Research in Microbiology 167 (2016) 325–333

www.elsevier.com/locate/resmic

Original article

Spatial and temporal variability of bacterial communities in high alpine water spring sediments

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Received 20 March 2015; accepted 21 December 2015
Available online 9 January 2016

Abstract

Water springs are complex, fragile and taxa-rich environments, especially in highly dynamic ecosystems such as glacier forefields experiencing glacier retreat. Bacterial communities are important actors in alpine water body metabolism, and have shown both high seasonal and spatial variations. Seven springs from a high alpine valley (Matsch Valley, South Tyrol, Italy) were examined via a multidisciplinary approach using both hydrochemical and microbiological techniques. Amplified ribosomal intergenic spacer analysis (ARISA) and electric conductivity (EC) measurements, as well as elemental composition and water stable isotopic analyses, were performed. Our target was to elucidate whether and how bacterial community structure is influenced by water chemistry, and to determine the origin and extent of variation in space and time. There existed variations in both space and time for all variables measured. Diversity values more markedly differed at the beginning of summer and then at the end; the extent of variation in space was prevalent over the time scale. Bacterial community structural variation responded to hydrochemical parameter changes; moreover, the stability of the hydrochemical parameters played an important role in shaping distinctive bacterial communities.

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Keywords: Water springs; Bacteria; ARISA; Fingerprints; Hydrochemistry; Alps

1. Introduction

Water spring ecosystems are particularly complex and sensitive due to their being ecotones between aquatic and terrestrial ecosystems, as they share characteristics and vulnerabilities of both [1]. Furthermore, water springs are refuges for sensitive organisms or relict species [2,3]. The size and patchy distribution of water springs within catchments creates a situation of low connectivity between them. Therefore, each water spring is a partially isolated, unique ecosystem [3]. All the above mentioned features make water springs ideal "natural laboratories for ecological studies" [4]. The hydrogeochemistry of spring water depends on three main variables: (i) the residing time of water in the aquifer; (ii) the reactivity of the rock substrate; and (iii) the pH and chemical composition of infiltrating waters [5]. Another feature that makes water spring unusual systems lies in water parameters (e.g. ionic enrichment and electric conductivity) that, in water springs, are mostly stable compared to those of surface-fed streams [6]. High mountain ecosystems, which are sensitive to natural or man-induced disturbances [7], often contain many water springs; therefore, at high altitudes, alpine water springs may experience dramatic consequences due to environmental changes.

The traditional tools used in water spring monitoring programs are based on measurement of chemical or physical parameters, whereas, when biotic components are included, studies usually focus on macrobenthos [8,9]. Studies on

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http://dx.doi.org/10.1016/j.resmic.2015.12.006
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microorganisms mainly deal with photosynthetic organisms like diatoms or microalgae [10,11]. Spitale et al., in 2012 [12], carried out an extensive study on spring biota in an adjacent region. They found that a classification system based on spring biota (although prokaryotes were not included) was coherent, to a certain extent, with the traditional system based on water current velocity at the spring mouth [12,13]. Few studies focused on bacterial communities in water springs, and most were carried out in hot springs [14], mainly due to their interest for biotechnology and possible ancestral hyperthermophiles [15]. As stated by Schimel 2001, until a few years ago, ecosystem modeling rarely took into account the microbial contribution to biogeochemical cycles, since their contribution was considered constant [16]. Bacterial communities are indeed relevant heterotrophic components in alpine water bodies, especially above the tree line: Their key role in stream respiration (a decrease in dissolved oxygen over time) is well known [17,18], as their activity enriches both sediment and the water of organic matter. Bacterial communities in alpine streams have shown high seasonality [19,20] and marked spatial diversity [21].

However, since those studies were carried out on riverine and lacustrine ecosystems, very little is known about spatial and temporal variation in microbial communities in water springs at high altitudes. Furthermore, in very few case studies was the focus on spring systems lying in oligotrophic high mountain environments such as alpine glacier catchments [3].

A rapid, inexpensive and reliable technique for detecting changes in bacterial community structure from environmental samples is amplified ribosomal intergenic spacer analysis (ARISA). This method has high resolution, since it is based on a genetic region (internal transcribed spacer or ITS), which is highly polymorphic in its length [22]. Therefore, it can provide a reliable reproducible image of variation in community structure even in very similar communities. In addition, there exist optimized protocols for data handling and parsing. The aim of the present study was to answer the following question: Does water origin and chemistry influence bacterial community structural shifts? And does bacterial community structure in water spring sediments depend on a spatial rather than on a temporal scale?

2. Materials and methods

2.1. Study site and sampling

The area of interest is located in the upper part of the Saldur Basin, corresponding to the Matsch Valley (total area 96 Km^2),

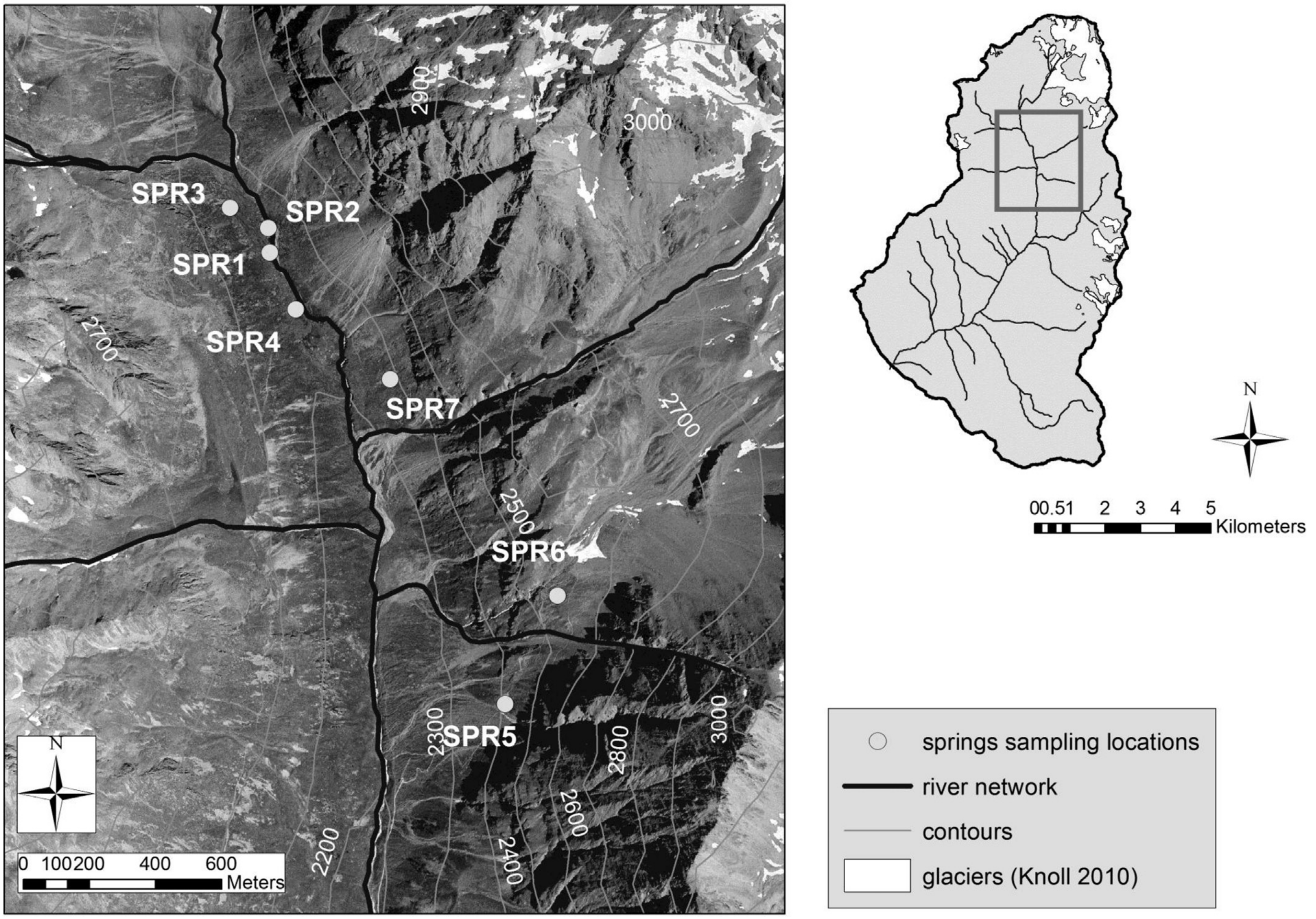

Fig. 1. Geographical locations of the water springs in the Matsch Valley.

a southeast-oriented valley in South Tyrol (Italy), which is a typical U-shaped valley with the bottom partially filled with sediments from screen (Fig. 1). The main water storage is the Matsch Glacier, which lies between 2700 and 3700 m above sea level (a.s.l.) with an extent of 2.3 km^2 (in 2013). Rock glaciers are also present, but their connectivity to the main channel is limited [23]. Average annual rainfall in the lower valley is about 550 mm, but it increases with altitude up to 1000 mm in the upper part (source: Hydrographic Office, South Tyrol). The Saldur Basin belongs to the Matsch Unit within the Ötztal-Stubai complex, and consists mainly of gneisses, mica gneisses and schists [24]. The investigated portion of the Saldur catchment lies entirely above the tree line (i.e. above 2200 m a.s.l.) with only recent vegetation colonization such as *Nardion strictae* at the bottom of the upper valley, while shrub vegetation Rhododendro-Vaccinion covers mainly the valley slopes. Seven springs (named with the prefix SPR- and progressive numbering) were selected according to logistic accessibility and spring type. While SPR1−4 was located at the bottom of the western valley slope, SPR5-7 was situated at higher elevations along the eastern valley slope, where infiltrating water possibly met the waterproof layer of the bedrock outcrop and exfiltrated to form the springs. The spring type was determined according to traditional classification parameters developed by Thienemann [13]. Five springs were classified as rheocrene, one as limnocrene and one was a transition between rheo- and limnocrene. Three of them were on a slope (SPR-1, -5 and -7) and the remaining four were on a flat area (Table 1). Four time points were chosen, between the snowmelt period to the early snowfall (corresponding to the months of June, July, August and September). This choice is due to the fact that only during summer were all seven springs accessible. For consistency, sampling was carried out during the same time interval defined from 12 am to 4 pm. Previous measurements showed that the main melt water contribution was from snow in June and July (recent and old snowmelt, respectively), from glacier ice in August and from rainfall in September [25]. Therefore, the assumption that a single day is representative of the monthly scale is legitimate. Using sterile vials, two samples of superficial benthic sediments (at a depth between 0 and 5 cm) were collected from the bottom of each water spring, for a total of 56 samples (2 replicates for 7 springs at 4 different time points). Vials were then stored at −80 °C until extraction. Water parameters were measured for each spring at each time point simultaneously with sediment sampling. Water samples from springs were collected in 50 ml high-density plastic bottles with a double cap, leaving no headspace. The samples were kept at 4 °C in the dark before isotopic analysis. Analyses of δ^2H and δ^{18}O were performed at the Laboratory of Isotope and Forest Hydrology of the University of Padova, Department of Land, Environments, Agriculture and Forestry, by an off-axis integrated cavity output spectroscope (model DLT-100 908-0008, Los Gatos Research Inc., USA) [26,27]. The instrumental precision (as an average standard deviation of 2094 samples) was 0.5‰ for δ^2H and 0.08‰ for δ^{18}O. The isotopic composition was calculated as δ^2H (in ‰) ratio of the sample to the Vienna Standard Mean Ocean Water (VSMOW) [28]. A derived measure of the isotopic values was the deuterium-excess (DE), defined as $\delta^2\text{H} - 8\delta^{18}\text{O}$, which is useful for determination of the provenance of water [29]. EC was measured in the field by a portable conductivity meter (WTW GmbH, Germany) with a precision of ±1 μScm^{-1}. pH from sediment was measured on 1 g (dry weight) in 10 mL of distilled water using a Crison Basic 20 pH meter. Separately, water samples were taken for elemental analysis; they were filtered with Whatman® 0.2 micron filters and sent to the Department of Agricultural Sciences (DIPSA) of the University of Bologna for elemental, total organic carbon (TOC) and total organic nitrogen (TON) analysis.

2.2. Molecular analysis

DNA was extracted from 0.25 to 0.50 g of sediments. The extraction was done with the Ultra Clean kit (MOBIO) following the manufacturer's instruction, with minor modifications. The protocol used for ARISA followed Cardinale et al. (2004) [30]. All PCRs were done in an Eppendorf MasterCycler™. PCR products were first screened on agarose gel in order to estimate product concentration and then sent to STAB Vida Lda. (Caparica, Portugal) for capillary electrophoresis using 40 s of injection time with a size standard LIZ-

Table 1
Description and location of each water spring.

Spring	Altitude	UTM coord.	Classification	Location and prominent features
SPR1	2379	N 629615,61 - E 5181043,32	Rheocrene	On a hillslope on the orographic right side, surrounded by gravel, high variation in discharge
SPR2	2361	N 629730,93 - E 5180982,00	Limnocrene	Fine sediment texture, surrounded by iron-rich schist, located in the middle of the valley, stable and slow discharge flow
SPR3	2356	N 629735,58 - E 5180908,73	Rheocrene	Similar to SPR-1, sandy sediments in the bottom, very close to the main river, water flow ended shortly after in the river
SPR4	2343	N 629814,68 - E 5180736,50	Rheo-Limnocrene	On a flat area formed by a moraine, characterized by evident bubbling of sandy sediments, mineral acidic soil
SPR5	2365	N 630605,65 - E 5179869,32	Rheocrene	On a hillslope on the orographic left side of the valley, mineral acidic soil, the highest EC values registered
SPR6	2502	N 630099,90 - E 5180524,30	Rheocrene	On a plateau on the orographic left side of the valley, mature acidic soil.
SPR7	2335	N 630448,01 - E 5179540,81	Rheocrene	On a hillslope on the orographic left side as SPR-5, surrounded by abundant vegetation, mature acidic soil

1200. Electropherograms were analyzed using Peak Scanner Software 1.0. We set fluorescence intensity for peak recognition of 0.01% and size limits of 150–1600 base pairs. The resulting matrix was eye-checked against electropherograms and adjusted accordingly.

2.3. Statistical analyses

The two matrices containing, respectively, the environmental parameters and the site by OTU of each sample were imported in an R environment for statistical analyses [31]. The Shannon (H′) index was calculated for each sample in order to compare variations in alpha diversity among the different springs during summer. Comparisons between variables from different springs or months were performed using the Mann–Whitney test. Principal component analysis (PCA) was carried out on the matrix containing only element measurements. This analysis was used to assess how elemental composition of the water from different springs varied in both space and time. PCA is a rigid rotation of multivariate data. It is a very efficient method, but it has weaknesses connected to data scales. Therefore data were normalized with the formula $(x - \bar{x})/\sigma$, where x is each value, $\bar{x}$ is the average for all the measurements of that parameter and σ is the standard deviation. Canonical correspondence analysis (CCA) was performed using the specific R function from the "vegan" package. CCA is a very popular ordination technique in community ecology. It fits sample points on an ordination space where axes are linear combinations of environmental variables, and it is possible to draw vectors starting from the origin and corresponding to each environmental variable alone. The data points that lie closest to the end of a vector correlate more closely with that environmental variable [32].

3. Results

3.1. Water parameters

Table SM1 shows chemical and physical water parameters measured in the seven springs at the four time-points. There existed pronounced variations in the concentration of the solutes among the springs. Spring SPR-4 showed the lowest average concentration for Ba, Ca, Mg, Na, S and Sr, while SPR-5 showed the highest average concentration for Ba, Ca, Li, Mg, S, Si, Sr and Zn. Carbon and nitrogen content showed distinct trends among the springs. Springs SPR-2 and -3 had the highest values at the beginning of the summer, and springs SPR-5 and -6 had the lowest values at the end of the summer. Fluctuation in the values also occurred throughout the summer. Only spring SPR-1 showed a continuous increase in TOC. C/N ratios also showed differences among the springs. SPR-4 showed the highest values throughout the summer (besides the month of June, the second highest), whereas SPR-6 constantly had the lowest values (Fig. 2). For most of the springs, a lower peak in the month of July was observed. TON showed patterns similar to TOC, alongside SPR-4 that had lower values during the entire summer (Fig. 2). Springs SPR-3 and SPR-6 had, respectively, the highest and lowest average carbon content, while the nitrogen content was comparable in all springs, alongside spring SPR-4, where it was the lowest (Fig. 2). Fig. 3 shows the PCA of the matrix obtained from the elemental composition data of the waters. The first two axes of variation explained, respectively, 43.1% and 17.3%, with a cumulative percentage of variation of 60%, with the third axes explaining a further 12% in variation. The higher loadings on the first axis were from Ca, Mg and Sr, while the highest loadings on the second axis were Ni, K and Mn. From this plot, it was possible to observe that spring SPR-5 was far different in elemental composition from the others. The pH was circumneutral for all springs, being more acidic for SPR-2 and slightly more basic for SPR-4 (Fig. 4). There was a general fluctuating trend; for all springs alongside SPR-2, the pH at the beginning of the summer was slightly more acidic. SPR-5 had distinctly higher values of deuterium, along with the lowest values of DE during the entire season. Table 2 shows p-values derived from the Mann–Whitney test for each pairwise comparison for DE. No significant differences were detected on the time scale in isotopic signatures, although some springs showed isotopic depletion according to snowmelt and others did not (data not shown). Pearson's r- and relative p-value were calculated among all environmental variables measured. Table 3 shows only strong and significant correlations (Pearson's $r > 0.75$ and $p < 0.01$).

3.2. ARISA analysis

The number of OTUs ranged from 20 to 250, with an average of 79.8 and a standard deviation of 37.6 (Table SM1). The Shannon index ranged from 2.98 to 5.08, with an average of 4.23 and a standard deviation of 0.41. The Shannon index (H′) showed an overall peak in August, but it had a wide range of values throughout the summer. In June, values of H′ in the springs located on the hill slope were greater than those at the bottom of the valley (with a p-value > 0.01, Mann–Whitney test; Fig. 5). The CCA plot shows a clustering pattern that responded to three main environmental drivers. One data cloud in the first quadrant contained SPR-2 and -3 and correlated with the TOC. Another quadrant correlated with TON, K and Zn and contained SPR-1 together. SPR-5 and -6 formed a cluster in the third quadrant that correlated with the pH, EC, S, Mg, Si, Cu and Li; SPR-7 had scattered distribution along the second and third quadrant. Spring SPR-4 clustered far apart from all others in the fourth quadrant. Samples from this spring correlated negatively with TON, Zn and K (Fig. 6). The variables measured were representative of the real gradient, because the eigenvalue of the first axes in all constrained analyses is always larger than the corresponding unconstrained axes. Table 4 shows the elements that significantly correlated with variation of the entire bacterial communities, outputted by the R function envfit(). There were three main environmental drivers. The first axes of the ordination plot had negative correlation (<-0.75, $p < 0.05$) with EC (and consequently with Ca, Cu, K, Ni, Sr and Zn). The second axis

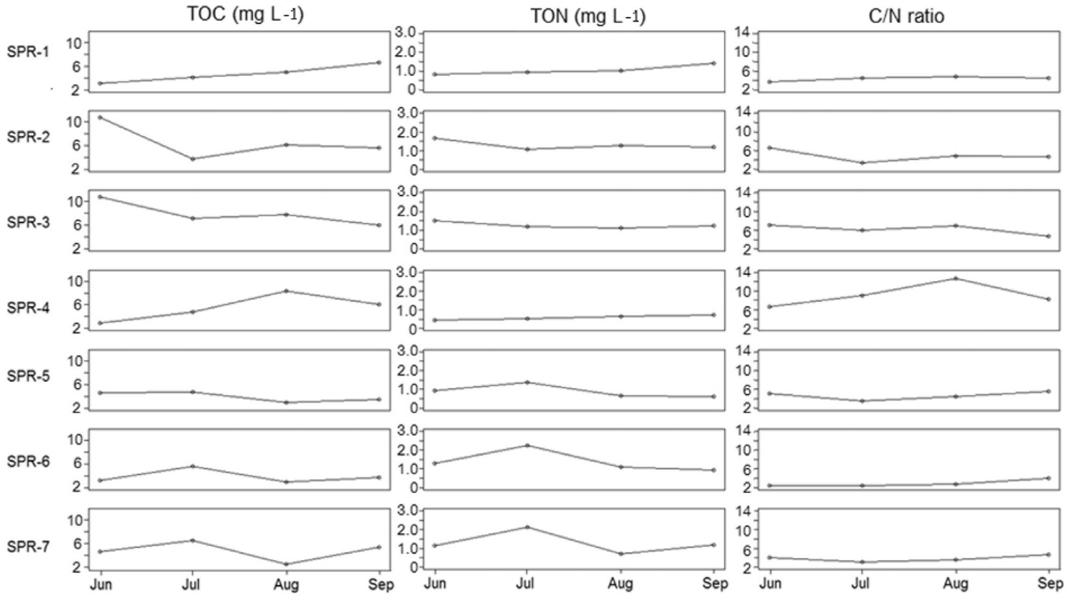

Fig. 2. Total organic carbon (TOC) and nitrogen (TON) for each water spring.

had positive correlation (>0.75, $p < 0.05$) with TOC and DE, while it had negative correlation with pH, Si, Li and ^{2}H.

4. Discussion

The seven springs analyzed in this work belong to a complex aquifer system with only little information available. The relatively contrasting geochemistry suggests that the water comes from different flow paths and possibly one aquifer in the valley bottom and another on the side of the valley. Hydrochemical factors have been shown to play a key role in determining the distribution of biota in crenic waters [33]. It is important to know how bacterial communities respond to those variations, as they are key players in almost all ecosystem processes. EC, isotopic ratios for ^{18}O and ^{2}H, TOC, TON and elemental analyses were applied to water samples, while PCR-based fingerprinting was performed on environmental DNA from sediment samples. There were two dimensions conceived in this study − a spatial and a temporal dimension, as different springs from the same valley were sampled four times throughout the summer.

In our survey, EC shows a high (Pearson's $r > 0.85$) and significant ($p < 0.01$) correlation with Ca, Li, Mg, S and Sr (Table 3); it is noteworthy that isotopic values showed a fairly high (Pearson's $r > 0.75$) yet significant ($p < 0.01$) correlation with EC. Another indication provided by EC is related to the time that water is stored as groundwater before being discharged into the surface as a spring or by feeding streams and lakes. Water accumulates ions until saturation during its period of permanence in contact with the rock substrate. The contact time of this subsurface water is probably longer for the springs on the left side of the valley, and by far the longest in spring SPR-5.

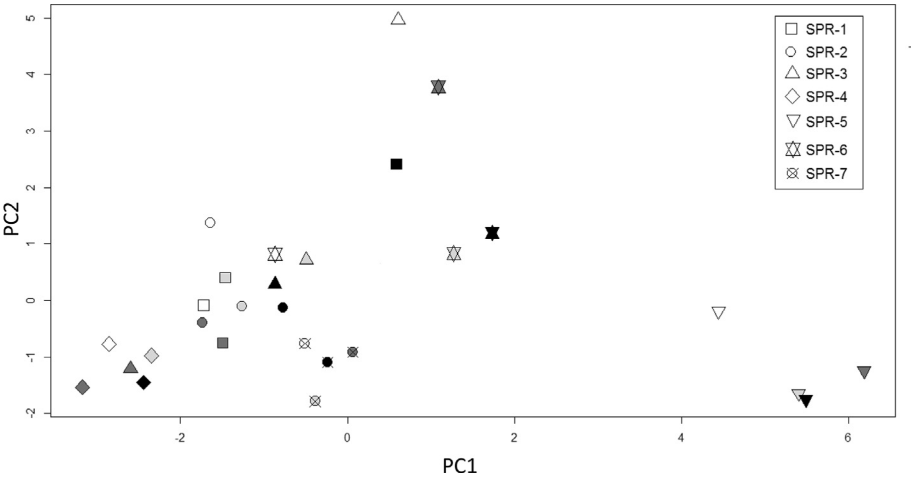

Fig. 3. Principal component analysis of the elemental composition of the waters. Colors represent each month: white, June samples; light gray, July; dark gray, August; black, September.

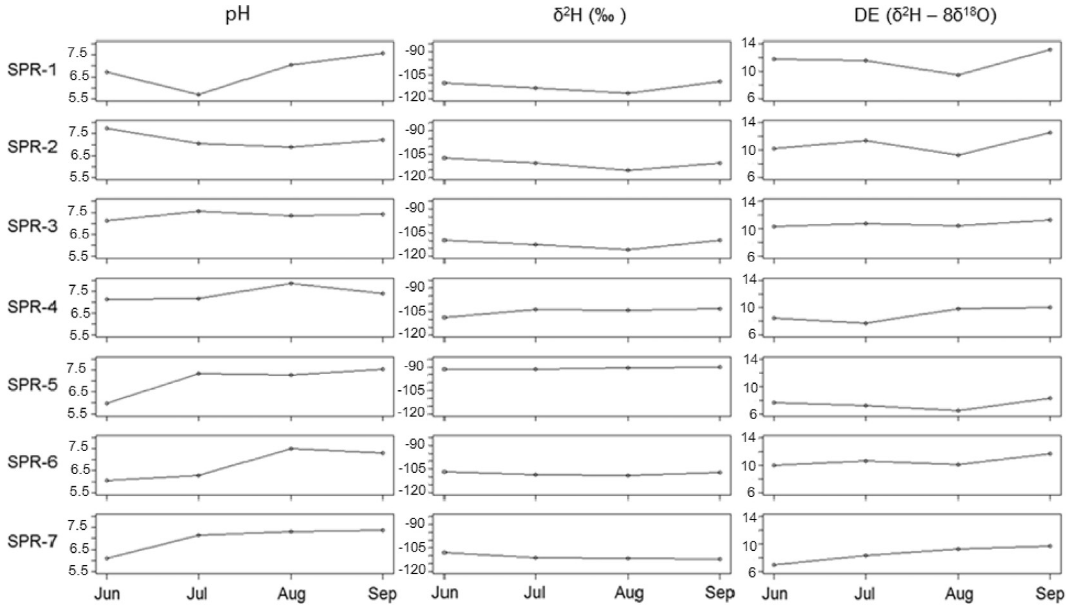

Fig. 4. pH and isotopic signature variations from each water spring throughout the summer.

Table 2
P-values according to Mann–Whitney test in values of DE.

	SPR1	SPR2	SPR3	SPR4	SPR5	SPR6
SPR2	0.486					
SPR3	0.343	1.000				
SPR4	0.114	0.114	**0.029**			
SPR5	**0.029**	**0.029**	**0.029**	0.057		
SPR6	0.486	0.886	0.686	**0.029**	**0.029**	
SPR7	0.057	0.057	**0.029**	0.486	0.200	**0.029**

In bold, significant p-values.

Table 3
Pearson's r correlation values among environmental parameters.

	^{2}H	^{18}O	Ca	Li	Mg	S	Si	Sr
^{18}O	**0.99**							
Ca	0.78	0.77						
Li	0.80	0.83	**0.92**					
Mg	0.76	0.76	**0.99**	**0.91**				
S	0.80	0.79	**0.97**	**0.91**	**0.97**			
Si	0.66[a]	0.63[a]	0.83	0.80	0.84	0.86		
Sr	0.73[a]	0.72[a]	**0.99**	**0.90**	**0.99**	**0.96**	0.84	
EC	0.77	0.76	**0.99**	**0.91**	**0.97**	**0.97**	0.82	**0.97**

[a] Pearson's r < 0.75, p-value < 0.01.

The concentration of the TOC, TON and elemental composition varied among the seven water springs exam. This may be due to the chemistry of the mineral substrate, but also to the discharge flow variation and to the sediment particle size (both described in Table 1).

Spring SPR-6 showed the highest concentration of nickel, with a range of 25–56 ppb. The concentration of silicon in the springs of the hill slope was always more than twice that of those at the bottom of the valley. This may be due either to a slightly different composition of the rock or to a higher content of diatoms (since silicates are important components of the frustule). Phosphorous was consistently below the detection limit, confirming previous studies that found phosphorus limitation in freshwater environments and in alpine springs as well [34].

The interpretation of the first axis of the PCA plot (Fig. 3) can stand in an ecological gradient of water content of the major cations (Ca^{2+}, Mg^{2+}, Sr^{2+}). The major cations are known to be present even in very diluted freshwaters and may be involved in the processes of attachment of bacterial cells to mineral surfaces [35]. The second axis is connected to oxidizing agents (Ni, K and Mn), and it could be related to the oxidizing activity of the water. Spring SPR-5 clusters far apart from the others, meaning that its elemental composition is very different. Samples from water springs SPR-4 and -7 cluster in close proximity to those in the PCA plot based on elemental composition, with little dispersion of the dots, highlighting the stability of the chemical composition through time. This is confirmed by the values of EC (Fig. 3, Table SM1).

Organic matter in a high alpine environment is usually scarce [36] and the little amount present may be in the form of small particulates and colloid materials which usually are not directly bio-available, since they are in polymeric form [37]. We expect that there is an increased microbial biomass at the end of the summer. It is known that, in rivers fed by ground water, microbial cell size and productivity are higher compared to glacier-fed rivers [36].

Differences in seasonal variation of isotopic and geochemical concentrations in spring water corroborate the assumption that there exist different water contributions and flow paths. The trend in isotopic depletion and geochemical enrichment in 2013 is in good agreement with spring monitoring since 2011 [38]. While isotopic depletion until August 2013 indicated increasing snowmelt contributions, isotopic enrichment revealed decreased snowmelt contributions in

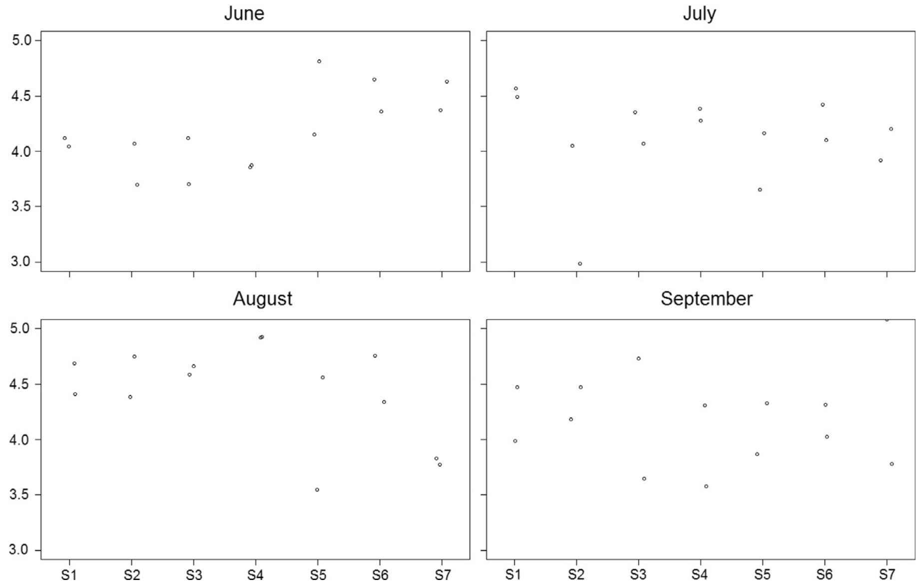

Fig. 5. Dot plots of values of Shannon diversity index (H′). Two replicates for each spring in each month were studied.

September. However, isotopic variations at SPR4 suggested the maximum contribution of snowmelt in June, with a gradual decrease over the season. In contrast, the EC concentration showed gradually increasing concentrations, instead of following isotopic behavior that had occurred during the previous years [38]. Therefore, the highest dilution with snowmelt water containing less solutes was anticipated, and already took place in June. Considering these spatial and temporal variations, SPR4 in the bottom of the valley and SPR5 on the hillslope have different water origins and/or potentially different flow paths compared to the surrounding water springs. Changes in hydrogeochemistry may be due to different chemical signatures of ice melting or to the water precipitation contribution. The isotopic ratios indicate that there is a change in the relative contribution on a spatial scale. The origin of the water in SPR-5 is clearly different from that of the others.

A high H′ reflects a wide genetic pool, thereby indicating a wide array of chemical reactions that can be potentially carried out by the bacterial community [39]. It is well known that bacterial community diversity does not follow the elevational

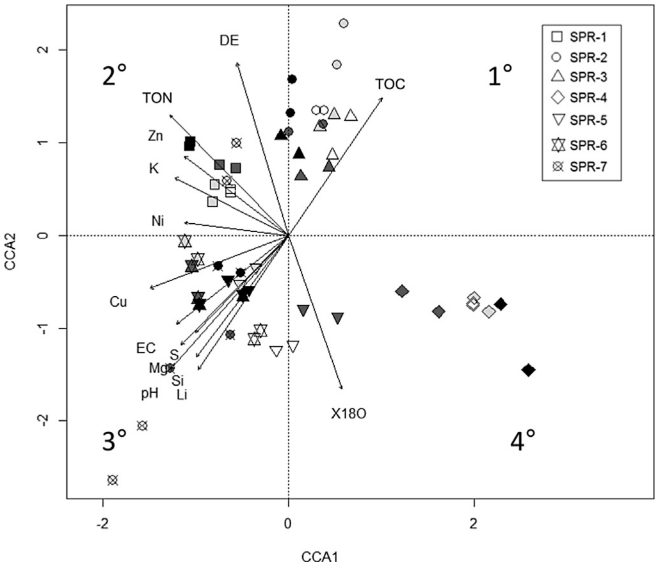

Fig. 6. Canonical correspondence analysis (CCA) showing the correlation between community and environmental data; due to multico-linearity, 8 environmental variables (namely ^{2}H, Ba, Ca, Fe, Mn, Mo, Na, Sr) are not shown in the plot. Colors represent each month: white, June samples; light gray, July; dark grey, August; black, September.

Table 4
Envfit() output showing correlation of environmental variables with each of the CCA axes.

	CCA1	CCA2	r2	Pr (>r)	
TOC	0.562	0.827	0.30	0.002	**
TON	−0.704	0.710	0.31	0.001	***
^{2}H	0.334	−0.943	0.23	0.002	**
^{18}O	0.324	−0.946	0.29	0.001	***
Ba	−0.909	0.417	0.04	0.338	
Ca	−0.766	−0.643	0.20	0.006	**
Cu	−0.934	−0.357	0.24	0.001	***
Fe	0.198	0.980	0.05	0.247	
K	−0.892	0.453	0.18	0.004	**
Li	−0.559	−0.829	0.28	0.001	***
Mg	−0.701	−0.713	0.25	0.002	**
Mn	−0.804	−0.595	0.00	0.984	
Mo	0.139	0.990	0.05	0.240	
Na	−0.269	0.963	0.01	0.799	
Ni	−0.993	0.119	0.12	0.038	*
S	−0.693	−0.721	0.20	0.007	**
Si	−0.607	−0.795	0.25	0.002	**
Sr	−0.809	−0.588	0.25	0.001	***
Zn	−0.797	0.604	0.19	0.007	**
EC	−0.785	−0.619	0.22	0.002	**
DE	−0.288	0.958	0.35	0.001	***
pH	−0.660	−0.751	0.34	0.001	***

Significance codes based on 999 permutations: 0 ***; 0.001 **; 0.05*.

gradient documented for other biota [40]. Indeed, it has been demonstrated that, in water systems, the trend is the opposite, with a growing diversity of bacterial communities at higher elevations [21]. In our case, we found greater richness in the springs at higher altitudes in June. This fact is in agreement with previous studies carried out on glacier streams [12,21,41,42], although our data show that this difference is not detectable later in the summer. Possible explanations for the higher beta diversity in high altitude springs in June may lie in the rapid turn-over dynamics of prokaryotes, and increased carbon availability in higher springs due to melting of glacier snow cover that mainly affects springs closer to the glacier terminus [41]. On the other hand, in late summer, different dynamics occur, such as the decrease in water deriving from snow cover melting, and the consequential increase in water from glacier ice melting and major eukaryotic competition and predation. These dynamics could affect the overall beta diversity of springs, canceling the differences between springs.

The change in water chemistry (EC, ionic strength, osmolarity, content of organic matter, metals, etc.) interacts with both the diversity and structure of bacterial communities to various extents [43,44]. A combination of biological (photosynthesis among them), chemical and physical (groundwater exchange) processes seem to play an important role in controlling diurnal fluctuation of EC in an alpine stream [45]. On the other hand, freshwater bacterial communities are known to follow seasonal fluctuations of water parameters on a yearly scale that are independent of the location, suggesting that closer springs do not always show similar trends [20]. An example of this behavior is SPR-4 where fingerprinting data reveal a community that is very different from all the nearby water springs. This may be due to an adaptation of the community to the stable conditions that characterize SPR-4, leading to a distinctive structure, a sort of “local” climax. Nonetheless, this situation is not visible in spring SPR-7, which also has stable hydrological parameters but a different ecology, as it is a proper rheocrene spring surrounded by vegetation.

In conclusion, our results suggest that the structure of bacterial communities in high mountain water spring sediments depends on the spring ecology, which is related to water origin and hydrochemical parameters. This is in agreement with previous knowledge about microbial communities of water bodies in high mountains. Interestingly, the spring in which hydrochemical parameter varied least showed a distinctive community, confirming that stability in water springs is one of the conditions that shapes the high degree of endemisms. Water spring environments host a large number of endemic species adapted to the specific conditions encountered there, and stability was shown to be among the most important features shaping spring biocenosis [3,10–12]. In this respect, our paper shows that microbial communities follow the same trend of macrobiota. Finally, multivariate analysis showed clusters made up of dots belonging to the same spring instead of the same month; hence, it can be concluded that the spatial scale tends to predominate over the temporal one, at least during the sampling period of choice.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors thank Dr. Erich- Ritter-Stiftung and Dr. Herzog-Sellenberg-Stiftung within the Stifterverband für die Deutsche Wissenschaft for financing the EMERGE project (CUP I41J11000490007). Funding also came from the Free University of Bozen/Bolzano Internal Funds TN5026 “Effects of climate change on high-altitude ecosystems”.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.resmic.2015.12.006.

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