



Structuring factors of the phytobenthos community along a mountain headwater (Kalkalpen National Park, Austria)

Romana Niedermayr¹ and Michael Schagerl^{2, *}

With 4 figures and 5 tables

Abstract: The phytobenthos community of a carbonate headwater and its springs were studied in the Kalkalpen National Park (Austria). The investigated rivulet Almbach is located at a traditionally farmed mountain pasture. Two springs, one of them fenced in to prevent livestock access, and two more sites from the headwater were sampled from May to October 2005. Whereas diatoms dominated the fenced spring, chlorophytes reached highest biomass at the spring with livestock access. A Discriminant Analysis revealed three functions based on nitrate, phosphorus and channel width, which classified 100 % of the sites correctly. Indicator taxa – regarded as highly indicative of a particular site when they are mainly found at that site and are present in a large number of its sample units – were mainly detected at the cattle-free sites. Both springs differed significantly from the rivulet sites and from each other concerning physico-chemical parameters, algal community structure, biomass and seasonal development. Canonical Correspondence Analysis was used to analyze the phytobenthos community pattern along environmental gradients. Phosphorus, benthic ash-free dry mass and pH had the highest influence on the first two axes, which explained 78 % of taxa-environmental variation. A negative impact of pasturing on the sensitive high mountain headwater system was detected because of reduced bank stability through trampling, decreased canopy cover and increased nitrogen input. These factors caused an atypical, chlorophyte-dominated phytobenthos community with both low algal diversity and evenness.

Key words: phytobenthos community, spring, karst, headwater, alga.

Introduction

Springs represent ecotones which link the phreatic zone to headwaters (Cantonati et al. 1996). The hydrology and physico-chemistry of springs are strongly affected by the geology and precipitation in their catchment areas. They feature characteristic stable physico-chemical conditions (Odum 1971), especially with regard to water temperature. High mountain springs and headwaters are sensitive ecosystems and highly threatened by human impacts such as withdrawal of water for drinking water supply, Alpine pasture farming and

the timber industry. Nonetheless, little research has been conducted on their phytobenthic communities. Commonly, dominant groups in high-mountain running waters are Cyanoprokaryota and diatoms (Kann 1978, Kawecka 1981, Pfister 1992 and Cantonati et al. 2001), which serve also as bioindicators for water quality (Rott et al. 1997, 1999). Kann (1978) studied the ecology and taxonomy of the phytobenthos of several mountain streams in Austria and highlighted that the cyanoprokaryote taxa *Chamaesiphon*, *Homoeothrix* and *Phormidium*, the chlorophyte *Gongrosira incrustans* (Reinsch) Schmidle, and the chrysophyte

Authors' addresses:

¹ DWS Hydro-Ökologie, Zentagasse 47, A-1050 Vienna, Austria.

² University of Vienna, Department of Limnology, Althanstraße 14, A-1090 Vienna, Austria.

*Corresponding author: email: michael.schagerl@univie.ac.at

Hydrurus foetidus (Villars) Trevisan are typical of calcareous summer-cold rivers. Sabater & Roca (1992) analysed diatom communities in Pyrenean springs and found that calcareous biotopes sheltered more cosmopolitan taxa than soft-water habitats which, in contrast, contained mainly boreal-alpine species. They also observed certain differences between diatom communities of springs and those of small streams. Studies on diatoms of high mountain springs and headwater streams in the Alps and the Himalayas revealed that epilithic samples were similar and mostly constant over the seasons; cosmopolitan taxa were the most abundant ones (Cantonati 1998, Cantonati et al. 2001, Cantonati & Pipp 2000). Diatom assemblage structures were mainly determined by geochemical factors such as the level of mineralization, pH and sulphate amounts. The vast majority of diatom taxa occurring in springs are also common in headwater streams; thus, they cannot be termed crenobionts (Round 1957, Cantonati 1998). Reichardt (1994), investigating calcareous tufa springs and headwater streams in Bavaria, also reported that crenobiont algae are subdominant to rare. Other studies focused on non-diatom communities (Pfister 1992, Cantonati et al. 1996, Cantonati 1999) and detected Cyanoprokaryota as being the dominant group in rheocrenes and high mountain streams.

We focused on the phytobenthos communities of two springs and an associated headwater within the Kalkalpen National Park (limestone Alps, Austria). In this area, more than 800 springs are known. Springs emerging from calcareous areas like the Kalkalpen offer special features because their bedrocks provide a high buffer capacity with stable pH conditions. Karst formations show typical sinkholes, complex fissures, subterranean furcations and caves. Thus, the water emerging from two closely located springs might have different drainage basins (Kralik 2006). Within the Kalkalpen National Park, some mountain pastures are still in use. In the course of a LIFE project (LIFE99NAT/A/5915), management measures were carried out on various disturbed Alpine springs to prevent them from further deterioration through livestock access. Their progress concerning water chemistry and hydrobiology has been monitored since 1991 (Pröll 2002). Additionally, studies on microbial activities (Thyr 2002) and zoobenthos (Weigand & Graf 2002) were carried out. Pokorný et al. (2008) studied the macroinvertebrate community of the Almbach and its associated springs.

We focused on a comparison between algal communities of two pasture springs, one of them without

livestock access. In view of already performed management measures, we examine whether significant differences can be found between the fenced spring, the spring with free livestock access and two sites further downstream. Our hypothesis is that excluding livestock led to the development of a special community including some crenophiles. Moreover, we extract relevant environmental variables that structure the algal communities. We investigate whether phyto-benthos taxa can be specified which characterize certain environmental conditions. In contrast to other studies, this investigation also includes quantitative analyses of phytobenthos communities.

Study area

The Kalkalpen National Park (Upper Austria), founded in 1998, covers an area of about 21 000 ha of the Sengengebirge and the Reichraminger Hintergebirge (Fig. 1). It is the largest forest protection area of Austria, consisting of 81 % forest, 11 % mountain pastures and rocks, and 8 % knee pines. The summit of the region is called Hoher Nock, with an altitude of 1963 m. Mean annual precipitation is about 1275 mm, with an average annual air temperature of 7.3 °C. According to Schön & Strauch (2004), the climate can be described as humid-temperate. The geology is characterized by limestone and dolomite; thus, weathering causes karst landscapes, subterranean streams and cave systems.

The study site "Ebenforstalm" is located at 1105 m altitude within the northern slope of the Reichraminger Hintergebirge. The inverse syncline of the area enabled the development of a humus layer (Haseke 2002) that tolerates livestock trampling. The clay fraction of the soil promotes water retention and, together with high local rainfall, the nearby peat-bog Ebenforst-Moor could develop around 8000 years ago. The brooklet Almbach covers a wide catchment area consisting of many small springs. The main spring was fenced in during the course of management measures (Weigand et al. 2002) to protect it against hoof tracks and cow droppings. Situated approximately 150 m downstream, a second spring has free livestock access. Water from these springs merge and cross the pasture before the Almbach enters a spruce forest and ultimately drains into a sinkhole known as Ebenforstponor. The whole stretch from the main spring to the ponor is about 900 m. The Ebenforstponor is connected to a spring at about 450 m altitude named Predigtstuhllquelle.

Material and methods

Four sites were sampled every three weeks from May to October 2005. Two springs (fenced EB 1, and EB 2 with livestock access) and two sites located a few hundred meters downstream (EB 3, EB 4) were investigated (Fig. 1). Water temperature ($^{\circ}\text{C}$), pH, electrical conductivity ($\mu\text{S cm}^{-1}$) and dissolved oxygen ($\text{O}_2\%$ and mg l^{-1}) were measured in the field (WTW-MultiLineP4 with TetraCon 325, SenTix 41, CelloX 325). At each site, channel width (b), water depth (z) and flow velocity (v) were recorded using a Marsh-McBirney Flow-Mate. The level of sun exposure was estimated on a relative scale from 1 to 5 (shaded to fully sun-exposed). Water samples were filtered through pre-combusted glass fibre filters (Ederol BM/C). The filters were used to determine the particulate matter (pelagic dry mass PDM, pelagic ash mass PAM). The filtrate was used for hydrochemistry, including nitrogen fractions (ammonium-nitrogen $\text{NH}_4^+\text{-N}$, nitrite-nitrogen $\text{NO}_2^-\text{-N}$ and nitrate-nitrogen $\text{NO}_3^-\text{-N}$) and phosphorus fractions (soluble reactive phosphorus SRP and total phosphorus TP; Table 1). Total alkalinity was analysed by endpoint titration to pH 4.3 (Methrom: Titrand 808, Dosimat 805, 814 USB Sample Processor).

For algal taxa lists, qualitative analyses were conducted. Choriotopes like pebbles, dead wood, bryophytes and muddy patches were considered. Living material was identified using a compound microscope (Polyvar Reichert-Jung). Identification followed Geitler (1932), Komarek & Anagnostidis (1999, 2001) for Cyanoprokaryota, Krammer & Lange-Bertalot (1986,

1988, 1991a, 1991b) for Bacillariophyceae, Komarek & Fott (1983) for Chlorococcales, Mrozinska (1985) for Oedogoniophyceae, Kadlubowska (1984) for Conjugatophyceae, and Ettl (1978) for Xanthophyceae. To quantify the phytobenthos, four replicates per site were taken by brushing off defined areas from pebbles (main substratum). Quantitative samples were split into three parts: (1) one fraction was fixed with 3% formaldehyde for cell counts and biovolume estimations using an inverted microscope (Nikon Diaphot) at 400 \times magnification according to Utermöhl (1958). Biovolumes of the various taxa were estimated using geometric formulae of the shapes of the respective cells. For rare taxa, biovolumes were taken from Hoehn et al. (1998). From the remaining part, permanent slides for diatom identification were prepared. Diatoms were digested using acid treatment (HCl , HNO_3 + H_2SO_4) and thereafter embedded in Naphrax (Brunel microscopes Ltd.). (2) From the second part, Chlorophyll-*a* concentrations were determined spectrophotometrically after extraction in cold 90% acetone (Hitachi U-2100; Lorenzen 1967). (3) Benthic dry mass (BDM) and benthic ash mass (BAM) were measured from the remaining part. Pelagic and benthic samples were filtered through pre-combusted filters, which then were used for gravimetric determination of dry mass (drying at 95 $^{\circ}\text{C}$) and ash mass (combusting at 550 $^{\circ}\text{C}$ for 3 h). Pelagic and benthic ash free dry mass (AFDM) were calculated as follows: $\text{DM} - \text{AM} = \text{AFDM}$.

Macroinvertebrates (Mi) were quantitatively collected by Hess samplers (100 μm mesh size) and immediately preserved in 90% ethanol (Pokorný et al. 2008). Individuals larger than 200 μm were sorted and counted, Plecoptera and Ephemerop-

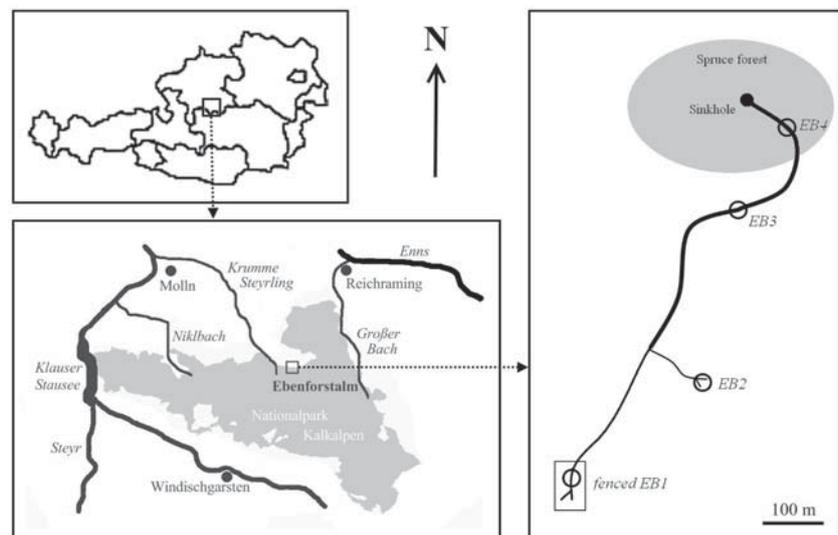


Fig. 1. Location of the sampling sites. Shaded area enclosing EB 4 designates forest area.

Table 1. Overview of methods used for nutrient analyses.

Parameter	unit	Analytical method	Reference
Soluble Reactive Phosphorus (SRP)	$[\mu\text{g l}^{-1}]$	Molybdenum blue method	Strickland & Parsons 1968
Nitrite-N ($\text{NO}_2^-\text{-N}$)	$[\mu\text{g l}^{-1}]$	Griess-reaction	DIN 38 405 – D 10
Nitrate-N ($\text{NO}_3^-\text{-N}$)	$[\mu\text{g l}^{-1}]$	Sodium salicylate method	Legler 1988
Ammonium-N ($\text{NH}_4^+\text{-N}$)	$[\mu\text{g l}^{-1}]$	Indophenol blue method	Krom 1980
Organic bound Nitrogen ($\text{N}_{\text{red}}\text{-N}$)	$[\mu\text{g l}^{-1}]$	Dissociation and Indophenol blue method	APHA 1995
Total alkalinity (Alkal)	$[\text{mval l}^{-1}]$	Titration to pH 4.3	–

tera were identified to the most accurate taxonomic level possible (larvae of Plecoptera: Hynes 1984, larvae of Ephemeroptera: Bauernfeind & Humpesch 2001).

Biological data were based on a total of 144 samples (4 replicates at each of the 4 sites on 9 dates). Phytobenthos and macroinvertebrate communities were characterized by the number of taxa S , the Shannon-Wiener Diversity Index H' and Shannon-Wiener Evenness V' (Pielou 1969); p_i is the proportion of the number of individuals of taxon i to the total of individuals.

$$H' = -\sum_i^S (p_i * \ln p_i) \quad V' = \frac{H'}{\ln S}$$

A Discriminant Analysis (DA) was performed to classify different sampling sites in terms of the environmental variables (SPSS 11; SPSS Inc., 2001). Some of the variables were ln-transformed to achieve normal distribution of the raw data. Spearman Rank Correlations were calculated to check for collinearity of the data set. If variables were highly correlated ($p < 0.001$; $r > 0.5$), redundant variables were removed from analysis. An Indicator Species Analysis (ISA) was carried out using PC-ORD for Windows 4.0 software (McCune & Mefford 1999) to find the significant indicative taxa ($p < 0.05$) for each sampling site. A species is regarded as highly indicative of a particular site when it is mainly found in that site and is present in a large number of its sample units (Dufrene & Legendre 1997). The mean abundance of that species in a particular site compared to all sites is calculated as a measure of specificity. The proportion of dates at this site at which that species has been found serves as a measure of reliability. Multiplication of the two values gives the indicator value (IV), the result being expressed as a percentage. Relationships between environmental variables and significant indicative taxa resulting from ISA were investigated by Canonical Correspondence Analysis (CCA, CANOCO for Windows 4; Ter Braak & Smilauer 1998). CCA was chosen because the gradient length of axis 1 was

about 2.3 standard deviations, and therefore a unimodal species response could be assumed (Ter Braak & Prentice 1988). For CCA, environmental variables were selected using the stepwise "forward selection" procedure including tests of significance. Monte Carlo analysis with 499 permutations was used to test the significance of the first four axes. Inter-set correlations of environmental variables with axes were examined to determine the environmental variables which correlate significantly with axes (Ter Braak 1995). Statistical significance was set at $p < 0.05$. With a sequential Bonferroni technique (Sachs 1992), probabilities for simultaneous tests were corrected.

Results

Hydrological and physicochemical parameters were highly affected by the geology and precipitation in the catchment area, yielding a high temporal and spatial variability of some parameters (Table 2). Both springs showed stable and similar pH and low water temperatures throughout the study period, but they differed significantly ($p < 0.05$) in chemical parameters. NO_3^- -N levels were highest at EB 2 ($1600 \mu\text{g l}^{-1}$), whereas, in contrast, maximum concentrations of SRP were measured at fenced EB 1 ($32 \mu\text{g l}^{-1}$). Within the pasture, the Almbach revealed an increase of channel width and oxygen concentration. Annual patterns of temperature, conductivity, flow velocity and channel depth were characterized by snow melt in May and a high-water event in August. Sun exposure was highest at unfenced EB 2, while shading increased with riparian vegetation growth at EB 1 and EB3 in summer.

Table 2. Hydrological and physico-chemical variables of the Almbach, Austria (means \pm 95 % confidence intervals C. I.). Irradiance is presented as median and quartiles Q_1 , Q_3 . Superior letters mark groups of significance.

		EB 1	EB 2	EB 3	EB 4
Channel width b	[m]	0.48 ± 0.07^A	0.69 ± 0.19^{AB}	0.99 ± 0.16^B	2.22 ± 0.49^C
Water depth z	[m]	0.07 ± 0.01^A	0.05 ± 0.01^A	0.10 ± 0.02^B	0.09 ± 0.01^B
Velocity flow v	[m s ⁻¹]	0.06 ± 0.01^A	0.13 ± 0.02^B	0.22 ± 0.07^B	0.19 ± 0.05^B
Temperature T	[°C]	6.8 ± 0.3^A	6.4 ± 0.08^A	13.5 ± 1.51^B	11.8 ± 1.35^B
Electric conductivity	[$\mu\text{S cm}^{-1}$]	345 ± 23^A	424 ± 15^B	310 ± 31^A	299 ± 30^A
pH	[$-\log H^+$]	7.4 ± 0.05^A	7.3 ± 0.05^A	8.0 ± 0.09^B	8.1 ± 0.3^B
Oxygen O ₂	[mg l ⁻¹]	10.2 ± 0.3^A	9.1 ± 0.3^A	9.0 ± 0.3^A	9.8 ± 0.5^A
Oxygen O ₂	[%]	95.5 ± 2.2	84.6 ± 2.6	97.4 ± 1.1	102.0 ± 2.1
Light	classes	2 (1.3) ^A	3 (3.4) ^A	3 (3.4) ^B	3 (3.3) ^B
Soluble reactive phosphorus SRP	[$\mu\text{g l}^{-1}$]	31.9 ± 2.4^B	4.9 ± 1.4^A	5.4 ± 0.8^A	5.3 ± 0.7^A
Nitrite-N NO ₂ ⁻ -N	[$\mu\text{g l}^{-1}$]	0.2 ± 0.1^A	0.2 ± 0.1^A	0.5 ± 0.1^B	0.6 ± 0.1^B
Nitrate-N NO ₃ ⁻ -N	[$\mu\text{g l}^{-1}$]	857 ± 84^A	1628 ± 167^B	184 ± 31^C	197 ± 29^C
Ammonium-N NH ₄ ⁺ -N	[$\mu\text{g l}^{-1}$]	6.35 ± 4.1^A	2.45 ± 1.4^B	8.87 ± 2.1^A	7.14 ± 2.6^A
Organic bound nitrogen N _{red} -N	[$\mu\text{g l}^{-1}$]	113 ± 24^A	75 ± 14^A	345 ± 39^B	347 ± 61^B
Total alkalinity	[mval l ⁻¹]	2.8 ± 0.3^A	3.3 ± 0.4^B	2.6 ± 0.3^A	2.7 ± 0.3^A
Pelagic dry mass PDM	[mg l ⁻¹]	8.6 ± 7.0	7.1 ± 6.1	13.2 ± 7.3	5.4 ± 2.3
Pelagic ash mass PAM	[mg l ⁻¹]	7.7 ± 6.7	6.3 ± 5.7	10.6 ± 7.0	3.5 ± 1.5
Pelagic ash-free dry mass PAFDM	[mg l ⁻¹]	1.0 ± 0.4^A	0.8 ± 0.4^A	2.6 ± 0.4^B	1.9 ± 0.8^B

Table 3. Results of the Discriminant Analysis. For better visibility, loadings > 1.00 or < -1.000 are indicated in bold; ln = variable was ln-transformed prior to analysis; abbreviation codes were used also for other statistical analyses.

Environmental variable	code	Function		
		1	2	3
Eigenvalue		32.79	21.02	2.43
Wilk's Lambda		0.000	0.013	0.292
Significance of function		<0.001	<0.001	0.013
Ln Distance to bank [m]	Diba	-0.366	0.303	0.170
Ln Channel width [m]	b	-0.329	-0.673	1.393
Ln Water depth [m]	z	-0.631	-0.247	0.117
Soluble reactive phosphorus [$\mu\text{g l}^{-1}$]	SRP	0.409	1.285	0.236
Ln Nitrate-N [$\mu\text{g l}^{-1}$]	$\text{NO}_3^- \text{-N}$	1.735	-1.656	0.886
Ln Nred [$\mu\text{g l}^{-1}$]	Nred	-0.407	0.327	0.081
Water temperature [$^{\circ}\text{C}$]	Temp	-0.376	-0.766	0.624
Electric conductivity [$\mu\text{S cm}^{-1}$]	Cond	-1.412	0.311	0.039
Alkalinity [mval l^{-1}]	Alkal	0.064	0.029	-0.253
Oxygen [mg l^{-1}]	O_2	-0.390	0.496	0.797
Ln Pelagic ash-free dry mass [mg m^{-2}]	PAFDM	-0.097	-0.278	-0.352
Ln Pelagic dry mass [mg l^{-1}]	PDM	0.650	0.354	0.229
Relation N/P *100	N/P	0.849	0.182	-0.220
Benthic dry mass [g m^{-2}]	BDM	1.122	1.883	-0.682
Benthic ash mass [g m^{-2}]	BAM	-0.478	-0.944	-0.563
Benthic ash-free dry mass [g m^{-2}]	BAFDM	-0.264	-0.570	1.028

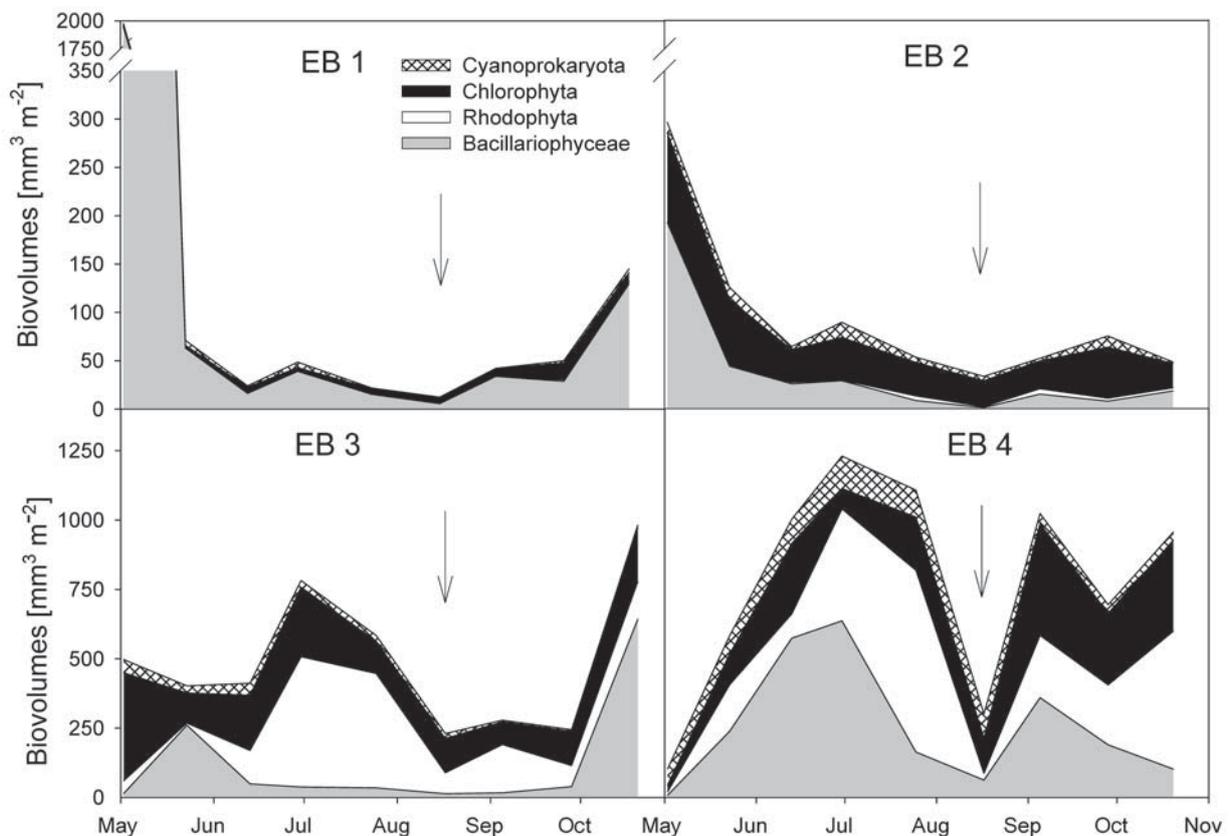
**Fig. 2.** Seasonal variability of algal biovolume and composition of the phytoplankton communities in two springs and the spring stream Almbach from May to October 2005. Data presented as geometric means of four replicates. Note different scalings. Arrows mark the high water event in August.

Table 4. Indicator Species Analysis with significant taxa ($p < 0.05$).

Site	Taxon	addition	Abbreviation code	Observed Indicator Value (IV)	Sig.
EB 1	<i>Diatoma mesodon</i>		Diame	100.0	0.001
	<i>Meridion circulare</i> var. <i>circulare</i>	small	Mercism	82.4	0.001
	<i>Cymbella</i> sp.		Cymb	81.2	0.001
	<i>Amphora inariensis</i>		Amphin	73.9	0.001
	Cyanoprokaryota	filamentous	Cyafi	52.6	0.016
	<i>Denticula tenuis</i>		Dente	43.7	0.024
EB 2	<i>Chamaesiphon</i> sp.	big	Chamb	51.0	0.003
	<i>Gyrosigma acuminatum</i>		Gyrac	33.3	0.036
EB 4	<i>Chamaesiphon</i> sp.		Chama	87.9	0.001
	<i>Chroococcales</i>	small	Chrsm	75.5	0.001
	<i>Phormidium</i> sp.		Phorm	75.5	0.001
	<i>Chroococcales</i>	middle	Chrmi	72.3	0.001
	<i>Oscillatoria</i> sp.	small	Oscsm	69.8	0.001
	<i>Nitzschia</i> sp.	15 μ m	Nitzty	62.1	0.005
	<i>Chantransia</i> -cells		Chant	61.3	0.004
	<i>Cocconeis placentula</i> var. <i>pseudolineata</i>	small	Coplsm	59.6	0.018
	<i>Achnanthes</i> sp., <i>Navicula</i> sp.	small	AcNasm	59.4	0.043
	<i>Chlorococcales</i>	big	Chlbg	48.3	0.012
	<i>Gongrosira incrustans</i>		Gonin	47.5	0.007
	<i>Achnanthes</i> sp., <i>Navicula</i> sp.	23 μ m	AcNami	43.4	0.049

Table 5. CCA summary, including correlation coefficients between the environmental variables and the first and second CCA axes for the phytobenthos indicator taxa. For better visibility, correlations > 0.400 or < -0.400 are in bold.

	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.400	0.114	0.091	0.041
Taxa-environment correlations	0.939	0.784	0.751	0.752
Cumulative percentage variance of taxa data	32.3	41.4	48.8	52.1
of taxa-environment relation	60.4	77.6	91.4	97.5

Environmental variable	Abbreviation code				
Soluble reactive phosphorus	SRP	0.792	-0.056	0.357	0.175
pH	pH	-0.407	-0.652	-0.253	0.050
Alkalinity [mval l ⁻¹]	Alkal	0.054	-0.029	-0.416	0.332
Benthic dry mass [g m ⁻²]	BDM	0.753	0.100	-0.092	-0.418
Shannon-Wiener-Index H' of Macroinvertebrates	Mi H'	0.527	0.037	-0.246	0.216

DA resulted in three significant functions which classified 100 % of the 4 sites correctly (Table 3). The first discriminant function explained 58 % of variance and separated spring sites EB 1 and EB 2 from the stream sites EB 3 and EB 4 (Fig. 3) with a high positive load of NO₃⁻-N and a negative loading of conductivity. Function 2 separated EB 1 from EB 2 due to a gradient of SRP and BDM (37 % of variance). Channel width and BAFDM loaded highly with function 3, which separated the two stream sites from each other. Remaining variables such as alkalinity or PAFDM ex-

erted only little influence on DA.

Qualitative phytobenthos analysis identified a total of 102 taxa, consisting of 88 Bacillariophyceae, 1 rhodophyte, 4 chlorophytes and 9 Cyanoprokaryota. Some ubiquitous such as *Cocconeis placentula* var. *lineata* (Ehrenberg) Van Heurck and the alkaliphilous species complex *Achnantheidium minutissimum* (Kützing) Czarnecki were found, but also species typical of springs (e.g., *Pinnularia appendiculata* (Agardh) Cleve in EB 1 and *Cymbella helvetica* Kützing in EB 2 (Niedermayr et al. 2009).

Quantitative analyses revealed 50 taxa. The seasonal variability and group composition of the phytobenthos at each sampling site is shown in Fig. 2. No significant difference was detected between EB 1 and the remaining sites, although total phytobenthos biovolumes differed significantly between EB 2, EB 3 and EB 4. At both springs, algal communities reached their biovolume maxima in May ($2000 \text{ mm}^3 \text{ m}^{-2}$ EB 1; $300 \text{ mm}^3 \text{ m}^{-2}$ EB 2), whereas biovolumes were lowest during summer ($< 100 \text{ mm}^3 \text{ m}^{-2}$). In contrast, biomass at the stream sites peaked in July and in October (about $1000 \text{ mm}^3 \text{ m}^{-2}$ at EB 3 and $1200 \text{ mm}^3 \text{ m}^{-2}$ at EB 4). After July, values decreased slightly, with the exception of a high water event in August, which considerably reduced phytobenthos biovolumes at the stream sites.

EB 1 was dominated by diatoms; at unfenced EB 2 also Chlorophyta came into play. At stream sites, dominant groups changed during sampling season (Fig. 2). EB 1 exhibited both highest Diversity Indices H' and Evenness V' ($H' = 2.2$; $V' = 0.8$), but more taxa were detected at EB 2. For stream site EB 3, lowest H' (1.4) and V' (0.5) were calculated. ISA yielded a total of 20 indicator taxa for all sampling sites investigated (Table 4). For EB 4, the highest number of indicator taxa were detected, comprising Cyanoprokaryota (5), Bacillariophyceae (4), Chlorophyta (2) and Rhodophyta (1) (Table. 4). With the exception of a filamentous cyanoprokaryote, exclusively diatoms were representative for EB 1, where *Diatoma mesodon* (Ehrenberg) Kützing, *Meridion circulare* var. *circulare* (Greville) Agardh (IV = 100 and 82), and *Cymbella* sp. (IV = 81) reached highest IVs. *Gyrosigma acuminatum* (Kützing) Rabenhorst and *Chamaesiphon* sp. were the only indicator species found at the unfenced spring EB 2. No indicator taxon could be detected for EB 3.

CCA was used to detect indicator species occurrences along the environmental data set (Monte Carlo test: $F = 11,25$; $p = 0.002$); forward selection reduced the environmental data set to 5 variables (SRP, pH, Alkal, BDM, $Mi H'$). In total, 98% of taxa-environmental variation was explained. The first two axes reached 78% (Table 5). A highly positive loading with SRP and BDM was calculated for the first CCA axis, which separated the spring site EB 1 from the other sites (Fig. 4A). pH was responsible for splitting EB 2 from the river sites along the

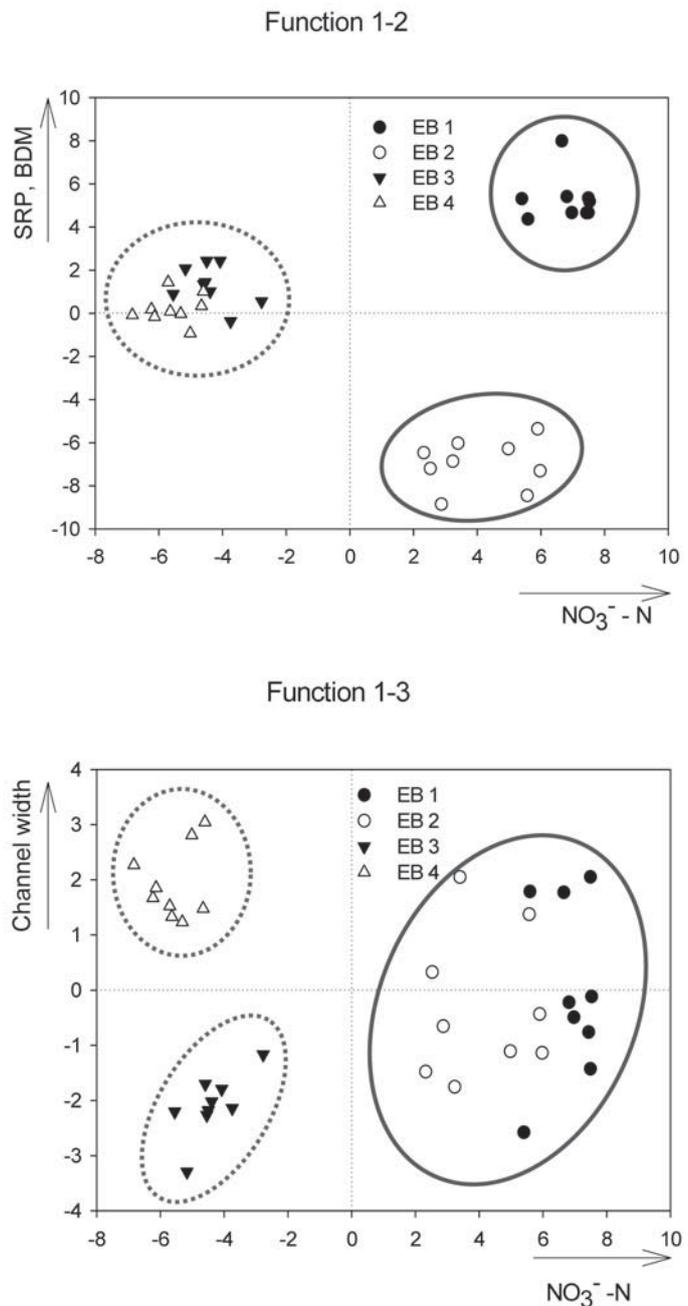


Fig. 3. Results of the three discriminant functions classifying the spring sites (EB 1 and EB 2) and the stream sites (EB 3 and EB 4).

second axis. *Denticula tenuis* Kützing, *Diatoma mesodon* and *Meridion circulare* var. *circulare* varied with SRP and BDM along the first axis. *Phormidium* sp., *Chamaesiphon* sp., the *Chantransia*-stages of *Batrachospermum* sp. and *Gyrosigma acuminatum* were highly associated with pH at axis 2. Alkalinity was an important environmental parameter for the distribution of *Gongrosira incrustans* (Reinsch) Schmidle.

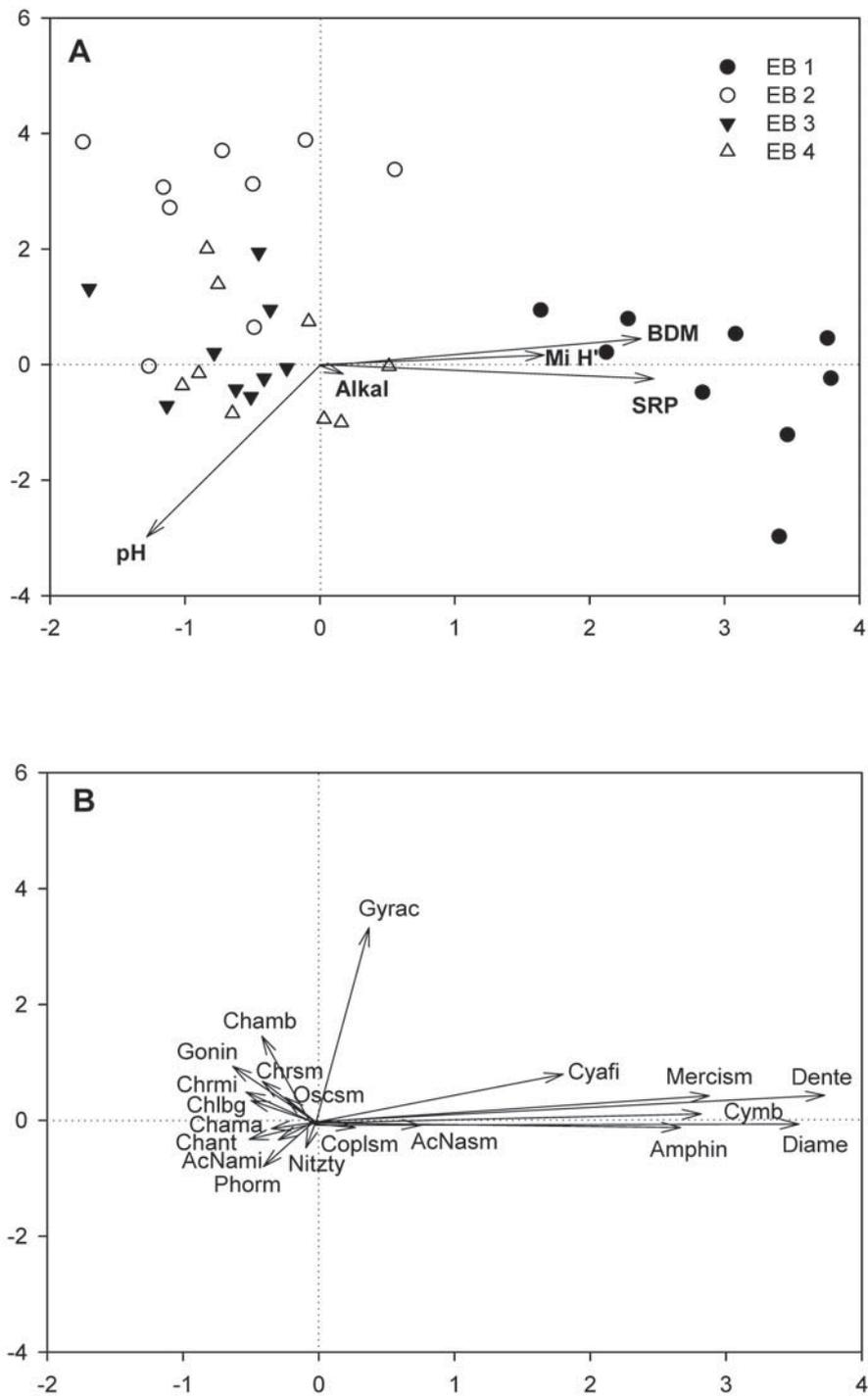


Fig. 4. (A) Biplot of the first two CCA axes for springs and stream sites and environmental variables. (B) Centroids of indicator taxa along the gradient axes 1 and 2. Abbreviation codes for environmental variables are given in Table 5, those for indicator taxa in Table 4.

Discussion

Snow melt and precipitation events diluted ions, which was indicated by decreased conductivity. In contrast to constant spring water temperatures, sampling sites

just a few metres downstream showed seasonal temperature changes typical of mountain streams. Significant differences in the selected hydrochemical parameters between the springs suggested that they are fed by groundwater from different areas. Elevated

SRP values at EB 1 probably originated from apatite layers in the bedrock, whereas cattle droppings are no doubt responsible for increased NO_3^- -N contents at EB 2. Interestingly, NO_3^- -N levels at the stream sites were already ten times lower than in EB 2 (Table 2), which could be related to nonpoint springs entering the Almbach and causing some dilution. Water input from nonpoint springs is reflected by the water depth and channel width increase along the Almbach stretch. Additionally, increased biofilm activity, most probably through phytobenthos nitrogen assimilation and microbial denitrification processes, might have played a role for the NO_3^- -N decrease (House et al. 2001). Battin et al. (2008) highlighted that biofilms typically dominate microbial life in ecosystems with high sediment-surface-area to water-volume ratios and high downstream transport. Furthermore, these authors pointed out that metabolic performance is highest in headwater streams, where most of the microbial biomass and metabolic processes are mainly associated with streambed surfaces, and where continuous surface-subsurface exchanges ensure replenishment of nutrients, substrates and oxygen, and the removal of metabolic wastes. Accordingly, BDM values were highest at stream sites, indicating a well-developed biofilm, exactly where low NO_3^- -N contents were measured. Very recently, Gesierich & Kofler (2010a, b) showed that algal assemblages of springs are mainly structured by NO_3^- -N amongst the nutrients, whereas the phosphorus supply seemed to be of minor importance.

Both springs reached their maximal algal biovolume in May, followed by a strong decrease persisting until the end of the study period. At fenced EB 1, the development of riparian vegetation led to almost complete shading, which limited phytobenthos growth. This agrees with the findings of Cantonati & Pipp (2000), who stated that light supply acts as a key factor for epilithic diatom communities in a mid-altitude carbonate rheocrene in the Southern Alps. A study of Cantonati (1999) focusing on non-diatom taxa also demonstrated irradiance to be the most important factor for quantitative changes in headwater streams. Ward & Dufford (1979) also stated that seasonal changes in algal communities in permanent springs are commonly controlled by light supply. This, however, is not the case at unfenced EB 2. Here, cattle grazed on the riparian vegetation, which in principle would increase light supply. However, bank and streambed stability were heavily disturbed by livestock trampling, dampening phytobenthos development by mechanical stress.

Undisturbed spring EB 1 was dominated by diatoms throughout the year, supporting Cantonati's (1998) results that diatoms are the most important component of the microflora in mountain springs. We found such a pattern only at the undisturbed EB 1. In contrast, at EB 2 with unrestricted livestock access, Chlorophyta and to a lesser extent Cyanoprokaryota were the most prominent algal groups. Both groups are apparently able to handle frequent disturbances and are sometimes also indicators for eutrophication. At stream sites, Rhodophyta reached high biomass and were exclusively represented by *Batrachospermum* sp., which strongly prefers clear, cold, fast-flowing, shaded streams rich in oxygen (Knappe et al. 1996).

Phytobenthos biomass was lowest at spring sites, where grazers reached highest abundances (see also Pokorny et al. 2008). Additionally, EB 1 and EB 2 exhibited higher V' than the stream sites; springs therefore sheltered numerous species with rather equal abundances. These findings are in accordance with Liess & Hillebrand (2004), who observed that grazing reduced algal species richness but increased V' . Steinman (1996) found that herbivores may reduce algal biomass if grazer densities and consumption rates are sufficient to exceed algal productivity (top-down control). Conversely, if rates of grazing or grazer densities are low, either grazer feeding morphology is not well adapted to dominant algal forms, or biomass accrual is constrained by limited resources irrespective of grazers (bottom-up control, Steinman 1996).

Interestingly, both sites with no livestock access (EB 1 and EB 4) exhibited a high number of indicator taxa, whereas EB 2 and EB 3 comprised only two indicator species in total. Frequent mechanical disturbances at EB 2 and EB 3 probably facilitated the development of ubiquitous like *Gyrosigma acuminatum* (Krammer & Lange-Bertalot 1986). This taxon also indicates some siltation at EB 2 caused by livestock trampling. EB 1 showed a *Diatoma-Meridion* association comparable to that previously described by Margalef (1949) to characterize calcareous springs and streams at medium altitudes (500–1500 m). Both taxa can be regarded as crenophiles according to Krammer & Lange-Bertalot (1991a). Rushfort et al. (1986) also found *Diatoma mesodon* as one dominating species in springs even in the Mount St. Helens Region (USA). Very recently, Gesierich & Kofler (2010b), in their broad survey, defined 5 groups of diatom assemblages, which were mainly defined by geology of the catchment area, pH and nutrients (especially NO_3^- -N). They demonstrated that *Achnantheidium minutissi-*

mum, *Diatoma mesodon* and *Meridion circulare* were quite abundant in some groups.

In recent years, many studies have evaluated the use of phytobenthos for bioindication purposes, and this approach is now part of assessment programs. Springs and their organisms, however, are still underexplored. The Austrian quality assessments program for rivers can be applied to extreme biotopes like bogs or springs, but the results have to be interpreted cautiously. There is a great need for studies focusing of such rare biotopes. This calls for developing a special sampling design for them because several guidelines of the Water Framework Directive in Austria for rivers cannot be fulfilled easily for these biotopes (e.g., a minimum of 20 m has to be sampled). For the Eastern Alps, a bioregional approach to differentiate between rheocrenes has been conducted very recently. This revealed that the distribution of the non-diatom community better reflects the bioregional approach for these environments than diatom taxa (Gesierich & Kofler 2010a). Among non-diatom taxa, cyanoprokaryotes seemed to be dominant in a broad range of rheocrenes (Gesierich & Kofler 2010a). Gesierich & Kofler (2010b) found that most of the frequent diatom taxa in rheocrenes were also common at lotic sites, and only 9 % could be classified as crenophiles.

Summarizing, we found significant differences in the phytobenthos with regard to community structure, biomass and seasonal development. We detected distinctions between fenced EB 1 and EB 2 with livestock access in terms of species composition. Weigand et al. (2002) reported an improvement of the spring's overall ecological situation after protection measures took place. The riparian vegetation recovered, the benthic substrate became coarser, and the proportion of zoo-benthic crenobionts increased. From these results, we assume that the dominance of Bacillariophyceae containing crenophilous species is evidence of recovery at EB 1. Finally, we show taxa preferences concerning specific pH, phosphorus or alkalinity values. Our study confirms the urgent need for management tools to protect mountain springs in pastured mountain areas. Otherwise, the phytobenthos might shift from a natural diatom-dominated community containing some crenophilous taxa towards a Chlorophyta- and Cyanoprokaryota-dominated scum – all induced by altered light conditions, eutrophication processes and reduced bank stability.

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