be considered as a further source of microbes. Concomitantly with these biomass patterns our data – although few during winter – indicate increased bacterial carbon production in the hypokryal and krenal streams shortly after the onset of snowmelt, hinting at favorable environmental conditions during this short period. Concurrently, our data from the hypokryal show peaks of DOC and  $\text{NO}_3\text{-N}$  concentrations, probably attributable to snowmelt. Unfortunately, we do not have comparative data from the metakryal because this site is hardly accessible during winter. However, pattern congruency of available data supports our view, which is furthermore corroborated by the numerous reports from others showing pulsed snowmelt-induced inputs of  $\text{NO}_3\text{-N}$  and other solutes (Campbell et al., 1995; Williams et al., 2001; Tockner et al., 2002). Snowpack  $\text{NO}_3\text{-N}$  from wet and dry nitrogen deposition can be remarkable ( $7$  to  $13 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) in the Central Alps during the end of snow accumulation when the lower tropospheric boundary layer extends upwards to glacial altitudes (e.g. Psenner and Nickus, 1986).

Hydrologic flow paths, which determine the opportunity for biogeochemical processes to alter snowmelt on their way to the stream (cf. Campbell et al., 1995; Brooks et al., 1999) and different timing of the snowmelt may explain the differential response of

microbial biofilms among stream types. During early snowmelt, for instance, only lower elevations have warmed sufficiently to produce snowmelt, and streams (i.e. metakryal and krenal sites) located within these landscape patches receive first inputs. As the season progresses, the source area is expanding as snowmelt occurs at higher altitudes and thereby affecting upper stream reaches (i.e. hypokryal and rhithral), but also conveying different loads to the lower streams (i.e. metakryal and krenal).

The recession period is a further hot moment for benthic microbial life in the glacial stream as illustrated by pattern congruency of chlorophyll *a*, bacterial biomass, exopolysaccharides and – to some extent – bacterial carbon production. Decreasing turbidity that enhances light availability, increased concentrations of NO<sub>3</sub>-N and other ions, and increasing channel stability all are factors favorable to in-stream primary production – an important contribution to microbial heterotrophs. These findings thus suggest predominantly endogenous processes to foster microbial life during recession, while exogenous processes may be more important during snowmelt.

Our results provide evidence towards the possible role of cations for microbial biofilms during snowmelt and particularly during recession. Notably calcium and magnesium are well known to enhance bacterial adhesion to substrates and to influence the tertiary structure of microbial exopolysaccharides (e.g. Geesey et al., 2001). We thus suggest that the copious production of exopolysaccharides during recession and early winter flow is supported by the high availability of calcium and magnesium. A well-developed exopolysaccharide matrix would translate into enhanced immobilization and storage of organic substrates and hence into an advantage during prolonged substrate deprivation (cf. Freeman and Lock, 1995; Battin et al., 1999). Furthermore, elevated exopolysaccharide to cell ratios in the glacial stream (notably in the metakryal) where flow velocity is high hints at the function of exopolysaccharides providing better adhesion and protection from erosion – a relationship that agrees with experimental findings (Battin et al., 2003b). This advantageous style of microbial life in a copious matrix is also reflected by elevated carbon production and by larger, more rod-like cells during late recession – a pattern that was particularly noticeable in the glacial stream

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sites.

Spatial and temporal patterns of microbial cell carbon content could also be attributable to resource dynamics. For instance, organic carbon of microbial/algal origin (as derived from fluorescence, McKnight et al., 2001) from glacial ice generally has better bioavailability than terrestrial organic carbon. This could explain the occurrence of larger cells in the metakryal during more favorable flow conditions. By contrast, smaller and coccoid-like cells during ablation hint at the glacial source of bacterial cells, which supports previous findings (Battin et al., 2001). After a prolonged period of snow cover, however, average bacterial cell volume can be as low as  $0.04 \mu\text{m}^3$  in the glacial stream (A. Wille, unpublished data) which may be a consequence of continuous starvation. These findings suggest that early winter could be important for microbial processes in the glacial stream, a notion that is in fact supported by observations from macroinvertebrate ecology (Schütz et al., 2001).

Tributaries (rhithral, krenal) were characterized by higher sediment organic matter, microbial biomass and bacterial carbon production. Assuming a growth efficiency of 30% (Benner, 1988), the average theoretical turnover time of benthic organic carbon ranges from 196 to 226 d in the tributaries, but from 229 to 581 d in the glacial streams. These turnovers are reasonably close to values reported from temperate streams (Fischer et al., 2002, and references therein), which suggests efficient use of available organic carbon, and emphasizes the role of microbial biofilms in the carbon fluxes for high-alpine streams with otherwise low retention capacity. In the context of alpine stream networks, rhithral and krenal streams may thus function as hotspots with disproportionately high microbial and biogeochemical reaction rates (sensu McClain et al., 2003) in an otherwise “cold” landscape. During increased hydrological linkage among streams — as indicated by the geochemical hysteresis curves — source-sink interactions between “hotspot” streams and channels with reduced retention may contribute to ecosystem functioning at the network scale.

Although we only used two domain-specific and three group-specific rRNA probes, our results show remarkable variation in the microbial community composition. Given

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the relatively close spatial proximity of the study streams, this variation suggests strong control of the physical template and provides evidence that landscape filters (sensu Poff, 1997) can even act at the microbial scale. In fact, detection rates of *Eubacteria* and  *$\alpha$ -Proteobacteria* clearly segregated the main glacial stream from both tributaries, and this pattern even persisted after the major storm event in September. Although this storm temporarily maximized among-stream linkage and increased the relative contribution of *Archaea* and members of the *Cytophaga-Flavobacterium* cluster to biofilm communities in all study streams, the main glacial stream was still characterized by elevated numbers of the  *$\alpha$ -Proteobacteria*. The higher detection rates of eubacterial cells in the tributaries likely reflect their elevated metabolic activity, a notion that is supported by the relationship between cell metabolic rate and rRNA content (e.g. Bouvier and del Giorgio, 2003). The adaptation of  *$\alpha$ -Proteobacteria* to very low inorganic and organic nutrient-source concentrations (Zavarzin et al., 1990) may render this group more competitive in the glacial stream.

In conclusion, our results have identified snowmelt and glacial ablation as important drivers in the structure and function of microbial biofilms in alpine streams. Growing awareness of alpine streams as sensitive indicators for global change has recently fostered the development of various models predicting the structure of macroinvertebrate communities in glacial streams (e.g. Smith et al., 2001; Füreder et al., 2001). Biofilms constitute a link between hydrogeochemistry and benthic invertebrates, and we thus consider our findings as a stimulus to complement these models by explicitly integrating the coupling between terrestrial/aquatic connectivity, hydrogeochemistry and microbial ecology of alpine streams.

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**Table 1.** Characterization of the Rotmoos study sites and bulk features of the sediment collected for microbial analyses. Given are means±SE.

Site	Location (m a.s.l.) <sup>b</sup>	Stream type <sup>a</sup> ( $\mu\text{m}$ )	Altitude ( $\text{g ml}^{-1}$ )	Median grain size (%w/w)	Bulk density	Organic matter
Site 1	Main channel	Metakryal <sup>c</sup>	2360	514±84	1.665±0.074	0.02±0.03
Site 2	Tributary	Rhithral	2320	467±38	1.678±0.034	0.18±0.05
Site 3	Main channel	Hypokryal <sup>d</sup>	2250	355±30	1.505±0.027	0.06±0.02
Site 4	Tributary	Krenal	2255	436±65	1.599±0.056	0.19±0.01

<sup>a</sup> according to Ward (1994)<sup>b</sup> meters above sea level<sup>c</sup> 0.3 km from glacial terminus<sup>d</sup> 3.0 km from glacial terminus

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**Table 2.** Summary statistics and repeated measures ANOVA for streamwater chemistry. Site (metakryal, rhithral, hypokryal, krenal) is the independent variable, hydrological periods (winter flow, snow melt, ablation, recession) are the repeated variables.

Parameter	Sites				ANOVA <i>F</i> -statistics <sup>a</sup>		
	Metakryal	Rhithral	Hypokryal	Krenal	Stream	Hydrologic period	Interaction
Temperature (°C)	1.32±0.29	3.91±1.11	3.60±0.70	4.42±1.19	9.386*	32.627***	2.163 <sup>ns</sup>
Conductance (μS cm <sup>-1</sup> )	115±23	186±14	114±13	70±4	46.784**	27.321***	7.671**
Ca <sup>2+</sup> (mg L <sup>-1</sup> )	13.87±2.55	23.84±1.82	14.16±1.64	6.70±0.54	75.483***	41.065***	7.356**
Mg <sup>2+</sup> (mg L <sup>-1</sup> )	3.21±0.91	5.68±0.71	2.75±0.45	1.54±0.17	42.776**	24.273***	4.453**
K <sup>+</sup> (mg L <sup>-1</sup> )	1.61±0.36	2.53±0.23	1.69±0.20	1.06±0.06	32.769**	60.772***	16.212***
Na <sup>+</sup> (mg L <sup>-1</sup> )	0.47±0.12	0.52±0.11	0.43±0.09	0.61±0.09	0.439 <sup>ns</sup>	5.998**	1.056 <sup>ns</sup>
SO <sub>4</sub> <sup>-</sup> (mg L <sup>-1</sup> )	19.09±3.93	18.52±2.22	14.90±1.75	12.52±0.71	1.978 <sup>ns</sup>	32.890***	11.313***
Cl <sup>-</sup> (mg L <sup>-1</sup> )	0.30±0.07	0.45±0.08	0.31±0.07	0.33±0.12	12.355**	27.659***	3.048*
NO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )	0.67±0.16	0.29±0.09	0.60±0.11	0.56±0.12	30.997**	188.72***	9.921**
DOC (mg L <sup>-1</sup> )	0.32±0.03	1.61±1.22	0.61±0.13	0.54±0.07	0.673 <sup>ns</sup>	0.939 <sup>ns</sup>	0.324 <sup>ns</sup>
FI <sup>c</sup>	1.71±0.08	1.58±0.03	1.56±0.04	1.52±0.04	NA <sup>b</sup>	NA	NA

<sup>a</sup> ns denotes  $P > 0.05$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

<sup>b</sup> NA, not analyzed because of missing values

<sup>c</sup> dimensionless inde

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**Table 3.** Correlation between discharge and selected chemical parameters in the hypokryal site (n=14, except for FI: n=10).

Dependent variable	Pearson's <i>r</i>	P
Conductance	-0.839	<0.001
Ca <sup>2+</sup> + Mg <sup>2+</sup>	-0.841	<0.001
Cl <sup>-</sup>	-0.725	0.003
Na <sup>+</sup>	-0.839	0.023
K	-0.601	<0.001
NO <sub>3</sub> -N	-0.512	0.074
DOC	-0.497	0.100
FI	-0.696	0.012
% glacial DOC	-0.692	0.025

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**Table 4.** Summary statistics (mean±SE) and repeated measures ANOVA for microbial biofilm parameters. Site (rhithral, krenal, metakryal, hypokryal) is the independent variable, hydrological periods (winter flow, snow melt, ablation, recession) is the repeated variable.

Parameter	Sites				ANOVA <i>F</i> -statistics <sup>a</sup>		
	Metakryal	Rhithral	Hypokryal	Krenal	Site	Hydrologic period	Interaction
Chlorophyll <i>a</i> (µg g <sup>-1</sup> DM)	0.01±0.01	0.45±0.16	0.02±0.01	0.86±0.39	41.05***	11.30***	0.496 <sup>ns</sup>
Bacterial abundance (10 <sup>6</sup> cells g <sup>-1</sup> DM)	4.38±0.99	96.3±21.4	18.70±4.76	154±34	111.88**	8.94**	0.489 <sup>ns</sup>
Cell length (µm)	0.94±0.07	0.85±0.03	0.81±0.03	0.89±0.02	0.974 <sup>ns</sup>	5.915*	1.146 <sup>ns</sup>
Cell volume (µm <sup>3</sup> )	0.11±0.02	0.08±0.01	0.08±0.01	0.10±0.01	1.419 <sup>ns</sup>	10.485**	0.980 <sup>ns</sup>
Cell circularity <sup>b</sup>	0.80±0.001	0.83±0.001	0.82±0.001	0.82±0.001	3.015 <sup>ns</sup>	3.459*	1.603 <sup>ns</sup>
Cell C-content (fg C cell <sup>-1</sup> )	18.35±1.32	17.17±0.83	15.58±0.66	21.24±1.60	12.05*	9.98**	2.31 <sup>ns</sup>
Bacterial biomass (µg C g <sup>-1</sup> DM)	0.080±0.001	1.68±0.39	0.287±0.075	2.95±0.53	103.91***	8.23**	0.853 <sup>ns</sup>
BCP (µg C h <sup>-1</sup> g <sup>-1</sup> DM)	0.03±0.01	0.41±0.07	0.15±0.03	0.34±0.07	62.38***	3.33 <sup>ns</sup>	1.05 <sup>ns</sup>
Exopolysaccharides (µg C g <sup>-1</sup> DM)	1.32±0.19	1.83±0.33	2.29±0.40	4.60±0.55	11.45*	1.55 <sup>ns</sup>	1.20 <sup>ns</sup>
Exopolysaccharides:cell (pg C cell <sup>-1</sup> )	0.66±0.14	0.03±0.01	0.25±0.06	0.4±0.01	32.59**	5.25*	0.441 <sup>ns</sup>

<sup>a</sup> ns denotes  $P > 0.05$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

<sup>b</sup> dimensionless

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**Table 5.** Detection rates (%) of fluorescence in situ hybridization (FISH) relative to total counts of DAPI-stained bacterial cells. Data are given as average±SE, (n=3).

Site	<i>Eubacteria</i>	$\alpha$ <i>Proteobacteria</i>	$\beta$ <i>Proteobacteria</i>	<i>Cytophaga- Flavobacterium</i>	<i>Archaea</i>
Metakryal	31.33±7.43	3.73±0.69	16.67±1.77	1.87±1.67	2.80±1.48
Rhithral	63.33±2.96	1.80±0.47	22.67±4.18	1.97±1.22	2.33±0.61
Hypokryal	41.67±2.61	2.90±0.15	19.67±1.20	1.00±0.44	1.70±1.11
Krenal	60.00±6.25	1.83±0.48	15.67±3.28	2.13±0.58	2.27±0.96

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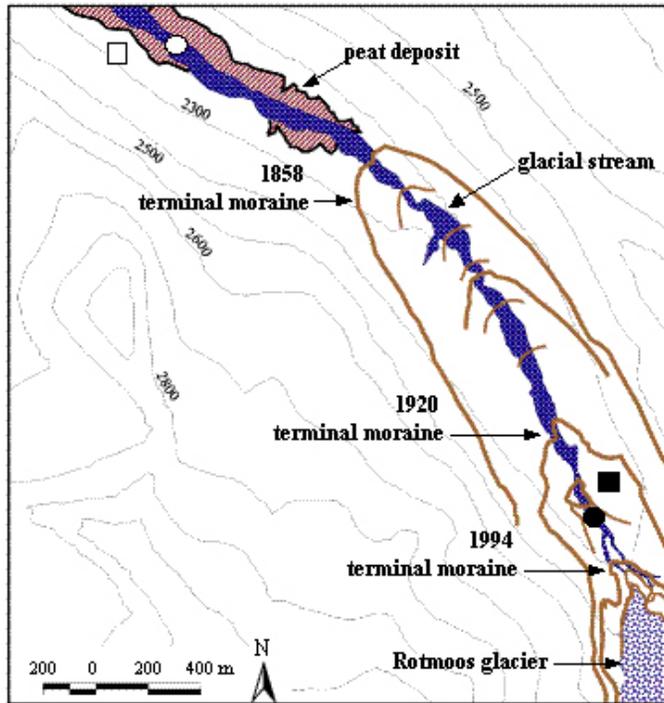
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○ hypokryal   □ krenal   ● metakryal   ■ rhithral

**Fig. 1.** Map of the Rotmoos catchment with the location of the study streams. Upstream sites include the hypokryal and rhithral, downstream sites include the metakryal and krenal. Contour lines refer to the altitude (meters above sea level).

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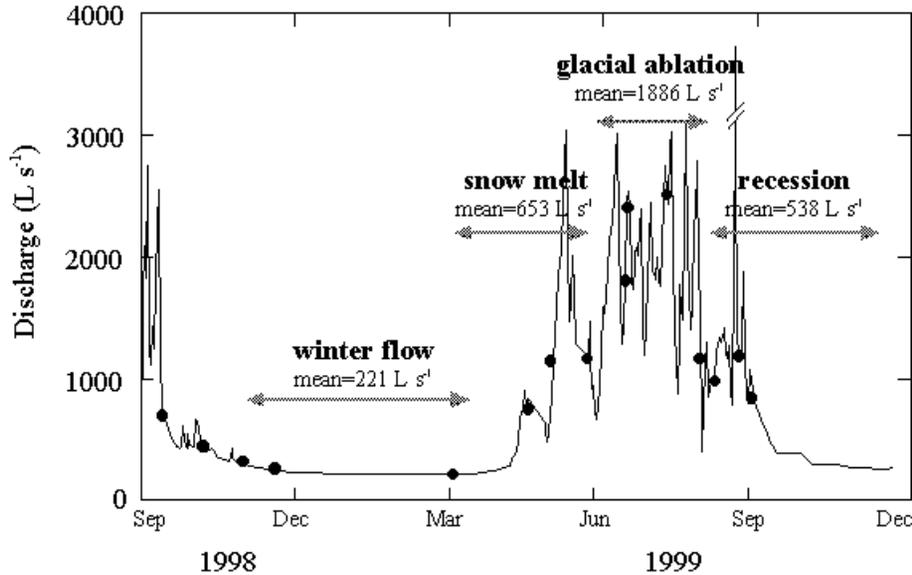
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**Fig. 2.** Stream annual hydrograph (hypokryal stream) illustrating hydrological periods characteristic of glacial streams.

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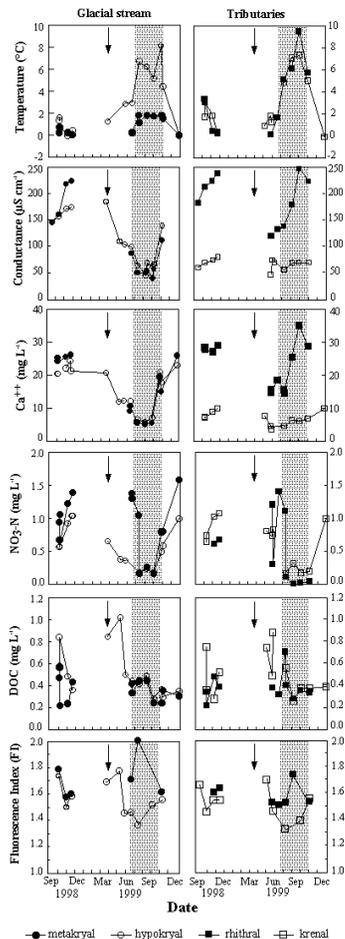
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**Fig. 3.** Temporal variation of streamwater temperature, conductance, calcium, nitrate and DOC concentrations in the glacial stream and the tributaries. Data are means±SE from the metakryal (●), hypokryal (○), rithral (■) and krenal stream (□). The arrow indicates the onset of the snowmelt and the gray shading indicates glacial ablation.

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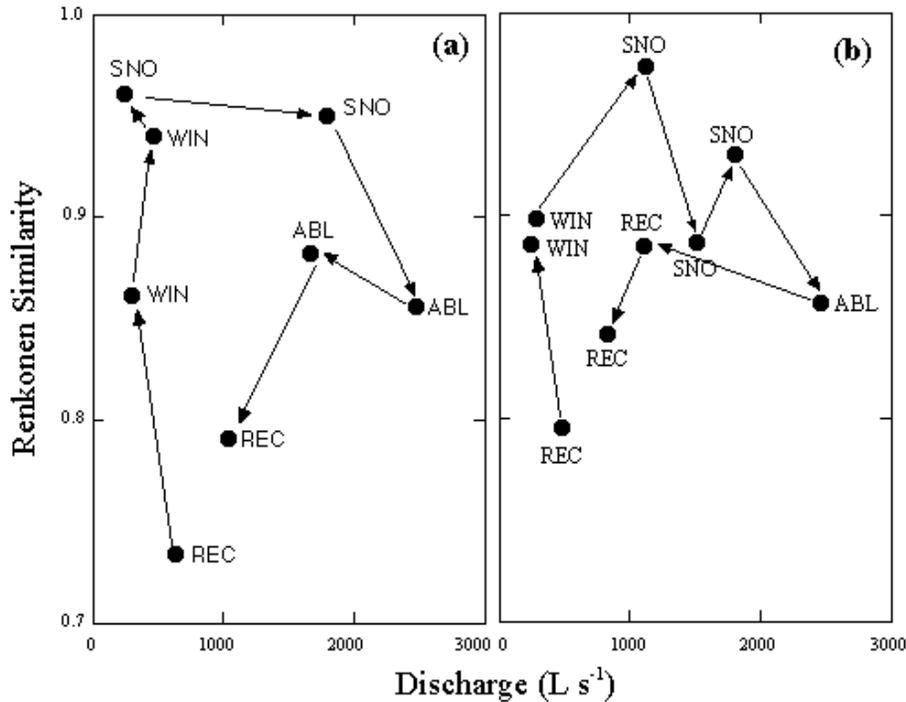
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**Fig. 4.** Hysteresis curves generated from pairwise comparisons (Renkonen Similarity index) of the hypokryal versus metakryal (a) and rhithral versus krenal (b) and based on streamwater ion concentrations. REC: recession, WIN: winter flow, SNO: snowmelt, ABL: ablation.

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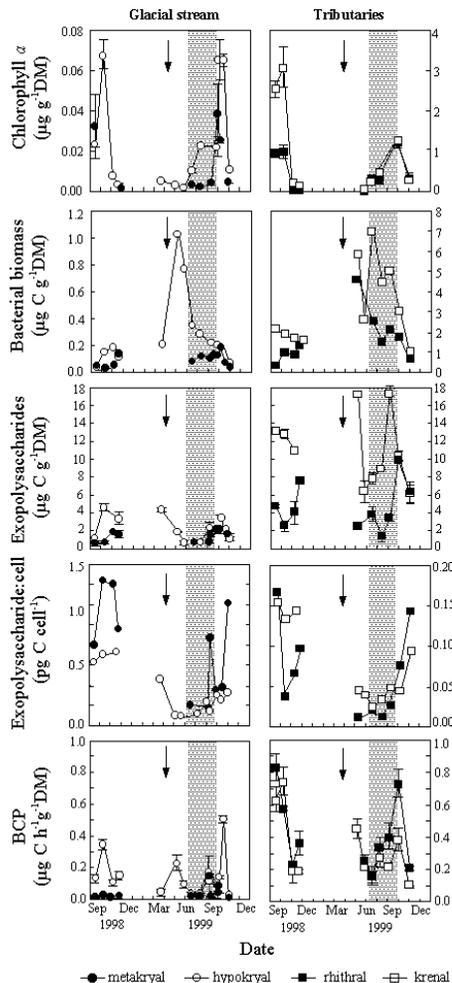
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**Fig. 5.** Temporal variation of various sediment biomass parameters and bacterial carbon production (BCP) in the glacial stream sites and the tributaries. Data are means±SE from the metakryal (●), hypokryal (○), rñithral (■) and krenal stream (□). The arrow indicates the onset of the snowmelt and the gray shading indicates glacial ablation.

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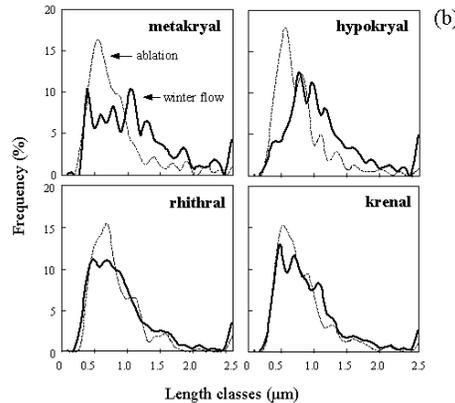
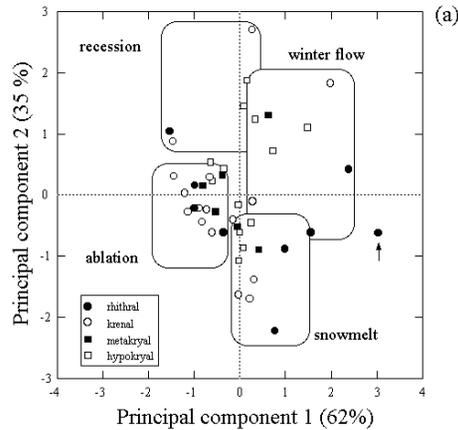
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**Fig. 6.** Principal component analysis of the bacterial cell size structure **(a)** and representative frequency analyses of bacterial cell length for ablation and winter flow **(b)**. Data are from the metakryal (●), hypokryal (○), rhithral (■) and krenal stream (□).

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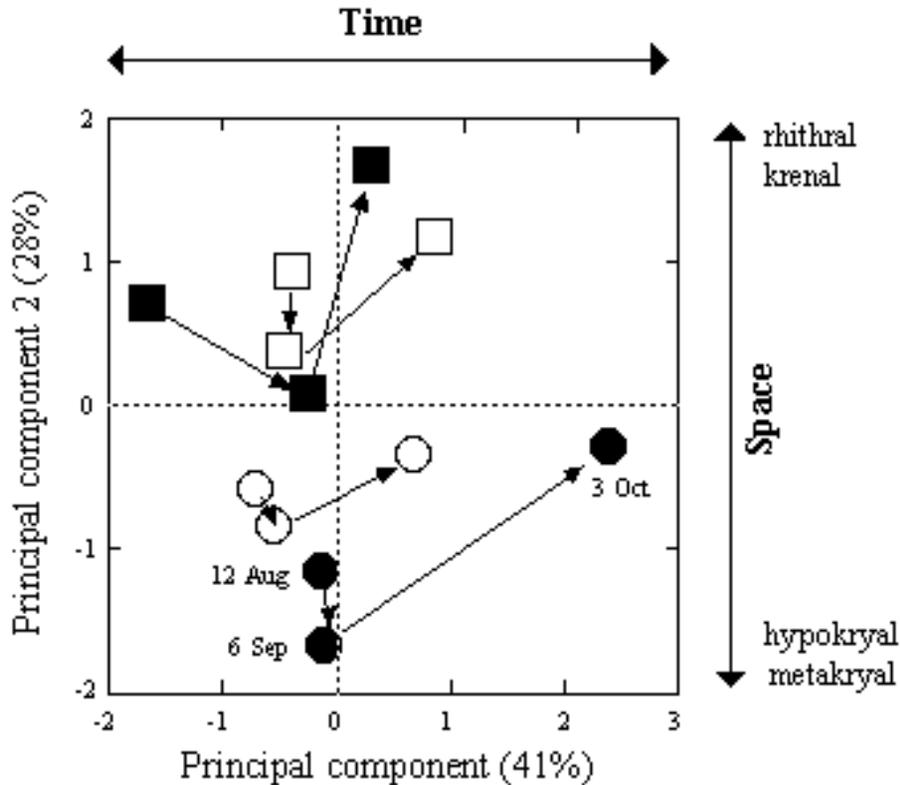
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**Fig. 7.** Principal component analysis of the data of whole cell fluorescence in situ hybridization on samples from 12 August, 6 September and 3 October. Arrows indicate the temporal trajectory of microbial communities. Data are from the metakryal (●), hypokryal (○), rhithral (■) and krenal stream (□).